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UNIVERSITÉ DE MONTRÉAL

AN APPROACH FOR STUDYING AND MODELING CROSS-BRIDGE BEHAVIOR IN SKELETAL MUSCLE CONTRACTION

par

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SOMMAIRE

Les muscles sont des effecteurs mécaniques des systèmes neuro-musculo-squelettiques sous le contrôle d'afférences du système nerveux central. Ils peuvent se contracter activement afin de produire une tension pour ainsi induire un mouvement au niveau des segments auxquels ils sont rattachés. Une bonne compréhension de la nature de la contraction musculaire et de ses propriétés est essentielle à l'étude du mouvement des membres (contrôle et aspects mécaniques), au développement de muscles artificiels ainsi qu'à la réadaptation de personnes ayant une atteinte au niveau du système neuromusculaire.

Selon la théorie de la contraction musculaire généralement acceptée des "filaments glissants/ponts transversaux" (cross-bridge, sliding-filament theory), les phénomènes mécaniques se produisant au niveau du muscle sont associés à l'ensemble des comportements des complexes d'actomyosine. Les techniques de mesure actuelles ne peuvent toutefois nous éclairer que partiellement sur les aspects mécaniques du complexe d'actomyosine. La modélisation peut donc être utilisée pour permettre l'avancement des connaissances en ce qui a trait aux propriétés de l'actomyosine afin de prédire les caractéristiques mécaniques du muscle. Des données provenant d'hypothèses basées sur des

études récentes portant sur la structure, la physiologie, la biochimie et la modélisation musculaire ont été incorporées dans un modèle en accord aver la théorie des filaments glissants, ceci dans le but d'étudier les caractéristiques dynamiques des ponts entre l'actine et la myosine. Le comportement de ces ponts a été caractérisé par deux états: un état sans production de force et un état avec production de force, ce dernier étant équivalent à l'état de "forte liaison" dans le modèle d'Huxley. Lors de l'état de production de force, les ponts entre l'actine et la myosine sont entraînés dans un processus mécanique continu qui est contrôlé par leur capacité à convertir l'énergie chimique provenant de l'hydrolyse de l'ATP en énergie mécanique. Une tension due à l'allongement des éléments élastiques des ponts se développe graduellement entre les filaments d'actine et de myosine. Le taux de développement de la tension dépend de réactions biochimiques, mais peut aussi être affecté par des changements mécaniques au niveau des ponts.

Les caractéristiques mécaniques d'une vaste population de ponts entre l'actine et la myosine sont représentées en termes du nombre de ponts formés lors de l'état de production de force et de l'allongement moyen de leurs éléments élastiques. Des expressions mathématiques ont été dérivées pour le taux moyen de développement de force afin de calculer l'étirement moyen des éléments élastiques des ponts et pour le taux moyen de transition de l'état de production de force à l'état sans production de force, et vice-versa, afin de calculer le

nombre de ponts lors de l'état de production de force. La validité du modèle est basée sur le fait qu'on puisse, à partir de celui-ci, faire des interprétations physiques et des prédictions quantitatives du comportement musculaire. Plus spécifiquement, il a été possible de prédire adéquatement et d'interpréter des comportements musculaires tels que les relations force/vélocité et taux de libération d'énergie/vélocité, les réponses transitoires à des changements brusques de longueur et de charge ainsi que les effets de la température sur les réponses musculaires transitoires.

Dans la présente étude, il a été démontré que le taux de développement de tension des ponts est probablement la variable la plus importante pour décrire les caractéristiques dynamiques du muscle. Ce taux ne dépend pas seulement de la vitesse de glissement des filaments mais est aussi influencé par les réactions biochimiques et les changements structuraux se produisant lors de l'interaction entre les molécules d'actine et de myosine. Il répond beaucoup plus rapidement aux changements que le taux de transition des ponts. Un lien entre les événements biochimiques et mécaniques qui se produisent lors d'une contraction musculaire est établi dans notre modèle par le fait que ces événements sont tous deux fonction de la vélocité angulaire de l'extrémité ("head") des ponts. Il est aussi démontré que la rigidité ("stiffness") musculaire, ou nombre de ponts dans l'état de génération de force, est contrôlée par des afférences nerveuses (concentration de Ca), la concentration d'ATP et le

taux de changement de la longueur du muscle alors que l'allongement des ponts est déterminé principalement par le taux de changement de la longueur du muscle. Cette dépendance constitue une boucle de rétroaction négative pour stabiliser le comportement du muscle. Il a aussi été observé, toutefois, que la tension musculaire peut osciller autour de la valeur d'une charge appliquée après un changement soudain de charge, agissant ainsi comme un oscillateur actif. Ceci suggère que d'autres facteurs, tels que le patron de recrutement des fibres musculaires, la coordination musculaire et la rétroaction réflexe peuvent être nécessaires pour la production de mouvement stables.

ABSTRACT

In neuromusculoskeletal systems, skeletal muscles are mechanical actuators under the control of central nervous system inputs. They can actively contract to generate tension and hence drive the connected limb segments. A solid understanding of the nature and properties of muscle contraction is essential to the study of limb movements (control and mechanics), the development of artificial muscles, and rehabilitation in neuromuscular disorders.

According to the widely accepted sliding-filament, cross-bridge theory of muscle contraction, muscle mechanics is the ensemble behavior of actomyosin complexes. Current measurement techniques can provide only limited information about the mechanics of the actomyosin complex. Modeling can be used to advance the understanding of actomyosin properties as well as to predict muscle mechanics. In order to address cross-bridge dynamics, several assumptions were made based on recent studies of muscle structure, physiology, biochemistry, and modeling, and incorporated into a model consistent with sliding-filament, cross-bridge theory. We characterized cross-bridge behavior with two states: a non-force generating state and a force generating state, which is equivalent to the strong binding state in Huxley's model. During the

force generating state, cross-bridges undergo a continuous mechanical process regulated by their ability to convert the chemical energy from ATP hydrolysis into mechanical work. Tension gradually develops between actin and myosin filaments by active head rotation of cross-bridges in the force generating state, resulting in the elongation of cross-bridge elastic elements. The rate of tension development depends on biochemical reactions, but also can be affected by mechanical changes in cross-bridges.

We represent the mechanics of a large population of cross-bridges in terms of the mean extension of cross-bridge elastic elements (cross-bridge elongation) and the number of cross-bridges in the force generating state. Mathematical expressions were derived for the mean rate of force development in order to calculate the mean extension of cross-bridge elastic elements and the mean rates of forward and reverse transition between the non-force generating and force generating states in order to calculate the number of cross-bridges in the force generating state. The validity of the model is based on its ability to make physical interpretations and quantitative predictions of muscle behavior. In particular, we were able to adequately predict and interpret such muscle behavior as the force-velocity and rate of energy liberation-velocity relations, the transient responses to sudden changes in length and load, and temperature effects on muscle transient responses.

Through this study, we have shown that the rate of cross-bridge force devel-

opment is probably the most fundamental variable needed to describe muscle dynamics. It not only depends on filament sliding velocity, but is also regulated by the biochemical reactions and structural changes during the interaction between myosin and actin molecules. It responds much more rapidly to changes than the cross-bridge transition rates. In our model, a link between biochemical and mechanical events which occur during muscle contraction is established by the fact that both are functions of the angular velocity of cross-bridge heads. Our study also shows that muscle stiffness or number of cross-bridges in the force generating state is under the regulation of neural input ([Ca]), [ATP] and the rate of change of muscle length while the cross-bridge elongation is determined mainly by the rate of change of muscle length only. This dependence constitutes a negative feedback loop for stabilizing muscle behavior. However, we also found that muscle tension sometimes oscillates about the value of an externally applied load after a sudden load change, behaving like an active oscillator. This suggests that other factors, such as muscle fiber recruitment patterns, muscle coordination and reflex feedback may be necessary for stable movements.

RESUME

Le muscle est un système mobile particulier qui a la capacité de convertir l'énergie chimique provenant de l'hydrolyse de l'ATP en travail mécanique et en chaleur. En termes de la structure d'un muscle, le complexe d'actine et de myosine (actomyosine) constitue le seul élément actif et essentiel du mécanisme de contraction musculaire. A.F. Huxley (1980) a suggéré que les réactions biochimiques et les processus mécaniques impliqués dans la contraction musculaire sont inextricablement liés ensemble, ce qui signifie que chacune des étapes de la série d'événements biochimiques permet d'accéder à l'étape suivante de la suite d'événements mécaniques et qu'à leur tour, les événements mécaniques influençent les événements biochimiques. A partir de cette hypothèse et de l'actuelle théorie de la contraction par glissement des filaments d'actine et de myosine, nous avons rassemblé des résultats expérimentaux relatifs à la structure, à la physiologie et à la biochimie des muscles et nous avons développé un nouveau modèle conceptuel nous permettant d'interpréter et de prédire le comportement des muscles d'un point de vue plus complet. L'élément clé dans ce modèle est relié à la mécanique des ponts d'union, c'est-à-dire à la capacité de convertir l'énergie chimique en travail mécanique. Cette capacité constitue

le lien entre les procédés mécaniques et biochimiques de la contraction musculaire puisqu'elle dépend non seulement de la suite d'événements mécaniques de développement de la force mais relève aussi des transformations biochimiques survenant lors de l'hydrolyse de l'ATP. Tout facteur, tel que la température ou le mouvement de glissement entre les filaments, influençant soit le processus chimique ou le processus mécanique, peut affecter cette capacité de convertir l'énergie chimique en travail mécanique (mécanique de ponts d'union) et, par conséquent, affecter la contraction musculaire.

Dans ce modèle conceptuel, nous avons supposé que les ponts d'union peuvent recevoir l'énergie libérée lors de l'hydrolyse de l'ATP. De même, nous avons supposé que chacune des têtes des ponts d'union, stimulée par cette énergie nouvellement reçue a la possibilité de pivoter activement dans un rayon allant de $\theta_r = 0^0$ à $\theta_e = 45^0$, où θ_r correspond à la position à laquelle le pont d'union se trouve à être en position perpendiculaire par rapport au filament d'actine. Il existe 2 cycles possibles pour un pont d'union une fois qu'il a reçu l'énergie libérée lors de l'hydrolyse de l'ATP (fig. 3.3): un cycle, appelé cycle de myosine, dans lequel il n'y aucune formation de lien entre les filaments de myosine et les molécules d'actine; et un cycle, appelé cycle d'actomyosine, dans lequel il y a liaison entre les filaments de myosine et un des sites d'attachement situé sur les molécules d'actine, le tout formant un lien très serré. Dans ce deuxième cycle, la tête des ponts d'union pivote en sens contraire de la force

d'amnite du complexe d'actomyosine, provoquant une déformation du lien créé par le pont d'union et produisant ainsi la tension musculaire. Nous avons représenté cette déformation à l'aide d'un élément élastique situé le long d'un axe

parallèle au filament d'actine et relié à l'extrémité du pont d'union (fig. 3.2). La tension produite par les ponts d'union est alors équivalente à la rigidité (k) multipliée par la grandeur de l'allongement de l'élément élastique (x). La rigidité k a été considérée constante. La zone effective de travail pour le développement de la force couvre une plage de déplacements angulaires des têtes des ponts d'union allant de θ_s où le pont d'union s'attache à la molécule d'actine jusqu'à θ_e. Pendant cette période de génération de force, un pont d'union transforme l'énergie chimique en travail mécanique. Par contre, la capacité du système à transformer l'énergie est limitée. Cette capacité dépend non seulement de la température et de la présence ou non de molécules d'actine mais elle est aussi fonction de la conformation réelle des ponts d'union et du taux de changement de cette conformation.

Suite à l'attachement d'un pont d'union à un site actif d'une molécule d'actine, alors que l'énergie libérée par l'hydrolyse de l'ATP est disponible, le système entre dans un état où le lien moléculaire est faible et c'est à ce moment que le processus mécanique qui est responsable du développement de la force débute. Puisque la capacité à transformer l'énergie chimique en travail

mécanique est très faible dans cet état de faible liaison, le pont d'union ne pourra générer de force considérable. Par contre, immédiatement après avoir laissé l'état de faible liaison, le pont d'union entre dans un état où le lien entre les molécules est considérablement plus fort et où les ponts d'union génèrent progressivement de plus en plus de force, au fur et à mesure que les têtes des ponts pivotent vers θ_e, et ce, grâce à une augmentation importante de la capacité à transformer l'énergie chimique en travail mécanique. Nous avons défini cette période du processus mécanique de la contraction musculaire pendant laquelle il y a un développement progressif de force produit par le pivotement des ponts d'union comme étant l'état de développement de force et nous avons regroupé ensemble toutes les autres périodes du processus mécanique afin de définir un état de non-développement de force. Après avoir atteint un angle de θ_e, le pont d'union, toujours en présence d'ATP, se sépare de son site d'attachement situé sur la molécule d'actine et transforme le reste de son énergie

chimique en chaleur. La liaison avec une nouvelle molécule d'ATP permet au pont d'union de recevoir à nouveau de l'énergie provenant de l'hydrolyse de l'ATP, initiant alors un nouveau cycle. En tenant compte de la consommation d'énergie lors de la contraction musculaire, nous avons supposé que le mouvement mécanique d'un pont d'union est lié au processus irréversible de l'hydrolyse de l'ATP, tel que démontré par une transition nette des ponts

d'union décrite dans des études portant sur la cinétique des ponts d'union. Nous avons également supposé que chaque pont d'union génère de la force d'une façon indépendante, à travers un élément élastique qui lui est associé. Enfin, les effets des événements biochimiques sur la mécanique des ponts d'union ont été modélisés comme étant l'effet de la transition entre l'état de développement de force et l'état de non-développement de force alors que les effets des événements mécaniques sur la mécanique des ponts d'union ont été modélisés comme étant la dépendance fonctionnelle du taux de développement de force sur la conformation du pont d'union et sur le taux de changement de cette conformation. Pour vérifier quantitativement notre hypothèse de départ et obtenir un aperçu de la nature physique de la contraction musculaire, nous avons dérivé une formulation mathématique afin de quantifier ce modèle conceptuel et de simuler le comportement d'un muscle.

Le muscle représente un système constitué de nombreux petits systèmes individuels répartis sur toute sa longueur puisque chaque pont d'union, situé à l'intérieur même de la moitié d'un sarcomère, peut avoir différentes propriétés et que chaque sarcomère à l'intérieur d'un même muscle peut travailler sous différentes conditions d'opération. Or, la modélisation des effets d'une telle distribution aurait engendré un modèle très compliqué. De plus, puisque nous n'avions que des données expérimentales basées sur des mesures effectuées avec des fibres musculaires entières, nous ne pouvions identifier les paramètres

du modèle d'un tel système. De façon à surmonter cette restriction, nous avons utilisé des valeurs moyennes pour représenter les effets moyens de ces événements produits par chacun des petits systèmes et agissant sur le comportement d'un muscle. Cette procédure se justifie en supposant que les sarcomères sont structurellement distribués en série et que la force générée par chaque sarcomère est comparable à celle produite par les sarcomères voisins. Nous avons donc simplifié le modèle en discutant de l'ensemble des comportements des ponts d'union en termes de ces valeurs moyennes. Avec cette approche, nous avons développé une section d'un modèle paramétrique et nous avons identifié les paramètres de ce modèle en utilisant les données disponibles.

Selon notre modèle conceptuel, la rigidité musculaire et la force peuvent être déterminées à partir du nombre de ponts d'union présents à l'état de développement de force et de la grandeur de l'allongement moyen des éléments élastiques associés à ces ponts. Le nombre de ponts d'union à l'état de développement de force est évalué à partir des taux de transition antérograde et rétrograde entre les deux états, F et G respectivement, alors que l'allongement moyen des éléments élastiques correspond à l'intégrale du taux moyen de développement de la force, $\overline{\dot{x}}$, sur toute la durée du cycle, puisque le taux de développement de force par les ponts d'union est directement proportionnel au taux de changement dans l'allongement de l'élément élastique. Nous avons donc trois variables essentielles: $\overline{\dot{x}}$, F et G.

Basé sur des études portant sur le muscle, nous avons exprimé le nombre de ponts d'union disponibles en fonction de la longueur musculaire, F et G en fonction des concentrations de calcium libre et d'ATP de même que du taux de changement de longueur du muscle; et enfin, nous avons exprimé x en fonction de F, de G, du taux de changement de longueur musculaire et de la valeur moyenne de la vitesse angulaire des têtes des ponts d'union $\overline{\dot{\theta}}$. $\overline{\dot{\theta}}$ peut être déterminé à partir des contraintes du système telles l'équation d'équilibre exprimant la puissance produite par le mouvement des ponts d'union, le taux de changement de l'énergie potentielle dans les éléments élastiques des ponts d'union et le taux d'échange d'énergie entre le muscle et son environnement. Nous avons d'abord élaboré notre modèle en nous limitant à une contraction musculaire constante. L'identification de la structure du modèle et de ses paramètres a été concrétisée lorsque les données obtenues par simulation correspondaient le mieux aux données expérimentales disponibles telles que la relation force/longueur (fig. 4.2), la relation entre la force et la concentration de calcium (fig. 4.3), la relation entre la force et la concentration d'ATP (fig. 4.4) et la relation entre la vitesse et le taux de libération d'énergie (fig. 4.5). Ensuite, nous avons adapté notre modèle, qui fut d'abord conçu pour une contraction musculaire constante, à des situations dynamiques, en remplaçant le taux de changement de la longueur musculaire dans F et G par la variable intrinsèque $\overline{\dot{\theta}}$ et en ajoutant un terme supplémentaire dans la formule expri-

mant $\overline{\dot{x}}$. Ce terme supplémentaire a également été identifié en comparant les données obtenues par simulation à la réponse transitoire du muscle lors d'un changement de longueur soudain d'une durée de 0.2 ms (figs. 5.1 et 5.2).

La validation de notre modèle a été effectuée en examinant la capacité du modèle à prédire des données expérimentales différentes telles que la relation force/vitesse (fig. 4.6), la réponse transitoire du muscle lors de changements de longueur soudains d'une durée de 1 ms (figs. 5.3 et 5.5) et la réponse transitoire du muscle lors de changements de charge soudains (figs. 5.7, 5.8 et 5.10). Nous avons également effectué des simulations avec notre modèle musculaire afin d'extrapoler au-delà des résultats expérimentaux déjà obtenus. Ces simulations nous ont procuré des résultats concernant la réponse transitoire du muscle lors de changements de longueur soudains d'une durée de 0.1 et 0.01 ms (figs. 5.3, 5.4 et 5.6) et lors de changements de charge soudains (fig. 5.9).

La flexibilité de notre modèle a été examinée par le biais de l'incorporation des effets de la température sur la réponse transitoire du muscle lors de changements de longueur soudains (figs. 5.11 et 5.12). Selon notre modèle conceptuel, la dépendance de la mécanique du complexe d'actomyosine face à la température constitue le plus important résultat expliquant le fait que la réponse mécanique d'un muscle soit dépendante de la température. Dans la formulation mathématique proposée, les propriétés de la mécanique des ponts d'union ont été exprimées par l'équation 4.18. Les paramètres A_{41} et A_{42} du

modèle représentent respectivement la vitesse maximale de pivotement d'un pont d'union lorsque celui-ci est lié à une molécule d'actine et le couple maximal mesuré lorsque la vitesse est nulle. Nous avons évalué ces deux paramètres selon les variations de la vitesse maximale de raccourcissement des fibres (V_m) et de la tension maximale mesurée lors d'une contraction tétanique (T_0), puisque V_m et T_0 représentent A_{41} et A_{42} à l'échelle macroscopique.

La validité de notre approche de modélisation et de notre hypothèse relative à la mécanique des ponts d'union est supportée par les prédictions obtenues avec ce modèle. Au travers de cette étude de modélisation, nous avons obtenu une image claire du comportement des ponts d'union pendant la contraction musculaire, ce qui accroît notre compréhension des propriétés musculaires et qui pourrait s'avérer être très utile lors de l'élaboration de futurs protocoles expérimentaux portant sur l'étude du comportement mécanique des muscles.

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NOMENCLATURE

Abbreviations

A	actin molecule
ADP	adenosine diphosphate
AMD	A·M·ADP
ATP	adenosine triphosphate
Ca	calcium ion
CB	cross-bridge
CBH	cross-bridge head
CPK	creatine phosphokinase
g	gram
HMM	heavy meromyosin
LMM	light meromyosin
M	myosin head
MD	M·ADP

MDP	M·ADP·P_i
mm	$\times 10^{-3}$ meter
mMol	$\times 10^{-3}$ mole
Mol	mole
ms	$\times 10^{-3}$ second
MT	M·ATP
N	newton
nm	$\times 10^{-9}$ meter
pN	$\times 10^{-12}$ newton
pCa	negative logarithm of free calcium concentraction
PCr	phosphocreatine
P_i	inorganic phosphate
s	second
SR	sarcoplasmic reticulum
S_1	head region of a myosin molecule
S_2	a fragment of heavy meromyosin
[·]	concentration
μM	$\times 10^{-6}$ mole

μm	$\times 10^{-6}$ meter

Superscripts

$-$	mean value of a distribution
$\ddagger$	highest free energy level
$\dagger$	intermediate free energy level

Subscripts

$*$	output power greater than base value
c	critical sliding rate
ij	numbering of model parameters
s	force-generating state
w	weak binding state
0	isometric state at optimal muscle length

Variables and Parameters

A_{ij}	model parameters
A_{01}	number of cross-bridges within a half-sarcomere
A_{03}	optimal length of a half-sarcomere

A_{41}	the maximum angular velocity of strongly attached cross-bridge heads
A_{42}	the maximum torque exerted by strongly attached cross-bridge heads
A_{43}	$\overline{\theta}$ during isometric contraction
A_{66}	critical sliding rate at which $\dot{\theta} = 0$
c_r	ratio of amount of energy converted to mechanical work from splitting one ATP molecule to e
c_1	energy liberation rate from muscle activation mechanism
e	amount of energy released by splitting one ATP molecule
E	total liberated energy
$\dot{E}$	energy liberation rate
F	forward cross-bridge transition rate
G	reverse cross-bridge transition rate
f_{a_i}	force exerted by a cross-bridge head on the ith actin site
f_{a_m}	maximum of f_a;
f_1	effect of filament sliding on F
f_2	effect of [Ca] on F

f_3	effect of [ATP] on F
F_m	muscle tension
F_{m_i}	muscle tension during a steady contraction
g_1	effect of filament sliding on G
g_2	effect of [Ca] on G
g_3	effect of [ATP] on G
H	heat
h_m	maximum extension of the hypothetical elastic element
k	stiffness of the hypothetical elastic element
l	length of a cross-bridge head
N_{am}	number of cross-bridge heads in the actomyosin cycle
N_d	number of detached cross-bridge heads in N_{am}
N_m	number of cross-bridge heads in the myosin cycle
N_r	number of forcible detachments occuring during a cross-bridge working stroke
N_s	number of strongly attached cross-bridge heads in N_{am}
N_w	number of weakly attached cross-bridge heads in N_{am}
P	output power of a cross-bridge head

P_h	output power of a cross-bridge head for heat generation
P_{h_a}	P_h in the actomyosin cycle
P_w	output power of a cross-bridge head for doing mechanical work
P_{w_s}	P_w of a strongly attached cross-bridge head
Q	output torque of a strongly attached cross-bridge head
R_w	efficiency of P_w
R_{w_s}	efficiency of P_{w_s}
S_{c1}	temperature scaling factor of V_m
S_{c2}	temperature scaling factor of F_{m_0}
t_a	duration of attachment of a cross-bridge before its forcible detachment during lenghtening
T_{am}	period of the actomyosin cycle
T_{am_s}	duration of strong binding state
T_{am_w}	duration of weak binding state
T_{am_1}	time needed for transition from detached to attached state
T_{am_2}	duration of heat generation state
T_b	time needed to bind with ATP
t_d	time between forcible detachment and re-attachment

T_m	period of the myosin cycle
T_{m_1}	time needed for hydrolysis of one ATP molecule
T_{m_2}	duration of $M \cdot D^{\ddagger}$ state
T_{m_3}	duration of $M \cdot D^{\ddagger}$ (45^0) state
T_1	minimum muscle tension during sudden shortening or maximum muscle tension during sudden lengthening
T_2	muscle tension at the end of early recovery phase
v_a	filament sliding velocity
V_m	maximum steady shortening rate
x	extension of the hypothetical elastic element
$\dot{x}$	rate of change of length of the hypothetical elastic element
X	length of a half-sarcomere
Z	total number of cross-bridge heads in the filament overlap region
θ	angular position of a cross-bridge head
θ_e	θ at the end of a working stroke
θ_r	θ at the begin of a working stroke
θ_s	θ at the begin of force development
$\dot{\theta}$	rate of change of θ

$\overline{\dot{\theta}}_d$	$\overline{\dot{\theta}}$ after forcible detachment and before re-attachment
$\ddot{\theta}$	rate of change of $\dot{\theta}$
$\triangle d$	relative distance between two adjacent actin sites
Ψ	distribution function

CHAPTER 1

INTRODUCTION

1.1 GENERAL

System modeling is the field of mathematical modeling of systems. In this field there are two major approaches that have been widely used, theoretical modeling and system identification. Theoretical modeling is an analytic approach, by which system models can be derived from a number of physical principles; the other is an experimental approach, by which system models can be developed by fitting recorded data. The primary aim of both approaches is to develop appropriate mathematical models. With mathematical models, a concise description of complex dynamic processes can be obtained, the values of certain variables and parameters can be estimated, proposed hypotheses for system physical structure or principles can be tested and confirmed or rejected, and experimental designs can be improved due to new insights into the behavior of the system. Because of these attractive characteristics system modeling

has been applied in diverse fields for control and prediction of system behavior, especially in studying the properties of biological systems. Most biological systems possess complex system structures, have a large operating range, but a limited number of measurable variables.

Skeletal muscles are complex biological machines, which can actively contract to generate tension. In neuromusculoskeletal systems they work as mechanical actuators to drive connected limb segments, utilizing the chemical energy from ATP hydrolysis and operating under the control of the central nervous system. Limb motion and mechanical behavior results from the temporal and spatial summation of the mechanical action of muscles on the skeleton. In order to understand and interpret the sequence of physiological events, as well as the intrinsic mechanisms of control and regulation involved in muscle contraction, it is important to understand the physical behavior of skeletal muscles and have appropriate mathematical representations of their dynamics.

From the point of view of system science, the neuromusculoskeletal system has a functional structure similar to that of a manipulator which can be hierarchically represented by the following three levels:

1) mechanical components such as the muscles and skeleton, which are the controlled targets of the higher level;

2) local controllers implemented by the neural circuits in the peripheral

system which realize different control strategies issued from the top level and provide fast compensation to achieve desired movements;

3) a decision maker which plans movements according to given commands, the current state of the environment and the knowledge of previous movements.

Although the mechanical variables at each joint can be measured during movement, very few neural signals can be reliably measured. Thus, it is very difficult to analyse the system as a complete entity through traditional experimental approaches. Fortunately, a number of system elements, such as skeletal muscles and muscle spindles, can be isolated and studied independently. Such experimental studies have provided certain details of the behavior of individual system elements. Using this knowledge, one can conceive a system configuration, develop suitable mathematical models for each of the system elements, and then simulate the total system behavior based on a limited number of experimentally observed system's inputs and outputs. This enables one to study proposed control structures, to examine interactions between system elements, and to study the behavior of variables of interest. Skeletal muscle is the fundamental element of the neuromusculoskeletal system. Motor function cannot be separated from muscle properties. Therefore, a first stage in studying control in the neuromusculoskeletal system is the investigation of the regulation

of muscle mechanics by neural inputs and mechanical loading.

Isolated skeletal muscles have been studied from the perspectives of structure, physiology and biochemistry, leading to the formulation of theories of excitation-contraction coupling, filament sliding and cross-bridge attachment and detachment. According to these theories, muscle contraction is induced by the cyclic interaction between actin and myosin molecules. However, because of the limited measurements that can be made at the molecular level many of the details of the cyclic interaction between actin and myosin molecules during muscle contraction have yet to be elucidated. System modeling has therefore been used as a tool to quanitatively describe and potentially advance our understanding of molecular mechanisms of muscle contraction by testing proposed hypotheses through the comparion between model prediction and experimental data.

1.2 LITERATURE OVERVIEW

In muscle modeling there are essentially two types of models. The most frequently used model is that derived by fitting curves to data obtained in experiments which characterize the macroscopic mechanical behavior of muscles. This approach originated with the work of A.V. Hill who proposed a

muscle model consisting of parallel and series elastic elements, a viscous element and an active force generating element (Hill, 1938). In Hill's formulation, the series elasticity resided partly in the tendon and partly in the muscle. It accounted for the response during isotonic quick-release. The parallel elastic component resided entirely in the sarcolemma and in the connective tissue surrounding the muscle fibers. The force-velocity relation was modeled as a nonlinear dashpot. The active part of the force-length relation and the time course of muscle tension during a twitch were represented by the active force generating element.

Hill modeled a muscle twitch by means of an energy supply for contraction that could be rapidly engaged following an action potential. Once activated, the contractile portion of the muscle generated tension gradually, stretching the elastic portion and overcoming the intrinsic viscosity. The energy supply was then quickly disengaged leading to the disappearence of the active state. Hill also assumed that a quick stretch did not affect the contractile element. Thus, the elastic element could stretch until its tension was equal to the force generated by the contractile element. Based on this idea, the time course of the active state of the contractile element was experimentally estimated by applying quick stretches at different times during the development of a twitch. Although other methods were later proposed for calculating the active state (Ritchie, 1954; McPherson and Wilkie, 1954; Gable *et al.*, 1968; Edman, 1970;

Inbar and Adam, 1976), they did not lead to any widely accepted model. In recent formulations of the Hill-type model, the active force generating element has been modeled as a function of muscle length and calcium concentration under isometric conditions (Otten, 1987). Velocity dependent behavior was modeled by using an approximation of the force-velocity relation to scale the isometric force. By treating the muscle as a black box, the parameters of this model could be chosen to give the best fit to empirical data obtained in experiments where an independent variable such as muscle length or concentration of free calcium in the sarcoplasm was varied. This type of model usually possesses a simple structure and does not require heavy computation.

Skeletal muscle is a biological motile system, converting the chemical energy from ATP hydrolysis into mechanical work and heat. Its behavior, like other mechanical actuators, relies heavily on the mechanism of energy tranformation. As with many other biological systems, it possesses a complex system structure and can function over a large operating range. Any modeling approach such as Hill's, which bypasses the intrinsic mechanisms of control and regulation of energy transformation and force generation, can only roughly approximate the mechanical behavior of muscle at the macroscopic level.

The first mathematical model of muscle behavior at the microscopic level was developed soon after the appearance of the sliding-filament, cross-bridge theory of A.F. Huxley (1957). This model consisted of two essential cross-

bridge states, attached and detached, and represented their cyclic characteristics by the transition rates between attached and detached states. With the assumption that one ATP molecule was consumed in an interaction cycle and that the rates of cross-bridge attachment and detachment were functions of the relative axial distance between a cross-bridge on the myosin filament and the nearest available actin binding site, this two-state model could predict the muscle tension and rate of energy liberation during steady shortening, observed by Hill (1938). The most important contribution made by this study to both muscle contraction theory and modeling, was to successfully link muscle macroscopic behavior to its microscopic behavior and show the possible dependence of cyclic actin-myosin interaction on the relative distance between the myosin head and the nearest actin binding site.

In 1964, Hill demonstrated a decrease in the rate of energy liberation when muscle shortened at high velocities. In order to accommodate this phenomenon, Huxley (1973) postulated that attachment took place in two stages. In the first stage, a cross-bridge could easily undergo a reversible detachment without any consumption of chemical energy. The original theory was further modified on the basis of experiments which studied the response of a muscle fiber to sudden changes in muscle length or tension (Huxley and Simmons, 1971). As described by Huxley in 1988, this consisted of postulating a sequence of attached states with progressively increasing affinity between

actin and myosin, which permitted the elastic component of the cross-bridge to undergo proportionately more extension. Several mathematical expressions were formulated to describe this progress (Huxley and Simmons, 1971; Huxley, 1973). However, they were not incorporated into the original model, presumably because of added complexity. In the original model (Huxley, 1957), the force generated by an attached cross-bridge was modeled as an elastic force that was directly proportional to the relative axial distance between the actin binding site and the myosin head. Consequently, the rate of change of the elastic force was directly proportional to the rate of change of the relative axial distance or the sliding velocity of the two filaments since the cross-bridge stiffness was assumed constant. Later, Huxley and Simmons (1971) postulated that the force depended not only on the relative axial distance but also on the current cross-bridge conformation, so that the rate of change of cross-bridge force became a function of both the sliding velocity and the current cross-bridge conformation. Although this assumption was not incorporated into the mathematical model, it would clearly have increased the model complexity since each additional attached state with a specified cross-bridge conformation would require an additional partial differential equation.

Eisenberg *et al.* (1980) combined recent studies of muscle kinetics with the theory proposed by A.F. Huxley in 1957 and 1971, and formulated a four-state model for muscle contraction. The model had two states for cross-bridge

attachment and two states for cross-bridge detachment. Immediately after detachment the cross-bridge went into a refractory state. This was followed by a nonrefractory detached state which preceded re-attachment. The two attached states were similar to the weak and strong binding states proposed by Huxley and Simmons (1971). Eisenberg *et al.* (1980) linked cross-bridge elasticity with a change in the chemical energy of an attached cross-bridge, employing the concept of free energy, i.e. the sum of the chemical energy and the mechanical energy stored in the elastic element due to its extension. According to T.L. Hill's theory (1974, 1975), the ratio of the forward transition rate to reverse transition rate between two adjacent states is related to the free energy difference between the two states. By choosing values for either the forward or reverse rate constant and specifying the free energy profiles, which regulated transitions between the proposed states, they were able to predict the portion of the force-velocity curve during muscle shortening and to approximate the muscle transient behavior observed by Huxley and Simmons (1971) during the recovery period after a rapid length change. Huxley (1973) had suggested that the decrease in the rate of energy liberation seen during high velocity shortening could be explained if the rate of transition from the first attached state to the nonrefractory state was quite high. However, the model of Eisenberg *et al.* (1980) could not account for this decrease even though the transition rate from the first attached state to the nonrefractory state

was relatively high near the extremes of extension of the cross-bridge elastic element. Thus, although the model gave qualitatively reasonable predictions, it was still deficient in certain quantitative aspects.

Huxley's model represents the stochastic behavior of cross-bridges over the range of mechanical interaction between actin and myosin. Essentially, muscle force, stiffness and energy liberation rate are functions of the cross-bridge attachment and detachment rates only. As pointed out by Huxley himself in 1988, his theory is incomplete since it contains no specific statement about the nature of the actin-myosin bond, the nature or location of the elastic element, or the mechanism for breaking the bond at the right time. The model does not consider either how the extension of the elastic element is regulated by biochemical events nor how biochemical processes can be influenced by mechanical factors. Without details of the cycling interaction between myosin and actin, the predictive capacity of the model and its scope of application are limited. For instance, the mechanical limits of extension of the cross-bridge elastic element, x_{max} and x_{min} should depend on the affinity of the actin and myosin, i.e. the maximum elastic deformation which a cross-bridge can undergo before being forced to detach. The number of attached cross-bridges at either limit, $x = x_{min}$ or $x = x_{max}$, should be equal to zero implying that forcible detachment must occur at the limits and that the rate of cross-bridge detachment should be very high. Otherwise, an unrealistic amplitude of

extension of the elastic element would result when the rate of change of muscle length is higher than a moderate value, as shown by the model simulations of Zahalak and Ma (1990).

Another important issue is the rate of cross-bridge force development. Since the cross-bridge is apparently an elastic structure, the rate of force development should be related to structural changes such as elastic deformation. If a linear spring is used to represent cross-bridge elasticity, then the time derivative of extension of the spring is directly proportional to the rate of cross-bridge force development. In 1980, A.F. Huxley suggested that the biochemical reactions and mechanical processes of muscle contraction are inextricably linked, such that each step in a series of biochemical events permits the next step in a series of mechanical events to take place and that in turn, the mechanical events influence the biochemical events. Since the rate of cross-bridge force development is most likely dependent on factors such as the ability of the cross-bridge to hydrolyze ATP, its capacity to convert chemical energy into work and its mechanical structure and constraints, in addition to the relative filament sliding velocity, it is a state variable which provides a means of combining the dynamics of mechanical and biochemical processes underlying force production.

Several conceptual models for control and regulation of cross-bridge force development have been proposed. One of these is the charge-transfer model of

Hatze (1990) in which the mechanism of myofilament force generation depends on intra-molecular charge transfer. Hatze assumes that after myosin binds to an actin site, the energy stored in the actomyosin complex is converted to electric potential energy by the transfer of electrons from the actin binding site of a cross-bridge to one of the moieties of the myosin head, $S_1 - 27\text{K}$. Force generation occurs simultaneously with electron transfer. A certain amount of work must be done during the charge-transfer process due to the presence of negative charge on the surface of the tropomyosin molecule. The transferred charges can return to their original locations resulting in a concomitant decrease in contractile force.

A second conceptual model is the self-induced translation model of Mitsui and Ohshima (1988). They assumed that a myosin head, energized by ATP hydrolysis, binds to an actin site at a fixed angle and that it maintains this orientation throughout attachment. After binding, there is a rearrangement of charges at the actin site which results in a repulsive force acting on the myosin head causing it to jump to an adjacent actin site by detaching and re-attaching thereby stretching the cross-bridge elastic element. These two models are quite recent and have not yet been developed to the extent of being able to predict muscle mechanical responses that can be compared with the experimental data.

For the last half century the cross-bridge, sliding-filament model of mus-

cle contraction has been frequently used as a framework for experimentation. However, experimental results from muscle studies have so far not provided direct evidence for many of the details of this theory. In 1983 Pollack discussed the uncertainty in some experimental interpretions and a number of observations which are not consistent with the main features or the extrapolated features of the cross-bridge, sliding-filament theory. He suggested that the following considerations should not be ruled out and might be worth incorporating into a new or modified theory.

1) The sliding of thick and thin filaments brings about passive length changes.

2) Cross-bridges project from thick filaments at right angles (as conceived originally); some or all may interconnect adjacent thick filaments, thereby serving as structural links that maintain the integrity of the filament lattice.

3) Contraction may not result from the simultaneous action of cross-bridges acting in parallel.

4) Active shortening, at least under some circumstances, may be cuased by thick-filament shortening.

5) The contractile process has a well-defined memory of past events.

Huxley (1988) also pointed out that there are yet a number of unanswered

questions regarding the details of the molecular mechanisms of contraction. For example:

1) Which structure is the elastic element in the cross-bridge?

2) What is the nature of this elasticity?

3) What structure undergoes the stepwise change?

4) Does the myosin head attach to a single actin monomer or two (or more) monomers within the this filament?

5) What kind of bonds hold the myosin head to the thin filament?

6) How does binding of ATP cause myosin to dissociate from actin?

7) What is the significance of the fact that each myosin molecule has two heads?

Fortunately, the evidence in favor of the cross-bridge, sliding-filament theory has continued to grow in recent years (Squire, 1990). We believe that the generality of the cross-bridge, sliding-filament theory of muscle contraction is presently the best working hypothesis. Our primary objective is to model the mechanical behavior of muscle at the molecular level, i.e. muscle micromechanics. Therefore we will not concern ourselves with the details of the intra-molecular behavior. Using the concepts of sliding filaments and cyclic

cross-bridge interactions, we focus our attention on cross-bridge behavior during the force generation process.

In our model, we first assume that cross-bridges are the fundamental force generating units and that they have linear elastic behavior. Muscle mechanics is the ensemble behavior of cross-bridge mechanics. There are two essential cross-bridge states, the non-force generating state and the force generating state. At the macroscopic level, muscle stiffness is proportional to the number of cross-bridges which are in the force generating state and is taken into account by the transition rates between the two states while muscle force is equal to the sum of the force generated by the individual cross-bridges. The force produced by a single attached cross-bridge is equal to the product of its stiffness and the extension of its elastic element which is predicted by the rate of force development. The rate at which a cross-bridge can develop force is a function of its structure, its ability to convert chemical energy into work and the energy exchange between muscle and the environment with which it interacts.

Recent data from cross-bridge structural studies using spectroscopic probes and X-ray diffraction support the idea that force development is accompanied by such structural changes as rotation and inter-domain motion of the cross-bridge head (Thomas, 1987; Vibert and Cohen, 1988). We assume that muscle force is mainly generated by the active rotation of attached myosin heads against the actin binding site, making angular velocity an important variable

for the determination of the rate of force development. We also assume that the ability of an attached cross-bridge to convert chemical energy into work is limited and a function of the structural change in an actomyosin complex. Thus we are able to link the mechanical events with biochemical reactions during cross-bridge force generation by studying their effects on the mechanical output power of cross-bridges. By balancing the power contributions of cross-bridges and externally applied forces with the mechanical work, the mechanical events observed at the macroscopic level can be directly related to those at the microscopic level.

1.3 SCOPE OF CURRENT RESEARCH WORK

Current theories and models of muscle contraction have so far failed to consider in detail the conversion of chemical energy derived from ATP hydrolysis to structural changes in the actomyosion complex. There has also been little treatment of the energy exchange between muscle and the environment with which it interacts during force development. To overcome this limitation a conceptual model is proposed, which is mainly concerned with aspects of the dynamics of cross-bridge behavior that have so far not been considered in previous models. It is assumed that cross-bridges have the ability to hydrolyze ATP, to store energy and to convert chemical or stored energy into mechanical

work by active head rotation, thereby resulting in muscle shortening against a load. The influence of mechanical events on biochemical events during the interaction between myosin and actin molecules can then be taken into account by assuming that the capacity to convert chemical energy into mechnical work or heat is limited and depends on the current angular position and angular velocity of the cross-bridge head. Consequently, force development is a continous process which follows an important physical principle in system dynamics: power balance. Biochemical processes, mechanical processes and the energy exchange between muscle and the environment with which it interacts can be linked through the power balance. With the detailed conceptual model, we can identify model structure and estimate model parameters directly from the experimental data, rather than guessing as in other cross-bridge models, thereby gaining a better understanding of the mechanism of muscle contraction.

In the following chapter, fundamental aspects of muscle behavior will be introduced. The most widely accepted theories of muscle contraction will be discussed along with experiments in muscle structure, biochemistry and physiology. The third chapter presents the theoretical analysis and development of the conceptual model. The consistency of the conceptual model with experimental results will be discussed. A mathematical structure will also be developed to facilitate the physical interpretion of these experiments in the context of the model. Muscle behavior in equilibrium states such as occur

during isometric, isotonic or isovelocity contractions will be treated first in the development of the model. In these states, it will be assumed that cross-bridge cycling rates are constant. The mathematical modeling, parameter estimation and prediction of muscle mechanical behavior for steady-state contractions will be presented in the fourth chapter followed by consideration of transient responses in the fifth chapter. Modeling results will be discussed in each chapter. The conclusions and the proposals for further work will be given in the sixth chapter.

CHAPTER 2

FUNDAMENTAL ELEMENTS OF MUSCLE BEHAVIOR

It has been widely accepted that skeletal muscle converts the energy from chemical reactions into mechanical work and heat, generating force through the cyclic interaction of two principal proteins, actin and myosin. In order to understand muscle behavior in a complete sense, it has been studied from the viewpoints of structure, physiology, and biochemistry. This includes biochemical studies of isolated muscle proteins in solution, physiological experiments on muscle mechanics and energetics, structural studies of muscle proteins using electron microscopy, X-ray diffraction and spectroscopic probes. In the following sections the fundamental findings of these studies will be summarized.

2.1 MUSCLE STRUCTURE

A skeletal muscle consists of a number of muscle fibers which are between 10 and 200 μm in diameter and between 2 and 100 mm in length. As an individual cell, a muscle fiber is surrounded by a plasma membrane, the sarcolemma, and contains an aqueous solution of organic and inorganic ions, the sarcoplasm. It is electrically separated from the other fibers. Other important structural components of muscle include T-tubules, sarcoplasmic reticulum, mitochondria and myofibrils (Fig. 1.1, Woledge *et al.*, 1985). The T-tubules are branched tubules running predominantly transversely into a muscle fiber and opening at the surface of the fiber, thus providing communication between deep portions of the fiber and the extracellular space. The sarcoplamic reticulum is a network of tubules surrounding each myofibril, isolated from the T-tubules, intracellullar space and extracellular space, and containing a high concentration of calcium ions. Calcium is released into the sarcoplasm in response to an action potential, activating the myofibrils and is subsequently taken up from the sarcoplasm by calcium pumps located in the sarcoplamic reticulum, producing muscle relaxation. Muscle mitochondria are structurally and functionally similar to those in other tissues. Their main function is to provide energy from oxidative phosphorylation of ADP. The myofibrils are about 1$-$2 μm in diameter and occupy most of the space within a muscle fiber. Myofibrils are composed of

two sets of hexagonally interdigitating myofilaments, thin and thick filaments.

Myofibrils have a banded appearance under a light microscope. These striated bands are called sarcomeres. There is a dark central region called the A-band which has the same length as the thick filament. In the center of the A-band is a lighter region which consists of myosin only, called the H-band. The remainder of the A-band constitutes the region of overlap between thin and thick filaments. Besides an A-band, a sarcomere also has two identical regions called I-bands that consist only of thin filaments. The Z-disc is a structure with a high refractive index located at the center of an I-band. It appears to be a structural membrane running through the whole cross-section of a muscle fibril that forms the mechanical interface for the two sets of thin filaments that are connected to it on either side. The region between adjacent Z-discs is defined as the sarcomere. It is the basic structural building block which is believed to possess the fundamental mechanical properties of muscle.

Within a myofibril cross-sectional area of one square micrometer there are 500 thick filaments and 1000 thin filaments which are interdigitated and arranged in a hexagonal pattern. A thin filament is composed of three protein molecules: actin, troponin, and tropomyosin, whereas a thick filament consists only of myosin molecules. Each thick filament is surrounded by six thin filaments, is about 1.6 μm long and contains about 300 myosin molecules. A myosin molecule consists of two parts, light meromyosin (LMM) and heavy

meromyosin (HMM) which can be further separated into S_1 and S_2 fragments. The S_1 fragment forms the head region of a myosin molecule and is about 19 nm long and 4.5 nm in diameter. The tail region or myosin rod is made up of the S_2 fragment which extends 43 nm from the head and LMM which forms the base of the thick filament. There is a flexible hinge region located between the LMM and HMM. Myosin molecules shown by electron micrographs or X-ray diffraction (Fig 1.5, Woledge *et al.*, 1985) have a 6/3 helical arrangement along the thick filament with a periodicity of 42.9 nm and an axial interval of 14.3 nm. There are a number of functional sites located on myosin heads, such as the site for binding actin, the ATPase site and other amino acid residues which play important roles in the actin-myosin interaction. Each thin filament is about 1 μm long and contains about 350 actin monomers and 50 molecules each of troponin and tropomyosin (Fig. 1.6, Woledge *et al.*, 1985). Actin monomers and tropomyosin molecules are polymerized and form separate strands. In a thin filament, two actin polymers are wound together with a pitch of 73 nm while the axial separation of adjacent molecules is about 5.46 nm. Two tropomyosin strands lie in the grooves between the actin strands and are bound to the actin molecules. Troponin complexes consist of three types of troponin molecules which can bind both to tropomyosin and actin. Troponin molecules have high affinity for calcium and can be classified functionally as troponin I, C, and T. Troponin I together with tropomyosin inhibits actin from

activating myosin ATPase, while troponin C combined with calcium shifts the tropomyosin chain aside, exposing actin to myosin and allowing interaction.

Structural studies have shown that the globular head region of a myosin molecule, which projects from the thick filament backbone, is free to move across the interfilament space and bind to actin. In the rigor state, the heads remain bound to thin filaments at an angle of 45^0, while in the resting state the heads are perpendicular to the thin filaments and may be free or weakly bound to actin. These physical links between the thin and thick filments are usually called cross-bridges. During muscle contraction, cross-bridge heads undergo rapid large-scale movement (Boredjo *et al.*, 1982; Cooke *et al.*, 1982; Huxley *et al.*, 1982; Burghardt *et al.*, 1983; Craig *et al.*, 1985; Applegate and Flicker, 1987), which is thought to consist of rotation and inter-domain motion (Huxley and Kress, 1985) based on evidence from optical and spin probes in detached myosin heads (Guth, 1980; Thomas and Cooke, 1980; Boredjo *et al.*, 1982; Barnett and Thomas, 1984). Cooke *et al.* (1982,1984) estimated that only about 20% of the cross-bridge heads are in the force generating state during contraction. Using X-ray diffraction, Kress *et al.* (1986) concluded that there was a sequence of underlying structural changes responsible for muscle force generation. The earliest structural change is the movement of tropomyosin strands which takes place very rapidly following the action potential. Subsequent structural changes associated with contraction, such as cross-bridge

attachment, then occur. Force development appears after a further delay.

Ultrastructural studies of cross-bridges have been made in intact muscle in different contraction states by utilizing the rapid-freeze technique. Recent data obtained from whole *A*-bands and from single actin filaments decorated with cross-bridges suggest that the axial spacing of the myosin subunit repeat (14.3 nm) is maintained both under isometric and high-velocity shortening contractions (Tsukita and Yano, 1988).

2.2 MUSCLE KINETICS AND ENERGETICS

The hydrolysis of ATP to ADP and inorganic phosphate provides energy for muscle contraction. There are two major ATPases in skeletal muscle, myosin and the calcium pump in the sarcoplamic reticulum. Muscle contraction is considered to result from a dynamic interaction between myosin and actin under the regulation of the biochemical events of ATP hydrolysis. Thus, understanding how myosin and the actomyosin complex hydrolyze ATP and use the chemical energy to do mechanical work is essential for the development of a complete hypothesis of the mechanism of muscle contraction at the molecular level.

By using purified actin and myosin in solution, Lymn and Taylor (1971) were able to show that the reason why myosin bound tightly to actin in the

rigor state was the absence of ATP since ATP greatly reduced the association constant between actin and myosin, and that the ATPase rate of myosin was quite low and could be accelerated by adding actin. Assuming that the basic properties of the isolated proteins and protein fragments in solution closely reflect the properties of these structures in the intact muscle contractile system, they related the events of enzyme breakdown of ATP with presumed cross-bridge mechanical behavior and proposed a biochemical model with an oar-like cycle (Fig. 1.8, Squire, 1986). In their model, hydrolysis of one ATP molecule is intimately linked to a cross-bridge cycle which is composed of a sequence of mechanical events: attachment, structural change, detachment and return of the cross-bridge to the starting point. The actual ATP hydrolysis occurs when the cross-bridge is detached from actin. The structural change after attachment leads to force generation in an activated muscle. Regulation of muscle contraction is mediated by Ca^{++}-dependent blocking and unblocking of the attachment of cross-bridges to actin.

Recently, it has been shown that some key features of the Lymn and Taylor model are inconsistent with experimental observations. First, the fact that the two states, M·ATP and M·ADP·P have very similar actin affinity and the fact that ATP hydrolysis can occur while a cross-bridge remains attached to actin (Stein *et al.*, 1979, 1984), are at odds with the notion of a co-ordinated oar-like cycle consisting of an irreversible sequence of attachment, force gen-

eration, detachment and recovery to the starting point with the consumption of one molecule of ATP. Secondly, it was found that M·ATP and M·ADP·P are able to bind to actin both in the presence and in the absence of Ca^{++} (Chalovich *et al.*, 1981), indicating that Ca^{++} may regulate force generation not by permitting attachment of a cross-bridge to actin, but rather through a different mechanism such as the transition from the weak binding state to the strong binding state. To accommodate these experimental findings with the two-state cross-bridge model (Huxley, 1973), several biochemical schemes have been proposed (Geeves, 1991). Goldman and Brenner (1987) present a scheme of possible elementary reaction steps for actomyosin ATPase. There is a predominant cyclic reaction pathway in which myosin splits ATP much more rapidly in the presence than in the absence of actin. Based on the actin affinity, the reaction steps are classified as two groups: weak binding and strong binding. The transition rate from weak binding to strong binding is regulated by calcium concentration (Brenner, 1987).

Apparently, the ATP hydrolysis during muscle contraction results in the actual output of mechanical work and heat generation. Observations of muscle energy liberation under different conditions can provide information about the mechanochemical coupling process, the number of completed cross-bridge cycles, and possible steps within a cycle. A noteworthy study involving measurement of the energy liberation rate during muscle shortening was made by

Hill (1938). He showed that the rate of energy liberation increases as muscle shortens faster. Using better equipment, Hill (1964) later showed that there was a slight decrease in the energy liberation rate when muscle shortened at rates higher than 0.4 V_m. A sharp decrease in the energy liberation rate was observed when muscle was stretched at low rates (Curtin and Davies, 1975). Further increasing the stretching rate produced a slow increase in the energy liberation rate which approached the isometric value when the stretching rate was around 0.8 V_m.

Huxley (1957) assumed a direct proportionality between the energy liberation rate and the cross-bridge turnover rate. However, Kushmerick *et al.* (1969) indicated that insufficient ATP was hydrolyzed during rapid shortening to account for the amount of energy liberated by muscle. Later, Homsher *et al.* (1981) monitored the energy balance by continuously measuring ATP consumption after rapid shortening and suggested that a relatively slow step in the biochemical reaction prevented many of the cross-bridges from completing the cycle and splitting ATP until after the end of shortening. This idea was also supported by the experiments of Irving and Woledge. They found that the rate of heat production above the isometric rate declined as the amount of shortening increased when muscle shortened at 0.5 V_m (1981b). Also, recovery of the ability to generate the same amount of heat in two sequential periods of rapid isovelocity shortening required a several hundred millisecond interval be-

tween shortening periods (1981a). They explained their data using a two-state model. The two states, A and B are relatively long-lived intermediates in the interaction of cross-bridges with actin sites. The transition rate from A to B is represented by either k_I or k_S according to whether the sliding velocity is equal or not equal to zero, respectively. The transition from B to A is coupled to the hydrolysis of ATP and also possibly dependent on the shortening rate. Its rate is denoted as k_R, where $k_I < k_R < k_S$. Therefore, A would be the predominant intermediate during isometric contraction and while B would be predominant during steady shortening due to an increase in the rate of reaction from A to B.

2.3 MUSCLE MECHANICS

Muscle mechanics at the macroscopic level has been well studied. The principal characteristics of muscle mechanical behavior are represented by the force at different muscle lengths (force-length relation), the force at different rates of change of muscle length (force-velocity relation), the transient responses to sudden changes in length or tension and the twitch and tetanic responses.

A single action potential propagating along an isometric muscle fiber, produces a rise in tension after some delay which reaches a peak and falls back to zero. This transient rise in tension is known as a twitch. With a train of

action potentials, muscle tension rises and remains at the new level. The tension is not constant, but has superimposed upon it, a ripple at the stimulation frequency. The higher the stimulation frequency the larger the tension and the smaller the ripple. This response is known as tetanus. The maximal value of tetanus is reached when muscle tension no longer increases with further increases in the stimulation frequency.

Given different muscle lengths, the isometric muscle tension will be different, provided that muscle activation level is kept constant. A force-length curve can be obtained by measuring muscle tension at a series of different lengths when a muscle is maximally tetanized. Tension in a resting muscle also changes with muscle length as the result of the passive mechanical properties of muscle connective tissue. The proportion of force contributed by active muscle contraction can be obtained by subtracting the passive effect from the total force-length curve. The active force-length curve (Gordon *et al.*, 1966) shows the existence of an optimal length, around which muscle tension can reach its maximum. The tension decreases whenever muscle length is shorter or longer than the optimal value. Based on the sliding filament, cross-bridge theory, Gordon *et al.* attributed the dependence of active muscle tension on length to a proportional relation between the number of cross-bridges available for tension development and the amount of overlap between the thin and thick filaments.

A viscous effect can be identified during muscle contraction since muscle force changes with velocity of shortening or lengthening. Two experimental approaches have been used to measure the force-velocity relation, isotonic and isovelocity length changes. In both cases, one end of a muscle is fixed and the other end remains free. After the muscle is tetanized, the free end is suddenly connected to an external load. Displacement of the free end is recorded during an isotonic experiment (Granzier *et al.*, 1989), while in an isovelocity experiment, the free end is forcibly moved at a fixed velocity and muscle tension is measured (Joyce *et al.*, 1969). When a tetanized muscle is allowed to shorten, the tension decreases with increasing velocity and reaches zero at the maximum steady shortening velocity. The maximum steady shortening velocity has been shown to be temperature dependent, but independent of initial muscle length and tension (Edman, 1979). On the other hand, when a tetanized muscle is forcibly stretched, the tension increases sharply for small increases in velocity, but begins to yield at loads of 1.6-1.8 times the isometric tension (Katz, 1939; Granzier *et al.*, 1989).

When in transition from one steady state to another steady state, a system always undergoes a dynamic change. For example, when applying a sudden length change to a tetanic muscle, there is a transient response in tension. Ford *et al.* (1977) conducted a series of tests to characterize muscle transient responses to sudden changes in length. They divided the transient response

into four phases, rapid change, early recovery, slow recovery and final recovery to the original state. During the first phase, both muscle tension and length change rapidly. The minimum tension in the shortening case or the maximum tension in the lengthening case, denoted as T_1, is reached at or before the end of the rapid change and has a nearly linear relation with the amplitude of a step change in muscle length, demonstrating that muscle possesses spring-like properties. They also showed that the ratio of T_1 to the isometric tension, F_{m_0} plotted against the amplitude of the step change was dependent on temperature and the rate of change of muscle length, but not F_{m_0}. Increasing the temperature or the velocity of the length changes reduces the slope of the T_1/F_{m_0} curve, which represents an apparent decrease in muscle stiffness. After the end of the length change, the second phase begins during which much of the tension change in the first phase is reversed. The tension at the end of this early recovery was symbolized by T_2. During recovery muscle tension normally passes through a local minimum if lengthening or a local maximum if shortening. The shape of the curve of T_2/F_{m_0} plotted against the amplitude of the length change varies little with temperature or velocity of the length change. However, the duration of the recovery decreases as temperature increases. During the third phase, the rate of recovery is significantly reduced or may even reverse, while during the final phase, muscle tension asymptotically approaches the isometric value.

Later, Ford *et al.* (1985) conducted another series of tests to study muscle transient responses to sudden changes in length during steady shortening. The muscle tension during steady shortening is denoted as F_{m_i}. Both $|T_1 - F_{m_i}|$ and T_2/F_{m_i} decreased with an increase in steady shortening velocity. They attributed these decreases to a decrease in the number of attached cross-bridges and also estimated muscle stiffness to be about 35% of the isometric value as F_{m_i} approached zero.

The experimental results of Julian and Morgan (1979a) suggest that muscle transient responses are mainly due to the mechanics of attached cross-bridges. In one of their experiments, muscle was subjected to a step length change just prior to tetanic stimulation. The time course of muscle force development was similar to that in an isometric state. In this experiment, filament sliding resulting from the stretch would not have influenced the majority of attached cross-bridges present in the final steady state since they would not have been formed until after the stimulation had begun. On the other hand, when the same step length change was applied during the tetanus in a second experiment, muscle tension approached a short-lived peak and then fell to a plateau.

Similar to the response to sudden changes in length, a tetanic muscle undergoes a transient response to a change in load before reaching an isotonic steady-state. The transient response can be influenced by a number factors, such as external load, muscle length, temperature, mass and viscosity (Podol-

sky, 1960; Joyce and Rack, 1969; Granzier *et al.*, 1989). It is almost the same as the response of a second-order mechanical system to an external change where smaller mass, heavier damping and slower change in external load facilitate the speed and smoothness of the transition from an isometric steady-state to an isotonic steady-state. With a given rate of change of muscle length, the actual filament sliding velocity is inversely proportional to the muscle length, which in turn directly affects cross-bridge behavior. The effects of temperature on the time course of muscle transient responses are manifested by the dependence of isometric muscle tension, steady shortening rate and the duration of the transient response on temperature (Podolsky, 1960). Quantitatively, Podolsky found that the temperature coefficient of the duration of the transient response during transition from an isometric to an isotonic steady-state was almost the same as the temperature coefficient of the ratio V_m/F_{m_0}. This suggests that the molecular mechanisms responsible for the temperature dependence of V_m and F_{m_0} are also implicated in the temperature dependence of the duration of the transient response.

We have outlined above what we think are primary elements needed for a complete model of muscle behavior. In the following chapters, more quantitative specifications for muscle micromechanics will be proposed based on experimental observations and the most widely accepted theories. Development of the conceptual model should not only be consistent with, but also

provide reasonable explanations for the experimental observations, while its mathematical formulations should be able to predict them quantitatively.

CHAPTER 3

THE CONCEPUTAL MODEL

Skeletal muscle has been studied from the perspectives of structure, physiology and biochemistry. The essential features of theories of excitation-contraction coupling, sliding-filaments and cyclic interaction of cross-bridges have been widely accepted. When an action potential propagates along the membrane of a single muscle fiber, calcium ions are released from the muscle sarcoplasmic reticulum, diffuse through the fiber and bind to troponin molecules. The binding of calcium ions to troponin molecules causes tropomyosin molecules to "shift", exposing sites on the actin filaments to which myosin heads can bind to form closed cross-bridge linkages. Cross-bridges are energized by the hydrolysis of ATP to form ADP and inorganic phosphate with the release of chemical energy. The energized cross-bridges attach to the exposed actin sites and generate tension between the thin and thick filaments. A cross-bridge remains attached until an ATP molecule binds to the cross-bridge head, providing energy for detachment and initiating a new cross-bridge cycle. As cross-bridges cycle,

thin and thick filaments can slide past one another in the process of muscle contraction. However, because of the limited ability to make measurements at the molecular level many of the details of the cyclic interaction between actin and myosin molecules during muscle contraction have yet to be elucidated. To overcome this limitation, a conceptual model of muscle contraction has been developed by synthesizing recent experimental results. This model is mainly concerned with aspects of cross-bridge dynamics (muscle micromechanics) that have so far not been considered in previous models.

3.1 MUSCLE CONTRACTILE MACHINERY

A muscle fiber consists of a series of regularly repeated units, the sarcomeres. Since a half sarcomere can be considered as the smallest working unit of skeletal muscle its properties should account for most muscle properties observed at the macroscopic level. Within the sarcomere, thin and thick filaments form a regular hexagonal array. Each thin filament consists of three protein molecules, actin, troponin, and tropomyosin. The thick filament is composed of only myosin molecules, whose heads project from its base and can attach to actin molecules to form closed cross-bridge linkages. Each thick filament is surrounded by six thin filaments. Muscle force is developed by the

interaction between actin and myosin molecules. Actomyosin complexes constitute the only essential active element of the muscle contractile machinery. Before discussing the details of the contractile process, a mechanical analog (Fig. 3.1) will be introduced to serve as a physical description of the active mechanochemical process of cross-bridge force development during muscle contraction.

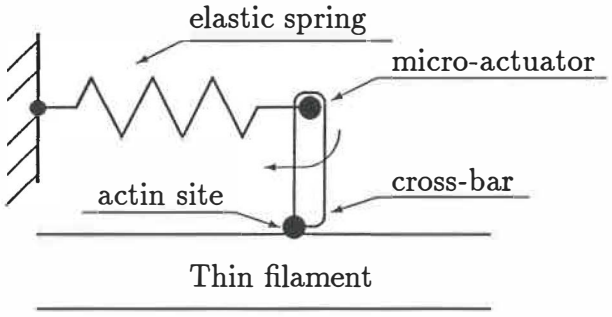


Figure 3.1 The mechanical analog of muscle contractile machinery.

A cross-bridge may be represented by a functionally equivalent micro-actuator, fixed to one end of a spring with a small bar which can rotate around an axis subject to certain constraints. Elongation of the spring is induced when the bar is obstructed during its rotation. The actuator is an energy convertor, which can transform chemical energy to mechanical work and heat.

3.1.1 Cross-bridge structure and mechanics

A myosin molecule consists of two parts, LMM and HMM, which can be further subdivided into the S_2 and S_1 fragments (Lowey *et al.*, 1969). The S_1 fragment is also called the cross-bridge head. A myosin molecule consists of two identical units, each with a cross-bridge head which can rotate about its point of attachment to S_2 with a range of motion from $\theta_r \equiv 0^0$ to $\theta_e \equiv 45^0$, where θ_r is the position in which the cross-bridge head is perpendicular to the thin filament. Rotation of cross-bridge heads from θ_r to θ_e is an active process, defined as the working stroke, while rotation back to θ_r takes place as a consequence of binding of an ATP molecule after releasing the products of ATP hydrolysis (Marston *et al.*, 1976).

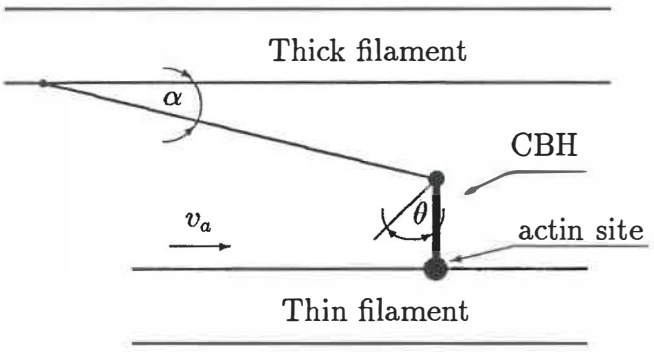
The cross-bridge head is an ATPase which increases its chemical energy by splitting ATP to ADP and P_i. The notation $MD^{\ddagger}$ is used to symbolize an energized cross-bridge head. The superscript, ‡ refers to the free energy level of a cross-bridge head. When the $MD^{\ddagger}$ attaches to an actin site, a closed cross-bridge linkage (thin filament base – AMD – S_2 – LMM – thick filament base) is formed (Fig. 3.2a). The notation AMD refers to an energized, attached cross-bridge head. The force generation of an AMD may begin at any angle θ_s within the range θ_r to θ_e. Rotation of AMD beyond θ_s induces deformation of the linkage or may cause forcible detachment if the force is greater than the

actomyosin affinity.

As a simple mechanical analog of the interaction between thin and thick filaments we introduce a hypothetical elastic element, which is presumed to be connected to the end of the cross-bridge head, but which we place along an axis parallel to the thin filament base, the x-axis in Fig. 3.2b, in order to simplify calculation. The quantities k and x are used to denote respectively, the stiffness and extension of this elastic element. The tension exerted by the overall deformation of the elements of the closed cross-bridge linkage is represented by a change in length of this hypothetical spring, while the remaining elements of the linkage are assumed to act as rigid bodies, although in reality deformation could occur anywhere in the linkage since it is composed of protein molecules which are not rigid. Recovery from the linkage-induced deformation is assumed to be rapid when the power driving the cross-bridge head goes to zero or when the linkage is broken by a forcible detachment.

When $\theta = \theta_s$, $x \equiv 0$ since no tension is generated between the thin and thick filaments. The elastic element will be extended as the attached cross-bridge head rotates during force generation. The effective working stroke for force development is represented by the angular displacement from θ_s to θ_e. The extension of the elastic element can be expressed in terms of θ_s and θ_e as

$$x = l(\sin\theta - \sin\theta_s) \tag{3.1}$$



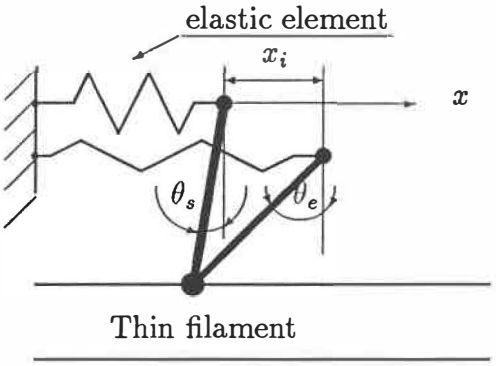


Figure 3.2 (a) The configuration of an attached cross-bridge at θ_r; (b) x_i is the projection of the structural change in two different configurations of an attached crossbridge to the axis which is used to represent the displacement of the proposed hypothetical spring.

where l is the length of the cross-bridge head. In a static state there two major external forces which act in opposite directions on the ith cross-bridge. One force, kx_i is exerted by the hypothetical elastic element, and the other force, f_{a_i} by the attached actin site. They balance each other and thus we have

$$f_{a_i} = kx_i \tag{3.2}$$

where k is assumed to remain constant.

X-ray diffraction studies of muscle structure have shown that the cross-bridges and actin binding sites are regularly spaced along the thick and thin filaments respectively. We assume that the number of actin sites and cross-bridges 'participating' in force development is proportional to the amount of overlap between the thin and thick filaments which varies with muscle length. If we further assume that cross-bridges act independently, then the number of cross-bridges involved in force generation will depend on the number of participating actin-myosin couples for a given calcium concentration provided that the supply of ATP is not a limiting factor. Muscle force generated in a half-sarcomere is taken as the ensemble average of the force developed by all the cross-bridges in a force generating state and can be expressed as

$$F_m = \sum_{i=1}^{N_s} f_{a_i} = k \sum_{i=1}^{N_s} x_i = kN_s\bar{x} \tag{3.3}$$

where N_s is the number of cross-bridges in a force generating state and $\bar{x}$ is the mean value of the ensemble x_i. Filament overlap, which determines the

number of cross-bridges that can contribute to force development, underlies the force-length relation (Gordon *et al.*, 1966).

Another property conferred by the regular arrangement of cross-bridge heads and actin binding sites is a uniform probability distribution of the relative axial distance between cross-bridges and nearest potential attachment sites on actin filaments in the region of overlap. This distribution is independent of muscle length (except in the case where there is no overlap between the thin and thick filaments) and results in a constant mean value of the relative axial distances.

3.1.2 Energy source and utilization

When a cross-bridge head is energized through the hydrolysis of an ATP molecule a certain amount of energy, e, can be released. Binding of ATP to the cross-bridge head and its cleavage to form $MD^{\ddagger}$ are rapid processes. A new ATP cannot bind to a cross-bridge head until the previous hydrolytic products have been released into the sarcoplasm. This only occurs when the chemical energy held by the cross-bridge drops to zero. The energizing process is usually linked to the cycle of ATP hydrolysis. We also assume that the cross-bridge working stroke is coupled to an irreversible cycle of ATP hydrolysis. Consequently, the interaction between actin and myosin molecules involved in muscle force generation will also be cyclical.

In 1934, Lohmann investigated the mechanism by which phosphocreatine (PCr) is involved in the resynthesis of ATP and showed that the transphosphorylation has a high equilibrium constant and is promoted by a specific enzyme, creatine phosphokinase (CPK). Carlson and Siger (1959, 1960) demonstrated that the activity of CPK in muscle was apparently sufficient to keep the reaction close to equilibrium. This implies that ATP concentration will remain approximately constant during a short period of muscular activity.

Studies of the kinetics of biochemical reactions involved in muscle contraction (Woledge *et al.*, 1985) have shown that the rate of ATP hydrolysis depends on the presence of actin, temperature, and the concentration of various ions in the sarcoplasm. In order to link biochemical and mechanical events during force development by an actomyosin complex, we assume that the ability of a cross-bridge to transform chemical energy to mechanical work or to heat is limited, and that it not only varies with temperature, ionic strength and the presence of actin, but also depends on the current mechanical state such as the conformation and angular velocity of the cross-bridge head. When an energized cross-bridge head rotates, it always tends to transform the chemical energy which it holds into mechanical work until it reaches the limit of its movement range where it transforms its remaining chemical energy into heat. This cross-bridge property can be mathematically expressed by

$$P = \begin{cases} R_w P_w & \text{if } \theta_e > \theta \geq \theta_r \\ P_h & \text{if } \theta = \theta_e \end{cases} \tag{3.4}$$

where P represents the rate at which a cross-bridge can transform its chemical energy into other forms of energy. $R_w P_w$ and P_h represent the rates of doing mechanical work and heat generation, respectively. It is important to recognize that the efficiency R_w varies with the instantaneous angular velocity of the cross-bridge head. From conservation of energy, the sum of the heat generated and work done by a cross-bridge head should be equal to e in an interaction cycle if there is no energy cost in the energy transformations.

3.1.3 Kinetics and mechanical processes

Once a cross-bridge is energized, its head begins to rotate actively forward to θ_e at a relatively low velocity. During its rotation it will undergo one of two processes—an actomyosin cycle or a myosin cycle (Eisenberg *et al.*, 1978) depending on whether or not it attaches to an activated actin site. This is consistent with observations using spectroscopic probes that myosin heads appear to have maximal rotational freedom in the detached phase of the cycle, probably facilitating attachment, and that considerable mobility is retained upon initial attachment to actin (Thomas, 1987).

During a myosin cycle, $MD^{\ddagger}$ does not attach to any activated actin site and therefore makes no contribution to muscle stiffness or force development. The

energy held by $MD^{\ddagger}$ drives it in slow rotation through its range of movement against the viscous resistance of the sarcoplasm and elastic resistance of the MD-S_2 joint. This cycle takes much more time than that of the actomyosin cycle since the ability of $MD^{\ddagger}$ to transform chemical energy to mechanical work or heat is quite low in the absence of activated actin. Most of the chemical energy is transformed into heat and hence $MD^{\ddagger}$ does little work during rotation. The heat generated may represent the fraction of muscle energy liberation referred to as resting heat (Wilkie, 1954) when muscle is in a resting state or the small portion of muscle energy liberation referred to as maintenance heat (Hill, 1938) when muscle contracts isometrically.

Four cross-bridge states, $M(45^0)$, MT, $MD^{\ddagger}$, and $MD^{\ddagger}(45^0)$ are used to represent the mechanical and chemical features of this kinetic scheme (upper loop in Fig. 3.3). The $M(45^0)$ state is the minimum energy state. Departing from this state, cross-bridges bind ATP to form MT. By cleaving ATP, cross-bridges are energized and recover back to θ_r, entering the $MD^{\ddagger}$ state. In this state they rotate until they arrive at θ_e, still with most of the chemical energy. This chemical energy is then transformed to heat in the $MD^{\ddagger}(45^0)$ state. The transition from $MD^{\ddagger}$ to $MD^{\ddagger}(45^0)$ is accompanied by a continuous change of cross-bridge conformation. The notation H represents the heat generated. The duration of the cycle period, T_m is equal to the sum of T_b, T_{m_1}, T_{m_2}, and T_{m_3}, where T_b, T_{m_1}, T_{m_2}, and T_{m_3} are the time intervals during which a cross-

bridge is in M(45^0), MT, $MD^{\ddagger}$ and $MD^{\ddagger}(45^0)$, respectively. The arrows in Fig. 3.3 represent the net transition directions between the proposed cross-bridge states.

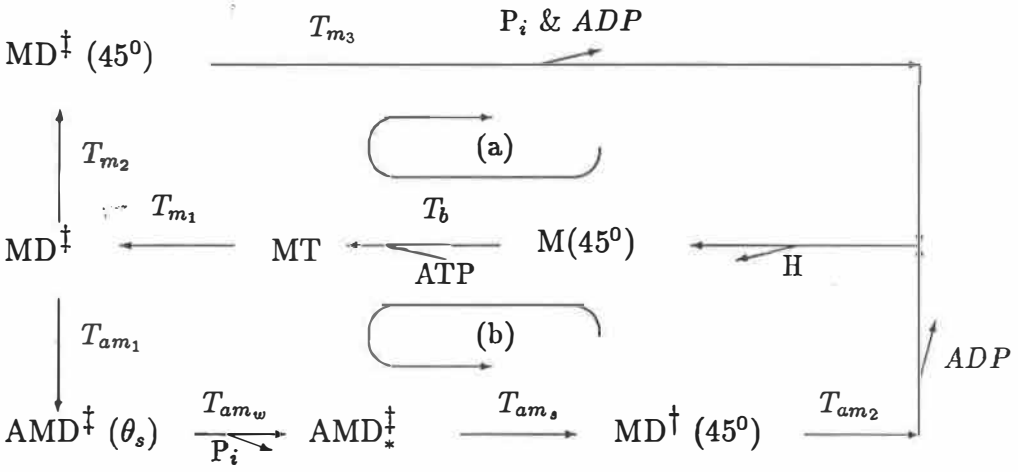


Figure 3.3 The sequential scheme of mechanical and biochemical processes on which the model is based: (a) myosin cycle. (b) actomyosin cycle.

During an actomyosin cycle, $MD^{\ddagger}$ attaches to an activated actin site to form $AMD^{\ddagger}$. This initial cross-bridge attachment constitutes a weakly attached state. In this attached state the ability of the cross-bridge to convert chemical energy to mechanical work or heat, as well as the affinity between

actin and the myosin is greatly amplified. The weakly attached state is short-lived since $AMD^{\ddagger}$ rapidly changes to a strongly attached state, $AMD^{\ddagger}_{*}$ where muscle force develops at a much faster rate due to amplification of the energy conversion rate. Tension between the thin and thick filaments can be sustained because of the structural support provided by the affinity between actin and myosin molecules. Both higher affinity and greater power output of a cross-bridge in the strong binding state make this state the major state of force generation. The energy conversion rate in the strong binding state is represented by $R_{w_s}P_{w_s}$ while P_{h_a} represents the energy conversion rate immediately following termination of the strong binding state. The subscript $*$ denotes an $AMD^{\ddagger}$ having a high energy conversion rate and affinity. The rotation of $AMD^{\ddagger}$ against the actin site initiates force production. However, we assume that it takes some time for the biochemical reactions involved in force generation to become fully activated. During this short period, muscle force would increase very slowly and would be negligible in comparison to the force developed later. This assumption is consistent with the observations of Kress *et al.* (1986) that the attachment of large numbers of cross-bridges to the actin filament occurs significantly before muscle tension develops. This is also similar to Huxley's suggestion that the cross-bridge attachment during muscle contraction may take place in two stages (Huxley, 1973), although it has a different basis since we have assumed that each cross-bridge mechanical cycle

is coupled to an irreversible cycle of ATP hydrolysis and the strong binding state is equivalent to a continuous transition through many intermediate state with diffrerent free energies.

$AMD_*^{\ddagger}$ rotates until arriving at θ_e where the cross-bridge becomes able to transform its remaining chemical energy into heat at the rate P_{h_a}. The cross-bridge terminates its force generation since there is no longer any power driving the rotation of its head. In this state, the affinity of the attached cross-bridge for the actin is quite low. Whether an attached cross-bridge can remain attached depends only on whether the force exerted on the actomyosin bond is less than the affinity. The elastic deformation in the actomyosin bond or some other structural change which leads to force generation, such as sliding of thin and thick filaments, could result in detachment. After delivering the products of ATP hydrolysis into the sarcoplasm and transforming all of its remaining energy to heat, a new ATP molecule can bind to the cross-bridge and another cycle can commence.

To draw attention to the sequential effects of the biochemical reactions on the cross-bridge mechanics during muscle force development, the period of an actomyosin cycle is divided into six subperiods, T_b, T_{m_1}, T_{am_1}, T_{am_w}, T_{am_s}, and T_{am_2}. During T_{am_1}, $MD^{\ddagger}$ attaches to an activated actin site and forms $AMD^{\ddagger}$; during T_{am_w}, $AMD^{\ddagger}$ releases P_i and goes from a weak to a strong binding state while both its output power and affinity are being amplified;

during T_{am_s}, $\text{AMD}^{\ddagger}_{*}$ develops force and does mechanical work; finally, during T_{am_2} ADP is delivered into the sarcoplasm and then the remaining chemical energy held by $\text{MD}^{\dagger}(45^0)$ is transformed into heat, but no mechanical work is done. Recovery to the original position θ_r occurs immediately after binding with a new ATP molecule. In the absence of ATP, the cross-bridge cannot be re-energized and thus is not able to recover its original conformation. All of the biochemical and mechanical events occuring during an actomyosin cycle are summarized in the lower loop of Fig. 3.3. The superscript † is used to represent the free chemical energy level of an energized cross-bridge head, which is lower than that represented by ‡.

The time spent in each cross-bridge state can be determined by the transition rates between adjacent biochemical states in the cycle. T_{m_1} and T_{am_w} depend only on the rates of biochemical reactions which in turn depend on muscle temperature and the sarcoplasmic concentration of the chemical species involved in the reactions. T_b is associated with the concentration of ATP in the sarcoplasm. T_{am_2} and T_{am_s} can be altered by cross-bridge mechanical properties, *i.e.* the angular velocity of AMD, since different angular velocities represent different amounts of chemical energy being transformed into mechanical work or heat. T_{am_1} can change as the result of sliding of thin and thick filaments which modifies the relative distances between $\text{MD}^{\ddagger}$s and activated actin sites.

From Fig. 3.2, it can be seen that the angular velocity of AMD may be altered by filament sliding which occurs when muscle is stretched or allowed to shorten. In this case, the period of an actomyosin cycle will change because T_{am_1}, T_{am_2} and T_{am_s} change. In addition, there will be mechanical energy exchange between the muscle and the environment. Consequently, muscle behavior is strongly influenced by filament sliding. In order to take into account the effects of such differences in operating conditions on cross-bridge mechanics, we have derived the following constraint equation based on the principle of power balance.

$$R_{w_s} P_{w_s} + f_a v_a = kx\dot{x} \tag{3.5}$$

where v_a is the rate of sliding of thin filaments past thick filaments when muscle is stretched and $\dot{x}$ is the rate of extension of the elastic element which can be obtained from Eq.(3.1) as follows,

$$\dot{x} = l(\dot{\theta}\cos\theta - \dot{\theta}_s \cos\theta_s) = l\dot{\theta}\cos\theta + v_a \tag{3.6}$$

Note that θ_s represents a virtual angle which can be altered by v_a, since θ_s depends on the relative axial distance between the energized cross-bridge and the potential actin binding site which changes when the filaments slide past one another. If there is no active rotation of a cross-bridge head, as when

muscle is passively shortening, $\dot{x}$ should be equal to zero so that we have $\dot{\theta}\cos\theta = \dot{\theta}_s \cos\theta_s$. The sliding velocity of the two filaments is equal to $l\dot{\theta}\cos\theta$, the projection of the tangential velocity of the tip of a cross-bridge head onto the base of the filaments. Consequently, the term $l\dot{\theta}_s \cos\theta_s$ in Eq.(3.6) can be replaced by $-v_a$.

It is evident from Eq.(3.5) that the rate of force development, which is directly proportional to $\dot{x}$, depends on the rate of utilization of chemical energy by the cross-bridges, the sliding velocity and the current tension; from the point of view of cross-bridge structure, it is a function of cross-bridge conformation, rate of conformational change and the relative sliding velocity, as shown by Eq.(3.6).

The affinity of the actomyosin bond is an important parameter. Not only does the maximum force which can be applied to an attached complex depend on the affinity, but also mechanical events such as cross-bridge attachment and detachment. We assume that compared to $\mathrm{AMD}^{\ddagger}_{*}$, the affinity in other cross-bridge states is negligible, although greater than zero. Even with a small affinity actin and myosin might remain attached throughout the entire cycle, although the only evidence for this comes from muscle kinetic studies using purified actin and myosin in solution (Geeves, 1991). The detachment of an attached cross-bridge always occurs if the force exerted on the complex is greater than the affinity, no matter how great. For instance, an attached cross-

bridge could be forcibly detached from actin when muscle is stretched even if it is in the $AMD_{*}^{\ddagger}$ state, because lengthening lowers $\dot{\theta}$ and raises $\dot{x}$ which may lead to a force larger than what can be sustained by the actomyosin affinity. After forcible detachment, the cross-bridge continues to rotate toward to θ_e without completing the current working stroke. It might, however, re-attach to another activated actin site and redevelop force before reaching θ_e. Furthermore, the low affinity at θ_e prevents cross-bridges from generating negative force when muscle shortening rate is significant.

A restriction on the range of movement of cross-bridge heads has been enforced by invoking a structural constraint which assumes that the backbone of the myosin molecule acts as a rigid rod. Under this constraint, an energized cross-bridge head would rotate around the tip of the stiff rod within a specified range in living muscle. In the rigor state this constraint could disappear, making the backbone more compliant and allowing cross-bridges to attach to actin without developing significant tension. Therefore, cross-bridges which would not form a closed linkage under normal physiological conditions, would now be able to attach to actin sites and undergo an extraordinarily large deformation in the absence of ATP. In contrast to Harrington's postulated active helix-coil transition for cross-bridge tension generation (1979), we treat the myosin rod as a passive element and assume that its mechanical properties may be altered by different chemical conditions.

3.2 CONSISTENCY WITH EXPERIMENT

In order to describe cross-bridge dynamics during skeletal muscle contraction, a conceptual model with several mathematical expressions has been developed, as outlined above. It is desirable that the model should be able to provide reasonable explanations for frequently observed behaviors, as well as a mathematical structure for quantitative predictions. Because of the vast amount of experimental data on muscle contraction, we have concentrated on several properties which were considered to be of primary importance rather than considering secondary effects such as those due to variations in muscle temperature or ionic concentration. Normally, muscle tension consists of active and passive components. In order to focus on cross-bridge behavior, we will neglect passive effects and assume that muscle is near its optimal length in the following discussion.

3.2.1 Isometric state

In an isometric state, muscle length is kept constant. The number of actin-myosin couples participating in force development within any half-sarcomere, Z, will be constant assuming that muscle properties within each sarcomere are identical throughout the muscle. Provided that there is sufficient ATP, the ratio of the number of cross-bridges in the actomyosin cycle to the number of

cross-bridges in the myosin cycle, N_{am}/N_m, will depend mainly on the calcium concentration in the sarcoplasm. The total number of cross-bridges in the actomyosin cycle, N_{am} is the sum of the number of attached cross-bridges in weak binding and strong binding states, N_w and N_s, and the number that detach after coming to the end of the working stroke or are about to attach to activated actin sites, N_d. We have

$$Z = N_m + N_{am} = N_m + N_w + N_s + N_d \tag{3.7}$$

If the length of a cross-bridge head is about 19 nm (Offer and Elliott, 1978), the maximum working stroke of the cross-bridge when projected onto the long axis of the thin filament has a length of about 13.4 nm which is close to the value at which T_2, muscle tension at the end of the early recovery after a sudden length change, decreases to zero (Ford *et al.*, 1977). The effective working stroke depends on θ_s which in turn depends on the relative axial distance between the cross-bridge location on the myosin filament and the nearest available actin binding site. Assuming that the probability distribution of the relative axial distances for an ensemble of actin sites and cross-bridges is uniform and independent of muscle length, both the distributions of θ_s and the effective working stroke of cross-bridges in the region of filament overlap will be uniform and independent of muscle length. If the probability distribution is rectangular, the mean value of the effective working strokes will be about

6.7 nm.

The relations of force-length and speed of unloaded shortening-length suggest that all cross-bridges act as independent force generators in parallel since muscle tension is directly proportional to the amount of overlap between the thin and thick filaments in the case of isometric contraction (Gordon *et al.*, 1966) and the speed of unloaded shortening is independent of the amount of filament overlap (Huxley and Julian, 1964). Since weakly attached cross-bridges make no significant contribution to muscle force generation or stiffness, the total elastic force in a half-sarcomere is given by $N_s k\bar{x}$ and the stored elastic energy by $N_s k\overline{x^2}/2$. The actual muscle stiffness associated with attached cross-bridges is equal to $N_s k$.

If calcium concentration is low, most cross-bridges would remain in the states where no significant force is developed. When a muscle fiber is activated following a neural input, the calcium concentration in the sarcoplasm increases leading to an increase in N_s. After entering into the strong binding state, an actomyosin complex begins to develop force with a time course dependent on the rate of energy transfer $R_{w_s} P_{w_s}$, but independent of calcium concentration. In most physically realizable torque generating systems, *e.g.* electromagnetic motors, the output torque decreases as angular velocity increases. Ford *et al.* (1985) demonstrated that muscle tension dropped to zero while muscle stiffness decreased only by about 65% of its value in the isometric state when

the rate of change of muscle length was near to the maximum steady shortening velocity. According to Eq.(3.3), both the mean value of elastic element extension and mechanical output power should decrease at the same time. Therefore we assume that R_{w_s} follows a concave downward parabolic curve as cross-bridge angular velocity increases while P_{w_s} remains essentially constant. The extension of the elastic element associated with the cross-bridge will go through a cyclical process: being zero at the moment of attachment, remaining near zero during the weak binding period, then proceeding to its maximum value under the constraints imposed by equations (3.5) and (3.6) during the strong binding state and finally dropping to zero again when the cross-bridge head reaches θ_e. The maximum value would be directly proportional to the effective working stroke. This process of force development produces muscle tension with dynamics that causes muscle force to rise and drop more slowly than the variation in the number of attached cross-bridges (Kress *et al.*, 1986) or muscle stiffness (Hatta *et al.*, 1988). It is also partly responsible for the lag between muscle force and changes in calcium concentration (Ridgway and Gordon, 1975), although we attribute this mainly to the finite transition rate from the non-force generating state to the force generating state.

When muscle contracts steadily, it acts as a static system. The number of cross-bridges in each state in our model will depend on the number of cross-bridges in the myosin and actomyosin cycles and the ratio of the state duration

to the mean value of the cycle period. Obviously, during steady contraction, the distribution of cross-bridges in different states remains constant.

3.2.2 Shortening state

During steady shortening, the relative motion between thin and thick filaments contributes to the process of muscle force development. Since this sliding is in the same direction as the active rotation of the cross-bridge head, it produces an effective increase in the angular velocity of attached cross-bridges. There are three major effects of this increase in cross-bridge angular velocity predicted by the conceptual model. First, the sliding allows the cross-bridge to reach the angular position θ_e with a shorter effective working stroke, equivalently shifting the virtual angle θ_s toward θ_e and thereby reducing the maximum attainable extension of the elastic element. The rate of change of the effective working stroke produced by this shift is represented mathematically by the second term on the right hand of Eq.(3.6). Second, the power output for force development changes since R_{w_s} is a function of angular velocity. This is consistent with experimental observations of muscle mechanical efficiency (Woledge, 1968). Third, the cross-bridge transition rate from $AMD^{\ddagger}_{*}$ to $MD^{\dagger}(45^{0})$ increases, which also implies that the duration of the strong binding state, T_{am_s} decreases. At the same time, the number of cross-bridges in the actomyosin cycle increases since the probability of a detached, energized

cross-bridge attaching to an activated actin site is increased by the filament sliding.

The mean energy liberation rate during steady shortening can be expressed as

$$\overline{\dot{E}} = (N_{am}/T_{am} + N_m/T_m)e + c_1 \tag{3.8}$$

where the first term on the right hand side represents the energy from the interaction of actin and myosin molecules while c_1 represents the energy from muscle activation mechanisms such as the Ca-releasing and Ca-reaccumulating processes (Homsher and Kean, 1978) which can be assumed constant for steady activation. Studies of muscle kinetics have shown that myosin ATPase activity is quite low compared with that of the actomyosin complex. Hence T_m should be much larger than T_{am}, in addition to being independent of v_a. The second term on the right hand side of Eq.(3.8) can therefore be neglected. The variation in energy liberation rate depends mainly on the changes in the number of cross-bridges in the actomyosin cycle and the mean value of their cycling rate. Assuming that the chemical energy used during T_{am_1} and T_{am_w} is much less than that used during T_{am_s} and T_{am_2} the amount of the chemical energy from splitting one ATP molecule would be used during T_{am_s} and T_{am_2}. Thus we have

$$T_{am_2} \approx (e - R_{w_s} P_{w_s} T_{am_s})/P_{h_a} \tag{3.9}$$

and

$$\begin{aligned} T_{am} &= T_{am_1} + T_{am_s} + T_{am_2} + \text{constant} \\ &= T_{am_1} + \left(1 - \frac{R_{w_s} P_{w_s}}{P_{h_a}}\right) T_{am_s} + \frac{e}{P_{h_a}} + \text{constant} \end{aligned} \tag{3.10}$$

Here R_{w_s}, T_{am_1} and T_{am_s} are dependent on v_a while P_{h_a}, P_{w_s} and e are constants.

During shortening, cross-bridge attachment will be a complex function of several variables, including the number of unattached cross-bridges available for attachment and the probability that a detached, energized cross-bridge will encounter an activated actin site during its working stroke. At low shortening velocities there will be a rapid increase in cross-bridge attachment since the probability of a detached, energized cross-bridge encountering an activated actin site will be increased by filament sliding. However, as the shortening velocity increases, cross-bridges might fail to attach to actin binding sites that pass by too rapidly.

T_{am_1} is dependent on the probability that a detached, energized cross-bridge will attach to an actin binding site. This probability increases as the result of filament sliding at low shortening velocities since more actin sites come within reach of the cross-bridge and on average a cross-bridge will spend less time rotating before attaching, thereby reducing T_{am_1}. As the shortening velocity increases further, T_{am_1} approaches a minimum because the facilitation of attachment by filament sliding reaches a limiting value. Assuming that the transition rate from $AMD^{\ddagger}_{*}$ to $MD^{\dagger}(45^0)$ is linearly dependent on shortening

velocity, then T_{am_s} will decrease in a continuous manner as shortening velocity increases. Higher shortening velocity results in cross-bridges arriving in the AMD†(45^0) state faster with more chemical energy, thereby increasing heat generation and T_{am_2}. P_{h_a} is less than P_{w_s}, but will be larger than $R_{w_s}P_{w_s}$ at certain shortening velocities since R_{w_s} is assumed to follow a concave downward parabolic curve as cross-bridge angular velocity increases. When $R_{w_s}P_{w_s}$ is larger than P_{h_a}, the second term on the right hand side of Eq.(3.10) will be negative, reducing T_{am}. It can be seen from Eq.(3.10) that as shortening velocity increases, T_{am} first decreases as T_{am_1} and T_{am_s} decrease, then reaches a minimum when the second term on the right hand of Eq.(3.10) has its minimum, and finally increases slightly when the second term on the right hand side of Eq.(3.10) increases due to an increase in T_{am_2}.

The refractory period is defined as the period during which cross-bridges are unable to attach to an activated actin site. T_{am_2} constitutes the variable portion of the refractory period. From Eq.(3.9), T_{am_2} would continually increase with increasing shortening velocity since more and more chemical energy must be transformed into heat at a finite transformation rate P_{h_a}. This leads to an increase in the number of detached cross-bridges which are not available for attachment. Therefore, after increasing at low shortening velocities the attachment rate would reach a maximum and then decrease when the shortening velocity is sufficiently high, due to a decrease in the probability of

attachment and an increased refractory period. This is in contrast to Huxley's model which predicted that the average rate of cross-bridge attachment would decrease continually with increased shortening velocity.

From Eq.(3.8), the mean energy liberation rate is proportional to the ratio of the number of cross-bridges in the actomyosin cycle and the mean cycling rate. Because the net cross-bridge transition has been modeled as an irreversible cycle, the number of cross-bridges in the actmyosin cycle is mainly dependent on the attachment rate. As disccussed above, N_{am} would increase first, reach a maximum and finally decrease as the shortening velocity increases. This behavior of the mean energy liberation rate-velocity relation is consistent with the experimental results observed by Hill in 1964.

Recent studies of muscle energetics strongly suggest that the rate of ATP hydrolysis can be used to account for the rate of energy liberation only in the case where the rate of ATP hydrolysis is constant, since cross-bridges act as temporary stores for the chemical energy (Homsher *et al.*, 1981). When an activated muscle is allowed to shorten at constant velocity from a prior steady isometric state, attached cross-bridges would do less mechanical work in developing force and reach to the $MD^{\dagger}(45^{0})$ state more rapidly than under isometric conditions. They must therefore convert a relatively large amount of chemical energy into heat at θ_e and hence the rate of heat production would increase. The more rapidly muscle shortens, the more cross-bridges there would

be in the $MD^{\dagger}(45^0)$ state and the higher the temporary rate of heat production. Initially the rate of heat generation would be high, but with time some of the cross-bridges in the $MD^{\dagger}(45^0)$ state would be redistributed to other states and the rate of heat generation would eventually decrease and reach a steady state.

If the shortening stops before reaching a steady state, the instantanous rate of ATP hydrolysis cannot account entirely for the muscle energy liberation rate. The ATP consumption during the recovery phase should be taken into account in the energy balance since cross-bridges are energy reservoirs. This mechanism could provide a reasonable explanation for experimental results that show the dependence of the extra energy liberated by rapidly shortening frog skeletal muscle on the extent of shortening (Irving and Woledge, 1981b). It could also explain the observation that when the muscle undergoes two consecutive periods of isovelocity shortening, energy liberation during the second period depends on the interval between the two periods (Irving and Woledge, 1981a). Our conceptual model is consistent with their two-state model for shortening-dependent energy liberation. In our case, $AMD_*^{\ddagger}$ and $MD^{\dagger}(45^0)$ are the two major states. The transition rate from the $AMD_*^{\ddagger}$ state to the $MD^{\dagger}(45^0)$ state depends on sliding velocity. Shortening results in an increase in cross-bridge transition from $AMD_*^{\ddagger}$ to $MD^{\dagger}(45^0)$. The more rapidly the muscle shortens, the more rapidly cross-bridges arrive at θ_e where chemical energy is transformed into heat, making $MD^{\dagger}(45^0)$ the predominant state.

Although the number of cross-bridges in the actomyosin cycle increases with filament sliding, the number of cross-bridges in the strongly attached state decreases since the increase in transition rate from $AMD_{*}^{\ddagger}$ to $MD^{\dagger}(45^{0})$ is more rapid than the increase in the attachment rate as the shortening velocity increases. This is responsible for the decrease in muscle stiffness (Ford *et al.*, 1985) and also partly for the decrease in muscle force (Hill, 1938) during shortening. The remainder of the force-velocity decrement results from the reduced duration and the reduced rate of cross-bridge force development, which lead to a reduction in the mean value of the elastic element extension. The decrease in R_{w_s} plays a more important role when muscle shortens at a velocity which is close to the maximum steady shortening velocity where muscle force is equal to zero, because muscle stiffness is not equal to zero. Compared to isometric contraction, a significant part of the chemical energy is used to do external mechanical work and the total consumption of chemical energy increases, of which a decreasingly smaller portion is used for force development and an increasingly larger portion converted to heat with increasing shortening velocity.

3.2.3 Lengthening state

Muscle tension rises and the rate of energy liberation goes down sharply when muscle is stretched at low velocities. Increased tension is mainly due to

a larger extension of the cross-bridge elastic elements and a greater number of cross-bridges in the strongly attached state as filaments slide in the direction opposite to the active rotation of attached cross-bridge heads. As the stretching velocity increases, the tension reaches a plateau while the energy liberation rate increases. The filament sliding during stretching will impede the active rotation of attached cross-bridge heads toward θ_e, producing a reduction in the angular velocity. The net effects are opposite to those during shortening. The effective value of θ_s will shift asymptotically toward θ_r, R_{w_s} will decrease continually toward a negative value, and the transition rate from $AMD_*^{\ddagger}$ to $MD^{\dagger}(45^0)$ will decrease. However, the total number of cross-bridges in the actomyosin cycle will follow a pattern almost the same as that during shortening since the probability of a cross-bridge attaching to an activated actin site is a function of the sliding velocity regardless of the direction. The duration of the refractory state will increase when the muscle is stretched since cross-bridges will tend to arrive at the end of their working stroke having expended less energy in doing mechanical work and will take longer to transform the remaining chemical energy to heat. However, the increased T_{am_2} will still be quite small compared to the sharp increase in the period of the actomyosin cycle.

When an externally applied force exceeds the muscle tension, lengthening occurs as the thin filament arrays originating from opposite sides of a sarcomere are pulled apart. In this case, two torques act on a strongly attached

cross-bridge head, the output of the cross-bridge actuator and the external torque exerted through the bound actin. Since the two torques act in opposite directions, an increase in the externally applied torque will reduce the cross-bridge angular velocity as well as R_{w_s}, allowing the filament sliding to have a more direct effect on the rate of cross-bridge force development as shown by Eq.(3.6) and thereby utilizing less chemical energy to develop force. The force that can be developed in an elastic element depends only on the magnitude of the larger torque. When the externally applied torque is larger than the intrinsically generated torque, attached cross-bridge heads will be forced to rotate in the opposite direction. This will reduce the angular velocity and increase the effective working stroke, thereby increasing the duration of the strongly attached state. The amount of torque at the actin binding site will also increase considerably due to resistance encountered in forcing the cross-bridge head to rotate in the negative direction. The longer working stroke will result in more cross-bridges in the strong binding state and the greater torque will produce a larger extension of the elastic element. As a result, muscle force will increase sharply. However, the rate of ATP hydrolysis will decrease because the period of the actomysoin cycle increases much more sharply than the number of cross-bridges in the actomyosin cycle.

The maximum extension of a cross-bridge elastic element is limited by the actomyosin affinity since forcible detachment will occur whenever the force

exerted on the thin filament is larger than the affinity. During its working stroke, a cross-bridge can move toward θ_e at a higher angular velocity after forcible detachment than in either the $AMD_*^{\ddagger}$ or $AMD^{\ddagger}$ states. It can re-attach to other activated actin sites on the way toward θ_e. The higher the rate of stretch, the higher the force development rate (allowing force to reach its maximum value within a shorter interval), the higher the forcible detachment rate and the less time spent in the strong binding state, reducing the number of strongly attached cross-bridges and the period for the actomyosin cycle. The average extension of attached cross-bridges would gradually approach its maximum because of a greater proportional increase in the force development rate than in the decrease in the duration of the strong binding state. If the increase in the average extension compensates for the decrease in the number of strongly attached cross-bridges, the muscle tension will remain approximately constant after an initial sharp increase, as in the experiments of Granzier *et al.* (1989).

As forcible detachment becomes more prominent with increasing rate of stretch, the cross-bridge cycling frequency will increase slightly since forcibly detached cross-bridges can move toward θ_e at a higher angular velocity than in either the $AMD_*^{\ddagger}$ or $AMD^{\ddagger}$ states. This slight decrease in T_{am} combined with the increase in the number of cross-bridges in the actomyosin cycle will cause a slow rise in the muscle energy liberation rate in contrast to the sharp

decrease which occurs at very low stretching velocities. This is similar to the experimental observations of Curtin and Davies (1975) and is consistent with the fact that the energy from external sources does most of the mechanical work during stretching, including development of muscle tension while the energy from ATP hydrolysis is almost all transformed into heat resulting in a lower R_{w_s} which may even become negative.

3.2.4 Transient responses to muscle length changes

Modeling muscle behavior under steady state conditions is insufficient for most useful applications due to the fact that muscle normally acts in a dynamic environment and is rarely in a steady state. In contrast to the muscle behavior in steady states, the following three important factors must be taken into account during transient responses to a change in the mechanical state:

1) The propagation of a mechanical disturbance along a muscle fibre.

2) The instantaneous distribution of cross-bridges in the force generating state.

3) The instantaneous rate of cross-bridge force development.

We will assume that under dynamic conditions the response of a muscle consists of four phases: rapid change, early recovery, slow recovery and recovery to the original state.

The experimental results of Julian and Morgan (1979a) suggest that muscle dynamics is mainly concerned with the dynamics of strongly attached cross-

bridges. In one of their experiments, muscle was subjected to a fast length change just prior to tetanic stimulation. The time course of muscle force development was similar to that in an isometric state. In this experiment, filament sliding resulting from the stretch would not have influenced the majority of strongly attached cross-bridges present in the final state since they would not have been formed until after the stimulation had begun. On the other hand, when the same fast length change was applied during the tetanus in a second experiment, muscle tension reached a short-lived peak and then fell to a plateau which was higher than that obtained in the first experiment. This steady-state difference was called 'permanent' extra tension.

In our conceptual model, tetanic stimulation would result in a large number of strongly attached cross-bridges due to a high calcium concentration in the sarcoplasmic reticulum. Each cross-bridge in the strong binding state would generate force that results in extension of its associated elastic element. Subsequent stretch of the muscle following tetanus would cause the elastic element of attached cross-bridges to extend at a rate dependent on the lengthening velocity, resulting in a rapid rise in force and in additional extension in the elastic element compared with that during isometric contraction. This extra extension, which depends mainly on the amount of stretch, would in turn slow the active rotation of the cross-bridge head toward θ_e because of the upper bound in the mechanical power output. Following the stretch the angular ve-

locity of some cross-bridges might even reach zero according to Eqs.(3.5) and (3.6), remaining frozen in one position. This would prolong the time during which extra tension was being generated. Normally, the forcible detachment would involve a certain delay. Only a few cross-bridges would forcibly detach during a rapid stretch whereas many might detach shortly after completion of the stretch, leading to a sharp drop in muscle tension. The delay involved in cross-bridge detachment might be very small and therefore have no significant effect on cross-bridge behavior during steady contraction. Its effect on force generation would only become important during rapidly changing mechanical disturbances. For example, an attached cross-bridge at θ_e could become a negative force generator due to this characteristic as the result of a sudden decrease in muscle length.

Ford, Huxley and Simmons (1977) conducted a series of tests to study muscle responses to transient mechanical disturbances. They measured the muscle tension during the response to a fast ramp change in length and noted the value, T_1 at the end of the rapid change phase and T_2 at the end of the early recovery phase. T_1 was almost linear with respect to the ramp amplitude when the amplitudes were small. The slope of this relation was believed to represent the muscle stiffness. For larger shortening ramp amplitudes, the change in T_1 was less than what would have been predicted by a strictly linear relation. When the ramp duration decreased the slope of the relation between

T_1 and ramp amplitude decreased.

According to our conceptual model, muscle tension depends on the number of cross-bridges in the strong biding state and the mean extension of their elastic elements. Irrespective of the number of cross-bridges in the strong binding state, a proportionately smaller change in T_1 would result if the change in x (amount of extension of the elastic element) became a progressively smaller fraction of the ramp change in length per half-sarcomere. Two terms in Eq.(3.6) contribute to the change in x. The integral of Eq.(3.6) over the period of the rapid change phase is equal to the change in x. Integrating the term v_a will give the ramp change in length per half-sarcomere. However, since the integral of $\dot{\theta}$ is not zero, it increases in proportion to the ramp amplitude and the ramp duration, resulting in a smaller change in x than the externally applied length change. Experimentally, the muscle shortening velocity and hence, v_a is kept below some upper limit which prevents muscle tension from dropping too quickly to zero during the ramp. Under this constraint, the integral of $\dot{\theta}$ would become larger either when applying the same ramp amplitude with a longer ramp duration or applying a larger amplitude ramp with the same duration since $\dot{\theta}$ increases as x decreases. Thus, T_1 would be dependent on the slope of the ramp.

As the amplitude of the shortening ramp increases, more cross-bridges in the strong binding state would reach the limit of their working stroke. Thus,

the rate of detachment would increase. The net effect would be fewer cross-bridges in the strong binding state after the rapid change phase since it occurs too rapidly for a significant number of cross-bridges to be converted from the weak binding to the strong binding state. On the other hand, the number of cross-bridges attaching in the weak binding state would increase since more actin binding sites would be available to each cross-bridge due to filament sliding. As a result, both the number of strongly and weakly bound cross-bridges would be a function of the extent of the ramp change.

Following a shortening ramp, recovery of muscle tension during the early recovery phase is mainly dependent on force re-development by the attached cross-bridges which remain in the strong binding state. Although the total number of attached cross-bridges might vary little (Ford *et al.*, 1974), there would be a progressive shift of attached cross-bridges from the weak binding state to the strong binding state which would play an important role in the slow recovery phase. With larger amplitude shortening ramps, more strongly attached cross-bridges arrive at the end of their working stroke without significantly stretching their elastic elements, resulting in lower muscle tension at the end of the early recovery phase. If the amplitude of a shortening ramp exceeds the maximum working stroke (13.4 nm) then all cross-bridges in the strong binding state would already be at the limit of rotation θ_e after the rapid change phase, so there would be no significant early recovery of muscle tension,

i.e. $T_2 \approx 0$. From the above explanation, it can be seen that T_2 would mainly depend on the number of cross-bridges in the strong binding state while the duration of the early recovery phase would mainly depend on the rate of cross-bridge force development. Furthermore, the transition rates take longer time to reach their steady state values than the force development rate.

In contrast to a shortening ramp, the elastic elements of attached cross-bridges would lengthen when a muscle is stretched. With small stretch amplitudes, the extra extension of the elastic elements would be almost equal to the length change. The tension at the end of a lengthening ramp, T_1 would be proportional to the extension of the elastic element and hence proportional to the amplitude of the ramp since the number of strongly attached cross-bridges would not increase significantly. At the end of the ramp, cross-bridge rotation would again be unimpeded, allowing attached cross-bridges to reach θ_e more rapidly and decreasing muscle tension. The larger the ramp amplitude, the greater would be the slowing of cross-bridge rotation due to extension of the elastic element and the more time the cross-bridges would take to reach θ_e. As a result, the period of early recovery would increase with ramp amplitude. As in the case of shortening ramps, the time taken for the tension to change from T_1 to T_2 would depend mainly on the cross-bridge force development rate. However, unlike shortening ramps, the tension at the end of early recovery, T_2 would be much less dependent on the size of the length change than T_1 due to

the smaller change in the number of strongly attached cross-bridges.

Since muscle fibers form an elongated elastic medium, their responses to transient mechanical disturbances will be propagated like a longitudinal wave. This could result in each sarcomere working at a different operating point, *e.g.*, different sarcomere length and different rate of change of sarcomere length, when a length or tension change is applied to the muscle. The effect on muscle behavior would become more pronounced as the magnitude and frequency of the externally applied change increased or when the muscle activation level was relatively low due to low muscle stiffness. In the early and slow recovery periods, for instance, this could lead to some sarcomeres shortening and others lengthening, becoming especially significant during the recovery phase to the original state where it could be responsible for tension 'creep' (Julian and Morgan, 1979b). Since muscle force is equal to the minimum force generated over any cross-sectional area, muscle length should go through a self-adjustment process to balance forces so that all half-sarcomeres eventually reach stable equilibrium lengths following a rapid mechanical change (Edman and Reggiani, 1982). In the cases muscle behavior can be fully represented by the cross-bridge dynamics of a half-sarcomere. However, the effect of sarcomere nonuniformity cannot always be neglected, especially during dynamic contraction. We recognize this fact, but do not address this issue in the current model.

3.3 DISCUSSION

A conceptual model has been proposed and it has been shown to be consistent with the results of certain key experiments. To clarify the relationship between this model and the Huxley theory of muscle contraction, we first list the major assumptions of the conceptual model.

1) The movement range of a cross-bridge head is fixed and equal to its maximum working stroke;

2) The actomyosin complexes normally are positive force generators which induce force by active rotation of cross-bridge heads;

3) The ability of a cross-bridge head to utilize chemical energy for tension development is limited and depends on the conditions obtaining;

4) The mechanical process of cross-bridge force development is linked to irreversible transitions between several cross-bridge biochemical states.

5) An attached cross-bridge head will transform its remaining chemical energy into heat after it arrives at the end of its working stroke;

6) An attached cross-bridge head will be forcibly detached if the the applied force is larger than the actomyosin affinity;

7) In a living muscle, the affinity of an actomyosin complex is highest when the myosin is strongly bound to the actin, the affinity is lowest between the energized cross-bridge and actin, and the affinity is intermediate in the weakly

bound actomyosin complex.

The results of Stein *et al.* (1979,1984) and Rosenfeld and Taylor (1984) have shown that a cross-bridge could remain attached to actin throughout the complete cycle of ATP hydrolysis. In particular, this is possible for some cross-bridges which do not generate force when muscle contracts steadily isometrically. The number of possible attached cross-bridge states in intact muscle fibres could be less than the number observed for isolated actin and myosin when cross-bridge tension exceeds actomyosin affinity. In order to address cross-bridge behavior in a general sense, the cross-bridge states can be grouped into two states: non-force generating and force generating. The forward transition rate from the non-force generating state to the force generating state is similar to the attachment rate in the original Huxley model while the reverse rate from force generating state to non-force generating state is similar to the detachment rate, as suggested by Brenner (1987).

In all cross-bridge models, an elastic element is always assumed to be associated with a cross-bridge, although its location may vary. What is different in our case is that we lump any deformation of the closed cross-bridge linkage into the elastic element in such a way that the inter-domain motion is not excluded (Vibert and Cohen, 1988). The extension of this elastic element can be used as the variable representing the force exerted between actin and myosin. The rate of extension is directly proportional to the actual rate of

force development in the cross-bridge. It should be noted that the extension of the elastic element does not represent the axial distance between the actin site and the myosin site, although during isometric contraction the maximum extension is directly proportional to the axial distance. The affinity provides an upper-limit for force development and prevents an attached cross-bridge from undergoing an unrealistically large distortion.

We have assumed that there is no significant extension of the elastic element when a cross-bridge attaches to an activated actin site. The tension in the elastic element develops progressively in a continuous manner according to the instantaneous ability of the cross-bridge to convert chemical energy into mechanical work after attachment. This is in contrast to the mechanism in which attachment or detachment acts only as an 'on' or 'off' switch for a cross-bridge to generate a fixed tension. The limited mechanical output power of an actomyosin complex results in a dependence of the force development period on the relative axial distance between the actin binding site and the position of the cross-bridge along the thick filament. The greater this distance, the longer the effective working stroke, and the more time that elapses in developing force before the cross-bridge arrives at the end of the working stroke, while the cross-bridge spends less time in the $MD^{\dagger}(45^{0})$ state where no force can be generated. Consequently, the transition rates between the non-force generating state and the force generating state or the rates of cross-bridge attachment and

detachment in the Huxley two-state model should be functions of the relative axial distance between the actin binding site and cross-bridge position.

When an attached cross-bridge reaches θ_e it immediately enters the $AMD^{\dagger}(45^0)$ state. If there is significant force, detachment could occur when the actomyosin affinity is low, although it would take a certain amount of time. Compression of the cross-bridge elastic element (producing negative force) could occur to a limited extent during the $AMD^{\dagger}(45^0)$ state as the result of very rapid shortening. Some cross-bridges could also be forced to exert negative tension even during their working strokes due to very rapid shortening (greater than the maximum steady shortening velocity) because of the limit of cross-bridge angular velocity. In this case, a sudden decrease in the sliding velocity could allow those cross-bridges to return to the normal strong binding state. This property could play an important role in the responses observed during the rapid change and early recovery phases of a transient response, as shown by the fact that the faster the length change, the faster the muscle tension reaches T_1 (Ford *et al.*, 1977). From the energetic point of view the incorporation of the $MD^{\dagger}(45^0)$ state into the conceptual model allows us to explain observations such as those reported by Hill (1964) and is also consistent with the two-state model for shortening-dependent energy liberation proposed by Irving and Woledge (1981b).

Our model also predicts that the transition rates between the non-force gen-

erating state and force generating state should depend on the rate of change of muscle length. This property has been demonstrated experimentally by a dependence of the number of attached cross-bridges on the rate of change of muscle length (Ford *et al.*, 1985). The most obvious reason is that the relative position of the actin binding site and the cross-bridge head changes with filament sliding. However, the effects of filament sliding on the two cross-bridge transition rates are intrinsically different and cannot be physically represented simply as functions of the relative axial separation of actin binding sites and myosin sites, as proposed in other cross-bridge models. We therefore make sliding velocity one of the independent variables. This is justified when we consider the interaction of muscle with other mechanical systems. Since the mechanical power output of a cross-bridge is limited, cross-bridge behavior can be altered by the energy transfer to or from the other systems. Muscles may interact with systems that have very different dynamic properties. The principle of power balance has been used to cope with this problem. Power balance is concerned with two variables, muscle tension and sliding velocity. Since we wish to predict muscle tension, sliding velocity becomes a causal variable. By specifying the structural change that occurs in a cross-bridge during muscle contraction, a dynamic cross-bridge model can be developed where the physical meaning at each step can be clearly interpreted with respect to power balance.

In most muscle kinetics schemes there are forward and reverse rates between any two adjacent states, which we specify with reference to the direction of the ATP hydrolysis cycle. The forward and reverse rates for some transitions are equal while others are not. The overall balance is in the same direction as the ATP hydrolysis cycle. For example, the main path in the kinetics scheme of Goldman and Brenner (1987) involves the states with actomyosin complexes. The forward rates and reverse rates on the path are equal except for the transitions from A·M·ADP·P_i or A·M′·ADP to A·M·ADP. The forward rate is several times greater than the reverse rate. This imbalance would produce a net transition of cross-bridges through the states over the main path. This net transition should mainly depend on the ratio of the forward rate to the reverse rate. From the point of view of force generation, the net transition of cross-bridges is all that we really need to know for our muscle model. Local loops such as the short loop involving M·ATP, M·ADP, A·M·ADP·P_i and A·M·ATP in the kinetics scheme of Goldman and Brenner (1987) may still exist, but may only be important, when isolated actin and myosin react in solution.

3.4 CONCLUSION

In our conceptual model, the biochemical and mechanical events associated with the actomyosin complex have been linked by their dependence on the an-

gular velocity of the cross-bridge head under the constraint of power balance. The key issue is how the rate of cross-bridge force generation is regulated by changes in muscle structure, biochemical reactions and mechanical energy exchange with the external environment. The maximum force that an attached cross-bridge can develop is limited by the affinity of actin and myosin. We have two major cross-bridge states, the non-force generating state and the force generating state. Filament sliding is an important element in the determination of cross-bridge dynamics, suggesting that cross-bridge transition rates should be functions of the rate of change of muscle length. Our conceptual model also provides a physical description for force generation during muscle contraction that lends itself naturally to a mathematical representation for cross-bridge behavior in a half-sarcomere.

CHAPTER 4

MODELING OF MUSCLE STEADY CONTRACTION

It has been generally accepted that the contraction of skeletal muscle is induced by the cyclic interaction between actin and myosin molecules that are regularly distributed along the thin and thick filaments respectively. The interaction between actin and myosin produces tension which in turn tends to reduce the relative distance between their respective locations on the two filaments. The energy for contraction comes from hydrolysis of ATP. Acting as an ATPase, the actomyosin complex splits ATP and thereby initiates and regulates the mechanical process of force development. In order to better understand the process of muscle contraction three major lines of investigation have emerged: studies of mechanical properties of muscle fibers, studies of the kinetics of actin and myosin interactions and studies of the structure of motile molecules. The kinetic and structural studies provide specific information about muscle behavior at the molecular level. With advanced techniques

such X-ray or laser diffraction, optical probes and 'caged' molecules, considerable progress has been made recently in understanding and modeling the process of muscle contraction (Goldman *et al.*, 1984a,1984b; Eisenberg and Hill, 1985; Huxley and Kress, 1985). Since most studies have been restricted to very specific conditions, a synthesis of various observations is needed in order to obtain a complete description of the mechanism of cross-bridge behavior during muscle contraction. In the previous chapter we have proposed a conceptual model to synthesize the mechanical, kinetic and structural aspects of muscle contraction within a half sarcomere. The development of the mathematical formulation which allows us to make quantitative predictions about muscle behavior during steady contractions is the main object of this chapter. The mathematical modeling of cross-bridge behavior during muscle transient responses will be subject of the following chapter.

4.1 MODELING APPROACH

Currently there are two major types of muscle models, the Hill model and the Huxley model. The Hill model is an empirical model which can predict the gross features of muscle mechanical behavior, but does not address muscle behavior from the microscopic point of view. This shortcoming is significant since muscle behavior is heavily dependent on the molecular mechanisms that

regulate the conversion of chemical energy to mechanical work. The Huxley model is based on an advanced theoretical understanding of muscle behavior at the molecular level (sliding filaments and cross-bridges). Although this type of model is not very practical due to the complexity of solving the partial differential equation upon which it is based (Brokaw, 1976; Zahalak, 1981), it offers an advantage over the Hill model since it associates muscle behavior at the macroscopic and microscopic levels.

In order to develop a realistic mechanochemical muscle model we have followed Huxley's approach. A key element in advancing this approach has been to link the rate of cross-bridge force development to the relative sliding velocity between the thin and thick filaments and concomitant structural changes occurring in attached cross-bridge heads under the regulation of the biochemical reactions.

In our conceptual model we proposed that muscle behavior depends mainly on the number of strongly attached cross-bridges, the elastic force which they generate and the rate of force development in each attached cross-bridge. The rate of force development of an actomyosin complex is regulated by the sliding velocity between the thin and thick filaments, the elastic force and the capacity to convert chemical energy into mechanical work, according to the principle of power balance. The rate of force development is an essential variable. Elastic force is the integral of the rate of force development over time. We have

separated cross-bridges into two major states, the non-force generating state and the force generating state during which we define a continuous mechanical process for force development. The non-force generating state includes all of the other cross-bridge states except the strong binding state. Since cross-bridges involved in force generation pass through an irreversible cycle, the forward and reverse transition rates between the non-force generating state and the force generating state can be used to find number of cross-bridges in the force generating state. These rates in turn specify the rate of muscle energy liberation if it is assumed that one ATP molecule is hydrolyzed per force generation cycle. We end up with three essential variables: the rate of cross-bridge force development and the forward and reverse transition rates.

Two computational methods for solving the original Huxley equation in the time domain have been described. Assuming the distribution of the relative axial distance between actin binding sites on the thin filament and cross-bridge sites on the thick filament to be Gaussian, Zahalak (1981) solved a set of ordinary differential equations for the first three moments of the distribution which represented muscle stiffness, elastic force and elastic energy, respectively. In the other approach Brokaw (1976) used stochastic methods to follow the time-history of individual cross-bridges in a large population. However, the computational cost for both methods is quite heavy. Furthermore, these approaches suffer from the fact that the cross-bridge attachment rate and/or

detachment rate must be given before simulations can be carried out. It would be preferable to be able to identify these rates from muscle experimental data.

Muscle constitutes a distributed system, not only because each cross-bridge within a half-sarcomere could have different properties, but also each sarcomere could be working under different operating conditions. Modeling of such distributed effects would lead to a complicated model. Since we have only experimental data based on measurements made with whole muscle fibres, we can not identify a distributed system model. In order to overcome this limitation, we use mean values to represent the average effects of those distributed events on muscle behavior, particularly since muscle sarcomeres are structurally distributed in series and hence the force generated by each sarcomere should be almost the same. This approach allows us to develop a lumped parameter muscle model and to identify the model parameters using available data.

As discussed in the previous chapter, muscle may act as a static system when it contracts steadily (constant ATP hydrolysis rate). Under such conditions, simulations using earlier models (Huxley, 1957; Brokaw, 1976; Einsenberg *et al.*, 1980; Zahalak, 1981) would suggest that the mean values of all variables concerned with cross-bridge behavior remain constant. We can therefore reduce the model complexity by discussing cross-bridge ensemble behavior in terms of these mean values. Since we are only interested in muscle mechanical behavior during contraction, the effect of cross-bridges in the myosin cycle

can be neglected as no significant contribution to force development is made by these cross-bridges. They contribute only to production of muscle resting heat. Finally, we need to represent variations in the mean values of the three essential variables as functions of independent variables.

4.2 CROSS-BRIDGE TRANSITION RATES

For motile biological systems, the induction of motion through the conversion of chemical energy to mechanical work is of primary importance. The chemical energy for muscle contraction comes from hydrolysis of ATP. Muscle kinetics, i.e. identification of the number of chemical states and the transition rates between these states, has been studied by observing the ATPase activity of myosin and actomyosin in solution. The biochemical behavior of actin and myosin in solution can be represented by a set of differential equations that relate the different chemical states. The model parameters, the transition rates between different states, can be directly estimated from recorded data. Such modeling has become more complex as the reactions have been studied in more and more detail, with the proposed chemical states for actomyosin ATPase now numbering 8, 10, or even 14 (Hibberd and Trentham, 1986).

Since actomyosin in solution neither generates force nor does mechanical work, the observed behavior could be quite different when thin and thick fil-

aments are organized in a structured system such as that found in a muscle fiber. In order to use studies of isolated muscle proteins in solution to describe their behavior in an organized structure such as a muscle fiber, A.F. Huxley (1980) suggested that a particular stage of the chemical reaction in an actomyosin complex permits the next step in its mechanical process, and vice versa, that each step in the mechanical cycle will influence the rate of ATP hydrolysis. This idea has been theoretically realized in our conceptual model which specifies how the biochemical reactions are coupled to the postulated mechanical behavior. There are two possible cyclical processes which constitute the ATP hydrolysis cycle for an energized cross-bridge, a myosin or an actomyosin cycle. In each cycle, cross-bridges sequentially proceed through a number of biochemical states. We model the myosin cycle as consisting of four states: M, MT, $MD^{\ddagger}$, $MD^{\ddagger}(45^0)$, while the actomyosin cycle consists of six states: $AM(45^0)$, MT, $MD^{\ddagger}$, $AMD^{\ddagger}(\theta_s)$, $AMD^{\ddagger}_{*}$, and $MD^{\dagger}(45^0)$. From the point of view of changes in muscle mechanical properties, the transition of cross-bridges from $AMD^{\ddagger}_{*}$ to $MD^{\dagger}(45^0)$ is most important. We therefore group the cross-bridge states into non-force generating and force generating states. The force generating state involves the transition of cross-bridges from $AMD^{\ddagger}_{*}$ to $MD^{\dagger}(45^0)$. The forward and reverse transition rates between the two grouped states are specified by F and G, respectively, as shown in Fig. 4.1. As discussed in the previous chapter F and G depend on the rate of change

ofg muscle length. We have chosen to use this representation rather than a dependence on cross-bridge position (Huxley, 1957) since it more accurately reflects the physical cause of their variation.

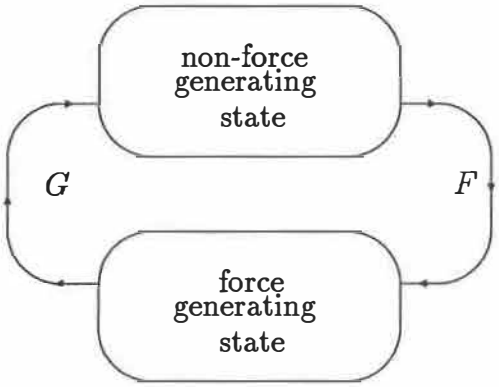


Figure 4.1 The diagram of a net cross-bridge transition cycle with the two states, the non-force generating state and the force generating state. F and G are the transition rates between the two states.

Studies of muscle kinetics and energetics have shown that the cross-bridge cycling rate or the time for hydrolysis of one ATP molecule varies with the concentration of free calcium and ATP in the sarcoplasm and the rate of change of muscle length. Because of the importance of these variables we define muscle length, concentration of free calcium and ATP as muscle independent variables and represent them by [Ca], [ATP] and X, respectively. In the previous chapter, we have assumed that muscle behavior could be represented by the ensemble behavior of cross-bridges in a half-sarcomere. Our model is concerned only

with the muscle mechanics of a half-sarcomere. However, sarcomere lengths may vary within a muscle fiber. We therefore use X to represent the average length of half-sarcomeres rather than the total muscle fiber length. In order to simplify the complex interactions of these variables on the regulation and control of cross-bridge behavior, we use the following mathematical expressions to represent the effects of each system variable on F and G,

$$F(v_a, [\mathrm{Ca}], [\mathrm{ATP}]) \equiv f_1(v_a) f_2([\mathrm{Ca}]) f_3([\mathrm{ATP}]) \tag{4.1}$$

and

$$G(v_a, [\mathrm{Ca}], [\mathrm{ATP}]) \equiv g_1(v_a) g_2([\mathrm{Ca}]) g_3([\mathrm{ATP}]) \tag{4.2}$$

Here f_1 and g_1, f_2 and g_2, f_3 and g_3 are mainly dependent on v_a, [Ca] and [ATP], respectively. The effects of system variables on F and G may not separable as assumed, but such interdependence could be taken into account by allowing certain model parameters to be functions of other system variables if necessary.

The instantaneous number of cross-bridges in the force generating state N_s can be determined by solving

$$\frac{dN_s}{dt} = F(Z - N_s) - GN_s \tag{4.3}$$

where $Z(X)$ is the number of cross-bridges in the region of actin/myosin overlap.

During steady contractions, we have a constant number of cross-bridges in the force generating state so that $\frac{dN_s}{dt} = 0$. Therefore, the number of cross-bridges in the force generating state during steady-state contraction can be represented by

$$N_s = \frac{FZ}{F+G} \tag{4.4}$$

Substituting Eqs.(4.1) and (4.2) into Eq.(4.4) we have the separable formulation,

$$N_s = Z(\frac{f_1}{f_1+g_1})(\frac{f_2}{f_2+g_2})(\frac{f_3}{f_3+g_3}) \tag{4.5}$$

with the constraint

$$f_1 f_2 g_3 + g_1 g_2 f_3 + (f_1 g_2 + g_1 f_2)(f_3 + g_3) \equiv 0 \tag{4.6}$$

We further define

$$N_i = \frac{f_i}{f_i + g_i} \qquad\qquad i = 1,2,3 \tag{4.7}$$

so that Eq.(4.5) becomes

$$N_s(X, v_a, [\mathrm{Ca}], [\mathrm{ATP}]) = Z(X)N_1(v_a)N_2([\mathrm{Ca}])N_3([\mathrm{ATP}]) \tag{4.8}$$

N_1, N_2 and N_3 act as scaling factors of $Z(X)$. The expression in Eq.(4.8) is similar to the representation of the contractile element in certain Hill-type models which use three serially arranged elements to account for the force-[Ca] relation, the force-velocity relation and the active portion of force-length

relation (Otten, 1987). This similarity gives credence to the idea that the effects of variations in the system variables on muscle contraction are properly represented by such scaling factors for steady-state muscle contraction since Hill-type models account adequately for such steady-state behavior.

Another advantage of this modeling approach is the ability to use existing data for model parameter estimation since in most experiments one state variable is altered in order to study its effect on steady states. Equation (4.7) constrains the parameters f_i and g_i to lie on straight lines for a given N_i. This leaves only one degree of freedom for the choice of g_i and f_i, and hence we have a total of three degrees of freedom for the choice of three pairs of f_i and g_i. If we then apply Eq.(4.6) to f_i and g_i for $i = 1, 2, 3$ we have one additional contraint so that the total number of degrees of freedom for the choice of the f_i and g_i is reduced to two. Physiological considerations may impose additional constraints on f_i and g_i.

In the following four sections we relate the cross-bridge transition rates between the two states to the effects of a single system variable on the generation of muscle tension under steady-state conditions. Mathematical expressions for f_i and g_i are developed based on this analysis. The symbols A_{ij} are used to denote model parameters.

4.2.1 Force-length relation

The relation between muscle tension and the degree of overlap between thin and thick filaments was studied by Gordon *et al.* (1966) for single muscle fibers. Their results showed that there is an optimal sarcomere length, at which muscle tension is maximal for a given activation level. When the sarcomere length is either longer or shorter than the optimal length, muscle tension is reduced. A similar observation was also made for whole muscle (Joyce *et al.*, 1969). They showed additionally that the force-length curves had a similar shape for different activation levels. We assume that this variation in muscle tension with muscle length results from variation in the number of 'participating' cross-bridges with the amount of filament overlap. The following parabolic function is used to approximate this nonlinear relation.

$$Z = A_{01} - A_{02}(X - A_{03})^2 \tag{4.9}$$

where A_{01} is the number of participating cross-bridges at the optimal length, A_{02} is the change in the number of participating cross-bridges for a unit change in sarcomere lenght, and A_{03} is the optimal length. The slope of the steady force-length curve represents the rate of change in muscle tension with muscle length, but not muscle stiffness because the mean extension of cross-bridge elastic elements remains almost the same and hence cross-bridge stiffness does

not play a role.

4.2.2 Force-velocity relation

As discussed in the previous chapter the variation of muscle force during length change is caused partially by changes in f_1 and g_1 and partially by the change of the mean extension of all elastic elements. Due to the assumption that a cross-bridge which contributes to muscle force generation undergoes an irreversible transition from the non-force generating state to the force generating state and then from the force generating state back to the non-force generating state after completely expending the energy from hydrolysis of one ATP molecule, we can discuss the effect of filament sliding on f_1 and g_1 separately. The rate g_1 affects mainly the duration of the force generating state within an ATP hydrolysis cycle while f_1 affects the remaining period of the cycle. Thus, we can set

$$g_1 = \frac{1}{g_2 g_3 T_{am_s}} \tag{4.10}$$

and

$$f_1 = \frac{N_1 g_1}{1 - N_1} \tag{4.11}$$

where N_1 is less than one and can be identified from the experimental data. Eq.(4.10) shows that T_{am_s} depends on g_1, but is also affected by g_2 and g_3. Once we know $g_2 g_3$, T_{am_s} and N_1, we can find f_1 and g_1 from Eqs.(4.11) and (4.10)

respectively. We assume that, in general, both f_1 and g_1 can be represented as polynomial functions of the rate of change of muscle length. Filament sliding in opposite directions has different effects on f_1 and g_1. For example, lengthening can result in a number of forcible detachments and re-attachments before a cross-bridge arrives to the end of its working stroke. This would significantly alter f_1 and g_1 with respect to their values during sarcomere shortening. This is also consistent with experimental observations which show a discontinuous point in the force-velocity relation at $v_a = 0$. Therefore, we would expect that both N_1 and the average extension of cross-bridge elastic elements undergo sudden changes at $v_a = 0$ and that different mathematical formulations would be needed to represent muscle behavior during lengthening and shortening.

4.2.3 Force-[Ca] relation

Muscle fibers consist of myofibrils encased in a membrane known as the sarcoplasmic reticulum (SR) that sequesters calcium in extracellular regions known as terminal cisternae. When an action potential initiated by a nerve impulse is conducted along a muscle fiber, depolarization of the membrane causes a transient release of calcium from the SR and the calcium concentration of the muscle sarcoplasm increases (Miledi *et al.*, 1977). The free calcium ions diffuse and bind to troponin molecules thereby activating actin binding sites. Meanwhile, calcium pumps located on the SR continuously remove calcium

from the sarcoplasm back into the SR (Robertson *et al.*, 1981). Therefore, the rate of change of [Ca] will be equal to the difference between the rate of calcium release and the rate of calcium removal. Assuming there is a fast equilibrium between a change in [Ca] and the number of troponin molecules that bind with Ca^{++}, an increase in [Ca] will result in an increase in the number of troponin molecules binding with Ca^{++} (Zot and Potter, 1987) and an increase in the affinity of actin for myosin binding (Wagner and Giniger, 1981; Chalovich and Eisenberg, 1982), resulting in more energized cross-bridges entering the force generating state. Consequently, muscle force will increase. This has been demonstrated by Yamada *et al.* (1989) who examined the [Ca]-dependence of actin-activated Mg-ATPase activity. The relation between Mg-ATPase activity and [Ca] is very similar to the relation between muscle tension and [Ca], which shows that there is a high correlation between cross-bridge ATPase activity and muscle tension as we suggested in our conceptual model. The steady force-[Ca] relation has been investigated in skinned muscle fibers (Julian and Moss, 1980) and can be represented by the following equation.

$$N_2 = \frac{f_2}{f_2 + g_2} = \frac{[\mathrm{Ca}]^{A_{21}}}{[\mathrm{Ca}]^{A_{21}} + A_{22}^{A_{21}}} \tag{4.12}$$

where A_{21} and A_{22} are the model parameters.

Since the variation in [Ca] directly influences only the number of cross-bridges in the force generating state, we assume that f_2 is a function of [Ca]

while g_2 is constant. From Eq.(4.12) we can then set

$$f_2 = [\mathrm{Ca}]^{A_{21}} \tag{4.13}$$

and

$$g_2 = A_{22}^{A_{21}} \tag{4.14}$$

When [Ca]$\approx$ 0, the number of cross-bridges in the force generating state is insignificant. Physiological considerations will constrain the choice of f_2 and g_2.

4.2.4 Force-[ATP] relation

The energy used for muscle contraction is produced by splitting ATP into ADP and P_i. In intact muscle there are two major ATPases: the actomyosin ATPase and the ATPase of the calcium pump located in the SR. ATP can originate from three sources (Margaria, 1976): free ATP in sarcomplasm, ATP produced by splitting creatine phosphate, and ATP produced by glucose catabolism. The instantaneous ATP concentration in the sarcoplasm is only sufficient for about eight muscle twitches (McMahon, 1984). Continuous muscle activity will cause a drop in [ATP] and a rise in both [ADP] and [P_i]. If ATP is resynthesized from the latter two sources the concentrations of creatine phosphate and glucose will decrease. Since glucose catabolism proceeds at a relatively slow rate, the major pathway for resynthesis of ATP must be

via creatine phosphate which itself can only be resynthesized by rephosphorylation of ADP. Therefore, [ATP] depends on both ATP-consuming processes and ATP-generating processes.

The structural differences of attached cross-bridge heads at θ_r and θ_e in the presence and absence of ATP, observed in electron microscopic studies (Craig *et al.*, 1985), and the dependence of muscle tension on [ATP] (Cooke and Bialek, 1979; Kawai and Halvorson, 1989) strongly suggest that the formation and breaking of actomyosin linkages should be functions of [ATP]. In the absence of ATP most cross-bridge heads remain attached at θ_e after their power stroke, producing the low level of muscle tension which is observed in rigor muscle. Muscle tension rises to a maximum as [ATP] increases to about 40 μMol ATP, then declines as [ATP] is raised further. It is 75% of its maximum at 1 mMol ATP, 64% at 5 mMol ATP, and 61% at 10 mMol ATP. This is possibly because G increases as [ATP] increases, so that N_3 or N_s decreases. Based on the experimental data of Cooke and Bialek (1979) N_3 can be expressed as

$$N_3 = \frac{f_3}{f_3 + g_3} = \frac{A_{31}[\mathrm{ATP}] + A_{34}}{A_{32}[\mathrm{ATP}]^2 + A_{33}[\mathrm{ATP}] + A_{34}} \tag{4.15}$$

Since N_3 is a scaling factor of $Z(X)$ it should only vary between zero and one. N_3 would be approximately equal to to one when muscle tension is maximum at [ATP] of about 40 μMol ATP. Physiological [ATP] is much higher than this.

Furthermore, we have

$$f_3 = (A_{31}[\mathrm{ATP}] + A_{34}) \tag{4.16}$$

and

$$g_3 = [\mathrm{ATP}]\{A_{32}[\mathrm{ATP}] + (A_{33} - A_{31})\} \tag{4.17}$$

The exponent of the [ATP] terms in Eq.(4.17) is one order higher than that in Eq.(4.16), which has physical significance for the mechanism of cyclic interaction between myosin and actin. It allows $N_s \geq 0$ when $[\mathrm{ATP}] = 0$ to account for the existence of a certain amount of tension in rigor muscle. In this case, the mechanical properties of all attached cross-bridges would be equivalently represented by that of a few cross-bridges in the force generating state.

4.3 AVERAGE RATE OF FORCE DEVELOPMENT

The average rate of force development of strongly attached cross-bridges which constitute the force generating state is the other important variable in muscle contraction. It can be equivalently represented mathematically as the average rate of change of extension of the hypothetical spring elements associated with the cross-bridge heads, which we denote by $\overline{\dot{x}}$. For most torque generators, the steady capacity to transform supplied energy into mechanical work can be represented by a relation between torque and the rate of change

of angular position of the rotor. For instance, many electromagnetic torque motors have an elliptical relation between torque and angular velocity. Muscle has similar behavior to that of mechanical actuators. In particular, since muscle tension drops more rapidly as a function of velocity than the decrease in its stiffness (Ford *et al.*, 1985), we are justified in assuming that the ability of cross-bridge to convert chemical energy into mechanical work is affected by the angular velocity of its head. We suppose that the torque that can be generated by strongly attached cross-bridge heads is an elliptical function of their average angular velocity which can be expressed as follows

$$\frac{\bar{\dot{\theta}}^2}{A_{41}^2} + \frac{Q^2}{A_{42}^2} = 1 \tag{4.18}$$

where Q is the mean torque of AMD$_*^\ddagger$s exerted on the base of the thin filament and $\bar{\dot{\theta}}$ is the average angular velocity of attached cross-bridge heads during force development. A_{41} is the maximum value of the angular velocity of AMD$_*^\ddagger$ for which AMD$_*^\ddagger$ makes an active contribution to muscle force while A_{42} is the maximum torque which can be generated by AMD$_*^\ddagger$. It follows that the mechanical output power is given by the relation

$$R_{w_s} P_{w_s} = Q\bar{\dot{\theta}} = \frac{A_{42}\bar{\dot{\theta}}}{A_{41}}\sqrt{A_{41}^2 - \bar{\dot{\theta}}^2} \tag{4.19}$$

Consequently, overall mechanical output power of AMD$_*^\ddagger$ is only a function of average angular velocity and hence $\bar{\dot{\theta}}$ is an independent system variable.

Since we are interested in the characteristics of an ensemble of distributed cross-bridges in order to derive a simple sliding-filament, cross-bridge model for muscle contraction, the variables in Eqs.(3.5) and (3.6) should be replaced by their respective mean values and rewritten as

$$R_{w_s} P_{w_s} + \overline{f_a} v_a = k \bar{x} \overline{\dot{x}_s} \tag{4.20}$$

and

$$\overline{\dot{x}_s} = l \overline{\dot{\theta} \cos\theta} + v_a \tag{4.21}$$

where the bar represents the mean ensemble value of a variable and the subscript s refers to the force generating state. Substituting Eq.(4.21) for $\overline{\dot{x}_s}$ into Eq.(4.20) we have

$$R_{w_s} P_{w_s} = l \overline{f_a \dot{\theta} \cos\theta} = l k \bar{x} \overline{\dot{\theta} \cos\theta} \tag{4.22}$$

Replacing the term on the left hand side of Eq.(4.22) by Eq.(4.19) we have

$$\frac{A_{42}}{A_{41}} \sqrt{A_{41}^2 - \overline{\dot{\theta}}^2} = l k \bar{x} \overline{\cos\theta} \tag{4.23}$$

which shows the relation between average angular velocity of AMD$_*^\ddagger$ and average elastic force, providing the link between cross-bridge mechanics and muscle mechanics.

A cross-bridge which has recently entered the strong binding-state exerts less tension, but has a higher rate of force development than a cross-bridge

which began its working stroke earlier. As a result, an instantaneous increase in the number of recent strongly attached cross-bridges will produce an increase in the mean rate of force development and a decrease in the mean extension of cross-bridge elastic elements. On the other hand, a cross-bridge nearing the end of its working stroke exerts high tension and has a low rate of force development. Its transition from the force generating state to the non-force generating state will also affect the mean rate of force development and the mean extension of cross-bridge elastic elements. In order to account for the effects occurring during the cross-bridge transitions between the non-force generating state and the force generating state, an additional term is needed in Eq.(4.21) since Eqs.(4.20) and (4.21) hold only for already strongly attached cross-bridges and do not include these effects. If $\psi(F, G, \bar{x})$ is used to account for this effect then

$$\dot{\bar{x}} = l\dot{\bar{\theta}}\overline{\cos\theta} + v_a + \psi(F, G, \bar{x}). \tag{4.24}$$

Assuming $\bar{\theta}$ does not change significantly, we can predict the average rate of force development and the mean extension of the elastic elements by solving Eqs.(4.24) and (4.23) given $\dot{\bar{\theta}}$, F and G since v_a is a measurable variable.

From the discussion in the previous chapter, we know that the angular velocity of attached cross-bridge heads can be influenced by the sliding motion between thin and thick filaments and hence we postulate a linear relation

between $\overline{\dot{\theta}}$ and v_a for steady contraction mathematically such that

$$\overline{\dot{\theta}} = A_{43} + A_{44} v_a, \tag{4.25}$$

where A_{43} is the value of $\overline{\dot{\theta}}$ in the isometric state.

4.4 MEAN ENERGY LIBERATION RATE

The expression for the mean muscle energy liberation rate is given by Eq.(3.8), from which it can be seen that the rate of muscle energy liberation depends mainly on the number and mean value of the turnover rate of cross-bridges in the actomyosin cycle since T_m is much greater than T_{am} (Geeves, 1991). Therefore, we can approximate the mean energy liberation rate by

$$\overline{\dot{E}} = \frac{eN_{am}}{T_{am}} + c_1. \tag{4.26}$$

During the actomyosin cycle, T_{am_1} and T_{am_s} vary independently with the sliding velocity of the thin and thick filaments while T_{am_2} depends on T_{am_s} according to Eq.(3.9). When $v_a \neq 0$, T_{am_1} is reduced since the time for an energized cross-bridge to move from its current position to the position where the activated actin site is located decreases. In addition, the sliding motion allows an energized cross-bridge to attach to an actin site which is located beyond its normal range of movement in the isometric state. This results in

a sharp increase in the number of cross-bridges in the actomyosin cycle. As sliding velocity increases T_{am_1} drops to its minimum value which is essentially the time required to complete attachment.

We use an exponential function plus a linear term to approximate T_{am1} as follows

$$T_{am_1} = A_{51} e^{A_{52}|v_a|} + A_{53}, \tag{4.27}$$

where $|\cdot|$ represents the absolute value. T_{am_1} is independent of the direction of the sliding motion since the effect of the angular motion of an energized, unattached cross-bridge head can be neglected compared to the effect of the sliding motion on cross-bridge attachment. T_{am_s}, on the other hand, behaves quite differently when muscle fibers are stretched or allowed to shorten. In steady-shortening,

$$T_{am_s} = \frac{\theta_e - [\overline{\theta}_s - T_{am_w} v_a / (l\overline{\cos\theta})]}{\dot{\theta}} = \frac{1}{G}, \tag{4.28}$$

where the term $\overline{\theta}_s - T_{am_w} v_a / (l\overline{\cos\theta})$ quantitatively describes the effective working stroke of an attached cross-bridge in the strong binding state as a function of the sliding velocity since $T_{am_w} v_a / (l\overline{\cos\theta})$ represents the angular displacement of the cross-bridge head during the weak binding state. According to Eqs.(4.2) and (4.14), the second equality in Eq.(4.28) holds only when there is no significant variation in [ATP]. For the purposes of this study we assume that [ATP] and [Ca] are normally both constant. Substituting Eqs.(4.27) and

(4.28) for T_{am_1} and T_{am_s} in Eq.(3.10), when $v_a \leq 0$ we have

$$\begin{aligned} T_{am} = \; & A_{51}e^{A_{52}|v_a|} + A_{53} + [1 - \frac{A_{42}\bar{\dot{\theta}}}{A_{41}P_{h_a}}\sqrt{A_{41}^2 - \bar{\dot{\theta}}^2}] \\ & [\frac{\theta_e - \bar{\theta}_s + T_{am_w}v_a/l)}{\bar{\dot{\theta}}}] + \frac{\mathrm{e}}{P_{h_a}} + c_2, \end{aligned} \tag{4.29}$$

and

$$c_2 = T_b + T_{m_1} + T_{am_w}, \tag{4.30}$$

where $T_b + T_{m_1}$ is an invariant portion of the total refractory period and T_{am_w} is constant. Stretching results in different cross-bridge behavior than shortening. We assume that the actomyosin affinity of a strongly bound cross-bridge is equal to the maximum tension that can be generated, f_{am}.

$$f_{am} = kh_m = kl(\sin\theta_e - \sin\theta_r) \tag{4.31}$$

If $f_a \geq f_{am}$, the cross-bridge will be forcibly detached. The time required for an attached cross-bridge to develop tension f_{am} is denoted by t_a and the time required for a forcibly detached cross-bridge to re-attach to another activated actin site is denoted by t_d. The term N_r represents the number of

re-attachments of a cross-bridge during its motion from θ_r to θ_e. We have

$$\begin{aligned} t_a &= \frac{h_m}{\dot{x}_s} \\ t_d &= \frac{\triangle d}{l\dot{\theta}_d + v_a} \\ N_r &= \frac{\theta_e - \theta_r}{\dot{\theta} t_a + \dot{\theta}_d t_d} \\ T_{am_s} &= t_a N_r = \frac{1}{G} \\ T_{am} &= A_{51} e^{A_{52}|v_a|} + A_{53} + (t_a + t_d) N_r \\ &\quad + \frac{e}{P_{h_a}} + c_3 \end{aligned} \tag{4.32}$$

where $c_3 = T_b + T_{m_1}$ and $\triangle d$ represents the relative distance between two adjacent actin sites to which an energized cross-bridge can attach. The symbol $\overline{\dot{\theta}_d}$ denotes the mean angular velocity of forcibly detached cross-bridge heads which is constant and independent of the sliding velocity. We also assume that $T_{am_2} = \frac{e}{P_{h_a}}$ in Eq.(4.32) since most of the energy for developing muscle tension comes from external sources while the chemical energy from ATP hydrolysis is dissipated in heat generation at θ_e except when the stretching rate is quite low, since for very low stretching rates cross-bridges continue to do positive mechanical work. In order to incorporate the process of repeated attachment and detachment into our two-state model, we take T_{am_s} to be the total time spent in the attached state during rotation of the cross-bridge head from θ_r to θ_e, conserving the inverse relation between T_{am_s} and G. It is worth noting

that during T_{am_w} cross-bridges can make a significant contribution to muscle tension when muscle is lengthened. Therefore these cross-bridges are treated as if in the force generating state.

In our model the number of cross-bridges in the actomyosin cycle is also a function of the filament sliding velocity, but essentially independent of the direction of filament sliding. In the isometric state, the number of cross-bridges in the actomyosin cycle, N_{am} depends almost exclusively on the amount of overlap between the thick and thin filaments provided that the concentration of calcium and ATP in the sarcoplasm is sufficiently high. Studies of muscle ultrastructure indicate that the movement range of a cross-bridge is about 13.4 nm. N_{am} should be directly proportional to the ratio of this movement range and the axial interval of 42.9 nm between adjacent cross-bridges. This ratio represents the fraction of the overlap region accessible to attachment. This region will be extended by filament sliding. The increase should be directly proportional to the sliding velocity and T_{am_1}, assuming that the angular velocity of a detached cross-bridge is relatively quite slow. N_{am} can then be expressed as

$$N_{am} = [l\cos(\pi/4) + \mid v_a \mid (T_{am_1} - A_{61} \mid v_a \mid)]A_{62} \qquad (4.33)$$

where A_{62} is the number of cross-bridges 'participating' in attachment in the axial interval of 42.9 nm. The first term on the right side of Eq.(4.33) represents

N_{am} in the isometric state. If N_{am} is known for isometric conditions then A_{62} can be estimated. The term with A_{61} is used to take into account the fact that the number of cross-bridges in the refractory state which attach to any activated actin site increases as sliding velocity increases.

Since an ATP hydrolysis cycle is closely coupled with the mechanical process of force generation there should be a high correlation between the mean energy liberation rate and the transition rates between the force and non-force generating states. During steady contraction, the fraction of cross-bridges in any state of the actomyosin cycle is equal to the fraction of the cycle period which cross-bridges spend in that state. Hence we have

$$\frac{N_s}{T_{am_s}} = \frac{N_{am}}{T_{am}} \tag{4.34}$$

for the number of cross-bridges in the strong binding state. Substituting Eq.(4.4) into Eq.(4.34) for N_s and replacing T_{am_s} by $1/G$ as in Eqs.(4.28) and (4.32), we have the following mathematical formulation for muscle energy liberation rate expressed as a function of F and G.

$$\overline{\dot{E}} = \frac{eN_{am}}{T_{am}} + c_1 = \frac{eZFG}{F+G} + c_1. \tag{4.35}$$

The first term on the right hand side of the above equation is very similar to that of Eq.(9) in Huxley's original model (Huxley, 1957) for the energy liberation rate during steady shortening and isometric contraction, although the mathematical expressions for F and G as functions of v_a are different.

4.5 MODEL PARAMETER ESTIMATION

Muscle acts as a static system during steady contraction and hence its behavior with respect to system independent variables can be described using algebraic equations. As shown above, muscle behavior depends on [Ca], [ATP], X and their derivatives. Most of the experiments on steady muscle contraction have involved altering one variable while keeping the others constant, e.g. studies of the force-length relation where [Ca], [ATP] and v_a are kept constant while X is allowed to vary. Normally, in such tests muscle length is maintained in the plateau region of the force-length curve in order to avoid changing the number of attachment sites.

Where available we have used data obtained from frog muscles at temperatures near 0^0C in order to standardize the parameters of the model. The model parameter estimation for steady contraction has been done by fitting the energy liberation rate-velocity relation predicted by the model with that obtained from experimental data. Model validation is based on the ability of the model to then predict the experimental force-velocity relation.

4.5.1 Isometric contraction

During isometric contraction the geometrical arrangement of cross-bridges and actin sites remains relatively unchanged. We have therefore assumed that

both the distributions of θ_s and of the effective cross-bridge working stroke in the region of filament overlap are uniform over the cross-bridge movement range and independent of muscle length. The mean value of the cross-bridge working strokes ($\overline{\theta}_s$) is then equal to half of the movement range or 0.3927 rad. Because of the stochastic nature of the diffusion and binding of chemical substances such as calcium and ATP it is very difficult to model the behavior of individual cross-bridges. Therefore, we have used a statistical approach in which we assume that value of θ associated with strongly attached cross-bridge heads has a rectangular distribution and hence $\overline{\cos\theta} = 0.9$. Although the distribution of θ is fixed, θ itself still varies from one cross-bridge to another.

During isometric contraction the same cross-bridges remain in the actomyosin cycle. They can interact with the same actin sites again and again. The length of a thick filament is about 1.6 μm of which approximately 0.15 μm in the centre of the filament is devoid of cross-bridges. Since each myosin molecule has two heads and there are 9 myosin molecules per 42.9 nm, the total number of cross-bridges per half thick filament will be approximately 300. At the optimal sarcomere length of 2.1 μm (Gorden *et al.*, 1966; Edman, 1979) there are about 800 actin molecules located within the filament overlap region in a half-sarcomere considering that the axial separation between actin sites is 5.46 nm and that there are 6 thin filaments surrounding each thick fila-

ment. The maximum movement range of a cross-bridge head is about 13.4 nm, which only covers 31.3% of the axial interval of 42.9 nm between cross-bridges. If all actin sites available for attachment there are about 250 actin sites accessible to the 300 cross-bridge heads, giving an upper limit to the maximum number of cross-bridges in the actomyosin cycle during isometric contraction (assuming that only one cross-bridge can attach to an activated actin site).

The mean extension of cross-bridge elastic elements should be constant for different values of muscle length, [ATP] and [Ca] in the isometric state since the mean value of the effective working strokes and the mechanics of strongly attached cross-bridges has been assumed to be independent of muscle length, [ATP] and [Ca]. If [ATP] and [Ca] are constant, F and G do not change because N_1 is fixed during isometric contraction. The variation in muscle tension observed with different muscle lengths is due to differences in the amount of overlap between thick and thin filaments or the number of 'participating' actomyosin couples as represented in Eq.(4.9). Therefore, Z(X) behaves in a similar manner to the muscle tension-length relation. According to the experimental data of Gordon *et al.* (1966), muscle tension is near zero when the sarcomere length is less than 1.27 μm or greater than 3.65 μm, and maximum at 2.1 μm.

We approximated the force-length relation of a half-sarcomere using two parabolas, one from 0.32 μm $\leq X \leq$ 0.525 μm and the other from

0.525 μm $< X \leq$ 0.91 μm. In our model this represents the number of cross-bridges which can contribute to force generation as a function of half-sarcomere length. We applied Eq.(4.9) separately over each region and obtained the following parameter estimates after fitting the data of Gordon *et al.* (1966): $A_{01} = 300$, $A_{03} = 0.525$ μm, $A_{02} = 2.068 \times 10^9 \text{cm}^{-2}$ for $0.32 \leq X \leq 0.525$ and $A_{02} = 7.12 \times 10^8 \text{cm}^{-2}$ for $0.525 < X \leq 0.91$. The model prediction for Z(X) is shown in Fig. 4.2. We assume that the number of 'participating' actomyosin couples is equal to its maximum if muscle length is around its optimal length.

The functional dependence of muscle tension on calcium concentration has been studied in skinned muscle fibers at optimal muscle length and fixed [ATP]. In this case Z(X), N_1, N_3 and the mean of extension cross-bridge elastic elements remain constant while N_2 changes with [Ca], resulting in a change in the number of strongly attached cross-bridges. We used the Levenberg-Marquardt procedure (Press *et al.*, 1988) for nonlinear model parameter estimation to fit the experimental data of Julian and Moss (1980) and found $A_{21} = 4$ and $A_{22} = 6.29$ pCa when $N_2 = 0.5$. These parameter values are within the ranges obtained from experimental data by Brandt *et al.* (1980) using Eq.(4.12), i.e. A_{21} between 1 and 6 and A_{22} between 5.5 and 6.5 pCa. Fig. 4.3 shows the model prediction for the steady muscle tension-pCa relation. By altering [ATP] while keeping [Ca] constant and muscle length at the optimal length, the effect of [ATP] on cross-bridge behavior can be stud-

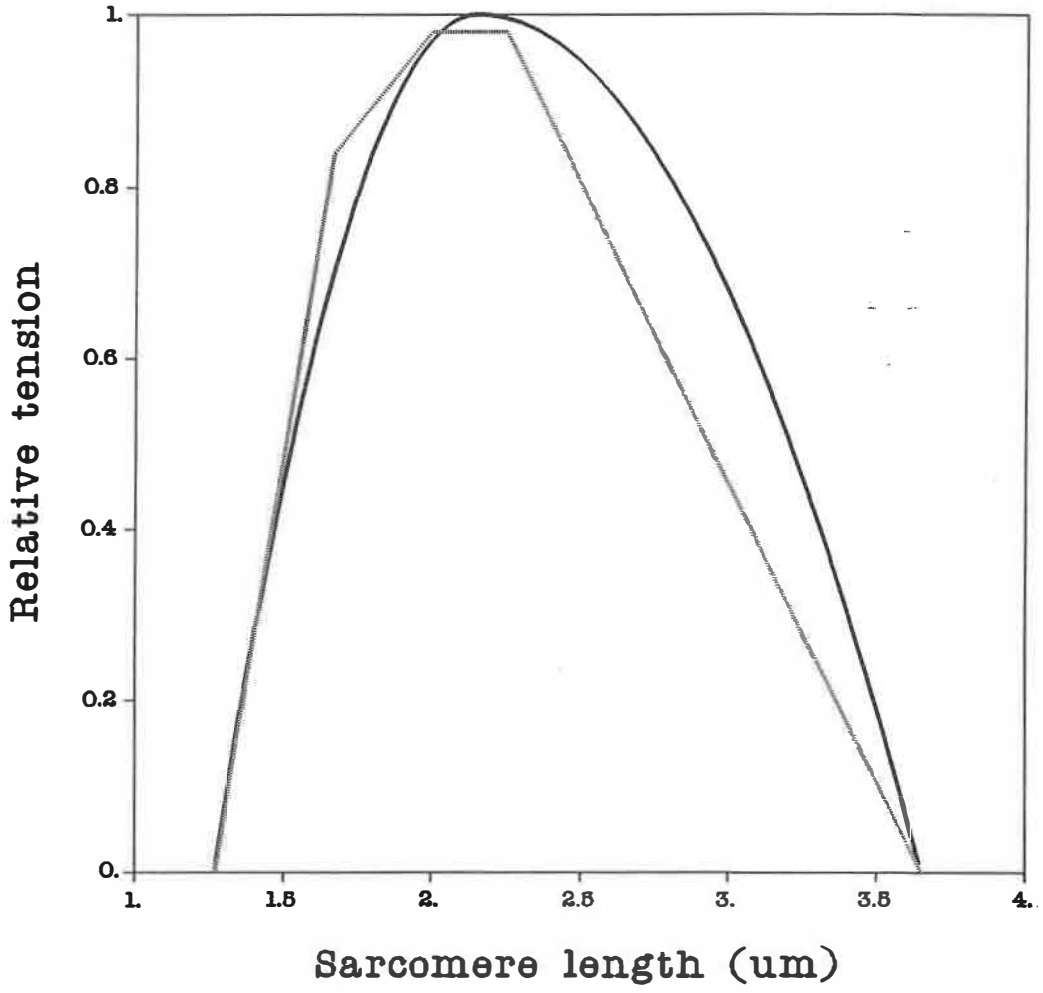


Figure 4.2 The predicted muscle tension-length relation. The dotted line represents the averaged data from Gordon *et al.* (1966).

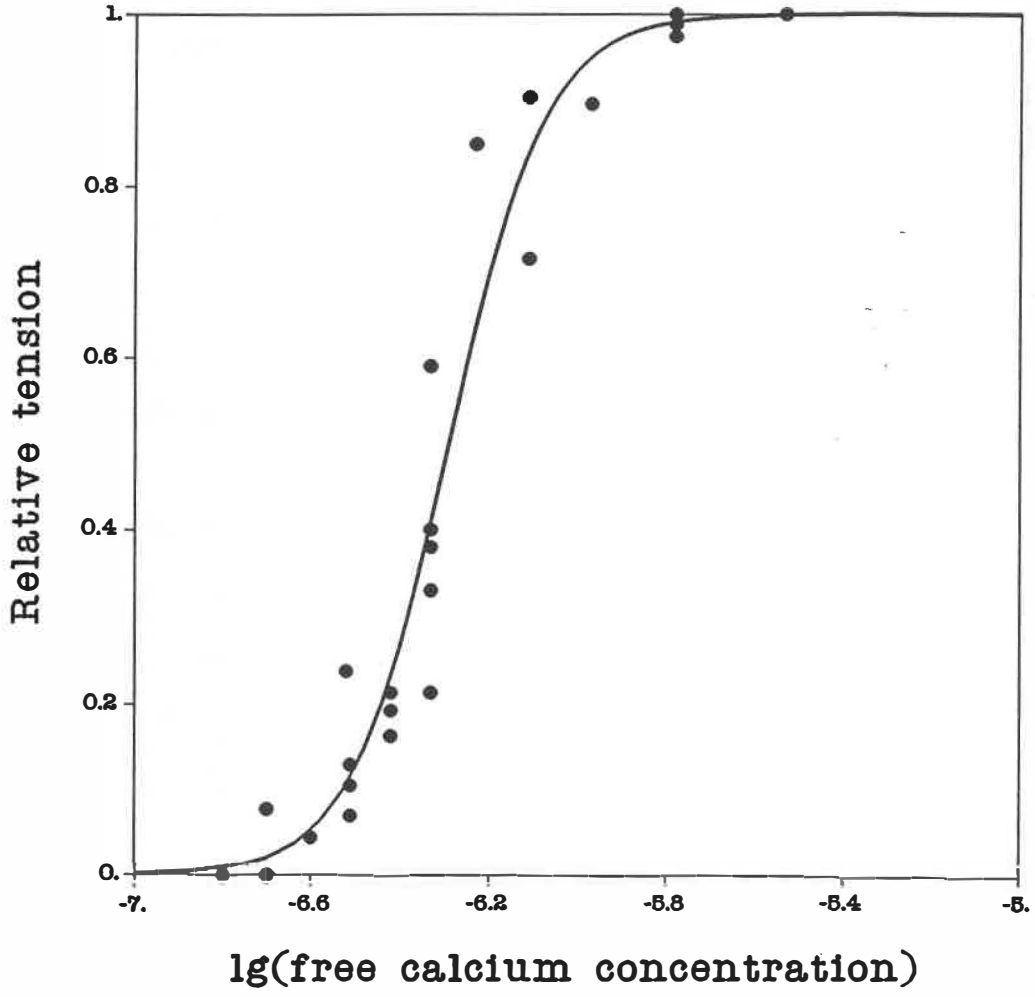


Figure 4.3 The predicted relation between muscle tension and logarithmic free calcium concentration. The dots are from Julian and Moss (1980).

ied, in particular how the transition rates between the force and non-force generating states depend on [ATP]. Data from the experiment of Cook and Bialek (1979) going from maximum tension at 50 μMol [ATP] to the tension at 10 mMol [ATP] were used to obtain the following parameter estimates: $A_{31} = 4.52$ s·Mol^{-1}, $A_{32} = 0.16$ s^{-1} · Mol^{-2}, $A_{33} = 7.28$ s·Mol^{-1}, and $A_{34} = 1$ s^{-1}. Fig. 4.4 shows the model prediction for the steady muscle tension-[ATP] relation. Muscle tension drops relatively rapidly when [ATP] increases from 50 μMol to 1 mMol, and then levels off. The tension at 5 mMol [ATP], the physiological concentration, is only about 55% of the maximum, which suggests that the number of cross-bridges in the force generating state should normally be less than 160 (due to the scaling effect of N_3 in Eq.(4.5)).

The actual number of cross-bridges in the actomyosin cycle, N_{am_0} was estimated by using Eq.(4.35) together with parameters derived from experimental data. Studies of muscle energetics have shown that 30-50% of the total energy liberated by an isometrically contracting muscle, E_0 comes from activation mechanisms such as the Ca-releasing and Ca-reaccumulating processes while the remaining portion comes from the interaction between the thin and thick filaments (Homsher and Kean, 1978). We chose mean values of 40% for the activation mechanism and 60% for the interaction of actomyosin complexes, respectively. In accordance with Edman's experimental data (1979) we took

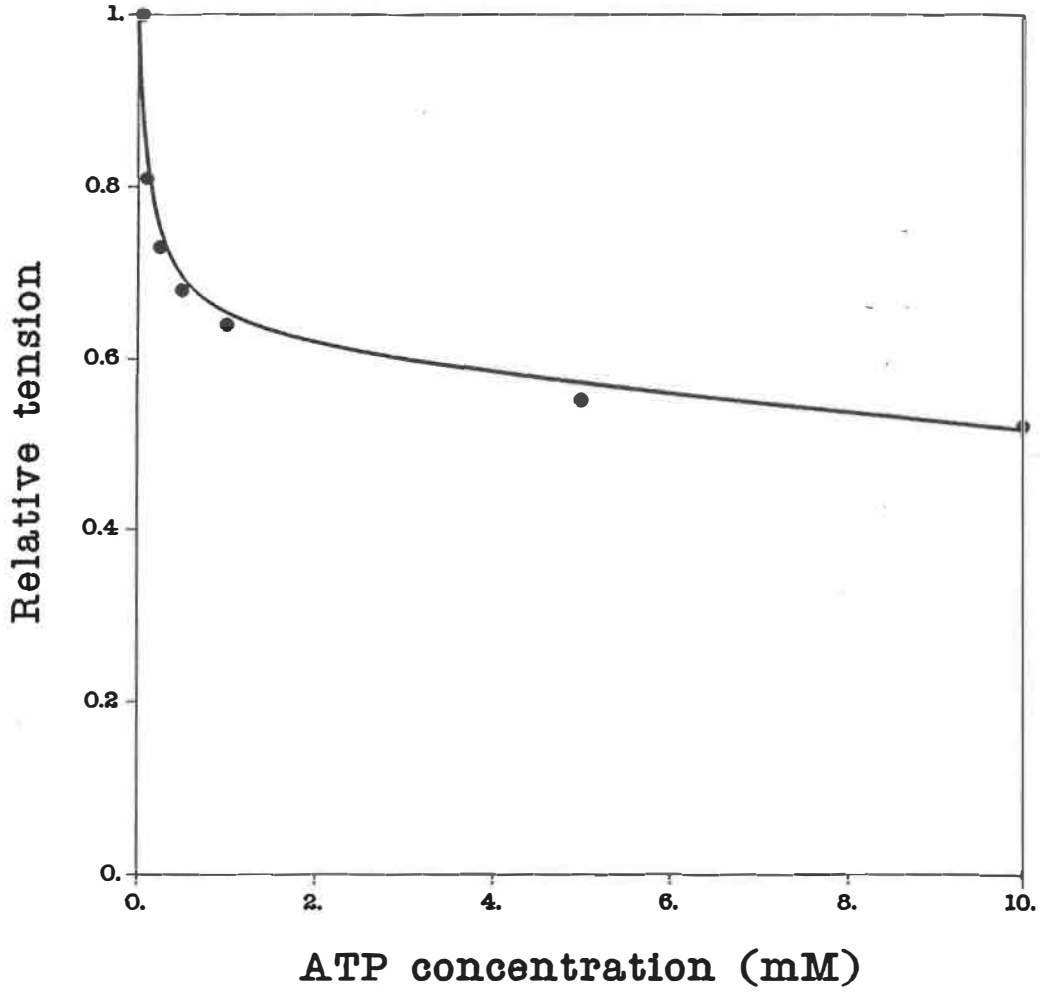


Figure 4.4 The predicted relation between muscle tension and ATP concentration. The dots are from Cooke and Bialek (1979).

the maximum shortening velocity to be $V_m = 2.1$ μm/s/half-sarcomere and the maximum isometric tension to be 22.5 N/cm^2. Since there are about 500 thick filaments within one square micrometer, we have the maximum isometric tension $T_0 \approx 449.7$ pN/thick filament. The amount of energy released by splitting one ATP molecule, e was set equal to 40 kJ/Mol or 64.2×10^{-21} N·m (Woledge *et al.*, 1985). The ATPase hydrolysis rate of isolated actomyosin has been reported to be about 4.5 e/s per cross-bridge, although the ATPase hydrolysis rate in intact muscle may be as high as 6.3 e/s per cross-bridge (Squire, 1986). The actomyosin cycle period is equal to the reciprocal of the ATP splitting rate which gives $T_{am_0} \approx 185$ ms. Using Hill's experimental results (1964), we have $(\overline{\dot{E}}_0 - c_1)/(T_0 V_m) = 0.0375 = eN_{am_0}/(T_{am_0} T_0 V_m)$, and hence $N_0 = 102$ cross-bridges. This is about one third of total number of cross-bridges in a half-sarcomere and is almost the same as the ratio of the maximum movement range of a cross-bridge head to the axial distance between myosin molecules. We then use the value for N_{am_0} to get $A_{62} = 7.59$ nm^{-1} in Eq.(4.33) under the isometric condition.

The actual muscle energy liberation rate, $\overline{\dot{E}}_0 = 0.0625 V_m T_0/(eA_{01}) = 3.06$ e/s per cross-bridge, is within the range 0.6-3.1 e/s per cross-bridge given by Goldman (1987). Assuming that muscle density is 1.0 g/cm^3 and that there are 500 thick filaments per square micrometer, we can also represent the ATP hydrolysis rate as $\overline{\dot{E}}_0 = 0.7015$ μMol·s^{-1}/g,

which is almost the same as the value obtained by Curtin and Davies (1975). Therefore, the ATP hydrolysis rate resulting from the interaction between actin and myosin molecules is equal to 0.4209 μMol·s^{-1}/g while $c_1 = 0.2806$ μMol·s^{-1}/g (in Eq.(4.35)).

If there are viscous forces in a muscle fiber, then the tension between the thin and thick filaments must be greater than zero when the rate of change of muscle length is equal to V_m. Therefore, we assume that the tension in cross-bridges does not drop to zero until shortening velocity reaches $1.05V_m$. From Eq.(4.18), we know that $\bar{\theta} = A_{41}$ when tension is zero. Therefore, substituting into Eq.(4.21) we have $A_{41} = 1.05V_m/(\overline{l\cos\theta}) = 0.12895$ rad/ms, where $V_m = 2.1$ μm/s and l=19 nm. Furthermore, we assume that the amount of energy from splitting one ATP molecule is just equal to the maximum elastic energy allowed by the actomyosin affinity of a strongly bound cross-bridge, and hence $0.5kx_m^2 = e$. Since $x_m = 13.4$ nm and $k = 0.715 \times 10^{-3}$ N/m the affinity or maximum elastic tension, $f_{a_m} = kx_m = 9.58$ pN. When muscle contracts isometrically, the effective working stroke is always less than or equal to x_m, and therefore, forcible detachment cannot occur.

Another important issue is how much of the energy from splitting one ATP molecule is used to develop the tension in an actomyosin complex. If c_{r_0} represents the ratio of the mean value of the energy for tension generation and the total energy released by splitting one ATP molecule during isometric

contraction, then from the principle of energy balance and Eq.(4.19) we have

$$c_{r_0} e = (\theta_e - \overline{\theta}_s)\bar{Q}_0 = \overline{\theta}_{s_0} \frac{A_{42}}{A_{41}} \sqrt{A_{41}^2 - \overline{\dot{\theta}}_0^{\,2}} \tag{4.36}$$

where $\overline{\theta}_{s_0}$ is the value of $\overline{\theta}_s$ in the isometric state. From Eqs.(3.3) and (4.23) we have

$$\frac{A_{42}}{A_{41}} \sqrt{A_{41}^2 - \overline{\dot{\theta}}_0^{\,2}} = lk\bar{x}\overline{\cos\theta} = l\overline{\cos\theta} T_0 / N_{s_0} \tag{4.37}$$

Reducing the above equations we get

$$N_{s_0} = \frac{l\overline{\cos\theta} T_0 \overline{\theta}_{s_0}}{c_{r_0} e}. \tag{4.38}$$

From Eq.(4.38), we can see that the number of cross-bridges in the force generating state is proportional to the isometric tension and inversely proportional to the amount of energy used to develop tension from splitting one ATP molecule. We take c_{r_0} as an unknown parameter to be estimated by fitting the experimental data from muscle steady shortening and isometric contraction. If we know c_{r_0} we can then calculate a number of model variables and parameters in the isometric state, such as N_{s_0} from Eq.(4.38), $\bar{x}_0$ from Eq.(3.3), $T_{am_{s_0}}$ from Eq.(4.34), $A_{43} = \overline{\theta}_0$ by dividing the mean working stroke by $T_{am_{s_0}}$, A_{42} from Eq.(3.36) and A_{44} from Eq.(4.25).

4.5.2 Steady shortening

When [Ca] and [ATP] are held constant all dependent variables associated with muscle behavior can be represented as algebraic functions of v_a. The

mean value of cross-bridge effective working strokes should also be a function of filament sliding velocity since $\overline{\theta}_s$ changes with v_a. Assuming that $\overline{\theta}_s$ decreases linearly as v_a increases and equals zero when $v_a = -V_m$, we can represent the effective working stroke by the following linear function of v_a

$$\begin{aligned} \theta_e - (\overline{\theta_s} - T_{am_w} v_a/(\overline{l\cos\theta})) &= \theta_e - \overline{\theta_{s_0}}(1 + v_a/V_m) + T_{am_w} v_a/(\overline{l\cos\theta})) \\ &= \overline{\theta_{s_0}} + A_{45} v_a \end{aligned} \tag{4.39}$$

The right hand side of the equation follows from the fact that $\theta_e = 0.7854$ rad, $\overline{\theta}_{s_0} = 0.3927$ rad and that T_{am_w} and $\overline{l\cos\theta}$ are constants. T_{am_w} can be obtained from the above equation.

$$T_{am_w} = (A_{45} + \overline{\theta_{s_0}}/V_m)\overline{l\cos\theta} \tag{4.40}$$

The Levenberg-Marquardt method was used to find the values of model parameters which gave the best fit to the experimental data for the energy liberation rate-velocity relation (Hill, 1964). The following estimates were obtained: $c_{r_0} = 0.6$, $A_{51} = 9.99$ ms, $A_{52} = 7.39$ μm/s, $A_{45} = 0.171$ s/μm, $A_{53} = 6.08$ ms, $P_{w_h} = 2.94 \times 10^{-6}$ pN·m/s and $A_{61} = 4.25 \times 10^3$ s^2/m.

With $c_{r_0} = 0.6$, we know that 60% of the total energy from splitting ATP can be used to develop muscle tension when muscle contracts isometrically, and $N_{s_0} = 78.66$ cross-bridges which is about 26% of the total number of cross-bridges and is consistent with the estimate of Parry and Squire (1979), that perhaps 20 to 30% of the myosin heads are attached. We have

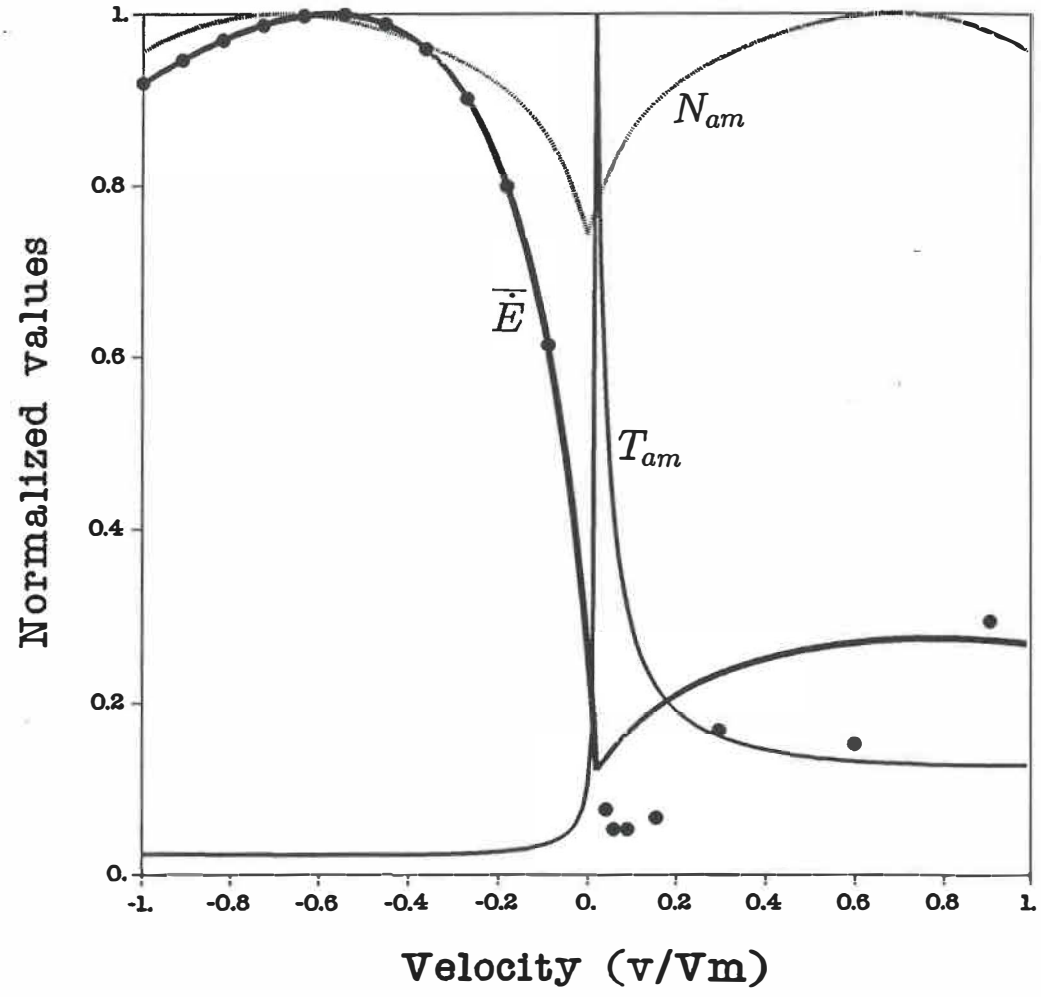


Figure 4.5 The model predictions for muscle energy liberation rate (thick line), actomyosin cycle period (thin line) and number of cross-bridges in the actomyosin cycle (dotted line) as functions of filament sliding velocity. The dots plotted in the shortening region are from Hill's model and in the lengthening region from Curtin and Davies (1975).

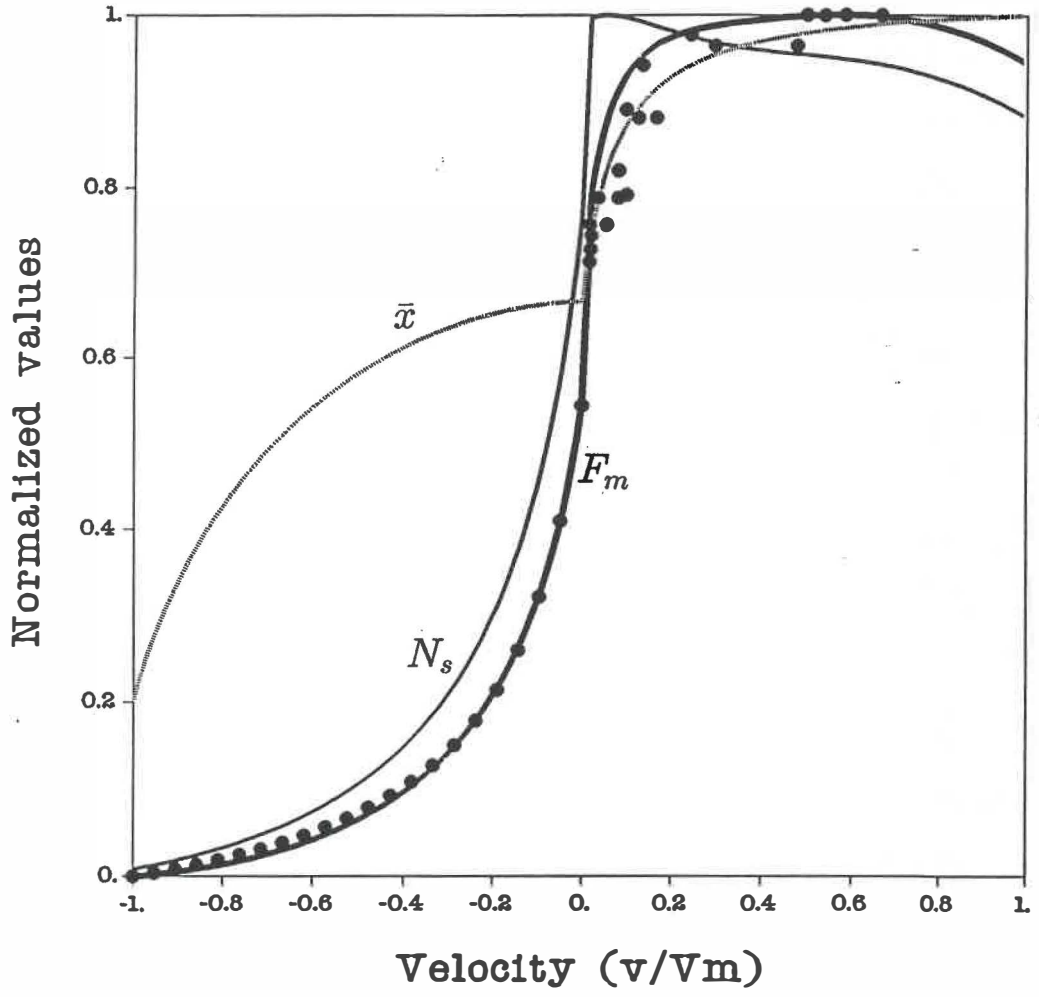


Figure 4.6 The model predictions for muscle tension (thick line), number of strongly attached cross-bridges (thin line) and mean extension in the elastic elements (dotted line) as functions of filament sliding velocity. The dots plotted in the shortening region are from Hill's model and in the lengthening region from Granzier *et al.* (1989).

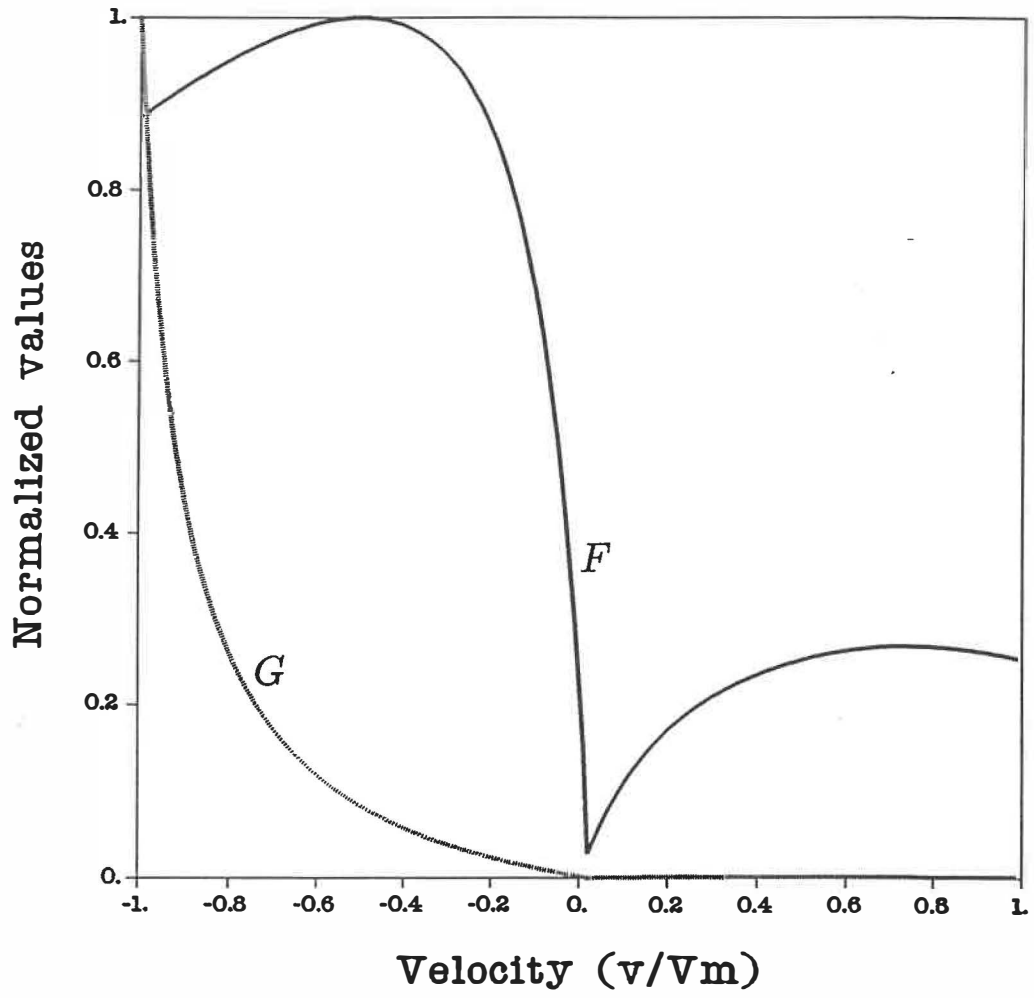


Figure 4.7 The model predictions for F (thin line) and G (dotted line) as functions of v_a.

$\bar{x}_0 = 8.036$ nm, $T_{am_{s_0}} = 143.1$ ms, $A_{43} = 2.744$ rad/s, $A_{42} = 97.79 \times 10^{-21}$ N·m and $A_{44} = 0.0572 \times 10^9$ rad/m. The model parameter estimation for shortening was done by fitting Hill's equation for the energy liberation rate-velocity relation (Hill, 1964). The model curve is shown in Fig. 4.5 together with points calculated from Hill's equation. The model predictions for the N_{am}-velocity and T_{am}-velocity relations obtained from Eqs.(4.33) and (4.29) are also shown in Fig. 4.5, respectively; $\bar{x}$-velocity, and N_s-velocity relations obtained from Eqs.(4.23), (4.25), (4.28) and (4.34) are shown in Fig. 4.6; and G-velocity and F-velocity relations obtained from Eqs.(4.28), (4.34) and (4.4) are shown in Fig. 4.7. All of the curves have been normalized by their maximum values. The model validation was examined by comparing the model prediction for the muscle force-velocity relation to Hill's equation (Hill, 1938). The thick line and dots in Fig. 4.6 show close agreement between the muscle force-velocity relation from our model and that from Hill's equation with $a/T_0 = b/V_m = 0.18$. The viscous coefficient, $A_{46} = 0.67 \times 10^{-6}$ N·s/m per thick filament was estimated by making the externally measured muscle tension equal to zero when muscle shortens at V_m, which is within the range of 0.3 to 0.8×10^{-6} N·s/m/thick filament reported by Ford *et al.* (1977). The intrinsic viscosity at the maximum shortening velocity is less than one percent of T_0 and is therefore negligible. With $A_{45} = 0.171 \times 10^6$ s/m and $T_{am_w} = 6.12$ ms in Eq.(4.40) we get unchangeable portion of the refractory

period is about 11.1 ms. The total refractory period changes almost linearly from 20 ms upto 33 ms as the shortening rate increases from zero to V_m. Since $\overline{\dot{x}} = 0$ during steady contractions, we can use Eq.(4.24) to estimate $\psi(F, G, \bar{x})$.

4.5.3 Steady lengthening

Muscle behavior during steady lengthening is strikingly different from that during steady shortening since the energy for cross-bridge force generation comes mainly from energy supplied externally rather than from splitting ATP, especially when the lengthening rate is larger than $0.06V_m$ (Curtin and Davies, 1975). When muscle is stretched, the filament sliding impedes active rotation of attached cross-bridge heads. High sliding velocities may result in backward rotation of attached cross-bridge heads. As shown by Eq.(4.19), lengthening inhibits utilization of energy from ATP hydrolysis. This is consistent with experimental observations that the energy remaining after subtracting the energy supplied externally from the total heat produced during lengthening, i.e. the energy from splitting ATP, is much less than that produced during isometric contraction (Abbott and Aubert, 1951; Hill and Howarth, 1959). The energy supplied externally becomes more and more predominant in cross-bridge force generation as the lengthening rate increases. It becomes the only energy source when $\dot{\theta} \leq 0$. Hence the stretching rate at which $\overline{\dot{\theta}} = 0$ represents a critical parameter in our model, A_{66}. We use one set of mathematical equations to

represent cross-bridge behavior with $\overline{\dot{\theta}} \geq 0$ and another set for $\overline{\dot{\theta}} < 0$.

During stretching, energy supplied externally is used to slow the normal active rotation of cross-bridge heads toward θ_e. The actual output torque of a cross-bridge is the sum of the torque generated by the active rotation of the cross-bridge and the torque used to slow the cross-bridge rotation. We know that a stretched muscle initially has a very high resistance to stretch, i.e. the amount of force required for a small change in lengthening velocity is quite high. However, as the lengthening velocity increases, smaller increments in force are needed to obtain the same change in velocity. Therefore, we assume that the cross-bridge head offers less resistance and the torque necessary to slow cross-bridge rotation asymptotically approaches a limit as the lengthening velocity increases. This is modeled by allowing A_{42} in Eq.(4.18) to be a function of lengthening rate

$$A_{42} = \begin{cases} A_{42_0} & if \quad v_a \leq 0 \\ A_{42_0} \dfrac{A_{63} v_a + 1}{A_{64} v_a + 1} & \text{otherwise} \end{cases} \tag{4.41}$$

where $A_{42_0} = 9.779 \times 10^{-20}$ N·m from steady shortening. During steady lengthening attached cross-bridge heads cannot reach θ_e or complete their current working stroke without forcible detachment if $\overline{\dot{\theta}} \leq 0$, due to the limited actomyosin affinity. In this case, attached cross-bridge heads rotate relatively slowly away from θ_e depending on the lengthening rate, but after forcible detachment they rotate toward θ_e at a much faster speed which we assume to

be a constant, $\bar{\dot{\theta}}_d$. We also assume that Eq.(4.25) holds for $\bar{\dot{\theta}} > 0$ or $v_a < A_{66}$ while for $\bar{\dot{\theta}} \leq 0$ we have the following linear relation

$$\bar{\dot{\theta}} = A_{65}(v_a - A_{66}) \tag{4.42}$$

When $v_a = A_{66}$, $\bar{\dot{\theta}} = 0$.

There have been fewer experimental studies of muscle behavior during steady lengthening than that during steady shortening, particulary with respect to the muscle energy liberation rate. Data obtained from different experiments varies significantly and there are no mathematical expressions equivalent to Hill's equations (Hill, 1938, 1964) for energy liberation rate and tension as functions of shortening velocity, although qualitative descriptions have been given (Woledge *et al.*, 1985). This is perhaps due to the complexity of muscle behavior and the difficulty in obtaining steady behavior during lengthening. We cannot use the same minimization procedure for model parameter estimation as for steady shortening. However, certain parameters which were estimated for steady shortening are independent of the direction of filament sliding and can be used for steady lengthening.

Eliminating the known parameters, we are left with only three parameters, A_{65}, A_{66} and $\bar{\dot{\theta}}_d$ that need to be estimated for the muscle energy liberation rate-lengthening velocity relation and two parameters, A_{63} and A_{64} for the muscle tension-lengthening velocity relation, during steady lengthening. We

used several criteria to define a rough contour to represent the energy liberation rate-lengthening velocity relation. First, the energy liberation rate should have a minimum when lengthening rate nears $0.06V_m$ in order to be consistent with experimental data and with the conceptual model. Second, we assume that it is also a continuous function of velocity for shortening and lengthening, although the first derivative is discontinuous at $v_a = 0$. Third, the energy liberation rate should rise and approach the isometric rate when the lengthening rate is about $0.8V_m$, according to the experimental results of Curtin and Davies (1975). Using these criteria we obtained $A_{65} = -0.001$ nm^{-1}, $A_{66} = 0.033$ μm/s and $\overline{\dot{\theta}}_d = 45$ rad/s. We then adjusted A_{63} and A_{64} to fit the experimental data for the muscle tension-velocity relation (Granzier *et al.*, 1989) and obtained $A_{63} = 10 \times 10^6$ s/m and $A_{64} = 6.5 \times 10^6$ s/m. The torque generated at $v_a = V_m$ is about $1.5A_{42_0}$. The model predictions for the energy liberation rate-velocity, N-velocity and T_{am}-velocity relations obtained from Eqs.(4.35), (4.33) and (4.32) are shown in Fig. 4.5; muscle tension-velocity, $\bar{x}$-velocity and N_s-velocity relations obtained from Eqs.(3.3), (4.23), (4.32) and (4.34) are shown in Fig. 4.6; and G-velocity and F-velocity relations obtained from Eqs.(4.32), (4.34) and (4.4) are shown in Fig. 4.7.

4.5.4 Formulations for F and G

Since F and G are essential to predicting muscle mechanical properties, we would like to express them as explicit functions of v_a. Because $v_a = A_{66}$ is a discontinuous point in cross-bridge behavior, we use two equations. One holds for the $v_a \leq A_{66}$ and the other for $v_a > A_{66}$. For $v_a \leq A_{66}$, we assume that

$$F(v_a) = \frac{(A_{11}v_a + A_{10})v_a + A_{17}}{A_{13}v_a + A_{12}} \tag{4.43}$$

and

$$G(v_a) = \frac{A_{14}v_a + A_{17}}{A_{16}v_a + A_{15}}. \tag{4.44}$$

By using the Levenberg-Marquardt minimization procedure, we get $A_{10} = -17.77\ \mu\text{m}^{-1}$, $A_{11} = -6.3 \times 10^{12}\ \text{s/m}^2$, $A_{12} = 0.396$, $A_{13} = -1.042 \times 10^6\ \text{s/m}$, $A_{14} = -20.857\ \mu\text{m}^{-1}$, $A_{15} = 0.143$, $A_{16} = 6.2 \times 10^4\ \text{s/m}$, and $A_{17} = 1\ \text{s}^{-1}$. For $v_a > A_{66}$,

$$F(v_a) = v_a(A_{71} + v_a(A_{72} + v_a(A_{73} + v_a A_{74}))), \tag{4.45}$$

where $A_{71} = 39.18\ \mu\text{m}^{-1}$, $A_{72} = -7.66 \times 10^{12}\ \text{s/m}^2$, $A_{73} = 5.79 \times 10^{18}\ \text{s}^2/\text{m}^3$ and $A_{74} = -1.85 \times 10^{24}\ \text{s}^3/\text{m}^4$.

$$G(v_a) = v_a A_{75}, \tag{4.46}$$

Where $A_{75} = 73.16\ \mu\text{m}^{-1}$. Fig. 4.8 compares the above equations for F and G with the representation of F and G in Fig. 4.7.

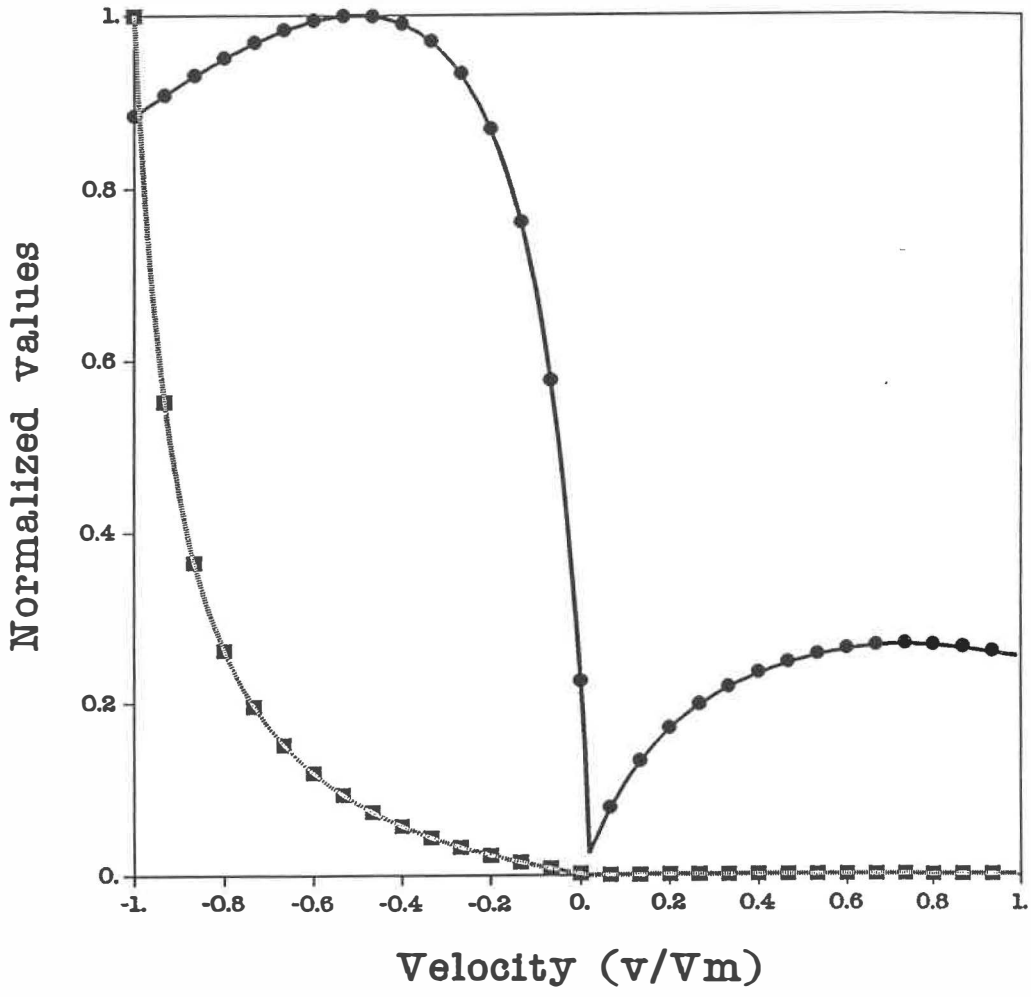


Figure 4.8 The model equations for F(v_a) (line) and G(v_a) (dotted line). The dots represent the F-v_a relation and the filled squares the G-v_a relation from Fig. 4.7.

4.6 DISCUSSION

Macroscopic muscle behavior is derived from the ensemble behavior of cross-bridges within a half-sarcomere. Consequently, muscle stiffness, tension and stored elastic energy have been represented by $N_s k$, $N_s k\bar{x}$ and $N_s k\overline{x^2}$, respectively. In order to predict these muscle variables, it was necessary to find mathematical formulations for N_s and the instantaneous distribution of x for different operating conditions. Following Huxley's approach, we have expressed N_s in terms of transition rates, F and G, between two states, the non-force generating state and the force generating state. The force generating state consists of a continuous mechanochemical process during which the cross-bridge contractile machinery drives the cross-bridge head to rotate while bound to actin, utilizing the energy from ATP hydrolysis. This stretches the cross-bridge elastic element and generates tension between the thin and thick filaments. The non-force generating state, on the other hand, includes most of the chemical states of the kinetics scheme, during which cross-bridges either attach to or detach from actin sites without converting chemical energy to mechanical work for tension development. Therefore, the transition rate F takes into account the regulation of biochemical reactions on the mechanical events of muscle force generation, while G accounts for the effects of the mechanical events on the biochemical reactions. The variation in the force development

rate, $\bar{\dot{x}}$ shows the effects of mechanical events of force generation on the ability of cross-bridges to convert chemical energy into mechanical work. We have lumped distributed effects by estimating mean values of ensemble variables, such as θ_s, $\dot{\theta}$, $\dot{x}$ and x. Using this approach we were able to characterize F, G and $\overline{\dot{x}}$ from experimental data. The variables $\overline{\dot{\theta}}$, $\bar{x}$, F and G have all been expressed as functions of v_a.

Assuming that the rate of energy liberation from muscle activation mechanisms is independent of filament sliding, the slight decrease in muscle energy liberation rate seen when the shortening velocity is sufficiently high, is the result of a drop in N_{am} and an increase in T_{am}. Both the drop in N_{am} and the increase in T_{am} could be caused by the increase in the duration of refractory period. In principle, Eq.(4.35) is very similar to Eq.(9) in the original Huxley model (1957) except that the expressions for F and G as functions of v_a are different. This results in a different energy liberation rate-velocity relation. Since F and G are computed rather than assumed, our model can provide a better approximation of experimental data. A rapid drop in the energy liberation rate when muscle is stretched slowly is a feature of our model which previous models have not been able to achieve. It results from a rapid increase of T_{am} or T_{am_s} since lengthening impedes the active rotation of a cross-bridge head to the end of its working stroke and thus reduces the conversion of chemical energy from ATP hydrolysis to mechanical work. As stretching rate is increased,

forcible detachment occurs and cross-bridge heads can rotate towards θ_e at a higher velocity than during attachment, thereby increasing the energy liberation rate. The F-velocity and G-velocity relations show the effects of filament sliding. The effect of changing [ATP] or [Ca] is to scale the transition rates can be seen from Eqs.(4.1) and (4.2). An interesting finding is that the F-velocity relation behaves in a similar manner to the experimental energy liberation rate-velocity relation indicating that the most important step for the conversion of chemical energy is the entry of cross-bridges into the force generating state. This implies that non-force generating states have little influence on the energy liberation rate and hence it is possible for a cross-bridge to reverse between attachment and detachment without consuming any chemical energy while in the non-force generating state.

The predicted value of N_s asymptotically approaches zero when the steady shortening velocity is close to maximum. However, when muscle is stretched, N_s first increases rapidly and then decreases slightly as the lengthening rate becomes greater than a critical value, $v_a = A_{66}$. This reversal of N_s may be the main reason for yielding in muscle tension. The discontinuous point in the experimental tension-velocity curve arises from the discontinuities in N_s and $\bar{x}$. The value of $\bar{x}$ in the isometric state ($\bar{x}_0$) is about 8.036 nm, which is greater than half of the maximum working stroke (6.7 nm). This means that the distribution is not uniform over the range from zero to x_m. This could be

caused by the effect of turnover rate. A cross-bridge with a larger x will have a lower $\dot{\theta}$ and need a longer cycle period.

Our modeling approach allows us to easily incorporate effects such as variation in temperature on muscle behavior. With our model the thermal effect on maximum steady shortening velocity could be represented by making A_{41} a function of temperature. The shift of the [Ca]-force curve to lower pCa has been attributed to the negative enthalpy of Ca^{++} binding to troponin C (Godt and Lindley, 1982). This can be modeled by assuming that A_{22} varies with muscle temperature. The relative increase in tetanic force with higher temperature would result mainly from an increase in the mean value of Ψ_x (the distribution of x) since the number of attached cross-bridges would remain approximately constant due to the fact that both F and G increase while the ratio of F to $F+G$ remains almost the same.

4.7 CONCLUSION

The force generating state is the major state in which cross-bridges produce elastic tension between the thin and thick filaments. Therefore, muscle mechanics is determined principally by the ensemble of cross-bridge mechanics in this state. Rather than attempting to model the behavior of individual cross-bridges, we used the mean ensemble values of variables such as the mean

rates of cross-bridge transitions and force development to represent the overall behavior of the ensemble. The mathematical expressions were derived and parameter values were determined using a non-linear model parameter estimation procedure to fit experimental data. The developed model not only provides a close agreement with the predictions from Hill's equations, but also accounts for muscle mechanical behavior during steady lengthening.

From this modeling study, we can assume that muscle intrinsic viscosity is negligible. Muscle mechanical behavior can be mainly represented by N_s and $\bar{x}$. The increase of the refractory period when shortening rate is relatively high is the cause of the drop in muscle energy liberation rate, which not only prevents cross-bridges from entering the actomyosin cycle, but also increases the actomyosin cycle period. Inhibition of ATP hydrolysis can occur due to lengthening through the energy conversion mechanism of the actomyosin complex. The mechanical and biochemical events during muscle contraction have been tightly coupled through the cyclic behavior of cross-bridges.

CHAPTER 5

MODELING OF MUSCLE TRANSIENT RESPONSES

The model which we developed in the previous chapter was able to successfully predict muscle tension-velocity and energy liberation rate-velocity relations. However, the model treated only steady-state relations and was therefore inadequate for most applications due to the fact that muscle contraction is normally dynamic. Because of our success in using F, G and $\overline{x}$ to represent muscle behavior during steady contractions, we have continued with this modeling approach to consider how these three variables change under dynamic conditions.

5.1 MODIFICATION OF STEADY-STATE MODEL

Any change in the muscle variables, $\dot{X}$, [Ca] or [ATP], would result in a transient response both in the number of cross-bridges in the force generating

state and the mean extension of the cross-bridge elastic elements. In our model, we attribute such dynamic behavior to changes in the instantaneous values of F, G and $\bar{x}$ or $\dot{\bar{x}}$. Thus we cannot use the $F - v_a$, $G - v_a$ and $\bar{x} - v_a$ relations identified in the previous chapter for steady state. In order to account for such dynamic responses we need to first study the functional dependence of the three state variables, F, G and $\dot{\bar{x}}$ on the muscle variables to determine what kind of model is needed.

5.1.1 Transition rates

The static dependence of F and G on the muscle variables, $\dot{X}$, [Ca] and [ATP] have the formulations shown by Eqs.(4.1) and (4.2). During steady contraction these muscle variables are constant so factors such as a time delay between a change in one of the variables and a subsequent change in muscle tension play no role. However, when one of these variables changes, as for example during a muscle twitch where [Ca] transiently increases and then decreases, the time delay becomes important.

From the viewpoint of system dynamics, instantaneous variations in F and G should be associated with $\ddot{X}$, $\dot{[Ca]}$ and $\dot{[ATP]}$ since F and G are functions of $\dot{X}$, [Ca] and [ATP]. If the muscle variables stabilize, muscle should reach a steady state and there should be no significant variation in either F or G. According to our conceptual model a delay between changes in [Ca] and muscle

tension during a muscle twitch (Ridgway and Gordon, 1975) can be interpreted as a lag in F while G remains constant. If changes in F parallel changes in [Ca], the number of cross-bridges in the force generating state and hence the muscle tension will increase and then decrease after reaching a maximal value. The time course of muscle tension during a twitch will look like the time course of [Ca] scaled and shifted in time. Since variations in [Ca] and [ATP] influence F and G, but do not directly affect $\bar{x}$, it should be sufficient to assume

$$\frac{dF}{dt} = \Phi_f(\dot{X}, [\mathrm{Ca}], [\mathrm{ATP}], \ddot{X}, [\dot{\mathrm{Ca}}], [\dot{\mathrm{ATP}}]) \tag{5.1}$$

and

$$\frac{dG}{dt} = \Phi_g(\dot{X}, [\mathrm{Ca}], [\mathrm{ATP}], \ddot{X}, [\dot{\mathrm{Ca}}], [\dot{\mathrm{ATP}}]) \tag{5.2}$$

where Φ_f and Φ_g may be rather complex functions of the system variables.

In this chapter, we will mainly study the effects of mechanical changes such as length changes and load changes on cross-bridge behavior, rather than deal with the dynamic effects of the variations in other variables such as [Ca] or [ATP]. Provided that [Ca] and [ATP] remain stable, f_2, f_3, g_2 and g_3 should remain constant since it has been assumed previously that [Ca], [ATP] and $\dot{X}$ have mutually independent influences on F and G. Either altering muscle length or muscle load will produce sliding of thin and thick filaments, which in turn influences cross-bridge behavior. Therefore, a sudden change in muscle length or load should induce a transient response in a steadily contracting

muscle. A portion of the overall variation in muscle mechanics comes from a change in the mean value of cross-bridge elastic element extension while the remainder comes from a change in the number of cross-bridges in the force generating state, which can be modeled by changing F and G.

According to our conceptual model, the acceleration of filament sliding would not have a direct effect on either F or G since G depends only only on the angular velocity of cross-bridge heads and the effective working stroke while F depends mainly on the probability that a cross-bridge will encounter an activated actin site, as well as the length of time spent in states that do not generate force, e.g. releasing the energy remaining at the end of the working stroke, binding to and hydrolyzing an ATP molecule. During responses to transient changes in the mechanical state of muscle, the filament sliding consists of two different motions in attached cross-bridges. One of them is the horizontal displacement caused by the rotation of a cross-bridge head and the other is the change in extension of the cross-bridge elastic element, as expressed in Eq.(3.6). During steady contractions, the mean rate of change of cross-bridge elastic element length is equal to zero, but it is quite large during transient responses and therefore contributes much more to the filament motion than the first term on the right hand side of Eq.(4.24), particularly when the sliding velocity is quite high since the maximum angular velocity of a strongly attached cross-bridge head is normally limited. The angular velocity

of cross-bridge heads affects G directly while the rate of change of the elastic extension does not. During steady contractions, the rate of change of the elastic extension is equal to zero, therefore the sliding rate is directly proportional to $\bar{\dot{\theta}}$, and hence we make G a function of v_a. However, during dynamic contractions G is not simply a function of v_a since much of the filament sliding is produced by changes in the extension of the elastic element. Therefore, it is necessary to make G a function of $\bar{\dot{\theta}}$ rather than v_a in order to reflect the intrinsic dependence of G on the angular motion of cross-bridge heads. $\bar{\dot{\theta}}$ is always between $-A_{41}$ and A_{41}, even during the phase of rapid change when the absolute value of the sliding velocity can be an order of magnitude greater than V_m. We replace v_a in Eqs.(4.44) and (4.46) by $\bar{\dot{\theta}}$ according to Eqs.(4.25) and (4.42) respectively and use the previously identified formulation of G for both steady and dynamic contractions.

For a steadily contracting muscle F is an implicit function of $\bar{\dot{\theta}}$ because of the linear relation between v_a and $\bar{\dot{\theta}}$. During transient changes in the mechanical state, filament sliding velocity may be an order of magnitude greater than V_m. At high sliding velocities, a cross-bridge would have a high probability of encountering an activated actin binding site, but attachment would be more difficult. This was shown in Fig. 4.5 where N_{am} drops for high filament sliding velocities. We assume that the transient effects of filament sliding do not alter

the relation between F and $\bar{\dot{\theta}}$ that holds for steady contractions. Then we have

$$IF = f_1(\bar{\dot{\theta}}) f_2([\mathrm{Ca}]) f_3([\mathrm{ATP}]) \tag{5.3}$$

and

$$IG = g_1(\bar{\dot{\theta}}) g_2([\mathrm{Ca}]) g_3([\mathrm{ATP}]) \tag{5.4}$$

Both F and G have a common point where their derivatives are discontinuous, $\bar{\dot{\theta}}_c = 0$. When $\bar{\dot{\theta}}_c = 0$, we have $\bar{x}_c = 9.05$ nm. In transforming the independent variable of f_1 and g_1 from v_a to $\bar{\dot{\theta}}$, we will use $\bar{\dot{\theta}}_c$ or $\bar{x}_c$ as reference points.

Cross-bridges should have a lower value of G at the beginning of muscle activation than in the steady state since cross-bridges are just starting their transition from the non-force generating state to the force generating state and require some time to reach a steady state. Thus, we propose that G should be modified as follows

$$G(\bar{\ddot{\theta}}, \bar{\dot{\theta}}) = \frac{IG(\bar{\dot{\theta}})}{1 + e^{A_{90}(A_{91} - v_a^2)[\bar{\ddot{\theta}}(\bar{\dot{\theta}} - \bar{\dot{\theta}}_0)]^2}} \tag{5.5}$$

where $IG(\bar{\dot{\theta}})$ represents the function G derived for steady contractions, where $\bar{\ddot{\theta}} = 0$. The parameters, A_{90} and A_{91} are chosen to match the rising rate of muscle tension before reaching steady contraction.

5.1.2 Force development rate

During steady contraction, an equilibrium is reached by the ensemble of cross-bridges and hence muscle mechanical variables and parameters also at-

tain equilibrium values. However, if the mechanical state suddenly changes there will be a transient mechanical response in the muscle. We could reasonably assume that during this transient response the inertia of the cross-bridge heads in the force generating state should affect cross-bridge mechanics since the average angular velocity, $\overline{\dot{\theta}}$ changes and $\overline{\ddot{\theta}} \neq 0$. The output power of an actomyosin complex could be a function of both $\overline{\dot{\theta}}$ and $\overline{\ddot{\theta}}$ rather than only of $\overline{\dot{\theta}}$, as we had assumed for the steady state. In vertebrate skeletal muscle each cross-bridge head consists of a portion of the myosin heavy chain (molecular weight 120 kilodaltons) and two light chains (molecular weight 40 kilodatons), an alkali light chain and a DTNB (5,5-dithiobis-2-nitrobenzoate) light chain (Squire, 1986). The total molecular weight is about 160 kilodaltons. Assuming that the mass is located in the center of a cross-bridge head, the moment of inertia is about 1.93×10^{-30} kg·m^2. Using our conceptual model we can make an estimation of the maximun angular acceleration.

It has been shown by Ford ***et al.*** (1977) that muscle tension drops to zero after applying a ramp length change with an amplitude of 6 nm/half-sarcomere and a ramp duration of 0.2 ms to an isometrically contracting muscle. Since muscle stiffness would not be zero, this implies that the mean extension of cross-bridge elastic elements must be zero. According to our proposed cross-bridge mechanics, attached cross-bridges would not generate any tension as $\overline{\dot{\theta}}$ nears A_{41}. We will ignore the possibility of negative extension of

the elastic elements since it would only reduce the estimated maximum angular acceleration. Therefore, we can assume that this $\bar{\dot{\theta}}$ reaches A_{41} within 0.2 ms. The average angular acceleration would be about 6.445×10^{5} rad/s^2. The torque required to overcome the inertia of a cross-bridge head at this angular acceleration would be approximately 1.24×10^{-24} N·m, which is negligible compared to the torque, $A_{42_0} = 97.8 \times 10^{-21}$ N·m when the angular velocity of cross-bridge heads is equal to zero. Consequently, Eq.(4.23) can be used for both dynamic and steady conditions in accordance with Huxley's original assumption that F and G are functions of x since Eq.(4.23) provides the link between $\bar{\dot{\theta}}$ and $\bar{x}$.

In contrast to steady-state conditions, muscle mechanical behavior changes continually during transient responses and thus both the state variables and their rates of change would be expected to vary. Normally, one would try to find mathematical expressions for the rates of change, i.e. equations for $\bar{\ddot{\theta}}$ and $\bar{\ddot{x}}$. Since we do not have sufficient information about the system behavior to accurately model these rates of change we have opted to use an iterative computation procedure in which we estimate $\bar{x}$ directly by integrating $\bar{\dot{x}}$, calculating $\bar{\dot{\theta}}$ from Eq.(4.23) based on the value of $\bar{x}$ in the current iteration and then approximating $\bar{\ddot{\theta}}$ and $\bar{\ddot{x}}$ by differentiating $\bar{\dot{\theta}}$ and $\bar{\dot{x}}$. In this way, we only need to modify Eq.(4.24) by adding an extra term to account for the effects of a transient change in the mechanical state of the muscle on cross-bridge

force generation. Eq.(4.23) is an important equation since it not only represents the regulation of muscle tension development, but also serves as a system constraint which allows us to estimate $\overline{\dot{\theta}}$ without deriving a mathematical expression for $\overline{\ddot{\theta}}$ and ensures that an equilibrium can be always achieved in a steady contracting state where $\overline{\dot{x}}$ equals zero.

The major difference between steady contraction and dynamic contraction from the point of view of cross-bridge mechanical behavior is that $\overline{\dot{\theta}}$ changes so $\overline{\ddot{\theta}}$ does not equal zero. Using $\overline{\ddot{\theta}}$ as a new independent variable we represent $\overline{\dot{x}}$ as

$$\overline{\dot{x}} = l\overline{\dot{\theta}\cos\theta} + v_a + \psi(\bar{x}, \overline{\dot{\theta}}) + \overline{\ddot{\theta}}\phi(\bar{x}, \overline{\dot{\theta}}, v_a). \tag{5.6}$$

The first three terms on the right hand side of Eq.(5.6) remain the same as in Eq.(4.24), while a fourth term, $\phi(\bar{x}, \overline{\dot{\theta}}, v_a)$, is added to account for the difference in the contribution of cross-bridges to muscle force development during steady and dynamic contractions. For instance, $\dot{x}$ would be negative if the rate of change of muscle length were less than $-1.05V_m$. The consequences of such rapid changes in length must be taken into account since the absolute value of the sliding velocity could be an order of magnitude greater than V_m. If $\dot{x}$ becomes negative then the force development rate also becomes negative and cross-bridge elastic element extension decreases. Once x reaches zero, further shortening will result in an attached cross-bridge exerting negative tension between the thin and thick filaments. An attached cross-bridge exerting negative

extension moving toward the end of its working stroke might again start generating force if the shortening rate were greatly reduced or became equal to zero. Although the extra term is used to account for the cross-bridge behavior during transient changes in the mechanical state of muscle, we do not yet know how it should be physically interpreted.

5.2 MODEL IDENTIFICATION

The modifications to F and G are relatively straightforward. We need only replace the independent variable, v_a with $\bar{\dot{\theta}}$ in the F-v_a and G-v_a relations which were identified for the steady state. After doing this we found that $A_{90} = 1.7 \times 10^{13}$ s^2/m^2 and $A_{91} = 1 \times 10^{-12}$ m^2/s^2 gave a 200 ms rise time to steady-state muscle tension during tetanus. The rise time increased with shortening velocity and decreased with lengthening velocity. Once muscle reaches to a steady state, the second term in the denominator of Eq.(5.5) will be equal to zero and consequently it will no longer influence G.

To extend the model equations for cross-bridge force development rate to account for dynamic responses, we first estimate $\psi(\bar{x}, \bar{\dot{\theta}})$ which represents the effect of cross-bridge transition between the non-force generating state and the force generating state on $\bar{\dot{x}}$ during steady contractions and then identify

$\overline{\ddot{\theta}}\phi(\bar{x}, \overline{\dot{\theta}}, v_a)$. We assume that

$$\psi(\bar{x}, \overline{\dot{\theta}}) = \bar{x}(A_{80} + A_{81}\overline{\dot{\theta}}) \tag{5.7}$$

and estimate A_{80} and A_{81} directly from Eq.(4.24) using the fact that $\overline{\dot{x}} = 0$ during steady contractions. We have

$$\psi(\bar{x}, \overline{\dot{\theta}}) = -(l\overline{\dot{\theta}\cos\theta} + v_a) \tag{5.8}$$

For steady shortening ($\bar{x} \leq \bar{x}_0 = 8.036$ nm), $A_{80} = 0.0059$ ms^{-1} and $A_{81} = 0.0383$ nm while $A_{80} = 0.0046$ ms^{-1} and $A_{81} = 827.6$ nm for steady lenghtening ($\bar{x} > \bar{x}_0$).

$\overline{\ddot{\theta}}$ varies during transient responses, while it is equal to zero during steady contraction. Therefore, we have taken $\overline{\ddot{\theta}}$ as an independent variable which acts as a scaling factor for $\phi(\bar{x}, \overline{\dot{\theta}}, v_a)$. The fourth term on the right side of Eq.(5.6) will disappear when the angular acceleration is equal to zero. Like cross-bridge behavior during steady contraction where the isometric force generation process is modified based on the direction of filament sliding motion, sudden shortening and lengthening will also modify the force generation process in different ways. For shortening, $\bar{x} \leq \bar{x}_0$ and we have

$$\phi(\bar{x}, \overline{\dot{\theta}}, v_a) = [A_{83}\overline{\dot{\theta}}(1 - e^{-A_{87}v_a^2})/\bar{x} + A_{84}v_a + A_{85}e^{-A_{86}\bar{x}}\overline{\dot{\theta}}] \tag{5.9}$$

while

$$\phi(\bar{x},\dot{\bar{\theta}},v_a)=[A_{92}(\dot{\bar{\theta}}_0-\dot{\bar{\theta}})\bar{x}(1-e^{-A_{93}v_a^2})+A_{94}v_a-A_{95}e^{-A_{93}v_a^2}(\dot{\bar{\theta}}_0-\dot{\bar{\theta}})/(A_{96}+\ddot{\bar{\theta}})] \tag{5.10}$$

for lengthening where $\bar{x}>\bar{x}_0$. The first two terms in Eqs.(5.9) and (5.10) represent the overall mechanical effects on cross-bridge force generation during the rapid change phase, *i.e.* the instantaneous effects of cross-bridge attachment and detachment. Both terms disappear when the sliding velocity is equal to zero and therefore they have little influence on the recovery of muscle tension. The third term in Eq.(5.9), on the other hand, is a function of $\bar{x}$ and takes into account the mechanics of those cross-bridges whose elastic elements are in negative extension during rapid shortening, but because they have not yet completed their working stroke and are able to return to the normal force generating state during the recovery phase of the transient response.

We fit the experimental data in Fig. 13 of Ford *et al.* (1977), under the assumption that there was no significant sarcomere inhomogeneity, by using a fifth-order polynomial to simulate step changes in muscle length with different amplitudes and durations. We used a fixed tension of one percent of the isometric tension to prevent the muscle from going slack during shortening ramps. The minimum tension during shortening and the maximum tension during lengthening were taken as T_1. The muscle tension when $\bar{x}$ had approximately reached its isometric steady-state value was taken as T_2. We obtained

$A_{83} = 6 \times 10^{-9}$ m^2/s^2, $A_{84} = 4.3177 \times 10^{-6}$ s^2, $A_{85} = 5.3 \times 10^{-12}$ nm·ms^2, $A_{86} = 0.47$ nm^{-1}, and $A_{87} = 1 \times 10^{-12}$ s^2/m^2 for shortening. For lengthening, $A_{92} = 3. \times 10^{-3}$ s^2, $A_{93} = 1 \times 10^{16}$ s^2/m^2, $A_{94} = 2.704 \times 10^{-5}$ s^2, $A_{95} = 600$ nm and $A_{96} = 47$ s^{-2} when $\bar{x} > 9.0$ nm, but $A_{95} = 350$ nm and $A_{96} = 300$ s^{-2} when $\bar{x}_0 < \bar{x} < 9$ nm since the recovery rate is relatively slower in this range. Fig. 5.1 compares the model predictions of T_1 and T_2 with the experimental data points for length changes with durations of 0.2 ms. The time course of the muscle transient responses are shown in Fig. 5.2.

5.3 SIMULATION RESULTS AND DISCUSSION

To validate a model one must not only be able to fit the experimental data which was used to identify the model, but must also be able to predict system behavior under a number of different operating conditions. Furthermore, a good model should not only be able to predict most of the properties of the system, but should also be formulated in a way that makes it convenient to incorporate new findings. In this section, we will present the results of simulations of dynamic responses under different operating conditions and show how temperature effects can be incorporated, as an indication of the flexibility of the model. Model simulations can also provide a better understanding of muscle properties, provided the model is sufficiently accurate.

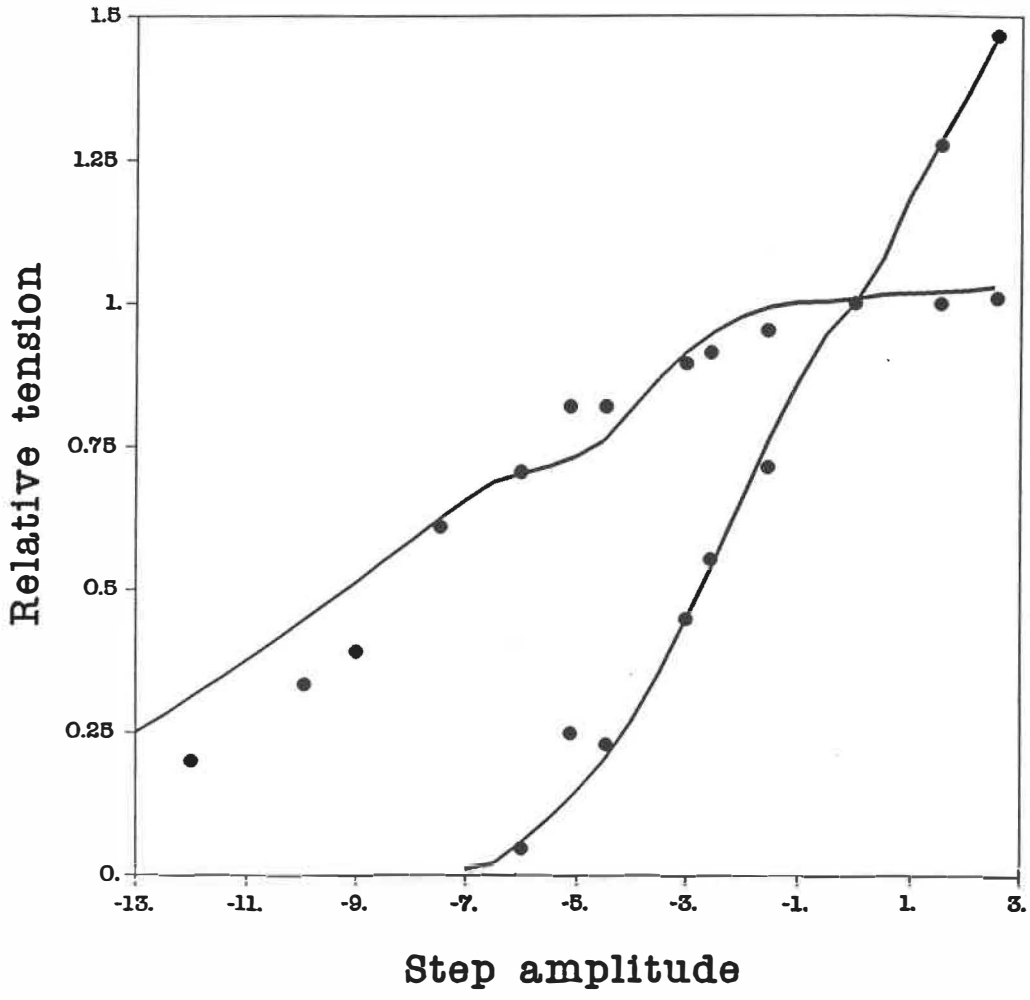


Figure 5.1 T_1 and T_2 as functions of step amplitude following sudden length changes of 0.2 ms duration. The dots are the experimental data from Ford *et al.* (1977).

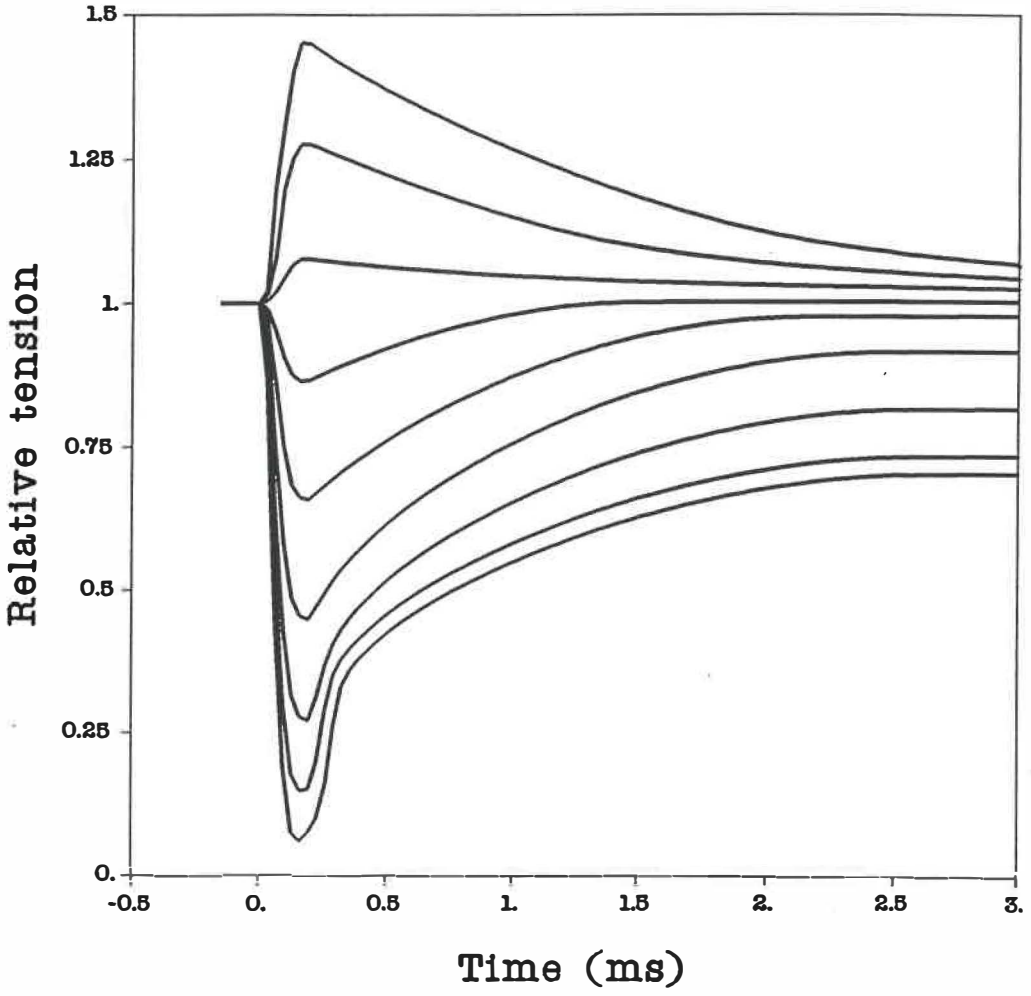


Figure 5.2 Time course of muscle transient responses to sudden length changes, 2.5, 1.5, 0.5, -1, -2, -3, -4, -5, -6 nm/half-sarcomere with 0.2 ms duration.

5.3.1 Transient responses to length changes

In the model identification above, we fit the model to data from experiments where the sudden changes in muscle length had a duration of 0.2 ms. In Fig. 19 of their paper, Ford *et al.* (1977) showed that T_1 and T_2 are not only dependent on the amplitude of the rapid change in muscle length, but also on the duration of the length change. With longer durations but the same amplitude, the slope of T_1 with respect to the change in length becomes flatter while T_2 changes little. This nonlinear behavior indicates that muscle mechanics cannot be accurately represented by a linear spring or even a combination of a number of linear springs and dashpots (Ford *et al.*, 1977). Nevertheless, our model predicts this muscle behavior as shown in Figs. 5.3 and 5.4 where the duration of the length change is 1 ms. In our simulations, we found that the cross-bridge elastic element extension changes much sooner than the number of cross-bridges in the force generating state. It took only about 3 ms for $\bar{x}$ to go from zero to $\bar{x}_0$, whereas the number of cross-bridges in the force generating state took about 200 ms to stabilize at N_{s_0}. T_1 was very sensitive to the rate of change of muscle length due to both the primary effect from the $\bar{\dot{x}}$-v_a relation and the secondary effect from the N_s-v_a relation. The difference in T_1 when length changes with different durations are applied is mainly due to the first two terms in Eqs.(5.9) and (5.10) suggesting that the more rapid

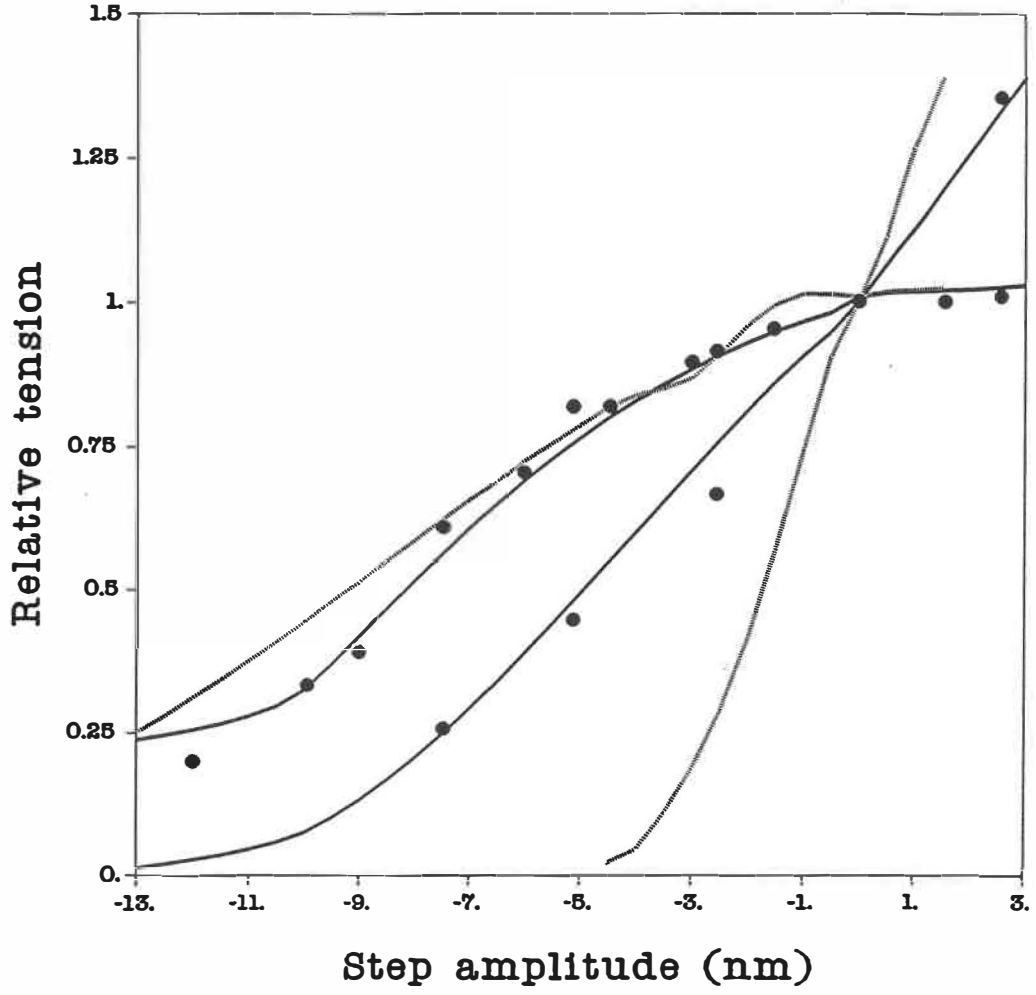


Figure 5.3 T_1 and T_2 as functions of step amplitude following sudden length changes of 1 ms (solid line) and 0.1 ms (dotted line) durations. The dots are the experimental data for T_1 and T_2 with 1 ms step duration from Ford *et al.* (1977).

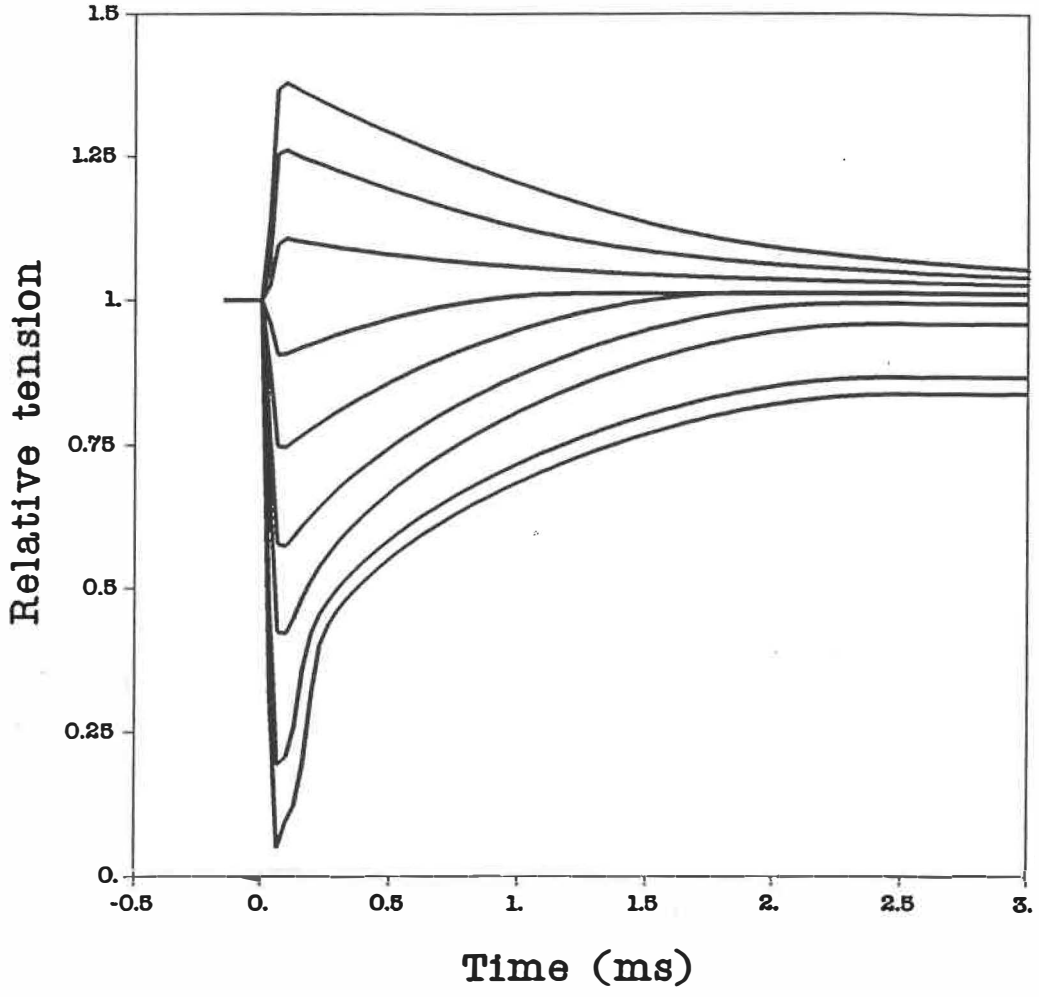


Figure 5.4 Time course of muscle transient responses to sudden length changes, 3, 2, 1, -1, -3, -5, -7, -9, -12 nm/half-sarcomere with 1 ms duration.

the shortening the more attached cross-bridges there are which develop large negative elastic element extension and hence the greater the drop in muscle tension. T_2 is much less sensitive to the change in duration than T_1. One reason is that F and G are slower to respond to mechanical disturbances than the cross-bridge force development rate. Thus, until the cross-bridge elastic element extension, $\bar{x} \approx \bar{x}_0$ (the point at which we estimate T_2), the change in force produced by the change in the number of cross-bridges in the force generating state is small relative to the change in force produced by the change in the elastic element extension, but thereafter changes in muscle tension are mainly due to changes in the number of cross-bridges in the force generating state. Therefore, for a given length change, the change in the number of cross-bridges in the force generating state or T_2 is relatively independent of v_a. This result also could explain why most cross-bridge models can predict muscle behavior during steady contractions using only F and G since muscle tension varies relatively slowly under those conditions.

We tried further reducing the duration of the length change. With a duration of 0.1 ms, a shortening of about 4 nm per half-sarcomere brought muscle tension to zero from its isometric value as shown in Figs. 5.3 and 5.5. This agrees with the extrapolation from experimental results (Ford *et al.*, 1977). We then set the duration to 0.01 ms. Since both the angular acceleration and velocity of a cross-bridge head are limited, a more rapid length change could

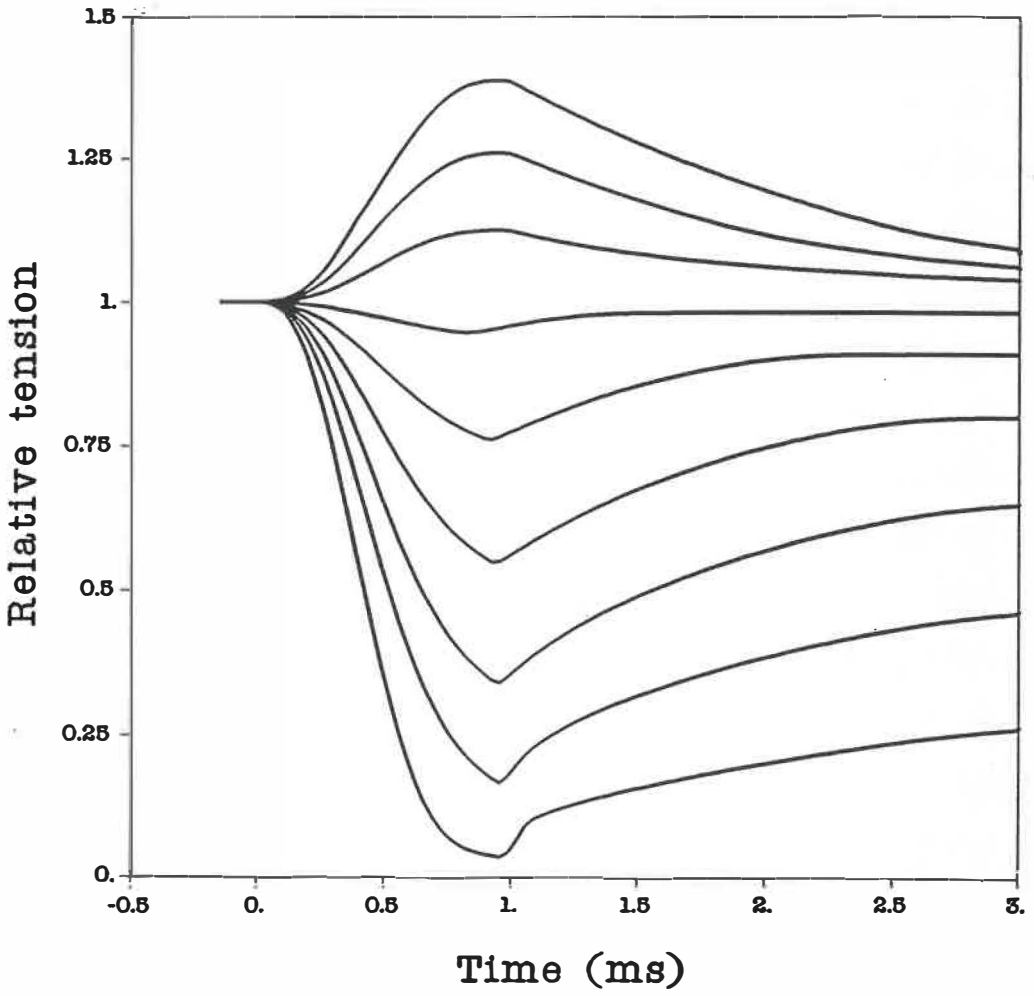


Figure 5.5 Time courses of muscle transient responses to sudden length changes, 1.5, 1, 0.5, -0.5, -1, -1.5, -2, -3, -4 nm/half-sarcomere with 1 ms duration.

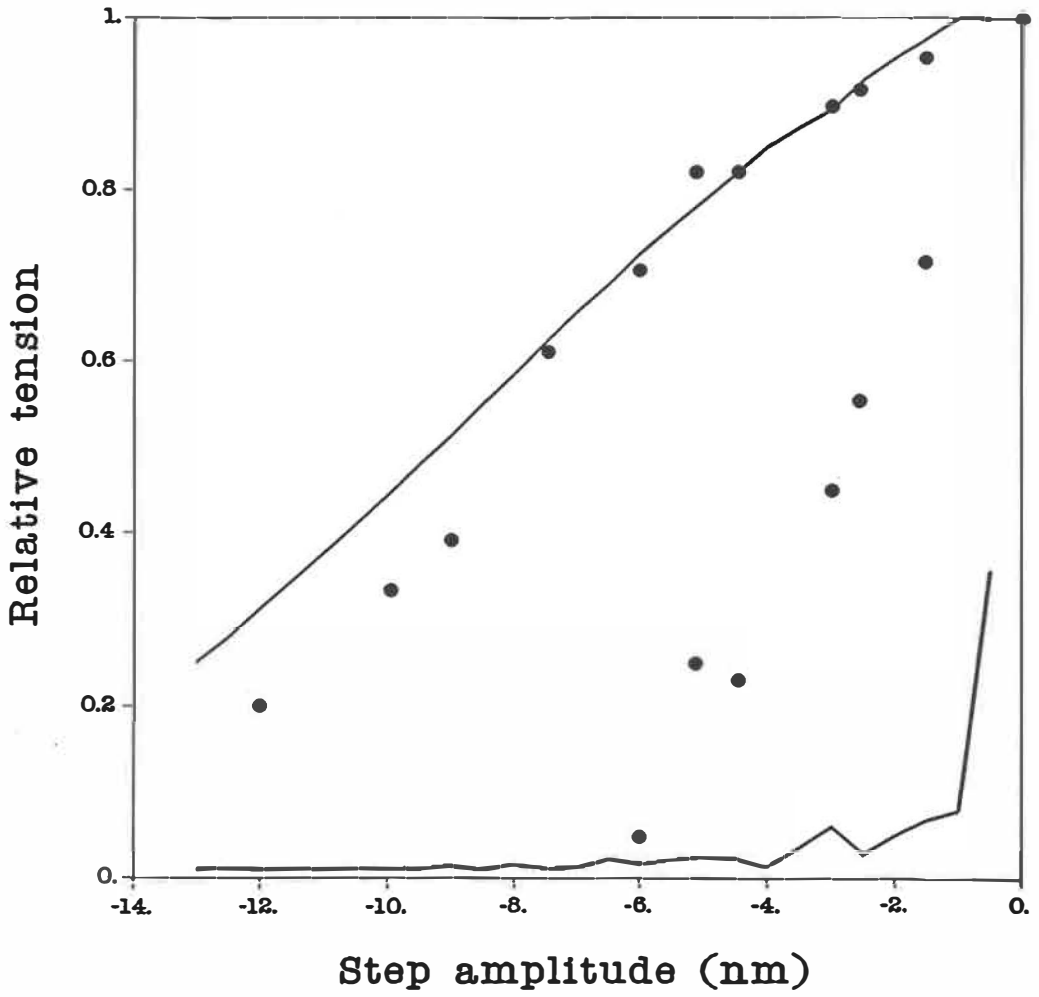


Figure 5.6 T_1 and T_2 as functions of step amplitude following sudden length changes of 0.01 ms ramp duration. The dots are the experimental data for T_1 and T_2 with 0.2 ms duration from Ford *et al.* (1977).

lead to more cross-bridges with negative tension. Therefore, the duration for a given length change was made sufficiently long that muscle tension always remained greater than a small tension (one percent of isometric tension) in order that muscle tension did not go to zero during shortening. We varied the step amplitude from 3 nm to -5 nm per half-sarcomere. Fig. 5.6 shows that the slope of T_1 with respect to step amplitude becomes steeper as the duration decreases, implying that the instantaneous muscle stiffness could not be directly measured by applying a rapid change in muscle length no matter how rapid the length change. As pointed out by Brenner (1990), the muscle stiffness measured by applying a sudden change in muscle length is only an apparent muscle stiffness.

Our simulations also show that the value of N_s at T_2 changes with different amplitudes of length change. When the length change is 13.5 nm per half-sarcomere or greater, T_2 and N_s are near zero, supporting our assumption that cross-bridge transition from the force generating state to the non-force generating state is dependent on the angular position of a cross-bridge head. Since the maximum working stroke is around 13.4 nm, a rapid length change of this amount per half-sarcomere would result in all currently strongly attached cross-bridges moving to θ_e where they can no longer exert tension between the thin and thick filaments.

5.3.2 Transient responses to load changes

Just as muscle responds to transient changes in length, it will also respond dynamically to a sudden change in the external load. However, changing muscle load, as opposed to muscle length will result in a different type of response. During length changes, muscle is allowed to shorten or forced to lengthen, but a sudden change in the external load will involve the effects of both shortening and lengthening (Granzier *et al.*, 1989).

When there is a sudden change in the external load, muscle tension behaves in an unstable manner, oscillating around the final value of the new external load. As the tension oscillates, muscle will shorten or be forced to lengthen. Sliding of the filaments acts as feedback to the muscle to reduce the difference between muscle tension and the external load. From the point of view of system dynamics, muscle possesses the properties of a negative feedback system. When muscle tension is greater than the applied external load, muscle is able to shorten and the shortening acts as feedback to reduce muscle tension and produce mechanical stability. On the other hand, if muscle tension is less than the applied load, muscle is forced to lengthen and thus muscle tension increases. This feedback behavior brings muscle to a new equilibrium state, but at the same time it may produce oscillation in muscle tension. As in other mechanical systems, there are a number of parameters which have effects on

the dynamic response of muscle. Through simulation we found that the frequency, amplitude and duration of the oscillations in muscle tension depend on amount of external load change, speed of the load change, muscle length, total mass and viscosity. All of these factors affect the rate of change of muscle length which in turn has a direct effect on muscle mechanical behavior. The greater the change in the external load and the faster it occurs, the more dramatic the oscillation in muscle tension; with longer muscle lengths, filament sliding velocity will be lower; greater viscosity produces faster damping of the tension oscillation. In certain situations the oscillation of muscle tension can disappear quickly and muscle reaches a steady contraction state, as shown in Figs. 5.7 and 5.8. On the other hand, the oscillation may persist and a steady state is never achieved (Fig. 5.9). In this case, the system behaves like an oscillator and muscle acts as an active element which can provide mechanical energy to the oscillation. Thus, oscillations can continue even if the system viscosity is greater than zero.

The simulated time course of muscle tension during transient responses is closer to the experimental results of Granzier *et al.* (1989), in which the oscillation in muscle tension is significant, than to those of Podolsky (1960). Since the dynamic properties of the apparatus are not given, it is difficult for us to recreate their experimental data through simulation in order to compare their experimental results with our model predictions. The sharp changes of

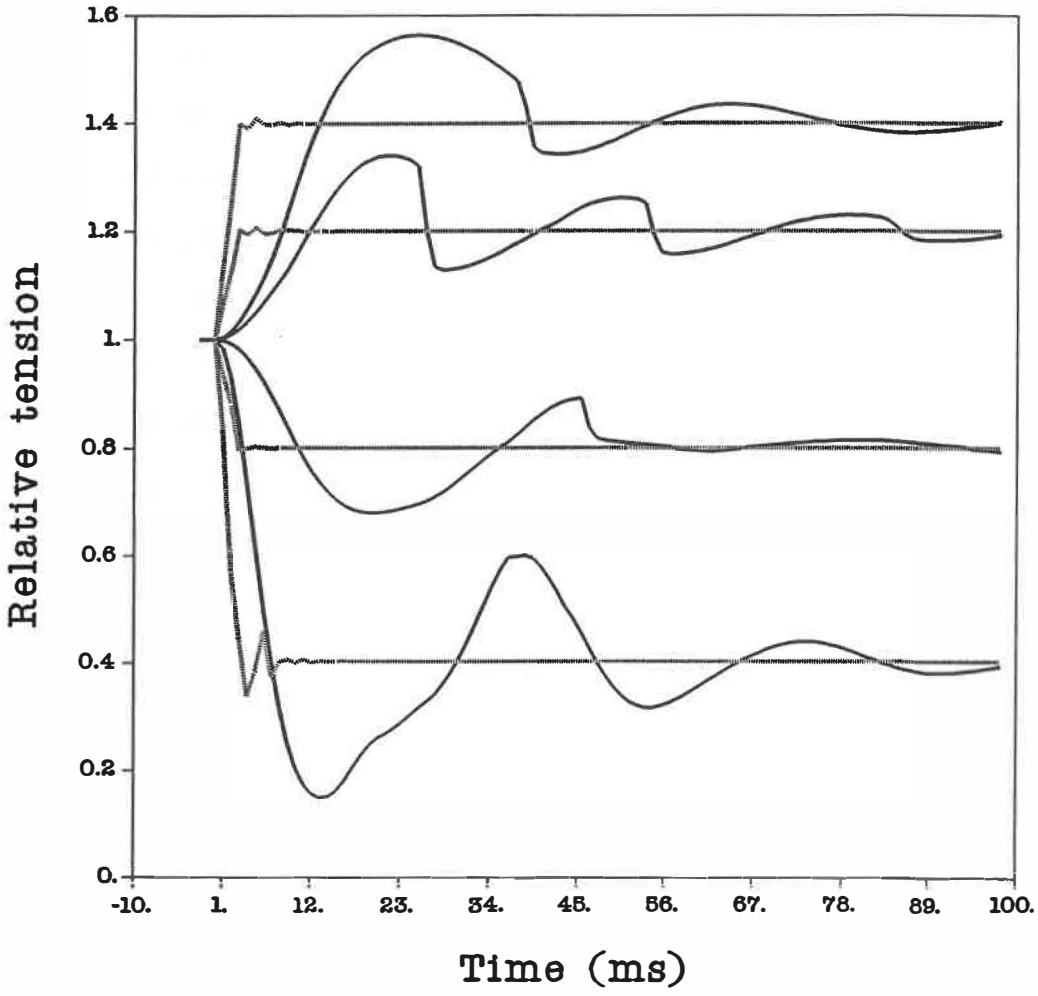


Figure 5.7 Time course of muscle tension in response to step changes in external load of 1.4, 1.2, 0.8 and 0.4 times tetanic tension with muscle length equal to 50 mm and a constant ratio between system mass and external load (solid line), and in response to 3 ms ramp changes with muscle length equal to 10 mm, a system mass equivalent to 0.01 tetanic tension and viscosity equal to $0.016F_{m_0}/V_m$ (dotted line).

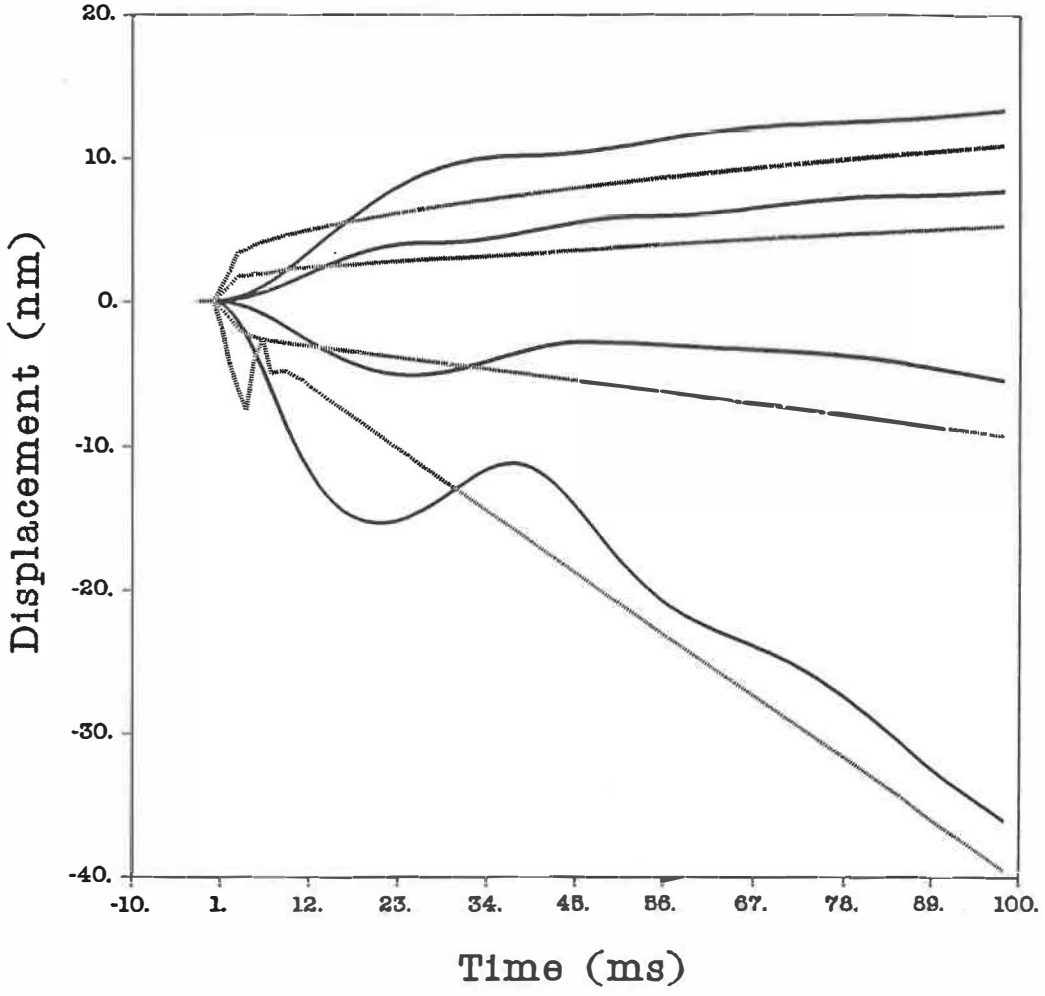


Figure 5.8 Time course of change of half-sarcomere length in response to step changes in external load of 1.4, 1.2, 0.8 and 0.4 times tetanic tension with muscle length equal to 50 mm and a constant ratio between system mass and external load (solid line), and in response to 3 ms ramp changes with muscle length equal to 10 mm, a system mass equivalent to 0.01 tetanic tension and viscosity equal to $0.016 F_{m_0}/V_m$ (dotted line).

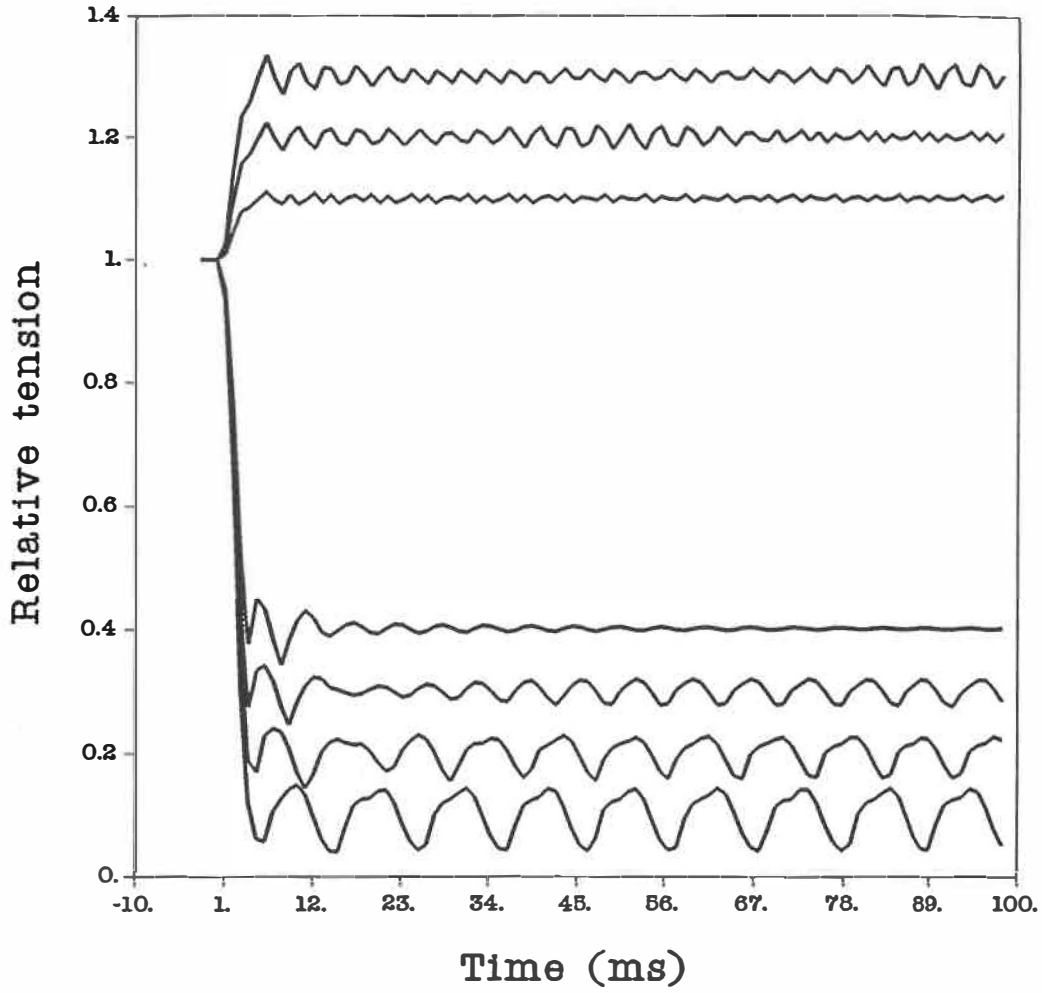


Figure 5.9 Time course of muscle tension during transient responses to 4.5 ms ramp changes in external load of 1.3, 1.2, 1.1, 0.4, 0.3, 0.2, 0.1 times tetanic tension with muscle length equal to 10 mm and a system mass equal to 0.05 tetanic tension.

the curves shown in Fig. 5.7 could be caused by model switching. However, from our simulations, we found that the actual duration of the change in external load and the system viscosity seem to affect muscle tension oscillation most significantly and directly. Whenever a final steady state is achieved in the simulations, the results always reproduce the experimental tension-velocity relation very well (Fig. 5.10).

5.3.3 Temperature effects

Muscle behavior is dependent on biochemical reactions, such as ATP hydrolysis, which are sensitive to variations in temperature. Many experiments have been conducted to determine how temperature affects muscle contraction and a number of physiological parameters have been shown to vary with termperature. In our model, the cross-bridge transition rates between the non-force generating state and the force generating state, F and G, and the rate of cross-bridge force development ($\bar{\dot{x}}$) have been used to represent muscle behavior. The incorporation of temperature effects on muscle transient responses into the muscle model is equivalent to modeling the functional dependence of these three state variables on temperature.

First, we assume that the temperature dependence of actomyosin mechanics is the major reason why muscle mechanical responses depend on temperature. Eq.(4.18) represents the mechanics of actomyosin in the force generating state.

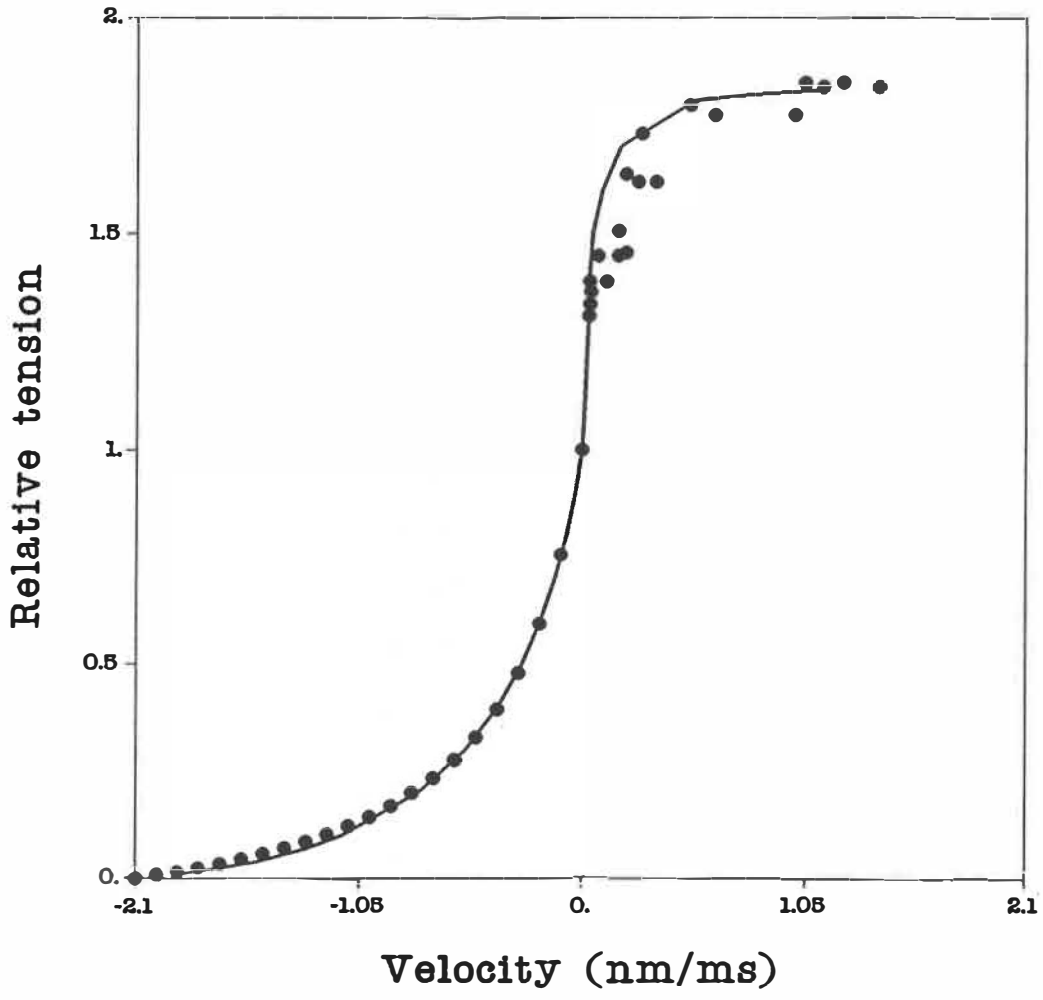


Figure 5.10 Relation between muscle tension and velocity for transient responses to sudden load changes under conditions where muscle steady-state behavior was stable.

Physically, A_{41} represents the maximum value of the angular velocity with which a strongly attached cross-bridge head can rotate while still actively contributing to muscle force, whereas A_{42} represents the maximum torque which can be generated by a cross-bridge head. If actomyosin mechanics varies with temperature, these two parameters should also change with temperature. If A_{41} increases, then V_m should also increase due to the dependence of v_a on $\bar{\dot{\theta}}$ during steady contractions, Eq.(4.21). Many experiments have shown that V_m increases directly as temperature increases. Therefore, we can model the effect of temperature on V_m through a dependence of A_{41} on temperature. Since the reaction rate of actomyosin ATPase and V_m are also strongly correlated, we assume that the energy liberation rate scales with V_m in such a way that the relation between energy liberation rate and velocity preserves its form if it is normalized with respect to V_m. In developing our model for cross-bridge mechanics, the functional dependence of F and G on cross-bridge angular velocity $\bar{\dot{\theta}}$ has been determined from the relation between energy liberation rate and velocity. Since this relation is simply scaled by V_m, it follows that F and G should also be scaled by V_m. Consequently, muscle will have a larger V_m, a shorter rise time for tension development and a shorter period for stablization of N_s due to the faster transition rates between the two states at higher temperatures.

F_{m_0} also depends on temperature, rising as temperature increases. In our

model, muscle tension is equal to $k\bar{x}N_s$, where N_s, the number of strongly attached cross-bridges, is directly proportional to the ratio of F to $F+G$. Both F and G will scale in a similar way with V_m keeping the ratio of F to $F+G$ constant. Therefore, any increase in F_{m_0} would more likely come from an increase in the force generated by each cross-bridge than in the number of strongly attached cross-bridges. Such an increase in cross-bridge force output would be reflected in a larger value of A_{42} which should vary in proportion to F_{m_0}. As shown by Eq.(4.23), $k\bar{x}$ is directly proportional to A_{42} and will scale with A_{42}. We took the temperature at which the experiments used in model identification were conducted, as a reference temperature. Two temperature scaling factors, $Sc1$ and $Sc2$, for V_m and F_{m_0}, respectively are required.

According to our conceptual model, the parameter A_{41} is directly proportional to V_m, guaranteeing that $\dot{\bar{x}}$ is positive when the rate of a steady sliding is less than V_m. From the power balance equation, Eq.(4.23) we can see that $\dot{\bar{\theta}}$ is directly proportional to A_{41} if $\bar{x}$ is fixed. This direct relation suggests that any change in V_m should produce a proportional change of both A_{41} and $\dot{\bar{\theta}}$, keeping their ratio constant. This is also true for the ratio of $k\bar{x}$ to A_{42}. These fixed proportionalities can be used in the original model to provide the basic constraints for incorporating the effects of changes in temperature. The procedure for computing the time sequence of values of $\bar{x}$ proceeds as follows. We first calculate $\dot{\bar{\theta}}$ using the original model with the initial value of $k\bar{x}$ scaled

by the inverse of $Sc2$, estimate F and G based on the new value of $\overline{\dot{\theta}}$ and then scale F, G and $\overline{\dot{\theta}}$ by $Sc1$ to get their true values. In the final step we substitute this value of $\overline{\dot{\theta}}$ into the expression for $\overline{\dot{x}}$, since v_a which represents the filament sliding velocity, appears in the expression for $\overline{\dot{x}}$. We then calculate $k\bar{x}$ by integrating $k\overline{\dot{x}}$. Computation of the next value in the time series begins again with the first step above. Therefore, all parameters in our model remain the same except the parameters in the expression for $\overline{\dot{x}}$, which are associated with $\overline{\dot{\theta}}$ and have been scaled by $Sc1$.

Model simulations were compared with experimental data on the effect of temperature on the transient mechanical response of muscle to a sudden change in length (Ford *et al.,* 1977). We used a fifth-order polynomial to simulate changes in muscle length with different amplitudes and durations as before, and a small fixed value ($0.01F_{m_0}$) as the tension necessary to prevent the muscle from going slack during shortening. The minimum tension during shortening and the maximum tension during lengthening at the end of applied rapid changes were taken as T_1, while muscle tension when $\bar{x}$ had approximately reached its isometric steady-state value was taken as T_2.

We simulated the effect of changing muscle temperature from 1^0C to 8.1^0C by modifying V_m and $k\bar{x}$. Values for V_m at 1^0C and 8.1^0C were obtained from the literature giving a temperature scaling coefficient, $Sc1$ of 1.65. We used our model to estimate $k\bar{x}$ in the isometric steady state at 1^0C and assumed

that the ratio of $k\bar{x}$ at 8.1^0C to its value at 1^0C was equal to the corresponding ratio for F_{m_0} which was obtained from the literature. This gave $Sc2 \approx 1.5$. Model equations and parameters were then modified as outlined above based on the values of $Sc1$ and $Sc2$.

Increasing V_m and $k\bar{x}$ to simulate an increase in muscle temperature affects muscle force generation as shown by the transient mechanical responses to ramp length changes in Figs. 5.11 and 5.12. The primary effect of increasing V_m is to produce an increase in the actively generated component of angular velocity of the cross-bridge heads. This leads to increases in F, G and $\bar{x}$, which in turn result in faster recovery of tension and therefore a larger value of T_2 (or N_s), for a given length change. Modifying $k\bar{x}$ can have different effects on the transient mechanical response, depending on whether k or $\bar{x}$ changes. Any change in the value of k simply scales the amplitude of the response, but does not affect the time courses of tension normalized with respect to F_{m_0} since the normalized tension only depends on N_s and $\bar{x}$. However, altering $\bar{x}$ can change both T_1 and the shape of the response. In particular, applying the same step change in muscle length produces a smaller change in relative tension when $\bar{x}_0$ is larger. The best fit to the experimental data of Ford *et al.* (1977), was obtained when approximately 34% of the change in F_{m_0} came from modifying k and the remainder from modifying A_{42} (Figs. 5.11 and 5.12). Our simulations also support the sugestion by Kuhn *et al.* (1979) that changes in temperature

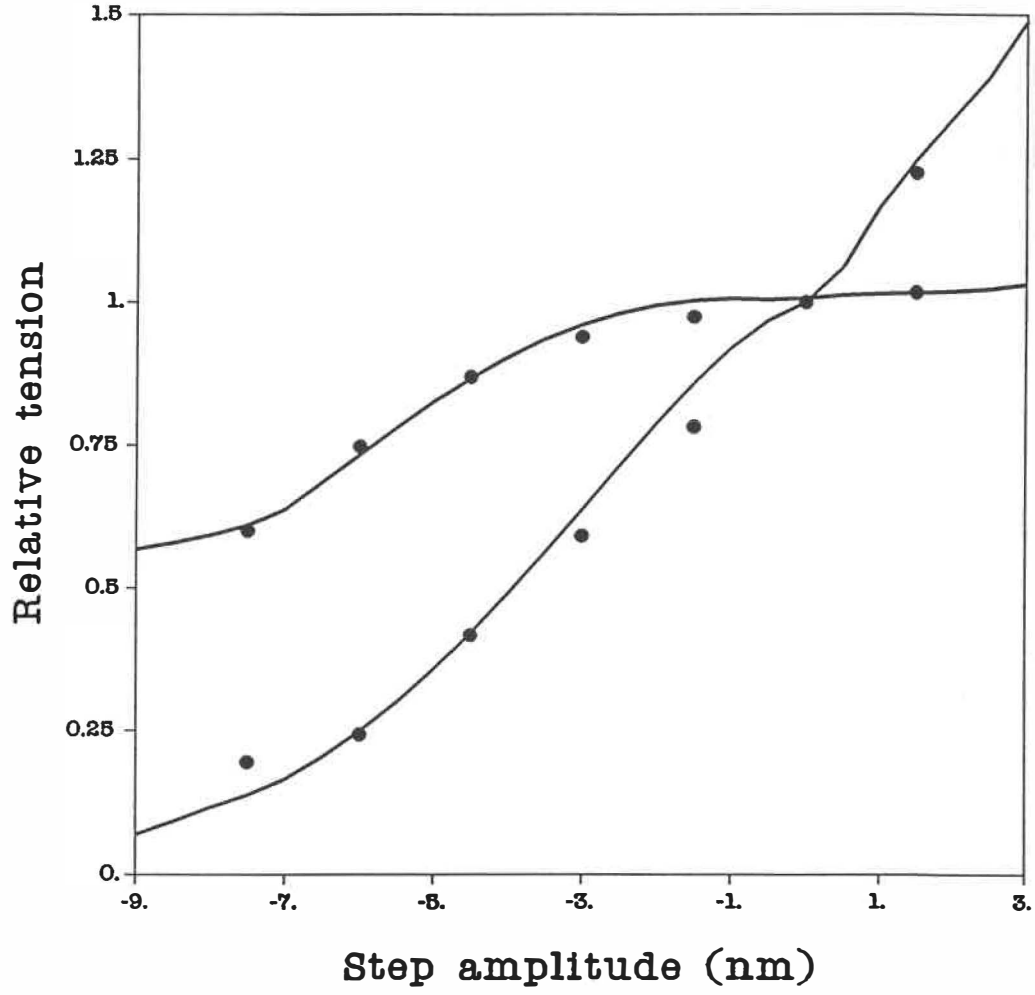


Figure 5.11 T_1 and T_2 as functions of step amplitude following sudden length changes with 0.2 ms ramp duration at 8^0 C (solid line). The dots are the experimental data from Ford *et al.* (1977).

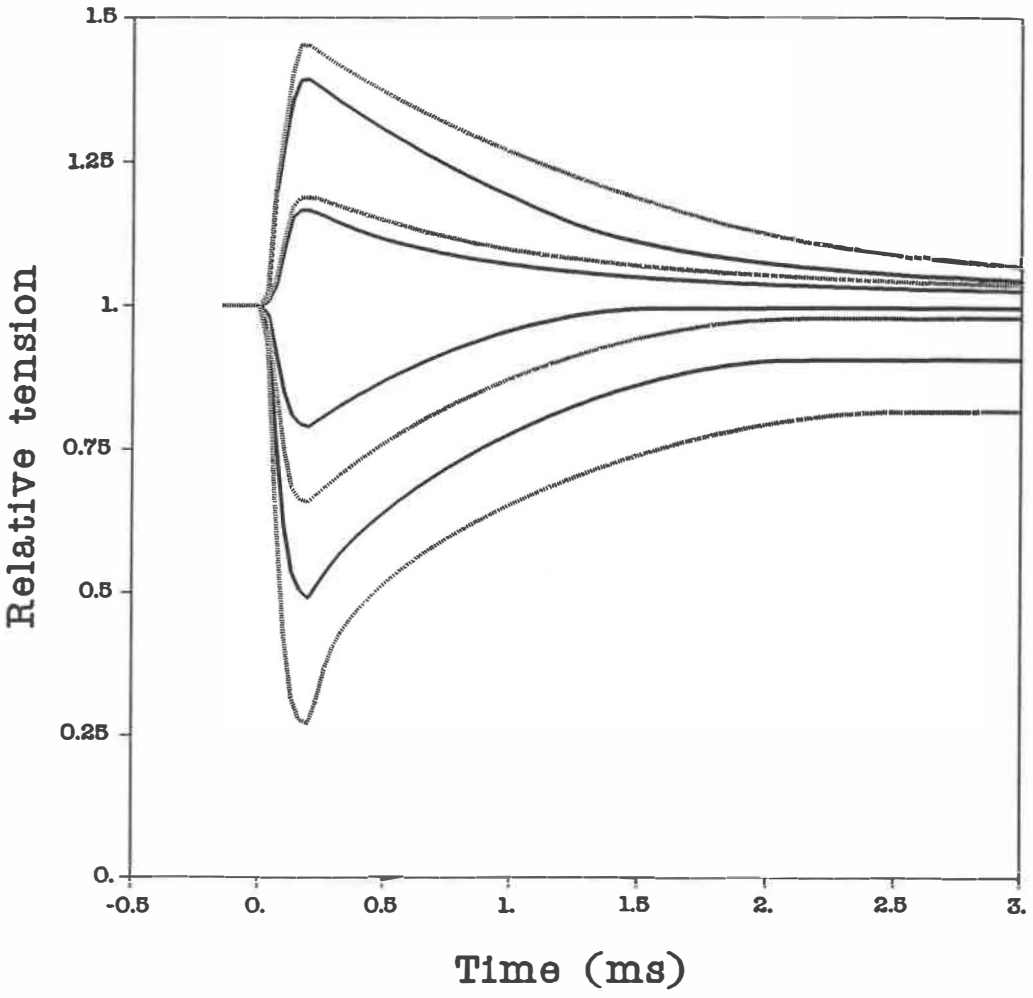


Figure 5.12 Time course of muscle transient responses to sudden length changes with 0.2 ms duration at 8^0 C (solid line) and at 1^0 C (dotted line) for 2.5, 1, -2, -4. nm amplitudes.

have little effect on N_{s_0}.

5.4 CONCLUSION

We have adapted the model which we identified for steady contraction states to account for dynamic muscle behavior by adding a single term to the equation for $\overline{\dot{x}}$ which is scaled by $\overline{\ddot{\theta}}$. The addition of this term allowed us to predict the mechanical effect of cross-bridge behavior on force generation during responses to transient changes in the mechanical state of the muscle. The modification of cross-bridge transition rates between the non-force generating state and the force generating state has been realized by replacing the independent variable v_a by $\overline{\dot{\theta}}$. The three state variables, F, G and $\overline{\dot{x}}$ are linked by the muscle intrinsic variable, $\overline{\dot{\theta}}$. Our simulations showed that $\overline{\dot{x}}$ plays a more important role than F or G during fast dynamic responses, whereas the reverse is true for slower processes. This may be the reason why the effect of $\overline{\dot{x}}$ on muscle behavior can be neglected in many cases.

Extending the muscle model to account for the effect of temperature on transient mechanical responses to sudden changes in length involved minor modifications to the state equations, consisting almost exclusively of a simple scaling of certain state variables. The scaling factors reflect the dependence of maximum steady shortening velocity, V_m and isometric tetanic tension, F_{m_0}

on temperature. Each scaling factor was set equal to the ratio of the value of the corresponding parameter at one temperature to its value at a chosen reference temperature. These simple modifications to the model were sufficient to achieve a good correspondence between simulations and experimental data.

CHAPTER 6

CONCLUSIONS AND FURTHER WORK

6.1 CONCLUSIONS

We have synthesized current ideas about muscle microstructure, physiology and biochemistry, extracting the most essential features of cross-bridge mechanics, in order to develop a conceptual model in which details are set out as to how an actomyosin complex utilizes the chemical energy derived from ATP hydrolysis during force generation. The major achievements of the conceptual model are

a) direct association of active structural change with tension development in an actomyosin complex;

b) using cross-bridge mechanics as a common regulated target to link biochemical and mechanical events occurring during cross-bridge force development and to provide a mechanism for interaction between biochemical reactions and mechanical events;

c) using the cross-bridge force development rate to account for the effects of cross-bridge mechanics, filament sliding and other mechanisms such as attachment and detachment on cross-bridge mechanics;

d) deriving a system constraint based on the principle of power balance which provides a simple way to estimate cross-bridge mechanics from muscle behavior at the macroscopic level.

We modeled muscle mechanical behavior using the mean values of variables associated with ensembles of cross-bridges to represent the cross-bridge behavior associated with a particular distribution at the macroscopic level. Consequently, we needed only three state variables, F, G, and $\overline{\dot{x}}$ to account for the mechanical properties of strongly attached cross-bridges during muscle force generation. The model

a) provides a means for understanding the mechanism of cross-bridge force development during muscle contraction;

b) has a simple structure and a low computational cost;

c) is identifiable from experimental data and therefore requires less guessing of parameters than other cross-bridge models;

d) can better account for the mechanical effects of changes in length, load, [Ca], [ATP] and temperature on cross-bridge behavior than previous models, giving good predictions of the muscle force-length, force-[Ca], force-[ATP], force-velocity and rate of energy liberation-velocity relations, as well as tran-

sient responses to sudden changes in muscle length or tension.

Our modeling study suggests that

a) cross-bridge mechanics is not only dependent on the chemical reactions which energize the cross-bridge, but also dependent on its head conformation and the rate of conformational change. The chemical energy from ATP hydrolysis is converted to mechnical work while the cross-bridge head is moving and to heat at the end of the working stroke;

b) attached cross-bridges at the end of their working stroke cannot produce significant tension between the thin and thick filaments, and thus are not in a force generating state;

c) the three state variables, F, G, and $\overline{\dot{x}}$ play different roles during different phases of muscle contraction. The first two variables have longer time constants and thus more profoundly affect the response to a relatively slow change in the mechanical state than the third variable, which is only important during the response to a rapid change in the mechanical state.

d) muscle mechanics can be simply represented by muscle stiffness and the mean extension of the cross-bridge elastic elements. Muscle stiffness varies with neural input ([Ca]), [ATP] and the rate of change of muscle length, while the mean extension is principally determined by the rate of change of muscle length, which forms an intrinsic feedback loop to regulate the operating point when muscle interacts with external systems. However, this feedback loop

could cause an oscillation in the system as shown by our model simulations for isotonic transient responses to sudden changes in external load, which is consistent with system control theory since muscle is an active element which possesses a negative feedback loop.

6.2 FURTHER WORK

From the viewpoint of muscle modeling, one could further improve model prediction by replacing lumped parameters with distributed parameters or incorporating other processes, such as excitation-contraction coupling (calcium dynamics), ATP catabolism and metabolism. Such an improved model could be applied to the study of muscle activation and coordination, which in turn could provide very useful information for the modeling of the neuro-musculo-skeletal system and the design of a functional neuromuscular stimulator for certain clinical disorders. Furthermore, we could gain a solid understanding of neuromuscular control through model simulation.

We could also develop an EMG-based muscle model by replacing one of the open model inputs, Calcium Concentration ([Ca]), by the Activation Level since Muscle Activation Level can be estimated from the averaged rectified EMG. In such a way, the model could be directly applied to study joint mechanics and neural control of the musculo-skeletal system, which would go

beyond what any traditional EMG-force model can achieve. It is because the recorded EMG merely represents information about the neural input to a muscle, but does not include the intrinsic effects of mechanical changes within the muscle on force generation.

During my model simulation, I found that the stability of muscle tension in the isotonic condition is very sensitive to a number of factors, such as change in external load, muscle length, mass and viscosity. These factors are critical in providing stable contraction in an intact system since they can compensate for potential instability that arises from the effect of the intrinsic feedback loop. The existence of muscle recruitment, coordination and different reflexes may play an important role in the system stability. Some patients, especially patients with spinal cord injury who have partly lost voluntary control of their legs may have highly disordered muscle coordination and reflexes. The model can be applied to study how to coordinate the artificial stimulation of leg muscles with the remaining voluntary control to reach some optimal or desired joint mechanics and how to use information about the H-reflex to facilitate system stability, during standing and walking.

How to make the model applicable to human muscles is another important research field. In my model certain muscle parameters are needed, such as actual muscle length, cross-section area, the maximum shortening velocity and the force-length relation. Estimation of these parameters for in-vivo human

skeletal muscles may be done noninvasively by combining experimental and system identification methods. As an example, muscle force is assumed to be generated by the active rotation of attached cross-bridge heads in my model. When muscle is allowed to shorten at the maximum shortening velocity, muscle force is equal to zero and the angular velocity of cross-bridge heads reaches its maximum. Therefore, the duration of force development is a function of the maximum angular velocity or the maximum shortening velocity. We could experimentally estimate the maximum shortening velocity from the duration of tension development or the rate of rise of tension beginning from a resting state or just after shortening at near maximum shortening velocity (in such cases the effect of excitation-contraction coupling is minimized).

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