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THE THREE-STATE MODEL FOR THE ELEMENTARY PROCESS OF ENERGY CONVERSION IN MUSCLE

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SUMMARY

A three-state model for the elementary process of energy conversion in striated muscle is analysed; in two of the three states, the crossbridge is attached to an actin site, while the third is a detached state. This model accounts for the mechanical properties of steady shortening and lengthening processes as well as those of isometric and isotonic transient processes in a quantitative way. Moreover, qualitative agreement is obtained for the total energy liberation from muscle. Biochemical properties are also computed for transient processes. Comparisons are made with other models with "three states".

INTRODUCTION

It is now established that the striated muscle shortens by relative sliding of the two interdigitating filaments, the thick and thin filaments. The sliding is thought to be the integrated result of elementary cycles in which ATP-splitting occurs. In the cycle, the crossbridge of the thick filament undergoes attachment to an actin site of the thin filament, moves, and is detached.

Models of muscular contraction are required to explain the following properties in a consistent way: (1) the tension-velocity relation and other mechanical properties of the steady shortening and lengthening processes; (2) transient mechanical responses after sudden change of external load or muscle length; (3) chemical energy, heat liberation and biochemical properties.

There are many models which can describe quantitatively mechanical properties of steady shortening process. On the other hand, few models have been presented for (2) or (3). Concerning the total energy liberation, Huxley [1] proposed a sort of three-state model to explain A. Hill's observation [2]: the dependence of the energy liberation upon shortening velocity V. Transient mechanical properties were discussed in detail by two groups: Huxley and Simmons [3] and Podolsky and Nolan [4]. These two models are different from each other, but they can both account for the observations; the Huxley-Simmons model is essentially a three-state one and the

Podolsky-Nolan model is a two-state one similar to the Huxley model [5]. It is very interesting that there is a common property in most of these models. That is the assumption of the existence of three distinguishable states of actomyosin molecule in the elementary cycle. This is supported also by a phenomenological treatment: semi-quantitative explanations were made for (1), (2) and some of (3) consistently from a model in which the elementary cycle with three states was assumed [6], and the dependences of the velocity of shortening and of the ATPase activity of isolated sarcomeres on the concentration of MgATP were analyzed by a similar model [7]. We may expect from these facts that (1), (2) and (3) will be described consistently and quantitatively by a three-state model or its extension.

The purpose of the present paper is not to give such a consistent theory but to take one step further to it. We will be concerned only with (1) and (2), because observed data for (3) may not be free from some ambiguity. T. Hill's theory [8] will be one of the guides for our investigation. However, we like to stress the difference between Hill's and our situations to avoid confusion. He tried to describe the molecular dynamics of the "force-generator", the actomyosin molecule, in terms of the free energy A of the actomyosin system in ATP solution. The free energy A was given as a function of the number n of ATP molecules and the relative position x between actin and myosin. The motion of the force-generator spontaneously occurred on x in the decreasing direction of A.

Strictly speaking, A is not only a function of x and n but also of ξ, the internal degrees of freedom of the force-generator other than x, including reaction coordinates for the chemical change ATP $\rightarrow$ ADP. The equilibrium thermodynamics says that a set of variables ξ, x and n changes spontaneously to the decreasing direction of A. This statement is, however, true only when the variables are macrovariables. Otherwise, the motion of the variables is greatly perturbed by thermal agitation such that the change of the variables occurs even in the increasing direction of A. This is the case that molecules evolve over potential barrier in a rate process. Hill assumed that each force-generator works independently, which leads to x and ξ being microvariables. The coupling between x and ξ in the dynamics of actomyosin molecule is unknown. Therefore, it is plausible that data of (1) and (2) which are related directly only to the change of the system on x give only incomplete information for the free energy A. For instance, the free energy A may vary largely without any appreciable change in x, if ξ can change almost independently. It means that the study of the motion of x cannot well clarify such a change in A. For the above reasons as well as others, the authors hesitate to use the free energy A for the description of the molecular dynamics of the force-generator on the coordinate x. Instead an effective potential $U(x)$, the mechanical potential, will be used for the purpose of the study of (1) and (2). The mechanical potential is defined by $-\mathrm{d}U(x)/\mathrm{d}x =$ the statistical average of the motive force from the force-generator at the position x. Perhaps, $U(x)$ may be substantially the same as Hill's $A(x)$. It is, however, noted that $U(x)$ should not be related directly to thermodynamic stability of the actomyosin system as well as to (3). Therefore, we will limit our discussion to (1) and (2) only and will not discuss (3) in detail. For the same reason, the thermodynamic detailed balance cannot always be assumed for the molecular dynamics on the mechanical potential U.

Now we assume that there are three states in the interaction of crossbridge

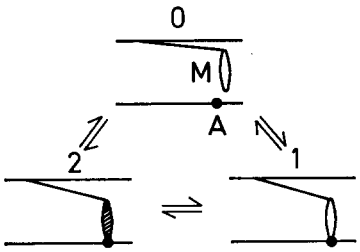


Fig. 1. Three states 0, 1 and 2 with transitions among them. M and A denote the crossbridge and the actin site, respectively.

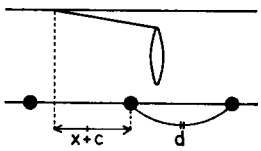
with actin site, two of which are attached ones with different mechanochemical properties and another is a detached state. These states are denoted as 1, 2 and 0, respectively, and are illustrated in Fig. 1. Furthermore, the following assumptions are made: (a) the mechanical potential energy U in the attached states depends on the relative distance x between crossbridge and actin site, whereas that for the detached state is independent of x; (b) tension is mainly produced in state 2; (c) rate constants for transitions among 0, 1, and 2 depend on the relative distance x: the condition of detailed balancing is present tentatively in the pair of transitions $i \rightarrow j$ and $j \rightarrow i$ except $2 \rightleftharpoons 0$; (d) attachment $(0 \rightarrow 1)$ takes place mostly in a limited range of x where state 1 is more stable than state 0 and transitions between attached states occur in a wider range of x than that of $0 \rightarrow 1$; (e) ATP decomposition is completed and the product is released in the transition $2 \rightarrow 0$ except in regions of x where state 0 is much more stable than state 2. ATP-splitting is not completed in the latter case. In our three-state model [6], the heat of shortening is related with ATP-splitting via the route $1 \rightarrow 0$. However, it will not be discussed here, because the present paper is concerned only with (1) and (2). At present, interpretations of the chemical energetics of muscular contraction are far from clear. The assumption of detailed balancing in (c) is only a tentative one to reduce the number of unknown parameters. This seems an acceptable assumption.

Detailed calculations are carried out for isometric and isotonic transient processes as well as for steady shortening and lengthening ones. As will be shown later, agreement between theory and experiment is excellent. Comparison of the present theory with other "three-state" models will be discussed.

FORMULATION OF A THREE-STATE MODEL

A. Elementary cycle

Though the attached states may be composed of multiple substates, they are characterized by a single potential $U(x)$ with $-d/2 < x \leqq d/2$. The length d denotes a unit interval between two nearest actin sites (the value of d is taken as 370 Å in this paper). Fig. 2 shows x and d. One of the simplest forms of the mechanical potentials which fulfill the assumptions (a) to (e) of the previous section is illustrated in Fig. 3. The functional form of each potential is determined both by the elasticity of myosin S-2 [3] and by actomyosin interaction. Since state 0 does not produce any tension, the mechanical potential is independent of x. Quadratic forms are assumed for U_1



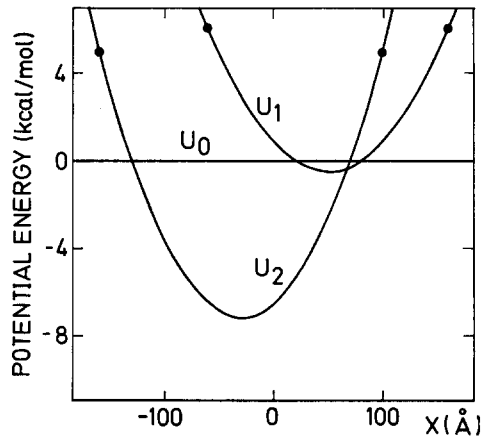


Fig. 2. Illustration of x and d. The value of c is determined in order for x to take $-d/2 < x \leq d/2$.

Fig. 3. Potential energies for the three states as a function of x. U_0, U_1 and U_2 denote, respectively, the potential energies of the states 0,1 and 2. Filled circles stand for the cut-off points (see Fig. 4).

and U_2 the equilibrium positions and curvatures of which are different from each other. Namely:

$$\begin{aligned} &\text{State-0;} \quad U_0(x) = 0 \\ &\text{State-1;} \quad U_1(x) = K_1(x-x_1)^2/2 - U_1^\circ \\ &\text{State-2;} \quad U_2(x) = K_2(x-x_2)^2/2 - U_2^\circ, \end{aligned} \tag{1}$$

where $K_1 = 0.75\,\text{dyne/cm}, K_2 = 1.00\,\text{dyne/cm}, x_1 = 50\,\text{Å}, x_2 = -30\,\text{Å}, U_1^\circ = 0.486$ kcal/mol, and $U_2^\circ = 7.201$ kcal/mol; the direction of x is defined as that of the lengthening. Similar curves of free energies $A(x)$ are given by T. Hill [8] in general discussions about mechanochemical conversion in muscle.

B. Rate constants

We require the detailed balancing for the pair of transitions other than $2 \rightleftharpoons 0$ associating with complete decomposition of ATP:

$$k_{ij}(x)/k_{ji}(x) = \exp\left[\{U_i(x) - U_j(x)\}/kT\right], \tag{2}$$

where k_{ij} denotes the rate constant of the transition from state i to j, k and T are the Boltzmann constant and absolute temperature, respectively. Temperature is constant (280 °K) throughout the present discussion.

1. 0 $\rightleftharpoons$ 1. The crossbridge is linked to an actin site when the two come near to each other. In the detached state, the position of the crossbridge will fluctuate in the direction of x because its stiffness is of the order of 1 dyne/cm [9], which results in random flight with an amplitude of about 30 Å. Therefore, the rate constant of attachment k_{01} may have a Gaussian form:

$$k_{01}(x) = k_1 \exp\{-K_1(x-x_1)^2/2kT\} \qquad k_1 = \text{constant} \tag{3}$$

The rate constant of attachment was discussed by Hill and White [10] from the

viewpoint of stochastic process. Furthermore, Hill [8] showed that one of the possible forms to k_{01} is a Gaussian one such as (3). According to condition (2),

$$k_{10} = k_1 e^{-U_1^\circ/kT} = \text{constant}$$

where we assume $k_1 = 100\ s^{-1}$ taking into account the above amplitude of the random flight of crossbridge.

II. 1 ⇌ 2. Since we have no molecular information about these transitions, the rate constant k_{12} is tentatively taken to be consistent with the results of Huxley and Simmons [3] and Julian et al. [11]. The functional forms assumed are as follows:

$$k_{12}(x) = \begin{cases} k_2, & x < (x_1+x_2)/2 \\ k_2 \exp\left[-K(x_1-x_2)\{x-(x_1+x_2)/2\}/kT\right], & (x_1+x_2)/2 \leqq x < x_1 \end{cases} \quad (4)$$

$$k_{21}(x) = k_2 \exp(C/kT), \qquad x_1 \leqq x \quad (5)$$

where

$$C = U_1^\circ - U_2^\circ - K(x_1-x_2)\{x_1-(x_1+x_2)/2\} + K_2(x_1-x_2)^2/2.$$

The parameters are $k_2 = 4000\ s^{-1}$ and $K = 0.23$ dyne/cm which are almost the same as those of Julian et al. [11]. Other rate constants are obtained at once from (2). The rate constants are shown in Fig. 4.

III. 2 ⇌ 0. The ATP molecule is completely decomposed and the product is released in the detachment process $2 \to 0$. The magnitude of k_{20} may be comparable with that of the rate constant of product release from actomyosin-ADP-P_i complex in vivo. This constant is 50–1000 s^{-1} according to Lymn's estimation [12]. Furthermore, Bárány [13] pointed out that the maximum velocity of sliding filaments in various muscles is generally proportional to actomyosin ATPase activity. From this, k_{20} must have a large value in the region of $x < x_2$ where the crossbridge produces resistive force. Therefore, we take the step function for k_{20},

$$k_{20} = \begin{cases} k_3, & x \geqq x_2 \\ k_4, & x < x_2 \end{cases} \quad (6)$$

where k_3 and k_4 are 75 s^{-1} and 1300 s^{-1}, respectively. The rate constant k_{02} of the reverse transition is assumed to be very small in the physiological condition and is not seen in Fig. 4. There are regions of x where this transition takes place without ATP splitting, as shown in section IV.

IV. Cut-off. The detached state becomes more stable than the attached where the potential energy of the former is lower than the latter. Our one-body model assumes that all the crossbridges are detached at $x = \pm d/2$. This assumption introduces critical points where the rate of detachment becomes so large that all the attached crossbridges are at once detached at the points. We assume such detachment occurs in state 1, provided that $|x-x_1| \geqq x_1^c$ with $x_1^c = 110$ Å, and similarly $|x-x_2| \geqq x_2^c$ with $x_2^c = 130$ Å for state 2. The critical points are shown in Fig. 3 as filled circles. It is to be pointed out that the critical points cannot be selected arbitrarily. Indeed, at and outside the points, the reverse transitions must be negligible, so that the difference of the potential energies between the detached and attached states must be large (> 4 kcal/mol). Since these detachments occur in a quite passive manner due to sliding motion of filaments, we assume that these are uncoupled from product release.

The rates of such rapid transitions are illustrated by the dashed lines in Fig. 4; k'_{20} denotes the rate constant of such a transition $2 \rightarrow 0$.

C. Kinetic equations for the three states

We denote the probability densities of the states 0, 1, and 2 at the distance x and time t as $n_0(x, t)$, $n_1(x, t)$ and $n_2(x, t)$, respectively. The thick and thin filaments have helical structures the periodicities of which differ from each other. The helical pitch of the thick filament is 429 Å and that of the thin filament is most probably in the neighbourhood of 2×370 Å [14]. Because of the difference in the pitches, it is reasonable in the 1st order approximation to assume the homogeneous distribution of crossbridges over the whole region of x. Hence, the kinetic equations for the probability densities can be written down as follows:

$$dn_i(x, t)/dt = \sum_{j \neq i} \{k_{ji}(x)n_j(x, t) - k_{ij}(x)n_i(x, t)\}; \qquad i = 0, 1 \text{ and } 2 \tag{7}$$

where

$$\sum_{i=0}^{2} n_i(x, t) = 1$$

The influence of the sliding motion to the time-dependence of $n_i(x, t)$ is included in the LHS of Eqn. 7 in the form $dn_i(x, t)/dt = \partial n_i(x, t)/\partial t - V\, \partial n_i(x, t)/\partial x$, where V denotes the shortening velocity. We can obtain from $\{n_i\}$ all the information about the mechanochemical properties of the system. For example we can calculate the following properties:

i. Tension per crossbridge, P(t)

$$P(t) = d^{-1} \int_{-d/2}^{d/2} dx \sum_{i=0}^{2} F_i(x)n_i(x, t) \tag{8}$$

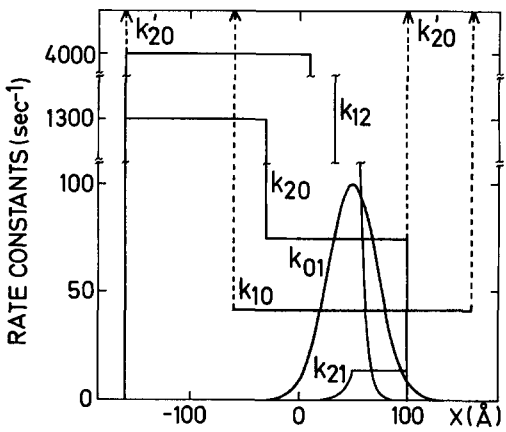


Fig. 4. Rate constants of transitions among the three states as a function of x. Dashed lines show the rates of rapid transitions ($1 \rightarrow 0$ and $2 \rightarrow 0$). Analytical expressions of these rate constants are given in the text.

where $F_i(x)$ is tension produced at x in state-i, which is defined by $F_i(x) = -dU_i(x)/dx$.

ii. ATPase activity per crossbridge, J(t)

$$J(t) = d^{-1}\int_{-d/2}^{d/2} dx k_{20}(x) n_2(x, t) \tag{9}$$

D. Method of calculation

(*1*) *Steady isometric process.* The process can be solved from Eqn. 7 by making $dn_i/dt = 0$, i = 0, 1 and 2.

(*2*) *Steady isovelocity process.* In this case $\{n_i\}$ depend only on x and Eqn. (7) becomes

$$-V dn_i(x)/dx = \sum_{j \neq i} \{k_{ji}(x) n_j(x) - k_{ij}(x) n_i(x)\} \tag{10}$$

These simultaneous ordinary equations can be numerically integrated by using the Runge-Kutta-Gill method.

(*3*) *Isometric transient process.* The time course of tension and ATPase activity after sudden change of muscle length are calculated. At $t = 0$, the muscle is suddenly stretched by the amount y (y becomes negative when the change is brought by sudden release). Then the initial distribution can be obtained from the steady state distributions $n_i^s(x)$ by the relations $n_i(x, 0) = n_i^s(x-y)$. For $t > 0$, the kinetic equations become:

$$\partial n_i(x, t)/\partial t = \sum_{j \neq i} \{k_{ji}(x) n_j(x, t) - k_{ij}(x) n_i(x, t)\}, \tag{11}$$

where x is only a parameter. These ordinary differential equations are solved by the Runge-Kutta-Gill method by dividing x into small pieces (in our calculation the length of the piece is 5 Å).

(*4*) *Isotonic transient process.* Assuming muscle tension balances with load instantaneously, we use $P(t)$ instead of load. At $t = 0$, tension P is suddenly changed from the steady isometric tension P_0 to another constant value P'. This causes sudden change of muscle length without appreciable change in the numbers of crossbridges in the attached states, the amount of which is denoted by z (the meaning of its sign is the same as in (3)). Hence the initial distributions of n_i are given by

$$n_i(x, 0) = n_i^s(x-z) \tag{12}$$

Here z can be determined from Eqns. (8) and (12):

$$P' = d^{-1}\int_{-d/2}^{d/2} dx \sum_i F_i(x) n_i^s(x-z) \tag{13}$$

For $t > 0$ the process is described by equations

$$\partial n_i(x, t)/\partial t - V \partial n_i(x, t)/\partial x = \sum_{j \neq i} \{k_{ji}(x) n_j(x, t) - k_{ij}(x) n_i(x, t)\}$$

with

$$P' = d^{-1}\int_{-d/2}^{d/2} dx \sum_i F_i(x) n_i(x, t) \tag{14}$$

Since these partial differential equations cannot be solved by the Runge-Kutta-Gill method, we follow the method given by Podolsky, Nolan, and Zaveler [15]. When t becomes large, this process will approach the steady isotonic (and isovelocity) process. The validity of their method can be checked by comparing the resultant isovelocity process to that calculated by the method given in (2).

RESULTS

A. Steady state

(*1*) *Tension-velocity relation and ATPase activity.* Calculation is carried out to steady shortening and lengthening process by utilizing Eqn. (10). In Fig. 5, there are illustrated relative tension (P/P_0) and ATPase activity as a function of relative velocity (V/V_{max}: V_{max} is the no-load shortening velocity). The dashed line indicates the experimental tension-velocity relation which is determined as follows. The shortening region is given by the Hill's relation

$$(P+a)\,(V+b) = \text{constant},$$

where $a/P_0 = b/V_{max} \cong 0.25$. For the lengthening process, our calculation should be compared with the observation by Curtin and Davies [16] for frog sartorius muscle at 0 °C; the resulting tension is observed under constant lengthening-velocity. On the other hand, Katz [17] studied lengthening process by applying a load greater than P_0 and observed the resultant length change.

As is clear from Fig. 5, our tension-velocity relation agrees very well with the observations. The peak of tension is obtained at $P/P_0 \cong 1.4$ and $V/V_{max} \cong -0.06$. The magnitude of our P_0 and V_{max} are:

$P_0 = 0.557 \cdot 10^{-7}$ dyne/crossbridge

$V_{max} = 44$Å/half sarcomere per ms.

The order of magnitude of P_0 is the same as that of the observations [18]. As for V_{max}, Curtin and Davies reported the value of about 2.0 L_0/s, where L_0 is the muscle

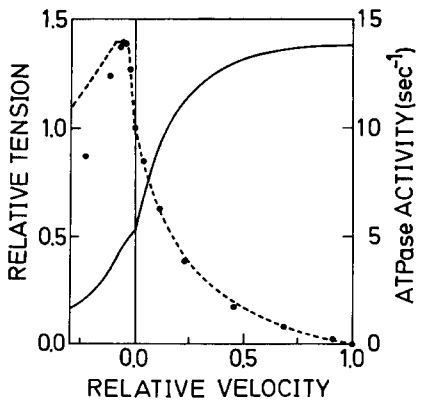


Fig. 5. Relative tension (●) and ATPase activity (—) as a function of relative velocity. Observed tension [16] is given as a dashed line.

length ($L_0 \cong 3$ cm). When a sarcomere length is about 2.5 μm, the observed V_{max} is approximately 25 Å/half sarcomere per ms.

We shall briefly comment on (3). The ATPase activity increases monotonically with V and this dependence on V also holds for lengthening process. On the other hand, it has been observed that the total energy liberation from shortening muscle begins to fall for $V/V_{max} \geqslant 0.6$ [2]. Moreover, the amount of split ATP seems to be constant per stretch [16]. Agreement between theory and experiment is rather qualitative, in contrast to the quantitative agreement in mechanical properties. The maximum efficiency is computed as 0.26 at $V/V_{max} = 0.16$ where the free energy of ATP is taken as 11 kcal/mol. The ATPase activity will be given the maximum in an intermediate velocity by the following modification. One way is to shift the left hand side cut-off point of state 2 in the positive direction of x, while others are made invariant. However, the mechanical properties deviated from those of the observations. Another way for improvement may be taking account of the two head-structure of myosin as discussed in our phenomenological theory [6].

(*2*) *Relative number of attached crossbridges.* The relationship between the attached crossbridges percent and the relative velocity is shown in Fig. 6. In isometric contraction about 13 % of the whole crossbridges are attached to thin filaments. Haselgrove and Huxley [19] studied the X-ray diffraction from frog sartorius muscle and reported that about 45 % of the crossbridges move out near thin filaments. However, all of the crossbridges near the thin filament may not always interact with actin sites. Indeed the diffraction pattern of X-ray from muscle remains unchanged for several seconds after stimulation has stopped [20].

The fraction of attached crossbridges may be proportional to the stiffness of muscle since series elasticity may exist in crossbridges interacting with actin sites [3, 21]. Fig. 6 shows that the percent of attached crossbridges decreases with shortening velocity and that crossbridges are still attached appreciably (≈ 4 %) at V_{max}. This result is compatible with the observed stiffness of frog skeletal muscle by Julian and Sollins [22]. But care must be taken for quantitative discussion because the force constants of state 1 and 2 may differ from each other.

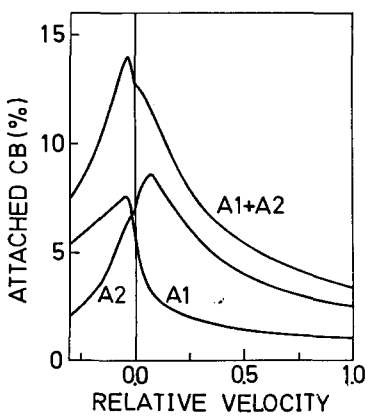


Fig. 6. The relationship between the percent of attached crossbridges and relative velocity. A1 and A2 denote the percent of states 1 and 2, respectively.

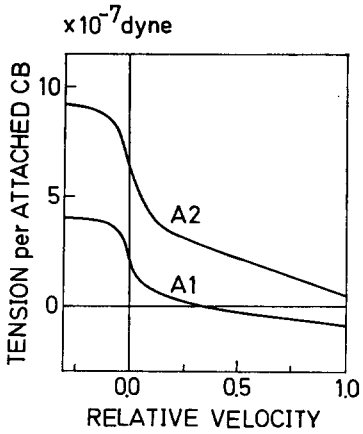


Fig. 7. Average tension per attached crossbridge vs. relative velocity. A1 and A2 denote the average tensions in states 1 and 2, respectively.

(*3*) *Tension per attached crossbridge.* We show in Fig. 7 the average tension per attached crossbridge. A1 and A2 denote the average tensions in state 1 and 2, respectively. Clearly, they have a remarkable dependence on shortening velocity. Huxley's model [5] also shows that the average tension depends on the velocity. A model proposed by Akazawa et al. [23] assumes phenomenologically that tension is a linearly decreasing function of V and has different gradients for shortening and lengthening processes. This assumption is consistent with our results in Fig. 7. Such a dependence of average tension results from the functional form of the mechanical potential $U(x)$ such as (1) and it will be studied further together with ATPase activity.

B. Transient processes

(*1*) *Isometric transient.* In Fig. 8 we illustrate calculated time courses of tension response to a sudden change of muscle length. There is a sudden change in tension at $t = 0$, the magnitude of which is denoted by T_1. After that the relaxation process of tension starts. In the case of sudden release, two steps of relaxation are remarkable; the one is the fast relaxation of tension to a level which is denoted by T_2, and the other is the succeeding slow relaxation to steady isometric tension (T_1 and T_2 are introduced by Huxley and Simmons [3] as the insert of Fig. 9). The former process is caused mainly from the transition $1 \rightarrow 2$ and the latter one is the relaxation toward the steady state. The relaxation time of the fast process becomes longer as the size of sudden release is decreased, which agrees with observations by Huxley and Simmons [3]. On the other hand, only a slow process of relaxation seems to exist for a sudden stretch of a small amount (< 30 Å per half sarcomere). It is caused by the rate constant for $1 \rightarrow 2$ being somewhat smaller in the lengthening change than in the shortening one. In this transient process, crossbridges in state 1 produce tension as well as in state 2. These tension responses are in reasonable agreement with data of frog skeletal muscle by Huxley and Simmons [3] and by Julian and Sollins [22].

Calculated T_1 and T_2 are shown in Fig. 9, where T_2 is at $t = 10$ ms after sudden release, meanwhile for sudden stretch T_2 is assumed to be 1 because the pro-

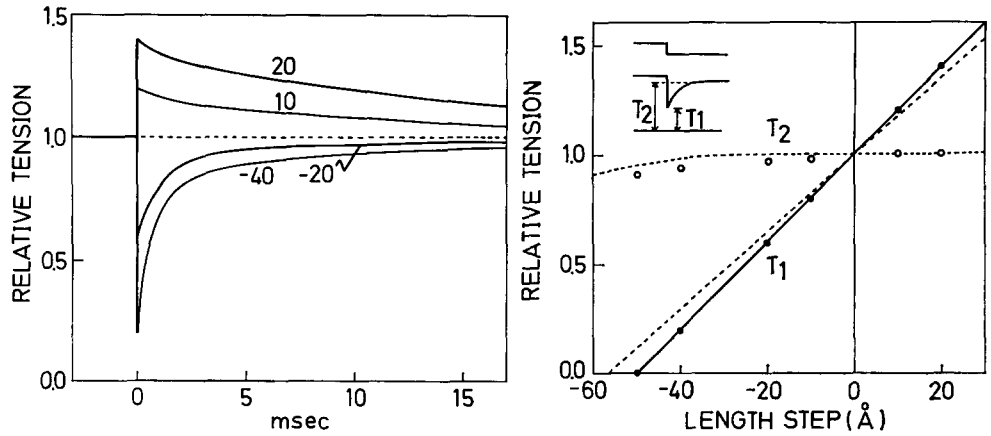


Fig. 8. The time-course of tension responses to sudden changes of muscle length. The figures in the graphs show the magnitudes (in Å) of sudden length change per half sarcomere.

Fig. 9. Calculated T_1 (●) and T_2 (○) as a function of the size of sudden length change. Observed values [22] are shown as the dashed lines. Definitions of T_1 and T_2 [3] are given in the insert.

cess is composed of only slow relaxation. Good agreement is seen between our calculation and the observation of Julian and Sollins [22] which is denoted by dashed lines.

The ATPase activity is illustrated in Fig. 10 for the sake of qualitative discussion. Sudden release of 40 Å/half sarcomere shows a large amount of ATP splitting in initial step. This property is related with the increase of k_{20} in the region $x < x_2$. It is interesting that a slight increase and decrease in ATPase activity occur for 20 Å-release and 20 Å-stretch, respectively.

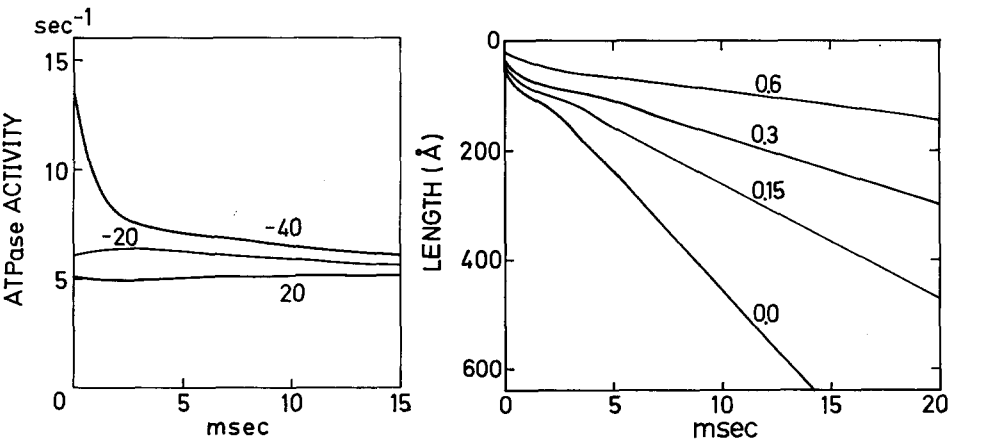


Fig. 10. The time-course of ATPase activity in isometric transients. The meaning of the figures is the same as that in Fig. 8.

Fig. 11. The time-course of length changes after sudden release of external load from P_0. The figure on each curve stands for the size of after-load in the unit of P_0.

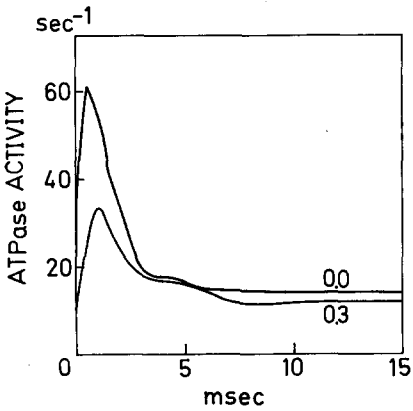


Fig. 12. The time-course of ATPase activity after sudden release of load: $P_0 \rightarrow 0.0P_0$ and $P_0 \rightarrow 0.3P_0$.

(*2*) *Isotonic transient.* In Fig. 11, computed time courses of length changes are shown after sudden reduction of external load from P_0. A few transient oscillations are followed by a steady shortening process. The duration of the transient response becomes longer as the change in load is reduced. These features agree qualitatively with observations by Civan and Podolsky [24]. Furthermore, these results are consistent with our previous conclusion that the damping constant of transient oscillation is an increasing function of load [6]. Gradients of the curves give shortening velocity. The P-V relation obtained from them coincides with that from steady isovelocity processes. This means that the present calculation is reliable. However, short-time behaviour of length change in Fig. 11 is less accurate than long-time behaviour because of our assumption, load = tension, which is valid only in steady state.

In Fig. 12 is given the ATPase activity in various isotonic transients. Large amount of ATP splitting is seen followed by a few small peaks.

DISCUSSION

(*1*) *Relation of rate constants and mechanical properties*

Simple forms of potential energies and rate constants assumed in this paper can well describe mechanical properties of muscle in steady state as well as in transient processes. We have tried various functional forms of potential energies and rate constants, so that some discussions can be made about the relation of rate constants and mechanical properties.

First, these properties in steady state and in later steps ($\geqslant$ 10 ms) of isometric and isotonic transient processes are mainly determined by rate constants (k_{01}, k_{10}, k_{20}, and k'_{20}) for attachment and detachment. The magnitude of V_{max} is sensitive to k_4 in k_{20}. Whereas the isometric tension P_0 depends mainly on the width of good region for attachment and on k_3 in k_{20}; if this width becomes small or k_3 becomes large, P_0 is reduced and vice versa.

The early transient stage is determined almost completely by the transitions within the attached states. This is indeed the case of the Huxley-Simmons model.

Observed fine structures in a short-time (within a few ms) behavior of tension response to sudden length change may be obtained by modifying rate constants k_{12} and k_{21} adequately. Cut-off of rate constants acts largely on steady lengthening process but only slightly on steady shortening process.

Potential energies of rather sharp curvature are necessary in order to get reasonable value of isometric tension P_0 (the force constant of Huxley-Simmons model as well as of the model of Julian et al. is smaller than ours by a factor of 4–5, and this results in smaller P_0 and smaller efficiency than ours).

The good region of attachment ($0 \rightarrow 1$) is about 60 Å in our model. It means that about 20 % of total crossbridges can attach to thin filaments ($\cong 60/d$). If the good region becomes wider, more crossbridges are in attached states. From the stiffness of myosin ($\sim$ 1 dyne/cm) we tentatively take the good region to be about 60 Å.

(2) Comparison with other three-state models

Huxley and Simmons [3] made calculations of tension response of isometric transient process based on a model having two attached steps. They did not discuss the later stage ($\geqslant$ 10 ms) of isomeric transient process as well as steady state. Recently Julian et al. [11] extended this model by including attachment and detachment rate constants. However, there are the following important differences between their model and ours. (1) They assumed that all the detached crossbridges have the same relative distance (which is $x = x_1$ in our notation). On the other hand, we assumed a homogeneous distribution of detached crossbridges over x by considering the difference of pitches between thick and thin filaments. These two kinds of assumption lead to different effects in various properties; for instance, in our model tension and the fraction of attached crossbridges can be calculated from potential energies and rate constants, but these quantities are arbitrary parameters in their model. (2) Their model does not take into account the rapid detachment of crossbridges from attached states in appropriate region of x. Therefore, there are still attached crossbridges at $x = \pm d/2$ in contraction with lengthening and shortening with high velocities, which is inadequate for a one-body model. Furthermore, the properties of lengthening process are hardly described by their model. (3) Transition of $0 \rightarrow 1$ is irreversible in their model, whereas ours is in general reversible. (4) The displacement of crossbridge on thin filament causing the sliding of muscular filament starts on unstable potential with respect to the movement [25]. The present model is consistent with this condition, because such a motion appears in state 2 after transition from state 1. However, their model is based on a mechanical potential with local stability.

A three-state model was given by Huxley [1] which can describe total energy liberation of steady shortening process observed by A. Hill [2]. This model also assumes a two-stage process for attachment. These two stages are denoted as i and ii, with 0 being the detached state. The following assumptions are made: (1) transition between 0 and i is rapidly and easily reversible, (2) transition from i to ii occurs only in a limited range of relative distance (there is no reverse transition), and (3) an elementary cycle is completed only if both stage i and stage ii have occurred, where an ATP is split in a cycle. These assumptions seem to be essentially similar to ours, but there are significant differences. In addition to these assumptions in the Huxley model [1], we found that some further assumptions were necessary, to describe the wide range of mechanical properties which were not included in the Huxley model.

These assumptions are as follows: (1) transition $0 \rightarrow 1$ occurs only in a limited region of x, (2) transitions between 1 and 2 occur in a wide region of x, and (3) an elementary cycle can be completed not only by $0 \rightarrow 1 \rightarrow 2 \rightarrow 0$ but also by $0 \rightarrow 1 \rightarrow 0$, especially in the lengthening process.

The main point of the present work is to show whether or not mechanical properties of muscular contraction can be described consistently by a three-state model, and we think that a positive answer has been obtained.

CONCLUSIONS

(1) A three-state model is presented which can describe consistently mechanical properties of steady shortening and lengthening processes as well as those of isometric and isotonic transient processes.

(2) Agreement between calculation and observation is rather qualitative in total energy liberation compared with the quantitative agreement in mechanical properties.

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