KINETIC MODEL FOR ISOMETRIC CONTRACTION IN SMOOTH MUSCLE ON THE BASIS OF MYOSIN PHOSPHORYLATION HYPOTHESIS

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ABSTRACT A kinetic model was proposed to simulate an isometric contraction curve in smooth muscle on the basis of the myosin phosphorylation hypothesis. The Ca^{2+}-calmodulin-dependent activation of myosin light-chain kinase and the phosphorylation-dephosphorylation reaction of myosin were mathematically treated. Solving the kinetic equations at a steady state, we could calculate the relationship between the Ca^{2+} concentration and the myosin phosphorylation. Assuming that two-head-phosphorylated myosin has an actin-activated Mg^{2+}-ATPase activity and that this state corresponds to an active state, we computed the time courses of the myosin phosphorylation and the active state for various Ca^{2+} transients. The time course of the active state was converted into that of isometric tension by use of Sandow's model composed of a contractile element and a series elastic component. The model could simulate not only the isometric contraction curves for any given Ca^{2+} transient but also the following experimental results: the calmodulin-dependent shift of the Ca^{2+} sensitivity of isometric tension observed in skinned muscle fibers, the disagreement between the Ca^{2+} sensitivity of myosin phosphorylation and that of isometric tension at a steady state, and the disagreement between the time course of myosin phosphorylation and that of isometric tension development.

INTRODUCTION

Rates of tension development and relaxation in smooth muscle are extremely slow compared with those of skeletal and cardiac muscles. Many investigators have supposed that this is caused by the difference in the Ca^{2+}-dependent regulatory mechanism for the contractile proteins in smooth muscle from that in striated muscles. Two mechanisms for the Ca^{2+} regulation in smooth muscle contraction have been proposed: a myosin-linked mechanism and an actin-linked mechanism (reviewed by Hartshorne and Siemankowski, 1981). The assumption in the former mechanism is that the contraction-relaxation cycle is related to the phosphorylation of myosin light chains by Ca^{2+}-calmodulin-dependent myosin light-chain kinase (MLCK) and dephosphorylation by Ca^{2+}-independent myosin light-chain phosphatase (MLCP). In the latter mechanism, it has been assumed that thin filaments are activated by Ca^{2+} binding proteins such as leiotonin or troponinlike proteins. The possibility of other regulation mechanisms also has been suggested (reviewed by Marston, 1982).

Because these hypotheses are based on the biochemical results obtained on isolated contractile proteins, it is difficult to determine whether or not these regulatory processes are the rate-limiting step for the isometric contraction in intact smooth muscle. To determine the rate-limiting process, myosin phosphorylation and isometric tension have been measured in intact muscle or skinned muscle fibers. Some of these reports have shown that myosin phosphorylation by MLCK is a prerequisite for tension development. Ca^{2+}-insensitive activation has been shown in skinned muscle fibers pretreated with ATPγS (Cassidy et al., 1979; Hoar et al., 1979; Kerrick et al., 1980). The thiophosphorylated myosin can not be dephosphorylated by MLCP. The isometric tension of skinned muscle fibers depended on the concentration of calmodulin which mediates the Ca^{2+} activation of MLCK (Cassidy et al., 1981; Ochiai et al., 1981; Sparrow et al., 1981; Arner, 1982). Inhibitors of calmodulin-dependent processes suppressed tension development in intact muscles (Hidaka et al., 1978) and skinned muscle fibres (Kerrick et al., 1980). Walsh et al. (1982*b*) prepared a Ca^{2+}-independent MLCK by chymotryptic digestion. This enzyme produced Ca^{2+}-independent tension development proportional to myosin phosphorylation in skinned fibers (Walsh et al., 1982*a*).

Although much evidence in favor of the myosin phosphorylation hypothesis has been accumulated, there are several points at issue. First, myosin phosphorylation preceded the tension development and declined during maintained contractions (Driska et al., 1981; Aksoy et al., 1982; Butler and Siegman, 1982; de Lanerolle et al., 1982; Silver and Stull, 1982). Second, high levels of phosphorylation have been reported in resting muscles, and its increase with tension development was small (Barron et al., 1980; de

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Lanerolle et al., 1980, 1982). Third, a disagreement between dose-response curves for myosin phosphorylation and isometric tension was observed in the methacholine-induced contraction of canine tracheal smooth muscle (de Lanerolle et al., 1982). A similar disagreement was reported for the relationships between the Sr^{2+}-induced myosin phosphorylation and super-precipitation of chicken gizzard actomyosin (Hirata et al., 1980).

This paper proposes a kinetic model that can simulate the experimental results described for the relationship between myosin phosphorylation and isometric tension. A simple model to account for transients in myosin phosphorylation during tonic contractions has been proposed by Peterson (1982*a*). Our model is composed of three parts: (*a*) Ca^{2+}-calmodulin-dependent activation of MLCK, (*b*) phosphorylation-dephosphorylation of myosin, and (*c*) conversion of myosin phosphorylation into the isometric tension. The basic assumption used in the model is the cooperative phosphorylation of two heads in myosin proposed by Persechini and Hartshorne (1981). The model could successfully simulate many experimental results and predict some unknown phenomena.

MODEL

Activation of Myosin Light-Chain Kinase

Myosin light-chain kinase is activated by Ca^{2+} (Frearson et al., 1976). Calmodulin (CM) mediates the Ca^{2+}-dependent activation of MLCK through the following two steps (Yazawa and Yagi, 1978; Dabrowska et al., 1978):

$$\text{step 1} \quad CM + nCa^{2+} \rightleftharpoons CM^* \cdot Ca_n^{2+}$$

$$\text{step 2} \quad MLCK + CM^* \cdot Ca_n^{2+} \rightleftharpoons MLCK^* \cdot CM^* \cdot Ca_n^{2+}.$$

Scheme I

When all of four binding sites on calmodulin are occupied by Ca^{2+}, the activated calmodulin (CM*) can bind MLCK (Blumenthal and Stull, 1980). MLCK and CM* form tight one-to-one stoichiometric complex (MLCK*) which is an active form of MLCK (Blumenthal et al., 1980; Hartshorne et al., 1980; Adelstein and Klee, 1981).

We assumed n independent, equivalent Ca^{2+} binding sites on calmodulin (Blumenthal et al., 1980), although positive cooperativity of Ca^{2+} binding was observed at low Ca^{2+} concentrations (Crouch and Klee, 1980). The concentrations of CM* and MLCK* at equilibrium are expressed by Eqs. 1 and 2:

$$[CM^*] = \frac{[CM]_0 - [MLCK^*]}{\left(1 + \frac{K_1}{[Ca^{2+}]}\right)^n} \tag{1}$$

$$[MLCK^*] = \frac{[MLCK]_0}{1 + \frac{K_2}{[CM^*]}} \tag{2}$$

where $[CM]_0$ and $[MLCK]_0$ represent the total concentrations of calmodulin and MLCK, respectively; K_1 is an apparent intrinsic dissociation constant for the Ca^{2+} binding site on calmodulin; K_2 is a dissociation constant for the CM*-MLCK* complex. Eliminating [CM*] and substituting for each concentration with $e = [MLCK^*]/[MLCK]_0$, $c = [Ca^{2+}]/K_1$, $\gamma_1 = [CM]_0/K_2$, $\gamma_2 = [MLCK]_0/K_2$, we obtained a dimensionless equation:

$$e^2 - \left[1 + \frac{\gamma_1}{\gamma_2} + \frac{1}{\gamma_2}\left(1 + \frac{1}{c}\right)^n\right] e + \frac{\gamma_1}{\gamma_2} = 0 \tag{3}$$

The solution of this equation gives the relative concentration of MLCK*, e ($0 \le e \le 1$).

The cytoplasm contains many enzymes that bind CM* (Klee et al., 1980). In the above model, it is assumed that the affinities of the other proteins to CM* are lower than that of MLCK. If all of affinities of these proteins to CM* are the same as those of MLCK, γ_2 must be substituted by

$$\gamma_2 = \frac{[MLCK]_0 + [P]_0}{K_2}, \tag{4}$$

where $[P]_0$ is the total concentration of proteins capable of binding CM*.

Phosphorylation and Dephosphorylation of Myosin

Myosin of smooth muscle possesses two globular heads like skeletal myosin, each of which contains two classes of light chains with molecular weights of 20,000 and 16,000–17,000 daltons (Hartshorne and Gorecka, 1980). The 20,000-dalton light chain is phosphorylated by MLCK (Frearson et al., 1976). Persechini and Hartshorne (1981) have shown that the phosphorylation of light chains in two heads is necessary for the actin-activated Mg^{2+}-ATPase activity of myosin isolated from smooth muscle. The phosphorylated myosin is dephosphorylated by MLCP (Onishi et al., 1979; Pato and Adelstein, 1980).

On the basis of this biochemical information, the following scheme was adopted to simulate the phosphorylation-dephosphorylation reaction for myosin:

$$M \underset{k_2}{\overset{k_1 e}{\rightleftharpoons}} M \sim P \underset{k_4}{\overset{k_3 e}{\rightleftharpoons}} M \sim P_2,$$

Scheme II

where M is myosin, M ~ P is one-head-phosphorylated myosin, M ~ P_2 is two-head-phosphorylated myosin; k_1, k_2, k_3, and k_4 represent rate constants, and e represents the relative concentration of MLCK* given by Eq. 3.

The following assumptions were made. (*a*) Myosin is activated by actin when both heads are phosphorylated by Ca^{2+}-calmodulin-dependent MLCK. (*b*) The phosphorylation reaction is an ordered process. (*c*) The phosphorylated myosin is dephosphorylated by Ca^{2+}-independent MLCP. (*d*) The activation of MLCK is rapid in comparison with the phosphorylation-dephosphorylation reaction for myosin. (*e*) The total myosin concentration, $[M]_0$, is sufficiently below the K_m (Michaelis constant) of myosin for both MLCK and MLCP, and the ATP concentration is sufficiently higher than the K_m of ATP for MLCK. Then, each reaction process can be represented by a pseudo–first-order reaction to the substrate myosin. (*f*) Fully phosphorylated myosin immediately interacts with actin filaments to form cycling cross bridges that produce an active state.

The transient state can be given as the solution of the following two differential equations:

$$\frac{dm_1}{dt} = -[(k_1 + k_3)e + k_2]\, m_1 - (k_1 e - k_4)\, m_2 + k_1 e \tag{5}$$

$$\frac{dm_2}{dt} = k_3 e m_1 - k_4 m_2, \tag{6}$$

where $m_1 = [M \sim P]/[M]_0$ and $m_2 = [M \sim P_2]/[M]_0$. Each component at

a steady state is expressed by

$$m_1 = \frac{1}{1 + \alpha_2 e + \dfrac{1}{\alpha_1 e}} \tag{7}$$

$$m_2 = \frac{1}{1 + \dfrac{1}{\alpha_2 e}\left(1 + \dfrac{1}{\alpha_2 e}\right)}, \tag{8}$$

where $\alpha_1 = k_1/k_2$ and $\alpha_2 = k_3/k_4$. The amount of phosphate incorporated in the light chain (mol phosphate/mol light chain), P_i, is

$$P_i = \frac{1}{2} m_1 + m_2 . \tag{9}$$

Isometric Contraction

Hill (1938) showed that an active skeletal muscle is represented by two-component system which consists of a contractile element governed by the phenomenological equation $(P + a)(v + b) = b(P_0 + a)$ and a series elastic component. Sandow (1958) developed this idea for simulating an isometric contraction curve. Assuming the time-dependent P_0 and a nonlinear elasticity of a series elastic component, he derived Eq. 10:

$$\frac{dp}{dt} = \frac{b(p + f)(p_a - p)}{\lambda(p + a)} \tag{10}$$

where t is the time, p is the isometric force of the muscle, p_a is the amount of active state at the time t, a and b are the constants related to the contractile element, f and λ are the constants related to the series elastic component; p, p_a, a, and f are normalized with respect to the maximum capacity to bear a load, P_0; b and λ are normalized with respect to the initial length of the muscle, L_0. Taylor (1969) applied this equation in computing the isometric tension curve by means of an analog computer, and could successfully simulate a wide range of phenomena associated with isometric contraction; e.g., potentiation of single twitch, tetanus, summation, and redevelopment of tension after a quick release.

If a series elastic component is neglected in smooth muscle, the isometric tension might be proportional to $[M \sim P_2]$. There is an evidence, however, that smooth muscles contain a series elastic component described by a single exponential function (Murphy, 1976). Because the force-velocity relationship of the contractile component in smooth muscle can be described by the Hill's phenomenological equation (reviewed by Murphy, 1976, 1980), we adopted Eq. 10 to convert the time course of the active state (m_2) into that of isometric tension. The active state, p_a, is defined by

$$p_a = \frac{m_2}{(m_2)_{max}} \tag{11}$$

where $(m_2)_{max}$ is the maximum value of m_2, which can be calculated using Eqs. 3 and 8 under the condition that $c \rightarrow \infty$.

Standard Values for Kinetic and Dynamic Parameters

All parameters in the model can be determined experimentally, although some of them would be exceedingly difficult to measure. At present there is no available data for all parameters from the same tissue of the same species. Thus the values for each parameter were estimated on the basis of data obtained from various muscles, most of which exhibit phasic contractions (Table I).

The kinetic parameters of MLCK have been obtained by using isolated myosin light chain as a substrate. Persechini and Hartshorne (1981) have suggested that when myosin is used as a substrate, one of two heads in unphosphorylated myosin is phosphorylated relatively easily, but the remained head is phosphorylated with more difficulty, that is, $k_1 > k_3$. If a pseudo–first-order reaction is assumed for the phosphorylation of myosin, its rate constant is described by $k_1 = k[MLCK]_0/Km$ in which k is a turnover number and Km is the Michaelis constant. Using the values of k = 22–66 s^{-1}, $[MLCK]_0 = 1.2 \times 10^{-6}$ M, and $Km = 5 \times 10^{-6}$ M reported on MLCK prepared from turkey gizzard muscle (Adelstein and Klee, 1981), k_1 was estimated to be on the order of 10 s^{-1}. The value of k_3 was assumed to be one-tenth of k_1.

There are a few reports on the kinetic properties of MLCP. Pato and Adelstein (1980) found two phosphatases, both of which can dephosphorylate myosin light chains. If $[M \sim P_2]$ is proportional to the degree of activation, the dephosphorylation rate of $[M \sim P_2]$, k_4, may approximate or be faster than the relaxation rate of isometric tension. Because the half decay time of isometric relaxation is several seconds (rat myometrium, Kato et al., 1982), k_4 was estimated to be on the order of 0.1 s^{-1}. Furthermore we assumed that $k_2 > k_4$; here $k_2 = 10k_4$.

Computer Detail

The equations derived in the kinetic model were programmed in FORTRAN. The program was run with a digital computer (ACOS-800, Nippon Electric Co., Tokyo, Japan). The differential equations were

TABLE I
STANDARD VALUES FOR KINETIC AND DYNAMIC PARAMETERS

Parameter		Units	Tissue	Reference
n	4	—	bovine brain	Blumenthal et al. (1980)
$[CM]_0$	10^{-5}	M	rabbit uterus	Grand et al. (1979)
$[MLCK]_0$	10^{-6}	M	turkey gizzard	Adelstein and Klee (1981)
K_1	10^{-5}	M	bovine brain	Crouch and Klee (1980)
K_2	10^{-9}	M	turkey gizzard	Adelstein and Klee (1981)
k_1	25.0	s^{-1}	turkey gizzard	Adelstein and Klee (1981)
k_2	5.0	s^{-1}	—	Assumed in this paper
k_3	2.5	s^{-1}	—	
k_4	0.5	s^{-1}	—	
a	0.2	—	e.g. guinea pig	Murphy (1976)
b	0.05	s^{-1}	*T. coli*	
f	0.01	—	canine trachea and	Stephens and Kromer (1971) and Arner (1982)
λ	0.05	—	rat arterial vessel	

solved by the Runge-Kutta-Gill method in the library program (MATHLIB, ACOS-800; Nippon Electric Co.). The computed results were displayed on the screen of a graphic display instrument (N6940, NEC), and hard copies were used in the figures.

SIMULATION RESULTS

Ca^{2+}-dependent Activation Curves

Solving Eq. 3, we obtained the relative concentration of active MLCK as a function of $[Ca^{2+}]$ (Fig. 1, curve 2). The activation curve of MLCK showed a 10-fold greater sensitivity to $[Ca^{2+}]$ than that of calmodulin (curve 1). The extent of the shift depended on the values of γ_1 and γ_2, especially $\gamma_1(=[CM]_0/K_2)$ if $[CM]_0 \gg [MLCK^*]$. Thus the large shift in Fig. 1 is caused by the large value of γ_1. This kind of $[CM]_0$-dependent pCa ($= -\log[Ca^{2+}]$)-MLCK activity relationship has been observed in isolated enzymes by Blumenthal and Stull (1980).

Because the pCa-*e* relationship was given, the relative concentration of two-head-phosphorylated myosin, m_2, at a steady state was calculated by using Eq. 8. The pCa-m_2 curve (Fig. 1, curve 3) slightly shifted towards lower $[Ca^{2+}]$ than the pCa-e curve. If m_2 is proportional to the actin activated Mg^{2+}-ATPase activity of myosin and the isometric tension in a steady state, the pCa-m_2 relationship corresponds to the Ca^{2+} activation curve for a muscle. Thus our model predicts that the pCa-Mg^{2+}-ATPase activity and pCa-isometric tension relationships will shift towards lower $[Ca^{2+}]$ than the activation curve for calmodulin (predicted also by Peterson's model, 1982*a*).

Effect of Parameter Variation on pCa-m_2 Relationship

The effect of variation in the parameters in Eqs. 3 and 8 on the pCa-m_2 relationship was investigated. The $[Ca^{2+}]$ needed for a half activation was defined by $[Ca^{2+}]_{0.5}$ as a indicator of Ca^{2+} sensitivity.

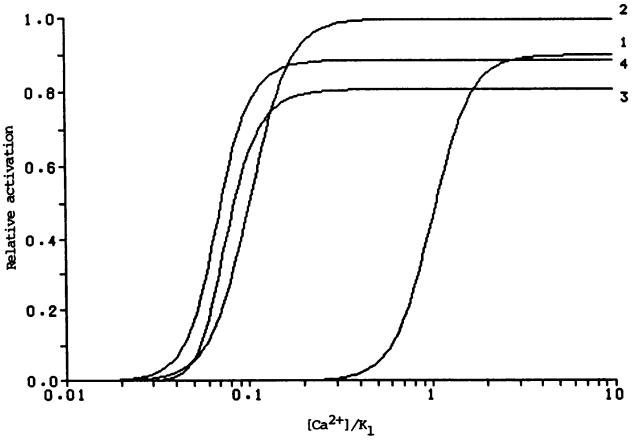


FIGURE 1 The Ca^{2+}-dependent activation curves of calmodulin, myosin light chain kinase, and myosin. *1*, $[CM^*]/[CM]_0$; *2*, *e*; *3*, m_2; *4*, P_i. The standard values listed in Table I were used.

The value of n determined the steepness of the curves. For example, for $n = 3$, the slope and the $[Ca^{2+}]_{0.5}$ decreased (Fig. 2, curve 2). It should be noted that the pCa-m_2 relationship can not be expressed by the simple form, $m_2/(m_2)_{max} = 1/[1 + (1/c)^n]$, which has been used for analysis of pCa-isometric tension curve (Peterson, 1982*a*; Arner, 1982).

A decrease in $[CM]_0$ lowered $(m_2)_{max}$ and increased $[Ca^{2+}]_{0.5}$ (Fig. 2, curve 3). The $[CM]_0$-dependent shift of the pCa-m_2 relationship was examined in detail (Fig. 3). At $[CM]_0 > 10^{-6}$ M, the curve shifted leftward without a change in $(m_2)_{max}$. The $[Ca^{2+}]_{0.5}$ decreased two-fold with a 10-fold increase in $[CM]_0$. At $[CM]_0 < 10^{-6}$ M, the extent of curve shift decreased and $(m_2)_{max}$ was markedly decreased. Sparrow et al. (1981) described the $[CM]_0$-dependent shift of the pCa-isometric tension curve in skinned muscle fibres of guinea pig *Taenia coli* by adding 0.012-7.2 μM calmodulin. About a three-fold decrease in $[Ca^{2+}]_{0.5}$ was observed for a 10-fold increase in $[CM]_0$ (between 0.048 and 0.48 μM). A similar result was reported for skinned muscle fibers of chicken gizzard and rabbit ileum by Cassidy et al. (1981). Ochiai et al. (1981) found that calmodulin elutes from the saponin-skinned fiber of rat myometrium. These results suggest that the Ca^{2+} sensitivity of in vivo contraction is higher than that observed in vitro. Fig. 1 shows that the contraction-relaxation cycle occurs at $[Ca^{2+}]$ lower than 2×10^{-6} M if the standard values are assumed. This is consistent with a recent report describing mitochondrial Ca^{2+} loading in saponin-skinned fibers: a physiologically maximal contraction takes place at free $[Ca^{2+}]$ of 10^{-6} M (Somlyo et al., 1982).

A decrease in $[MLCK]_0$ had a similar effect as a

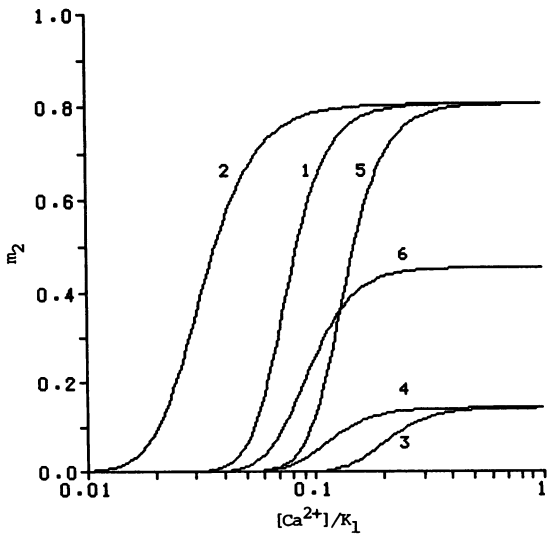


FIGURE 2 Effect of the parameter variation on the pCa-m_2 relationship. The standard values were used except the following values. *1*, control; *2*, $n = 3$; *3*, $[CM]_0 = 10^{-7}$ M; *4*, $[MLCK]_0 = 10^{-5}$ M, $k_1 = 2.5\ s^{-1}$, $k_3 = 0.25\ s^{-1}$; *5*, $K_2 = 10^{-8}$ M; *6*, $k_3 = 0.5\ s^{-1}$.

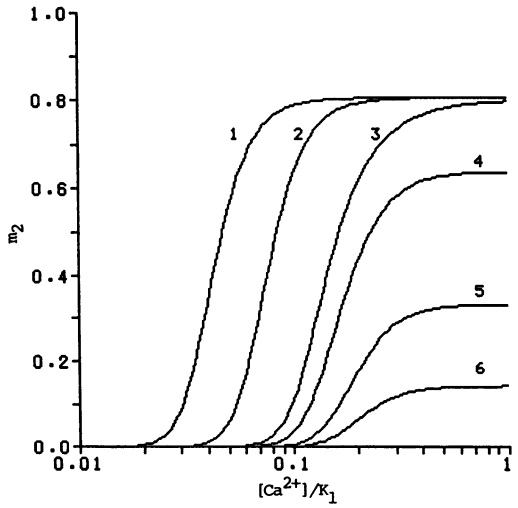


FIGURE 3 Effect of the calmodulin concentration on the pCa-m_2 relationship. The standard values were used except the following values for $[CM]_0$ (M). *1*, 10^{-4}; *2*, 10^{-5}; *3*, 10^{-6}; *4*, 5×10^{-7}; *5*, 2×10^{-7}; *6*, 10^{-7}.

decrease in $[CM]_0$ (Fig. 2, curve 4). The decrease of $[MLCK]_0$ is accompanied by a decrease in k_1 and k_3, which are proportional to $[MLCK]_0$. If other proteins bind CM*, γ_2 in Eq. 4 should be used. When γ_2 was changed over the range of 10 to 1,000, the pCa-m_2 relationship did not show a significant shift (data not shown).

An increase in K_2, that is, a decrease in affinity of MLCK to calmodulin caused a rightward shift of the pCa-m_2 curve (Fig. 2, curve 5). This may be the quantitative description for the hypothesis proposed by Conti and Adelstein (1980) on the cAMP-dependent regulation of smooth muscle contraction: cAMP-dependent protein kinase phosphorylates MLCK, which reduces the interaction between calmodulin and MLCK with an inhibition of contraction. Indeed, the addition of the catalytic subunit of cAMP-dependent protein kinase caused the rightward shift of the pCa-isometric tension curve in skinned muscle fibers (Sparrow et al., 1981) and in the pCa-ATPase, and pCa-phosphorylation curves for actomyosin (Silver et al., 1981).

The pCa-m_2 relationship was affected not only by the degree of activation of MLCK, but also by the rate constants for myosin phosphorylation. Both decreases in α_1 and α_2 caused $(m_2)_{max}$ to decrease without a marked change in $[Ca^{2+}]_{0.5}$ (Fig. 2, curve 6 on α_2, and cf. Fig. 4 *A*).

Disagreement Between pCa-m_2 Curve and pCa-P_i Curve

The Ca^{2+} sensitivity of myosin phosphorylation can be calculated by using Eq. 9. As shown in Fig. 1 (curve 4), the pCa-P_i relationship shifted to slightly lower $[Ca^{2+}]$ than did the pCa-m_2 relationship. The disagreement between the pCa-P_i curve and the pCa-m_2 curve resulted from the assumption that two-head-phosphorylated myosin alone was active. Thus, the ratio of the rate constant of MLCK to that of MLCP, α_1 and α_2, affected the maximum value of phosphorylation, $(P_i)_{max}$, and the pCa dependence as expected from Eqs. 7 and 8. A decrease in α_1 or α_2 lowered $(m_2)_{max}$ and $(P_i)_{max}$ (Fig. 4 *A*). When the relative values of m_2 and P_i to the maximum were plotted, the disagreement between both relationships became clear (Fig. 4 *B*). The extent of disagreement increased with the increase of α_1 or the decrease of α_2. It is noted that the pCa-m_2 curve is steeper than the pCa-myosin phosphorylation curve.

Evidences for the myosin phosphorylation hypothesis is given by an agreement between the Ca^{2+} sensitivities for isometric tension development and myosin phosphorylation (e.g., Hoar et al., 1979). Our model predicts a disagree-

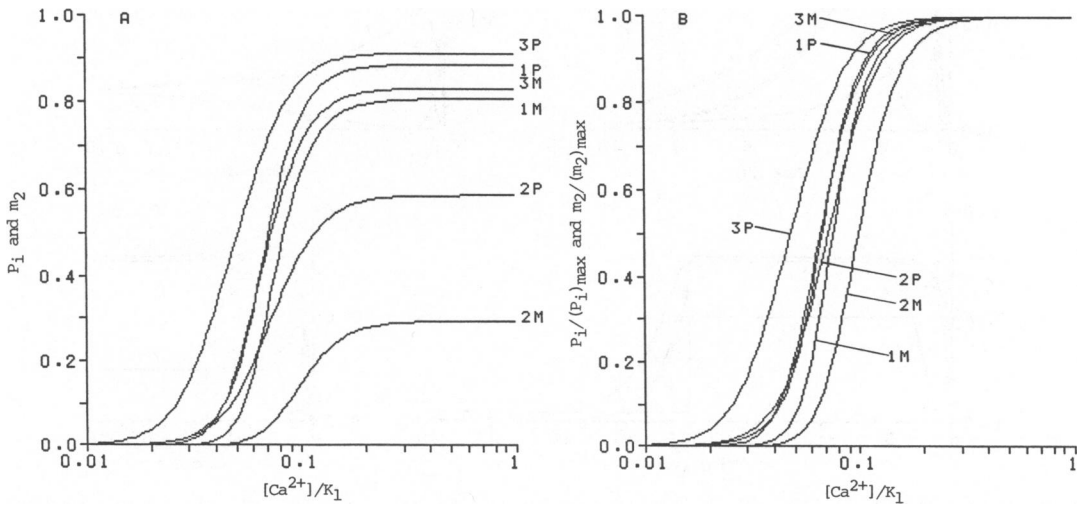


FIGURE 4 Disagreement between the Ca^{2+} sensitivity of myosin phosphorylation and that of isometric tension. (*A*) P represents myosin phosphorylation, P_i. *M* represents m_2. (*B*) Normalized curves: $P_i/(P_i)_{max}$ and $m_2/(m_2)_{max}$. The standard values were used except the following values. *1*, control: $\alpha_1 = 5$, $\alpha_2 = 5$; *2*, $\alpha_2 = 0.5$; *3*, $\alpha_1 = 50$.

ment between both relationships, but the difference may be too small to measure. Recently de Lanerolle et al. (1982) observed a statistically significant difference between the dose-response relationship of myosin phosphorylation and that of isometric tension in tracheal smooth muscle stimulated with methacholine: the leftward shift of myosin phosphorylation. This result agrees with our prediction if methacholine regulates the intracellular free $[Ca^{2+}]$ only. Hirata et al. (1980) have been opposed to the myosin phosphorylation hypothesis because the Sr^{2+} sensitivity of myosin phosphorylation is much higher than that of superprecipitation. This result can be explained by our model if we assume that Sr^{2+} influences α_1 and/or α_2 as well as the activation of MLCK.

A high level of myosin phosphorylation in the resting muscle with little increase in the level of phosphorylation upon tension development have been observed: P_i changed from 0.31–0.52 to 0.52–0.88 in porcine carotid arteries (Barron et al., 1980), and from 0.29 to 0.62 in canine tracheal muscle (de Lanerolle et al., 1982). These results can be explained by using Fig. 4. An increase in $[Ca^{2+}]/K_1$ from 0.04 to 0.1 caused a fourfold increase in the incorporated phosphate (from 0.21 to 0.84, Fig. 4 *A*, curve 3P) and a 10-fold increase in isometric tension (from 8 to 85% of the maximum tension, Fig. 4 *B*, curve 3M).

Isometric Contraction for Various Ca^{2+} Transients

If a Ca^{2+} transient is given, we can calculate the time courses of myosin phosphorylation, the active state, and isometric tension development by using Eqs. 3, 5, 6, 9, and 10. Recently, the Ca^{2+} transient during a single contraction or a contracture in smooth muscle has been measured by means of Ca^{2+}-sensitive reagents (Kometani and Sugi, 1978; Fay et al., 1979; Neering and Morgan, 1980; Morgan and Morgan, 1982). Referring to these reports, we assumed a simple Ca^{2+} transient consisting of a linear rising phase and a single exponential falling phase:

$$[Ca^{2+}] = [Ca^{2+}]_r + ([Ca^{2+}]_p - [Ca^{2+}]_r)t/t_p, \qquad 0 \le t \le t_p$$

$$[Ca^{2+}] = [Ca^{2+}]_r + ([Ca^{2+}]_p - [Ca^{2+}]_r)\exp\{-k_f(t - t_p)\}, \qquad t_p \le t$$

where t_p is the time to peak; $[Ca^{2+}]_r$ and $[Ca^{2+}]_p$ are the Ca^{2+} concentrations at the resting state and at the peak, respectively; k_f is the rate constant of the falling phase.

Neering et al. (1980) have reported a t_p of 0.2–0.8 s and a half decay time of 2–2.5 s in dog antral circular muscle. Using this Ca^{2+} transient, we computed the time courses of myosin phosphorylation, active state and isometric tension (Fig. 5 *A*). The myosin phosphorylation during the initial phase preceded the increase of the active state, then after some latency the isometric tension developed. The onset of isometric tension was approximately coincident with the peak of Ca^{2+} transient. The time course of myosin phosphorylation was the same as that of the active state except for the initial rising phase. The peak of myosin phosphorylation preceded that of isometric tension. At the peak of isometric tension, the $[Ca^{2+}]$ returned to approximately the resting level. This relationship between the Ca^{2+} transient and the isometric tension curve agrees with the experimental result reported by Neering and Morgan (1980) and is similar to the observation in molluscan anterior byssus retractor muscle by Kometani and Sugi

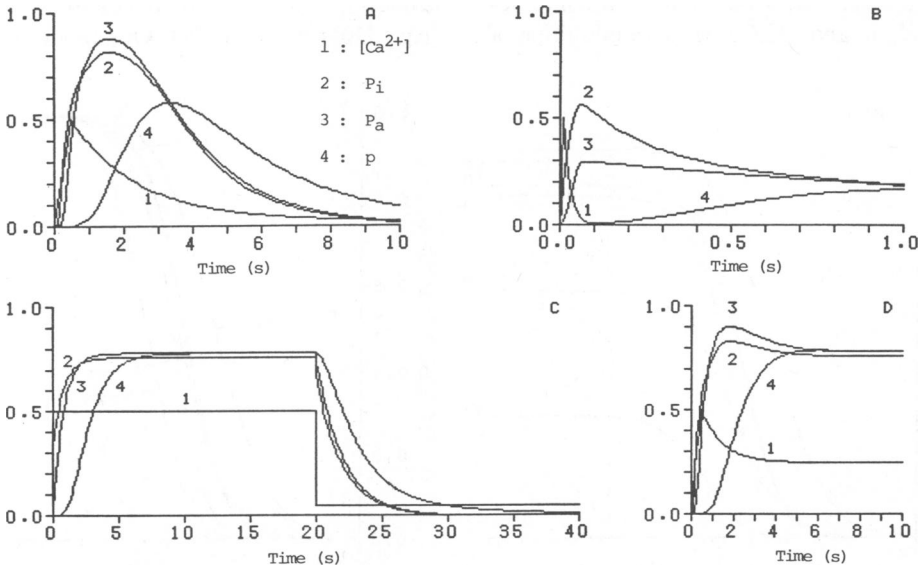


FIGURE 5 The time courses of myosin phosphorylation, active state and isometric tension for various Ca^{2+} transients. *1*, $[Ca^{2+}]$ in arbitary unit; *2*, P_i; *3*, p_a; *4*, *p*. The standard values for the kinetic parameters were used except *B*. The following values for the Ca^{2+} transient were used. $[Ca^{2+}]_r = 10^{-7}$ M, $[Ca^{2+}]_p = 10^{-6}$ M. (*A*) $t_p = 0.5$ s, $k_f = 0.5\ s^{-1}$; (*B*) $[Ca^{2+}]_p = 10^{-5}$ M, $t_p = 0.01$ s, $k_f = 50\ s^{-1}$, $k_1 = 50\ s^{-1}$, $k_3 = 10\ s^{-1}$, $b/\lambda = 5\ s^{-1}$; (*C*) a step change in $[Ca^{2+}]$; (*D*) $t_p = 0.5$ s, $[Ca^{2+}]_p = 2 \times 10^{-6}$ M, $k_f = 1.0\ s^{-1}$, $[Ca^{2+}]$ at steady contraction $= 10^{-6}$ M.

(1978). The relationship between the active state and isometric tension is in agreement with the experimental result obtained in rabbit *T. coli* (Gordon and Siegman, 1971).

On the other hand, a shorter and faster Ca^{2+} transient has been observed in isolated toad stomach muscle (Fay et al., 1979): t_p is <10 ms and k_f is ~37 s^{-1}. Strikingly, the tension development began after the Ca^{2+} transient. Assuming the appropriate values for parameters, our model can simulate this kind of result (Fig. 5 *B*). Here we adopted the large values of k_1, k_3, b/λ, and $[Ca^{2+}]_p$, compared with those used in Fig. 5 *A*. According to our model, the slow onset of tension development is due to the series elastic component. If the active state were measured by a quick-stretch method, its time course might be shown by curve 3 in Fig. 5 *B*. However, Fay's (1977) measurements of the time course of stiffness and resistance to quick-stretch showed that isometric force and stiffness increased simultaneously. The time course of myosin phosphorylation is needed to see if agreement exists between theoretical and experimental results in this parameter.

Fig. 5 *C* shows the calculated responses to a step change in $[Ca^{2+}]$ that approximates stimulation and isometric tension development in skinned muscle fibers. Interpreting these contractures induced by the external concentration change of ions or reagents, we should consider the possibility of diffusionlimiting process. We have shown that the rate-limiting step of isometric tension change in the Ca^{2+}-induced contracture under high potassium depolarization is the diffusion process of Ca^{2+} in the extracellular space when a thick preparation is used. Even in thin preparations the diffusion of Ca^{2+} in the unstirred layer at the preparation surface affects the rate of tension change (Kato et al., 1982).

A tetanus induced by electrical stimulation is suitable for the present purpose because it does not contain the extracellular diffusion process except for those of O_2 and the other metabolites. A spontaneous or electrically stimulated single contraction of myometrium can be regarded as a tetanus induced by a burst of action potentials (Mironneau, 1973). Janis et al. (1980) have reported that myosin phosphorylation precedes maximal tension development of spontaneous contraction in rat uterine smooth muscle and the dephosphorylation precedes the complete relaxation. Butler and Siegman (1982) have compared the time courses of isometric tension, myosin phosphorylation, and active state of the tetanus induced by alternating current stimulus in the rabbit *T. coli*. The myosin phosphorylation preceded the isometric tension development, and the dephosphorylation showed the same time course as the decay of the active state, but was quicker than that of relaxation as predicted in Fig. 5 *C*.

Fig. 5 *D* shows the response to the Ca^{2+} transient possessing an overshoot. An overshoot also occurred in myosin phosphorylation, but not in isometric tension. The similar overshoot of myosin phosphorylation has been observed in tracheal muscle (Silver and Stull, 1982; de Lanerolle et al., 1982; Gerthoffer and Murphy, 1983), in rabbit *T. coli* (Butler and Siegman, 1982) and in swine carotid (Aksoy et al., 1982; Dillon et al., 1981). This relationship between the Ca^{2+} transient and isometric tension may be useful to explain that for the agonist elicited tension in vascular smooth muscle reported by Morgan and Morgan (1982).

Effect of Parameter Variation on Isometric Contraction

Fig. 6 shows the effect of kinetic and mechanical parameters on the rates of tension development and relaxation. Increasing the rate constant of MLCK (k_3) caused an increase in the tension development rate (Fig. 6 *A*). Decreasing the rate constant of MLCP (k_4) slowed relaxation rates (Fig. 6 *B*). When there is a series elastic component, the value of b affects the rate of tension change as expected from Eq. 10. A decrease in b prolonged the latency and markedly slowed the rates of tension develop-

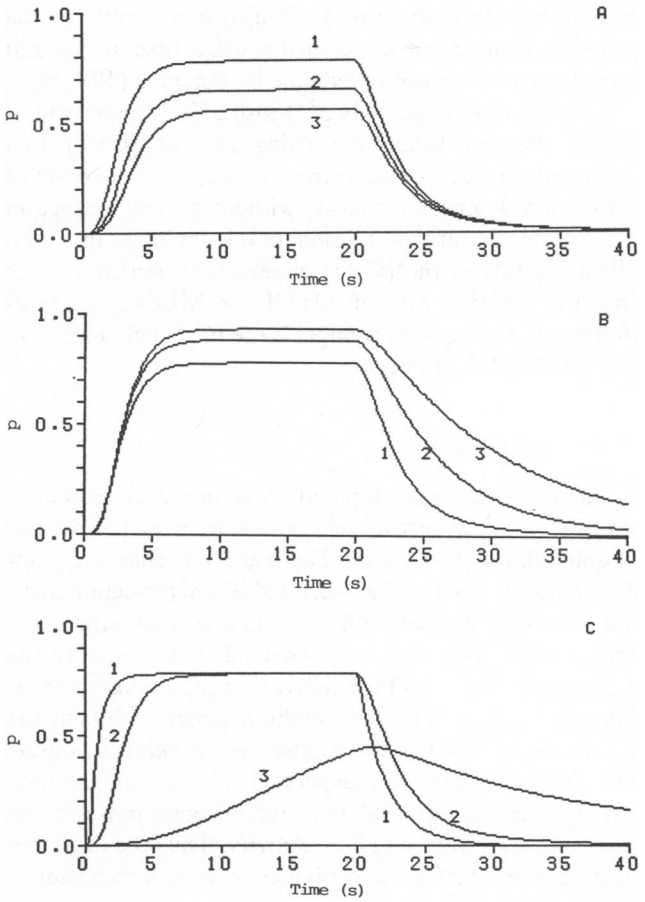


FIGURE 6 Effect of the kinetic parameter on the rates of isometric tension development and relaxation. The same step change as in Fig. 5 *C* was used for the Ca^{2+} transient. The standard values were used for the kinetic parameter except the following values. (*A*) Effect of $k_3(s^{-1})$. *1*, 2.5; *2*, 1.0; *3*, 0.5. (*B*) Effect of $k_4(s^{-1})$. *1*, 0.5; *2*, 0.2; *3*, 0.1. (*C*) Effect of b (s^{-1}). *1*, 10; *2*, 1; *3*, 0.1.

ment and relaxation (Fig. 6 *C*). Because b is expressed by av_0/p_a, a small value of b reflects a slow cycling rate of the cross bridge (v_0) if a is not tissue dependent. When the value of b approximated that in skeletal muscles, the time course of isometric tension closely followed that of the active state (Fig. 6 *c*, curve 1).

Changes in a Ca^{2+} transient also have an influence on the rates of tension change. When $[Ca^{2+}]_p$ increased, both the half time to peak tension and the half decay time during relaxation slightly increased (Fig. 7 *A*). This may be one of the explanations for the $[Ca^{2+}]$-dependent rate of tension development observed in skinned fiber (Peterson, 1982*a*). In general, tension development and relaxation in skinned muscle fiber are very slow: on the order of minutes compared with seconds in intact muscle. Our previous paper has shown that the rate of tension change in the Ca^{2+}-induced contracture strongly depends on not only the preparation thickness but also the external $[Ca^{2+}]$ if the relationship between the isometric tension at a steady state and the external $[Ca^{2+}]$ is nonlinear (Kato et al., 1982, Fig. 10). This result suggests that the slow rates of tension change in skinned muscle fiber may result from diffusion limitations. Recently, the Ca^{2+}-diffusion limitation on isometric contraction in skinned muscle fiber of arterial smooth muscle has been overcome by Peterson (1982*b*).

The rate of change in $[Ca^{2+}]$ also affected the rate of tension change. When the rising rate of $[Ca^{2+}]$ (k_r) decreased, the latency from the stimulaton to the onset of tension development increased without a great change in the maximum rate of tension development (Fig. 7 *B*). When the rate in the $[Ca^{2+}]$ change is sufficiently lower than the reaction rate of MLCK or MLCP, the rate-limiting step for tension changes is the $[Ca^{2+}]$ change, as in Ca^{2+}-induced contractures.

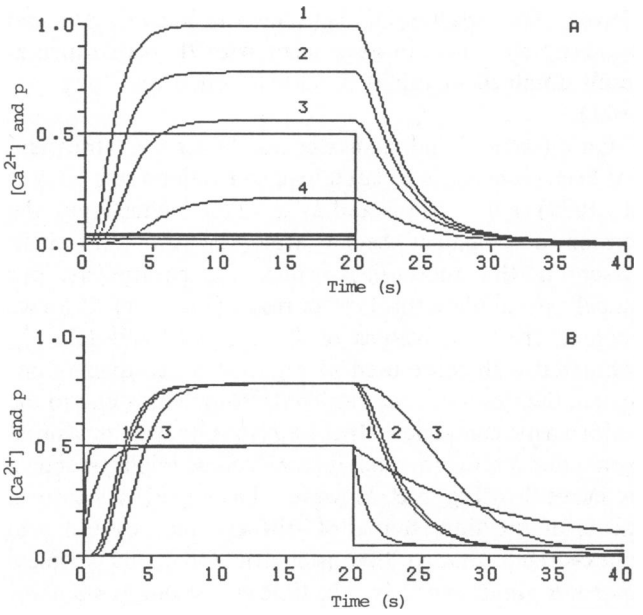


FIGURE 7 Effect of the Ca^{2+} transient on the rates of isometric tension development and relaxation. The standard values were used for the kinetic parameter. $[Ca^{2+}]_r = 10^{-7}$ M, $[Ca^{2+}]_p = 10^{-6}$ M. (*A*) Effect of $[Ca^{2+}]_p(M)$. *1*, 10^{-5}; *2*, 10^{-6}; *3*, 8×10^{-7}; *4*, 6×10^{-7}. (*B*) Effect of k_r and k_f (s^{-1}). *1*, step changes; *2*, $k_r = 5$, $k_f = 1$; *3*, $k_r = 1$, $k_f = 0.1$. Ordinate, $[Ca^{2+}]$ in arbitrary unit and p.

DISCUSSION

A kinetic model was proposed to simulate an isometric contraction in smooth muscle on the basis of the myosin phosphorylation hypothesis. There are four characteristics of this model. (*a*) The Ca^{2+}-calmodulin-dependent activation process of myosin light chain kinase was mathematically treated. This treatment enabled us to simulate the pCa-myosin Mg^{2+}-ATPase activity or pCa-isometric tension relationships. The calmodulin-dependent shift of the pCa-isometric tension curve observed in skinned muscle fibers was quantitatively explained. (*b*) It was assumed that two-head-phosphorylated myosin alone possesses an actin-activated Mg^{2+}-ATPase activity. Thus the disagreement between the Ca^{2+} sensitivities of myosin phosphorylation and isometric tension at a steady state was predicted. (*c*) Isometric tension was predicted from myosin phosphorylation by using Sandow's two-component model. The existence of a series elastic component caused isometric tension development to fall behind the time courses of myosin phosphorylation. (*d*) All parameters in the model can be experimentally determined, some of which would be difficult to measure. Using the appropriate values observed in various smooth muscle and the assumed values for some parameters, we could simulate a corresponding isometric contraction curve. Consequently we conclude that the slow rate of isometric tension change in smooth muscle compared with in striated muscles is caused by (*a*) the existence of the myosin phosphorylation process, (*b*) the slow cycling rate of cross bridges, and (*c*) the slow change in the Ca^{2+} transient.

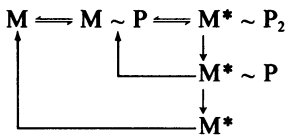
We used many assumptions to simplify the model that can be improved. First, the activation of MLCK was assumed to be rapid compared with myosin phosphorylation. Because of the strong affinity of MLCK for CM*, the dissociation of the complex might be very slow. Thus the inactivation process of MLCK may be a rate-limiting step in myosin dephosphorylation after a Ca^{2+} removal. However, Adelstein and Klee (1981) have suggested that the initial step in inactivating MLCK is the dissociation of Ca^{2+} from MLCK* complex. As one possibility, the large gap between the Ca^{2+} transient and isometric tension development observed by Fay et al. (1979) may be caused by the slow inactivation of MLCK. Further analysis on an isolated enzyme system is needed.

A second assumption was that myosin phosphorylation is a pseudo–first-order reaction. This assumption is right only if the myosin concentration is sufficiently lower than the *Km* of myosin for MLCK. The myosin content of

smooth muscle has been estimated to be ~20 mg/g wet tissue (Cohen and Murphy, 1978) which corresponds to ~40 μM, assuming a molecular weight of 470,000. The *Km* of isolated myosin light chain for MLCK prepared from turkey gizzard has been measured to be 5 μM (Adelstein and Klee, 1981). These values do not satisfy the condition for the pseudo–first-order assumption. If the *Km* for myosin as a substrate is larger than that for the light chain, this assumption may be useful. Furthermore, it was assumed that the same MLCK catalyzes both phosphorylation reactions for unphosphorylated and singly phosphorylated myosin. This kind of competitive reaction of one enzyme for two different substrates leads to more complicated kinetic equations (Segel, 1975). Even if a more complicated model is used, the characteristic behavior of the model may be qualitatively similar to that described here. MLCK was assumed to be soluble in the cytoplasm. The recent report by Dabrowska et al. (1982) suggested that MLCK can bind tightly to actin in vitro, and estimated that there are 1.5–2.0 molecules of MLCK per thin filament in vivo. If this is true, it is difficult to explain the experimental result that most of the myosin can be phosphorylated during full activation of intact smooth muscle. This is one of the remaining problems together with uncertainties about properties of MLCP.

Sandow's two-element mechanical model was used to calculate the isometric tension. His model required that the values of a and b ($=av_0/p_a$) are independent of the extent of activation, p_a. Arner (1982) reported that a is independent of $[Ca^{2+}]$ and that both v_0 and p_a decrease with a decrease in $[Ca^{2+}]$, but not at a constant v_0/p_a in K^+-induced contractions of guinea-pig *T. coli* and in contractions of skinned fibers. On the other hand, Dillon and Murphy (1982) reported that both a and b fall with a decrease in $[Ca^{2+}]$ in K^+-depolarized swine carotid artery, although most of these changes were not statistically significant (Murphy, personal communication). Thus further improvement for simulation of mechanical response is necessary, although Sandow's model is practically useful to simulate the isometric contraction.

The proposed model can explain many experimental results but not all. Murphy's group has found that myosin phosphorylation declined, occasionally to the resting level, during the course of a maintained contraction in the swine carotid media (Driska et al., 1981; Aksoy et al., 1982). The slow decline of phosphorylation during the tension development and maintained contracture has been observed in bovine tracheal muscle (de Lanerolle et al., 1982; Silver and Stull, 1982) and in rabbit *T. coli* (Butler and Siegman, 1982), but not in canine tracheal muscle (de Lanerolle et al., 1980). To explain these results, a "latch bridge" was proposed which resulted from the dephosphorylation of a cycling cross bridge (Dillon et al., 1981; Aksoy et al., 1982). This corresponds to the attached, noncycling cross bridge proposed by Siegman et al. (1976) for the guinea pig *T. coli*. Considering these results, our model can be improved by the following way:



Scheme III

where M* ~ P and/or M* represent the latch bridge. If this scheme is adopted, a new model is needed to describe the relationship between cross-bridge formation and isometric tension development on the basis of the cross-bridge theory.

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