J. Theor. Biol. (1977) **64**, 517–534[1]

Kinetic Model of Regulation of Muscle Protein Activity

V. I. DESHCHEREVSKY

Institute of Biological Physics of the Academy of Sciences of the USSR, Pushchino, USSR

(*Received* 1 *July* 1975, *and in revised form* 24 *March* 1976)

The widely accepted steric model of calcium regulation of actin-myosin interactions in vertebrate muscles has to be completed to fit the kinetic data. It should be supposed that: (1) the thin filaments consist of functionally independent units, containing seven actin sites regulated by one troponin-tropomyosin complex; (2) actin sites become available for myosin heads only due to fluctuations of tropomyosin position; (3) binding of calcium to troponin results either in the shift of the tropomyosin equilibrium position or in the weakening of its interactions with actin strand so that the probability of effective fluctuations increases; (4) link formation between myosin head and some of the available actin site fixates the tropomyosin in such a position that the other six actin sites of the same functional unit become available for myosin too.

The model gives linear kinetic scheme for the transitions of a functional unit between nine states (a "turned off" state, and eight "turned on" ones with different occupancy by myosin heads). The dependences of the apparent rate constants of actomyosin formation and dissociation upon the myosin head and substrate concentrations are obtained from the Lymn-Taylor scheme. The frequency of the actomyosin complexes dissociation is assumed to give the ATPase rate.

The model fits the kinetic data on the ATP hydrolysis by myosin subfragment-1 with regulated or unregulated actin as a cofactor under various conditions. It shows a sharp dependence of activation upon the apparent affinity of the actin and myosin sites. Therefore, the model appears to be applicable to myosin controlled systems.

1. Introduction

The simple steric model for calcium regulation of actin-myosin interactions in muscles is strongly supported by X-ray data on living muscle (Haselgrove, 1973) and on reconstructed thin filaments (Hanson, Lednev, O'Brien & Bennett, 1973). According to this model, the tropomyosin strand blocks sterically active sites of actin (A-sites) in the absence of calcium. Binding of

calcium to troponin results in a shift of the tropomyosin to the center of the actin groove, this making actin sites available for myosin. Thus, it is assumed that actin is "turned off" in the first case and "turned on" in the second one.

However, the data on the kinetics of the ATP hydrolysis by regulated actomyosin cannot be explained in terms of such a simple model. It is known that at a low substrate concentration the system is completely activated even in the absence of calcium. On the contrary, even at saturating calcium concentrations the system can exhibit a biphasic behavior, i.e. it is not completely activated under such conditions. These results were obtained by Bremel & Weber (1972) and Bremel, Murray & Weber (1973) in the experiments with the single active site moiety of the myosin molecule (S-1). Thus, all the cooperative phenomena should be ascribed to the interactions within the actin filaments. When interpreted in terms of the steric model, they mean, firstly, that tropomyosin can be shifted from "off" position without calcium participation and, secondly, that the binding of calcium to troponin cannot by itself shift tropomyosin to such a position in which it does not interfere at all with myosin heads.

To explain the behavior of the system, Bremel *et al.* (1973) proposed a complex picture of protein-protein interactions within the thin filaments which is a contrast to the simplicity of the steric model. In the present work the latter is completed with the assumption which makes it possible to explain the kinetic data and, on the one hand, seems to be physically reasonable. It is assumed, namely, that the fluctuations in the tropomyosin position play a significant role in the operation of the regulatory system.

2. Formulation and Mathematical Treatment of the Model

The consideration is based on the idea of Bremel & Weber (1972) according to which the thin filament consists of independent functional units (FU's). Each FU contains l A-sites, regulated by one troponin-tropomyosin complex and operates as a single whole. The following assumptions are made.

(1) Tropomyosin has a discrete or continuous number of positions on the surface of actin strand which it passes through due to thermal agitation. Some of the positions are compatible with the link formation between A-sites and S-1 and others are not. Binding of calcium to troponin increases the probability for tropomyosin to be in the position of a first type. In this case the FU is "turned on", otherwise it is "turned off".

(2) The binding of S-1 to any A-site of FU makes impossible all the positions of tropomyosin in which it interferes with S-1, i.e. a "turned off" state of FU.

(3) Each A-site in a "turned on" FU operates independently of the others.

(4) The cyclic mechanism, suggested by Bukatina & Deshcherevsky (1972) for the actomyosin ATPase, is applicable for the ATP hydrolysis by S-1 with a "turned on" actin as a cofactor. According to this mechanism, each enzymatic turn of ATP splitting is coupled with the actomyosin association- dissociation cycle, and can be adequately described by two apparent rate constants of actomyosin complex formation κ and dissociation ν. A cyclic mechanism of the actomyosin ATPase can be derived from Lymn & Taylor's (1971) scheme, which is used in the present consideration to obtain the dependences of κ and ν on the S-1 and MgATP concentrations. However, it is more convenient in the initial formulation to treat the apparent rate constants κ and ν as independent parameters.

According to these assumptions, an FU has $l+2$ kinetically significant states. In the "relaxed" state, R, all its A-sites are "turned off" and incapable of combining with S-1. In $l+1$ "active" state a_i ($i = 0, 1, \ldots, l$) all l A-sites are "turned on" and i of them are combined with myosin. The apparent equilibrium constant $1/r$ for the transition from R to a_0 increases with calcium concentration, i.e. the equilibrium constant r for the inverse transition decreases when the system is activated by calcium. A linear kinetic chain can be written for the transitions of an FU between these states:

$$R \underset{r}{\rightleftharpoons} a_0 \overset{l\kappa}{\underset{\nu}{\rightleftharpoons}} a_1 \overset{(l-1)\kappa}{\underset{2\nu}{\rightleftharpoons}} \ldots \overset{(l-i+1)\kappa}{\underset{i\nu}{\rightleftharpoons}} a_i \overset{(l-i)\kappa}{\underset{(i+1)\nu}{\rightleftharpoons}} \ldots \overset{\kappa}{\underset{l\nu}{\rightleftharpoons}} a_l. \qquad (1)$$

The probability of S-1 attachment to an FU in the state a_i is proportional to the number of free "turned on" A-sites, l-i, and the probability of S-1 detachment from a_i is proportional to the number of acto-S-1 complexes, i. Therefore, the apparent rate constants of the transitions of a_i into a_{i+1} and a_{i-1} are evidently $(l-i)\kappa$ and $i\nu$, respectively.

Scheme (1) yields the following correlations between the steady-state concentrations of the FU's of all types:

$$R = ra_0,\ a_1 = lga_0,\ \ldots,\ a_i = \frac{l-i+1}{i} ga_{i-1},\ \ldots,\ a_l = \frac{1}{l} ga_{l-1} \qquad (2)$$

where $g = \kappa/\nu$. If A is the total concentration of A-sites then A/l is that of the FU's. The latter can be expressed through a_0 by the use of equation (2):

$$\frac{A}{l} = R + \sum_{i=0}^{l} a_i = a_0 \left\{ r + \left[1 + lg + \frac{l(l-1)}{2!} g^2 + \ldots \right] \right\} =$$

$$= a_0\{r + [1+g]^l\} \qquad (3)$$

The overall concentration of acto-S-1 complexes in all the FU's, a, can be expressed in a similar manner:

$$a = \sum_{i=0}^{l} ia_i = a_0 lg \left[1+(l-1)g+\frac{(l-1)(l-2)}{2!}g^2+ \ldots \right] = a_0 lg[1+g]^{l-1} \quad (4)$$

It may be noted for elucidation that the expressions in the square brackets in equations (3) and (4) are binomial rows $[1+g]^l$ and $[1+g]^{l-1}$, respectively. The substitution in equation (4) for a_0 of its expression from equation (3) gives:

$$a = A\frac{g(1+g)^{l-1}}{r+(1+g)^l} \quad (5)$$

The number of acto-S-1 complexes dissociating in 1 s, ω, equals va. In the steady-state it equals the number of S-1 attachments to A, $\kappa\alpha$, where α is the concentration of free "turned on" A-sites. By the use of equation (5) we obtain

$$\omega = A\kappa\frac{(1+g)^{l-1}}{r+(1+g)^l} \quad (6)$$

and

$$\alpha = A\frac{(1+g)^{l-1}}{r+(1+g)^l} \quad (7)$$

According to the assumption (4), ω gives a steady-state rate of actomyosin ATPase. If the total concentration of S-1 exceeds significantly A then the free concentration of S-1 does not change with substrate and calcium levels. In this case κ is constant and v depends only on MgATP concentration. The dependences of the actomyosin complex fraction, a/A, and the relative ATPase rate, $\omega/\kappa A$, on the equilibrium constant g are given in Fig. 1.

3. Specification of the Model

Applications of the model to concrete actomyosin systems imply that r, κ and v are known functions of calcium, actin, myosin and substrate concentrations. However, the behavior of the model can be analysed qualitatively before a detailed evaluation of the parameters is made. The decreasing of the "inhibition" constant r corresponds to calcium activation. The equilibrium constant g of the actin and myosin sites association decreases with substrate concentration, i.e. the right hand movement along the abscissa of Fig. 1 corresponds to the increase of MgATP concentration. Therefore, we may conclude that the curves of Fig. 1 indicate the qualitative agreement of the theory with the behavior of real regulated actomyosin systems. The

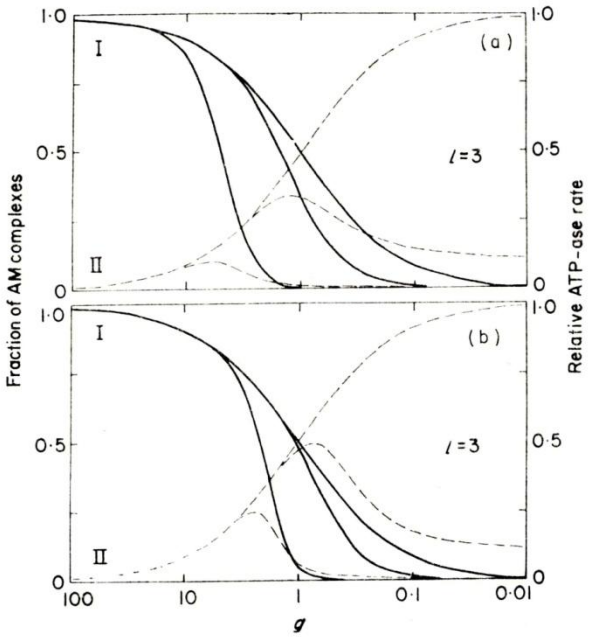

FIG. 1. Activation of actomyosin system as a function of the apparent equilibrium constant g of actomyosin formation. I (———), fraction of AM complexes a/A^{ϕ}, II (-----), relative ATPase rate $\omega/\kappa A$, calculated from formulas (5) and 6), respectively, at $r = 0$ (upper curves of each family), $r = 10$ (middle curves) and $r = 2000$ (lower curves). Parameter r increases when Ca^{2+} concentration decreases.

theory gives biphasic dependence of ATPase on the substrate concentration in some region of the values of r. The maximum rate rises and its position shifts to the right (to higher substrate concentrations) with decrease of r (with increase of Ca^{++} concentration). At low MgATP levels (high values of g) the ATPase rate does not depend on r. At $r = 0$ the curve becomes hyperbolic which corresponds to an unregulated actomyosin. The non-linearity of the curves in Fig. 1 depends on the number of A-sites in an FU, l, which plays the role of a cooperation parameter.

4. Interpretation of the Apparent Rate Constants κ and ν in Terms of Lymn-Taylor's Scheme

In what follows we shall use, as a rule, the notation M instead of S-1 for myosin head to preserve the symbol S for the substrate (MgATP). The notation P is used for both products (MgADP and inorganic phosphate).

Various complexes of actin and myosin sites with each other and with the substrate and products are denoted as MS, MP, AM, AMS, and AMP. The kinetic diagram suggested by Lymn & Taylor (1971) for ATP hydrolysis by unregulated actomyosin is given in Fig. 2 (notations and numeration of the steps are changed in comparison with the original publication and the rates

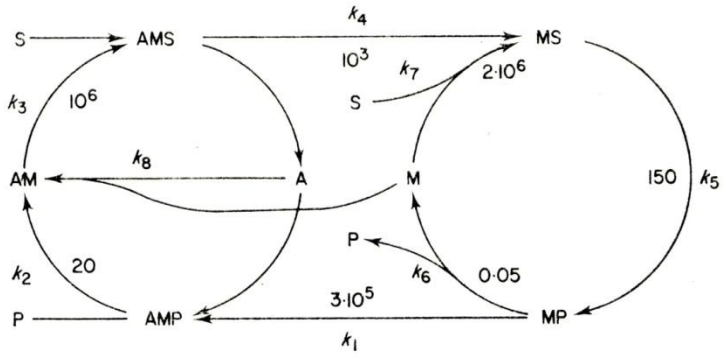

FIG. 2. Lymn-Taylor's (1971) scheme of actomyosin ATPase. A = actin, M = myosin, S = substrate (MgATP), P = products (ADP+phosphate). The values of monomolecular (K_2, K_4, K_5 and K_6) and bimolecular (K_1, K_3 and K_7) rate constants are given in s^{-1} and $m^{-1}\ s^{-1}$, respectively.

of all the reversal processes are regarded as negligible). We shall assume that this mechanism is applicable to ATP hydrolysis by S-1 with a "turned on" actin as a cofactor, though the values of all rate constants need not be the same in both cases. According to the scheme, the relative steady-state concentrations of M, MS and MP are expressed as follows:

$$\frac{[MS]}{[MP]} = \frac{K_6 + K_1\alpha}{K_5} < \frac{K_6 + K_1 A}{K_5} \qquad (8)$$

$$\frac{[M]}{[MP]} = \frac{K_6}{K_7 S + K_8\alpha} < \frac{K_6}{K_7 S} \qquad (9)$$

In accordance with the notations of the preceeding sections, α is the free A-site concentration and A is the total one. By using the values of the rate constants given by Lymn & Taylor (see Fig. 2) we obtain the following estimations

$$\frac{[MS]}{[MP]} < 0{\cdot}1 \text{ at } A < 50\ \mu\text{M}, \text{ and } \frac{[M]}{[MP]} < 0{\cdot}1 \text{ at } S > 0{\cdot}25\ \mu\text{M} \qquad (10)$$

in which S is the free MgATP concentration. These estimations mean that, under usual experimental conditions, the free myosin and myosin-substrate

concentrations are small in comparison with the concentration of the myosin-product complex. Therefore, we shall assume that all myosin, not bound to actin, is in the form of MP complex, and the apparent first order rate constant of the actomyosin complex formation may be expressed as

$$\kappa = K_1 \mu \tag{11}$$

where μ is the concentration of M-sites not bound to actin. The apparent rate constant ν of actomyosin dissociation may be expressed as an inverse life-time of actomyosin complexes of all kinds, i.e. AMP, AM and AMS, so that

$$\nu^{-1} = (K_2)^{-1} + (K_3 S)^{-1} + (K_4)^{-1} \approx (K_2)^{-1} + (K_3 S)^{-1}$$

and

$$\nu = \frac{K_2 K_3 S}{K_2 + K_3 S} \tag{12}$$

5. Interpretation of the Steady-state Acto-HMM ATPase Kinetics in Terms of "the Cyclic Model" and Evaluation of κ and ν

The two-step kinetic mechanism was proposed by Bukatina & Deshcherevsky (1972) as the simplest interpretation of the steady-state kinetic data (Eisenberg & Moos, 1970; Rizino, Barouch, Eisenberg & Moos, 1970; Barouch & Moos, 1971). It is suggested in this scheme, that the hydrolysis of the ATP molecule is coupled with the whole myosin-actin association-dissociation cycle. The simplest cycle of two significant states is sufficient to describe the situation:

$$\mathrm{A} + \mathrm{M} \underset{\nu}{\overset{K_1}{\rightleftharpoons}} \mathrm{AM} \tag{13}$$

If actin is in a large excess, the number of ATP molecules, hydrolysed by one myosin site in 1 s, and its reciprocal are expressed as

$$\omega = \frac{\nu K_1 A}{\nu + K_1 A} \text{ and } \omega^{-1} = \nu^{-1} + (K_1 A)^{-1} \tag{14}$$

so that the double reciprocal plot of the relationship between the ATPase rate and the actin concentration must give a straight line and can be easily compared with experimental data to obtain the values of K_1 and ν. It is evident that κ in our scheme is expressed as $K_1 \mu$, where μ is the free M-site concentration.

By comparing equation (14) with the experimental plots of Eisenberg & Moos (1970) we obtain the dependences of ν and K_1 on MgATP concentration at two concentrations of KCl [Fig. 3(a)]. The rate constant K_1 does

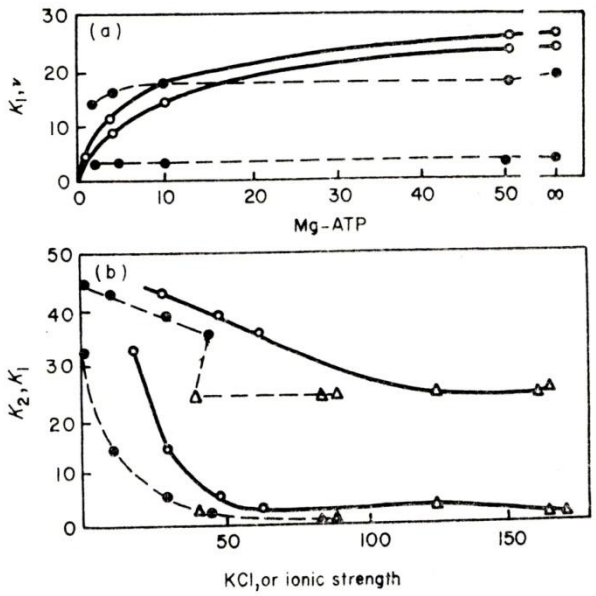

FIG. 3. Analysis of the steady-state kinetic data on ATP hydrolysis by unregulated acto-HMM in terms of the "cyclic" model. (a) the dependences on MgATP concentration of the apparent rate constant of AM dissociation, ν, (solid) and association, K_1 (dashed curves) in the absence of KCl (upper curves of each type) and at 20 mM KCl (lower ones); the abscissa: μmol of MgATP, the ordinate: s^{-1} for ν and 10^5 m^{-1} for K_1. (b) the dependences on the ionic strength (solid) and KCl concentration (dashed lines) of the rate constants of the production dissociation K_2 (the upper pair of curves) and AM complex formation K_1 (the lower one); circles and triangles were obtained from the data of Rizino *et al.* (1970) and Eisenberg & Moos (1968) respectively; the abscissa: KCl concentration or total ionic strength in mmol, the ordinate: s^{-1} for K_2 and 10^5 m^{-1} s^{-1} for K_1.

not depend on the substrate concentration, while ν is strongly influenced by small concentrations of MgATP. The last dependence may be approximated by formula (12) at an appropriate choice of the parameters, which is $K_2 = 25$ s^{-1} and $K_3 = 4{\cdot}6 \times 10^6$ $м^{-1}$ s^{-1} in the absence of KCl and $K_2 = 24$ s^{-1} and $K_3 = 4{\cdot}0 \times 10^6$ $м^{-1}$ s^{-1} for 20 mM of KC1. It can be seen that the parameters decrease with KCl concentration. The dependences of K_1 and K_2 upon KCl concentration and the total ionic strength obtained from the data of Rizino *et al.* (1970) and Eisenberg & Moos (1968) are plotted in Fig. 3(b) as circles and triangles, respectively. The total ionic strength in these two experiments was achieved by various combinations of the ingredients, which gives the possibility to conclude that the rate constant K_2 is influenced by the total ionic strength while K_1 is sensitive to KCl

concentration rather than to the total ionic strength. The values of the rate constants obtained by the analysis of steady-state data in terms of "the cyclic model" differ from the transient kinetics determinations. The discrepancy can be ascribed to different pH and temperature conditions in the experiments of Lymn & Taylor (1971) and Eisenberg & Moos (1970). However, it may reflect also different abilities of the intermediate and relaxed myosin-product complexes (for examples E* . ADP . P_i and E . ADP . P_i of Trentham *et al.*, 1972) to react with actin.

6. Calculations of the Steady-state Kinetic Curves and Comparison with Bremel's *et al.* (1972, 1973) Data on ATPase of Regulated Acto-S-1

It is assumed, firstly, that a two-step cyclic mechanism is applicable to ATP hydrolysis by S-1 with "turned on" A-sites in regulated actin filaments as a cofactor and, secondly, that the apparent rate constants κ and ν in this case can be approximated by formulas (11) and (12). If S-1 is in a large excess the situation is simple enough to be used for estimation of the parameters. The value of κ increases linearly with the free M-site concentration whereas ν cannot exceed K_2. Thus, at high levels of M-sites $g >> 1$ and the inhibition constant r may be neglected as compared with $(1+g)$[7], and formula (6) is reduced to

$$\omega = A\frac{\kappa}{(1+g)} = A\frac{K_1 M}{1+K_1 M/\nu} \qquad (15)$$

where M is the total M-site concentration. At a high level of MgATP $\nu = K_2$, and we can rewrite equation (15) as

$$\frac{A}{\omega} = \frac{1}{K_1 M} + \frac{1}{K_2} \qquad (16)$$

i.e. the inverse values of the S-1 concentration and the ATPase rate normalized against the total actin concentration are related linearly. By comparing this expression with the curves in Fig. 4 from Bremel *et al.* (1973), and using the linear region at small values 1/M for regulated acto-S-1 we obtain $K_2 = 160 - 190\ s^{-1}$ and $K_1 = (2 - 2{\cdot}8) \times 10^6$ $м^{-1}\ s^{-1}$ for regulated acto-S-1, $K_2 = 160 - 190\ s^{-1}$ and $K_1 = (0{\cdot}2 - 0{\cdot}3) \times 10^6$ $м^{-1}\ s^{-1}$ for unregulated acto-S-1. The theory predicts also a linear relationship between the inverse values of the concentration of free M-sites and the substrate concentration at which the ATPase rate is maximal. This gives the possibility to estimate the values of the rate constant K_3 and the inhibition constant r at a high level of calcium ions. The condition of the extremum of the ATPase rate

[1] This article was published in Journal of Theoretical Biology, Vol. 64(3), Deshcherevsky V. I ., Kinetic model of regulation of muscle protein activity, pp. 517–534, Copyright Elsevier (1977).

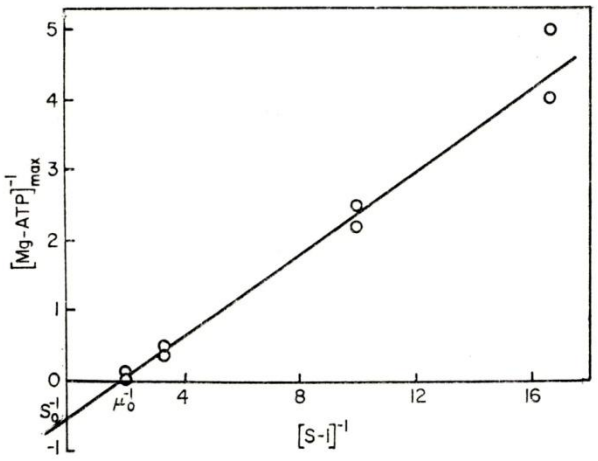

FIG. 4. Correlation between [S-1] and optimal substrate concentrations recalculated from Fig. 3(b) of Bremel *et al.* (1973). The inverse of [S-1] (abscissa) and of optimum MgATP concentrations (ordinate) are given in 10^4 м$^{-1}$. The cross-points with the abscissa and ordinate correspond to $\mu_0 = 55$ μм and $S_0 = -180$ μм.

dependence on the substrate concentration at a constant κ, i.e. at a significant excess of M-sites, can be written according to equation (6):

$$\frac{d}{dg}\left[\frac{A\kappa}{\omega}\right] = 1 - \frac{(l-1)r}{(1+g)^l} = 0 \qquad (17)$$

At $l = 7$ (seven A-sites in a "functional unit") this gives $g_{max} = \sqrt[7]{(6r)} - 1$

By substituting instead of $g_{max} = (\kappa/v_{max})$ its expression through equations (11) and (12) we obtain:

$$\mu^{-1} = K_1[\sqrt[7]{(6r)} - 1]^{-1} \times [(K_2)^{-1} + (K_3 S)^{-1}] \qquad (18)$$

The correlation between the M-site concentration and the position of the ATPase maximum predicted by equation (18) does exist. The double reciprocal plot of $[S\text{-}1]^{-1}$ against $[MgATP]_{max}^{-1}$, which was reconstructed from the set of curves in Fig. 3 of Bremel *et al.* (1973), is given in Fig. 4. By comparing formula (18) with this plot and using most probable values of K_1 and K_2 from the preceeding estimation ($2{\cdot}4 \times 10^6$ м$^{-1}$ s^{-1} and 175 s^{-1} respectively) we obtain: $K_3 = -K_2/S_0 = 1 \times 10^6$ м$^{-1}$ s^{-1} and $r = 1/6 \times (1 + K_1\mu_0/K_2)^7 = 8$ at 10^{-4} м Ca^{2+}.

The dependences of the steady-state rate of ATP hydrolysis by regulated acto-S-1 on the substrate concentration were calculated for the conditions under which the experiments of Bremel & Weber (1972) and Bremel *et al.* (1973) were performed. A special method of calculations was worked out for

cases where myosin, actin and substrate concentrations are of the same order of magnitude. In this method the apparent equilibrium constant $g = \kappa/\nu$ is not calculated as a function of given experimental conditions but its values are chosen arbitrarily and for each of them the rate of ATP hydrolysis, on the one hand, and the total substrate concentration, on the other hand, are calculated. The procedure of calculations for each given g consists of the following operation:

(1) Calculation of the concentrations of the AM complexes, a, and free "switched on" A-sites, α, according to formulae (5) and (7).

(2) Calculation of the free M-site concentration, $\mu = M - a$, and the apparent rate constant of A and M association, $\kappa = K_1\mu$.

(3) Calculation of the ATP hydrolysis rate in the cyclic interaction of A and M, $\omega = \kappa\alpha$, and of the apparent rate constant of AM dissociation $\nu = \kappa/g$.

(4) Calculation of the free substrate concentration in accordance with equation (12): $S = K_2\nu/[K_3(K_2 - \nu)]$.

(5) Calculation of the bound nucleotide concentration s

$$s = \frac{K_a S}{K_d + K_a S} M \qquad (19)$$

The ratio in equation (19) equals the fraction of M-sites occupied by the nucleotide, i.e. (MS + AMS + MP + AMP)/(M + AM + MS + AMS + MP + AMP). Its expression through the elementary rate constants can be obtained from the Lymn-Taylor scheme (Fig. 2). If [MS] and [AMS] are regarded as negligible then we can obtain the expressions of [AM], [M] and [AMP] through [MP] from the steady-state equations $d[MP]/dt = d[M]/dt = d[AMP]/dt = 0$. From here the ratio we need can be calculated directly. It has the form of equation (19) with

$$K_a = K_3(1 + K_1\alpha/K_2) \quad \text{and} \quad K_d = K_1\alpha + K_3K_6/K_7 \simeq K_1\alpha + K_6 \qquad (20)$$

(6) Calculation of the total nucleotide concentration $N = s + S$. The "actomyosin" ATPase rates normalized against the total A- or M-site concentrations, are plotted against $\lg N$ (Figs 5 and 6). For comparison, the experimental points from the corresponding curves of Bremel *et al.* (1973) are given in the same figures.

To obtain the agreement between the calculated and experimental curves the parameters K_1, K_2, and K_3 were varied about the values obtained according to equations (16) and (18). The final values of these constants are given in the corresponding figures. It can be seen that the calculated curves fit satisfactorily the experimental points except for the range of low substrate concentrations. This disagreement can be eliminated by supposing that the

real apparent rate constant of substrate dissociation K_d is of the order of value of 1 s^{-1} instead of 0·1 s^{-1} which is obtained from formula (20) for the initial parts of all the curves in the case when $\mu >> A$. In this formula the reversibility of the step of substrate association with M-site is not taken into consideration. However, according to the data of Bagshaw & Trentham (1973), the rate constant of M-ATP dissociation does not exceed 0·02 s^{-1}, therefore, there is no reason for the apparent rate constant K_d to be of the order of 1 s^{-1}. A more probable explanation is suggested by Bremel (1974) according to which the discrepancy can be conditioned by the nonhomogeneity of S-1 preparations, which is not taken into consideration in the present calculations.

It may be assumed that variations of the model parameters from figure to figure reflect a natural variability of different enzyme preparations. However, this variability does not allow us to speak about the quantitative accordance of the model with the real behavior of the system, though any experimental curve can be fitted by an individual choice of the parameters.

One set of the rate constants is sufficient to describe various experimental situations. In nine cases from fourteen [the curves of Figs 5(b) and (c) and 6(c) and (d)] the following rate constants are specified for regulated and "turned on" A-site and S-1 of myosin:

$K_1 = 2{\cdot}6 \times 10^6$ м$^{-1}$ s^{-1}, $K_2 = 180$ s^{-1}, $K_3 = 1{\cdot}6 \times 10^6$ м$^{-1}$ s^{-1},
and $K_6 = 0{\cdot}05$ s^{-1}.

The same rate constants are suited for unregulated actin except for the constant K_1 which is in this case $0{\cdot}66 \times 10^6$ м$^{-1}$ s^{-1} [Fig. 5(a)] and $0{\cdot}55 \times 10^6$ м$^{-1}$ s^{-1} [Fig. 5(b)]. It should be noted, that the rate constant of the product dissociation, K_2, which must be ATPase rate limiting according to Lymn & Taylor's scheme, is tenfold that given by Eisenberg & Moos (1970) for acto-HMM and used by Lymn & Taylor (1971). Thus, this step was not ATPase rate limiting both in the case of regulated and unregulated acto-S-1. The calcium dependent inhibition parameter, r, in the absence of Ca^{++} and with 100 μm of calcium, has the values of 2000 [lower curves of Fig 6(c) and (d)] and 8–12 [Figs 5(c) and 6(a) and (b); upper curves of Figs 5(a) and 6(c) and (d) and lower curve of Fig. 5(b)], respectively. In the case of unregulated actin, r equals zero [lower curve of Fig. 5(a) and upper one (b)].

7. Discussion

The behavior of the model shows all features of the steady-state ATPase kinetics of regulated acto-S-1, which were demonstrated by Bremel *et al.* (1972, 1973).

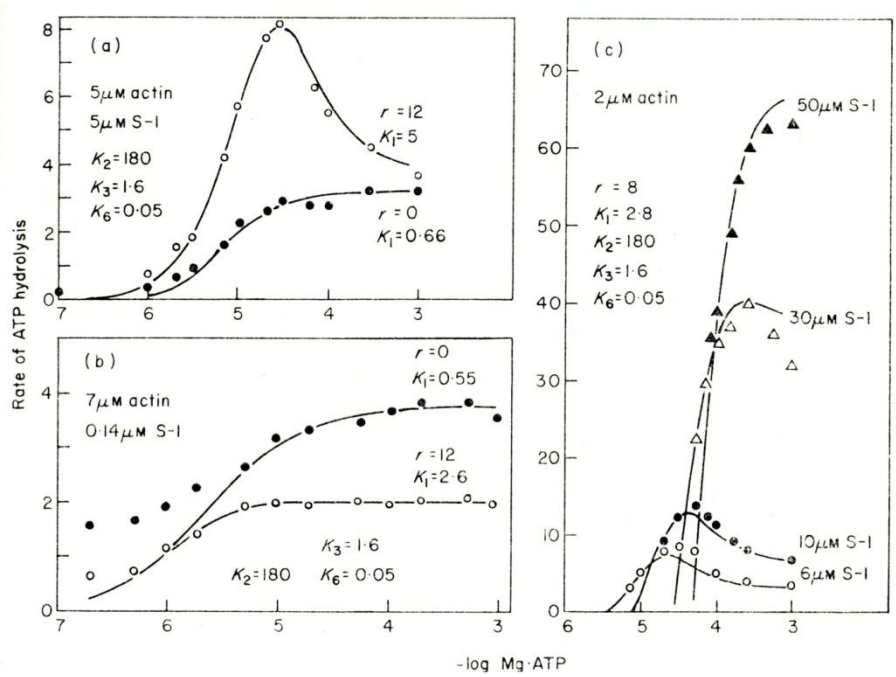

FIG. 5. ATP hydrolysis rate as a function of substrate concentration. Solid curves are computed at the values of the parameters, shown at the curves. Circles and triangles on (a), (b) and (c) correspond to the experimental points of Figs 1, 2 and 3(a), respectively, of Bremel *et al.* (1973). The ATPase rate is expressed as a number of turns in 1 s of S-1 [(a) and (b)] and actin (c).

(1) At low substrate levels and sufficient S-1 concentration calcium does not activate the system [Fig. 6(c)].

(2) At moderate S-1 and actin concentrations ATPase rate shows a biphasic dependence on [MgATP] both in the absence and presence of calcium [lower curve of Fig. 6(c) and upper curve of Fig. 5(a), respectively].

(3) The biphasic behavior disappears in two extremal cases of very low and very high S-1 concentrations [Figs 5(b) and (c), and 6(a) and (b), respectively].

(4) The optimal substrate concentration increases with S-1 concentration [Fig. 5(c)].

The theory predicts [formulae (18)] a shift of the optimum position to the range of higher MgATP concentrations when the system is activated by calcium at a constant S-1 concentration (i.e. when r decreases from 2000 to 10).

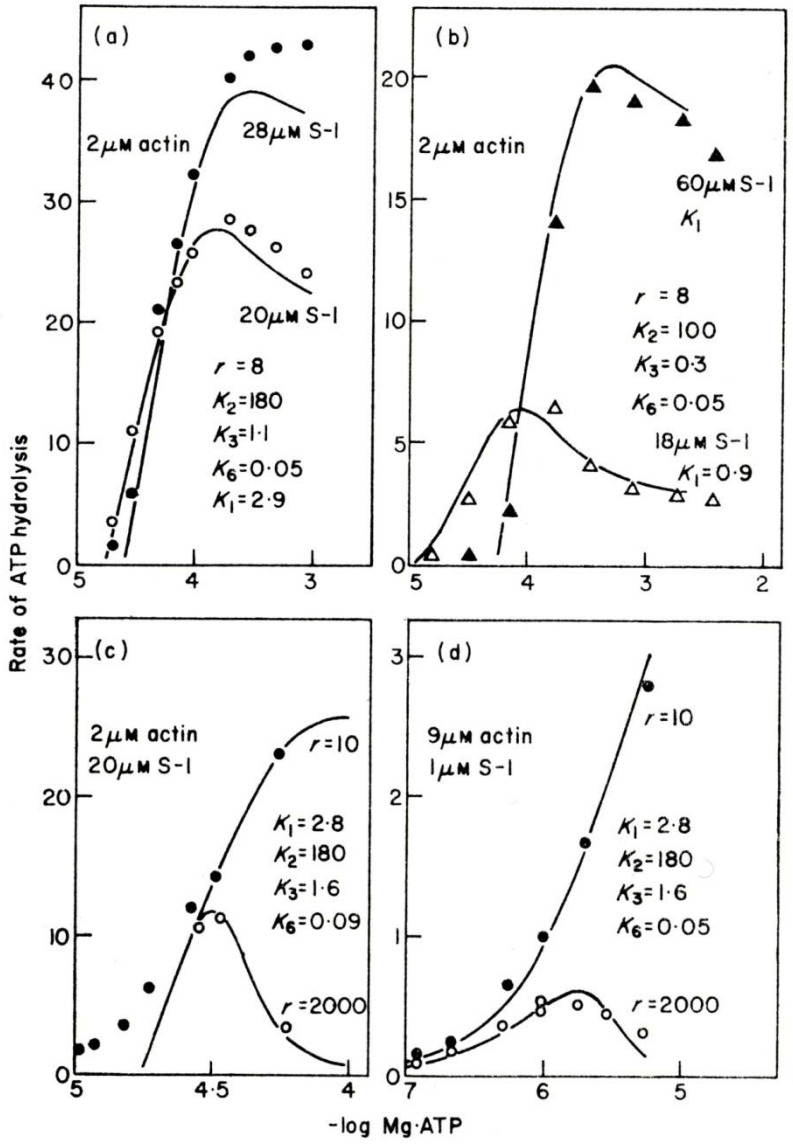

FIG. 6. The same as in Fig. 5(a) and (b) correspond to Fig. 6(a) and (b) of Bremel *et al.* (1973), (c) and (d) to Fig. 3(a) and (b) of Bremel & Weber (1972). The ATPase rate is expressed as a number of turns in 1 s of actin [(a), (b) and (c)] and S-1 (d).

All these features have a simple explanation in terms of the model. The distribution of FU's between "on" and "off" states is governed by two factors. The first one is calcium dependent probability of initiation of the "on" state, and the second one is the probability of its stabilization by acto-S-1 complexation. It is essential that the first process has a "low power" as compared with the second one, i.e. calcium itself can activate only a small part of all FU's. The complete activation is achieved only due to actin- myosin link formation. Binding of calcium to troponin is of no importance in the last case. At a low substrate concentration most of the A-sites are combined with M so that the behavior of the system does not depend upon the calcium concentration. In this case the ATPase rate is limited by the frequency of AM dissociation which increases with MgATP concentration. At a high substrate level the rate of dissociation achieves its maximal value, K_2. Besides, if the M-site concentration is low, most of FU's will have no A-sites combined with M. In the absence of calcium, less than one thousandth of these FUs are in the "on" state, so that the ATPase rate is limited by the frequency of A and M association. This mechanism gives a bisphasic dependence of the ATPase rate upon the substrate concentration.

At a high level of calcium the situation is similar except for the fact that about one tenth of free FUs are in the "on" state in this case. Therefore, the actomyosin ATPase has a measurable rate even at a low M to A ratio. Its dependence on the substrate concentration is hyperbolic in this case because AM complexation is insufficient to change the distribution of FU's between "on" and "off" states.

At a higher concentration of M-sites the same degree of AM dissociation is achieved with higher substrate levels. The rate of association varies directly as the M-site concentration and can be increased infinitely, while the rate of dissociation is limited by the value of K_2. Thus, at a very high concentration of M-sites the degree of dissociation can be insufficient for the ATPase rate to achieve its maximum even at an infinite rise of substrate concentration. This also results in disappearance of a biphasic behavior.

It can be concluded, that the essential properties of regulated acto-subfragment-1 can be interpreted in terms of the model. Consequently, it is not necessary to assume the interaction between the A-sites in an FU which results in the potentiation of their cofactor activity when some of them are bound to M-sites. In terms of our model any "turned on" A-site of a regulated thin filament has a constant cofactor activity which is significantly potentiated as compared with that of the A-site in an unregulated F-actin, or more accurately, the former has a several-fold larger rate constant of association with the M-site [Fig. 5(a) and (b)]. It may be assumed that the

electrostatic repulsion between F-actin and myosin head is partly screened by tropomyosin.

Two possible molecular interpretations of the suggested kinetic scheme are illustrated by Fig. 7(a) and (b). The angle position of tropomyosin on the F-actin strand is compatible with the AM link formation, if φ exceeds a threshold value φ_t. In the absence of calcium tropomyosin is at φ_0. Two situations are possible when troponin binds Ca^{++}. In the first case [Fig. 7(a)] the tropomyosin equilibrium position shifts to φ_c, but the steepness of the potential well is the same as in the absence of calcium. In the second case [Fig. 7(b)] the equilibrium position is not changed but the potential well becomes more smooth. In both cases the positions with $\varphi > \varphi_t$ become more probable, i.e. the binding of calcium to troponin shifts the distribution of FU's to the "on" state. The first possibility is more preferable in the light

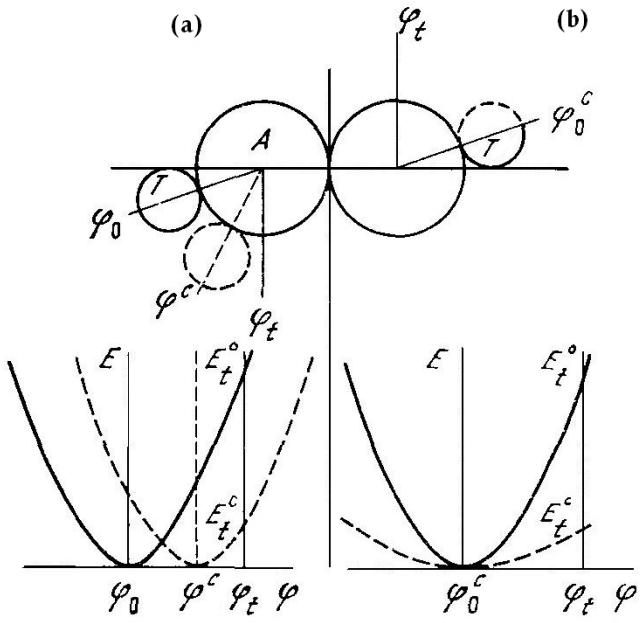

FIG. 7. Two versions of a calcium-dependent change of the tropomyosin (T) potential well on the actin (A) strand surface. Solid and dashed lines correspond to troponin free of and occupied by calcium, respectively, E = potential energy, φ = angle variable, φ_0 and φ^c are equilibrium positions of T in the absence and presence of calcium, respectively. At $\varphi \geq \varphi_t$ the A-sites become available for myosin, the corresponding threshold levels of energy in the absence and presence of calcium being E_t^0 and E_t^c, respectively, (a) and (b) the shift and the smoothing of the potential well.

of X-ray experiments on reconstructed thin filaments (Hanson *et al*., 1973), though the second one cannot be excluded completely. In any case, myosin binding to actin must result in an additional displacement of tropomyosin. Chaplain & Sacharjan's (1974) data on insect flight muscle, in which the cross-bridge formation initiates a significant increase in the intensity of the 19·3 nm layer-line, can be regarded as an indirect evidence for the model. The mechanism described here depends much on the apparent affinity of actin for myosin. For example, the increasing of g from 1 to 2 at r equal to 2000 results in more than a tenfold activation of the system [the fraction of AM complexes increases from 0·03 to 0·4, as can be seen from Fig. 1(b)]. Thus, the model seems to be applicable to myosin controlled systems in which the actin filaments contain tropomyosin, but show no sensitivity to calcium.

The apparent affinity of actin for myosin and the degree of activation of a living muscle must decrease with the velocity of contraction. Preliminary estimation shows that the rate of energy production (Hill, 1964) can be explained by the suggested model.

Many points of the theory are open for criticism. The model does not take into consideration a very probable difference in the interaction of relaxed and intermediate M . ADP. P complexes with the A-site which was postulated by Eisenberg & Kielley (1973). The results of Inoue, Shikegawa & Tonomura (1973) can also be regarded as indications of such a difference, which can result, in terms of our model, in the nonlinear dependence of the apparent rate constant of association K_1 upon the free M-site concentration. Secondly, the independent operation of FU's by itself seems to be hardly probable because of the tendency of tropomyosin molecules to form the continuous strand along the whole thin filament. Finally, it may seem strange that even at saturating levels of calcium the free of myosin FU's spend in the "on" state only a small fraction of time. However, such a kind of calcium regulation has some advantages in comparison with the "all or none" mechanism. It is more efficient because a smaller amplitude of a calcium-induced shift of the tropomyosin strand is necessary to give a practically complete activation at a sufficiently high M-site concentration. An additional shift of tropomyosin in this case is paid by the energy of myosin-actin interaction. This must result in a co-operative character of calcium binding to troponin which makes the whole system more labile so that a smaller change of calcium concentration is necessary for its operation.

I am deeply indebted to Professor E. E. Shnol and Mrs. E. A. Umnova from the Scientific Computing Center (NIVC) for the help in calculations. I am also grateful to Dr R. I. Sel'kova for the correction of the English version of the manuscript.

REFERENCES

BAGSHAW, C. R. & TRENTHAM, D. R. (1973). *Biochem. J.* **133,** 323.
BAROUCH, W. W. & MOOS, C. (1971). *Biochim. Biophys. Acta.* **234,** 138.
BREMEL, R. D. (1974). Private communication.
BREMEL, R. D. & WEBER, A. (1972). *Nature New Biol.*, **238**, 97.
BREMEL, R. D., MURRAY, J. M. & WEBER, A. (1973). *Cold Spring Harbor Symp. quant. Biol.* **37,** 267.
BUKATINA, A. E. & DESHCHEREVSKY, V. I. (1972). *Biofizica*, **17,** 738.
CHAPLAIN, R. A. & SACHARJAN, S. (1974). *FEBS Lett.* **42,** 50.
EISENBERG, E. & MOOS, C. (1968). *Biochemistry.* **7**, 1486.
EISENBERG, E. & MOOS, C. (1970). *J. Biol. Chem.* **245,** 2451.
EISENBERG, E. & KIELLEY, W. W. (1970). *Biochem. Biophys. Res. Comm.* **40,** 50.
EISENBERG, E. & KIELLEY, W. W. (1973). *Cold Spring Harbor Symp. quant. Biol.* **37,** 445.
HANSON, J., LEDNEV, V., O'BRIEN, E. J. & BENNETT, P. M. (1973). *Cold Spring Harbor Symp. quant. Biol.* **37,** 311.
HASELGROVE**,** J. C. (1973). *Cold Spring Harbor Symp. quant. Biol.* **37,** 341.
HILL, A. V. (1964). *Proc. R. Soc. bond. Ser.* **B159**, 297.
INOUE, A., SHIKEGAWA, M. & TONOMURA, Y. (1973). *J. Biochem.* **74,** 923.
LYMN, R. W. & TAYLOR, E. W. (1971). *Biochemistry.* **10,** 4617.
RIZINO, A. A., BAROUCH, W. W., EISENBERG, E. & MOOS, C. (1970). *Biochemistry.* **9**, 2402.
TRENTHAM, D. R., BARDSLEY, R. C., ECCLESTON, J. F. & WEEDS, A. G. (1972). *Biochem. J.* **126,** 635.

JOURNAL OF THEORETICAL BIOLOGY, Vol. 64, №3, 7 February 1977

Contents

Solimano, F., Beretta, E. and Piatti, E., The Tree Graphs Theory for Enzymatic Reactions: a Theorem for the Reactions Among the Non-enzymatic Species……401
Kootsey, J. M., The Steady-state Finite Cable: Numerical Method for Non-linear Membrane 413
Ho, S. P. and Kostin, M. D., Kinetics and Diffusion in Multienzyme Systems……...421
Chizmadjev, Yu. A. and Aityan, S. KH., Ion Transport Across Sodium Channels in Biological Membranes ……429
Hewitt, J. A., On the Influence of Polyvalent Ligands on Membrane Curvature……455
Wright, R. H., Odor and Molecular Vibration: Neural Coding of Olfactory Information ……473
Crill, P. A., The Photosynthesis-light CURVE: A Simple Analog Model……503
Deshcherevsky, V. I., Kinetic Model of Regulation of Muscle Protein Activity……517
Homer, L. D. and Small, A., A Unified Theory for Estimation of Cardiac Output, Volumes of Distribution and Renal Clearances from Indicator Dilution Curves ……535
Shinagawa, Y., Donnan Potential in the Stationary State……551
Ortega Blake, I., A Molecular Mechanism for Transcription of a Closed DNA Molecule ……557
Vansickle, J., Analysis of a Distributed-parameter Population Model Based on Physiological Age……571
Lapidus, I. R., Rosen, B. and Hepperle, R., Secondary Structure of QβRNA……587
LETTERS TO THE EDITOR
Green, P. B., On Growth and the Cell Cycle in Roots……597
Blum, J. J., On the Geometry of Four-dimensions and the Relationship Between Metabolism and Body Mass ……599

Printed in Great Britain by The Whitefriars Press Ltd., London and Tonbridge