TENSION FLUCTUATIONS IN CONTRACTING MYOFIBRILS AND THEIR INTERPRETATION

JULIAN BOREJDO, *Polymer Department, Weizmann Institute of Science, Rehovot, Israel and Cardiovascular Research Institute, University of California, San Francisco, California 94143 U.S.A.*

ABSTRACT A self-consistent cycling steady-state model of contracting muscle is postulated to relate the autocorrelation functions of force fluctuations to the kinetic constants governing the operation of the cross-bridges. The fluctuations in the concentration of various intermediates in the model are due to the probabilistic nature of the transitions between states. It is shown that the decay rate of the autocorrelation of fluctuations in force is dependent on, and only on, the two rate constants governing transitions between attached states, and hence that the experimental autocorrelation functions can be used to estimate these rate constants. The model relates the time behavior of fluctuations in the concentration of any pair of enzymatic intermediates through the cross-correlation functions of fluctuations, and thus suggests a way to establish experimentally whether coupling exists between enzymatic and mechanical events during muscle contraction.

INTRODUCTION

In the last 40 years great progress has been made toward understanding contraction in "molecular" terms. However, some fundamental difficulties stand in the way of a thoroughly satisfying explanation of contraction. One of these is to establish the "cross-bridge theory" by decisive experimentation. According to this theory, appendages of myosin molecules known as "cross-bridges," driven by their own ATPase chemistry, cyclically deliver mechanical impulses to actin filaments; the time average of such impulses is the contractile force.

It is not difficult, in principle, to think of ways in which the cyclic operation of one cross-bridge might be sensed. For example, by x-ray diffraction or fluorescence polarization methods one might follow cyclic behavior of its attitude in space, or by a chemical probe one might record the cyclic reappearance of a particular enzymatic intermediate. But most practical instruments are insensitive to single molecules. When one resorts to observing large ensembles of cross-bridges operating asynchronously, i.e., nonentrained cross-bridges, one records ensemble averages, unchanging in time. The fundamental difficulty, then, is that such averages convey no information allowing one to deduce that there are underlying cyclic events, and to establish their kinetic behavior; time-independent averages are also to be expected if there is no cycling at all. In this paper it will be shown that an escape from the dilemma may be to record and to analyze fluctuations in cross-bridge-related parameters during the steady state of muscle contraction. Plausible alternatives, e.g., "relaxation" methods, are mathematically related to the fluctuation approach through the fluctuation-dissipation theorem of statistical physics (cf. Stevens, 1975).

Fluctuation (or correlation) spectroscopy, the experimental technique relying on the extraction of the kinetic information from the random fluctuations of measured signals, has

been increasingly recognized as an important biophysical tool. The measurement of voltage and current fluctuations has in fact been routinely used to study the behavior of ionic channels in membranes (Katz and Miledi, 1972*a*,*b*; Fishman, 1973; Varveen and DeFelice, 1974), and more recently, the measurement of concentration fluctuations has been successfully adapted to the study of reaction kinetics of simple chemical reactions (Magde et al., 1974; Feher and Weissman, 1973). The theoretical work of Chen and Hill (1973) and Hill (1974, 1975) has paved the way for the application of fluctuation analysis to active muscle. Chen and Hill (1973) and Chen (1973) considered multistate systems in which the transition between any two states follows first-order kinetics. They have derived the power spectra and the autocorrelation functions of fluctuations in the concentration of any intermediate in equilibrium systems in terms of the rate constants, and later Chen (1975*a*) has extended the analysis to systems in a "cycling" steady state.

This paper is an attempt to compute the stochastic behavior of a cycling steady-state model of muscle with the aim of relating the parameters characterizing this stochastic behavior (such as the autocorrelation functions) to the phenomenological coefficients governing the kinetics of the model. The muscle model is based on the available structural, physiological, and biochemical data. The modeling procedure simulates the time evolution of the entire ensemble of cross-bridges and is an economical alternative to the Monte Carlo approach (Brokaw, 1976; Borejdo and Morales, 1977). The model predicts the shape of the correlation functions of fluctuations in the concentrations of all chemical intermediates, as well as force and ATPase rate. It is shown that the force autocorrelation function depends mainly on two rate constants governing transitions between the two attached (force generating) states. The cross-correlation functions relating the time dependence of fluctuations in the concentration of any pair of enzymatic intermediates, as well as force and ATPase activity, are computed. Thus the model shows how the experimental data can be interpreted to extract the specific kinetic constants, and how to decide whether coupling exists between enzymatic and mechanical events during muscle contraction.

KINETIC MODEL OF MUSCLE

Mechanochemical Cycle

The reaction scheme in Fig. 1 *A* constitutes our biochemical assumptions; it is similar to the actomyosin cycles proposed by others (Chock et al., 1976; Sleep and Taylor, 1976; White and Taylor, 1976). We begin by noting that because the cross-bridges are fixed in the myofilament lattice the fluxes are independent of "concentration" of actin sites in this structure (Hill et al., 1975). We further assume that the concentrations of ATP, ADP, and phosphate (denoted hereafter as T, D, and P, respectively) are fixed, either by creatine kinase regeneration or (in the case of P) because the system is small relative to the container. Therefore, we assume that we deal only with "isomerization" reactions.

By the approach below we would now proceed to deduce the steady-state behavior of the model in Fig. 1 *A*. However, available information about the actomyosin cycle in solution suggests certain simplifications. Specifically, in vivo binding of ATP to actomyosin (step 6, Fig. 1 *A*) is probably very rapid, and is followed by fast dissociation (step 7) (Lymn and Taylor, 1971). We can therefore assume that the concentrations of AM and AMT are

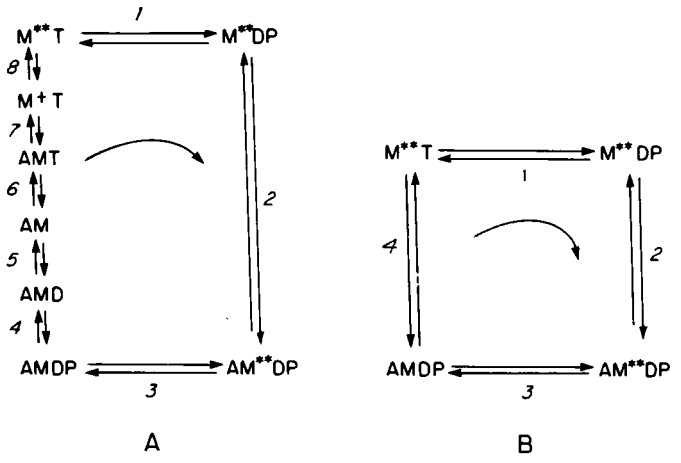

FIGURE 1 (*A*) Complete actomyosin ATPase cycle. Numbers identify individual transitions, M, A, T, D, and P represent myosin, actin, ATP, ADP, and orthophosphate, respectively. Superscripts of M represent different conformational states of myosin. For simplicity, free A, T, D, and P are not depicted, but of course must be included in the apparent "isomerization" rate constants. (*B*) Simplified actomyosin ATPase cycle. In the text various intermediates are identified by state numbers as follows: M**T, 1; M**DP, 2; AM**DP, 3; AMDP, 4.

negligible compared with those of other intermediates. For simplicity, and because little is known about the sequence of product desorption, we shall not differentiate between dissociation of P and ADP from actomyosin and so shall lump steps 4 and 5 together under the heading of product dissociation. Further, it has recently been suggested that the presumed intermediate M^+T may not be necessary to account for the experimental results (Johnson and Taylor, 1978). These considerations lead to the simpler model shown in Fig. 1 *B* which will serve as a basis for our discussion.

We recognize that experimental evidence can also be interpreted by suggesting that in the state M**DP myosin head finds itself in a conformation unable to bind to actin (Eisenberg et al., 1972; Eisenberg and Kielley, 1972; Fraser et al., 1975), and that the transition to a new state (M^fDP) in which myosin is able to bind to actin is rate limiting in the overall cycle (Chock et al., 1976). For reasons of simplicity this state is not included in the present model. Because the total number of cross-bridges in the dissociated states is greater than the number of attached cross-bridges (see below), the model is consistent with the observations that led to the postulation of the refractory state.

The transition to the state M**DP is followed by binding of the cross-bridge to actin. Reedy et al. (1965) showed that the visible cross-bridge (in current terms the S-1 moiety of myosin) makes a roughly 90° angle with the filament axis in relaxation and a 45° angle in rigor; furthermore, there is evidence that when the S-1 moiety is bound to actin while in a state analagous to AM**DP its attitudinal angle, θ, is still 90° (dos Remedios et al., 1972; see also Eisenberg and Hill, 1978). We will therefore identify state AM**DP with 90° conformation. No states with θ intermediate between 45° have been observed, but in the popular Huxley-Simmons (1971) hypothesis it is assumed that in thrust S-1 "rolls" on actin, thus passing perhaps through a succession of attitudinal states from 90 to 45°. The final

attitudinal state, corresponding to the rigor conformation (=45°, Reedy et al., 1965; Borejdo and Putnam, 1977) is identified here with the state AMDP.

The Rate Constants

In biochemical models such as the one illustrated in Fig. 1, each state exists at, or near, its minimum free energy, and so only the transitions from the minimum of one state to the minimum of another can be measured. Therefore, in solution, there is only one kinetic cycle; this feature is common to all purely biochemical models (Lymn and Taylor, 1971; Sleep and Taylor, 1976). However, in vivo, the state of a cross-bridge must be specified by: (*a*) the occupants of the N-(nucleotide) and A-(actin) sites of the S-1 moiety; (*b*) the attitudinal angle, θ, of S-1 with respect to the fiber axis; and (*c*) the axial stretch or compression, x, suffered by an elasticity which we assume, following Huxley and Simmons (1971), is residing in the cross-bridge structure, e.g., in S-2. The magnitude of an equilibrium constant describing transitions between two states depends upon their free energies. In the most general case of considering a state to be specified by the three foregoing variables, the multiplicity of rate constants would be so enormous as to preclude simulation and modeling, so approximations have to be made. There is evidence (dos Remedios et al., 1972) that θ is the same for all detached states irrespective of N-site occupancy, and (actually without evidence) most current workers assume tacitly that for attached states occupancy determines θ; in this way one of the three variables disappears. On the other hand, following the theoretical formalism of Hill (1974, 1975), there is general recognition that x cannot be neglected for attached states. Basically, this requirement arises because of the mismatch in the axial spacings of thick and thin filaments. At any overlap, this structural feature causes the tip of each S-1 to be at a variable axial distance from the nearest binding site on the adjacent thin filament.

Hill's formalism demands that, for reasons of self-consistency, the transitions between states involving attached states vary with x in a nonarbitrary fashion. The ratio of rate

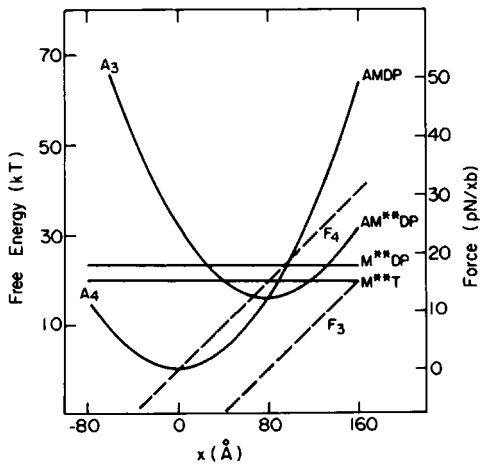

FIGURE 2 Free energy profile for the cross-bridge model shown in Fig. 1 *B*. The abscissa shows the axial position of the cross-bridge. The broken lines indicate forces associated with either attached state (force = dA/dx). The expressions for free energy profiles are: $A_3/kT = 16 + (x\text{-}80)^2/400$; $A_4/kT = x^2/400$ when x is expressed in ångströms.

constants defining transitions between states i and j at any x must be related to the free energy difference $A_i - A_j$ between these states (Hill, 1977). The force developed by a cross-bridge in a given attached state at a given x must be equal to the gradient of free energy of that state at that x. Further, the product of the "isomerization equilibrium constants" around the ring of the kinetic scheme must equal unity, so if we write the ring product in terms of intrinsic equilibrium constants it must equal $e^{\Delta/RT}$, where $\Delta = \mu_{ADP} + \mu_{P_i} - \mu_{ATP}$, where μ is a chemical potential, R is the gas constant, and T is absolute temperature (Hill et al., 1975). It is therefore essential to specify the free energy curves for the various states in the model of Fig. 1, from which force and self-consistent rate constant dependence on x can be deduced. Dr. T. L. Hill (National Institutes of Health) has generously provided me with a set of appropriate x-dependent free energy and rate constant curves suitable for the model of Fig. 1. A complete set of free energy curves and corresponding force functions is shown in Fig. 2. Fig. 3 gives a set of rate constants consistent with the free energy curves. Thus, for example, at $x = 80$ Å, when there is no difference between free energies of attached states AM**DP and AMDP ($A_3 = A_4$), the rate constants k_3 and k_{-3} that define the transitions between these states are equal (cf. Fig. 3).

Numerical Methods

The specification of the kinetic scheme and assignment of the rate constants determine the behavior of the model. The set of linear differential equations describing the system can be solved at each x and then integrated numerically to average over x. Because of the complexity of x-dependence it was preferred here to adopt an alternative method in which the behavior of the system is simulated on the digital computer. Simulations of contractile behavior have often been made on two-state models using the Monte Carlo method, i.e., by tracing the

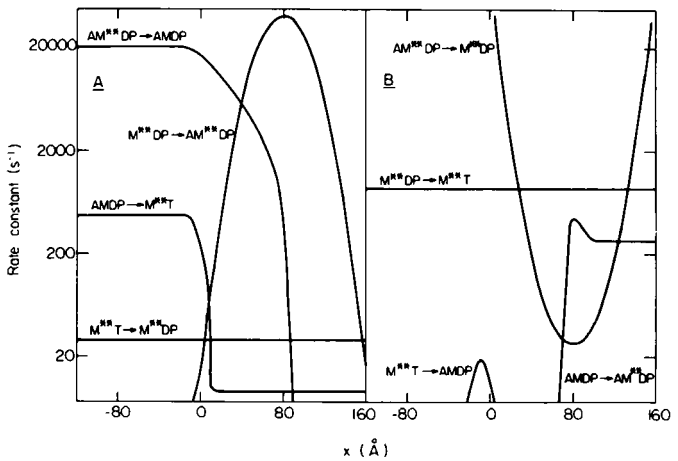

FIGURE 3 Dependence of the rate constants on the axial position of the cross-bridge. (*A*) Forward rate constants. (*B*) Reverse rate constants. The expressions for various rate constants (kindly supplied by Dr. T. L. Hill) are: M**T → M**DP, $k_1 = 28\ s^{-1}$; M**DP → M**T, $k_1 = 842\ s^{-1}$; M**DP → AM**DP, $k_2 = 1{,}052 \exp\{-[(x - 80)^2/800] + 3.7\}s^{-1}$; AM**DP → M**DP, $k_{-2} = 1{,}052 \exp\{+[(x - 80)^2/800] - 3.7\}s^{-1}$; AM**DP → AMDP, k_3 is not an analytical expression; AMDP → AM**DP, k_{-3} is not an analytical expression; AMDP → M**T, $k_4 = 456\ s^{-1}$ for $x < -10$ Å, $8.9\ s^{-1}$ for $x > 10$ Å; and M**T → AMDP, $k_{-4} = k_4/\exp\{3 + [x^2/400]\}$.

individual histories of a limited number of cross-bridges (e.g., Brokaw, 1976; Borejdo and Morales, 1977). When a cross-bridge is permitted to be in any one of the many states (e.g., 4 as proposed here) suggested by chemical information, the application of the Monte Carlo method soon exceeds the capabilities of a small computer such as ours. So, in this work we are introducing a new approach suggested to us by Dr. H. M. Martinez of the University of California, San Francisco. As usual, the evolution of the jth molecular species during the interval, $t \rightarrow t + \Delta t$, is given by the recursion

$$N_j(t + \Delta t) - N_j(t) = \delta_{j-1,j} - \delta_{j,j-1} - \delta_{j,j+1} + \delta_{j+1,j} \tag{1}$$

where

$$\delta_{j-1,j} = N_{j-1}(t) k_{j-1,j} \Delta t \tag{2}$$

and N_j is the number of cross-bridges in state j before transition. Deterministically, δ is a mean value, expressible in terms of a rate constant as in Eq. 2. What we do here to simulate stochastic behavior is to assume that an actual (as opposed to mean) δ is a member of a Poisson distribution. This distribution is characterized by only one parameter, namely the mean, so an actual δ is drawn by the computer from members of a Poisson distribution whose mean is that given by the deterministic expression, i.e., the frequency of drawing a particular size δ is the Poissonian probability of that δ (Knuth, 1969).

Validity of the stochastic algorithim has been tested by applying it to the following reactions whose steady-state response or power spectra were known a priori: radioactive decay, unimolecular isomerization reaction (Varveen and DeFelice, 1974), and four-state cycling steady-state reaction that exhibits "peaking" of the power spectrum (Chen, 1975*a*). In all cases accurate agreement with the expected results has been obtained.

The force developed by the model is computed as follows:

$$F(t) = f_3(t) + f_4(t), f_j(t) = \int F_j(x)\, N_j(x, t) \mathrm{d}x, j = 3,4. \tag{3}$$

In discrete approximation this reduces to:

$$F(t) = \sum_{j=3}^{4} \sum_{i=1}^{i=I} F_j(x_i)\, N_j(x_i, t). \tag{4}$$

N_j for each x is computed using recursion formula, Eq. 1, and F_j for each x is obtained from Fig. 2. The rate constants are assigned to each x using Fig. 3 as follows: the abscissa is subdivided into I equal intervals and the rate constant appropriate for each interval is taken as equal to the average value of the constant in that interval. The number of cross-bridges undergoing a cycle at a given value of x is assumed equal for each x interval.

The ATPase rate and the fraction of bound cross-bridges are:

$$R(t) = \sum_i [\delta_{4,1}(x_i, t) - \delta_{1,4}(x_i, t)] \tag{5}$$

$$S(t) = \sum_{j=3}^{4} \sum_i N_j(x_i, t). \tag{6}$$

The mean values of F, R, and S are obtained by solving analytically the kinetic system of Fig. 1 at every x. It can be shown that:[1]

$$R = \frac{\prod_{j=1}^{4} K_j - 1}{\sum_{j=1}^{4} \frac{1}{k_{-j}} \phi_j(k)} \tag{7}$$

where

$$\begin{aligned} \phi_1 &= 1 + K_2 + K_2K_3 + K_2K_3K_4 \\ \phi_2 &= 1 + K_3 + K_3K_4 + K_3K_4K_1 \\ &\vdots \\ \phi_4 &= 1 + K_1 + K_1K_2 + K_1K_2K_3 \end{aligned}$$

$$\text{and, } K_j = \frac{k_j}{k_{-j}} \tag{8}$$

are the equilibrium constants at given axial displacement x.

By hypothesis the steady-state force per cross-bridge and fraction of attached cross-bridges at given displacement x are:

$$F = \frac{F_3\xi_3(K) + F_4\xi_4(K)}{\sum_{j=1}^{4} \frac{1}{k_{-j}} \phi_j(K)} \tag{9}$$

$$S = \frac{\xi_3(K) + \xi_4(K)}{\sum_{j=1}^{4} \frac{1}{k_{-j}} \phi_j(K)} \tag{10}$$

where

$$\xi_3(K) = \frac{1}{k_{-2}} + \frac{K_2}{k_{-1}} + \frac{K_1K_2}{k_{-4}} + \frac{K_1K_2K_4}{k_{-3}} \tag{11}$$

$$\xi_4(K) = \frac{1}{k_{-3}} + \frac{K_3}{k_{-2}} + \frac{K_2K_3}{k_{-1}} + \frac{K_1K_2K_3}{k_{-4}}. \tag{12}$$

Fig. 4 illustrates the mean steady-state distribution of cross-bridges between various states at different axial position x, and the mean force per cross-bridge at different x. It is apparent that only those cross-bridges whose axial displacement is 20 Å $\leq x \leq$ 120 Å contribute with any significance to the force development. Beyond this range of x, most of the cross-bridges (>95%) are in state 1 (relaxed configuration).

The computed concentrations of all intermediates as well as force and ATPase rate

[1]These are special cases of general formulae for cycling first-order reactions of N species that were obtained by conventional methods. The diagram method of Hill (1977) can be used to obtain the same result.

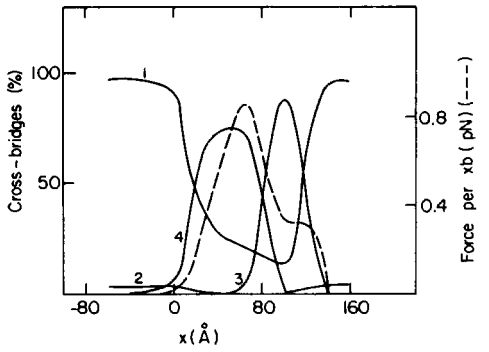

FIGURE 4 Distribution of cross-bridges among various states at different axial distances x in a model of Fig. 4 *B* with the rate constants of Fig. 6. The broken line is force developed by cross-bridges cycling at a given value of x.

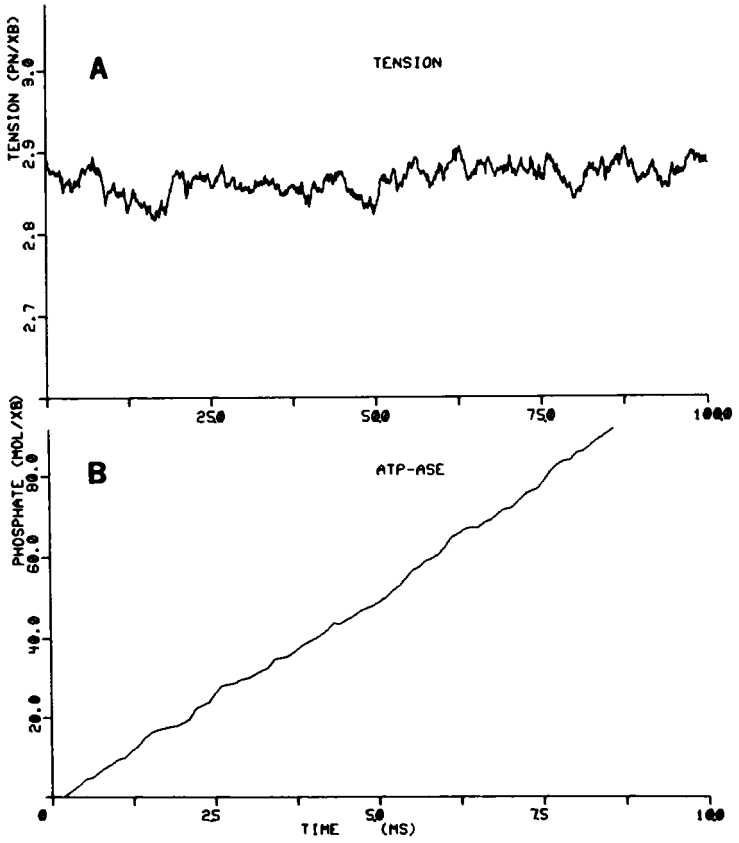

FIGURE 5 (*A*) Isometric tension as a function of time for the kinetic model with the rate constants shown in Fig. 6. (*B*) Steady-state ATPase rate.

generated at each time step are stored in random files in blocks of 1,024 points on the computer disk. The deviations of each file are computed to create the sequence of numbers with zero mean. The amplitude spectra were computed by applying a fast Fourier transform algorithm (FFT, Cooley-Tukey algorithm; Rabiner and Gold, 1975) to each block of 1,024 points. 100 spectra were squared and averaged to obtain the power spectrum of fluctuations. The spectra were smoothed by computing the autocorrelation functions of the original data sequence and applying a triangular Barlett window to the first and last 256 points of the autocorrelation function, as previously described (Borejdo and Morales, 1977). The autocorrelation function was Fourier-transformed to yield the smoothed power density spectrum. The cross-power spectra were computed by taking FFT of both data sequences and multiplying one resulting sequence by the complex conjugate of the other. The cross-correlation function is an inverse Fourier transform of the cross-power spectrum. The reported results always refer to the steady state of the system. The initial ($t = 0$) conditions consist in having all the cross-bridges in the M**T state, i.e., they are in a relaxed condition. Under isometric conditions the steady state was well established in 0.3 s.

All computations were performed on a Data General Eclipse S/200 computer (Data General Corp., Westboro, Mass.). Typical parameters used in calculations were: total number of cross-bridges, 50,000; the range of x, $-40 \leq x \leq 140$; $I = 10$, 10^{-5} s $\leq \Delta t \leq 10^{-4}$ s.

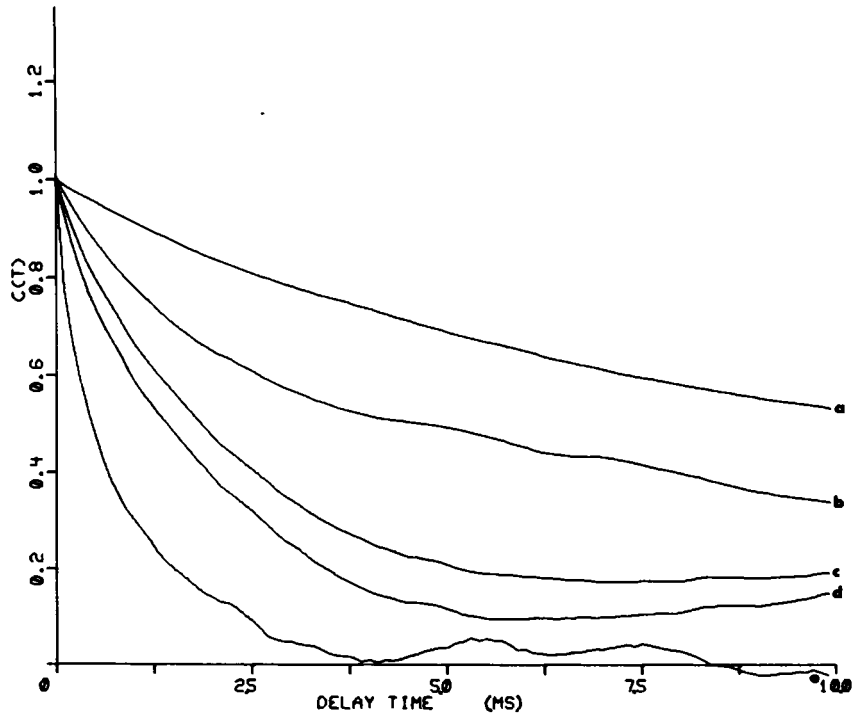

FIGURE 6 Normalized autocorrelation functions of the fluctuations in the concentration of various intermediates, force, and ATPase. (*a*) ATPase rate. (*b*) State 1. (*c*) State 4. (*d*) Mechanical force. (*e*) State 2.

Stochastic Behavior of the Model

Fig. 5 *A* shows the time evolution of tension computed through Eq. 3. The mean isometric force per cross-bridge and the root mean square fluctuation in force are 2.867 ± 0.038 pN. This compares favorably with the value of 4.5 pN estimated on the basis of published structural and physiological measurements by Oplatka (1972), 2 pN per attached cross-bridge estimated by Huxley and Simmons (1971), and 1.6 pN calculated by Brokaw (1976) using the Monte Carlo simulation algorithm.

Fig. 5 *B* shows the time evolution of the flux (ATPase) during the steady state. The normalized autocorrelation functions of fluctuations in concentration of states 1, 2, 4, force, and ATPase are shown in Fig. 6. Some of these autocorrelation functions are shown on the extended time scale in Fig. 7.

In general, the decay rate of the autocorrelation function is related to the combination of all the rate constants in the chemical scheme (Hill, 1975). However, it can be shown that in our scheme the decay of the autocorrelation function of force fluctuations is dominated by a pair of specific rate constants. Fig. 8 shows the effect of different rate constants on the decay time of the force autocorrelation function. Curve *e* in Fig. 8 is the autocorrelation function obtained from the model with the rate constants of Fig. 3. Scaling the rate constants k_3 and k_{-3} down 10 times for every x-value (and thus preserving the self-consistency of the model because their ratio, and hence the difference in free energies between attached states, is unchanged) results in the force autocorrelation function shown as curve *a*, decaying significantly slower than the original autocorrelation function. In sharp contrast, the change in k_1 and k_{-1}, k_2 and k_{-2}, or

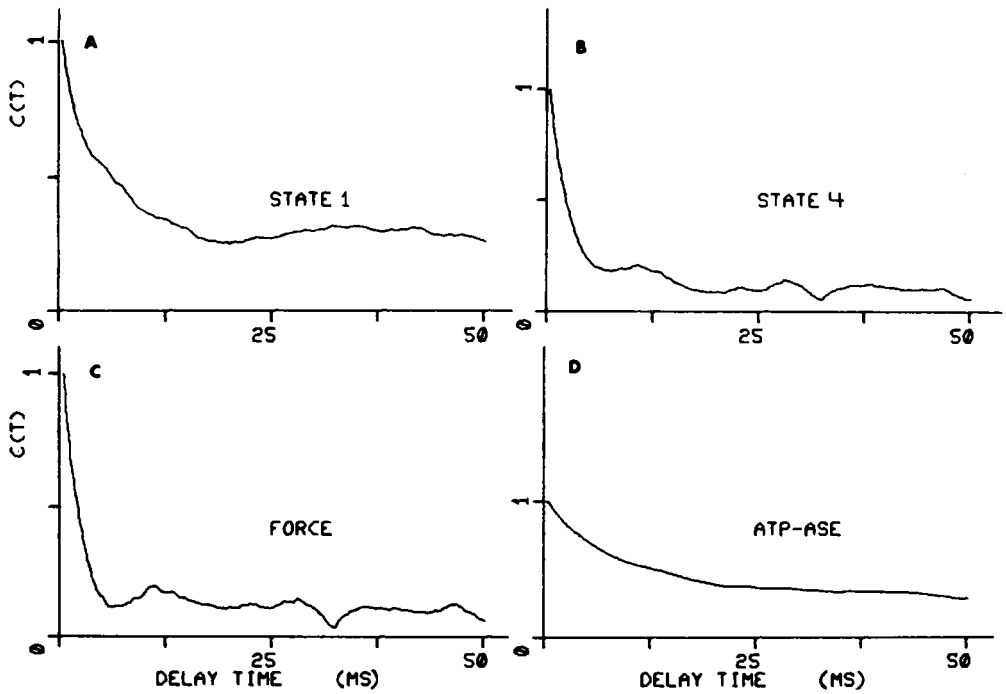

FIGURE 7 Autocorrelation functions of fluctuations in concentration of cross-bridges in state 1 (*A*), state 4 (*B*), and of mechanical force (*C*), and ATPase rate (*D*), shown on a slow time scale.

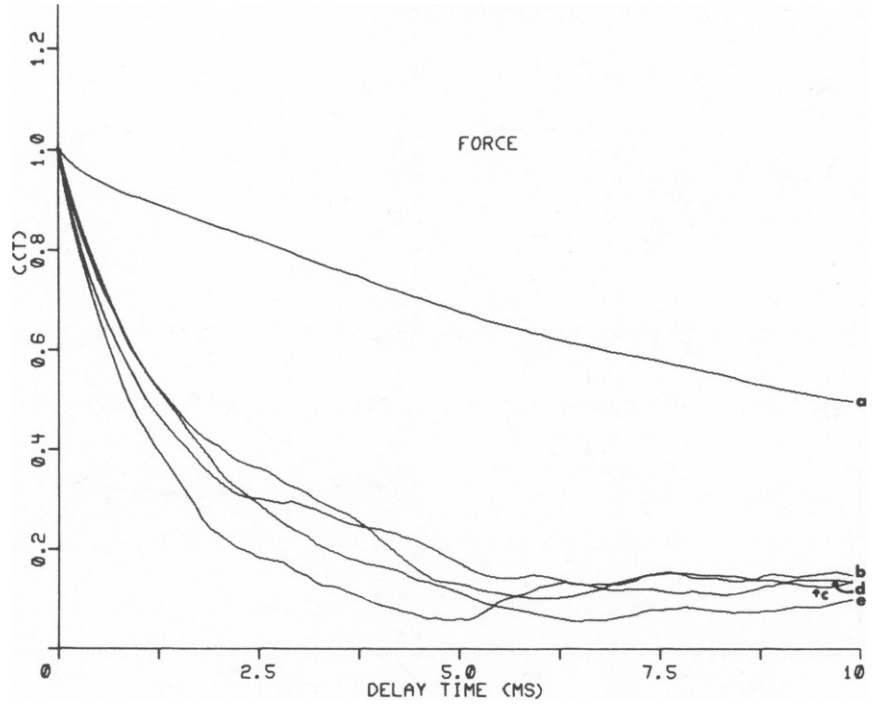

FIGURE 8 The influence of different rate constants on the autocorrelation function of fluctuations in mechanical force. Curve *e* is the original autocorrelation function with the rate constants such as the ones shown in Fig. 6. In curve *a* the shape of the k_3 and $k_{-3} - x$ dependence has been preserved but the magnitudes have been scaled down 10 times for each x. In curve *b* the same has been done to the rate constants k_4 and k_{-4}. In curve *c* the rate constants k_1 and k_{-1} have been similarly modified; in curve *d* the rate constants k_2 and k_{-2} have also been decreased by a factor of 10. The curves have been normalized.

k_4 and k_{-4} by the same factor of 10 has no significant effect on the decay time of force autocorrelation function as shown by curves *c*, *d*, and *b*, respectively. Thus in the case of force fluctuations it is the rate constants describing the transitions between attached states that are necessary and sufficient to completely determine the decay rate of the force autocorrelation function. (In general, the decay of the autocorrelation function of fluctuations in the concentration of a given intermediate is dominated by the rate constants characterizing the transitions to and from this intermediate.) This property of the force autocorrelation function is of practical value in determining the rate constants from the stochastic experimental data. In general, eight independent measurements monitoring concentrations of different intermediates in the cycle of Fig. 1 are necessary to evaluate the rate parameters of the reactions comprising the kinetic system (Chen, 1975*b*). Fig. 8 demonstrates, however, that the individual rate constants k_3 and k_{-3} can at least be estimated by making a single type of measurement.

The cross-correlation function that relates the time behavior of one type of intermediate in the model to the behavior of other intermediates is another useful measurable quantity because it provides information about the degree of coupling between different enzymatic and mechanical states of the cross-bridges. Fig. 9 *A* shows the cross-correlation function between the ATPase rate and the mechanical force. It is apparent that the two are strongly correlated

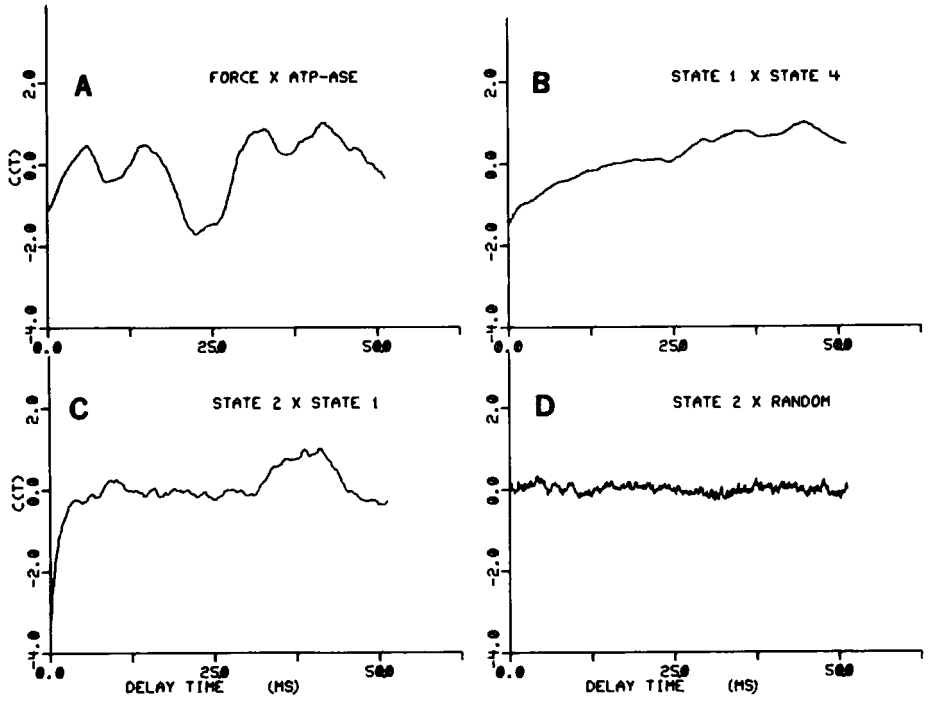

FIGURE 9 Cross-correlation functions of fluctuations in the occupancy of different enzymatic states of Fig. 4 *B*. (*A*) Mechanical force and the steady-state ATPase rate. (*B*) State 1 × state 4. (*C*) State 1 and state 2. (*D*) State 2 × random signal with the rectangular probability distribution. The rate constants are as in Fig. 6. In this figure the correlation functions are normalized such that the ordinates are directly comparable.

in a millisecond time scale (cross-correlation function of two uncorrelated signals is zero everywhere, cf., Fig. 9 *D*). Strong correlations of varying shapes were observed between any pair of intermediates. Figs. 9 *B* and *C* are further examples of cross-correlation: between fluctuations in the concentration of state 1 and state 4, and state 1 and state 2.

It should be emphasized that to make the chemical model mathematically tractable we have dealt with a system of isomerizations. This has been achieved by assuming that the concentrations of such ligands as T, D, and P are strictly constant. In this way nonlinearities (products of concentration variables) in the mathematical descriptions have been avoided. Such simplification is accomplished at a price. Obviously, we are missing the effect of fluctuations in [T], [D], and [P], but the avoidance of nonlinearities will not influence results dramatically, the greatest effect being only on the magnitude of fluctuations in the concentration of the relevant chemical intermediate, mechanical force, or ATPase rate.[2]

[2]Another consequence of the linear approximation is that in equilibrium sustained oscillations will not occur (Hearon, 1963; Chen and Hill, 1973; Chen, 1973), although they can appear in steady state (Chen, 1975*b*). Oscillations may then result in the "peaking" of the power density spectra. Mathematically such peaking arises because the eigenvalues of the matrix constructed from the set of linear differential equations describing the kinetic system can be complex. Since for linear kinetic systems the real parts of the eigenvalues are always negative (Hearon, 1963), the special solution of the system (the autocorrelation function) may show damped oscillations, and thus the power spectrum may exhibit peaking.

DISCUSSION

The motivation behind the present attempt to simulate the time evolution of the series of enzymatic intermediates characterizing the state of myosin cross-bridge, muscle force, and ATPase activity, was the advent of experimental techniques that make it practical to measure fluctuations in the cross-bridge related parameters during muscle contraction (Borejdo and Morales, 1977). At present these techniques are limited to the frequency range that is slower by a factor of 20 than the range that was shown here to contain important information about kinetics of the system. Nevertheless, with the prospect of faster measurements in sight (Borejdo et al., 1979), it seems necessary to provide the theoretical basis for the interpretation of such data.

The present approach introduces a number of simplifications that allow one to numerically treat the muscle as a linear kinetic system. An important feature of this approach is that it is possible to assign a nonarbitrary shape and magnitude to the x-distribution of the rate constants governing the transitions between different states in the kinetic cycle. A stochastic simulation algorithm used to compute the time evolutions of the simplified system is characterized by a considerable time efficiency. In contrast to Monte Carlo simulations, the algorithm pays no attention to the behavior of the individual cross-bridge and instead emphasizes the statistical properties of the entire ensemble of cross-bridges in a given state. The resulting time efficiency allows one to explore the stochastic behavior of all the intermediates in the cycle and their relative time relationship expressed through the cross-correlation functions.

The Fourier analysis of fluctuations yielded the autocorrelation functions of fluctuations in concentrations of all intermediate cross-bridge populations, force, and ATPase activity. It was found that the rate of decay of the autocorrelation function of fluctuations in the concentration of given cross-bridge population is determined chiefly by the rate constants governing the transitions to and from this population. In the case of muscle force fluctuations, that means that once such fluctuations can be recorded with the sufficient time resolution, the rate of transition between force generating (attached) states can be determined. It is important to point out that the rate constants determined through the fluctuation approach are equal to the "isometric" rate constants extrapolated from the perturbation experiments that monitor the transients in muscle length or tension (associated with the relaxation of the number of cross-bridges into the second force-generating state) following rapidly applied change in force (Podolsky, 1960; Civan and Podolsky, 1966) or length (Huxley and Simmons, 1971). This is a consequence of the fluctuation-dissipation theorem (cf. Stevens, 1975) that pronounces the equivalence between the time dependence of the autocorrelation function of steady-state fluctuations in the concentration of given molecular species and the relaxation of applied concentration perturbation. The advantage of the fluctuation approach is that it can be applied in the steady state without perturbing the system and thus without the need to extrapolate the perturbed rate constants to the isometric case.

The deduction about the rate constants based on the above approach suffers because the conclusions are model dependent. Further, one obtains information only about x-averaged rate constants. Of course, the analysis of experimental isometric and isotonic transients for the purpose of extracting kinetic data also involves averaging over x.

The demonstration that the cross-correlation functions are nonzero (Fig. 9) is a direct

consequence of the assumption that the mechanical and enzymatic events are coupled to each other. This need not always be the case; it is possible to suggest models which make no such assumption. By making a detailed prediction of the shape of the specific cross-correlation functions, the model suggests a way to experimentally confirm or refute the assumption that the enzymatic and mechanical events are coupled in the process of muscle contraction.

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