HEAD ROTATION OR DISSOCIATION?

A Study of Exponential Rate Processes in Chemically Skinned Rabbit Muscle Fibers When MgATP Concentration is Changed

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ABSTRACT The mechanical response of fully activated muscle bundles (one to five fibers) to sinusoidal length perturbation (~0.4% L_0) was studied as a function of MgATP concentration. The frequency response (0.25–167 Hz; corresponding to 1 ms time resolution) of chemically skinned rabbit muscle fibers was resolved into three exponential rate processes, (A), (B), and (C). At 20°C, the apparent rate constants associated with the fast exponential lead ($2\pi c$ = 388–588 s^{-1}) and the oscillatory work ($2\pi b$ = 59–116 s^{-1}) both increase with increment of the MgATP concentration from 1 to 5 mM, and they both saturate for further increase. Over the whole range of MgATP concentrations the slow exponential lead ($2\pi a$ = 9–7 s^{-1}) remains constant. The effect of MgATP on processes (B) and (C) can be interpreted in the context of the biochemical evidence, in which MgATP enters the cross-bridge cycle after the desorption of the product, and the binding of MgATP to rigorlike cross-bridges promotes a rapid dissociation of actomyosin (Lymn and Taylor, 1971. *Biochemistry.* **10**:4617–4624.). The effect is not predicted by a model for force generation in which head rotation dominates the fast component ("stage 2" of Huxley and Simmons, 1971. *Nature (Lond.).* **233**:533–538. and 1973. *Cold Spring Harbor Symp. Quant. Biol.* **37**:669–680.), and head dissociation dominates the slow component ("phase 4" of Huxley, 1974. *J. Physiol. (Lond.).* **243**:1–43; Julian et al., 1974. *Biophys. J.* **14**: 546–562.).

A recent publication by Abbott and Steiger (1977) argued that the time-course of early tension relaxation ("phase 2" of Huxley, 1974) after a step length change in muscle consists of two phases (rate constants K_1 and K_2), and that neither of them are correlated with the force generation stage or head rotation as originally proposed by Huxley and Simmons (1971). Abbott and Steiger further argued that K_2 (above) and K_3 (rate constant for the delayed tension) are both correlated with head dissociation reactions based on their temperature sensitivity.

If the interpretation of Abbott and Steiger is correct, then the corresponding rate constants of the fast exponential lead (labeled $2\pi c$ by Kawai et al., 1977) and of the oscillatory work ($2\pi b$), resolved by sinusoidal analysis, will be correlated with MgATP concentration. This is expected because binding of this molecule precedes head dis-

sociation in biochemical studies (Lymn and Taylor, 1971). The experimental evidence presented in this report is in clear accord with this expectation.[1]

Rabbit psoas muscle is employed for this purpose, because many biochemical studies utilize this material. Rabbit psoas bundles were tied to wooden sticks at body length before they were cut free from the muscle. They were skinned chemically in a saline containing 5 mM EGTA, 2 mM MgATP, 180 mM K propionate, and 5 mM imidazole (pH 7.00) for 24 h at 4°C, as described by Wood et al. (1975) for biopsies of human muscle. After the chemical skinning procedure, the preparation can be kept at −20°C in an iso-ionic glycerol saline (skinning saline + 6 M glycerol) for many months without any loss in activity. However, for the purpose of this report, fresh preparations 1–5 days old were used. Small bundles (one to five fibers) were dissected and mounted in an apparatus described by Kawai et al. (1977) and bathed in relaxing saline (same as the skinning saline). The sarcomere length was measured by optical diffraction techniques (Kawai and Kuntz, 1973) and adjusted to 2.5–2.7 μm. Saline surrounding the preparation was vigorously stirred to regulate temperature in the bath (20 ± 0.5°C) and to maintain homogeneous concentrations of ionic constituents.

Fibers were presoaked in the desired experimental saline without added Ca (solution conditions in Fig. 1 legend) for at least 3 min. They remained relaxed in this saline and registered little stiffness. The fibers were then activated by injecting $CaCl_2$ into the saline to achieve a final concentration of 2.4 mM (free Ca is approximately 100 μM). The solutions were all adjusted to maintain pH 7.00. As soon as steady tension had developed, the computer was triggered and complex-stiffness data $Y(f)$ (a frequency response function relating length to tension and defined by Kawai et al., 1977) was collected at 16 frequencies, ranging from 0.25 to 167 Hz in 22 s. The peak-to-peak amplitude of the length oscillation was about 0.4% of L_0 (muscle length), which corresponds to ±26 Å movement per half sarcomere. The preparation was subsequently relaxed and this procedure was repeated for different MgATP concentrations, with ionic strength and Na concentration kept constant. Typical complex-stiffness data at 1 mM MgATP (1S) and at 5 mM (5S) are displayed as Nyquist plots in Fig. 1 A. Fig. 1 B is a plot of the phase vs. frequency of the same data.

In active muscles we have established (Kawai and Brandt, 1975; Kawai and Orentlicher, 1976; Kawai et al., 1977) that tension response to sinusoidal length change can be resolved into the sum of three exponential rate processes (A), (B), and (C) over the frequency range used (see Eq. 1 below). These three processes represent active cycling of cross-bridges, since all of them disappear when the muscles go into rigor or into relaxation. We find that these three processes are universally present in various striated muscle types, including intact and skinned crayfish single fibers, frog semitendinosus muscles, and chemically skinned rabbit psoas preparations.

The transfer function of the sum of three exponential rate processes is

$$\underset{\text{(A)}}{} \quad \underset{\text{(B)}}{} \quad \underset{\text{(C)}}{}$$

$$Y(f) = H + Afi/(a + fi) - Bfi/(b + fi) + Cfi/(c + fi) \qquad (1)$$

where a, b, and c are the characteristic frequencies ($a < b < c$) associated with processes, and 2π time of these are the apparent rate constants; A, B, and C are their re-

[1] A preliminary account of the present results was presented earlier (Kawai and Orentlicher, 1976). Effect of MgATP on rate constants was first noticed in skinned crayfish muscle preparations (Kawai and Brandt, 1975, 1977).

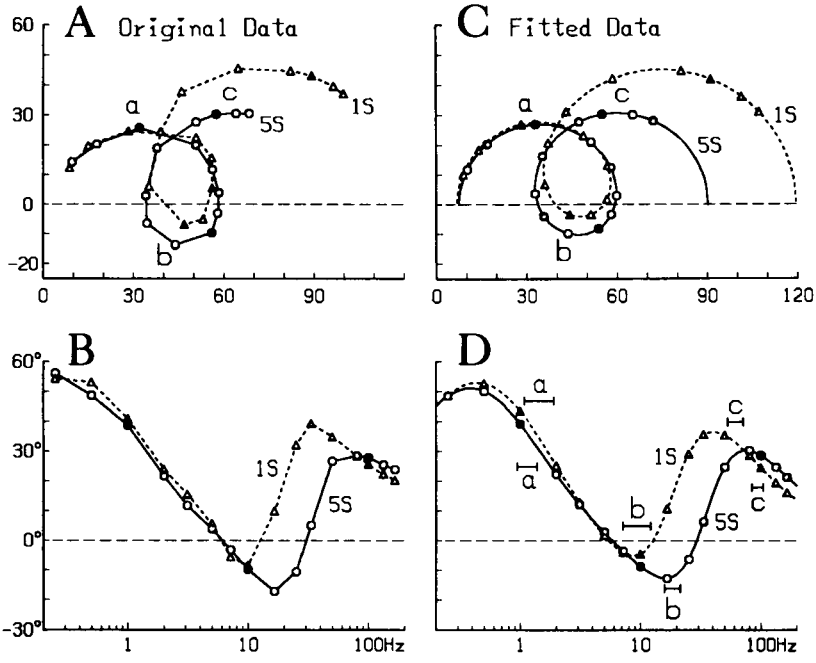

FIGURE 1 A, B: The complex stiffness data $Y(f)$ at 1 mM MgATP (1S) and 5 mM (5S) are shown as Nyquist plots in A, and phase vs. frequency plots in B. Symbols represent actual measurements, and lines are drawn to connect them. Frequencies are (from left) 0.25, 0.5, *1*, 2, 3.13, 5, 7.14, *10*, 16.7, 25, 33, 50, 80, *100*, 133, 167 Hz (filled symbols are in italics). Isometric tension for 1S was 1.33 M dyn/cm^2, and for 5S was 1.14 M dyn/cm^2. C, D: The same sets of data are fitted to Eq. 1, and predicted values of $Y(f)$ are shown in the figure. In A and C, abscissa is the elastic modulus (real part of $Y(f) \cdot L_0$/area) and ordinate is the viscous modulus (imaginary part). Units in M dyn/cm^2. *a*, *b*, and *c* indicate characteristic frequencies corresponding to rate processes (A), (B), and (C). In B and D, the abscissa is frequency, and the ordinate is phase (*arg* (Y)). In D, horizontal bars labeled *a*, *b*, and *c*, represent locations of these parameters along the abscissa and their 95% confidence limits. The labels *a*, *b*, *c* over the bars belong to 1S and those under the bars to 5S. Note that process (A) is unchanged when MgATP concentration is increased, while (B) and (C) become faster. Solution condition for 1S was (in mM) 2.4 CaATP, 1.02 MgATP, 4.9 ATP, 7 phosphate, 20 creatine phosphate (all sodium salts), 4.8 NaCl, 26 Na_2SO_4, 5 imidazole, 74 U/ml creatine phosphokinase, pCa 4.0, pH 7.00. For 5S, 4.1 mM of Na_2SO_4 is replaced with Na_2MgATP, with pCa, pH, ionic strength (225 mM), Na^+, and free ATP concentrations kept constant. Temperature was 20 ± 0.5°C. A bundle of four fibers (1 day old) from the experiment of 12/22/75, preparation 1, records 5 and 12.

spective magnitudes, f is frequency, $i = \sqrt{-1}$, and H is a constant. This description of the complex-stiffness is obviously limited to the frequency range of use (0.25–167 Hz), which corresponds to the time interval of 1–700 ms in step analysis techniques.

It is important to correlate the above rate processes to those identified by step analyses. For this purpose the oscillatory work (B) serves an unambiguous marker, because of its unique polarity (negative sign in Eq. 1) and its universal presence in variety of active striated muscles (crayfish, frog, rabbit, insect, heart, and so on). The nega-

TABLE I
FITTED PARAMETERS AND CONFIDENCE RANGES

	S = 1 mM	S = 5 mM
H, $M\ dyn/cm^2$	7 ± 4	8 ± 2
A, $M\ dyn/cm^2$	67 ± 5	59 ± 3
B, $M\ dyn/cm^2$	59 ± 6	55 ± 4
C, $M\ dyn/cm^2$	104 ± 9	79 ± 6
$2\pi a, s^{-1}$	9.0 ± 2.6	7.1 ± 1.4
$2\pi b, s^{-1}$	59 ± 16	116 ± 18
$2\pi c, s^{-1}$	388 ± 59	588 ± 64

Confidence ranges (approximately 95%) are shown after $\pm$, and they were obtained by Fisher's F-statistics. Degrees of freedom are 23 in all cases.

tive polarity of (B) causes a delayed tension rise (decay) on step length increase (decrease), and thus it corresponds to K_3 of Abbott and Steiger (1977); or to phase 3 of Huxley (1974), Heinl et al. (1974), and Ford et al. (1977).[2]

The rate process (C) is defined as the next faster process than (B) and it is an exponential lead. Hence, it corresponds to phase 2 of Huxley and Simmons (1971) or Huxley (1974). It is still to be determined whether this is better treated as the sum of two exponential leads (K_1 and K_2 of Abbott and Steiger, 1977), four closely spaced ones (Ford et al., 1977), or in some other way.[3]

The slow exponential lead (A) resides between DC (0 Hz) and the oscillatory work (B). Thus its identification is not presently controversial, and process (A) corresponds to phase 4 of Huxley (1974), or K_4 of Abbott and Steiger (1977).

The above comparisons are useful when correlating data from a variety of muscle tissues under various experimental conditions. Even though mapping sinusoidal data to step data is not quantitatively exact, because of the nonlinearities associated with the rate processes, their qualitative features, such as polarity of the processes, and their relative order cannot change. The present rate processes obviously related to the length transients on step load change, such as observed by Podolsky (1960) or by Civan and Podolsky (1966). Since the relationship between length and tension transients is discussed by Huxley (1974) in detail, no further elaboration, at present, is needed.

The parameters of Table I were found after fitting the data of Fig. 1 A and B, and the calculated values of $Y(f)$ at corresponding frequencies are shown in Fig. 1 C and D. The characteristic frequencies a, b, and c are entered in Fig. 1 D as horizontal bars that indicate their 95% confidence ranges. It is seen from the plots that the phase is at a minimum and at a maximum around the characteristic frequencies b and c, respec-

[2] The phase 3 exhibits a plateau when magnitude of B is small, as shown by many of the step analysis data.

[3] If the phase 2 splits into multiple exponential rate processes, (C) should correspond to the slower components. The same argument holds for the "stage 2" of Huxley and Simmons (1971, 1973), and thus there is no ambiguity that (C) corresponds to the component on which Huxley and Simmons based their model.

tively. It is also seen from this data that both b and c increase as the MgATP concentration is elevated from 1 to 5 mM, and that process (A) remains constant (Fig. 1 C, D: Table I) within the confidence range. With the further increase in MgATP concentration up to 20 mM, there is little further change in any of the rate constants.[4]

This effect of MgATP does not depend on the choice of fitting function, (Eq. 1) nor depend on the fact that the data is collected only up to 167 Hz (corresponding to 1 ms time resolution in step analysis). This is because (*i*) 95% confidence ranges of the fitting parameters are narrow (Table I and Fig. 1 D) and (*ii*) without reference to the fitting equation the effect is clearly demonstrated in Fig. 1 B by the shift of the minimum (around 10 Hz) and maximum (around 50 Hz).

It might be supposed that the present phenomena are observed because of some nonlinear response of muscle to length change. The tension responses to step length changes are reported to be asymmetrical by Huxley and Simmons (1971), White and Thorson (1972), Heinl et al. (1974), Abbott and Steiger (1977), and Ford et al. (1977). In this sense the rate constants calculated after Eq. 1 must be sensitive to the total excursion of the length oscillation. This argument, however, does not invalidate the present conclusions, because the total excursion is kept constant, which permits a relative comparison of the data from various conditions such as different MgATP concentrations. Furthermore, the nonlinearity was detected at each frequency in a few experiments, and it is less than 3% in nonlinear power at frequencies around b (oscillatory work, cf. Steiger, 1971), and less than 0.3% at other frequencies including a and c. This magnitude of nonlinearity is small enough to validate sinusoidal analyses.

The dependence of the rate constants of MgATP concentration is not due to ATP depletion or ADP buildup in the core of the muscle preparations, since (*i*) an adequate buffering system (20 mM creatine phosphate, 74 U/ml creatine phosphokinase) was present and a large excess of ATP (total of 8.3–12.3 mM) is supplied; (*ii*) change in free ATP (not bound to Mg or Ca) concentration per se in the range 1–10 mM does not make any appreciable difference in the results, and (*iii*) change in the number of the fibers in a bundle between one and five does not make any difference in the results.

It is possible to interpret the effect of MgATP on the rate constants in the context of biochemical schemes such as those of Lymn and Taylor (1971), Chock et al. (1976), or Taylor and Sleep (1976). In all of these schemes substrate (MgATP) molecules bind to the myosin heads, which are in rigorlike linkages with actin, and this binding of MgATP promotes a rapid dissociation of myosin from the actin:

$$\text{A–M} + \text{S} \rightleftharpoons \text{A–MS} \rightleftharpoons \text{A} + \text{MS}$$

where S is substrate (MgATP), M is myosin, and A is actin.

When substrate concentration is low, the first reaction is rate-limiting, and thus the rate for overall transitions is proportionate to its concentration. At sufficiently high concentration, the second or other sequential reaction limits the transition rate. Since apparent rate constants b and c both increase with the substrate concentration and

[4] $2\pi b$ saturates at about 140 s^{-1}, and $2\pi c$ at 650 s^{-1}.

saturate, it is assumed that substrate binding and head dissociation reactions are represented in both of these observed processes (B) and (C).

The substrate effect on the apparent rate constant $2\pi c$ is not predicted by the model of Huxley and Simmons (1971, 1973), nor by an expanded version of this model by Julian et al. (1974). Examination of these models shows that they predict the slower processes (primarily (A) and in part (B)) will be affected by the dissociation reactions (cf. Huxley, 1974), while the fast process (C) will be unaffected. The present data are more consistent with the model of Abbott and Steiger (1977), in which head dissociation reaction dominates the faster rate constants K_2 and K_3.

In conclusion, the present studies show that both fast processes (B) and (C) represent substrate binding to actomyosin and the subsequent dissociation of the myosin head from actin, and conversely process (A) is not limited by dissociation reactions in the range of physiological substrate concentrations. This conclusion is based on the experimental evidence, and it does not depend on a particular model, nor the equations to which data are fitted.

The author wishes to thank Drs. P. W. Brandt and M. Orentlicher for their helpful suggestions in revising the manuscript, and to Dr. D. S. Wood for his preparations of chemically skinned rabbit muscle bundles.

The present work is supported by a grant from the National Science Foundation (PCM 76-00441) and grants to the H. Houston Merritt Clinical Research Center (NS 11766 and MDA).

Received for publication 13 August 1977 and in revised form 15 December 1977.

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