DIFFERENCES IN THE TRANSIENT RESPONSE OF FAST AND SLOW SKELETAL MUSCLE FIBERS

Correlations Between Complex Modulus and Myosin Light Chains

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ABSTRACT Sinusoidal analysis of the mechanochemical properties of skinned muscle fibers under conditions of maximal activation was applied to fibers from several rabbit skeletal muscles (psoas, tibialis anterior, extensor digitorum longus, diaphragm, soleus, semitendinosus). This investigation distinguished between two general classes of fibers, which on the basis of their myosin light chain complements could be classified as fast and slow. In fast fibers (e.g., psoas) we identified the presence of at least three exponential processes (A), (B), (C) of comparable magnitudes. In slow fibers (e.g., soleus) we identified the presence of at least four exponential processes (A)–(D) of very different magnitudes; magnitudes of processes (A) and (B) are very small compared with those of (C) and (D). The apparent rate constants are 8–29-fold slower in slow fibers. Because our sinusoidal characterization takes ≤22 s and does not involve chemical denaturation or other means of disruption of the myofilament lattice, it allows the different physiological classes of fibers to be characterized and then studied further by other techniques. The perfect correlation between physiological and molecular properties as assayed by gel electrophoresis after sinusoidal analysis demonstrates this and justifies its use in distinguishing between fiber types.

INTRODUCTION

In the past we have employed a transient analysis procedure using sinusoidal waveforms to characterize the kinetics of the cross-bridge cycle in fast skeletal muscles from several sources (Kawai and Brandt, 1980; Kawai, 1982). In these investigations we found, in active muscles, the presence of at least three exponential processes, which we designated (A), (B), and (C) in the order of slow to fast. Since these processes are absent in situations where there is no sizable cross-bridge cycling (rigor or relaxed state), and since their magnitudes and/or rate constants are functions of Ca^{2+} and $MgATP^{2-}$, which promote cycling, we concluded that the three processes arise from chemomechanical reactions of actively cycling cross-bridges. It can be shown by mathematical manipulations that the three processes (A), (B), and (C) correspond to phases 4, 3, and 2, respectively, of step analysis results of Huxley and Simmons (1971) or Huxley (1974).

In an attempt to further characterize these processes and associate them with known biochemical steps in the myosin cross-bridge cycle, we began an investigation of fibers from fast and slow twitch muscles. In particular, we want to know whether these processes are common to both fast and slow fibers and whether their response to variations in the levels of Ca^{2+}, $MgATP^{2-}$, and other ionic conditions are the same. In this report fibers were subjected to frequency response analysis under maximal activating conditions, and then classified as fast or slow on the basis of their myosin light chain complements (MLC) as revealed by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS). The frequency response data fell into two general classes: one identical to that previously observed in fast fibers, and a second that showed lower rate constants for all processes and reduced magnitudes of two slower processes. The MLC analysis demonstrated a perfect correlation between the fast response with fast MLC and the slow response with slow MLC. A preliminary account of the present study was presented at a Biophysical Society meeting (Kawai et al., 1983).

METHODS

Preparation of Fibers for Analysis

Fiber bundles (~20 mm in length and 1 mm in diam) tied to bamboo sticks at body length were excised from the psoas, extensor digitorum longus (EDL), tibialis anterior, semitendinosus, soleus, and diaphragm of

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3–5 kg New Zealand white rabbits killed by inhaling CO_2. The bundles were chemically skinned in a solution containing 5 mM K_2EGTA, 2 mM Na_2MgATP, 5 mM Na_2K_2ATP, 132 mM potassium propionate, 6 mM imidazole (pH 7.0) at 0°C, a skinning condition modified from Eastwood et al. (1979). Increase in free ATP to 5 mM facilitated integrity of the fibers. After 24 h in skinning solution, single fibers ~8 mm in length were dissected from the bundles and used for experiments. Fibers stored at 0°C in this solution for as long as 3 wk showed no changes in their mechanochemical properties and were used for experiments.

Transient Analysis of Fibers

Single fibers adjusted to a sarcomere length between 2.5 and 2.6 μm were activated in a standard solution containing (in millimoles per liter): 6 $K_2CaEGTA$, 5.1 Na_2MgATP, 5.17 Na_2K_2ATP, 8 potassium phosphate, 15 Na_2CP (creatine phosphate), 80 unit/ml CPK (CP kinase), 30 NaCl, 26 KCl, and 10 morpholinopropane sulfonic acid at 20°C. Depending on the experiment, a variation in the Na_2MgATP concentration was made and was substituted by NaCl/KCl so that the ionic strength was maintained at 200 mM and Na^+ concentration at 80 mM. The pCa of the solution was 5.0, and pH was adjusted to 7.00. After a constant tension developed, sinusoidal oscillations with peak-to-peak amplitude of 0.25% of fiber length were initiated by computer control as described earlier (Kawai and Brandt, 1980; Kawai, 1982). Data on tension and length time courses were collected and processed on-line to yield complex stiffness. The complex stiffness was then normalized for the size of the fiber (length and cross-sectional area) to yield the complex modulus. Its absolute value (dynamic modulus) is an indicator of the muscle stiffness at each frequency, and its argument (phase shift) is an indicator of the direction of net work flow. Various components of the complex modulus were displayed on a graphics terminal. This display enabled an experimenter to judge the fiber type. A frequency range of 0.067–250 Hz was used for slow twitch fibers (Fig. 1). A somewhat narrower range was used for fast twitch fibers (Fig. 2).

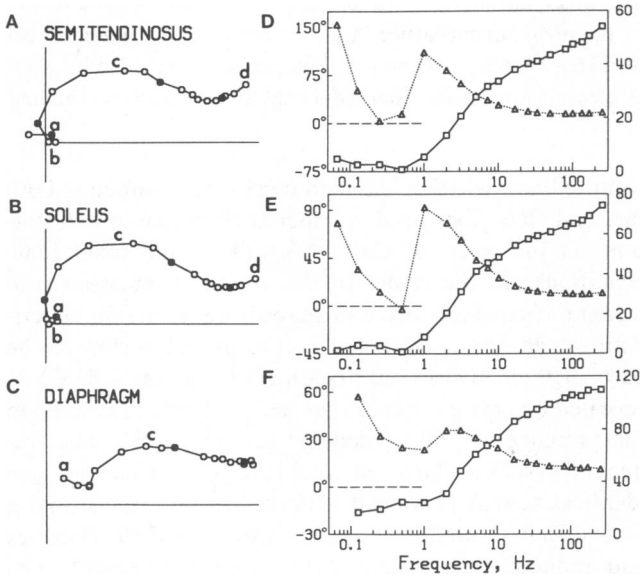

FIGURE 1 Complex modulus Y_M from slow twitch fibers of semitendinosus (*A, D*), soleus (*B, E*), and diaphragm (*C, F*). The data are shown in Nyquist plots (—○—), phase shift (equals arg $[Y_M]$) vs. frequency plots (· △ ·), and dynamic modulus (equals $|Y_M|$) vs. frequency plots (—□—). The center ordinates are phase shift in degrees, and the right ordinates are dynamic modulus in Mdyn/cm². In Nyquist plots (*A, B, C,*) axes are not labeled for visual simplicity. The abscissa is elastic modulus (real part of Y_M) and the ordinate viscous modulus (imaginary part of Y_M). Letters *a, b, c, d* represent characteristic frequencies and identify exponential processes. Frequencies used are (counterclockwise) 250, 167, 133, *100*, 80, 50, 33, 25, 17, *10*, 7.1, 5, 3, 2, *1*, 0.5, 0.25, *0.125*, 0.067 Hz (frequencies in italics correspond to ●). All were single fibers and activated in the standard solution (5 mM MgATP), except for *A*, which was activated in 10 mM MgATP.

SDS-Polyacrylamide Gel Electrophoresis (PAGE) Analysis of Contractile Proteins

Following transient analysis of single muscle fibers, they were prepared for electrophoresis in the following way. Fibers were removed from the apparatus, washed in a solution containing (in millimoles per liter): 100 NaCl, 25 Tris, 5 $MgCl_2$, 2 EGTA, 5 dithiothreitol, 0.5% (wt/vol) Triton X-100, 1 phenylmethylsulfonyl fluoride (pH 7.5), then dissolved in sample diluting buffer (25–50 μl), which contained 2% (wt/vol) SDS, 125 Tris, 2 EDTA, 5 dithiothreitol, and 10% (wt/vol) glycerol, with the pH adjusted to 6.8. As a precaution against proteolysis, both the wash and sample diluting solutions were supplemented with a broad spectrum of protease inhibitors (2 μg/ml each of leupeptin, antipain, chymostatin, and 10 units/ml of aprotinin). The samples were frozen at −20°C and shipped from New York to Durham on dry ice. They were then heated to 100°C for several minutes, cooled, and analyzed for their myosin light chain content by electrophoresis (at 18°C) in the presence of SDS on 10.5% polyacrylamide gels with a 30 to 1 ratio of acrylamide to *N,N'*-dimethylene-*bis*-acrylamide as described by Laemmli (1970). These were stained with silver (see Oakley et al., 1980). Gels were run on a Hoefer Scientific SE600 (Hoefer Scientific Instruments, San Francisco, CA) using a 20-well comb and 0.75 mm spacers. Storage and shipment of the dissolved fibers in the SDS solution supplemented with protease inhibitors at −20°C preserve the same patterns (for at least 6 mo) as observed on the gels electrophoresed immediately after sampling. Thus storage and shipment did not affect electrophoretic properties of myosin light chains (compare Fig. 3 with published gel patterns of Bronson and Schachat, 1982).

RESULTS

Sinusoidal Analysis Reveals Two Classes of Fibers

Since there is potentially a mixture of different fiber types in any muscle bundle (Pette and Schnez, 1977; Zeman and Wood, 1980), only single fibers were used for most of the experiments reported here. To assure that both fast- and slow-twitch fibers are analyzed, a variety of muscles whose histochemistry and/or biochemistry were described have been used (cf. Weeds et al., 1975; Pette and Schnez, 1977; Dhoot and Perry, 1979; Bronson and Schachat, 1982; Salviati et al., 1982).

Fibers were activated and complex modulus data were collected as described in Methods. The Nyquist plots shown in Figs. 1 *A–C*, 2 *A–D*, and frequency plots in Figs. 1 *D–F*, 2 *E–H* are results from such analyses. The transient kinetic behavior of slow fibers (based on MLC complements) from semitendinosus, soleus, and diaphragm can be seen in Fig. 1 and compared with fast fibers from diaphragm, EDL, tibialis anterior, and psoas in Fig. 2. It is evident from these figures that the complex modulus can be classified into two groups by the appearance of the Nyquist plots or by the position of the frequency plots.

In Nyquist plots the fast fibers (Fig. 2 *A–D*) have three clearly defined exponential processes (A), (B), (C)

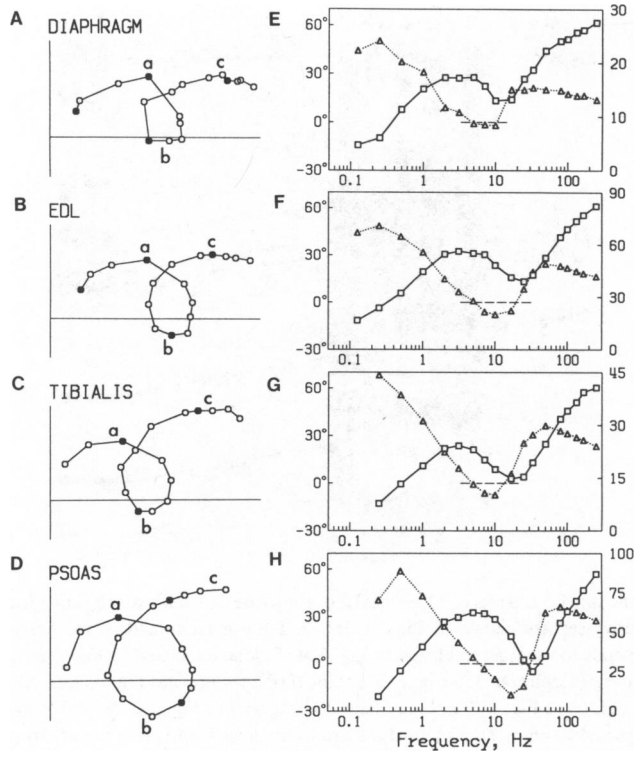

FIGURE 2 Complex modulus from fast twitch fibers of diaphragm (*A, E*), extensor digitorum longus (*B, F*), tibialis anterior (*C, G*), and psoas (*D, H*). The data are shown as in Fig. 1. All were single fibers, except for *C*, which was a two fiber-bundle. They were activated in the standard solution.

(marked *a, b, c*) as reported by us earlier on flexor/extensor muscles of crayfish walking leg, frog semitendinosus (Kawai et al., 1977), and rabbit psoas (Kawai, 1978). Some of the Nyquist plots have distorted circular arcs (eg., Fig. 2 *A, C*); this is due to noise contamination. Process (B) is an exponential delay and known as the oscillatory work component (Pringle, 1967): it is an ability to generate net work on an oscillating environment and different from a unidirectional work (cf. Cox and Kawai, 1981).

In contrast, the Nyquist plots of slow fibers (Fig. 1 *A–C*) exhibit a large process (C), but processes (A) and (B) are reduced in magnitudes to the point that they are almost unidentifiable without time consuming experiments (measurements at lower frequencies) and without careful examination of the data. The fact that the Nyquist plot loops near the origin (Fig. 1 *A, B*) indicates that both processes (A) and (B) are present. The presence of a dip in frequency vs. dynamic modulus plot (Fig. 1 *D, E;* at 0.5 Hz) is also consistent with the presence of the loop in the Nyquist plots. The diameter of the loop is approximately the magnitude of the process (B); it is about 10% of process (C) (Fig. 1 *A, B*). Process (A) has a magnitude of similar order. In Fig. 1 *C* process (B) is not clearly identifiable. However, the fact that two frequency points (0.5 Hz, 1 Hz) almost superimpose implies that (B) may be present (see also Fig. 1 *F*). In addition, a fourth process (D) is identifiable in the high frequency range (Fig. 1 *A, B;* and probably in Fig. 1 *C*).

In frequency plots of slow fibers (Fig. 1 *D–F*) characteristic frequencies, which give peaks and valleys in dynamic modulus and phase shift plots, are shifted to the left by ~30-fold compared with fast fibers (Fig. 2 *E–H*). The left shift indicates that corresponding apparent rate constants are slower by an equivalent degree. All of these processes were absent when each fiber was relaxed or brought into rigor condition. The apparent rate constants and tension in the standard activating condition is summarized in Table I. Confidence of such measurement depends on the relative magntidue, i.e., confidence limit is larger when the relative magnitude is smaller (see Appendix 2 of Kawai and Brandt, 1980, for this discussion). Hence confidence of $2\pi a$ and $2\pi b$ of slow fibers is not as good as that for $2\pi c$ or for the rate constants of fast fibers. Also Table I shows that the tension is lower in slow-twitch fibers, presumably due to

TABLE I
SUMMARY OF THE RATE CONSTANTS AND TENSION

Fiber type	$2\pi a$	$2\pi b$	$2\pi c$	Tension
	$1/s$	$1/s$	$1/s$	$kdyn/cm^2$
Slow fibers				
semitendinosus	0.55 ± 0.04 (7)	2.5 ± 0.2 (8)	27 ± 3 (8)	600 ± 60 (8)
soleus	0.87 ± 0.18 (6)	3.1 ± 0.4 (10)	31 ± 3 (10)	580 ± 90 (10)
diaphragm	—	4.7 (1)	38 (1)	1,140 (1)
Fast fibers				
diaphragm	5.2 ± 0.4 (8)	49.3 ± 3 (8)	510 ± 30 (8)	560 ± 80 (8)
EDL	4.4 ± 0.6 (3)	92 ± 2 (3)	820 ± 100 (3)	860 ± 200 (3)
tibialis anterior	5.3 ± 0.2 (6)	81 ± 9 (6)	910 ± 50 (6)	700 ± 110 (6)
psoas	6.5 ± 0.2 (27)	92 ± 3 (27)	780 ± 30 (27)	1,290 ± 70 (27)
Average				
slow fibers	0.70 ± 0.09 (13)	2.9 ± 0.3 (19)	27 ± 2 (19)	620 ± 60 (19)
fast fibers	5.9 ± 0.2 (44)	83 ± 3 (44)	750 ± 30 (44)	1,050 ± 70 (44)
fast:slow	8	29	28	1.7

Rate constants were estimated from frequencies that gave maximum or minimum viscous moduli after multiplication by 2π. All data were obtained at the standard activating conditions. SEM is shown after ±, and the number of fibers are given in parentheses.

the presence of more mitochondria in the fiber's cross section (Gauthier, 1970). The tension in general is rather low in Table I because of the activating solution had high concentrations of poly anions (MgATP, free ATP, CP, phosphate); in our experience, all of these ions contribute to lower the Ca-activated tension.

In the above description of complex modulus we designated various processes of slow fibers as indicated in Fig. 1 *A–C*. This designation is in accord with our earlier method of mapping exponential processes from a variety of muscles (Kawai and Brandt, 1980). Although small in magnitude, oscillatory work (an exponential delay) is undoubtedly present in slow muscles, hence it is not difficult to identify this as process (B). Process (A) is an exponential advance that is present between 0 Hz and process (B), hence there is no question of its identity in slow muscles. Process (C) is another exponential advance whose optimal frequency is the next faster than (B), hence the big arc in the middle of the Nyquist plot is labeled *c*. Process (D) is a new process and only the beginning of it can be seen in the high frequency range. This mapping method is more of a phenomenological one than the one based on mechanisms, which must be kept in mind when muscles with very different complex moduli are compared.

Molecular Analysis of the Fibers

Each fiber characterized by sinusoidal analysis was also characterized for the expression of fast or slow myofibrillar proteins. Because it is well-established that fast muscles have characteristic MLC that are distinctively different from those of slow muscles (Lowey and Risby, 1971; Weeds et al., 1975; Pette and Schnez, 1977; Schachat et al., 1980; Julian et al., 1981; Salviati et al., 1982), fiber type can be readily identified by an analysis at the molecular level. This was done by determining whether fast or slow MLC were present in the fibers. The results are shown in Fig. 3. Lanes *a–c* are the same fibers analyzed in Fig. 1 *A–C*, respectively; lanes *d–g* are the same fibers analyzed in Fig. 2 *A–D*, respectively. Loading of protein from lane to lane varies because the sizes of the fibers varied. As shown in Fig. 3, all fibers in Fig. 2, which show a fast mechanical response, have the three fast light chains (two alkali light chains, LC_{1f} and LC_{3f}, and a DTNB- or p-light chain, LC_{2f}; Freason and Perry, 1975), while the fibers in Fig. 1, which show a slow mechanical response, have the distinctively different slow light chains (two alkali light chains LC_{las} and LC_{lbs}, and p-light chain LC_{2s}).

That the correlation between the two classes of kinetic behavior and fast or slow fiber type based on MLC is a general rule, is supported by additional correlative studies. This included fifteen psoas, two EDL, two tibialis anterior, six soleus, nine semitendinosus, and four diaphragm fibers. Of these, all fibers from psoas, EDL, tibialis anterior, and three of the diaphragm fibers show typical fast frequency response and Nyquist plots (such as in Fig. 2) and exhibit fast MLC; whereas all fibers from soleus, semitendinosus,

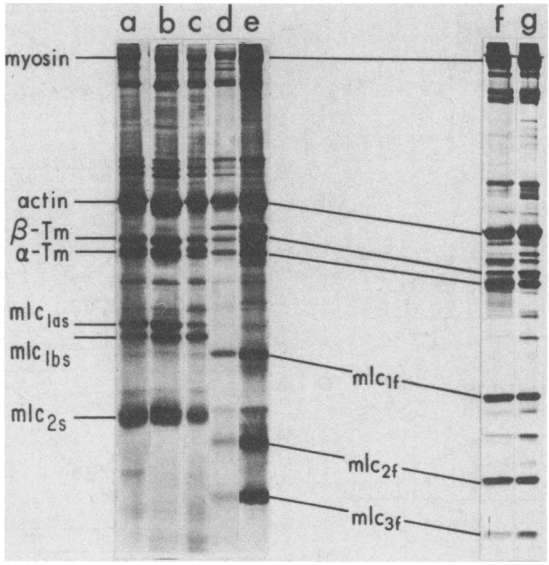

FIGURE 3 Results of SDS-PAGE of the same fibers whose physiological responses are shown in Figs. 1 and 2. Lane *a* (semitendinosus) corresponds to the fiber analyzed in Fig. 1 *A, D;* lane *b* (soleus) to Fig. 1 *B, E;* lane *c* (diaphragm) to Fig. 1 *C, F;* lane *d* (diaphragm) to Fig. 2 *A, E;* lane *e* (EDL) to Fig. 2 *B, F;* lane *f* (tibialis anterior) to Fig. 2 *C, G;* and lane *g* (psoas) to Fig. 2 *D, H*. Lanes *a–e* are from one gel, and lanes *f, g* are from another gel. Various protein components are identified: myosin indicates myosin heavy chain, T_m indicates tropomyosin, and *mlc* indicates myosin light chain. Subscript s denotes slow and f denotes fast. For further identifications of constituent proteins see Schachat et al. (1980) and Bronson and Schachat (1982).

and one from diaphragm show slow frequency response and Nyquist plots (such as in Fig. 1) and exhibited slow MLC. Thus, correlation obtained between the two methods was perfect. Therefore, we can safely conclude that the frequency response and Nyquist plots represented in Fig. 1 are characteristic of slow muscle fibers, whereas those shown in Fig. 2 are characteristic of fast muscle fibers.

In this series of experiments, as well as those shown in Table I, we only found fast fibers in psoas, EDL, and tibialis anterior, and slow fibers in soleus and semitendinosus, in spite of the reported fiber-type mixing as shown by earlier works that used histochemical technique (e.g., Pette and Schnez, 1977; Zeman and Wood, 1980). However, the current result is consistent with those using electrophoresis for identification of slow or fast fibers (Salviati et al., 1982; Bronson and Schachat, 1982). These studies found that 100% of psoas fibers are fast; 100% semitendinosus, 94–99% of soleus, and 26–37% of diaphragm fibers are slow. The current result is also consistent with that of Weeds et al. (1975) who reported that 99.6–100% of psoas fibers are fast type based on an ATPase stain.

Additional Mechanochemical Properties of Fast and Slow Fibers

In our earlier studies, which used psoas fibers, we found a striking effect of MgATP on both the magnitude and the rate constants of processes (B) and (C) in the range of

0.25–20 mM (Kawai, 1978, 1979, 1982). While the same striking effect was observed in all fast fibers studied here, very different and unexpected results were observed in slow fibers. In slow fibers, the MgATP (0.1–20 mM) effect on the rate constants and tension was almost absent. The only noticeable effect was that a portion of the Nyquist plot sometimes fell on the second quadrant in the high millimolar range (e.g., Fig. 1 *A*). The general lack of MgATP sensitivity in slow fibers, however, does not include the low micromolar range (<100 μM). In this range slow fibers showed a clear dependence on the MgATP concentration, and their complex modulus function approached that of rigor as MgATP was further reduced. The rate constants also diminished at the same time. The gradual approach of the complex modulus to that of rigor in the micromolar concentration range in slow fibers was similar to what we have reported earlier in psoas (Cox and Kawai, 1981); MgATP dependence of all fast fibers tested in this report was just the same as that of psoas in all concentration ranges.

Another difference is the nonlinearity involvement in the periodic force time courses. In our earlier studies on psoas, we found that the sine function of the force time course was minimally distorted when the peak-to-peak length oscillation was kept at an amplitude of ≤0.25%. In our sensitive technique, however, a small distortion can be detected near the optimum frequency of oscillatory work, and peaks at a frequency where dynamic modulus is at a minimum (Kawai and Brandt, 1980; Kawai, 1982). The relative nonlinear amplitude was in the order of 0.11 (meaning relative linear amplitude is 0.994: square sum is unity) in psoas. While these characteristics of nonlinearity are common of all fast fibers shown in Fig. 2, slow fibers exhibits a larger nonlinearity at frequencies where oscillatory work is just detectable (nonlinearity sometimes approaches 0.6). This implies that the delayed tension (equivalent to oscillatory work) would be much more asymmetric if a step length change was imposed on slow twitch fibers. In slow fibers, nonlinearities at other frequencies are sufficiently small (~0.01) to justify linear approximation.

DISCUSSION

The observations in the present study show that there are both quantitative and qualitative differences in the frequency response function (complex modulus) between fast and slow skeletal muscle fibers (Figs. 1 and 2). These differences in physiological response correlate one-to-one with the presence of fast and slow MLC complements (Fig. 3). The difference in Nyquist plots is so striking that the fiber type can be readily identified in an experiment that lasts only for 22 s. For slow fibers data were collected in the range 0.067–250 Hz (Fig. 1), which required significantly longer experimental time, but, if the purpose of the experiment had been to physiologically identify fiber types, the usual frequency range (0.25–250 Hz) would have sufficed. Thus our analysis technique provides a novel and sensitive tool for determining whether a fiber is slow or fast while maintaining its mechanochemical properties, allowing a wide variety of further experiment to be considered on an already well-characterized fiber. Our technique is an additional tool to already established physiological methods of characterizing fiber types. These are analysis of strontium or calcium vs. tension curves (Kerrick et al., 1976) and force-velocity measurements correlated with SDS PAGE (Julian et al., 1981).

The apparent rate constants of the three processes (A), (B), and (C) are all slower in slow fibers (Table I). Because the rate constants reflect aspects of the cross-bridge cycle, this is to be expected. What is surprising is the magnitude of the differences. The rate constants are as much as 8–29-fold less in slow fibers. These numbers contrast with a difference of three times in ATPase rates and the maximum shortening velocity (V_{max}) between fast and slow fibers (Barány, 1967; Close, 1972; Julian et al., 1981). However, our number compares better with the 16 time difference in myofibrillar ATPase rate of fast and slow muscles from chicken (Marston and Taylor, 1980, p. 582). One precaution needed in these comparisons is that the transient analysis procedure using either step or sinusoidal waveform is rather sensitive to faster reactions, while the ATPase rate (and possibly V_{max}) is limited by slower reactions in the cross-bridge cycle. Thus the measured 8–29-fold differences in apparent rate constants from transient kinetic analysis can be interpreted to mean that the faster rate constants may be different by this degree, however, they do not imply that the rate-limiting step in the actomyosin ATPase reaction or V_{max} are that much different. The fact that the magnitudes of processes (A) and (B) are much reduced in slow fibers (Fig. 1 *A–C*) indicates that the number of cross-bridge reactions involved in these processes are likewise reduced.

It is interesting that these differences in exponential processes correlates with the difference in fast and slow MLC complements. This is consistent with the importance of myosin and its light chains in ATP hydrolysis and energy transduction mechanisms. However, our current observation alone cannot rule out the possibility that differences in other proteins in contractile machinery may cause or contribute to the physiological differences. In fact, there are an increasing number of reports of the presence of additional myofibrillar proteins (isoenzymes), which differ in rabbit's slow and fast muscles. This includes the thin filament proteins troponin and tropomyosin (Dhoot and Perry, 1979; Bronson and Schachat, 1982; Salviati et al., 1982; Briggs and Schachat, 1983), and the thick filament proteins, myosin (Lowey and Risby, 1971; Freason and Perry, 1975), and C-protein (Callaway and Bechtel, 1981). However, as the rabbit skeletal muscle fibers used here are maximally activated in the saturating Ca^{2+}, and as the contraction of skeletal fibers is controlled by thin filament regulatory mechanism (Ebashi and Endo, 1968), variation in thin filament proteins probably does not contribute to

the differences observed here (this argument excludes the higher order interactions). Thus our tentative conclusion is that differences in myosin isoenzymes are responsible for the difference in apparent rate constants between fast and slow fibers, and it remains a subject of further investigation.

In spite of their differences, note that the three exponential processes, (A), (B), and (C), which were previously observed on fast fibers from crayfish walking leg, frog semitendinosus, and rabbit psoas (Kawai et al., 1977; Kawai, 1978), are also present in slow fibers. (In comparing these observations note that, in frog, semitendinosus is a fast muscle, whereas in rabbit it is a slow muscle.) The universal presence of these three processes in skeletal muscles implies that they result from fundamental properties of cycling cross-bridges. This possibility is strengthened by our previous observation that these processes are absent in psoas fibers that are brought into relaxation or rigor (Kawai and Brandt, 1980). This conclusion is again confirmed in the present study in all fast and slow rabbit skeletal muscle fibers.

There are, however, other physiological differences between fast and slow fibers detectable by the sinuosidal analysis procedure. These are MgATP sensitivity and nonlinearity. Both of these differences probably reflect a difference in the step which rate limits the exponential processes between fast and slow fibers. The reason is as follows.

In psoas fibers we have accumulated evidence that shows processes (B) and (C) involve MgATP binding reaction to rigorlike actomyosin cross-bridge in the range of 0.25–20 mM MgATP (Kawai, 1978, 1979, 1982). This interpretation can be extended to include other fast fibers studied in this report, because we observed the same MgATP sensitivity in fast fibers. However, the interpretation may not be extended to include slow fibers, because of the apparent absence of MgATP sensitivity (in the range 0.25–20 mM) in slow fibers. In this respect an observation by Marston and Taylor (1980) may be of interest. They found that the rate constants of MgATP binding to S1-actin complex is almost the same in fast and slow muscles from chicken (difference is 1.3 times, p. 587). If then, a consistent conclusion to their findings and our own observations is that, in fast muscles, MgATP binding rate limits processes (B) and (C) in the millimolar range, whereas in slow muscles, some other reaction, which is slower than the binding, rate limits processes (B) and (C). In other words, chemical reactions directly involved in processes (B) and (C) are different in fast and slow muscle fibers. The observation by Heinl et al. (1974) that the asymmetric profile of the length dependence of the rate constant of phase 2 (equivalent to our process (C) is opposite between slow and fast fibers by using tortois and frog fibers can also be interpreted to mean that the cross-bridge reaction involved in phase 2 may be different in two fiber types. Our preliminary results (M. Kawai, unpublished), which used step length changes on slow and fast rabbit muscles, also support this conclusion.

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