Shortening Velocity in Single Fibers from Adult Rabbit Soleus Muscles Is Correlated with Myosin Heavy Chain Composition

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Extensive variations exist in the heavy and light chain components of myosin in vertebrate striated muscles. In the present study, we have characterized a specific contractile property, velocity of shortening, and protein subunit composition of single fibers from adult rabbit soleus muscles. Maximum velocity of shortening ($V_{\text{max}}$) was measured using the slack test method, and the myosin composition of these same fibers was determined using an ultrasensitive sodium dodecyl sulfate-polyacrylamide gel electrophoresis system. While most fibers were found to have velocities between 0.5 and 1.0 muscle length/s, several had velocities distributed between 1.33 and 2.99 muscle lengths/s. The fibers in the slower group had myosin subunits that were solely of the slow type; however, those in the faster group contained both fast and slow heavy chains and light chains. The velocity of shortening measured in fibers having both myosin types was highly correlated with the myosin heavy chain composition, with velocity increasing as the proportion of fast-type heavy chain increased. Variations in light chain composition, particularly fast and slow myosin light chain 1, appeared to occur independently of the variations in heavy chain composition, suggesting that some myosin molecules consist of mixtures of slow- and fast-type subunits.

The possible roles of the heavy and light chain subunits of the contractile protein myosin in regulating the kinetics of interaction with actin are, for the most part, unclear. Changes in the subunit composition of the myosin molecule have been clearly demonstrated to occur during development (Rubinstein and Holzter, 1979; Whalen et al., 1981; Winkelmann et al., 1983; Crow et al., 1983), following denervation (Carraro et al., 1981), during regeneration (Carraro et al., 1983), and in association with some disease states (Rushbrook et al., 1982; Bandman, 1984, 1985). However, the specific relationships between the composition of the myosin molecule and variations in contractile function in terms of tension development and shortening velocity remain uncertain (see, for example, the review by Jolesz and Sréter, 1981). To date, only a few studies (e.g., Pette et al., 1979; Unsworth et al., 1982) have attempted measurements of both the myosin subunit composition and contractile properties in the same whole muscle preparations; however, the mixed fiber populations of whole muscles can in many cases obscure specific correlations between performance and protein subunit composition. We therefore developed an experimental system in which we are able to measure contraction parameters and protein composition in the same single fiber preparation. Previously, maximum shortening velocities measured in rabbit fast-twitch muscle fibers were found to be reversibly reduced by partial extraction of myosin LC1 (Moss et al., 1982, 1983). In the present paper, we report quantitative descriptions of the relationships between naturally occurring variations in the myosin heavy chain and light chain compositions and the maximum shortening velocities of single fibers from adult soleus muscles of the rabbit. Our results indicate that 1) fast- and slow-type myosins frequently occur within the same adult soleus fiber; 2) within a single fiber having mixed myosin types, the ratio of total fast to total slow light chain is sometimes different from the ratio of fast to slow heavy chains; and 3) shortening velocity in soleus fibers is highly correlated with the proportion of total heavy chain that is of the fast type.

A brief report of these findings was made at the Annual Meeting of the Biophysical Society (Greaser et al., 1985).

EXPERIMENTAL PROCEDURES

Small bundles of fibers were dissected from soleus muscles of adult New Zealand rabbits (approximately 3 kg of body weight), tied at slightly stretched lengths to glass capillary tubes, placed in 50% (v/v) glycerol-containing relaxing solution (Moss et al., 1982), and stored at −22 °C for 2–21 days before use. On the day of an experiment, single fibers were dissected from the bundles, and segments of these fibers were mounted in an experimental apparatus similar to that described previously (Moss et al., 1983). The solutions that were used to relax and activate the fiber segments contained 7.0 mM EGTA, 1.0 mM free Mg2+, 20 mM imidazole, 6.28 mM total ATP, 14.5 mM creatine phosphate, pH 7.0, and sufficient KCl to yield a final ionic strength of 180 mM. Relaxing solution had a free Ca2+ concentration of 10−4 M (i.e., pCa 9.0), and the Ca2+ concentration in the activating solution was 10−5 M, which was found to elicit maximum tension generation in these fibers. The computer program of Fabiato and Fabiato (1979) was used to calculate the final concentrations of each metal, ligand, and metal-ligand complex, based on the stability constants listed by Godt and Lindley (1982). The apparent stability constant for Ca-EGTA was corrected to 15 °C (Fabiato and Fabiato, 1979) and for the effects of ionic strength (Martel and Smith, 1974). Sarcomere length was adjusted to 2.5–2.6 μm, as determined by direct microscopy (Moss, 1979), by adjusting the overall length of the segment. Measurements of $V_{\text{max}}$ were made during Ca2+ activation at 15 °C using the slack test method (Moss et al., 1982) in which the duration of

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1 The abbreviations used are: LC1, LC2, and LC3, fast-type myosin light chain 1, 2, and 3, respectively; LC1 and LC2, slow-type myosin light chain 1 and 2, respectively; MHC-f and MHC-s, fast-type and slow-type myosin heavy chains, respectively; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; pCa, −log [Ca2+]; $V_{\text{max}}$, maximal velocity of shortening.

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unloading shortening was recorded as a function of the amount of slack introduced at one end of the segment (Fig. 1). After measurement of \( V_{\text{max}} \), each fiber was placed in 10 \( \mu l \) of 1% (w/v) SDS sample buffer and stored at \(-22^\circ C\) for up to 1 week prior to the gel run or at \(-80^\circ C\) if the samples were stored longer (Giulian et al., 1983). The polyacrylamide gels and running protocols were similar to those described by Giulian et al. (1983) except that the acrylamide concentrations were 3.5% (w/v) in the stacking gel and 10% (w/v) in the running gel. Glycerol was added to the gel matrix to a final concentration of 10% (v/v), as described by Carraro and Catani (1983). Sample loads were kept small (equivalent to a 0.5-mm length of fiber) in order to enhance resolution of the closely situated heavy chain bands. Following the gel run, the gels were silver-stained as previously described (Giulian et al., 1983). The gels were scanned on a laser scanning densitometer, and the areas under the peaks were integrated. The relative amount of fast heavy chain present was calculated as the ratio of the area of the fast heavy chain peak to the sum of the areas of the fast and slow peaks.

RESULTS AND DISCUSSION

Examples of the force traces obtained during the slack test on one fiber segment are shown in the inset of Fig. 1. The amount of slack introduced was then plotted versus the duration of unloaded shortening (Fig. 1), and \( V_{\text{max}} \) was calculated from the slope of a straight line fitted to these data (Moss et al., 1982). The results of the \( V_{\text{max}} \) determinations indicate that individual fibers from the adult mammalian soleus muscle (generally considered to be slow twitch; Close, 1964) had a wide range of velocities (Fig. 2). The majority of fibers (27 of 33) had velocities ranging from 0.51 to 0.99 muscle length/s with a mean of 0.76 \pm 0.14 S.D. However, the remaining fibers had \( V_{\text{max}} \) values between 1.33 and 2.99 muscle lengths/s. The velocities of these fibers formed a continuum in this range rather than falling into one or two discrete populations. The myosin heavy and light chain compositions were then determined for the same individual fibers on which the velocity measurements were made. Fig. 3 shows the myosin heavy chain (MHC) region from one such gel. In this instance, the fibers were arranged on the gel in order of increasing velocity from lane 1 (0.57 muscle length/s) to lane 6 (2.02 muscle lengths/s). The fibers having the lowest \( V_{\text{max}} \) values (lanes 1–4) were found to contain only the slow-type myosin heavy chain (MHC-s). The fibers in lanes 5 and 6 had higher shortening velocities (1.33 and 2.02 muscle lengths/s, respectively) and were also found to contain myosin heavy chains of the fast type (MHC-f). The slow- and fast-type MHC bands were identified on the basis of co-migration with the predominant MHC bands observed in samples from the slow-twitch soleus and fast-twitch psoas muscles, respectively. A plot of \( V_{\text{max}} \) as a function of MHC-f content (Fig. 4) shows that there is a strongly positive correlation between these two variables. \( V_{\text{max}} \) increased from a mean value of 0.76 muscle length/s (\( n = 27 \)) in fibers in which no MHC-f was detected to a value of 2.99 muscle lengths/s in a fiber containing only MHC-f. The data points of Fig. 4 are well fit by a straight line (correlation coefficient of 0.94), and the slope of this line is significantly different from zero (\( p < 0.05 \)). Thus, while it is commonly believed that fast-twitch fibers contain myosin of the fast-type and slow-twitch fibers contain slow-type myosin, the present study is the first demonstration on the single fiber level of a quantitative functional relationship between a physiological descriptor of muscle performance (i.e., \( V_{\text{max}} \)) and variations in myosin heavy chain composition.

The myosin heavy and light chain compositions of the population of fibers having a mean \( V_{\text{max}} \) value of 0.76 muscle length/s were uniformly of the slow type. Myosin light chain composition was also examined in the fibers having \( V_{\text{max}} \) values between 1.77 and 2.99 muscle lengths/s (Fig. 5). The

**Fig. 1.** Determination of \( V_{\text{max}} \) for a single muscle fiber. When peak isometric tension was generated in a maximally calcium-activated fiber, a rapid (1 ms) length change (dL) was imposed which transiently caused the fiber to slacken and tension to fall to zero. The fiber then shortened for a period of time (dt), during which the imposed slack was taken up. Once the slack was taken up, tension started to increase (inset). The fiber was subsequently relaxed in a solution of pCa 9.0 and was then re-extended to its original length. During succeeding activations, length changes of various amplitudes were imposed. The amount of length change was then plotted against the duration of unloaded shortening, and \( V_{\text{max}} \) was calculated as the slope (dL/dt) of a straight line fitted to this data. The data for a given fiber was accepted only if the correlation coefficient for the straight line fit was greater than 0.96. Inset, three superimposed length (upper) and tension (lower) records obtained during and immediately after length changes were applied to an activated fiber. Data from these records are included in the slack test plot. Note that as the amplitude of the length step was increased, so also did the duration of unloaded shortening.

**Fig. 2.** Distribution of maximum shortening velocities in adult soleus muscle fibers. The height of each vertical bar represents the number of fibers for which \( V_{\text{max}} \) was found to occur in each 0.1 muscle length/s increment.
The myosin heavy chain region of an SDS-PAGE gel. Each lane represents a segment of a different single fiber. The fibers were arranged on the gel in order of increasing $V_{\text{max}}$, in muscle lengths/second: lane 1, 0.57; lane 2, 0.58; lane 3, 0.63; lane 4, 0.68; lane 5, 1.33; lane 6, 2.02. The stacking gel was 3.5% (w/v) acrylamide, and the running gel was 10%. Acrylamide: bisacrylamide ratios were described previously (Giulian et al., 1983).

Relationship between $V_{\text{max}}$ and the relative amount of fast-type myosin heavy chain. The point corresponding to 0% MHC-f represents the mean ± S.D. of the 27 fibers that had exclusively MHC-s. Each of the other points represents $V_{\text{max}}$ and MHC data from an individual single fiber. ML/s, muscle lengths/second.

SDS-PAGE of single soleus muscle fibers showing both the heavy chain and light chain regions. The fibers are arranged in order of increasing velocity of shortening, in muscle lengths/second: lane 1, 1.77; lane 2, 2.25; lane 3, 2.46; lane 4, 2.99. The slow and fast types of myosin heavy chains are not clearly resolvable under these conditions which are identical to those of Fig. 3 except that the stack is 6% (w/v) acrylamide and the separating gel is 12%. A is actin, and a-e represent LC_{1s}, LC_{1f}, LC_{2s}, LC_{2f}, and LC_{3s} respectively.

Relationship between various myosin light chain bands on the gels were identified on the basis of the migration of myosin light chain standards run on similar gels. These fibers were found to contain light chains of both the fast and slow types. The ratio of LC_{1f} to LC_{1s} did not appear to vary appreciably in these fibers, with LC_{1f} comprising approximately 30% of the total LC_{1}. LC_{3}, which is usually associated with fast-twitch fibers (Lowey and Risby, 1971), was found in each of these fibers, and the amount present increased slightly in the fibers having greater shortening velocities. The LC_{2} composition of these fibers varied substantially, although not in proportion to the variations reported above for the myosin heavy chain compositions.

For the fiber for which $V_{\text{max}}$ was 1.77 muscle lengths/s was found to have equal proportions of LC_{2s} and LC_{2f}, while the fastest fiber (i.e. $V_{\text{max}}$ = 2.99 muscle lengths/s) contained virtually 100% LC_{2f}, with a band corresponding to LC_{2s} being just detectable on the gel. For the remaining fibers in the high velocity range, LC_{2s} accounted for approximately 20% of the total LC_{2}, and this proportion was invariant as a function of shortening velocity. Thus, with the possible exception of LC_{3s}, there appears to be no consistent correlation between $V_{\text{max}}$ and the proportions of different light chains that are present. This does not exclude the possibility that light chain composition may modulate $V_{\text{max}}$ to some degree, but simply suggests that variations in light chain composition are an unlikely explanation for the approximate 3-fold variation in $V_{\text{max}}$ observed in adult soleus fibers.

Examination of the gels of the fibers having mixed myosin types (Figs. 3 and 5) indicates that the proportions of slow-type (or fast-type) light chains are not necessarily equivalent to the proportions of slow-type (or fast-type) heavy chains that are present. This is especially true of LC_{1 slow:fast ratios,
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which are relatively invariant in these fibers. This suggests that the genes for the myosin heavy chains, LCH, and possibly LC, are not expressed coordinately in these particular soleus fibers and furthermore that there very likely are fast light chains associated with slow heavy chains and vice versa. The ability to derive such conclusions relies heavily on our use of single muscle fibers for SDS-PAGE analysis, since we are able to state with certainty that the observed variations in protein composition are not the result of the heterogeneity between fibers that would be expected in whole muscle preparations.

In conclusion, our results suggest that maximum shortening velocity in soleus muscle fibers from adult rabbits is related primarily to the relative proportions of fast- and slow-type myosin heavy chains that are present. It was previously demonstrated (Julian et al., 1981) that Vmax of newborn and adult psoas and adult soleus rabbit muscle fibers was not well correlated with the specific myosin light chain composition of individual fibers. Likewise, we have found that, although the light chain content of adult soleus fibers does vary, Vmax correlates better with the heavy chain content. The main determinant of shortening velocity in muscle appears to be the rate constant for detachment (g) of the myosin heads from actin (Huxley, 1957), although recent reports (Goldman et al., 1982, 1984) suggest that the rate-limiting step in the cross-bridge cycle occurs just prior to detachment. In a fiber containing both fast- and slow-type myosins, the slow-type myosin would be expected to have a disproportionately greater influence upon Vmax, since cross-bridge heads containing MHC-s would comprise a substantial internal load to the muscle due to their slow rates of detachment. Because of their much greater rates of detachment, heads containing MHC-f would be expected to have a lesser effect on Vmax. A prediction of this scheme is that the relationship between Vmax and per cent MHC-f (Fig. 4) should not be linear, but rather the rate of increase of Vmax would increase in proportion to MHC-f content. In terms of curve fitting to the data of Fig. 4, a straight line is an adequate fit considering the number of data points at the higher velocity values; however, it is possible to obtain a slightly better fit to the data with an exponential curve, and this is consistent with the suggestion of a greater effect of MHC-s on Vmax. Independent of any specific model, the observed continuum in Vmax as a function of myosin heavy chain composition is strong evidence that, in muscle fibers containing both myosin types, Vmax is the result of summed contributions from both types. Were this not the case, we would have expected two discrete fiber populations with respect to Vmax: one containing purely one type of myosin heavy chain and the other containing fibers with various mixtures of the two heavy chain types.

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