The Presence of Two Skeletal Muscle α-Actinins Correlates with Troponin–Tropomyosin Expression and Z-line Width

FREDERICK H. SCHACHAT, AMY C. CANINE, MARGARET M. BRIGGS, and MARY C. REEDY
Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT Two species of α-actinin from rabbit fast skeletal muscles were identified with a monospecific antisera. Designated α-actinin\textsubscript{1f} and α-actinin\textsubscript{2f}, their distribution in muscles does not correlate with histochemically defined fast fiber type. Rather, the presence of each correlates with Z-line width and with the expression of different thin filament Ca\textsuperscript{2+}-regulatory complexes. α-Actinin\textsubscript{1f} is expressed with troponin T\textsubscript{1f}-αβ tropomyosin, and α-actinin\textsubscript{2f} with troponin T\textsubscript{2f}-α2 tropomyosin. CNBr peptide maps show that the fast α-actinin species differ in primary structure. In contrast, the slow α-actinin is indistinguishable from α-actinin\textsubscript{1f}. Further evidence for the similarity of α-actinin\textsubscript{1f} and slow α-actinin comes from electron microscopic studies which show that fibers that express these species exhibit thick Z-lines. So, unlike other contractile proteins, the multiple forms of α-actinin do not reflect the distinction between fast- and slow-twitch muscles.

Multiple forms of most of the major skeletal muscle contractile proteins have been identified in mammalian muscles. In the case of myosin (11), troponin T (7), α-tropomyosin subunits (4), and C-protein (6), these homologous species are thought to be part of the molecular basis responsible for the differences between fast- and slow-twitch muscle. Similarly, Suzuki et al. (33, 34) described two porcine skeletal muscle forms of α-actinin: one found in fast muscle and the other in slow muscle. In the studies described here, two forms of α-actinin in fast skeletal muscles of rabbit are characterized. Identified with a monospecific antisera on immunoblots, they differ extensively in primary structure and have been designated α-actinin\textsubscript{1f} and α-actinin\textsubscript{2f}.

α-Actinin is a major component of the Z-line in skeletal muscle (5). Because differences in Z-line structure have been associated with the two histologically and ultrastructurally defined types of fast fibers (9, 10, 12), the relationship between Z-line width, histochemical fiber type, and the expression of these two fast α-actinin species was investigated. No simple correlation between histochemically defined fast fiber type and the expression of either fast α-actinin species was found. Instead, Z-line width appears to be at least partly determined by the α-actinin species expressed. In addition to this morphological correlate, comparison of the pattern of fast α-actinin expression with that of the two major forms of the fast skeletal thin filament protein troponin T (described by Briggs et al. [3]) shows that the presence of each α-actinin is linked to the presence of a different fast troponin T.

An investigation of the relationship between the two forms of fast α-actinin and slow α-actinin by CNBr peptide mapping reveals no differences in the CNBr fragments of α-actinin\textsubscript{1f} and α-actinin\textsubscript{2f}. Because there is commonly more homology among fast myofibrillar proteins than between fast and slow homologs, the differences between the peptide maps of the two fast α-actinin species, coupled with the similarity of one of the fast α-actinin species and slow α-actinin, was unexpected. It suggests that the selective pressures that resulted in the multiple forms of α-actinin differed from those that led to the multiplicity of other myofibrillar proteins.

MATERIALS AND METHODS

Reagents: The monospecific antisera to α-actinin was a gift from Dr. Keith Burridge and was characterized as previously described (18). Second antibodies for immunoblot reactions were obtained from either Tago Inc. (Burlingame, CA) or Vector Laboratories, Inc. (Burlingame, CA). Trasylol was purchased from Mobay Chemical Corp. (Pittsburg, PA) and Tris-HCl and Tris base were from Sigma Chemical Company (St. Louis, MO). Pepstatin A, leupeptin, antipain, and chymostatin were purchased from the Peptide Institute (Osaka, Japan). Glutaraldehyde was purchased from Tousimis (Rockville, MD). All other chemicals were reagent grade. Nitrocellulose paper was purchased from Schleicher & Schuell, Inc. (Keene, NH).

Myobrin and Single Fiber Preparations: Dissection of muscles and single muscle fibers and preparation of myofibrils has been described previously (4, 30, 31).

Gel Electrophoresis: Polyacrylamide gel electrophoresis in the pres-
Fiber Typing: Fiber typing was performed by histochemical staining of serial cross-sections as described in Bronson and Schachat (4). The following muscles were taken from each rabbit: soleus, psoas, diaphragm, and tongue. The muscles were exposed and flushed with Ringer’s solution (120 mM NaCl/1.6 mM KCl/4.3 mM NaHCO3/1.4 mM KH2PO4/1.5 mM MgSO4/15 mM morpholine propane sulfonic acid/5 mM glucose, pH 7.0). When possible, sarcomere length was controlled by holding the limb in a flexed or extended position during primary fixation. Fixation was performed in situ; the primary fixative (3% glutaraldehyde, 0.2% tannic acid, 1% paraformaldehyde, in Ringer’s) was injected into the muscle mass and allowed accurate comparison of structural features to be made from micrographs, since these could be recorded from the same section, so all fibers were identically fixed, processed, of the same thickness, and subjected to the same stain conditions. In addition, different fibers in the section could be photographed without changing the magnification or even the focus. In the case of diaphragm, intact blocks composed of several fibers were also made to allow comparison of the different fiber types.

Measurements of Z-line thickness were performed on the 12,000× negatives using a Scherr-Tumico (New York, NY) optical comparator with a 10× objective. The Z-line was defined as extending to its fuzziest border, that is, the position where its density decreased sharply. For soleus, 25 Z-lines from five fibers were measured; for psosas, 28 Z-lines from five fibers were measured; for tongue, 21 Z-lines from three fibers; and, for diaphragm, 31 Z-lines from eight fibers.

RESULTS

α-Actinin Heterogeneity in Rabbit Fast Skeletal Muscles

When myofibril preparations from rabbit fast skeletal muscles are analyzed by SDS PAGE (Fig. 1, lanes a–h), two protein bands with Mr’s characteristic of α-actinin (~100,000) are found. Exhibiting Mr’s of 100,000 and 104,000, they are labeled α-actinin1f and α-actinin2f in Fig. 1, where the subscripts 1 and 2 indicate order of mobility, and the subscript f indicates they are from fast muscles. Their identity as α-actinin species was confirmed by immunohistochemical studies described below.

It is clear in Fig. 1 that the proportion of these two species varies in the fast muscles studied. Psosas, adductor magnus, and rectus femoris (lanes a, b, and c, respectively) exhibit α-actinin2f as their predominant α-actinin species. In contrast, the intrinsic musculature of the tongue overwhelmingly exhibits α-actinin1 (lane h). And several muscles from the hindlimb, gracilis, tibialis anterior, extensor digitorum longus (EDL), and plantaris express both species.

The identification of α-actinin1f and α-actinin2f was confirmed by immunoblot analysis of the same myofibril samples from psosas and tongue used in Fig. 1. Fig. 2 shows that both α-actinin2f and α-actinin1f are recognized as α-actinin by a monospecific sera raised against chicken gizzard α-actinin (lanes a and b, respectively).

Because the proportions of α-actinin1f and α-actinin2f varied in the fast muscles and α-actinin is known to be susceptible to proteolysis (26), two studies were undertaken to determine whether α-actinin2f is a proteolytic fragment of α-actinin1f. First, a time-course degradation study was performed, and second, peptide maps of the two proteins were compared.

Myofibrils from psosas and the intrinsic musculature of the tongue were chosen as sources of α-actinin2f and α-actinin1f, respectively, for the time-course degradation study. Samples of fresh muscle were dissected and homogenized in myofibril preparation buffer within 10 min of killing an animal. Crude myofibrils were prepared from aliquots taken immediately after homogenization (t = 0) and at 15 and 45 min. This preparation was completed and samples placed in SDS diluting buffer within 90 s. The results of SDS PAGE analysis of those samples is shown in Fig. 3. It is apparent in the psosas samples (lanes a, d, and g) that α-actinin2f is present in the earliest time and is not lost when incubated in the myofibril preparation buffer. Similarly, α-actinin1f is present in the time preparation at the earliest time and shows no evidence used has been shown to result in <4% lattice shrinkage in X-ray diffraction-monitored bundles of insect flight muscle (25).
FIGURE 1 Identification of α-actinin₁f and α-actinin₂f by SDS PAGE in myofibrils from several fast rabbit skeletal muscles: psoas (a); adductor magnus (b); rectus femoris (c); gracilis (d); tibialis anterior (e); EDL (f); plantaris (g); intrinsic musculature of the tongue (h); and, the mixed muscle diaphragm (i). The two fast forms of α-actinin are resolved by SDS PAGE on an 8.5% polyacrylamide gel. They are labeled α-actinin₁f and α-actinin₂f, and their Mr's were determined using myosin heavy chain (Mr 210,000), the α, β, and γ subunits of RNA polymerase (Mr's 165,000, 155,000, 39,000, respectively), glycogen phosphorylase (Mr 97,600), bovine serum albumin (Mr 68,000), and ovalbumin (Mr 43,000) as standards. An α-actinin species labeled α-actininₙ is shown in myofibrils from the slow muscle soleus (j).

FIGURE 2 Immunoblot reaction of the different α-actinin species with a monospecific anti-α-actinin serum. Duplicate sets of myofibril samples were transferred to nitrocellulose. One transfer was stained with amido black, and the other reacted with the monospecific anti-α-actinin serum. Stained and immunoblot reactions are shown: lanes a and a' are from psoas (>85% α-actinin₁f); lanes b and b' are from the intrinsic musculature of the tongue (>85% α-actinin₁f); and, lanes c and c' are from the slow muscle soleus (α-actininₙ). SDS PAGE was performed on an 8.0% polyacrylamide gel. Of degradation to either α-actinin₂f or any other species during the incubation (lanes b, e, and h). From these observations, we conclude that both fast α-actinin species are stable and do not interconvert by any proteolytic or posttranslational event occurring in the preparation of myofibrils.

Additional evidence that the two fast α-actinin species are different proteins is provided by CNBr peptide maps. SDS PAGE gel bands containing the different species were excised and treated with CNBr as described by Pepinski (23). When the CNBr fragment maps are compared (Fig. 4), none of the 23 fragments generated from α-actinin₁f (lane a) co-migrate with those of α-actinin₂f (lane b). Because CNBr cleavage occurs at Met residues, the differences in these maps indicate that extensive differences exist in the primary structure of the two fast α-actinin species.

Slow α-Actinin Is Not Resolved from α-Actinin₁f by SDS PAGE

The same procedures used to analyze fast α-actinin above were also applied to the slow muscle soleus. As can be seen in Fig. 1, lane j, the α-actinin species present in slow myofibrils, labeled α-actininₙ, co-migrates with α-actinin₁f. Its identity was confirmed by reaction with the monospecific antisera.
In contrast, the intrinsic musculature of the tongue, in which α-actinin, is predominant, is almost exclusively FOG in fiber type. Although these two observations suggest a relationship between fiber type and α-actinin expression, the presence of significant quantities of α-actinin, in fibrosis, the presence of significant quantities of α-actinin, in FG muscles, shows that both fast α-actinin species can be found in FG muscles. Specifically, in plantalis, whose fiber type distribution is virtually indistinguishable from that of psoas (Table I), α-actinin, composes 57% of the α-actinin in fast fibers (after correcting for SO fiber content [Table I]), confirming the presence of α-actinin, in FG fibers. So, α-actinin, may be the predominant species in either FG or FOG muscle fibers, while α-actinin, appears to be prevalent only in a restricted set of FG muscles. The absence of a one-to-one correlation between fiber type and α-actinin expression is further demonstrated by the presence of both α-actinin species in individual fibers (Fig. 5).

Because this pattern of α-actinin expression was analogous to that previously described for α, and αβ troponin T (2, 3, 31), the ratio of the different fast α-actinin species was compared with that of the two major fast troponin T species to determine if their expression was linked. Three sets of myofibril samples, each from a different animal, were analyzed using 8% SDS PAGE to separate the α-actinin species and 11% SDS PAGE to resolve TnT and TnT, as shown in Fig. 6. Fig. 7 shows the densitometric ratio of the fast α-actinin species plotted against the ratio of fast TnT species from these preparations. At the boundaries, the correlation between the expression of TnT and α-actinin, and that of TnT and α-actinin, is strong (for example, in tongue and psoas, respectively). At intermediate values the relationship is monotonic. The variation in the proportion of the α-actinin species in tibialis anterior, EDL, and plantaris from animal to animal is evident in Fig. 6. This is not due to proteolysis or differential solubility in SDS, but, as shown for the troponin T species, is an inherent aspect of contractile protein expression in these muscles (31).

**Peptide Maps of α-Actinin**

Because SDS PAGE did not resolve α-actinin, and α-actinin, CNBr fragments from these proteins were compared to determine how similar they are. Fragments were generated as described above for the two fast α-actinins and then compared by SDS PAGE. Fig. 8 shows that the CNBr peptides of α-actinin, (lane a) and α-actinin, (lane b) co-migrate. There-

**α-Actinin** Is Principal in Fast Fibers of Tongue

**Table I. Tabulation of Fiber Type, α-Actinin, and Z-line Width Distributions in Several Muscles of the Rabbit**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>FG</th>
<th>FOG</th>
<th>SO*</th>
<th>α-Actinin1f</th>
<th>Ratio for fast fibers</th>
<th>Z-line width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erector spinae</td>
<td>79</td>
<td>11</td>
<td>10</td>
<td>0.07</td>
<td>0.01</td>
<td>ND*</td>
</tr>
<tr>
<td>Psoas</td>
<td>90</td>
<td>4</td>
<td>6</td>
<td>0.14</td>
<td>0.09</td>
<td>666 ± 140</td>
</tr>
<tr>
<td>Plantaris</td>
<td>76</td>
<td>17</td>
<td>8</td>
<td>0.60</td>
<td>0.57</td>
<td>ND</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>45</td>
<td>29</td>
<td>26</td>
<td>0.76</td>
<td>0.67</td>
<td>994 ± 120</td>
</tr>
<tr>
<td>Soleus</td>
<td>&lt;0.3</td>
<td>1</td>
<td>99</td>
<td>NA†</td>
<td>NA†</td>
<td>1,360 ± 200</td>
</tr>
<tr>
<td>Tongue</td>
<td>&lt;0.3</td>
<td>99</td>
<td>1</td>
<td>0.89</td>
<td>0.89</td>
<td>1,110 ± 130</td>
</tr>
</tbody>
</table>

*Histochemical tabulations are as described in Materials and Methods.

† In order to correct for the co-migration of α-actinin and α-actinin, the fraction in the preceeding column was corrected for the fraction of SO fibers using the equation (fraction of α-actinin - fraction of SO fibers)/[1 - fraction in the preceeding column was corrected for the fraction of SO fibers].

‡ ND, not determined.

### α-Actinin1f and α-Actinin2f Are Not Specific to Either Fast Fiber Type

Two types of fast-twitch fibers, fast-twitch glycolytic (FG) and FOG, have been distinguished by histochemical, physiological, metabolic, and ultrastructural criteria. The results of histochemical analyses presented in Table I show that psoas, in which α-actinin, is predominant, is 90% FG in fiber composition. In contrast, the intrinsic musculature of the tongue, in which α-actinin, is predominant, is almost exclusively FOG in fiber type. Although these two observations suggest a relationship between fiber type and α-actinin expression, the presence of significant quantities of α-actinin, in tibialis anterior, plantaris, and EDL, other predominantly FG muscles, shows that both fast α-actinin species can be found in FG muscles. Specifically, in plantaris, whose fiber type distribution is virtually indistinguishable from that of psoas (Table I), α-actinin, composes 57% of the α-actinin in fast fibers (after correcting for SO fiber content [Table I]), confirming the presence of α-actinin, in FG fibers. So, α-actinin, may be the predominant species in either FG or FOG muscle fibers, while α-actinin, appears to be prevalent only in a restricted set of FG muscles. The absence of a one-to-one correlation between fiber type and α-actinin expression is further demonstrated by the presence of both α-actinin species in individual fibers (Fig. 5).

Because this pattern of α-actinin expression was analogous to that previously described for α2 and αβ troponin T (2, 3, 31), the ratio of the different fast α-actinin species was compared with that of the two major fast troponin T species to determine how similar they are. Fragments were generated as described above for the two fast α-actinins and then compared by SDS PAGE. Fig. 8 shows that the CNBr peptides of α-actinin, (lane a) and α-actinin, (lane b) co-migrate. There-

**α-Actinin1f and α-Actinin2f Are Indistinguishable**

Because SDS PAGE did not resolve α-actinin, and α-actinin, CNBr fragments from these proteins were compared to determine how similar they are. Fragments were generated as described above for the two fast α-actinins and then compared by SDS PAGE. Fig. 8 shows that the CNBr peptides of α-actinin, (lane a) and α-actinin, (lane b) co-migrate. There-

**Figure 4** CNBr-peptide maps of α-actinin1f and α-actinin2f. The CNBr peptides of α-actinin1f from tongue (lane a) and α-actinin2f from erector spinae (lane b) are compared by SDS PAGE on a 16% polyacrylamide gel. The CNBr peptides of troponin T (described in Briggs et al. [3]) were used as Mr standards. Numbers on the right refer to kilodaltons.

**Figure 5** Electrophoretic evidence that both α-actinin species are expressed in the same single fiber. Several single fibers from plantaris (lanes a–j) dissected and prepared for electrophoresis as described in Schachat et al. (30, 31) were analyzed by SDS PAGE on 8.5% polyacrylamide gels. As is evident, both α-actinin, and α-actinin, are present in individual single fibers from this muscle.
Comparison of the ratio of the two fast α-actinin species with that of the two major fast troponin T species in several fast muscles of the rabbit. The ratios of α-actinin to total α-actinin are plotted against TnT1f/(TnT1f + TnT2f) from several fast muscles. The ratios were determined by densitometry and planimetry of SDS polyacrylamide gels including those shown in Fig. 5. Each point represents a determination on myofibrils from a single muscle. Ad, adductor magnus; EDL, extensor digitorum longus.

**Effect of the Different α-Actinin Species on Sarcomere Morphology**

α-Actinin is a major component of the skeletal muscle Z-line (5, 27). Gauthier (12, 13) proposed that differences in Z-line width correlated with the difference between fast- and slow-twitch fibers. In guinea pig muscles, she found that FG fibers had thin Z-lines, whereas SO fibers had thick Z-lines, and, in rat diaphragm, FOG fibers could have either thin or thick Z-lines. Because this relationship is similar to that described for the distribution of α-actinin species and histochemical fiber type, electron microscopic studies were performed on fibers from rabbit muscles that expressed α-actinin2f and α-actinin1f/s.

α-Actinin1f/s is present in SO fibers in soleus, in FOG fibers from tongue and diaphragm, and also in FG fibers in diaphragm, whereas α-actinin2f is found in the FG fibers of psoas. Electron microscopic analysis on fibers from these muscles indicates that there may be a simple relationship between the expression of α-actinin2f and thin Z-lines and α-actinin1f/s and thicker Z-lines. The relative Z-line widths of fibers from these muscles from the same animal were determined in fixed fiber bundles that were co-embedded to equalize the effects of sectioning, staining, and variation in magnification. Inspection of Fig. 9 and the tabulated data in Table I shows that the width of Z-lines in SO fibers from soleus (Fig. 9A) is twice that of FG fibers from psoas (Fig. 9B). Fibers from the FOG intrinsic musculature of the tongue also exhibit thick Z-lines.
FIGURE 9  Electron microscopic observations on fibers from psoas, tongue, diaphragm, and soleus. These fibers were co-embedded before sectioning, and all micrographs were taken from the same section as described in Materials and Methods. Differences in the relative widths of Z-lines are apparent: the Z-lines in the FG fiber from psoas (B) are thinner than those in the SO fiber from soleus (A), the FOG fiber from the intrinsic musculature of the tongue (C), and the oxidative fiber from diaphragm (D). The position of the Z-line (Z), I-band (I), A-band (A), and H-zone (H) are indicated in the fiber from psoas (B). x 9,600.

(Fig. 9 C). However, in contrast with the proposal that thick Z-lines are related to fiber type, all fibers from diaphragm, regardless of type, exhibit Z-lines thicker than those of psoas. In the case of psoas, soleus, and tongue, fiber type assignments were made statistically, based on the purity of fiber type composition (each muscle is at least 90% a single fiber type) and the uniformity of ultrastructural features in the many fibers studied from each muscle. Because diaphragm is composed of a mixture of fiber types (Table I), both statistical and ultrastructural parameters must be used to show Z-line width is independent of fiber type. The tightness of the distribution of Z-line width, calculated by dividing the variance by the mean, is as sharp in diaphragm, where all fiber types are included, as in any of the muscles of a single fiber type. This argues that there are not several groups of fibers with different mean Z-line widths in diaphragm, but rather a population of fibers with a distribution of Z-line widths about a single mean. In addition, although ultrastructural parameters cannot be used precisely to identify FG fibers (10, 11), as recognized by Gauthier (12) and quantitated by Eisenberg and co-workers (9, 10), FG fibers differ from SO and FOG fibers in the size, number, and shape of their mitochondria. This is a reflection of the difference in their capacity for oxidative phosphorylation (24). In Fig. 9 this difference is apparent. The FG fiber from psoas (Fig. 9 B) exhibits smaller and fewer mitochondria than the SO fiber from soleus or the FOG fiber from tongue (Fig. 9, A and C, respectively). In Fig. 10 these same criteria can be used to distinguish two fibers from diaphragm. The fiber in A is FG, and that in B is oxidative. Both have virtually identical Z-line widths. So, on the basis of both direct measurements and statistical arguments, FG fibers in diaphragm have thick Z-lines, and there is a relationship between Z-line width and the α-actinin species present, not histochemical fiber type.

DISCUSSION

The recognition of extensive heterogeneity in the major skeletal muscle contractile proteins has led to difficulty with two
continuum of fast fibers due to the expression of these two species can be expressed in the same fiber. Additional evidence for the absence of fiber-type specificity for the fast and slow a-actinin species comes from the fact that both a-actinin species in the same fiber adds to the evidence for the continuous, rather than discrete, nature of skeletal muscle fiber types. More specifically, with regard to the continuous nature of fast fiber diversity, it is possible that the continuum of Z-line widths observed in fast fibers by Eisenberg and Kuda (10) is a consequence, at least in part, of the expression of both a-actinin species in different ratios in individual fast fibers.

The contemporaneous expression of a-actinin1f with troponin T1f and a-actinin2f with troponin T2f in adult fast muscles indicates that the presence of each a-actinin correlates with the presence of a different thin filament Ca2+-regulatory complex: a-actinin1f with troponin T1f - aB tropomyosin and a-actinin2f with troponin T2f - a2 tropomyosin. The role of troponin–tropomyosin in the regulation of skeletal muscle contraction (8) suggests a physiological consequence of the expression of different fast troponin–tropomyosin combinations: fine control of the actomyosin ATPase. Experimental evidence for this proposal comes from the studies of Brandt et al. (1) where differences in the Ca2+-dependence of force generation in fast fibers correlates with different patterns of troponin–tropomyosin expression. It is possible that this variation may be the molecular basis of the continuum of contractile properties found in fast motor units by Henneman and his colleagues (14).

Many aspects of the heterogeneity of fast a-actinin reported here are similar to the observations on fast troponin and tropomyosin. Two species of a-actinin in fast muscles, a-actinin1f and a-actinin2f, were identified. They differ in Mr, and the absence of any co-migrating CNBr fragment implies they are the products of different mRNAs. As is true for the fast troponin T and tropomyosin species, their distribution in fast muscles is not consistent with either a-actinin species being FG or FOG type specific. a-Actinin1f can be the predominant a-actinin in either FG or FOG fibers, whereas a-actinin2f is prevalent only in several FG muscles. Additional evidence for the absence of fiber-type specificity for the fast a-actinin species comes from the fact that both a-actinin species can be expressed in the same fiber.

Based on the expression of troponin T1f with aB tropomyosin and troponin T2f with a2 tropomyosin in fast muscle fibers, we proposed that, at the molecular level, there is a continuum of fast fibers due to the expression of these two troponin–tropomyosin combinations in different ratios in individual muscle fibers (31). The expression of both fast a-actinin species in the same fiber adds to the evidence for the continuous, rather than discrete, nature of skeletal muscle fiber types. More specifically, with regard to the continuous nature of fast fiber diversity, it is possible that the continuum of Z-line widths observed in fast fibers by Eisenberg and Kuda (10) is a consequence, at least in part, of the expression of both a-actinin species in different ratios in individual fast fibers.
between α-actinin from the slow muscle soleus and α-actininfr by either SDS PAGE or CNBr fragment mapping. Studies on Z-line morphology also suggested that α-actininfr is more closely related to α-actinin, than to α-actininsfr. Fibers that express either α-actininfr or α-actinin have significantly thicker Z-lines than those that express α-actininsfr. The relationship of α-actininfr and α-actininsfr and the two fast-twitch glycolytic species isolated from rabbit by Kobayashi et al. (15, 16) is less clear. These proteins, I-actinin and II-actinin, were found in erector spinae and psosas, respectively. They could be distinguished from slow α-actinin immunologically and by partial proteolytic mapping, and their Mrs differed by 12,000. These observations are not consistent with either the distribution or properties of α-actininfr and α-actininsfr. Using both immunoblot and SDS PAGE analyses of freshly prepared myofibrils from both erector spinae and psosas, we have not found different α-actinin species in those muscles; α-actininfr composes more than 85% of the total α-actinin in both. Also, α-actininfr is virtually identical with slow α-actinin, and the difference in Mr, between α-actininfr and α-actininsfr is only 4,000. In addition, we find no Coomassie Blue-staining band that corresponds to the II α-actinin species in freshly prepared myofibrils.

The most unexpected observation of this study is the similarity of α-actininfr and α-actinin. While the simplest explanation for the peptide mapping data is that α-actininfr and α-actinin are identical, we are hesitant to suggest this solely on the basis of peptide maps. Nonetheless, their similarity alone is sufficient to argue that the differences between fast and slow myofibrillar proteins observed in myosin, troponin, tropomyosin, and C-protein are not characteristic of all contractile proteins. Clearly, these observations identify a protein of the myofibrillar lattice whose homologous forms have not evolved along the fast/slow dichotomy. There is evidence to suggest a great deal about the mechanisms that coordinate synthesis of myofibrillar proteins.

We wish to thank Mr. Brad MacDonald who initiated studies on the fast and slow forms of rabbit skeletal muscle α-actinin in the laboratory.

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REFERENCES

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