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Biochemistry

Titin: Major myofibrillar components of striated muscle

(gel electrophoresis/immunofluorescent staining/smooth muscle)

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ABSTRACT **Electrophoretic analyses of protein components of striated muscle myofibril purified from various vertebrate and invertebrate species revealed that proteins much larger than myosin heavy chain are present in significant amounts. To define possible roles of these heretofore unidentified proteins, we purified a combination of two uncommonly large proteins, designated as titin, from chicken breast myofibrils. Chemical and immunological studies indicated that titin is distinct from myosin, actin, and filamin. Specific titin antibody crossreacts with similar protein in both skeletal and cardiac myofibrils of many vertebrate and invertebrate species. Immunofluorescent staining of glycerinated chicken breast myofibrils indicated that titin is present in M lines, Z lines, the junctions of A and I bands, and perhaps throughout the entire A bands. Similar staining studies of myofibrils from other species suggest that titinlike proteins may be organized in all myofibrils according to a common architectural plan. We conclude that titin is a structurally conserved myofibrillar component of vertebrate and invertebrate striated muscles.**

The composition and organization of vertebrate skeletal myofibril have been the subject of intense studies (for recent reviews, see refs. 1 and 2). Detailed investigations of the properties of purified myofibrillar proteins have provided the molecular basis for understanding muscle contraction (3) and a framework to explore nonmuscle contractility (4, 5).

Because our studies on the structure and function of a different contractile protein (6–8) had led to the unexpected observation that filamin is abundant in smooth muscle and a wide range of nonmuscle cells but is essentially absent in striated muscle (6, 7), we decided to undertake a comparison of the total protein compositions of striated and smooth muscle. In this investigation we found that the high molecular weight (M_r > 200,000) components in these two types of muscle differed significantly. Besides the virtual absence of filamin in striated muscle, each type of striated muscle that we studied was found to contain a group of three extremely large polypeptides (M_r > 400,000) that are not present in smooth muscle. By using chicken breast muscle as a source of striated muscle, we have studied the chemical and immunological properties and the distribution in myofibril of a combination of two of these polypeptides. This paper gives a general survey of our results. A preliminary report was presented at the 18th American Society for Cell Biology meeting (9).

MATERIALS AND METHODS

Details of experimental procedures are described in appropriate figure legends. All myofibrils were purified according to the procedure of Etlinger *et al.* (10). ^{125}I-Labeled goat anti-rabbit IgG was a gift of W. Mandy. Antifilamin and other immunochemical and chemical reagents were prepared as described (6–8).

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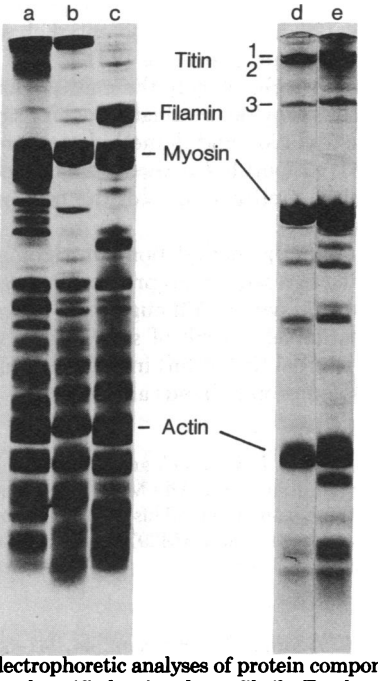

FIG. 1. Electrophoretic analyses of protein components of muscle homogenates and purified striated myofibrils. Fresh striated muscles [chicken breast muscle (lane a) and chicken cardiac muscle (lane b)] and smooth muscle [chicken gizzard muscle (lane c)] were blended in ice cold buffer (0.5 g of muscle per ml of 0.6 M KCl/5 mM EDTA/100 mM Tris·HCl, pH 7.0). The homogenates were solubilized in $NaDodSO_4$ and electrophoresed on 4% polyacrylamide gels as described (7). Sufficient sample was used in lanes a and b to show a trace protein band with the same mobility as filamin. Purified striated muscle myofibrils [chicken breast muscle (lane d) and rabbit back muscle (lane e)], prepared according to Etlinger *et al.* (10), were solubilized by mixing an equal volume of well-suspended myofibrils with sample buffer (100 mM Tris·HCl/10 mM EDTA/40 mM dithiothreitol/10% $NaDodSO_4$/20% glycerol at pH 8.0), followed by heating in a boiling water bath for 2 min. Samples (50 μg) were electrophoresed on 3.2% polyacrylamide gels (0.5 × 10 cm; acrylamide/bisacrylamide = 50:1; electrophoresis buffer: 0.1% $NaDodSO_4$/25 mM Tris glycine, pH 8.8) at 100 V for 2 hr. All gels were stained with Coomassie blue (7).

RESULTS

Protein Composition of Muscles and Myofibrils. When striated and smooth muscles from chickens were homogenized and analyzed by sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis according to Fairbanks *et al.* (11), the overall gel patterns (Fig. 1, lanes a, b, and c) were very similar because major contractile proteins such as actin and myosin are abundant in both types of muscle. However, striking differences near the top of these gels were easily detected: filamin, a major high molecular weight protein present in gizzard smooth muscle (Fig. 1, lane c), was absent or greatly diminished

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

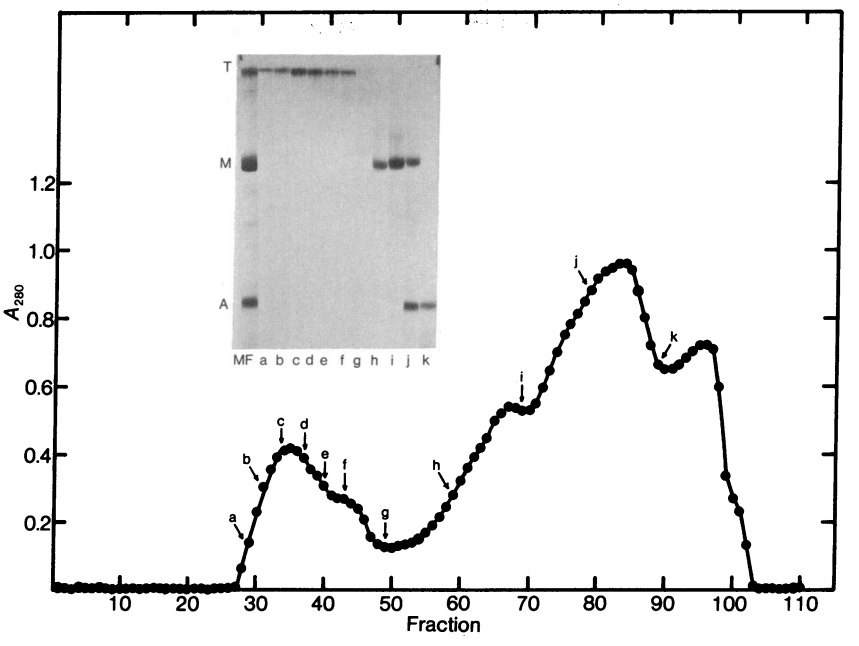

FIG. 2. Purification of chicken breast titin by agarose gel filtration in the presence of $NaDodSO_4$. Freshly prepared chicken breast myofibrils (200 mg) were solubilized by the addition of an equal volume of hot sample buffer (0.2 M Tris·HCl/10 mM EDTA/10% $NaDodSO_4$/0.1 mM phenylmethylsulfonyl fluoride at pH 8.0). The sample was heated with stirring in a boiling water bath for 3 min and then centrifuged at 27,000 rpm for 1 hr at room temperature in a Beckman type 30 rotor to remove a small amount of insoluble material. The clear supernatant (12 ml) was applied to a Bio-Gel A-50m column (2.5 × 90 cm) equilibrated with elution buffer (0.1 M Tris glycine/5 mM EDTA/0.1% $NaDodSO_4$/0.5 mM dithiothreitol at pH 8.8). Fractions (4.5 ml each) were collected at a rate of 15 ml/hr and were analyzed for protein by absorbance at 280 nm (●) and by gel electrophoresis. A typical elution profile is shown here. (*Inset*) Gel patterns of selected fractions, indicated by arrows above the elution profile. MF, solubilized myofibril; T, titin; M, myosin heavy chain; A, actin.

in striated muscles [skeletal (lane a) and cardiac (lane b)]. In contrast, significant amounts of proteins that remained near the origin of the gels of striated muscle were absent in smooth muscle (Fig. 1, lane c). When purified chicken and rabbit skeletal myofibrils were analyzed with high-porosity polyacrylamide gels according to a modified procedure of Etlinger *et al.* (10) [chicken (lane d) and rabbit (lane e)], all solubilized proteins entered the gels and there were three major bands above the myosin heavy chain: a closely spaced doublet and a singlet band with faster mobility (labeled as 1, 2, and 3, respectively). Filamin was completely absent. The presence of these proteins in the purified preparations of myofibril suggests that they are associated with the myofibril. These gel patterns are reproducible under various dissociating conditions. The unusually low electrophoretic mobilities of these three bands suggest that they are extremely large polypeptides. By using crosslinked myosin heavy chain as standards and assuming that the empirical relationship between mobility and molecular weight is still valid in this molecular weight range, we were able to estimate from preliminary experiments that each of the doublet bands (1 and 2) has $M_r \approx 1 \times 10^6$ and that band 3 has $M_r \approx 5 \times 10^5$. These values probably represent the upper limits of size of the polypeptides. These bands all together represent 5–8% of total staining intensities of myofibrillar proteins.

We were intrigued by the apparently exclusive occurrence of filamin in smooth muscle and these large polypeptides in striated muscle, and we have therefore initiated systematic studies of these heretofore unidentified striated muscle proteins. As a first step, we investigated the properties of a combination of proteins forming the doublet bands (M_r 1 × 10^6). For convenience of discussion, we shall tentatively name the combination "titin"* with the clear understanding that each protein(s) in the titin doublet may or may not be a subunit of the same protein.

Purification of Titin. Protein aggregation has as yet prevented us from purifying titin in native forms. However, their gigantic size has made it possible to obtain chemically pure titin by gel filtration (2% agarose) of $NaDodSO_4$-solubilized chicken breast myofibrils. Fig. 2 shows a typical elution profile, in which pure titin appears in the leading edge of the first peak (fractions 29–37). Myosin heavy chain and actin were eluted as the second and third peaks, respectively. The resolution was adequate for estimation of the relative content of bands 1, 2, and 3 as a group in myofibrils. Protein analyses of approximately 20 elution profiles showed that, surprisingly, titin and the protein in band 3 together account for 15–22% of total myofibrillar proteins based on absorbance at 280 nm, and 10–15% based on Lowry protein determinations (12). These values are at least twice those estimated by stain intensity of polyacrylamide gels (see above). The cause for the variation among preparations is not clear. The discrepancies resulting from different analytical techniques could well be related to their chemical compositions. However, it is apparent that titin (and band 3) are major chicken myofibrillar proteins. Preliminary experiments with chicken cardiac and rabbit skeletal myofibrils demonstrated a similar abundance of these extremely large polypeptides.

Immunological Properties of Titin. To explore the possible roles of titin in myofibril structure, we produced antibodies by injecting rabbits with purified chicken breast titin obtained from the gel filtration experiments described above. The specificity of the antiserum was demonstrated by double immunodiffusion tests (Fig. 3 *inset*) and by radioimmunostaining techniques (13) (Fig. 3). As shown in Fig. 3 *inset*, titin antiserum gave a single precipitin line with titin in 8 M urea buffer, but gave no precipitin line with either the 8 M urea buffer alone

* Titin: Derived from Titan (Greek, a giant deity. Anything of great size.)

or with chicken breast myosin in the same buffer.† Furthermore, we found that chicken breast myosin antiserum formed a single precipitin line with myosin and also with total myofibrillar proteins solubilized in 8 M urea, but not with titin or the urea buffer (data not shown). These results demonstrated that titin and myosin are antigenically distinct. Radioimmunostaining tests confirmed and supplemented this conclusion. When ^{125}I-labeled antibody was used to stain (localize) myofibrillar proteins fractionated on polyacrylamide gels, it was found that more than 99% of the radioactive counts in the gels was present in the titin bands and no other protein bands had detectable counts above background (Fig. 3). The specific binding of antibody to titin bands is also indicated by the selective increase of Coomassie blue stain of these bands in antibody-treated gels, as shown by the upper photograph of gels below the corresponding counting profiles in Fig. 3. In control experiments, in which preimmune serum or filamin antiserum was used, no binding was detected. In several parallel experiments in which fluorescent antibodies were substituted for ^{125}I-labeled antibody, we observed labeling of both band 1 and band 2 of titin (data not shown).

Based on these and other immunological tests, we conclude that titin antiserum is highly specific to band 1 and band 2, and that it does not crossreact with myosin, actin, or filamin and probably other chicken breast myofibrillar proteins as well.

Localization of Titin in Chicken Breast Myofibril. With this specific titin antibody, we proceeded to localize titin in chicken breast myofibrils by indirect immunofluorescence techniques (6, 8). In these experiments, glycerinated myofibrils were prepared by stretching the muscle stripes to 130% of the excised length and tying them to plastic rods before glycerination (Fig. 4). We found that glycerinated myofibrils yielded far superior phase contrast micrographs and more reproducible labeling patterns than those prepared by blending relaxed muscle according to the procedure of Etlinger *et al.* (10).

We observed that the titin staining patterns of glycerinated chicken breast myofibrils varied significantly in configuration and relative intensity in any given preparation and appeared to be related to, yet not necessarily determined by, sacromere lengths. Several representative pairs of phase micrographs and fluorescence micrographs are shown in Fig. 4, arranged in the order of decreasing sacromere length (Z line to Z line distance). Of these patterns, that in Fig. 4*f* is the most frequently observed. The phase micrograph shows clearly structural features such as A bands, I bands, Z lines, and H zones. The corresponding fluorescence micrograph appears banded along the sacromere, thus indicating that titin antibody reacts with myofibril in two specific regions: (*i*) the junctions of A and I bands of each sacromere (these junctions are brightly stained) and (*ii*) the central region of the A band (designated as mid-A band) (presumably the H zone or M line is less intensely labeled). In addition, throughout the entire A band, there is a weak and diffuse staining. The staining observed was specific because the controls (preimmune serum) were negative (Fig. 4*e*). Furthermore, affinity-purified titin antibody yielded patterns indistinguishable from those observed by using the IgG fraction of titin antiserum (unpublished observations).

Although staining patterns observed at other sacromere lengths appear at first to be distinct, on close examination they are found to share the same labeling scheme as described above for Fig. 4*f*. However, there are significant variations in the relative dimensions, shapes, and intensities of the stained regions. For example, Fig. 4*a* (sacromere length of 2.6 μm) has very narrow and dim A-I junction labeling, yet the H zone labeling is wide and bright. This pattern, however, is rarely observable. Fig. 4*b* shows a more common pattern at the same sacromere length. The A-I junction labeling is wider and brighter than that in Fig. 4*a*. Note also that the mid-A fluorescent band is much narrower than the wide phase lucent H zone in the corresponding phase micrograph, suggesting that the M line is labeled here. Fig. 4 *c* and *d* shows two examples of shorter sacromeres (2.5 μm) with the mid-A band appearing blurred. An easily detected special feature in these long sacromeres is the labeling of Z lines (Fig. 4 *a*, *b*, and *c*). Such labeling is either absent or difficult to observe in the much shorter sacromeres (e.g., in Fig. 4*f*).

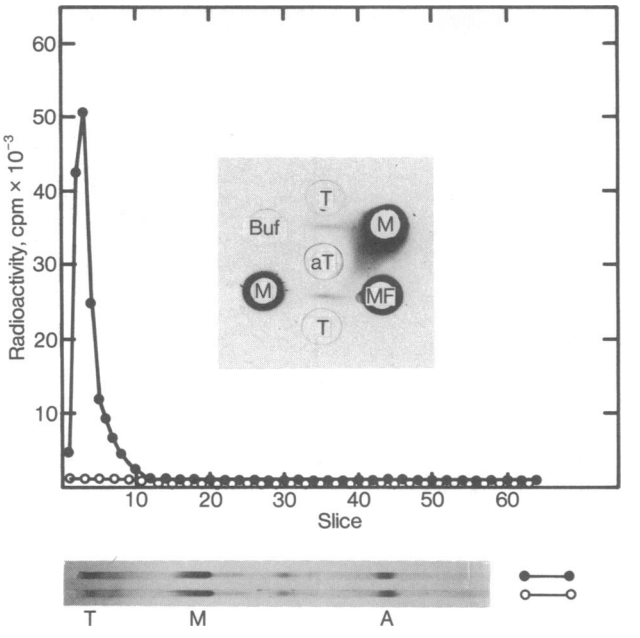

FIG. 3. Characterization of titin antiserum by immunodiffusion and radioimmunostaining. Preparation of antiserum: Chicken breast titin antibody was produced in rabbits by multiple-site injection of 300 μg of titin (emulsified with Freund's complete adjuvant) per rabbit. Initial intramuscular and subcutaneous injections were followed by similar booster injections (except that Freund's incomplete adjuvant was used) at the 4th, 8th, and 13th week. Weekly bleedings were started 1 week after the first booster injection. (*Inset*) Double immunodiffusion test: The slide (1% agarose in 0.3 M sodium phosphate/20 mM NaN_3 at pH 8.8) was developed at room temperature for 36 hr, washed, and stained with amido black as described (6). aT, titin antiserum; T, titin (1 mg/ml); M, chicken breast myosin (8 mg/ml); MF, chicken breast myofibril (5 mg/ml); BUF, buffer (8 M urea/0.05 M Tris acetate, pH 7.8). All samples (except aT) were dialyzed against the urea buffer before being applied to the wells (20 μl per well). The dense staining around the M and MF wells is due to residual myosin that has resisted washings. Radioimmunostaining: Samples of chicken breast myofibrils (50 μg) were solubilized and electrophoresed on small (0.2 × 16 cm) polyacrylamide gels at 50 V for 5 hr (see Fig. 2). The gels were washed with three changes of 25% isopropanol/10% acetic acid to remove $NaDodSO_4$, followed by three changes of wash buffer (0.15 M NaCl/0.05 M sodium phosphate, pH 7.4). Gels were soaked for 15 hr with antititin IgG at 0.1 mg/ml (●) or antifilamin IgG at 0.1 mg/ml (○) plus bovine serum albumin at 1 mg/ml. The gels were then washed extensively before soaking in ^{125}I-labeled goat anti-rabbit IgG (0.1 mg/ml; specific activity: 3.5 × 10^5 cpm/μg) plus bovine serum albumin at 1 mg/ml for 15 hr. The gels were again extensively washed, then stained with Coomassie blue, photographed, and sliced into 2-mm sections for counting. The distribution of radioactivities was plotted, and photographs of the Coomassie blue-stained gels are shown below the counting profiles. T, titin; M, myosin heavy chain; A, actin.

† Urea was added to solubilize titin. We have so far been unable to detect any precipitin line between titin antiserum and solubilized myofibril. The reason is unclear.

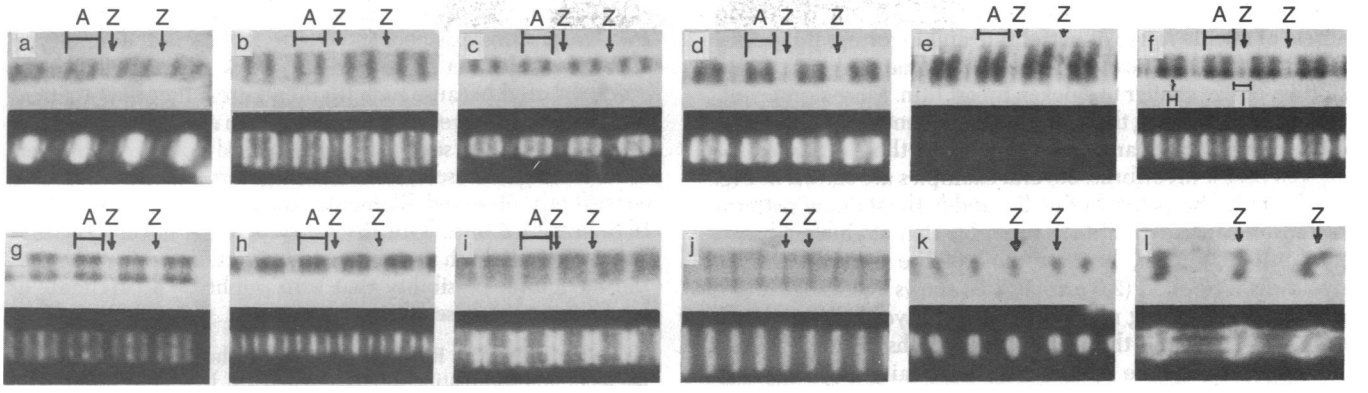

FIG. 4. Indirect immunofluorescent localization of titin in glycerinated chicken breast myofibrils. Myofibril preparation: Chicken breast muscle fibers were stretched to 130% of the excised length, tied to plastic rods, and glycerinated for 2 weeks according to Etlinger *et al.* (10). Myofibrils were prepared from these fibers by homogenization in a VirTis homogenizer. The washed myofibrils were allowed to settle at room temperature onto cover glasses. Many myofibrils adhered to the plate surface and remained so throughout the staining process. Supercontracted myofibrils (*j*) were prepared by rinsing myofibrils on cover glasses with a contraction solution (5 μM ATP/0.1 M KCl/1 mM $MgCl_2$ at pH 7.0) for 10 min. Immunofluorescent staining: These myofibrils were fixed with 2.5% glutaraldehyde and then reduced with $NaBH_4$ at 0.5 mg/ml according to Weber *et al.* (14) to prevent any possible antibody-induced reorganization. (However, similar results were obtained with an unfixed preparation.) The fixed myofibrils were treated with titin antibody at 0.1 mg/ml (*a, b, c, d, f, g, h, i, j, k, l*) or preimmune IgG at 0.1 mg/ml (*e*), washed, and then stained with Lissamine rhodamine-conjugated goat anti-rabbit IgG at 0.1 mg/ml as described (6, 8). Pairs of phase micrographs (upper panels) and fluorescence micrographs (lower panels) of the same fields were taken with a Zeiss universal microscope equipped with epi-fluorescence optics and a ×100 planachromate objective lens. (*a–j*) Staining patterns of intact myofibrils. (*k–l*) Staining patterns of KI-extracted myofibrils. Glycerinated myofibrils prepared on cover glasses were extracted with KI buffer (0.6 M KI/0.1 M Tris·HCl/3 mM EGTA/5 mM $Na_2S_2O_3$/1 mM dithiothreitol at pH 7.5) at room temperature for about 10 min. The extracted myofibril preparation was then washed, fixed, and stained with titin antibody as described above. Sacromere length (Z-to-Z distance): *a*, 2.6 μm; *b*, 2.6 μm; *c*, 2.5 μm; *d*, 2.5 μm; *e*, 2.3 μm; *f*, 2.3 μm; *g*, 2.2 μm; *h*, 2.1 μm; *i*, 1.8 μm; *j*, 1.3 μm. A, A band; H, H zone; I, I band; Z, Z line.

In Fig. 4 *g* and *h*, mid-A bands are brighter and straighter than A-I junction bands. In Fig. 4*i* where thin filaments almost reach the M lines, resulting in narrow I bands, the A-I junctions are very bright and the mid-A band is much dimmer. Fig. 4*j* shows a supercontracted myofibril prepared by adding Mg^{2+}/ATP to myofibrils to induce contraction (15). The phase dense contracture bands are brightly stained, as if two A-I junction bands from adjacent sacromeres were fused together.

The interpretation of these labeling patterns is complicated by the fact that the antiserum used is directed toward both bands 1 and 2. However, despite the uncertainty concerning the distribution of individual bands of titin, several general conclusions can be reached: (*i*) The labeling of M and Z lines suggests that these transverse structures contain titin. (*ii*) The A-I junction bands behave as though the titin-containing structure is coupled to the ends of thick filaments, because the distance between the pair of labeling bands bordering each A band does not change with sacromere length. (*iii*) The observed variation of labeling pattern and intensity could result from structural or accessibility changes of titin-containing structure in sacromeres of different lengths. Such variations are highly reminiscent of similar changes of myosin labeling patterns reported by Pepe (16) and by Lowey and Steiner (17).

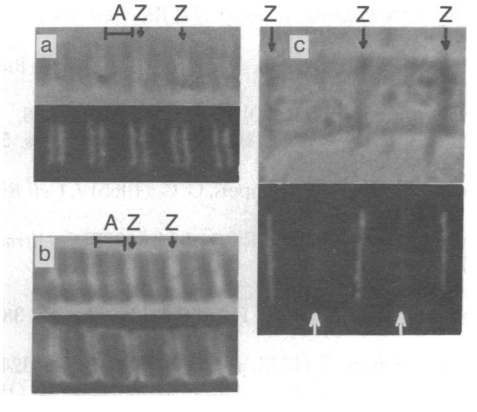

FIG. 5. Immunofluorescent localization of titinlike material in chicken cardiac and insect myofibrils. Chicken cardiac myofibrils (*a* and *b*) and cricket leg myofibrils (*c*) were prepared and stained with chicken breast titin antibody as described in Fig. 4. Arrows in *c* indicate the position of faint bands. Sacromere length: *a*, 1.9 μm; *b*, 1.9 μm; *c*, 4.4 μm.

We have also studied the distribution of titin when various selective extraction procedures known to alter certain structures of myofibrils are used. An informative experiment for the present purpose is the potassium iodide extraction which removes the majority of actin, myosin, and their associated regulatory proteins (18). As shown in Fig. 4*k*, a drastic change of phase pattern was affected by the 0.6 M KI buffer solution. The only discernible structures after this extraction are the irregularly spaced phase dense bands which are derived from, yet thicker than, the original Z lines. Titin antibody labeled both edges, but not the very center, of these thick Z bands (Fig. 4*k*). The double bands are frequently seen closed at both ends into a flattened circle (Fig. 4 *k* and *l*). Occasionally, a small portion of the KI-extracted myofibrils was stained brightly in the region between the Z bands in the form of roughly parallel threads connecting adjacent bands (Fig. 4*l*). Preliminary gel analysis showed that the titin doublet is the major component of the remaining extracted myofibril preparation. However, small and significant amounts of myosin and actin still remain (data not shown). Our results support the idea that titin is a structural component of myofibrils. Furthermore, since nearly all thick and thin filament-associated regulatory proteins are solubilized and removed along with the majority of actin and myosin by extraction procedures, the observation that titin is more resistant indicated that titin is probably not a regulatory protein. Our results, of course, do not rule out the possibility that titin can interact with these other myofibrillar proteins.

Detection and Localization of Titinlike Proteins in Striated Muscles of Other Organs and Species. We have performed a preliminary survey of the occurrence of titinlike proteins in other organs and species. Our results indicate that

titin is widespread in striated muscles: skeletal and cardiac muscle of a wide range of vertebrate and invertebrate species contain immunologically crossreacting material and polypeptides of size similar to chicken breast titin. More significant, perhaps, is the finding that immunofluorescent staining patterns of these muscles are analogous to some of those observed for chicken breast myofibrils. Several examples are shown in Fig. 5 to illustrate this point. In Fig. 5 *a* and *b*, the staining patterns of chicken cardiac myofibrils resemble that of chicken breast myofibril in Fig. 4*i*, because most of the purified cardiac myofibrils were short (2.0 μm). Fig. 5*c* shows a staining pattern of insect (cricket) leg myofibrils. These myofibrils are much larger (4.4 μm) and the phase micrographs show no clearly defined A bands. The Z lines have been stained by titin antibody. In addition, there is a dim band located halfway between adjacent Z lines, probably corresponding to the mid-A labeling band of chicken breast myofibrils. This pattern is similar, but not identical, to that of supercontracted chicken myofibrils in Fig. 4*j*. The overall intensity of Fig. 5*c* is much weaker, presumably due to partial crossreactivity between insect and chicken antigens.

Our observations on the crossreactivity of titin, and the similarity of staining patterns of different muscles, suggest that titin may be chemically conserved and, furthermore, that titin may be organized in a similar fashion in striated muscles in both vertebrates and invertebrates.

DISCUSSION

We have shown that there exists in chicken breast muscle a group of three unidentified major components of which at least two are chemically and immunologically distinct from myosin, actin, and filamin. With the use of specific antibody to two extremely large polypeptides (titin), we have been able to make two other important observations. First, using immunofluorescent localization techniques, we have shown that titin appears to be a bona fide structural component of myofibrils. Second, we found that materials antigenically crossreacting to chicken titin are widespread in striated muscles of various vertebrate and invertebrate species. Moreover, these titinlike proteins may be organized in all myofibrils according to a common architectural plan.

The precise molecular weights of titin polypeptides remain to be determined by other methods. However, they are likely to be among the largest chains discovered. We have therefore named them collectively as titin. Perhaps due to their uncommonly low electrophoretic mobilities and their susceptibility to proteolytic degradation, titin has escaped serious attention despite extensive studies of skeletal muscle structure in the past two decades. Several reports have alluded to their existence (10, 19, 20).

We do not know the detailed structural arrangement of titin in myofibrils. Our extraction experiments suggest that they are unlikely to be exclusively either thin or thick filament-associated regulatory proteins such as tropomyosin or C-protein (19). Studies by others (10, 21) on the protein composition of isolated native thin and thick filaments have shown no evidence of titin and are therefore consistent with this view. Based on our labeling studies, titin appears to be a component of transverse structures such as M or Z lines. In addition, we speculate that titin may be related to some of the more obscure features of myofibrils such as the N lines of I-bands (22), S filaments (15), gap filaments (23–25), T filaments (26), and M filaments (27, 28). The possible existence in vertebrate muscle of a third type of filament distinct from thin and thick filaments has been much debated because such filaments are either hypothetical or have been observed only occasionally in electron microscopic studies and have no supporting biochemical evidence. However, in insect flight muscle, some investigators (e.g., ref. 29) have consistently observed filaments connecting the end of thick filaments to Z lines. Our discovery of these novel structural components in both vertebrate and invertebrate muscle raises the intriguing possibility that titin might be a candidate as components of these elusive filaments.

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1. Mannherz, H. G. & Goody, R. S. (1976) *Annu. Rev. Biochem.* **45**, 427–465.
2. Squire, J. M. (1975) *Annu. Rev. Biophys. Bioeng.* **4**, 137–162.
3. Huxley, H. E. (1972) in *The Structure and Function of Muscle*, ed. Bourne, G. H. (Academic, New York), Vol. 1, pp. 301–387.
4. Korn, E. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 588–599.
5. Clarke, M. & Spudich, J. A. (1977) *Annu. Rev. Biochem.* **46**, 797–822.
6. Wang, K., Ash, J. F. & Singer, S. J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4483–4486.
7. Wang, K. (1977) *Biochemistry* **16**, 1857–1865.
8. Heggeness, M. H., Wang, K. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3883–3887.
9. Wang, K. & McClure, J. (1978) *J. Cell Biol.* **79**, 334a.
10. Etlinger, J. D., Zak, R. & Fischman, D. A. (1976) *J. Cell Biol.* **68**, 123–141.
11. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2616.
12. Lowry, D. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
13. Burridge, K. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4457–4461.
14. Weber, K., Rathke, P. C. & Osborn, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1820–1824.
15. Huxley, H. E. & Hanson, J. (1954) *Nature (London)* **173**, 973–974.
16. Pepe, F. A. (1975) *J. Histochem. Cytochem.* **23**, 543–562.
17. Lowey, S. & Steiner, L. A. (1972) *J. Mol. Biol.* **65**, 111–126.
18. Szent-Györgyi, A. G. (1951) *J. Biol. Chem.* **192**, 361–369.
19. Offer, G., Moos, C. & Starr, R. (1973) *J. Mol. Biol.* **74**, 653–676.
20. Porzio, M. A. & Pearson, A. M. (1977) *Biochim. Biophys. Acta* **490**, 27–34.
21. Morimoto, K. & Harrington, W. F. (1973) *J. Mol. Biol.* **77**, 165–175.
22. Franzini-Armstrong, C. (1970) *Tissue Cell* **2**, 327–338.
23. Locker, R. H. & Leet, N. G. (1975) *J. Ultrastruct. Res.* **52**, 64–75.
24. Carlsen, F., Fuchs, F. & Knappeis, G. G. (1965) *J. Cell Biol.* **27**, 35–46.
25. Sjöstrand, F. J. & Jagendorf-Elfavin, M. (1967) *J. Ultrastruct. Res.* **17**, 348–378.
26. Hoyle, G. (1967) *Am. Zool.* **7**, 435–449.
27. Knappeis, G. G. & Carlsen, F. (1968) *J. Cell Biol.* **38**, 202–241.
28. Luther, P. & Squire, J. (1978) *J. Mol. Biol.* **125**, 313–324.
29. Ullrick, W. C., Toselli, P. A., Chase, D. & Dasse, K. (1977) *J. Ultrastruct. Res.* **60**, 263–271.