Highly Homologous Filamin Polypeptides Have Different Distributions in Avian Slow and Fast Muscle Fibers

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ABSTRACT The high molecular weight actin-binding protein filamin is located at the periphery of the Z disk in the fast adult chicken pectoral muscle (Gomer, R. H., and E. Lazarides, 1981, *Cell*, 23: 524–532). In contrast, we have found that in the slow anterior latissimus dorsi (ALD) muscle, filamin was additionally located throughout the I band as judged by immunofluorescence with affinity-purified antibodies on myofibrils and cryosections. The Z line proteins desmin and α-actinin, however, had the same distribution in ALD as they do in pectoral muscle. Quantitation of filamin and actin from the two muscle types showed that there was approximately 10 times as much filamin per actin in ALD myofibrils as in pectoral myofibrils. Filamin immunoprecipitated from ALD had an electrophoretic mobility in SDS polyacrylamide gels identical to that of pectoral myofibril filamin and slightly greater than that of chicken gizzard filamin. Two-dimensional peptide maps of filamin immunoprecipitated and labeled with ^{125}I showed that ALD myofibril filamin was virtually identical to pectoral myofibril filamin and was distinct from chicken gizzard filamin.

Filamin is an actin-binding protein originally isolated from avian smooth muscle (48, 55) and is closely related to an actin-binding protein isolated from mammalian leukocytes (21). In its native state filamin exists as a dimer with a molecular weight of 500,000, and binds to F-actin filaments and cross-links them, forming a gel under a large variety of conditions (7, 10, 11, 22, 23, 35, 38, 44, 51, 57, 59). Antibodies to filamin stain stress fibers, membrane ruffles, and microspikes in a variety of nonmuscle cells grown in tissue culture (56). In adult chickens filamin is located on Z lines of thigh (5), pectoral (16), and heart muscle (32).

We recently used quantitative immunoprecipitation and two-dimensional peptide mapping of proteins labeled with ^{125}I to compare myoblast and mature myotube filamins (17). Chick fibroblast and purified adult gizzard filamin are virtually identical and are closely related to chick myoblast filamin. Mature myotube and adult myofibril filamins are closely homologous but have many peptides different from the other three types. Mature myotube and myofibril filamins have, and are synthesized as, a lower molecular weight variant compared with myoblast, fibroblast, and gizzard filamins. These results have suggested that myoblast and mature myotube filamins are distinct gene products.

It is well known that adult skeletal muscle fibers are not homogeneous. Two main physiological classes are known, fast and slow (8, 25, 39, 41). Fast and slow skeletal muscles can be distinguished on the basis of contraction speed (8, 25, 41) and type of innervation (1, 8, 24, 25, 45, 46). Differences also exist with respect to protein composition. Fast and slow muscles have characteristic α-actinins (43, 53), tropomyosin subunits (6, 8, 37), troponins (13), myosin heavy and light chains (3, 4, 25, 26, 36, 45, 52), and C-protein subunits (47). In addition, levels of metabolic enzymes are different (12, 36, 41, 50). Fast and slow muscles have different T-tubule and sarcoplasmic reticulum distributions (39, 42, 54), and differences in Z line thickness (39, 46, 54). In the chicken the anterior latissimus dorsi (ALD)[1] is a slow muscle and the posterior latissimus dorsi and pectoralis are fast muscles. In this study we show that although ALD and pectoral muscle filamins are highly homologous polypeptides, ALD filamin and pectoral filamin have different distributions within myofibrils.

MATERIALS AND METHODS

Cell Culture: Chicken embryonic fibroblasts and embryonic myogenic cells were grown as previously described (15, 17).

Myofibril Preparation: Strips of muscle were tied to wooden applicator sticks and glycerinated in 50% glycerol, ½ × PBS and 5 mM EGTA at −20°C for 1 yr. Myofibrils were obtained by homogenization of glycerinated muscle in a Lourdes blender (Lourdes Industries, Hauppauge, NY) at top speed

[1] *Abbreviation used in this paper:* ALD, anterior latissimus dorsi.

THE JOURNAL OF CELL BIOLOGY · VOLUME 97 SEPTEMBER 1983 818-823

for 2 min in PBS/5 mM EGTA at 0°C. Fresh skeletal myofibrils were prepared from adult chicken pectoral muscle by trimming a piece of muscle free of fat and connective tissue, and homogenizing it in a Lourdes blender at top speed for 30 s in ice-cold C buffer (100 mM KCl, 12 mM NaCl, 4.9 mM K_2HPO_4, 3.6 mM KH_2PO_4, 0.72 mM NaH_2PO_4, 5 mM EGTA, pH 7.0, at room temperature). Myofibrils were then purified by filtering the homogenate twice through two layers of cheesecloth and then centrifuging it at 1,500 *g* for 5 min in a Sorvall HB-4 rotor (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments, Div., Newtown, CT). The supernatant was discarded and myofibrils were resuspended in C buffer.

Cryosections: Tissue samples for cryosections were taken from freshly killed chickens and frozen in liquid nitrogen. Skeletal muscle samples were stretched with forceps while freezing. 2–4-μm-thick sections were cut on a Tissue Tek II cryostat (Miles-Yeda, Miles Laboratories, Inc., Elkhart, IN) at −20°C using frozen samples embedded in O.C.T. compound (Tissue-Tek). Sections were picked up onto coverslips and fixed at room temperature for 10 min in C buffer containing 2% formaldehyde (Mallinkrodt Inc., Paris, KY). Sections were then rinsed in C buffer at room temperature for at least 10 min. Alternatively, sections were fixed in 95% ethanol at room temperature for 10 min and then rinsed in C buffer.

Antibody Preparation: Filamin antibodies used in this study were prepared in rabbits using either native chicken gizzard filamin as antigen or filamin further purified by electrophoresis on SDS polyacrylamide gels prior to use as antigen (16, 17). Serum was precipitated at 50% ammonium sulfate saturation at 0°C and the partially purified IgG was dialyzed against PBS in the presence of 10 mM sodium azide. The final protein concentration was ~20 mg/ml, assuming $E^{280}_{1mg/ml} = 1.4$. The IgG was further purified by dialysis against 10 mM $NaPO_4$, pH 7.5, for 2 d and then removal of the insoluble material by centrifugation at 10,000 *g* for 15 min. 1 ml of the supernatant was then passed through a column containing 4 ml of Whatman DE-52 ion exchange resin (Whatman Chemical Separation Inc., Clifton, NJ) equilibrated and run in the above buffer. The purified IgG was collected in the column flow-through peak; this was typically 4 ml containing 1 mg/ml protein. The IgG fraction was then dialyzed against PBS/10 mM NaN_3. Affinity-purified antiserum was prepared as described (16) using either antiserum described above. Typical protein concentrations were 0.2 mg/ml. Antisera to desmin and α-actinin were prepared as previously described (15, 19).

Immunofluorescence: Buffers for immunofluorescence were PBS/5 mM EGTA for glycerol-extracted myofibrils and cryosections and buffer C for fresh myofibrils. Coverslips were incubated with antibody for 1 h, rinsed in buffer at room temperature 15–30 min, incubated in a 1:50 dilution of fluorescein isothiocyanate-labeled goat anti-rabbit IgG (Miles-Yeda) for 1 h, and then rinsed in the appropriate buffer at room temperature for 30 min. Both antibody incubations were at 37°C for glycerinated myofibrils and cryosections and at room temperature for fresh myofibrils. Coverslips were mounted on slides in PBS/5 mM EGTA/90% glycerol (glycerinated myofibrils and cryosections) or C buffer/90% glycerol (fresh myofibrils). Microscopy and photography were performed as described (15, 19).

Sample Preparation: Myofibrils were prepared from pectoral or ALD muscle as described above. However, instead of being resuspended in buffer C, pelleted myofibrils were resuspended in 1% SDS, 20 mM Tris/HCl, 130 mM NaCl, 5 mM EGTA, pH 7.5, and boiled for 1 min at a concentration of ~10 mg wet wt of myofibrils per milliliter.

Adult chicken gizzard was trimmed free of fat and connective tissue and homogenized for 20 s at top speed in a Lourdes blender in ice-cold 20 mM Tris/HCl, 130 mM NaCl, 5 mM EGTA, pH 7.5, at a concentration of ~1 mg wet weight gizzard/ml; 20% SDS was added to a final concentration of 1%, and the extract was then boiled for 1 min. Chicken gizzard filamin was purified as described by Wang (55) with the modification that all buffers contained an additional 1 mM EGTA. Adult chicken pectoral muscle actin was purified according to the method of Spudich and Watt (49). For immunoprecipitation, cell cultures were rinsed at room temperature with PBS, scraped into 1% SDS, 20 mM Tris/HCl, 130 mM NaCl, 5 mM EGTA, pH 7.5, and boiled for 1 min; typically, cells from one 100-mm culture plate were solubilized in 1 ml.

Immunoprecipitation: Myofibrils solubilized in 1% SDS were diluted with 4 vol of ice-cold 1.25% Nonidet P-40 (Particle Data Inc., Elmhurst, IL), 20 mM Tris/HCl, 130 mM NaCl, 5 mM EGTA, pH 7.5, to a final detergent concentration of 0.2% SDS and 1% Nonidet P-40. The solution was then clarified at 10,000 *g* for 10 min in a SS34 rotor. 2 ml of the above supernatant containing ~0.2 mg total protein was added to 60 μl of affinity-purified IgG. After gentle rocking at 4°C for 2 h, 150 μl of a 10% wt/vol solution of fixed *Staphylococcus aureus* was added (29, 30). Prior to incubation with the samples, the bacteria were washed twice in 20 mM Tris/Cl, 130 mM NaCl, 5 mM EGTA, pH 7.5, resuspended to 10% wt/vol in the same buffer containing 1% SDS, and then placed in a boiling water bath for 1 min. Then, after pelleting in an Eppendorf centrifuge (Brinkmann Instruments, Inc., Westbury, NY) for 4 min, the bacteria were washed three more times in precipitation buffer (20 mM Tris/HCl, 130 mM NaCl, 5 mM EGTA, 0.2% SDS, 1% NP-40).

The bacteria were rocked with the immunoprecipitation solutions for 2 h at 4°C and then pelleted in a Sorvall J6 centrifuge using a JS5.2 rotor (E. I. DuPont de Nemours & Co., Inc.) for 15 min at 2,000 *g*. The pellets were resuspended at 4°C in precipitation buffer and washed by pelleting three times in an Eppendorf centrifuge for 4 min. Immunoprecipitates to be run on SDS polyacrylamide gels were resuspended in 50 μl sample buffer (1% SDS, 0.5% β-mercaptoethanol, 20 mM Tris/HCl, pH 6.8, 0.2 mM EDTA, 10% glycerol, 0.005% bromphenol blue), boiled for 2 min, and cleared of bacteria by centrifuging for 5 min in an Eppendorf centrifuge. Immunoprecipitates to be iodinated (see below) were washed additionally twice in 100 mM sodium phosphate, pH 7.5, after having been washed in precipitation buffer, and then resuspended in 100 μl of 0.5 M sodium phosphate, pH 7.5, containing 1% SDS, boiled for 15 s, and cleared of bacteria by being spun twice for 5 min in an Eppendorf centrifuge.

Iodination: Proteins were iodinated in solution following the method of Greenwood et al. (20). 0.3 mCi of carrier-free ^{125}I (O33H, New England Nuclear, Boston, MA) in 10 μl of 0.5 M $NaPO_4$, pH 7.5, was added to the 100 μl of immunoprecipitate in 0.5 M $NaPO_4$, pH 7.5, 1% SDS, followed by 20 μl of 1 mg/ml chloramine T (Sigma Chemical Co., St. Louis, MO). The reaction proceeded for 2 min at room temperature and was stopped by the addition of 10 μl of 10 mg/ml sodium metabisulfite. 30 μl of 5 × SDS sample buffer was added, the mixture was boiled for 15 s and then loaded on an SDS polyacrylamide gel and electrophoresed. After the dye front was removed, the gel was stained and destained normally, and the filamin bands were then cut out.

Peptide Mapping: Polyacrylamide gel slices containing ^{125}I-labeled proteins were washed for 48 h in two changes of 10% methanol and then for 6 h in 100% methanol at room temperature; they were then dried under vacuum. Following the method of Elder et al. (14), digestion of labeled proteins in gel slices was carried out by adding 400 μl of 0.05 mg/ml protease in 200 mM ammonium bicarbonate to each dried gel slice for 12 h at 37°C. 600 μl of freshly prepared protease solution was then added and the digestion was allowed to proceed for an additional 12 h, after which time the eluted peptides were lyophilized. The protease used was trypsin-*N*-tosyl-L-phenylalanine chloromethyl ketone (Millipore Co., Bedford, MA). Two-dimensional peptide mapping was performed on cellulose 20 × 20 cm Chromatogram sheets (Eastman 13255, American Scientific Products Div., American Hospital Supply Corp., McGaw Park, IL). The electrophoresis buffer for the first dimension was 11.4:10:379 acetic acid/formic acid/water, and the chromatography buffer for the second dimension was 5.5:3.3:1:3 butanol/pyridine/acetic acid/water. Autoradiography was done on Kodak AR-5 film (Eastman Kodak, Rochester, NY) using, for ^{125}I, DuPont Cronex Lightening-Plus intensifying screens (E. I. DuPont de Nemours & Co., Inc.).

Quantitation of Protein: Purified chicken gizzard filamin or pectoral muscle actin was quantitated using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA). Ovalbumin (A5503, Sigma Chemical Co.) was used to construct a curve of optical density 595 vs. weight of protein. To determine the amount of protein in 1% SDS extracts, the assay was performed using 1 ml of the diluted Bio-Rad dye reagent mixed with 5 μl of sample in 1% SDS and 1 ml of water. Ovalbumin was boiled in 1% SDS and then used to construct a standard curve.

Gels stained with Coomassie Blue were scanned with a Joyce Loebl MK III C densitometer (National Instrument Co., Baltimore, MD), using a 0–1 OD wedge. Several scans were made across each band, and the traces were recorded on paper. The area under each peak was cut out and weighed, and an average was taken. Scans of known quantities of filamin or actin (as determined above) were used to construct a standard curve for each gel. These curves were then used to correct for differences in Coomassie Blue binding between the two proteins and for nonlinearity in Coomassie Blue binding.

Polyacrylamide Gel Electrophoresis: SDS PAGE was based on a modification of the method of Laemmli (33) as described by Hubbard and Lazarides (27). Gels contained 10% acrylamide and 0.13% *N,N'*-methylene bisacrylamide or 12.5% acrylamide and 0.10% *N,N'*-methylene bisacrylamide.

RESULTS

Immunofluorescence

Immunofluorescence using antiserum affinity purified over purified chicken gizzard filamin showed that filamin was exclusively located on the Z line in myofibrils from adult chicken pectoralis major (Fig. 1, *a* and *b*), posterior latissimus dorsi (data not shown), and heart (32). However, myofibrils from the ALD showed filamin staining on the I bands in

addition to the Z line (Fig. 1 *d*). These results were obtained with myofibrils from glycerol-extracted muscle samples or myofibrils from fresh muscle, using antiserum made to either native or SDS-denatured chicken gizzard filamin.

The location of filamin at the I bands in the ALD could also be seen in cryosections of adult muscle. Fig. 2 shows longitudinal cryosections of ALD muscle stained with affinity-purified filamin antiserum. In this muscle, filamin was located both on the I bands and Z lines while in the pectoral muscle it was located only on the Z line. Immunofluorescence on myofibrils and cryosections of the fast skeletal muscle posterior latissimus dorsi and heart also showed filamin located only at the Z line. These distributions were seen with either fixed or unfixed tissue (not shown) and in all myofibrils from the ALD.

In the ALD the distribution of filamin is not accompanied by a similar distribution of other Z-line–associated proteins. The Z-line–associated intermediate filament proteins desmin and vimentin are located at the Z line in glycerol-extracted ALD myofibrils (19, see also Fig. 1 *f*) or cryosections of ALD (Fig. 2 *d*). The actin-binding protein α-actinin, another Z-line–associated protein, is also located exclusively at the Z line in glycerol-extracted (Fig. 1 *h*) or fresh ALD myofibrils or cryosections of ALD (Fig. 2 *f*) (19).

We have previously shown that in glycerol-extracted pectoral or thigh muscle extracted with 0.6 M KI, filamin is located at the periphery of the Z disk (16), as is the case for desmin (18) and vimentin (19). Cryosections cut transverse to the myofibrils in fresh ALD showed an even distribution of fluorescence (Fig. 3 *a*), indicating that filamin was distrib-

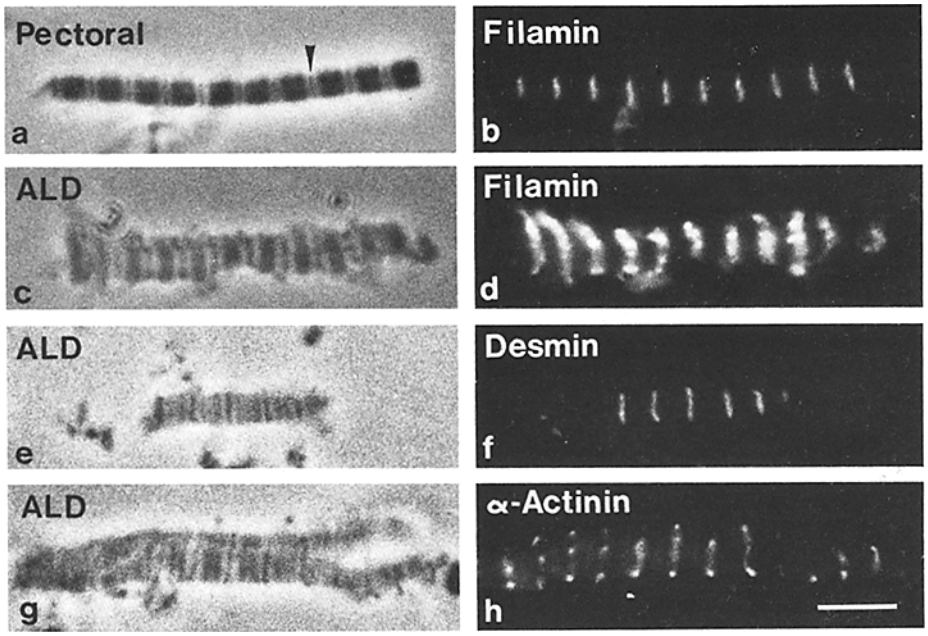

FIGURE 1 Immunofluorescence on freshly prepared myofibrils. *a*, *c*, *e*, and *g* are phase images and *b*, *d*, *f*, and *h* are the corresponding fluorescence images. (*a* and *b*) A pectoral muscle myofibril stained for filamin; (*c* and *d*) an ALD muscle myofibril also stained for filamin; (*e* and *f*) an ALD muscle myofibril stained for desmin; and (*g* and *h*) a similar myofibril stained for α-actinin. Arrowhead indicates Z line. Bar, 5 μm.

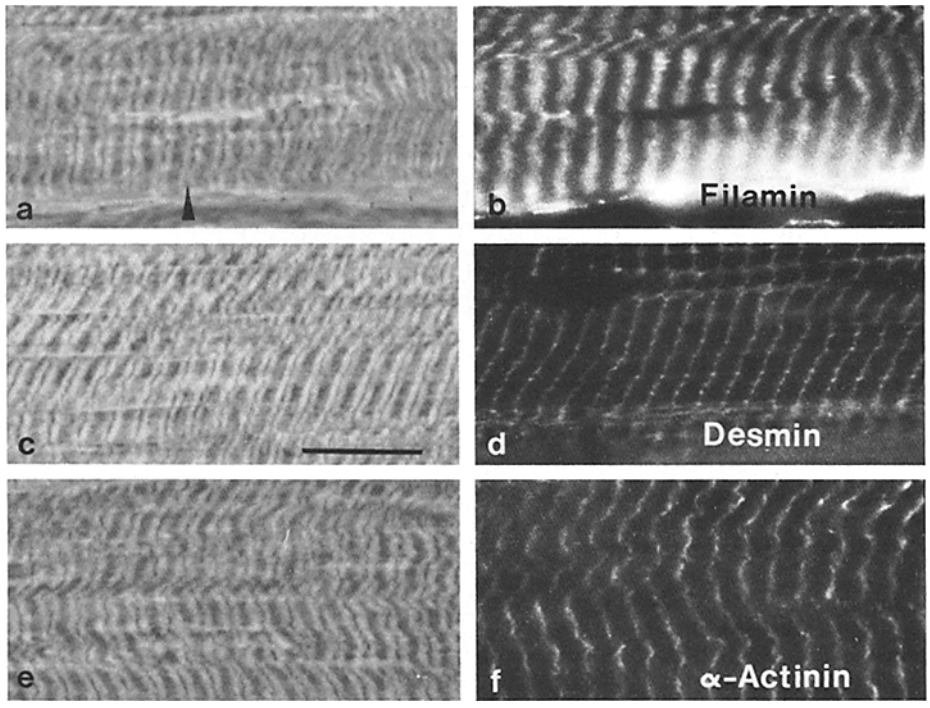

FIGURE 2 Longitudinal cryosections of fresh ALD muscle stained for filamin, desmin, and α-actinin by immunofluorescence. *a*, *c*, and *e* are phase images and *b*, *d*, and *f* are the corresponding fluorescence images. (*a* and *b*) A section stained for filamin; (*c* and *d*) a section stained for desmin; and (*e* and *f*) a section stained for α-actinin. Arrowhead indicates Z line. Bar, 10 μm.

uted throughout the I band and possibly the Z line. Similar sections stained for desmin showed an open lattice distribution (Fig. 3 *b*), indicating a distribution of this antigen at the periphery of the myofibril as previously shown for leg muscle (34). In glycerinated and then KI-extracted thigh muscle, α-actinin is located in the interior of the Z disk (18); a similar distribution was seen in transverse cryosections of fresh ALD (Fig. 3 *c*).

Quantitation of Filamin and Actin

By scanning gels of filamin quantitatively immunoprecipitated from known quantities of total protein, we can determine the amount of filamin in a tissue (17). Using this method (Fig. 4) we found that there was 7.0 mg filamin/g total protein in adult chicken ALD myofibrils, as compared with 0.88 mg filamin/g total protein in adult pectoralis major myofibrils.

To determine filamin to actin ratios, known quantities of protein from tissues and myofibrils were run on SDS polyacrylamide gels along with known quantities of purified actin. The results are shown in Table I. When it was assumed that all of the protein in the 42,000-mol-wt band was actin, gizzard, pectoral myofibrils, and ALD myofibrils all had roughly equivalent levels of actin, ~10% of total protein, while fibroblasts and myoblasts had significantly lower quantities. Gizzard had the highest level of filamin, 3% of total protein. In fibroblast and ALD myofibrils filamin represented ~1% of the total protein. Myoblasts had an even lower level while pectoral myofibrils had the lowest measured level of filamin, less than a tenth of one percent of total protein.

Characterization of ALD Filamin

The electrophoretic mobility of the filamin immunoprecipitated from ALD was slightly greater in SDS polyacrylamide gels than that of purified chicken gizzard filamin (Fig. 4). We have observed this increased mobility in filamin immunoprecipitated from pectoral muscle or cultured adult myotubes (17).

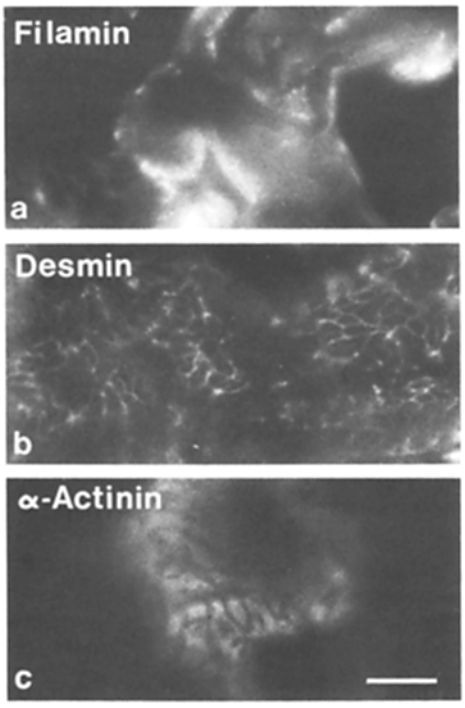

FIGURE 3 Transverse cryosections of fresh ALD muscle stained for filamin (*a*), desmin (*b*), and α-actinin (*c*). Bar, 5 μm.

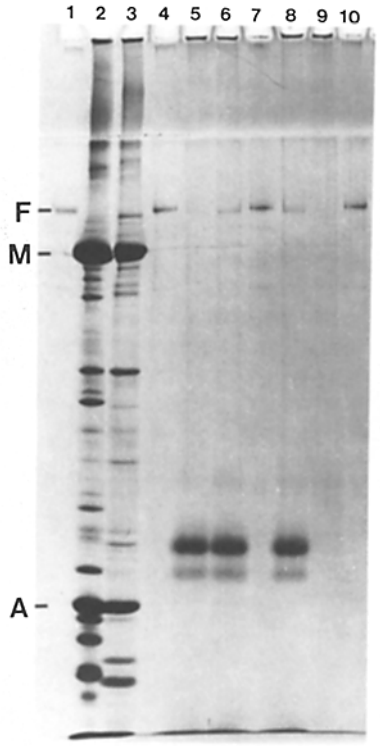

FIGURE 4 (*A*) Coomassie Blue-stained 10% polyacrylamide SDS gel showing filamin immunoprecipitated from fresh ALD myofibrils. Molecular weight markers are purified gizzard filamin (*F*) at 250,000 mol wt in lanes *1*, *4*, *7*, and *10*, myosin heavy chain (*M*) at 200,000 mol wt, and actin (*A*) at 42,000 mol wt. Lane *2* shows pectoral muscle myofibrils and lane *3* shows ALD muscle myofibrils; ~40 μg of protein was loaded in each lane. Lanes *5*, *6*, and *8* are immunoprecipitations of filamin from 17, 35, and 70 μg, respectively, of total ALD myofibril protein using antisera affinity purified with purified gizzard filamin. Lane *9* is an immunoprecipitation as in lane *8*, using PBS instead of affinity-purified antiserum.

TABLE I

Quantitation of Actin and Filamin in Tissues and Myofibrils

Tissue	mg actin/g total protein*	mg filamin/g total protein*	mol actin/mol filamin‡
Gizzard	130	30	52
Fibroblast	41	10	48
Myoblast	18	2.0	108
Pectoral Myofibrils	122	0.88	1,650
ALD Myofibrils	106	7.0	164

* Values are the result of three separate determinations and have a standard deviation of 15% of the mean.
‡ Assuming molecular weights of 500,000 for filamin and 42,000 for actin.

To further characterize filamin from ALD, two-dimensional peptide maps were performed. Immunoprecipitations, iodinations, and proteolytic digestions were performed in parallel for all samples. Filamin was immunoprecipitated from SDS-solubilized fresh adult chicken gizzard, fresh adult chicken pectoral myofibrils, and fresh adult chicken ALD myofibrils. Filamin was iodinated in solution in the presence of 1% SDS (see Materials and Methods). Maps of tryptic peptides (Fig. 5, *a–c*) show that gizzard (Fig. 5 *a*) and pectoral myofibril (Fig. 5 *c*) filamins were related to each other but had many differences. The maps of ALD myofibril filamin (Fig. 5 *b*) show that it was virtually identical to pectoral muscle filamin when comparing Fig. 5 *b* and *c*. Of 45 tryptic peptides (determined by different exposures of the film) in the pectoral myofibril filamin map (Fig. 5 *c*), all matched the spots in the corresponding map of ALD filamin (Fig. 5 *b*). However, four spots differed somewhat in intensity (arrows, Fig. 5, *b* and *c*). These spots are in the lower area of the peptide map, having low R_f values in the chromatography step, and are therefore probably hydrophilic (29).

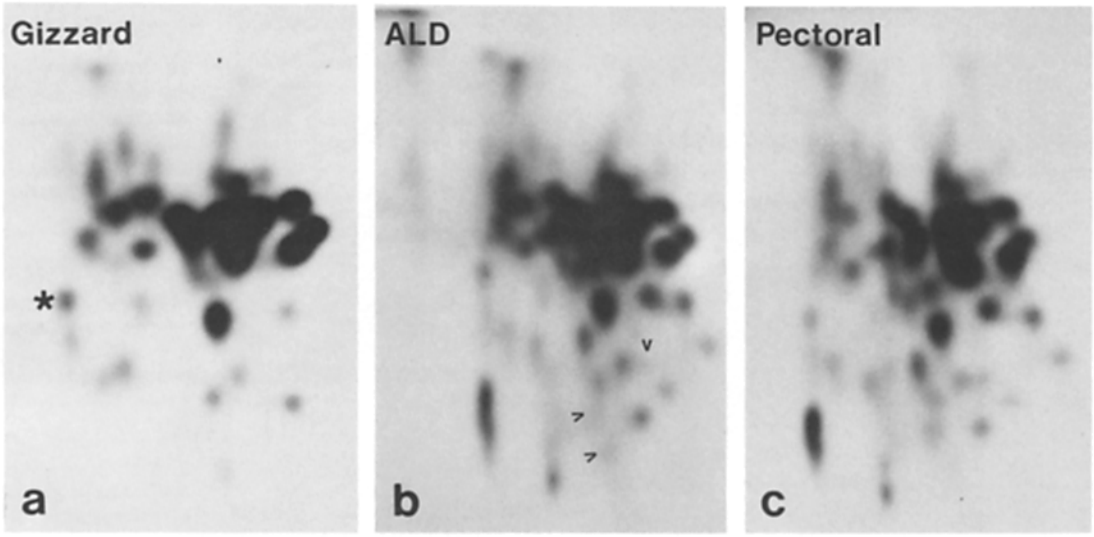

FIGURE 5 Two-dimensional peptide maps of filamin from gizzard (*a*), ALD myofibrils (*b*), and pectoral myofibrils (*c*). Filamin was immunoprecipitated, iodinated in the presence of 1% SDS, and digested with trypsin-*N*-L-phenylalanine cloromethyl ketone. Peptide maps were typically of 0.1 μg filamin containing 5,000 cpm and were exposed at −70°C with intensifying screens for about 48 h. In *a*, * indicates an example of a peptide present in gizzard but not ALD or pectoral myofibril filamin. Arrows in *b* indicate spots that differ in intensity between ALD and pectoral myofibril filamins (*b* and *c*).

DISCUSSION

We have observed here a difference in the location of the actin-binding protein filamin within the contractile apparatus of the sarcomere. In fast muscle, filamin is located at the periphery of the Z disk (16) while in a slow muscle it is additionally located in the I band (Fig. 1).

Figs. 1 and 2 show that filamin was distributed evenly throughout the length of the I band rather than being in a specific subset of the I band, such as the A-I junction or the N line. However, we do not know whether the filamin distribution extends past the A-I junction to the end of the thin filaments. We observed the distribution of filamin at the I band in all the myofibrils throughout ALD myofibers, rather than, for instance, only those myofibrils at the periphery of the myofiber.

In addition to being evenly distributed along the length of the I band in ALD myfibrils, filamin was evenly distributed along the diameter of the I band. Fig. 3*b* shows that desmin can clearly be seen to occupy a position only on the periphery of ALD myofibrils, and Fig. 3*c* shows that α-actinin can be seen to occupy only the interior part of the diameter of the myofibril. Similar cryosections stained for filamin showed neither type of staining but only even patches generally larger than the diameter of a myofibril. This indicates that filamin was located neither at the periphery of the I band in ALD nor at specific sites in the core of the I band, but rather evenly throughout the I band. However, the exact ultrastructural location of filamin within the I band region of ALD is unknown. We do not know whether filamin is randomly binding to all of the thin filaments of the contractile apparatus or to a subset of these thin filaments.

If we assume that there are approximately 400 actin monomers per thin filament in each half of an I band (28, 40), and that the vast majority of the actin in a myofibril is in the I band, we see from the results of the gel scans (Table I) that there is one filamin dimer for every four thin filaments in the I band in pectoral muscle. With the same assumptions, we see that there are approximately 10 filamin dimers per four thin filaments in the ALD. If, however, a substantial fraction of myofibril actin is in the Z disk, the amount of filamin per thin filament would increase correspondingly.

Since the maximal value for filamin binding to actin is on the order of 1 filamin/10 actins (21, 48, 57), the 10 filamin dimers that are binding to four thin filaments could bind to approximately 100 adjacent actin monomers on a single thin filament. Thus, given the level in which it is present, filamin could occupy only a fraction of the length of the I band in ALD, rather than be evenly distributed as we observed by immunofluorescence.

Using scans of rabbit pulmonary macrophages, run on SDS polyacrylamide gels, Stossel and Hartwig (51) found actin and actin-binding protein concentrations to be 10.4 and 1.8%, respectively, of total protein concentration, giving a ratio of ~68 mol actin/mol actin-binding protein. This is in rough agreement with our figures for gizzard and fibroblast filamin (Table I), which gave a ratio of one filamin dimer per four complete turns of the actin filament helix (28). This is well below the maximal binding value of approximately one filamin dimer per 10 actin monomers reported for filamin and actin-binding protein (21, 48, 57).

Little or no gizzard-type filamin was present in the ALD myofibrils, as was shown by the absence of its characteristic electrophoretic variant in immunoprecipitates (Fig. 4) and by the absence of characteristic peptides in peptide maps (Fig. 5). The type of filamin in ALD was virtually identical to the type of filamin in fast skeletal muscle (Fig. 5). Although some spots differed in intensity, there was a complete match of spots in the two-dimensional tryptic peptide maps. The differences in spot intensity could be due either to an artifact of the labeling and mapping procedure or to differences in levels of posttranslational modification of the peptides. The similarity of fast and slow muscle filamin is in contrast to the differences between fast and slow muscle forms of other actin-binding proteins.

The mechanism by which filamin binds to the I bands of ALD myofibrils is presently unknown. One way by which

this may be accomplished involves the deposition of filamin on I band thin filaments during myogenesis. We have previously shown that filamin is not present and is not synthesized in myogenic cells during the time in which sarcomeres are first forming (16). During the development of a slow muscle, given the proper innervation (1, 8, 24, 25, 45), synthesis of the two filamin forms may overlap rather than be temporally separated as it is in fast muscle, and hence the adult muscle form may bind to assembling actin filaments.

The reason for the difference in the distribution of filamin between ALD and pectoral myofibrils is also unknown. We might hypothesize that the presence of filamin in the I band of a slow muscle is related to some of the observed physiological differences between slow and fast muscles. Although it has been hypothesized that the lower ATPase activities of slow muscle myosin may be directly responsible for the slower twitch times (2, 4), the filamin in the I band of a slow muscle may, by cross-linking actin filaments, disrupt the semicrystalline contractile apparatus enough to affect contraction speed.

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