IDENTIFICATION OF A FILAMIN-LIKE PROTEIN IN CHICKEN HEART MUSCLE

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1. Introduction

The protein composition and structural organization of skeletal and heart myofibrils are the objects of intensive studies (reviews [1,2]). Much attention has been given to the study of high relative molecular mass muscle and muscle-like proteins ($M_r > 200\,000$) in various types of muscle, tissues and cells [3–10]. Filamin, a large actin-binding protein (M_r 250 000–270 000) was first isolated from chicken gizzard smooth muscle [3–6]. An analogous protein denoted as 'actin-binding protein' (ABP) has been isolated from macrophages and fibroblasts [7,11]. A large actin-binding protein has also been identified in substantial amounts in platelets and in small amounts in kidney and liver [3,8]. However, filamin was not detected in skeletal and heart striated muscles [3,8,9]. Filamin was identified in skeletal muscle [10], containing a small amount of this protein [10]. In myofibrils isolated from skeletal muscle the filamin-like protein has been shown to be located in the Z-line region of the sarcomere [10].

This work reports the identification of a filamin-like protein in a second type of striated muscle, the heart muscle. It has been shown that chicken heart muscle extract subjected to SDS–polyacrylamide gel electrophoresis (SDS) contains a protein with a similar electrophoretic mobility as filamin isolated from chicken gizzard. Indirect immunofluorescence has indicated that purified antibodies to chicken gizzard filamin interact specifically with chicken heart muscle myofibrils, the antibodies to filamin being localized predominantly in the Z-line region of the muscle sarcomere. Immunocytochemical analysis has also shown that the filamin-like protein is located in the Z-line region of the heart muscle sarcomere.

2. Materials and methods

Filamin was prepared by a combination of methods including the main steps described previously and with chromatography on a column of Sepharose 4B (2.6 × 100 cm) and on two columns of DEAE-cellulose [4,5]. Chicken gizzard actin was isolated from acetone powder [12]. Myosin from smooth muscle was prepared from the by-products remaining after isolation of filamin [5].

The extract from chicken heat muscle was made from 20 g homogenized purified meat in a 40 ml buffer containing 0.3 M KCl, 0.5 mM $MgCl_2$, 0.05 M imidazole, 2 mM Na_2ATP, 0.5 mM dithiothreitol, 0.1 mM PMSF, $pH_{20°C}$ 6.9. The homogenate was clarified at 50 000 × g for 20 min and the supernatant was examined by SDS–polyacrylamide gel electrophoresis [13]. Myofibrils from chicken heart muscle were prepared according to [14] and stored in 50% glycerine with a buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 2 mM EGTA, 0.1 mM dithiothreitol, 0.01 M Tris–HCl (pH 6.8 at +4°C).

Antiserum against filamin was made by injecting a mixture of 1 ml antigen solution, 0.5 mg/ml, plus an equal volume of Freund's complete adjuvant into foot pads of rabbits. After 30 days, injections with the antigen were repeated (0.3 mg), but without the adjuvant. Filamin, purified both chromatographically and by preparative electrophoresis was used as the antigen [15]. The results were the same and did not depend on the antigen preparation.

The IgG fraction from rabbit antiserum was isolated by precipitation with 50% ammonium sulfate. Purified antibodies to filamin were obtained by affinity chromatography on a column with filamin–Sepharose (1–1.5 mg filamin/ml gel). The antifilamin IgG fraction was preliminary passed through columns

with myosin–Sepharose and actin–Sepharose. The purified antibodies at 0.3 mg/ml were stored frozen at −70°C. After thawing, the antibodies to filamin were cross-reacted by the double immunodiffusion technique with purified filamin.

For immunofluorescence staining, glycerinized heart myofibrils were prepared by homogenization in a VirTis homogenizer. The myofibrils were then sorbed onto glass and treated first with antibodies to filamin at 0.02 mg/ml, washed and then stained with fluorescein-conjugated goat anti-rabbit IgG at 0.1 mg/ml [16].

Observations were done with a Zeiss Photomicroscope III equipped with epifluorescence illumination and a Planapo 40x oil immersion objective.

Immunocytochemical localization of filamin in chicken heart tissue was done as in [17]. To this end, slices prepared from heart tissue for routine electron microscopy after fixation with 4% formaldehyde were first treated with antibodies to filamin and then with goat anti-rabbit IgG which were conjugated to horseradish peroxidase. For staining, a solution of 3,3-diaminobenzadine and hydrogen peroxide was added to the slices. The resulting peroxidase reaction yields a dark product which is readily detected in the electron microscope in the form of characteristic granules. In this study a JEM 100 CX microscope was used.

3. Results and discussion

3.1. *Protein composition of the chicken heart muscle extract*

If fresh chicken heart muscle is homogenized in a buffer with 0.3 M KCl, 2 mM Na_2ATP, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.05 M imidazole and 0.1 mM PMSF (pH 6.9) (buffer for filamin extraction from chicken gizzard) [5] and the resulting extract examined by electrophoresis we get a picture of protein distribution presented in fig.1. It is seen in fig.1a that there is a band(s) in the upper part of the gel with a lesser electrophoretic mobility than that of myosin. This band corresponds to protein(s) of M_r 250 000–270 000 and practically coincides with the electrophoretic mobility of filamin isolated from chicken gizzard (fig.1b). A densitometric analysis of the gel with protein from the chicken heart muscle extract (fig.1d) indicates that the protein(s) of M_r 250 000–270 000 constitutes ~1–3% of the total protein of the extract.

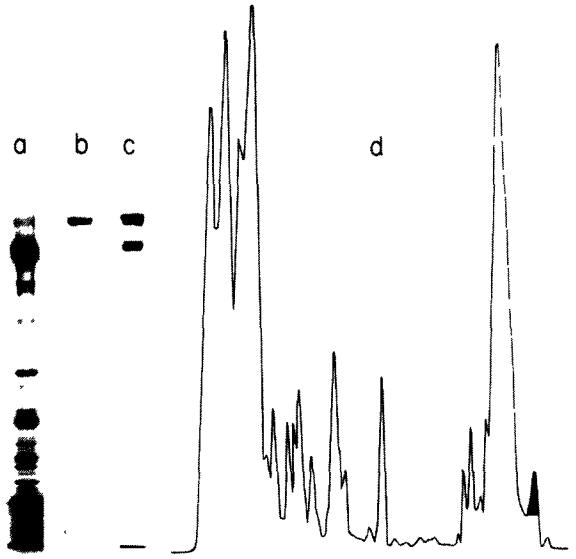

Fig.1. Electrophoretic analysis of the protein extract from chicken heart muscle: (a) extract; (b) purified filamin; (c) filamin and myosin; (d) densitogram of (a).

3.2. *Immunofluorescence detection of filamin in heart myofibrils*

In the next series of experiments we identified a filamin-like protein in individual heart myofibrils using antibodies to filamin isolated from smooth muscle (chicken gizzard). The antibodies were purified by affinity chromatography on filamin–Sepharose. These antibodies were preliminary passed through sorbents containing myosin and actin. Fig.2 demonstrates the staining of glycerinized heart myofibrils by antibodies to smooth muscle filamin. It is seen in fig.2a that the heart myofibrils are stained by the antibodies. At the same time the staining disappears if the antibodies to filamin were preincubated with purified filamin (fig.2b). An analogous result is also obtained on treatment of myofibrils with non-immune IgG (fig.2d). But, on the contrary, if the antibodies to filamin were preincubated with actin and myosin from chicken gizzard, the fluorescence pattern does not change (fig.2c). Thus, the result obtained is a direct indication that in heart myofibrils there is material which specifically interacts with purified antibodies to smooth muscle filamin.

3.3. *Localization of filamin in heart myofibrils*

To localize the material specifically reacting with the antibodies to filamin, the same glycerinized myo-

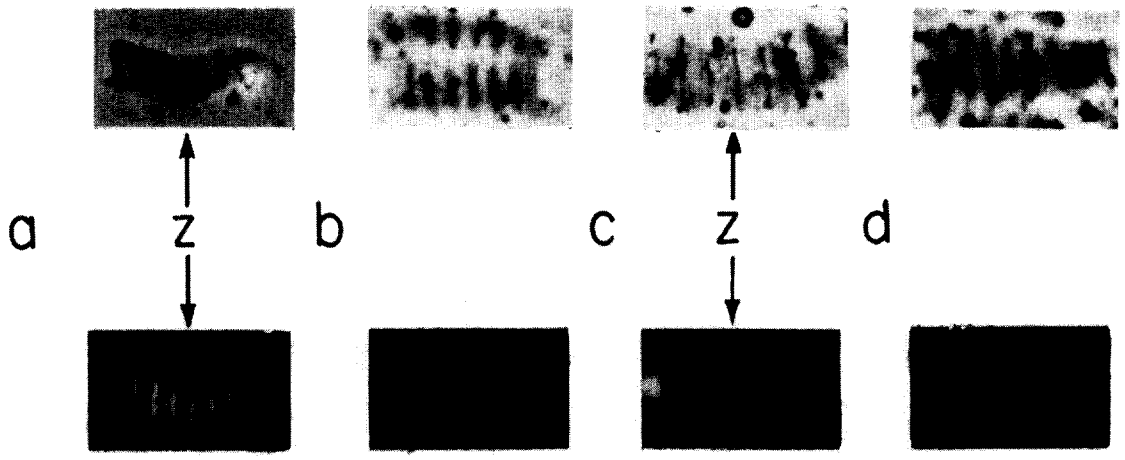

Fig.2. Localization of filamin-like protein in glycerinized chicken heart myofibrils by indirect immunofluorescence: top row, phase contrast; bottom row, immunofluorescence; Z, Z-line, ×1100; (a) myofibrils incubated with antibodies to filamin; (b) the same as (a) but the antibodies were preincubated with purified filamin; (c) the same as (a) but the antibodies were preincubated with purified actin and myosin; (d) myofibrils incubated with non-immune IgG.

fibrils were studied by indirect immunofluorescence (fig.2, bottom) and phase contrast methods (fig.2, top). Phase micrographs reveal a characteristic myofibril Z-line structure. Fluorescence analysis shows that staining by filamin antibodies is not uniform, but takes place in one specific region corresponding to the Z-line revealed on phase micrographs. A weak staining in the middle of the sarcomere can also be detected in fig.2a,c. Similar results were obtained for the localization of filamin in skeletal myofibrils [10]. It should be noted that in our experiments we usually got glycerinized heart myofibrils in which we could not clearly distinguish the A band by the phase contrast method. This is connected with the fact that the chicken heart at the moment of slaughter is in systole, i.e., contracted.

For a more precise localization of filamin in the heart muscle sarcomere we applied the immunocytochemical method of protein localization. Fig.3 shows the results of immunocytochemical of filamin in slices of chicken heart muscle. The characteristic structure of the muscle sarcomere, the Z-line, is clearly seen in the electron micrographs. After treatment of the slices with antibodies to filamin the reaction products are revealed in the form of black granules. It is seen in fig.3b that granularity appears in a definite part of the sarcomere, i.e., in the Z-line region. At the same time no granularity is observed in the control experiment (fig.3a) when the slices are treated with non-immune IgG. Thus, the filamin-like protein in the heart muscle is located predominantly on the borders of the sarcomere. The result obtained is in good accordance with immunofluorescence localization of filamin in striated muscle [10] and with section 3.2.

4. Conclusion

A filamin-like protein in a second type of striated muscle (heart muscle) has been identified. A protein(s) has been detected in chicken heart muscle with an M_r = 250 000–270 000 protein(s), the same as that of the filamin monomer from chicken gizzard. The amount of this protein(s) constitutes ~1–3% of the total amount of chicken gizzard heart muscle protein.

Studies on the composition and localization of the filamin-like protein in heart myofibrils by indirect fluorescence and immunocytochemical methods have shown that:

1. The purified antibodies to filamin from chicken gizzard (smooth muscle) specifically interact with heart myofibrils;
2. The interaction of antibodies to filamin proceeds in a limited region of the heart sarcomere, in the Z-line region.

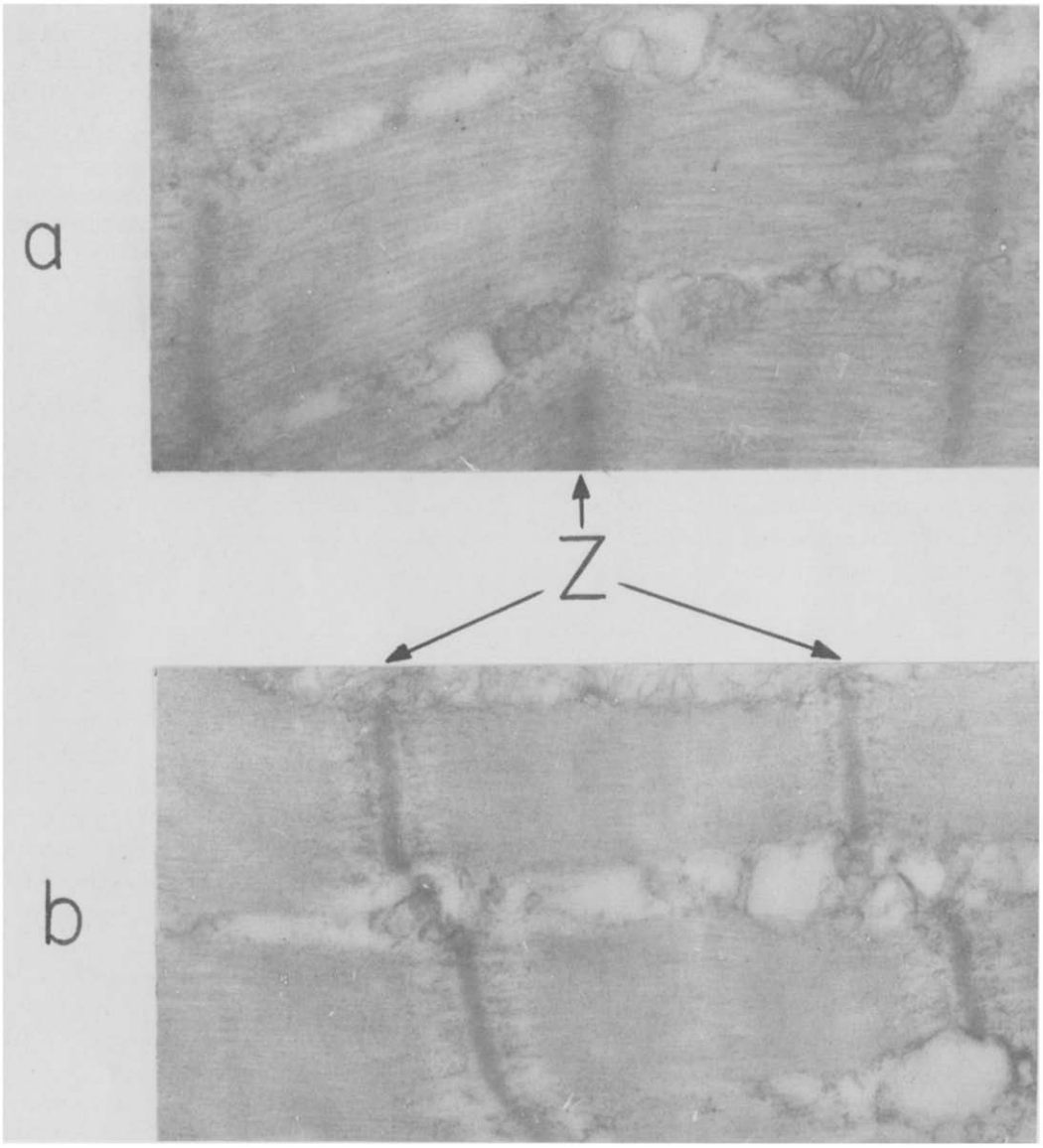

Fig.3. Localization of filamin-like protein on slices from chicken heart muscle by the immunocytochemical method: Z, Z-line; ×40 000; (a) slices treated with non-immune IgG; (b) slices treated with antibodies to filamin.

Thus, a new protein has been detected at the ends of the chicken heart muscle sarcomeres having common immunological determinants with the high relative molecular mass actin-binding protein from smooth muscle, filamin.

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