Vinculin localization in cardiac muscle

V.E. Koteliansky and G.N. Gneushev

Institute of Experimental Cardiology, USSR Cardiology Research Center, Academy of Medical Sciences, Cherepkovskaya Str., 15A, 7, Moscow 121500, USSR

Received 14 June 1983

Vinculin isolated from chicken cardiac muscle crossreacts with antibodies against smooth muscle vinculin. Antibodies to vinculin were used for localization of vinculin in cardiac muscle by indirect immunofluorescence method. In cardiac muscle vinculin was localized in intercalated discs and near plasma membrane at the cell periphery between external myofibrils and sarcolemma. It was suggested that vinculin plays an important role in myofibril–sarcolemma interaction in cardiac muscle.

Cardiac muscle *Vinculin* *Sarcolemma* *Immunofluorescence* *Sarcolemma–myofibril interaction*

1. INTRODUCTION

One of the most significant problems in cell biology is understanding of the molecular mechanism of plasma membrane–cytoskeleton interactions. Vinculin [actin-binding protein (M_r 130000)] plays a key role in anchoring of the actin filament bundles to plasma membrane [1–6]. Initially vinculin was found in chicken gizzard smooth muscle [1,7]. In [8,9] vinculin was isolated from Hela cells and platelets. Vinculin was also identified in a variety of cell types and tissues [1–5]. Indirect immunofluorescence and immunoelectron microscopy has shown that vinculin is concentrated in different objects at the termini of actin filaments and is associated with inner face of plasma membrane: in focal contacts of cultured cells, in zonula adherence of intestinal epithelium, in intercalated discs of cardiac muscle and dense plaques of chicken gizzard smooth muscle [1–5]. Vinculin distribution coincides with localization of the important extracellular protein, fibronectin [2,10].

During Rous sarcoma virus-induced cell transformation vinculin may be one of the preferred substrates for the transforming protein $p60^{src}$ (protein kinase) and contains 8-fold more phosphotyrosine in transformed cells than in uninfected cells [11]. Transformation of cells to a malignant state is accompanied by dramatic changes in cell morphology and reorganization of cytoskeleton and extracellular matrix [12].

There are at least two types of linkages between actin filaments and membranes in cardiac muscle: in the intercalated discs region and sarcolemma –myofibril attachment sites. We suggested that vinculin may be involved in sarcolemma–myofibril interaction. Here, we study the role of vinculin in myofibril–sarcolemma interaction of cardiac muscle.

2. MATERIALS AND METHODS

Chicken gizzard smooth muscle and chicken cardiac muscle vinculins were isolated according to [7]. Antibodies to chicken gizzard smooth muscle vinculin used here were obtained by immunization of rabbits with vinculin which was purified by preparative sodium dodecyl sulfate–polyacrylamide gel electrophoresis [13]. Crude immunoglobulin G (IgG) fraction from immune serum was precipitated by ammonium sulfate (50% saturation), and purified antibodies to vinculin were obtained by affinity chromatography on a vinculin –Sepharose column. The antibodies to vinculin were characterized by standard immunological

Published by Elsevier Science Publishers B.V.

techniques and in all tests interacted only with vinculin.

For immunofluorescence staining glycerinized cardiac myofibrils [14] and unfixed cryostat sections (3–6 μm) of chicken and rat cardiac muscle were used. Samples were treated with antibodies to vinculin at 0.02–0.05 mg/ml, washed, then stained with fluorescein-conjugated goat anti-rabbit IgG at 0.1 mg/ml. Observations were done on a Zeiss epifluorescence photomicroscope III with a 63 × oil phase and 40 × objectives.

Enzyme-linked immunosorbent assay (ELISA) was used for comparing vinculins from chicken smooth and cardiac muscles [15].

3. RESULTS AND DISCUSSION

It was shown in [5] that vinculin is present in intercalated discs of cardiac muscle. Highly purified vinculin was isolated from chicken cardiac muscle and cross-reactivity of the polyclonal antibodies to chicken gizzard smooth muscle vinculin with vinculin from chicken cardiac muscle was determined by ELISA-procedure (fig.1). It was demonstrated that antibodies cross-reacted with both vinculins and the value of cross-reactivity was at least 90%. Thus, chicken gizzard smooth muscle and chicken cardiac muscle vinculins have similar antigenic determinants and structural organization. Cardiac muscle vinculin like smooth muscle vinculin causes a decrease of the low shear viscosity of F-actin (not shown).

To localize vinculin in cardiac muscle glycerinized chicken cardiac myofibrils and cryostat sections of chicken and rat cardiac muscle were studied by indirect immunofluorescence with the affinity purified antibodies to chicken smooth muscle vinculin. Antibodies to vinculin do not react with glycerinized myofibrils (fig.2a,b). It means that vinculin is not integral myofibrillar protein. Fluorescence analysis of cryostat sections of chicken and rat hearts demonstrates three types of vinculin localization in cardiac muscle:

(i) Vinculin is located in specific cardiomyocyte contact region; intercalated discs (fig.2c);
(ii) The periphery of the cells was stained by antivinculin; vinculin's spots are periodically distributed along the cell margins (fig.2d).
(iii) In longitudinal sections antivinculin staining is periodical and corresponds to the I bands of sarcomeres around the Z-line region (fig.2e).

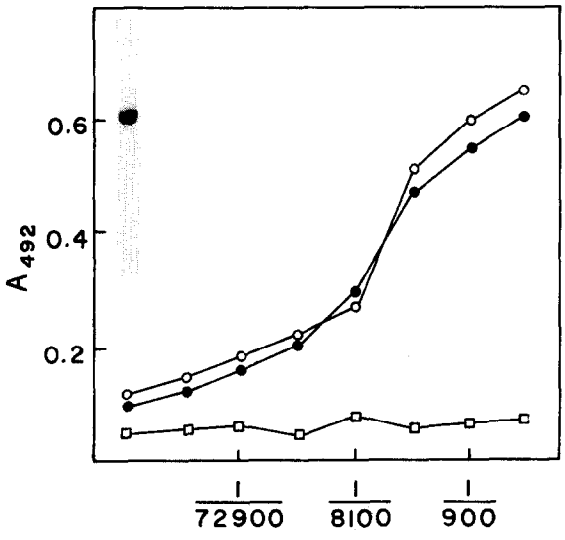

Fig.1. Binding of antibodies against chicken gizzard smooth muscle vinculin to chicken cardiac and smooth muscle vinculins (ELISA-procedure). The wells were coated with vinculin from chicken gizzard smooth muscle (○), vinculin from chicken cardiac muscle (●), and albumin (□). In the left upper corner, an electrophoretogram of the purified chicken cardiac muscle vinculin.

Vinculin was not found on internal myofibrils. Thus, additionally to the reported localization of vinculin in intercalated discs [5] we have found a new type of vinculin localization in cardiac muscle; the protein overlies the I bands around the Z-line region of external myofibrils near sarcolemma. It is important that vinculin, not being an integral myofibrillar protein, is periodically distributed along the cardiomyocyte myofibrils with the same periodicity as I bands of sarcomeres containing actin filaments. It is generally assumed that in all objects tested, vinculin is involved in the attachment of actin bundles to the membrane [1–5]. However, in vitro vinculin can interact with F-actin. Thus, we suggest that in cardiac muscle vinculin plays an important role in linkages between contractile structures (external myofibrils) and membranes of sarcolemma. Vinculin is the first non-membrane component which can link sarcolemma and myofibrils. Sarcolemma membranes do not simply cover the myofibrils bundles but physically associate with external myofibrils through the periodically distributed structures containing vin-

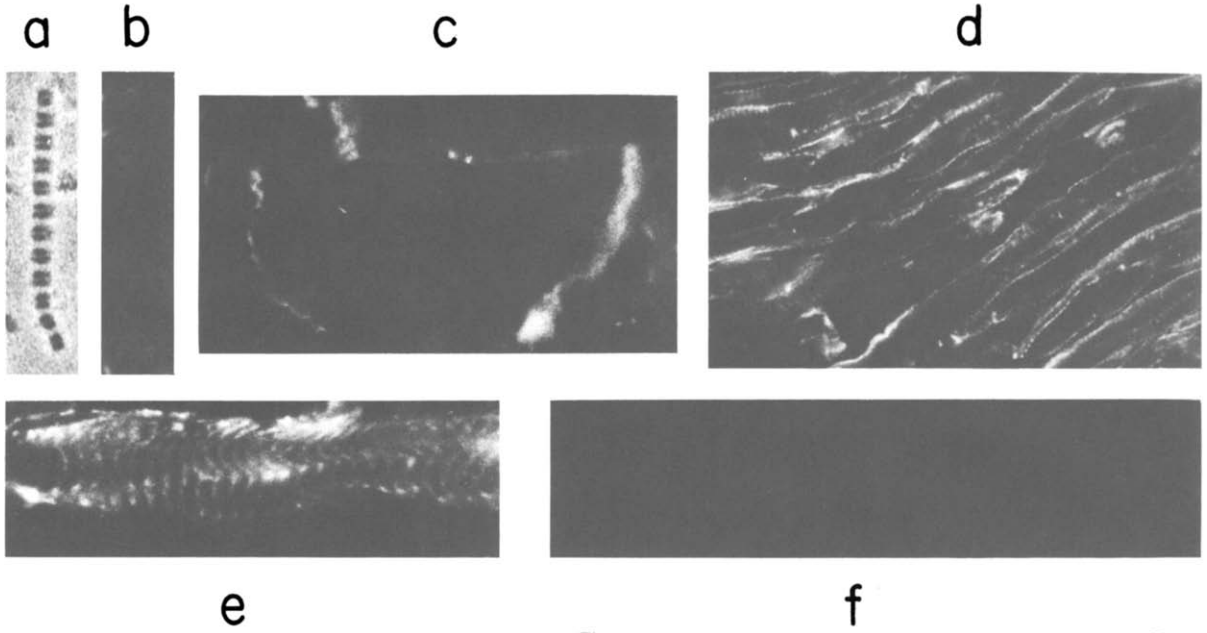

Fig.2. Localization of vinculin in cardiac muscle by indirect immunofluorescence: (a,b) glycerinized chicken cardiac muscle myofibrils, (a) phase contrast, (b) immunofluorescence, × 396; (c) part of rat cardiac muscle cryostat section in intercalated disc's region, × 1656; (d) longitudinal section of chicken cardiac muscle (general view), × 92; (e) part of longitudinal section of chicken cardiac muscle with periodically distributed fluorescence, × 1012; (f) longitudinal section of chicken cardiac muscle incubated with non-immune IgG or antivinculin antibodies pre-adsorbed with vinculin, × 368.

culin. These myofibril–sarcolemma attachment sites containing vinculin were named costameres [18].

Costameres were demonstrated in skeletal muscle [18]. Existence of these structures suggests that sarcolemma being linked with external myofibrils may move during muscle contraction. The movement can be important for regulation of the process.

REFERENCES

[1] Geiger, B. (1979) Cell 18, 193–205.
[2] Burridge, K. and Feramisco, V.R. (1980) Cell 19, 587–595.
[3] Geiger, B., Tokuyasu, K.T., Dutton, A.H. and Singer, S.J. (1980) Proc. Natl. Acad. Sci. USA 77, 4127–4131.
[4] Geiger, B., Dutton, A.H., Tokuyasu, K.T. and Singer, S.J. (1981) J. Cell Biol. 91, 614–628.
[5] Tokuyasu, K.T., Dutton, A.H., Geiger, B. and Singer, S.J. (1981) Proc. Natl. Acad. Sci. USA 78, 7619–7623.
[6] Siliciano, J.D. and Craig, S.W. (1982) Nature 300, 533–535.
[7] Feramisco, J.R. and Burridge, K. (1980) J. Biol. Chem. 255, 1194–1199.
[8] Burridge, K. and Feramisco, J.R. (1982) Cold Spring Harbor Symp. 46, 587–597.
[9] Koteliansky, V.E., Gneushev, G.N., Glukhova, M.A., Smirnov, V.N., Venyaminov, S.Yu. and Muszbek, L. (1983) Eur. J. Biochem., in press.
[10] Singer, J.J. and Paradiso, P.R. (1981) Cell 24, 481–492.
[11] Sefton, B.M., Hunter, T., Ball, E.H. and Singer, S.J. (1981) Cell 24, 165–174.
[12] Vasiliev, J.M. and Gelfand, I.M. (1977) Int. Rev. Cytol. 50, 159–274.
[13] Lazarides, E. and Weber, K. (1974) Proc. Natl. Acad. Sci. USA 71, 2268–2272.
[14] Etlinger, J.D., Zak, R. and Fishman, D.A. (1976) J. Cell. Biol. 68, 123–141.
[15] Engvall, E. and Perlmann, P. (1972) J. Immunol. 109, 129–135.
[16] Jockusch, B.M. and Isenberg, G. (1981) Proc. Natl. Acad. Sci. USA 78, 3005–3009.
[17] Wilkins, J.A. and Lin, S. (1982) Cell 28, 83–90.
[18] Pardo, J.V., Siliciano, J.D. and Craig, S.W. (1983) Proc. Natl. Acad. Sci. USA 80, 1008–1012.