Isolation and Some Properties of Macrophage α-Actinin: Evidence That It Is Not an Actin Gelling Protein†

Jonathan P. Bennett,‡ Ken Scott Zaner,* and Thomas P. Stossel

ABSTRACT: We have isolated an actin-binding protein from rabbit alveolar macrophages which by virtue of its physical properties we classify as a nonmuscle α-actinin. The protein consists of two subunits of M_r 103 000 and has a Stokes' radius of 7.26 nm and a sedimentation coefficient of 6.83×10^{-13} s^{-1}. Under the electron microscope, rotary-shadowed molecules appeared as short rods with an average length of 39.9 nm. We have examined the nature of the interaction of macrophage α-actinin with F-actin. The binding of radioiodinated macrophage α-actinin to F-actin is calcium sensitive. At a low concentration of free calcium (less than 10^{-9} M), the binding affinity is 4.2×10^6 M^{-1} and is relatively unaffected by changes in temperature, while in the presence of 0.1 mM Ca^{2+}, binding is reduced more than 5-fold. The stoichiometry of binding suggests that α-actinin binds all along the length of the actin filaments. The affinity of $^{45}Ca^{2+}$ for macrophage α-actinin is 4×10^6 M^{-1} with a capacity of four calcium ions per molecule. Although macrophage α-actinin has calcium-inhibitable actin gelation activity at 7 °C, its effect on the apparent viscosity of F-actin decreases with increasing temperature, and at 37 °C, no gel point is observed. Therefore, at the temperature at which macrophages function in vivo, α-actinin probably does not promote the isotropic gelation of actin.

α-Actinin was originally isolated from skeletal muscle (Ebashi & Ebashi, 1965), and its presence in nonmuscle cells was later identified by immunofluorescence microscopy (Lazarides & Burridge, 1975). Nonmuscle α-actinin was subsequently isolated from HeLa cells and platelets (Burridge & Feramisco, 1981; Rosenberg et al., 1981), and in contrast to the muscle protein, it was found to display a calcium sensitivity in its interaction with F-actin. Burridge and Feramisco have suggested that a number of other calcium-sensitive actin-binding proteins previously identified in nonmuscle cells may belong to the class of nonmuscle α-actinins, including actinogelin from Ehrlich tumor cells (Mimura & Asano, 1979), *Acanthamoeba* 85K protein (Pollard, 1981), and *Dictyostelium* 95K protein (Fechheimer et al., 1982; Condeelis & Vahey, 1982).

Because muscle and nonmuscle α-actinins increase the viscosity of F-actin solutions (Maruyama & Ebashi, 1965; Burridge & Ferramisco, 1981), it has been proposed that nonmuscle α-actinins are gelation proteins which contribute to the calcium-sensitive gelation of actin in cytoplasmic extacts and possibly to the formation of isotropic networks in the cortical cytoplasm of eukaryotic cells. However, macrophages have been shown to contain a 540-kilodalton (kDa) actin-binding protein, which accounts for the majority of the actin cross-linking activity present in crude cell extracts (Hartwig & Stossel, 1975; Brotschi et al., 1976), and gelsolin, which confers calcium sensitivity on the actin gelation process (Yin & Stossel, 1979). Although it appeared that these two proteins were sufficient to account for the sol–gel transformations observed in macrophage extracts, the report that platelets (which also contain both the 540-kDa actin-binding protein and gelsolin) contained α-actinin prompted us to examine whether this additional calcium-regulated actin-binding protein was also present in macrophages. We find that there is a protein, which we identify as α-actinin by its physical properties, in rabbit alveolar macrophages at comparable levels to actin-binding protein. However, although this protein changes the apparent viscosity of F-actin in a calcium-dependent manner at 7 °C, it has little effect at physiologic temperatures. In this paper, we describe the isolation and characterization of an α-actinin-like protein from rabbit alveolar macrophages.

Experimental Procedures

Purification of Macrophage α-Actinin. Alveolar macrophages were obtained by intratracheal lavage of New Zealand white rabbits which had been injected intravenously with 1.2 mL of Freund's complete adjuvant containing BCG vaccine (4% v/v) 2 weeks prior to sacrifice (Myrvik et al., 1961). The cells were washed twice in 0.15 M NaCl and treated with 5 mM diisopropyl fluorophosphate (Amrein & Stossel, 1980). After two further washes, 20 mL of packed cells was resuspended in 180 mL of ice-cold 0.1 M KCl, 10 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 mM $MgCl_2$, and 10 mM imidazole hydrochloride, pH 7.5, and 20 mL of 10% (w/v) Triton X-100 was added slowly while stirring. This buffer was designed to maintain the integrity of the cytoskeleton after solubilization. After 5 min at 0 °C, the solubilized cells were centrifuged for 20 min at 12000*g* at 4 °C. The supernatant was discarded, and the pellets containing the cytoskeletal proteins were resuspended in 2 mM EGTA, 1 M $MgCl_2$, and 10 mM imidazole hydrochloride pH 7.5, and incubated for 30 min at 37 °C in a shaking water bath. After centrifugation (20 min, 12000*g*, 4 °C), the supernatant was applied directly to a 20-mL column of hydroxyapatite (Bio-Gel HTP) which had been equilibrated in the same buffer. The column was washed with 100 mL of 25 mM potassium phosphate, pH 7.5, and then the α-actinin-containing fraction was eluted with 100 mL of 90 mM potassium phosphate, pH 7.5. Solid ammonium sulfate (30 g/100 mL) was added to the eluate, and the precipitated protein was collected by centrifugation (20 min, 12000*g*, 4 °C). The protein was resuspended in 10 mL of column buffer consisting of 0.1 M KCl, 0.1 mM EGTA, 2 mM NaN_3, and 10 mM imidazole hydrochloride, pH 7.5. After clarification

† From the Hematology-Oncology Unit, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114. *Received March 1, 1984.* This work was supported by U.S. Public Health Service Grants HL19429 and HL00912 and by grants from the American Heart Association and the Council for Tobacco Research. J.P.B. held a NATO Postdoctoral Fellowship from the U.K. Science and Engineering Research Council.

‡ Present address: Laboratory of Molecular Biology, MRC Centre, Cambridge, U.K. CB22QH.

0006-2960/84/0423-5081$01.50/0

by centrifugation (20 min, 12000g, 4 °C), the sample was applied to a 90 × 2.5 cm column of 6% agarose (Bio-Gel A-5m, 200–400 mesh) and chromatographed in the same buffer. α-Actinin was eluted as the principal protein peak, was separated from any other remaining contaminants, and was routinely identified by gel electrophoresis.

Physical Characterization Techniques. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out in 7.5% acrylamide slab gels using a discontinuous buffer system (Laemmli, 1970). Molecular weight standards (Bio-Rad Laboratories) used were myosin (M_r 200 000), β-galactosidase (M_r 116 250), phosphorylase b (M_r 92 500), bovine serum albumin (M_r 66 200), and ovalbumin (M_r 45 000). To determine the subunit molecular weight of macrophage α-actinin, the protein was coelectrophoresed in the same lane as the standards and the positions of the Coomassie Blue staining peaks were obtained by densitometry. Molecular weight was determined graphically from a plot of log M_r vs. distance migrated.

The Stokes radius was determined by gel filtration on a 90 × 1.5 cm column of Sepharose 6B which had been calibrated with standards (Pharmacia Fine Chemicals) consisting of thyroglobulin (a = 8.50 nm), ferritin (a = 6.10 nm), catalase (a = 5.22 nm), and bovine serum albumin (a = 3.55 nm). The void volume and the total column volume were measured with Blue Dextran and tris(hydroxymethyl)aminomethane (Tris) base, respectively, and interpolation to determine the Stokes radius of α-actinin was carried out according to Laurent & Killander (1964).

Sedimentation velocity measurements were carried out by using a Beckman Model E analytical ultracentrifuge. Macrophage α-actinin was concentrated by using collodion bags (Schleicher & Schuell) in 0.1 M KCl, 1 mM EGTA, 2 mM NaN_3, and 10 mM imidazole hydrochloride, pH 7.5. Measurements were made by using schlieren optics at 42 040 rpm and 20 °C for six samples at protein concentrations between 0.5 and 1.6 mg/mL. Measured sedimentation rates were corrected to standard conditions and extrapolated to zero α-actinin concentration (Schachman, 1957).

Amino acid analysis was performed on samples of both macrophage and smooth muscle α-actinin which had been dialyzed extensively against glass-distilled water, lyophilized, and hydrolyzed in 6 N HCl in sealed tubes at 105 °C for 24 h. The hydrolysates were analyzed in a Beckman 121-MB amino acid analyzer.

For electron microscopy, a sample of α-actinin (200 μg/mL in 0.1 M KCl, 1 mM EGTA, 2 mM NaN_3, and 10 mM imidazole hydrochloride, pH 7.5) was diluted 1:2 with glycerol and sprayed onto mica. After drying and rotary shadowing with platinum/carbon at a 10° shadow angle according to Tyler & Branton (1980), we photographed replicas at 34000× magnification in a Philips 301 electron microscope and photographically enlarged them to 340000× for measurement of molecular dimensions.

Gel-Point Determination by Falling-Ball Viscometry. Varying concentrations of macrophage α-actinin or 540-kDa actin-binding protein were copolymerized with 1 mg/mL G-actin in 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM ATP, and 10 mM imidazole hydrochloride pH 7.5. In some samples, $CaCl_2$ (final concentration 2 mM) was also added. Immediately after the addition of G-actin, samples were drawn up into 100-μL pipets and sealed at the bottom. Samples were allowed to polymerize for 2 h (at which point control samples had reached a constant apparent viscosity) either in a cold room (7 °C), at room temperature (21 °C), or in an incubator regulated at 37 °C. The time taken for a stainless-steel ball to fall 2 cm through the tube was measrued at the same temperatures in tubes inclined at 15° to the vertical.

Binding of α-Actinin to F-Actin. Radioiodinated Bolton–Hunter reagent (New England Nuclear) was used to radiolabel macrophage α-actinin to a level of 3 × 10^5 cpm/μg. In a control experiment, an excess (0.2 μg/μg of protein) of the unlabeled parent compound of Bolton–Hunter reagent, 3-(*p*-hydroxyphenyl)propionic acid *N*-hydroxysuccininmide ester (Sigma), was allowed to react with α-actinin by the procedure used for radiolabeling (Bolton & Hunter, 1973). The reacted protein was found to have an activity similar to that of unreacted α-actinin in gel point measurements as described above, implying that its ability to react with F-actin was not significantly altered.

For binding experiments, different concentrations of ^{125}I-labeled α-actinin were incubated for 3 h at either 4 or 37 °C with actin (0.25 mg/mL) which had previously been polymerized in a buffer comprising 0.1 M KCl, 2 mM $MgCl_2$, 2.5 mM EGTA, 0.5 mM ATP, and 10 mM imidazole hydrochloride pH 7.5 (total volume 0.5 mL). For some experiments, 0.1 mM $CaCl_2$ was used in place of the EGTA. The samples were centrifuged for 1 h at 240000g at the same temperature as the preceding incubation. Control experiments showed that α-actinin alone did not sediment under these conditions. The supernatants were removed and the pellets were rinsed briefly in 0.1 M KCl before being resuspended in 1% (w/v) sodium dodecyl sulfate. Radioactivity was measured of the trichloroacetic acid insoluble material in triplicate 0.1-mL samples of both pellet and supernatant. The amount of F-actin in the pellet was determined for each experiment by taking samples through the same procedure and measuring the protein content of the acid-insoluble pellet by the Lowry protein assay.

Binding of Calcium to Macrophage α-Actinin. This was determined by equilibrium dialysis (Yin & Stossel, 1980). Samples (0.3 mL) of α-actinin (0.5 mg/mL) which had been dialyzed overnight into 0.1 M KCl, 50 μM EGTA, 2 mM $MgCl_2$, and 20 mM imidazole hydrochloride, pH 7.00, made up in double-distilled water, were dialyzed with shaking for 24 h at 4 °C against 15 mL of the same solution together with various concentrations of $CaCl_2$ containing ^{45}Ca (New England Nuclear) to a specific activity of 0.1 μCi/nmol. The concentration of free Ca^{2+} in each sample was calculated by using a computer program (Perrin & Sayce, 1967). After dialysis, radioactivity in 20-μL aliquots of the solutions inside and outside each dialysis bag was measured by scintillation counting (in quintuplicate), and the protein concentration inside the dialysis bag was measured for each sample.

Other Procedures. Rabbit skeletal muscle actin was prepared by the method of Spudich & Watt (1971). Macrophage 540-kDa actin-binding protein was prepared as previously described (Hartwig & Stossel, 1979). Smooth muscle α-actinin was prepared according to Feramisco & Burridge (1980) from rabbit postpartem uterus. Protein concentration was measured either by the method of Lowry et al. (1951) with bovine serum albumin as standard or by that of Bradford (1976) with bovine γ-globulin as standard.

Results

Purification of Macrophage α-Actinin. We purified macrophage α-actinin by differential extraction of Triton-solubilized cells, followed by chromatography on hydroxyapatite and by gel filtration. This procedure was based on steps in the published methods of Rosenberg et al. (1981), Suzuki et al. (1976), and Feramisco & Burridge (1980) and is described in detail under Experimental Procedures. The successive stages

Table I: Protein Yield during the Purification of α-Actinin from Macrophages

fraction	total protein (mg)
(A) cell homogenate (from 20 mL of packed cells)	2307
(B) pellet after Triton solubilization	573
(C) supernatant after low ionic strength extraction	124
(D) fraction eluted from hydroxyapatite	12
(E) peak fractions from 6% agarose gel filtration	6

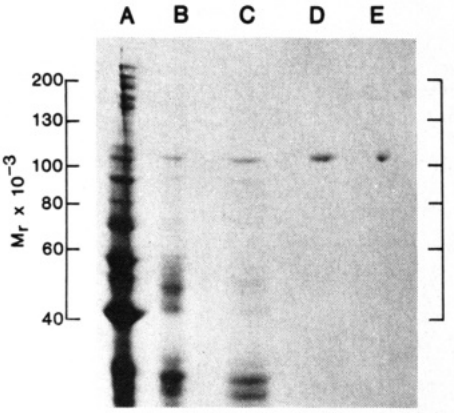

FIGURE 1: Stages in the purification of α-actinin from rabbit alveolar macrophages. The figure shows polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate of (A) cell homogenate, (B) pellet after Triton solubilization, (C) supernatant after low ionic strength extraction, (D) fraction eluted from the hydroxyapatite column, and (E) pooled peak fractions from 6% agarose gel filtration.

of purification are documented in Table I and Figure 1. We estimated that α-actinin comprised 0.5–1.5% of the protein in cell homogenates, on the basis of measurements by quantitative densitometry of polyacrylamide gels (data not shown). This suggests that we recovered purified α-actinin in approximately 20% yield.

Physical Properties of Macrophage α-Actinin. The subunit molecular weight of macrophage α-actinin in sodium dodecyl sulfate was determined by electrophoresis in polyacrylamide slab gels, in which α-actinin migrated as a single polypeptide with an apparent molecular weight of 103 000. We estimatead the native molecular weight of α-actinin from the Stokes radius and the sedimentation coefficient. The Stokes' radius was found to be 7.26 nm, determined by gel filtration on a calibrated 6% agarose column. The sedimentation coefficient ($s^0_{20,w}$) was 6.83×10^{-13} s^{-1}, determined by analytical ultracentrifugation in a buffer containing 1 mM EGTA. The amino acid composition of the purified protein (Table II) resembled that of a sample of rabbit smooth muscle α-actinin which was analyzed in parallel. We used these data to estimate a value of 0.725 cm^3 g^{-1} for the partial specific volume of the protein (Cohn & Edsall, 1943). Combining these numbers using the equation of Siegel & Monty (1966), we calculate a molecular weight for macrophage α-actinin of 209 000, indicating that the native molecule is a homodimer.

Assuming a degree of solvation for α-actinin of 0.45 (Suzuki et al., 1976), the frictional ratio (f/f_0) is 1.57, corresponding to a prolate ellipse with an axial ratio of about 10:1. The radius of gyration calculated from the hydrodynamic data is 10.9 nm. This conclusion agrees with the appearance of the molecule visualized in the electon microscope by low-angle rotary shadowing. Macrophage α-actinin appeared as short rods (Figure 2) with an average length of 39.9 ± 2.4 nm (mean ± standard deviation, $n = 70$) and a width of 5–8 nm. This corresponds to a radius of gyration of 11.5 nm.

Table II: Amino Acid Analysis of Rabbit Macrophage α-Actinin and Rabbit Smooth Muscle α-Actinin

amino acid	residues (mol %) macrophage α-actinin	residues (mol %) smooth muscle α-actinin
aspartic acid	10.9	11.1
threonine	5.0	4.8
serine	7.6	8.6
glutamic acid	13.7	13.7
proline	3.0	1.7
glycine	9.4	10.4
alanine	7.9	8.7
valine	5.8	5.0
methionine	1.9	1.9
isoleucine	4.6	4.9
leucine	9.0	9.3
tyrosine	2.5	2.2
phenylalanine	3.3	3.3
lysine	6.6	7.0
histidine	2.2	2.2
arginine	6.6	5.1

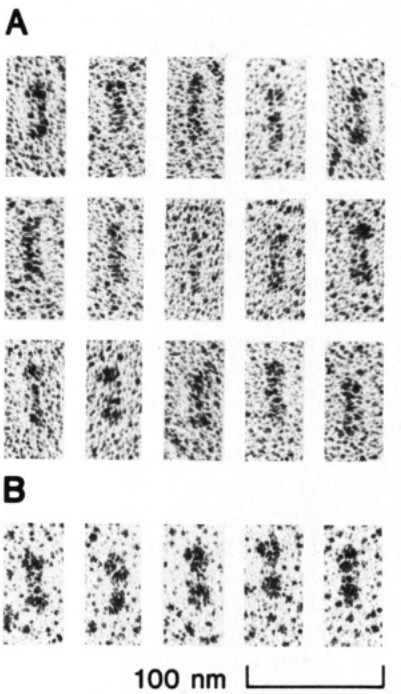

FIGURE 2: Molecules of macrophage α-actinin visualized by rotary shadowing and electron microscopy. The micrographs illustrate representative molecules from samples prepared (A) in the presence of 1 mM EGTA or (B) in the presence of 1 mM $CaCl_2$.

We looked for evidence of a calcium-dependent structural change in the molecule. There was no significant difference in the sedimentation rate of macrophage α-actinin at a protein concentration of 1 mg/mL when we compared the effects of buffers containing EGTA (1 mM) or $CaCl_2$ (0.1 mM). Similarly, there was no difference in the morphology of rotary-shadowed samples prepared from solutions containing EGTA (1 mM) or $CaCl_2$ (1 mM).

Effect of Macrophage α-Actinin on the Apparent Viscosity of F-Actin. We compared the effects of macrophage α-actinin and 540-kDa actin-binding protein on 1 mg/mL actin by using falling-ball viscometry (Maclean-Fletcher & Pollard, 1980). Because of previous reports that the interaction of skeletal muscle α-actinin with F-actin is temperature sensitive (Goll et al., 1972; Jockusch & Isenberg, 1981), we carried out this study at three different temperatures.

Figure 3 shows that macrophage 540-kDa actin-binding protein and α-actinin (in the presence of EGTA) at similar

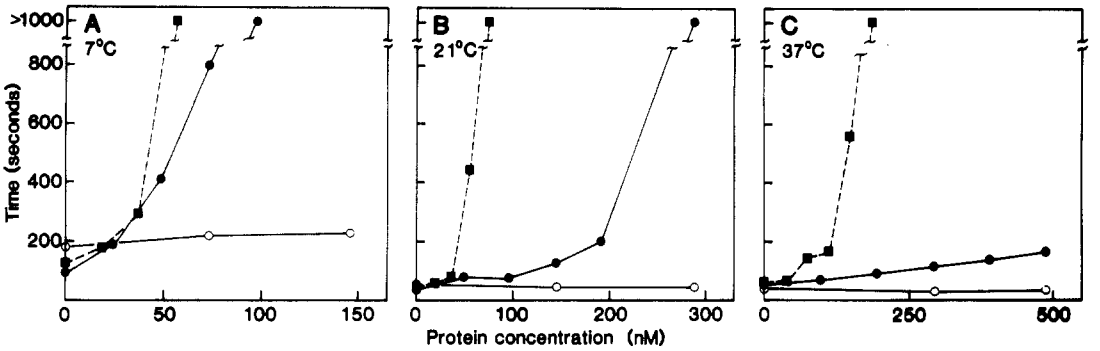

FIGURE 3: Gel-point activity of macrophage α-actinin and 540-kDa actin-binding protein, measured at three temperatures. Differing amounts of the two proteins were copolymerized with 1 mg/mL actin at each temperature in the rpesence of either 1 mM EGTA or 1 mM $CaCl_2$. The time taken for a steel ball to travel 20 mm in a falling-ball viscometer was recorded for each sample after polymerization was complete. The temperatures used were (A) 7, (B) 21, and (C) 37 °C; note that the protein concentration scale is different for each graph. The points indicate (●) α-actinin in the presence of EGTA, (○) α-actinin in the presence of $CaCl_2$, and (■) 540-kDa actin-binding protein in the presence of EGTA. The activity of the 540-kDa actin-binding protein was also measured in the presence of $CaCl_2$ in the same experiment and did not differ significantly from the activity in the presence of EGTA; these data are not included in the figure for reasons of clarity.

concentrations both cause an abrupt increase in the apparent viscosity of F-actin (i.e., a gel point) at 7 °C. At 21 °C, α-actinin was severalfold less active in this assay, while at 37 °C no gel point was observed at all at concentrations up to 0.5 μM. We observed a much smaller change with temperature in the gel point for the 540-kDa actin-binding protein, which presumably reflects a change in the average filament length of F-actin.

The effect of α-actinin on the apparent viscosity of F-actin at each temperature was completely abolished by addition of calcium (see Figure 3). In contrast, the interaction of the 540-kDa actin-binding protein with F-actin was unaffected by calcium (data not shown).

Binding of Macrophage α-Actinin to F-Actin. Binding of ^{125}I-labeled α-actinin to F-actin at 37 °C in the presence of EGTA (2.5 mM) and $CaCl_2$ (0.1 mM) is shown in Figure 4A. The same data are presented in the form of Scatchard plots in Figure 4B. In the illustrated experiment (and in some but not all others), the binding of α-actinin at the lowest concentrations tested deviated significantly from the best-fit line for the remaining data points, and these points were ignored when calculating the binding parameters. It is not clear to us whether this is indicative of some degree of positive cooperativity in the binding of macrophage α-actinin to F-actin or whether it merely reflects a technical difficulty in accurate measurement at the condition of least binding.

The maximum capacity of F-actin for α-actinin is high, corresponding to one α-actinin per two to three actin monomers. At 37 °C, the binding affinity in the presence of EGTA ($[Ca^{2+} < 10^{-9}$ M) is 4.2×10^6 M^{-1}, and in the presence of $CaCl_2$ (0.1 mM), it falls more than 5-fold to 7.7×10^5 M^{-1}. We also measured the binding of α-actinin to F-actin at 4 °C (data not shown) and obtained a value for the binding affinity in the presence of EGTA of 2.5×10^6 M^{-1}.

Binding of Calcium to Macrophage α-Actinin. The binding of $^{45}Ca^{2+}$ to α-actinin is presented in Figure 5A and redrawn in Figure 5B as a Scatchard plot. The best-fit line to the Scatchard plot indicates an affinity of 4×10^6 M^{-1} with maximal binding capacity corresponding to four calcium ions per α-actinin molecule, or two calcium ions per polypeptide chain.

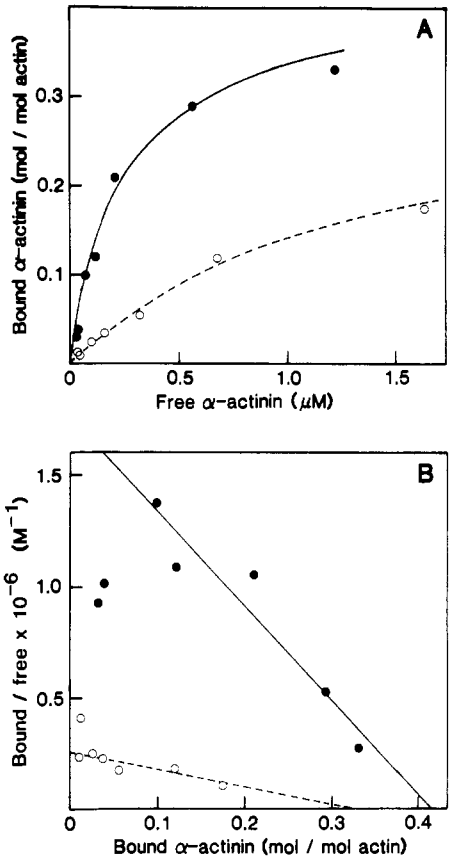

FIGURE 4: Binding of ^{125}I-labeled macrophage α-actinin to F-actin at 37 °C, measured by centrifugation. Data shown are from experiments in the presence of (●) 2.5 mM EGTA and (○) 0.1 mM $CaCl_2$. Data are plotted directly in (A) and as Scatchard plots in (B). The lines drawn in (B) did not take into account the points which obviously deviated from the rest of the binding data. The binding curves in (A) correspond to the lines in (B), and it is apparent that the extent of the deviation from these curves at low α-actinin concentrations is small and may simply reflect experimental inaccuracies.

Discussion

The physical properties of a calcium-sensitive actin-binding protein from macrophages are summarized in Table III. The protein is rod shaped and consists of two subunits each of M_r 103000. These properties are very similar to those previously reported for skeletal muscle α-actinin [see, for example, Suzuki et al. (1976)]. The macrophage protein differs from the muscle protein principally in the calcium sensitivity of its binding to F-actin. In this property, it resembles a number of other proteins which are also rod-shaped dimers with approximately 100-kDa subunits; such proteins have been isolated from tissue culture cells (Burridge & Ferramisco, 1981), blood platelets (Rosenberg et al., 1981; Landon & Lomucki, 1983), tumor cells (Mimura & Asano, 1979), liver cells (Kuo et al., 1982), kidney cells (Kobayashi & Tashima, 1983), and am-

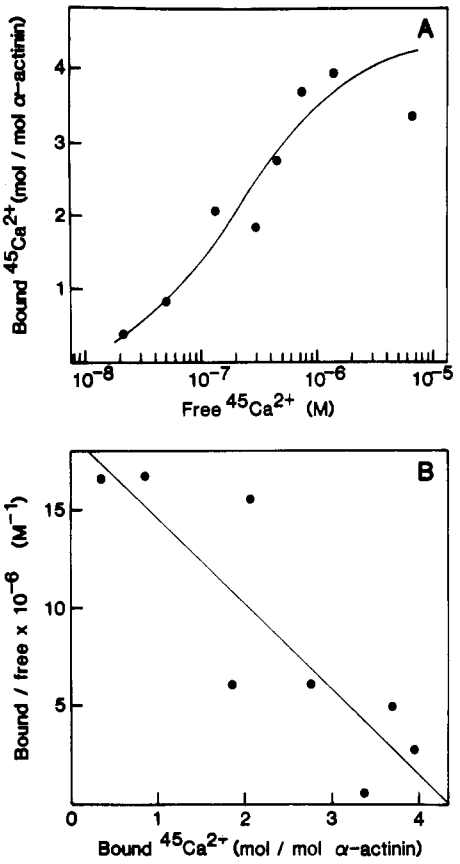

FIGURE 5: Binding of $^{45}Ca^{2+}$ to macrophage α-actinin at 4 °C, measured by equilibrium dialysis. Data are plotted directly in (A) and as a Scatchard plot in (B). The binding curve in (A) corresponds to the best-fit line to the points in (B), calculated by linear regression.

Table III: Summary of the Physical Properties of Rabbit Macrophage α-Actinin

parameter	value
Stokes' radius, a (nm)	7.26
sedimentation coefficient, $s^0_{20,w}$ (s^{-1})	6.83×10^{-13}
partial specific volume, $\bar{V}$ ($cm^3\ g^{-1}$)	0.725
molecular weight, M_r	
by electrophoresis in the presence of sodium dodecyl sulfate	103 000
from $s^0_{20,w}$, $\bar{V}$, and a	209 000
radius of gyration (nm)	
from electron micrographs	11.5
from M_r, $\bar{V}$, and $s^0_{20,w}$	10.9
binding to F-actin	
K_a ($[Ca^{2+}] < 10^{-9}$ M) (M^{-1})	4.2×10^6
K_a ($[Ca^{2+}] = 10^{-4}$ M) (M^{-1})	7.7×10^5
B_{max} ($[Ca^{2+}] < 10^{-9}$ M) (mol of α-actinin/mol of actin)	0.42
calcium binding	
K_a (M^{-1})	4×10^6
B_{max} (mol of Ca^{2+}/mol of α-actinin)	4.3

oebae (Pollard, 1981; Fechheimer et al., 1982; Condeelis & Vahey, 1982). It has been proposed that these proteins are all members of a distinct class of nonmuscle α-actinins (Burridge & Ferramisco, 1981). On the basis of its similar properties, we classify the macrophage protein described in this paper as a nonmuscle α-actinin.

Although previous investigators have demonstrated that binding of nonmuscle α-actinin to F-actin is inhibited in the presence of calcium, we report here the measurement of the binding parameters. The present paper also includes an examination of the calcium-binding sites of nonmuscle α-actinin and an investigation of the change with temperature of the interaction of the protein with F-actin.

Many workers have proposed that nonmuscle α-actinins act as cytoplasmic "gelation proteins". This arises from their ability to increase the apparent viscosity of F-actin, as determined by falling-ball viscometry in experiments like that of Figure 3. The cross-linking of actin filaments into extensive isotropic networks is indicated by a sharp gel point, such as that observed for the 540-kDa actin-binding protein (Figure 3). Our failure to observe a gel point at 37 °C for α-actinin argues against it being a gelation protein, at least at 37 °C in macrophages. The data of Figure 3C show no gel point at α-actinin concentrations up to 0.5 μM, and in other experiments, we have observed no gel point even at 1 μM when the binding of α-actinin to F-actin is close to saturation (Figure 4). These data are consistent with the fact that most of the actin cross-linking activity present in crude cell extracts can be accounted for by the 540-kDa actin-binding protein.

It is important to consider possible causes for the striking difference in the temperature-dependent behavior of α-actinin and actin-binding protein. The decrease in gelation activity of α-actinin is not simply due to a decrease in affinity of both actin binding sties for actin with increasing temperature, since we find that the binding affinity is higher at 37 °C than at 4 °C. However, since the binding assay would be somewhat insensitive to a change in affinity of only one binding site, it is possible that one of the binding sites has a lower actin-binding affinity as the temperature rises. This could radically decrease the ability of α-actinin to cross-link F-actin at higher temperatures since at least two actin-binding sites are required.

Alternatively, the difference in behavior of α-actinin and actin-binding protein (ABP) may be a manifestation of the properties of F-actin in solution and be reflected by the differences in structure of the actin assemblies which the respective cross-linking proteins produce. Electron microscopy has demonstrated that actin-binding protein cross-links actin filaments into an open orthogonal network (Niederman et al., 1980), in contrast to the parallel arrays which are formed by α-actinin (Podlubnaya et al., 1975; Jockusch & Isenberg, 1981). Since the F-actin filaments are elongated rods which in solution have the rheologic characteristics of a topologically constrained system (Zaner & Stossel, 1983), there will be a thermodynamic drive for lateral aggregates to form at low temperatures (Flory, 1952). α-Actinin may enhance this tendency at low temperatures and increase the apparent viscosity, as measured by the falling-ball viscometer, to the extent of an apparent gelation (Zaner & Stossel, 1982). The much lower thermodynamic drive for F-actin to form lateral aggregates at higher temperatures would largely remove the gelation activity of α-actinin. In contrast, ABP appears able to cross-link actin in an isotropic state, perhaps due to its elongated structure (Hartwig & Stossel, 1981), and should be influenced to a much lesser degree by an increase in temperature.

Added in Proof

We call attention to a recent publication reporting that calcium decreases the binding of brain α-actinin to F-actin (Duhaiman & Bamburg, 1984).

Acknowledgments

We thank Dr. John Hartwig for carrying out the electron microscopy.

Registry No. Ca, 7440-70-2.

References

Amrein, P. C., & Stossel, T. P. (1980) *Blood 56*, 442.

Bolton, A. E., & Hunter, W. M. (1973) *Biochem. J. 133*, 529.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248.
Brotschi, E. A., Hartwig, J. H., & Stossel, T. P. (1978) *J. Biol. Chem. 253*, 8988.
Burridge, K., & McCullough, J. (1980) *J. Supramol. Struct. 13*, 53.
Burridge, K., & Feramisco, J. R. (1981) *Nature (London) 294*, 565.
Cohn, E. J., & Edsall, T. J. (1943) in *Proteases, Amino Acids and Peptides*, p 375, Van Nostrand-Reinhold, Princeton, NJ.
Condeelis, J., & Vahey, M. (1982) *J. Cell Biol. 94*, 466.
Duhaiman, A. S., & Bamburg, J. R. (1984) *Biochemistry 23*, 1600–1608.
Ebashi, S., & Ebashi, E. (1965) *J. Biochem. (Tokyo) 58*, 5.
Fechheimer, M., Brier, J., Rockwell, M., Luna, E. J., & Taylor, D. L. (1982) *Cell Motil. 2*, 287.
Feramisco, J. R., & Burridge, K. (1980) *J. Biol. Chem. 255*, 1194.
Goll, D. E., Suzuki, A., Temple, J., & Holmes, G. R. (1972) *J. Mol. Biol. 67*, 469.
Hartwig, J. H., & Stossel, T. P. (1975) *J. Biol. Chem. 250*, 5696.
Hartwig, J. H., & Stossel, T. P. (1979) *J. Mol. Biol. 134*, 539.
Hartwig, J. H., & Stossel, T. P. (1981) *J. Mol. Biol. 145*, 563.
Jochkusch, B. M., & Isenberg, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3005.
Kobayashi, R., & Tashima, Y. (1983) *Biochim. Biophys. Acta 745*, 209.
Kuo, P. F., Mimura, N., & Asano, A. (1982) *Eur. J. Biochem. 125*, 277.
Laemmli, U. K. (1970) *Nature (London) 227*, 680.
Landon, F., & Olomucki, A. (1983) *Biochim. Biophys. Acta 742*, 129.
Laurent, T. C., & Killander, J. (1964) *J. Chromatogr. 14*, 317.
Lazarides, E., & Burridge, K. (1975) *Cell (Cambridge, Mass.) 6*, 289.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.
Maclean-Fletcher, S. D., & Pollard, T. D. (1980) *J. Cell Biol. 85*, 414.
Maruyama, K., & Ebashi, S. (1965) *J. Biochem. (Tokyo) 58*, 13.
Mimura, N., & Asano, A. (1979) *Nature (London) 282*, 44.
Myrvik, Q. N., Leake, E. S., & Farviss, B. (1961) *J. Immunol. 86*, 128.
Niederman, R., Amrein, P. C. & Hartwig, J. H. (1983) *J. Cell Biol. 96*, 1400.
Perrin, D. D., & Sayce, I. G. (1976) *Talanta 14*, 833.
Pollard, T. D. (1981) *J. Biol. Chem. 256*, 7666.
Rosenberg, S., Stracher, A., & Burridge, K. (1981) *J. Biol. Chem. 256*, 12986.
Schachman, H. K. (1957) *Methods Enzymol. 4*, 32.
Siegel, L. M., & Monty, K. J. (1966) *Biochim. Biophys. Acta 112*, 346.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866.
Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M., & Stromer, M. H. (1976) *J. Biol. Chem. 251*, 6860.
Tyler, J., & Branton, D. (1980) *J. Ultrastruct. Res. 71*, 95.
Yin, H. L., & Stossel, T. P. (1979) *Nature (London) 281*, 583.
Yin, H. L., & Stossel, T. P. (1980) *J. Biol. Chem. 255*, 9490.
Zaner, K. S., & Stossel, T. P. (1982) *J. Cell Biol. 93*, 987.
Zaner, K. S., & Stossel, T. P. (1983) *J. Biol. Chem. 258*, 11004.