

Purification and Characterization of an *Acanthamoeba* Nuclear Actin-binding Protein

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Abstract. Immunolocalization of monoclonal antibodies to *Acanthamoeba* myosin I showed a cross-reactive protein in nuclei (Hagen, S. J., D. P. Kiehart, D. A. Kaiser, and T. D. Pollard. 1986. *J. Cell Biol.* 103:2121-2128). This protein is antigenically related to myosin I in that nine monoclonal antibodies and three polyclonal antibodies are cross-reactive. However, studies with affinity-purified antibodies and two-dimensional peptide maps show that the protein is not a proteolytic product of myosin I. We have used cell fractionation and column chromatography to purify this protein. It is a dimer of 34-kD polypeptides with

a Stokes' radius of 4 nm. A polyclonal antisera generated against the purified protein confirms the nuclear localization seen with the cross-reactive monoclonal antibodies. The 34-kD protein binds actin filaments in an ATP-insensitive manner with a K_d of $\sim 0.25 \mu\text{M}$ without cross-linking, severing, or capping. No ATPase activity was detected in the presence or absence of actin. It also binds to DNA. These unique properties suggest we have discovered a new class of actin-binding protein. We have given this protein the name NAB for "nuclear actin-binding" protein.

NUMEROUS actin-binding proteins have been purified from the cytoplasm of many cells, including *Acanthamoeba castellanii* (Stossel et al., 1985; Pollard and Cooper, 1986). The proteins fall into five general groups including actin filament-cross-linking proteins, actin filament-capping proteins, actin filament-severing proteins, actin monomer-binding proteins, and cytoplasmic myosins. These groupings cross species lines although the analogous proteins in different species may vary in size. In this paper we describe the first example of a new class of actin-binding protein. It is antigenically related to myosin I, but its characteristics and nuclear localization suggest it represents a novel type of actin-binding protein.

This protein was discovered through a curious immunological cross-reactivity with the globular myosin I from *Acanthamoeba castellanii*. *Acanthamoeba* has two well-characterized types of myosin (Korn and Hammer, 1988). Myosin II (Maruta and Korn, 1977; Pollard et al., 1978) is a typical nonmuscle myosin with two heads and a coiled coil tail composed of two 171-kD heavy chains and two classes of light chains. Like other myosins it has actin-activated ATPase activity and forms bipolar filaments. Myosin I (Pollard and Korn, 1973) is a globular monomeric protein with an NH_2 -terminal head domain that resembles other myosin heads and a unique COOH-terminal 50-kD domain (Jung et al., 1987) that includes an ATP-insensitive actin-binding site (Lynch et al., 1986).

Portions of this work were presented at the 1985 Annual Meeting of the American Society for Cell Biology and have appeared in abstract form (1985. *J. Cell Biol.* 101:158a. [Abstr.]).

Although staining *Acanthamoeba* with a polyclonal antibody suggested that myosin I is excluded from the nucleus (Gadasi 1980), Hagen et al. (1986) found that several monoclonal antibodies made against *Acanthamoeba* myosin I stained the nucleus. They went on to show that the reaction of these antibodies with the nucleus was due to cross-reaction with a 29-kD protein rather than with myosin I. In this paper we describe the purification and initial characterization of this nuclear protein. We show that it binds both actin and DNA and that its characteristics suggest it represents a new class of actin-binding proteins.

Materials and Methods

Purification of Proteins

Acanthamoeba castellanii were grown in liquid culture to $>10^6/\text{ml}$. Typically 30 liters yielded ~ 900 g packed cells. The cells were washed once in 50 mM NaCl and then homogenized at 0°C in 2 vol sucrose extraction buffer (10% sucrose, 20 mM imidazole-HCl, pH 7.5, 1 mM ATP, 1 mM EGTA, 1 mM DTT, 0.1 mM benzamidine, 0.1 mM PMSF) by 12 passes in a teflon/glass homogenizer (Potter-Elvehjem, VWR, Baltimore, MD) rotating at 3,000 rpm at 0°C . The homogenate was centrifuged for 10 min at 4°C at 5,000 rpm in a rotor (JA-10; Beckman Instruments, Inc., Fullerton, CA). The pellets were taken and nuclei were purified using a modification of a protocol by Kumar et al. (1984). The pellets were resuspended and washed four times in STMDK buffer (0.75 M sucrose, 40 mM Tris-HCl, pH 7.1, 4 mM MgCl_2 , 50 mM KCl, 1 mM DTT) using 3-10 passes with a homogenizer (Dounce; Kontes Glass Co., Vineland, NJ) between washes to resuspend the pellets. The wash volume used was $\sim 5-8$ times the pellet volume and the nuclei were pelleted at 2,500 rpm in a rotor (JA-10; Beckman Instruments, Inc.) for 10 min at 4°C after each wash. These washed loose pellets were resuspended in a minimal volume of STMDK buffer and loaded onto a two-step gradient made by layering 8 ml of 1.3 M sucrose in STMDK

buffer on top of 8 ml of 1.7 M sucrose in STMDK buffer. About 30 ml of resuspended pellet was layered onto each 16-ml gradient and then centrifuged in a rotor (J-6; Beckman Instruments, Inc.) at 5,000 rpm for 20 min at 4°C. The pellet contained purified nuclei. The major contaminants observed by phase-contrast microscopy were whole unlysed cells, seen at a concentration of 1–5 cells per 100 intact nuclei. There were no other contaminating organelles seen by light microscopy.

The nuclei were then extracted for 1 h in high salt extraction (HSE) buffer (0.7 M NaCl, 30 mM KCl, 5 mM MgCl₂, 50 mM Tris-HCl, pH 8, 0.5 mM DTT, 0.1 mM benzamidine) and then pelleted in a rotor (JA-20; Beckman Instruments, Inc.) at 15,000 rpm for 15 min at 4°C. The supernatant was fractionated with solid ammonium sulfate. The protein precipitating between 1.5 and 2.5 M was resuspended in and dialyzed against HSE buffer.

The dialyzed ammonium sulfate pellet was run through a 50-ml column of hydroxylapatite equilibrated with HSE buffer. The optimal purification was obtained when the nuclear actin-binding (NAB)¹ protein passed directly through the column in HSE buffer. This was possible with lots of HTP hydroxylapatite powder from Bio-Rad Laboratories (Richmond, CA). Better yields are obtained from HTP lots from which single-stranded DNA could be eluted at low (150–175 mM) phosphate concentrations (this information is printed on each bottle of HTP and varies considerably from batch to batch). Samples of hydroxylapatite preparations from four other suppliers and a batch of “home-made” hydroxylapatite have been tried. All of these hydroxylapatites retained NAB protein in HSE buffer. The bound NAB protein could be eluted with a phosphate gradient, but it was not as pure as when it flowed directly through a hydroxylapatite column and was partially degraded during the exposure to phosphate. The flow through from the HTP column was collected and precipitated by adding solid ammonium sulfate to 3 M. The precipitated protein was resuspended in a minimal volume of HSE buffer and run over a column of Sephacryl S-300 equilibrated with HSE buffer. Using ELISA assays and SDS-PAGE the appropriate fractions were pooled and dialyzed against TD buffer (2 mM Tris-HCl, pH 8, 0.5 mM DTT). The dialyzed sample was loaded on a 10-ml heparin-agarose (Sigma Chemical Co., St. Louis, MO) column equilibrated in TD buffer, washed with 20 ml TD buffer, and eluted with a 40-ml linear gradient of 0–1 M NaCl in TD buffer. The purified protein was dialyzed and stored in TD buffer at 4°C.

Actin was purified from rabbit skeletal muscle by Dr. Dhan Bhandari (Johns Hopkins Medical School, Baltimore, MD) using the method of MacLean-Fletcher and Pollard (1980).

Immunological Methods

NAB protein was purified through the S-300 column and resolved by SDS-PAGE. The gel was stained with aqueous Coomassie blue. Bands containing 5–10 µg of NAB protein were excised from the gel, homogenized in a homogenizer (Dounce; Kontes Gass Co.) with Freund's complete adjuvant, and injected into rabbits according to the protocol of Fujiwara and Pollard (1976). Specific antibodies were purified from serum by their affinity for NAB protein immobilized on nitrocellulose (Olmsted, 1981; Pollard, 1984).

Monoclonal antibodies were produced by Kiehart et al. (1984). ELISA assays were done with horseradish peroxidase-labeled goat anti-mouse or goat anti-rabbit second antibody and *O*-phenylenediamine as the substrate according to the manufacturer's (HyClone Laboratories, Logan, UT) protocol.

Biochemical Methods

Protein concentrations were estimated using the Bradford (1976) reagent purchased from Bio-Rad Laboratories. Ovalbumin was used as a standard. Gel electrophoresis was done using the method of Laemmli (1970) and gels were stained with Coomassie brilliant blue or silver as described by Wray et al., (1981). Proteins were electroblotted onto nitrocellulose as described by Towbin et al. (1979). The nitrocellulose filter was blocked in Tween buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween, 1 mM thimerosal) with 0.6% BSA, and incubated in a Tween-BSA buffer with 10 µg/ml of monoclonal antibody or 1:1,000 dilution of polyclonal antibody for 1 h at room temperature. After three 10-min washes in Tween buffer, the blot was incubated in ¹²⁵I-goat anti-mouse antibody (HyClone Laboratories) in Tween-BSA buffer for 1 h at room temperature. After three 10-min washes the blots were dried and autoradiographs were made. The second antibody (goat anti-mouse) was labeled using chloramine T as previously described (Kiehart et al., 1984) and used at a concentration of (~)10⁶ cpm/ml. In some cases peroxidase-linked goat anti-rabbit or goat anti-

1. *Abbreviation used in this paper:* NAB, nuclear actin binding.

mouse (HyClone Laboratories) was used as the second antibody. After a 1-h incubation and two Tween buffer washes, the blot was washed twice with 10 mM Tris-HCl, 150 mM NaCl and then developed using 0.03% hydrogen peroxide and 1 mg/ml 4-chloro-naphthol in 20% methanol for 5–15 min for optimal contrast.

Two-dimensional peptide mapping was done by electrophoresis and chromatography on thin-layer plates using the method of Elder et al. (1977) as described in Cooper et al. (1986). About 5 µg myosin-IA, myosin-IB, or NAB were run on SDS-PAGE, stained with Coomassie blue, and cut from the gels. After drying and iodination the slices were digested with L-1-p-tosylamino phenylethyl chloromethyl ketone-trypsin (Worthington Biochemical Corp., Freehold, NJ). The peptides were separated on 10-cm thin-layer chromatography plates or, for better resolution, on 20-cm plates. Iodinated peptides were detected by autoradiography.

Fluorescent Antibody Staining

Immunolocalization was done in a manner similar to that described by Hagen et al. (1986). Cells attached to coverslips were fixed, permeabilized with acetone, and stained with drops of 1:200 dilutions of rabbit sera for 1 h at room temperature. Coverslips were washed with PBS and then a 1:500 dilution of rhodamine-labeled second antibody (HyClone Laboratories) that was preadsorbed with acetone-extracted *Acanthamoeba*. After a final wash the coverslips were mounted in 85% glycerol, 10% PBS, 5% *n*-propyl-galate and photographed with a microscope (E. Leitz, Wetzlar, FRG) with a vertical illuminator.

Actin-binding Experiments

Various concentrations of NAB protein were incubated with 3 µM F-actin in 20 mM imidazole, pH 7, 1 mM ATP, 1 mM EGTA, 1 mM MgCl₂ at room temperature for 10 min. Samples of these reaction mixtures were used to construct a standard curve for NAB protein using a filter-binding antibody assay. Samples were adsorbed to nitrocellulose in a dot-blot filtering apparatus (Schleicher & Schuell, Inc., Keene, NH). The nitrocellulose filter was removed, blocked in Tween-BSA buffer for 15 min and handled as a Western blot as described above. After autoradiography, each hot spot was cut out and counted in a gamma counter. The reaction mixtures were centrifuged for 20 min at 100,000 rpm in a rotor (TL 100; Beckman Instruments, Inc.) at 20°C. Supernatant samples (5–10% of the total volume) were taken with care not to disturb the pellet. These samples, taken in duplicate or triplicate, were loaded onto the dot blotter, washed with Tween-BSA buffer, and processed with the standards.

Southwestern Blots

DNA-binding assays were done in a manner similar to that described by McKay (1981). Proteins were separated by SDS-PAGE (Laemmli, 1970) and then electroblotted to nitrocellulose paper (Gelman Sciences, Inc., Ann Arbor, MI) in a Tris/glycine buffer. The blots were blocked for 1 h in SW buffer (10 mM Tris-HCl pH 7.1, 2× Denhardt's Solution [Maniatis et al., 1982], 1 mM EDTA, 50 mM NaCl) then incubated for 1 h at room temperature with *Acanthamoeba* genomic DNA (Rimm et al., 1989). The DNA was labeled with [³²P]dCTP by nick translation using a kit supplied by Bethesda Research Laboratories (Gaithersburg, MD) and diluted to a final concentration of 10⁶ cpm/ml in SW buffer. The blots were then washed three times for 10 min with SW buffer with 150 mM NaCl, dried, and autoradiographed.

Results

Evidence for a Nuclear Protein that Cross Reacts with Antibodies to Acanthamoeba Myosin I

Hagen et al. (1986) originally identified a polypeptide that migrated at 29 kD by SDS-PAGE as the nuclear antigen that cross reacts with antibodies to the ~130-kD myosin I. We found that the 29-kD species is actually a common breakdown product of a larger protein that migrates at ~34 kD. We have purified and characterized this 34-kD protein. Based on its localization and properties we call it the nuclear actin-binding or NAB protein. It is rather protease sensitive

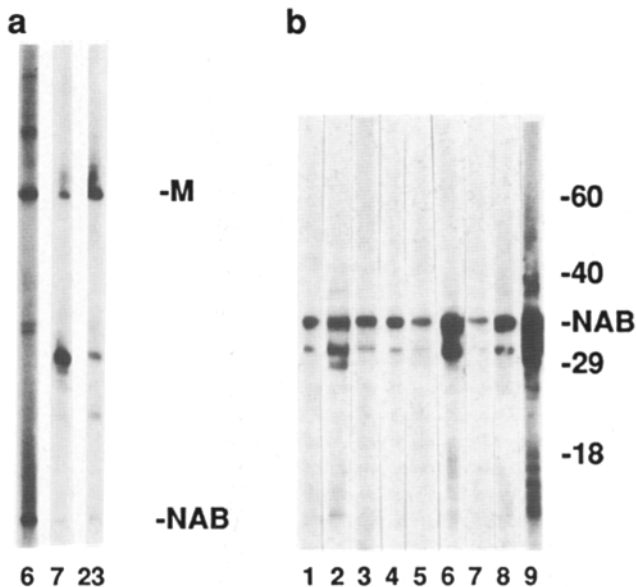


Figure 1. Antibodies that bind to myosin I also bind to NAB protein. Proteins were separated by SDS-PAGE, blotted onto nitrocellulose, reacted with primary antibodies followed by secondary antibodies conjugated to horseradish peroxidase, and developed as described in Materials and Methods. (a) Three independently isolated polyclonal antibodies to myosin I—labeled (lane 6) JH-6, (lane 7) JH-7, and (lane 23) JH-23—react with both myosin I (*M*) and NAB protein (*NAB*). The blot strips shown are 10:1 dilutions of whole *Acanthamoeba* cells. (b) Reaction of nine monoclonal antibodies to myosin I with strips that were blotted from gels of isolated nuclei from *Acanthamoeba*. Lane numbers correspond to monoclonal antibody numbers (M1.X). The gel resolves only proteins that migrate with molecular masses between 10 and 85 kD, so myosin I is not seen.

and the method used for preparing the nuclei can change the apparent molecular weight. The fact that purified NAB protein also generates similar antigenic fragments as it breaks down confirms that the different molecular weight bands seen on the blots do not represent multiple subunits or iso-types.

Myosin I and NAB protein share many epitopes since nine monoclonal antibodies and three polyclonal sera were cross-reactive (Fig. 1, *a* and *b*). All of the polyclonal sera react with NAB protein resolved by SDS-PAGE although all react more strongly with myosin I (Fig. 1 *a*, *M*). The nine monoclonal antibodies react with at least eight epitopes on myosin I (Hagen et al., 1986). Their reaction with the 34-kD NAB protein (Fig. 1 *b*) shows that the two proteins share a number of epitopes. Note that the monoclonal antibodies also bind to various breakdown products below the major reactive band at 34 kD, including 32- and 29-kD bands. The fact that different monoclonal antibodies identify different degradation products confirms that the antibodies recognize multiple epitopes.

Purification of NAB Protein

NAB protein is substantially enriched in *Acanthamoeba* nuclei prepared by the method of Kumar et al. (1984) such that it may be seen as a distinct band on a Coomassie blue-stained gel (Fig. 2, lane *N*) representing ~1% of the total nuclear protein (data not shown). The purification of the NAB

protein was followed by ELISA assays using monoclonal antibody M1.5.

The NAB protein was completely extracted from the nuclei by 0.7 M NaCl. Ammonium sulfate fractionation was used to enrich for NAB protein and to concentrate the nuclear extract. NAB protein does not bind to Bio-Rad HTP hydroxylapatite (Fig. 3) and voided fractions show substantial purification (Fig. 2, cf. lanes 2 and 4). The flow through was concentrated by ammonium sulfate precipitation and dialyzed back into the high salt buffer, since NAB protein precipitates irreversibly in low salt at this stage. After gel filtration on an S-300 column in HSE buffer, the pooled NAB fractions (Fig. 4) could be dialyzed into a low salt buffer without precipitation (Fig. 2, lane 5). For final purification, the pooled, dialyzed fractions from the S-300 column were chromatographed on a heparin-agarose column and eluted with a gradient of NaCl (Fig. 5). The final product is essentially pure NAB protein in spite of the presence of multiple bands on Coomassie blue-stained gels (Fig. 2, lane 6) since immunoblots identified the major contaminants at 32 and 29 kD as proteolytic products of the NAB protein.

The yield of purified NAB protein, 200–600 μ g/kg packed cells, has been limited by several factors. First, 1 kg *Acanthamoeba* yields only ~30–50 g purified nuclei which can

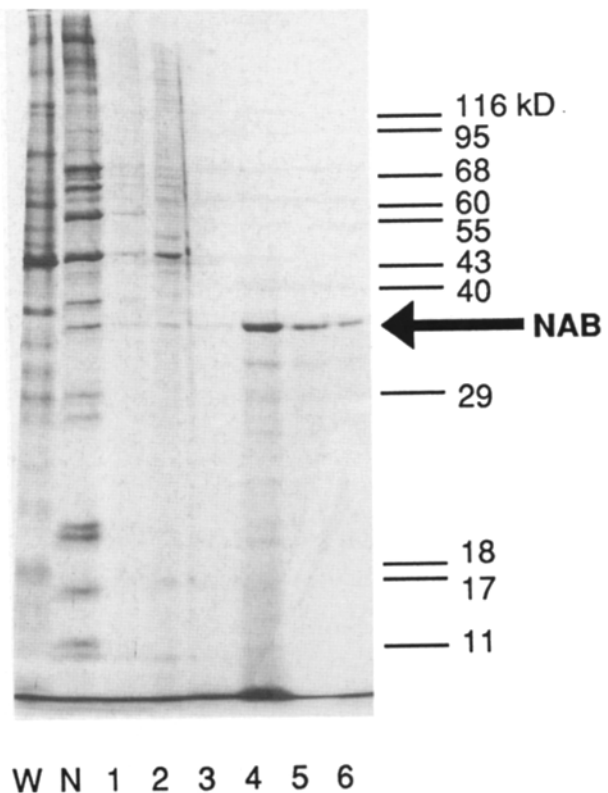


Figure 2. SDS-PAGE of fractions obtained at each step in the purification of NAB protein. (Lane *W*) Whole *Acanthamoeba*; (Lane *N*) purified nuclei; (lane 1) high salt nuclear extract; (lane 2) 1.5–2.5 ammonium sulfate precipitate; (lane 3) hydroxylapatite column flow through peak; (lane 4) 3 M ammonium sulfate precipitate of HTP column flow through; (lane 5) NAB protein peak from S-300 column; (lane 6) NAB protein peak from heparin-agarose column. Note that immunoblots of purified protein suggest that bands at ~32 and 29 kD are proteolytic fragments of NAB protein.

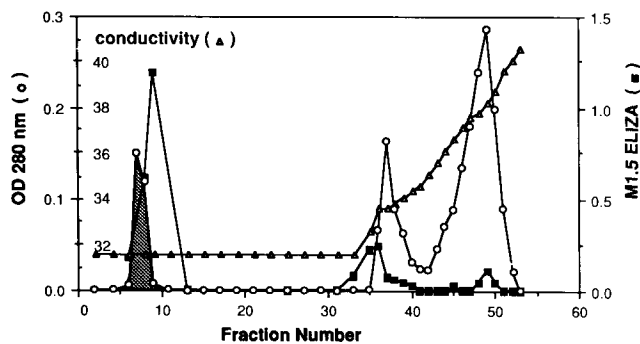


Figure 3. Hydroxylapatite chromatography of a nuclear extract. A 50-ml column of fresh Bio-Rad HTP equilibrated with HSE buffer was loaded with nuclear extract and 8-ml fractions were collected. After loading and washing with one column volume of HSE buffer, the column was eluted with a linear gradient of 0–0.5 M potassium phosphate in HSE buffer. ELISA assays for NAB protein using monoclonal antibody M1.5 were done with 10- μ l samples. Absorbance at 280 nm (○); NAB protein as assayed by ELISA assay (■); conductivity of the buffer (Δ). Pooled fractions (6–10) are shaded.

be used for starting material. Second, affinity methods such as columns of immobilized monoclonal antibodies were compromised by the fact that the NAB protein dissociated from the antibodies only under denaturing conditions. Consequently we were forced to use conventional chromatographic methods with the inevitable losses at each step. Third, NAB protein is highly susceptible to proteolysis especially in the presence of phosphate, where even highly enriched NAB protein is completely degraded in 12–24 h. Consequently, we could not use phosphate to elute the fraction of the NAB protein bound to the hydroxylapatite column since it is substantially degraded before we can dialyze out the phosphate. The extent of degradation is unaffected by conventional protease inhibitors. This problem has forced us to use only Bio-Rad HTP with specific properties as defined in Materials and

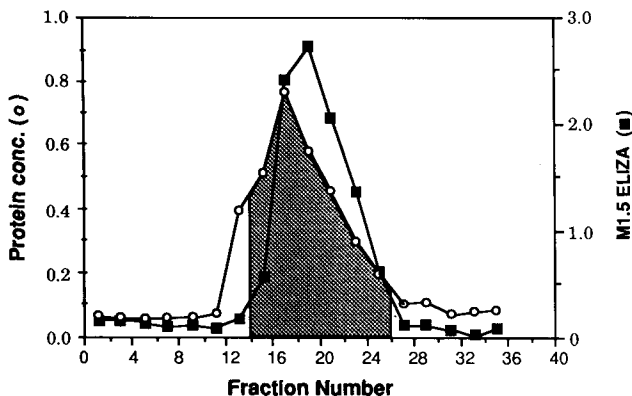


Figure 4. Sephacryl S-300 chromatography of the concentrated, pooled fractions from the hydroxylapatite column. The pooled fractions from the hydroxylapatite column were precipitated with ammonium sulfate and loaded on a 1.5 \times 50-cm column of Sephacryl S-300 equilibrated in HSE buffer. Bradford assays and ELISA assays were done on 10- μ l samples of every other 3.5-ml fraction. Protein concentration (○) is shown in arbitrary units. NAB protein (■) as detected by ELISA assay with monoclonal antibody M1.5. Pooled fractions (14–25) are shaded.

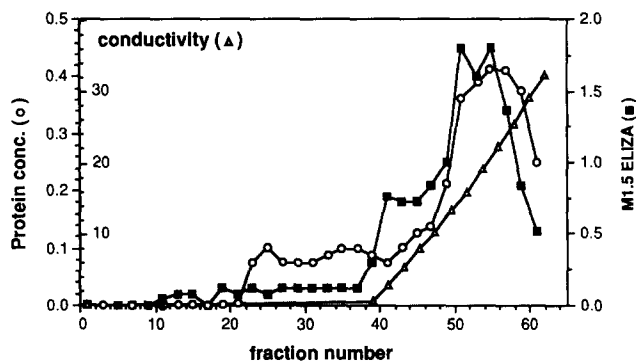


Figure 5. Heparin-agarose chromatography of enriched NAB protein. Pooled fractions from the S-300 column were dialyzed into TD buffer and loaded onto a 10-ml column of heparin-agarose equilibrated with TD buffer as described in Materials and Methods. The column was washed with 20 ml and eluted with a 40-ml linear gradient of 0–1 M NaCl in TD buffer. Bradford assays and ELISA assays were done on 50- μ l samples of every other 1-ml fraction. Protein concentration (○) is shown in arbitrary units. NAB protein (■) as detected by ELISA assay with monoclonal antibody M1.5. Conductivity of the buffer (Δ) in mMhos. Individual peak fractions were dialyzed into TD buffer for storage.

Methods to maximize yield. We have also been unable to use phospho-cellulose as an ion exchanger for the final purification step since it has a similar unexplainable degradative effect on this protein. Finally, NAB protein has a propensity

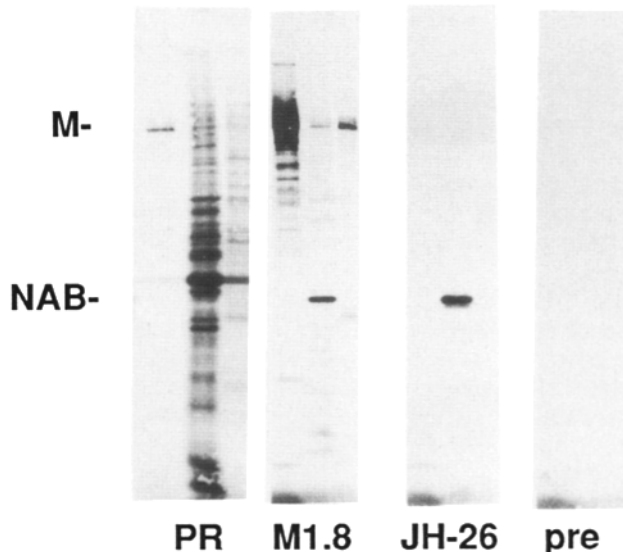


Figure 6. Tests for antibody cross-reactivity show that NAB protein is related to myosin I but is not a degradation product. Each panel shows an electrophoretogram of three lanes containing (from left to right) purified myosin I, a nuclear extract, and a high speed *Acanthamoeba* supernatant separated on an SDS-8% polyacrylamide gel. (PR) Ponceau red stain for total protein; (M1.8) a monoclonal antibody to myosin I which cross reacts with NAB protein; (JH-26) an affinity-purified rabbit polyclonal antibody to NAB protein; and (pre) preimmune serum from rabbit JH-26. All antibody reactions were visualized by horseradish peroxidase reactions on a 4-chloronaphthol substrate. M, myosin I heavy chain; NAB, NAB protein. Note that M1.8 reacts with NAB protein but that anti-NAB protein (JH-26) does not react with myosin I.

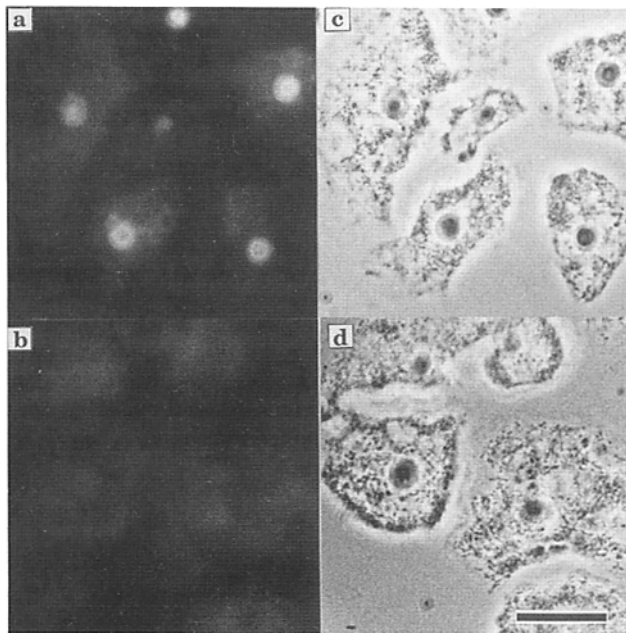


Figure 7. Fluorescent antibody localization of NAB protein. (a) An NAB protein-specific affinity-purified polyclonal antibody, JH-26, shows NAB protein is primarily localized in the nucleus. The primary antibodies were detected with rhodamine-labeled goat anti-rabbit antibodies. (b) Preimmune serum. Phase-contrast micrographs of the same fields are seen in c and d, respectively. Bar, 10 μm .

to precipitate out of the solution if the salt concentration is too low. We think that this may be due to DNA-binding and have found that if high salt is used throughout the majority of the purification, the enriched protein can be dialyzed into low salt after the S-300 column.

Immunological Studies

Affinity-purified polyclonal antibodies to NAB protein react with a 34-kD polypeptide in isolated nuclei, but not with purified myosin I or any other peptide in cytoplasmic extracts (Fig. 6). This shows that NAB protein has unique epitopes not found on myosin I and confirms that it is confined to the nucleus. In contrast, monoclonal (and polyclonal) antibodies to myosin I react with both myosin I and NAB protein (Fig. 6). The concentration of myosin I is much higher in the cytoplasm than in isolated nuclei, while NAB protein is only in nuclei.

Affinity-purified polyclonal antibodies to NAB protein stain the nuclei of *Acanthamoeba* (Fig. 7) exactly like the monoclonal antibodies to myosin I (Hagen et al., 1986). The staining is diffuse throughout the nucleoplasm, but faint or absent from nucleoli. Since these anti-NAB protein antibodies do not cross react with myosin I, there is little or no staining of the cytoplasm where myosin I is located (Gadasi and Korn, 1980).

Peptide Maps

On two-dimensional peptide maps, myosin IB (Fig. 8 a) shares few if any peptides with the NAB protein (Fig. 8 b). The overall patterns are very different even though the thin-layer plates were run under identical conditions. In contrast,

myosin IA is nearly indistinguishable from myosin IB (data not shown).

Physical Properties

The physical properties of NAB protein suggest that it is an elongated dimer in its native state. Stokes' radius was estimated to be 4 nm by gel filtration on Sephadex G150. Sedimentation through a sucrose gradient, however, gave a sedimentation coefficient of 3.6 S. The molecular mass calculated from the Stokes' radius and the sedimentation coefficient was $\sim 65,000$ D. Given its mobility in SDS-PAGE, we propose that the native protein is an elongated dimer of two 34-kD subunits. Like many nuclear or DNA-binding proteins, NAB protein has a basic isoelectric point of 8.7 in 6 M urea and thus a net positive charge at neutral pH.

Functional Studies

Since NAB protein is antigenically related to myosin I, we

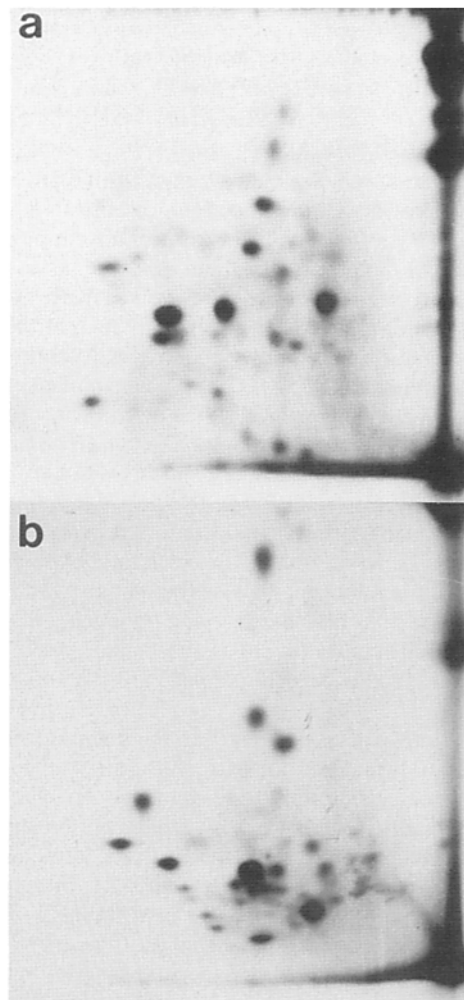


Figure 8. Two-dimensional peptide maps of myosin IB and NAB protein. Autoradiograms of peptide fingerprints of iodinated peptides of (a) myosin IB and (b) NAB protein separated on thin-layer chromatography plates. The samples were spotted on the lower right hand corner of the plate and then electrophoresed horizontally and chromatographed vertically. Myosin IA is nearly identical to myosin IB (not shown).

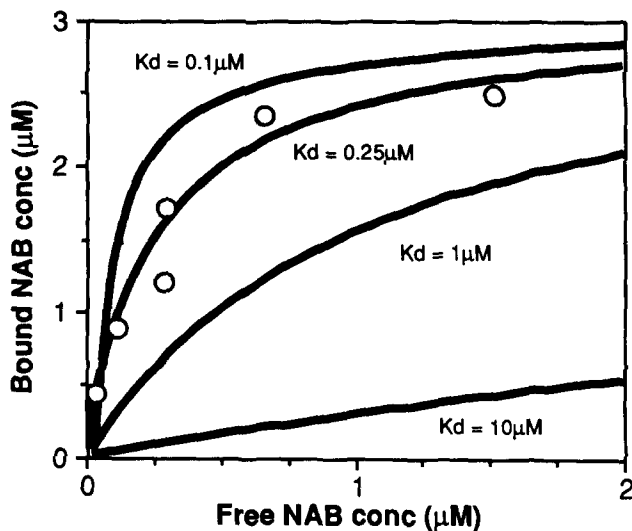


Figure 9. Binding of NAB protein to 3- μ M actin filaments. NAB protein was reacted with F-actin and the free NAB protein concentration was measured after pelleting the complex of F-actin and NAB protein. Bound NAB protein concentration was determined by subtracting free concentration from total concentration. Solid lines show theoretical curves of binding isotherms with several K_d values, assuming one NAB protein binding site per actin monomer.

began our characterization by looking for myosin-like properties. Unlike myosins, NAB protein has no detectable K^+ /EDTA or Mg^{++} ATPase activity in either the absence or presence of actin. On the other hand, sedimentation assays showed that NAB protein binds to F-actin even in the presence of ATP. The binding profile (Fig. 9) suggests that the protein binds F-actin at a stoichiometry of one 34-kD peptide per actin monomer with a K_d of $\sim 0.25 \mu M$. Scatchard plots of this data are nonlinear, suggesting that the binding may be complex. Additionally, NAB protein blotted onto nitrocellulose binds ^{125}I -labeled G-actin (data not shown).

The presence of NAB protein during the polymerization of actin has no effect on the viscosity as assessed by falling ball assays. This is a sensitive assay to detect actin monomer sequestration, actin filament capping (Isenberg et al., 1980; Cooper et al., 1984), actin filament severing (Cooper et al., 1986), and actin filament cross-linking (Pollard et al., 1986). Consequently, NAB protein does not appear to have any of these activities.

Given the localization of NAB protein in the nucleus, the high salt concentration required for extraction from the nuclei and its basic isoelectric point we tested for DNA binding. Labeled *Acanthamoeba* DNA binds to many proteins in an *Acanthamoeba* nuclear extract after separating proteins by SDS-PAGE and electroblotting as described by McKay (1981). When purified proteins are assayed in a similar fashion, NAB protein binds labeled DNA while actin, myosin I, and α -actinin do not (Fig. 10). Other experiments show that the protein cosediments with both single- and double-stranded DNA bound to Sepharose beads (data not shown). The specific DNA sequence that NAB protein binds, if it exists, has not yet been identified.

Discussion

Although NAB protein was originally discovered by its anti-

genic cross-reactivity with myosin I, its relationship to other myosins is probably rather distant. NAB protein does not have most of the characteristic properties that define myosins such as actin-activated ATPase and ATP-sensitive actin binding resulting in force production. The monoclonal antibodies that cross react have all been localized to a 30-kD COOH-terminal proteolytic product of myosin I (Hagen et al., 1986). This is the region of the myosin I molecule that has a primary structure (Jung et al., 1987) completely unrelated to conventional two-headed myosins (Warrick and Spudich, 1987). It is notable that this region of myosin I contains an actin-binding site, with micromolar affinity, that is ATP insensitive like NAB protein (Lynch et al., 1986). It is possible that these antibodies recognize some features of the actin-binding site and are cross-reactive on that basis alone, suggesting that the relationship to myosin is quite distant or that both NAB protein and the COOH-terminal domain of myosin I have a common ancestor.

NAB protein is the first actin-binding protein to be localized exclusively to the nucleus of any cell. Another actin-binding protein, cofilin, is predominantly in the cytoplasm of normal mouse fibroblast cells but becomes associated with actin filament bundles in the nucleus after the cells are stressed by heat shock or treatment with dimethyl sulfoxide (Nishida et al., 1987).

Although the function of NAB protein in the cell is not yet known, its ability to bind both actin and DNA makes it conceivable that it plays some role in attaching DNA to an actin-containing matrix. Immunohistology of diverse tissues suggests that the NAB protein is not unique to *Acanthamoeba*. Antibodies to NAB protein stain nuclei in skeletal muscle, glial, and retinal epithelial cells (Dr. Joseph Madsen, Massachusetts General Hospital, Boston, MA, personal communication). In all cases its diffuse localization in the nucleus is suggestive of a matrix-like structure. Although there is no evidence to show that actin is a component of the nuclear ma-

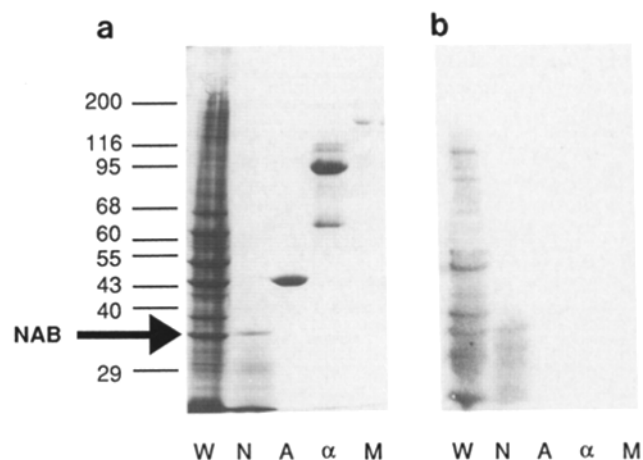


Figure 10. Southwestern blot shows that *Acanthamoeba* DNA binds to NAB protein and not to actin or other actin-associated proteins. The protein fractions were separated by SDS-PAGE, electroblotted onto nitrocellulose, and reacted with ^{32}P -labeled *Acanthamoeba* genomic DNA. (Lane W) whole nuclear extract; (lane N) partially purified NAB protein; (lane A) actin; (lane α) α -actinin; (lane M) myosin IA and IB. (a) Gel stained with Coomassie blue; (b) an autoradiogram detecting labeled DNA on a blot of an identical gel.

trix or chromatin-supporting system of *Acanthamoeba*, it is interesting to note that a specific isoform of actin has been purified from the *Acanthamoeba* nucleus (Kumar et al., 1984). Actin has also been found in nuclear extracts of several types of cells (LeStourgeon, 1978) and a distinct nuclear isoform has been isolated from Novikoff hepatoma cells (Bremer et al., 1981). Nuclear actin has also been isolated from *Xenopus* oocytes where 25% of the nuclear actin is found to be stably associated with an insoluble gel containing chromosomes and nucleoli (Clark and Merriam, 1977).

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