Isolation of a Minor Species of Actin from the Nuclei of *Acanthamoeba castellanii*†

A. Kumar,* Raziuddin, T. H. Finlay, J. O. Thomas, and W. Szer

ABSTRACT: Actin was extracted from isolated nuclei of *Acanthamoeba castellanii* and purified to homogeneity under nondenaturing conditions by diethylaminoethylcellulose and Sephadex G-100 chromatography. The pure protein has the same molecular weight as cytoplasmic *Acanthamoeba* actin and a very similar amino acid composition. Isoelectrofocusing shows that nuclear actin is slightly more acidic than the major cytoplasmic species, and comparative analysis of peptides from tryptic and cyanogen bromide digests shows that both actins are very similar but not chemically identical. In an assay that is specific for most actins, the inhibition of DNase I through the formation of a 1:1 G-actin–DNase I complex, the nuclear and cytoplasmic actins are equally effective. By use of a similar procedure for the purification of both actins, it is estimated that the amount of nuclear actin is about 1.5% of the amount of cytoplasmic actin, a major protein of the amoeba. It is concluded that a minor isoelectric species of actin associates selectively with the nuclei of *A. castellanii*.

Actin is a major cytoplasmic protein of nonmuscle cells that is involved in cell movement and in the maintenance of cell structure and shape [for a review, see Korn (1982)]. It is the most abundant protein in *Acanthamoeba castellanii*, comprising nearly 10% of total amoeba protein (Gordon et al., 1976; Jantzen, 1981). Although it was originally thought that *Acanthamoeba* contains a single species of cytoplasmic actin (Gordon et al., 1977), two minor species of actin have recently been detected by autoradiography of isoelectrofocusing gels of total amoeba proteins labeled in vivo or in vitro with [^{14}C]acetate (Jantzen, 1981). The isoelectric points of the minor species differ from that of the major actin species by about 0.05 pH unit, one being more acidic and one more basic. Neither of the minor species was detectable by staining with Coomassie blue. Analysis of the *Acanthamoeba* genome demonstrated the presence of at least three actin genes, one of which, actin gene I, has been sequenced (Nellen & Gallwitz, 1982). The protein sequence of the cytoplasmic actin has also been determined (Vandekerckhove et al., 1984) and is in complete agreement with the nucleotide sequence of actin gene I.

In view of our interest in nuclear proteins involved in the metabolism of hnRNA[1] (Thomas et al., 1981; Raziuddin et al., 1982; Thomas et al., 1983), we have isolated heterogeneous nuclear ribonucleoprotein particles (hnRNP) from the nuclei of *Acanthamoeba* by standard extraction procedures (Samarina et al., 1968; Beyer et al., 1977; Martin et al., 1978). We have noticed that the nuclear extract contains, in addition to the typical hnRNP proteins, a large amount of a protein with a molecular weight of about 42 000 that comigrates in denaturing gels with authentic cytoplasmic *Acanthamoeba* actin. It does not cross-react with antibodies to the immunologically interrelated core hnRNP proteins. In this communication we describe the purification of this protein to homogeneity, and we present evidence that it is a minor species of *Acanthamoeba* actin with a slightly more acidic p*I* than the major cytoplasmic actin.

Materials and Methods

Growth of Cells and Isolation of Nuclei. *A. castellanii* (Neff strain) was grown in suspension in 2.0-L flasks at 28 °C with shaking in a medium (1.0-L volume) containing 0.75% proteose peptone, 0.75% yeast extract, 1.5% glucose, 2 mM KH_2PO_4, 0.5 mM $MgCl_2$, 0.027 mM $CaCl_2$ and 0.064 mM ferric citrate supplemented with 1 mg of vitamin B_1, 0.2 mg of D-biotin, and 1 μg of vitamin B_{12} per 1.0 L. Cells were routinely harvested after 5 days at a density of about 1.2×10^6 cells/mL. The cells (8.0 L of culture) were washed twice in a buffer containing 20 mM Tris-HCl, pH 7.1, 2 mM $MgCl_2$, 0.5 mM DTT, and 0.25 M sucrose and homogenized in 2 volumes of the same buffer supplemented with 0.5 mM phenylmethanesulfonyl fluoride in a Potter-Elvehjem Teflon homogenizer (about four strokes). The resulting homogenate was diluted twice with the same buffer supplemented with 25 mM KCl (buffer A) and centrifuged at 1500*g* for 15 min. The top layer of the biphasic pellet containing the nuclei was suspended in buffer A, pelleted at 800*g* for 10 min, and washed 4 times in the same buffer. The nuclei were suspended in 30 mL of buffer A, and 10-mL portions were layered on a discontinuous sucrose gradient (10 mL of each 1.7, 1.3, and 0.5 M sucrose in buffer A) and centrifuged for 20 min at 4000 *g*. The material in the 1.3 M sucrose layer was collected, diluted 1:1 with buffer A, and pelleted. In some experiments the nuclei were washed with buffer A containing 0.5% (v/v) of the nonionic detergent NP-40 followed by a wash with buffer A containing 1% (v/v) of magic detergent mixture (6.6% Tween-80 and 3.3% sodium deoxycholate; Penman, 1966). Detergent washing had no effect on the yield and properties of nuclear actin. At each step of the preparation the nuclei were checked by phase-contrast microscopy and ethidium bromide staining. They appeared unbroken and free of cytoplasmic debris.

Polyacrylamide gel electrophoresis in the presence of 0.1% $NaDodSO_4$ was carried out on 10% (w/v) slab gels ($1.2 \times 140 \times 180$ mm) as described (Laemmli, 1970). Isoelectric focusing (Winter et al., 1977) was done on 4% (w/v) polyacrylamide column gels (3×10 mm) by using 2% ampholine (LKB), pH 3.5–10, in the presence of 8.0 M urea.

The DNase I inhibition assay by actin was carried out as described by Blikstad et al. (1978) using calf thymus DNA (Sigma) at 1.0 A_{260} unit/mL and DNase I (Worthington) at 1–2 μg/mL.

† From the Departments of Biochemistry (A.K., R., J.O.T., and W.S.) and Obstetrics and Gynecology (T.H.F.), New York University School of Medicine, New York, New York 10016. *Received April 23, 1984.* This work was supported by grants from the National Institutes of Health.

[1] Abbreviations: hnRNA, heterogeneous nuclear RNA; hnRNP, heterogeneous nuclear RNA–protein complexes; $NaDodSO_4$, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography.

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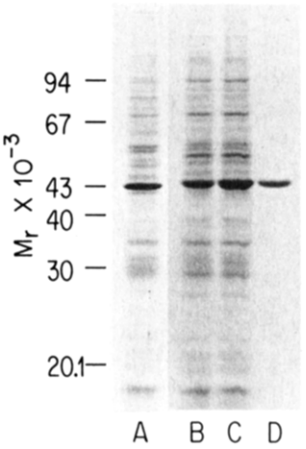


FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis of nuclear extracts. Lanes: (A) extract from nuclei isolated without detergent washing; (B) extract from detergent-washed nuclei; (C) same as in (B) but supplemented with 3 μg of cytoplasmic actin; (D) purified cytoplasmic actin (4 μg). The positions of molecular weight markers ($M_r \times 10^{-3}$) are indicated on the left.

Amino acid analysis was performed in a Durum D-500 automatic analyzer after hydrolysis of the protein sample (0.1–0.2 nmol) for 21 and 48 h (Lai et al., 1978).

Comparative HPLC Peptide Mapping. Protein samples of nuclear and cytoplasmic actin were reduced and carboxymethylated as described by Kibbelaar et al. (1979). The proteins (60 μg each) were digested with trypsin according to the procedure of Bremer et al. (1981) except that the total time of incubation was 12 h. The final product was suspended in a 0.1% solution of trifluoroacetic acid at 0.5 mg/mL and precipitated material removed by centrifugation in a Beckman microfuge. The supernatant was analyzed by HPLC using a VYDAC C-18 (Beckman) reversed-phase column (0.46 × 30 cm) equilibrated with a 0.1% solution of trifluoroacetic acid containing 5% (v/v) of 95% acetonitrile (see the legends to the figures). For cyanogen bromide digestion, the protein samples (25–45 μg each) in 70% formic acid were treated with cyanogen bromide (4.0 mg/mL, in 90% formic acid) at room temperature in the dark for 18 h at a reagent to protein ratio of 3:1 (w/w). The material was diluted with water, and the cyanogen bromide and formic acid were removed by repeated (3 times) lyophilization. The peptides were analyzed as described above.

Cytoplasmic actin was purified to homogeneity from the postnuclear supernatant (see above) as described by Gordon et al. (1976). Another sample of pure *A. castellanii* cytoplasmic actin was a generous gift from Dr. E. D. Korn.

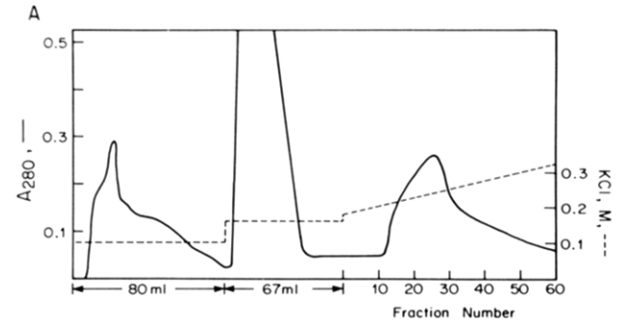
Results

Purification of Nuclear Actin. Pelleted nuclei from 1×10^{10} amoeba (8.0 L of culture) were extracted by gentle stirring at 4 °C for 1 h with 5 volumes of a buffer containing 20 mM Tris-HCl, pH 8.1, 20 mM NaCl, 1 mM $MgCl_2$, 1 mM DTT, and 0.5 mM PMSF (buffer B). The nuclei were pelleted, the extraction procedure was repeated twice, and the combined extracts were clarified by centrifugation for 10 min at 10000 *g*. This is essentially the standard procedure that has been employed for the extraction of monomeric 30–50S hnRNP particles from the nuclei of a variety of eukaryotic cells (Samarina et al., 1968). As seen from the $NaDodSO_4$–polyacrylamide gel of the extracts (Figure 1), a protein band of molecular weight about 42000 that comigrates with cytoplasmic amoeba actin is the major component of the extract. The pattern of the gel is virtually the same with detergent-washed nuclei (Figure 1). Several protein bands in the M_r 30000–40000 range are the typical hnRNP core proteins found in all eukaryotic cells examined (LeStourgeon et al., 1981). The pH 8.1 extract was layered on 3 mL of 30% sucrose cushions in buffer B and centrifuged for 15 h at 30000 rpm in the Ti 60 rotor. Four-milliliter portions from the bottom of the tubes were collected, adjusted to 2 M KCl with solid KCl (to dissociate hnRNP particles), stirred for 2 h at 4 °C, and clarified by centrifugation at 12000*g* for 10 min. Subsequent steps were carried out as described by Gordon et al. (1976) for the purification of cytoplasmic *Acanthamoeba* actin. The supernatant was dialyzed against buffer G (3 mM imidazole, 0.1 mM $CaCl_2$, 0.5 mM ATP, 0.75 mM β-mercaptoethanol, pH 7.5) for 5 h (two changes). In buffer G, actin assumes the monomeric or G (globular) form. The dialysate was chromatographed on a DEAE-cellulose column (1 × 30 cm) equilibrated with buffer D (same as buffer G, except the concentration of imidazole was 10 mM) containing 0.1 M KCl. Before and after sample application the column was washed with 50 mL of buffer G to prevent exposing actin to 0.1 M KCl during adsorbtion to the resin. The column was washed stepwise with buffer D containing 0.1 and 0.16 M KCl and then eluted with 120 mL of a linear gradient from 0.18 to 0.32 M KCl in buffer D. Eluted fractions were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 2). A nearly homogeneous protein peak that comigrates with authentic cytoplasmic *A. castellanii* actin eluted at 0.20–0.25 M KCl. Fractions no. 13–36 were pooled, precipitated with ammonium sulfate, and dialyzed against buffer G. For further purification, the sample was passed through a Sephadex G-100 column equilibrated with the same buffer [cf. Gordon et al. (1976)] and dialyzed against buffer G containing 0.01% sodium azide. The yield of several preparations varied from 0.55 to 0.8 mg of protein/8.0 L of culture. The yield of cytoplasmic actin purified from the same amount of cells grown to the same cell density is 37–50 mg (Gordon et al., 1976).

Analysis of Nuclear Actin. The molecular weight of nuclear actin as determined by $NaDodSO_4$–polyacrylamide gel electrophoresis is about 42000 (Figure 2). The amino acid composition of the protein (Table I) is very similar to that of cytoplasmic actin [calculated from Nellen & Gallwitz (1982) and Vandekerckhove et al. (1984)]. About one residue of 3-methylhistidine, a typical component of many other actins, was detected as well as one residue of a methylated lysine, which is unique for *Acanthamoeba* actin (Gordon et al., 1976).

A characteristic property of monomeric actin is the formation of a tight 1:1 complex with DNase I that inhibits completely the activity of the enzyme (Lazarides & Lindberg, 1974). While the physiological role of this inhibition is not established, it provides a simple and selective assay for actin (Blikstad et al., 1978). As shown in Table II, both cytoplasmic actin and nuclear actin are equally effective in inhibiting DNase I at approximately 1:1 molar ratios.

Taken together, all these experiments demonstrate that the M_r 42000 protein purified from the nuclei is authentic *Acanthamoeba* actin. Isoelectrofocusing of nuclear and cytoplasmic actins and a mixture of the two (Figure 3) shows that the major species of nuclear actin is slightly more acidic than cytoplasmic actin. The same pattern was seen in five preparations of nuclear actin whether nuclei were washed with detergents or not (see Materials and Methods). All preparations contained small amounts (about 5–10%) of two additional components, one slightly more acidic and one identical in p*I* to cytoplasmic actin (Figure 3c).



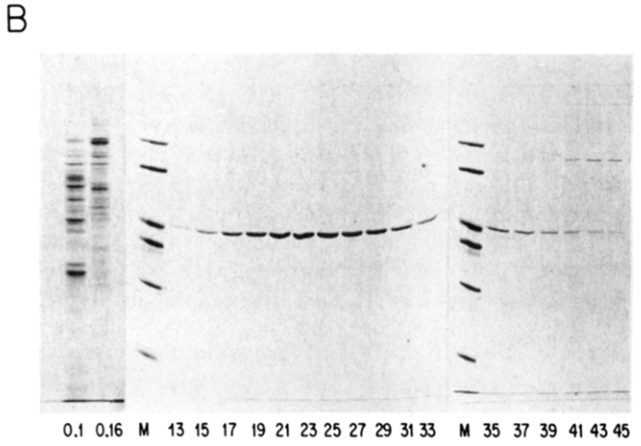


FIGURE 2: DEAE-cellulose chromatography of the nuclear extract. (A) Elution pattern monitored at 280 nm. The concentrations of KCl (in buffer D) in the column washes and in the elution gradient are indicated; in the region of the gradient, 2.0-mL fractions were collected. (B) $NaDodSO_4$–polyacrylamide gel electrophoresis of column eluates. The first two lanes are aliquots of pooled 0.1 and 0.16 M KCl washes, respectively; the next 17 lanes are aliquots from gradient eluates; the numbers correspond to the fraction numbers in panel A. The actin peak elutes in buffer D at 0.20–0.25 M KCl. Lanes M are molecular weight markers ($M_r \times 10^{-3}$): 94, 67, 43, 40, 30, and 20.1.

Table I: Amino Acid Composition of Cytoplasmic and Nuclear Actins (Two Samples of Nuclear Actin from Different Preparations Were Analyzed)

	no. of residues		
		nuclear actin	
amino acid	cytoplasmic actin[a]	I	II
Lys	19	22	18
Lys(Me)$_n$ (total)[b]	1	ND[c]	0.8
His	9	9	11
3-Me-His[b]	1	ND	1.5
Arg	18	16	17
Trp	4	3	ND
Asx	30	33	31
Thr	26	24	24
Ser	24	22	23
Glx	39	39	41
Pro	19	16	18
Gly	30	31	34
Ala	29	29	28
Cys	4	ND	ND
Val	23	26	25
Met	15	10	13
Ile	27	23	24
Leu	29	28	26
Tyr	15	16	16
Phe	13	14	15

[a] Calculated from known sequence (Nellen & Gallwitz, 1982; Vandekerckhove et al., 1984). [b] Determined with standards of N^{ϵ}-methyllysine, $N^{\epsilon},N^{\epsilon}$-dimethyllysine, and 3-methylhistidine. [c] ND, not determined.

Analyses of tryptic peptides from digests of nuclear and cytoplasmic actins by HPLC are shown in Figure 4. The

Table II: Inhibition of DNase I by Actins[a]

additions	% inhibition
cytoplasmic actin, 0.7 μg/mL	56
cytoplasmic actin, 1.4 μg/mL	98
nuclear extract, 2.5 μg/mL	48
nuclear actin, 1.0 μg/mL	59
nuclear actin, 1.5 μg/mL	96

[a] The amount of enzyme used (1.0 μg/mL) gave a plateau in hyperchromicity (37–38% increase in A_{260}) within 2.5–3 min at 18 °C. The percent inhibition was calculated from the hyperchromicity in the presence of actin after 5 min at 18 °C. The molecular weight of pancreatic DNase I is 31 000.

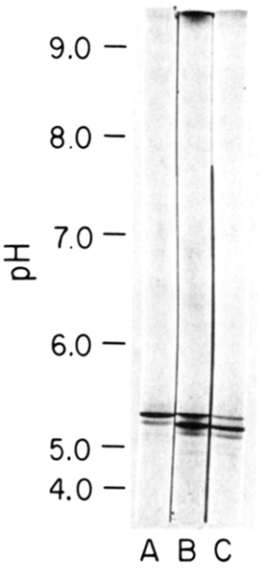


FIGURE 3: Isoelectrofocusing gels of (A) cytoplasmic actin, (B) a mixture of cytoplasmic and nuclear actin, and (C) nuclear actin. 10 μg of each species was applied to each column.

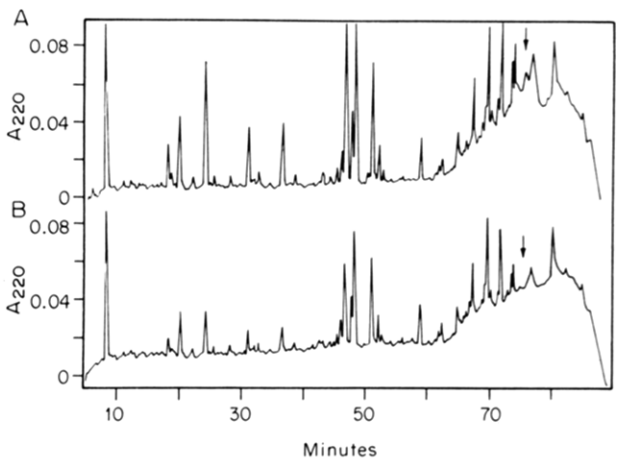


FIGURE 4: HPLC analysis of tryptic peptides from 0.9 nmol of (A) cytoplasmic actin and (B) nuclear actin. The column was eluted at a flow rate of 0.75 mL/min with linear gradients of acetonitrile: 5–30% for 60 min and 30–60% for 20 min. Absorbance was monitored at 220 nm; full scale is 0.1 absorbance unit. The patterns shown are representative of patterns obtained from three different digestions. The arrow in (A) indicates a unique peptide in the digest of cytoplasmic actin at min 74.

number of tryptic peptides calculated from the sequence of cytoplasmic actin (Nellen & Gallwitz, 1982; Vandekerckhove et al., 1984) is 33, including five di- and three tripeptides. HPLC traces of the digests of both actins show that all the larger peptides are clearly distinguishable from the back-

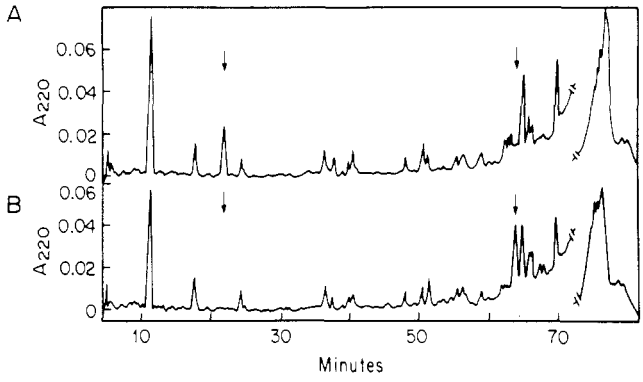


FIGURE 5: HPLC analysis of cyanogen bromide peptides from 0.7 nmol of (A) cytoplasmic actin and (B) nuclear actin. The column was eluted and monitored as described in the legend to Figure 4, except that past min 74 full scale is 0.2 absorbance unit. The patterns shown are representative of patterns obtained from two different digestions. Arrows indicate the positions of unique peptides.

ground. Except for one peptide at min 74 (see the arrow in Figure 4) that is present in the cytoplasmic but not in the nuclear digest, both tracings are nearly identical.

The HPLC patterns of cyanogen bromide digests of cytoplasmic and nuclear actins are shown in Figure 5. From its known sequence, there should be 16 cyanogen bromide peptides in the digest of cytoplasmic actin including one tripeptide that may not be distinguishable from the background resulting from unspecific degradation. As seen from Figure 5, the one essential difference between the two patterns is a shift in the position of one peptide from min 22 in the cytoplasmic tracing to min 64 in the nuclear one (see the arrows).

Discussion

A minor species of actin, somewhat more acidic than the major cellular cytoplasmic actin, was purified to near homogeneity from the nuclei of *A. castellanii*. The purification procedure, except for the preparation of the extract from purified nuclei, is identical with that employed for the purification of cytoplasmic actin (Gordon et al., 1976). Therefore, the average yields of nuclear actin are likely to be within the same range as those of the cytoplasmic species. The average amount of pure actins isolated from 8.0 L of culture grown to a density of 1.2×10^6 cells/mL (0.67 mg of the minor species and 43 mg of the major species; see Results) indicates that the minor species comprises about 1.5% of the total and could have remained undetected if nuclei were not first separated from the cytoplasm in the isolation procedure.

There is only a small difference in isoelectric points between the two forms of actin, the nuclear one being more acidic by 0.05–0.1 pH unit (Figure 3). From the known content of basic and acidic amino acids of cytoplasmic actin, we have calculated that the substitution of one positively charged residue for a neutral one will suffice to bring about a change in p*I* within this range. In line with this, analysis of tryptic peptides of both species (Figure 4) shows one additional peptide in the digest of cytoplasmic actin. Analysis of the cyanogen bromide digests shows the same number of peptides from both species, the only difference between the two being a shift in the position of one peptide (Figure 5). The observed differences could be due to posttranslational modifications, or the nuclear species could be the product of an actin gene different from gene I that encodes the major species (Nellen & Gallwitz, 1982).

Although actin has been found in nuclear extracts of several organisms (LeStourgeon, 1978), its in vivo nuclear localization has been uncertain. The presence of actin in isolated nuclei could be a result of either equilibration of cytoplasmic actin between the cytoplasm and the nucleoplasm or its adherence to the nuclear envelope [for a detailed discussion, see LeStourgeon (1978)]. More recently, a distinct species of nuclear actin slightly more acidic than cytoplasmic actin has been isolated from the chromatin of Novikoff hepatoma ascites cells (Bremer et al., 1981). Another example of subcellular sorting of isoactins is the selective association of γ actin with skeletal muscle mitochondria (Pardo et al., 1983). This investigation shows that the presence of actin in the nuclear extract of *A. castellanii* is not a result of equilibration of cytoplasmic actin since a chemically distinct species of actin selectively associates with the nuclei.

It is known that the isolation of nuclei by aqueous procedures results in a large loss of nuclear proteins. It has been estimated that about 50% of nuclear proteins are lost into the medium within about 4 min (Paine et al., 1983). Proteins that remain in the nucleus after aqueous isolation and washing are mainly components of particulate structures, e.g., hnRNP. Consequently, it is likely that the nuclear actin we isolate by the low-salt extraction procedure along with hnRNP particles exists in vivo primarily in the filamentous form.

The function of nuclear actin is not known. Sedimentation of the nuclear extract from *A. castellanii* on isopycnic sucrose gradients (unpublished experiments) demonstrates that hnRNP particles band at a higher density than most of the nuclear actin, which, along with other proteins in the extract, bands at a density expected for free proteins. Extraction of nuclear actin along with hnRNP particles in this investigation could be fortuitous.

Registry No. DNase I, 9003-98-9.

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N-Terminal and C-Terminal Segments of Actin Participate in Binding Depactin, an Actin-Depolymerizing Protein from Starfish Oocytes†

Kazuo Sutoh* and Issei Mabuchi

ABSTRACT: A one to one complex of actin monomer and depactin (an actin-depolymerizing protein isolated from starfish oocytes) was cross-linked with the zero-length cross-linker 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) to generate a covalently cross-linked complex of actin and depactin. Cross-linking sites of depactin in the actin sequence were determined by mapping products after partial cleavages of the actin–depactin cross-linked complex. The peptide mappings have revealed that acidic residues in the N-terminal segment of actin (Asp-1, Glu-2, Asp-3, Glu-4, and Asp-11) are candidates for one of the cross-linking sites of depactin while lysine and/or acidic residues in its C-terminal segment (Lys-358, Glu-360, Asp-362, Glu-363, and Lys-372) are candidates for another cross-linking site of depactin. Since EDC can cross-link amino and carboxyl groups only when they are in direct contact, it is very likely that some of those residues in the N-terminal and C-terminal segments of actin participate in binding depactin.

Actin plays pivotal roles in muscle and nonmuscle cells. In muscles, interactions between actin and other proteins such as myosin and tropomyosin are the basis of their contraction and relaxation. In nonmuscle cells, actin is a major constituent of both their contractile systems and their cytoskeletal structures. In these nonmuscle cells, many types of "actin-binding proteins" regulate the supramolecular structure of actin [as a review, see Craig & Pollard (1982)]. The primary structure of actin is highly conserved (Elzinga et al., 1973; Elzinga & Lu, 1976; Vandekerckhove & Weber, 1978a–d), and therefore, actin from various sources can bind a large number of proteins isolated from various muscle and nonmuscle cells. It seems that the surface of the actin molecule is covered with a large number of binding sites specific for these proteins. In a sense, actin is a protein which has highly evolved so that it can bind a large number of proteins.

In echinoderm eggs, actin is a constituent of contractile and cytoskeletal structures such as microvilli or the contractile ring. Several actin-binding proteins which might function in organization and disorganization of these structures have been isolated (Kane, 1976; Ishimoda-Takagi, 1978; Mabuchi & Hosoya, 1982; Mabuchi, 1981, 1983). One approach to an understanding of the molecular mechanism by which these actin-binding proteins exert their effects on the supramolecular structure of actin would be to locate their binding sites in the primary as well as three-dimensional structures of actin.

A simple method has been developed to identify actin segments which participate in binding various proteins, and this method has been applied to locate binding sites of myosin (Sutoh, 1982a) and DNase I (Sutoh, 1984). The same method has also been employed to locate the actin–actin contact site responsible for self-association of subunits into F-actin (Sutoh, 1984). Since X-ray diffraction studies on actin–DNase I crystals are now in progress (Suck et al., 1981; Sakabe et al., 1983), it will soon become possible to locate binding sites for various proteins in the three-dimensional structure of actin once their locations in the primary sequence of actin are identified by the above method.

In this paper, we identified actin segments participating in binding depactin, an actin-depolymerizing protein isolated from starfish oocytes (Mabuchi, 1981, 1983).

Materials and Methods

Proteins and Reagents. Depactin was prepared as previously described (Mabuchi, 1983). Actin was prepared according to the method of Spudich & Watt (1971). It was further purified by chromatography on Sephadex G-150. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC)[1] was purchased from Nakarai Chemical Co. (Tokyo, Japan). BNPS-skatole was from Pierce Chemical Co. (Rockford, IL). *N*-[7-(Dimethylamino)-4-methyl-5-courmarinyl]maleimide (DACM) was from Wako Chemical Co. (Tokyo, Japan).

Cross-Linking of the Depactin–Actin Complex. G-Actin (1 mg/mL) in 2 mM Tris-HCl, 0.1 mM ATP, and 0.1 mM $CaCl_2$ (pH 8.0) was labeled with DACM at a molar ratio of 1.0:0.8 (actin monomer:DACM) for 5 min at 0 °C. Under the conditions, Cys-373 of actin was selectively labeled with the fluorescent dye (Sutoh, 1982a). The DACM-labeled actin in the above solvent was mixed with depactin in 0.1 M KCl,

† From the Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan (K.S.), the Department of Biology, College of Arts and Sciences, University of Tokyo, Komaba, Tokyo, Japan (I.M.), and the Department of Cell Biology, National Institute for Basic Biology, Okazaki, Aichi, Japan (I.M.). *Received April 19, 1984.* This work was supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan.

[1] Abbreviations: EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; BNPS-skatole, 2-[(2-nitrophenyl)sulfenyl]-3-methyl-3-bromoindolenine; DACM, *N*-[7-(dimethylamino)-4-methyl-5-coumarinyl]-maleimide; DTT, dithiothreitol; $NaDodSO_4$, sodium dodecyl sulfate; CNBr, cyanogen bromide; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

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