G-ACTIN–TUBULIN INTERACTION

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1. Introduction

Actin is one of the major cytoskeletal proteins of eukaryotic cells. Structural organization and functions of cellular actin depend on its interaction with other proteins. Numerous actin-binding proteins have been found in non-muscle cells: e.g., F-actin-gelating factors, DNase I, profilin, spectrin, villin, gelsolin, serum and brain actin-depolymerizing proteins (review [1]; [2–5]).

To detect new G-actin-binding proteins in mammalian brain we used affinity chromatography of brain extract on actin–Sepharose. We have found that the main protein of brain extract that binds to immobilized actin is tubulin. We have also shown that tubulin is able to interact with G-actin in solution.

2. Materials and methods

Actin was purified from rabbit skeletal muscle according to [6], except that ATP concentration in the buffers was 0.5 mM instead of 0.2 mM.

Bovine brain tubulin was purified by a polymerization–depolymerization procedure [7] (modified as in [8]) and by chromatography on phosphocellulose (Whatman P11) [9]. Tubulin thus prepared was used as a standard for electrophoresis and peptide mapping. For sedimentation experiments it was necessary to obtain tubulin in actin-depolymerizing buffer (buffer A) containing 2 mM Tris–HCl (pH 7.5), 0.5 mM ATP, 0.2 mM $CaCl_2$ and 0.5 mM 2-mercaptoethanol. In this case a procedure for tubulin purification was modified as follows: microtubules at the final depolymerization step were suspended in the buffer A, microtubule protein was dialysed (2 × 1.5 h) against buffer A, and phosphocellulose chromatography was carried out in the same buffer.

Actin was immobilized on BrCN-activated Sepharose 4B (Pharmacia) as in [10]. BSA was immobilized by the same procedure, but 0.1 M Na-phosphate buffer (pH 8.5) containing 0.5 M NaCl was used for protein solubilization instead of triethanolamine–HCl buffer. 'Empty' Sepharose was obtained by the same treatment of BrCN-activated Sepharose 4B as for BSA immobilization, but in the absence of any protein.

Bovine brain extract for affinity chromatography was prepared as follows. Bovine brains <1 h after slaughter, were homogenized in 2 vol. cold solution containing 10 mM imidazole–HCl (pH 7.5), 0.5 mM ATP, 0.1 mM $CaCl_2$ and 0.75 mM 2-mercaptoethanol (the buffer for non-muscle actin extraction [11]). Homogenate was clarified by centrifugation for 40 min at 100 000 × *g* and 2°C, dialysed for 3 h and clarified once again. The resulting brain extract in buffer A was applied to 1 ml affinity columns with actin–Sepharose, BSA–Sepharose or 'empty' Sepharose. Extract (20–30 ml) was passed through each column over 2 h at 20°C. Then the columns were washed overnight with buffer A, eluted with 5 ml 0.6 M KCl in buffer A, washed with ~10 ml 0.6 M KCl in buffer A and with ~10 ml buffer A and finally eluted with 5 ml 40% formamide in buffer A.

SDS-electrophoresis was performed by the method in [12] in 15% polyacrylamide slab gel at the acrylamide/methylene-bisacrylamide ratio of 100:1.

One-dimensional peptide maps were obtained as in [13]. A tested protein (0.3 mg/ml) was treated with γ-chymotrypsin (Sigma, type II) (0.05 mg/ml) in solution containing 10% glycerol and 0.1% SDS at 37°C for 3, 5 or 15 min. The resulting peptides were ana-

Abbreviations: BSA, bovine serum albumin; SDS, sodium dodecyl sulphate

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lysed by SDS-electrophoresis in a 20% polyacrylamide slab gel.

Analytical ultracentrifugation was carried out using a Spinco E Beckman ultracentrifuge.

Protein concentration was determined as in [14] using BSA as a standard.

3. Results

To reveal brain proteins that interact with G-actin we applied brain extract in buffer A to a G-actin–Sepharose column. The column was then washed with buffer A and step-eluted with 0.6 M KCl in buffer A and with 40% formamide in buffer A. Formamide was used as in [15] for elution of proteins that, like DNase I, formed a tight complex with G-actin. The eluted proteins were analysed by SDS gel electrophoresis (fig.1). KCl (0.6 M) released from the column several proteins with M_r-values of 30 000–60 000. Formamide eluted a protein with the electrophoretic mobility of tubulin and a small amount of polypeptide with the electrophoretic mobility of actin. To elucidate whether the major formamide-eluted protein is indeed tubulin we compared one-dimensional chymotryptic peptide maps of the formamide eluate and of purified brain tubulin. These maps were identical, whereas BSA digestion in a control experiment resulted in a completely different peptide pattern (fig.2).

To test specificity of tubulin binding to the actin column, we applied brain extract to a BSA–Sepharose column and to a column with 'empty' Sepharose (BrCN-activated Sepharose processed as for protein immobilization but in the absence of proteins). The protein with the same electrophoretic mobility as tubulin did bind to BSA–Sepharose and even to 'empty' Sepharose, but was completely eluted from both the columns with KCl (fig.1). Thus, KCl-sensitive binding of tubulin to Sepharose columns appears to be non-selective. Probably some tubulin aggregated in the low ionic strength buffer and was retained mechanically by the resin itself. Tubulin binding to actin–Sepharose differed, however, in that a significant amount of tubulin remained bound to the column

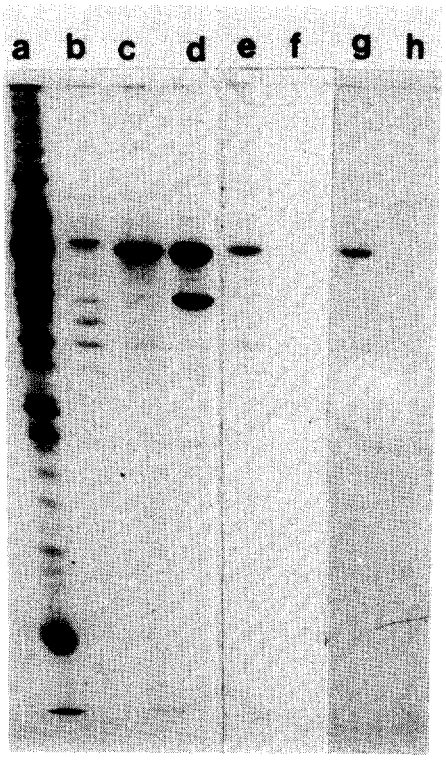

Fig.1. SDS electrophoresis of fractions obtained after affinity chromatography of brain extract: (a) brain extract; (b) KCl eluate and (c) formamide eluate from actin–Sepharose; (d) actin and tubulin standard; (e) KCl eluate and (f) formamide eluate from BSA–Sepharose; (g) KCl eluate and (h) formamide eluate from 'empty' Sepharose.

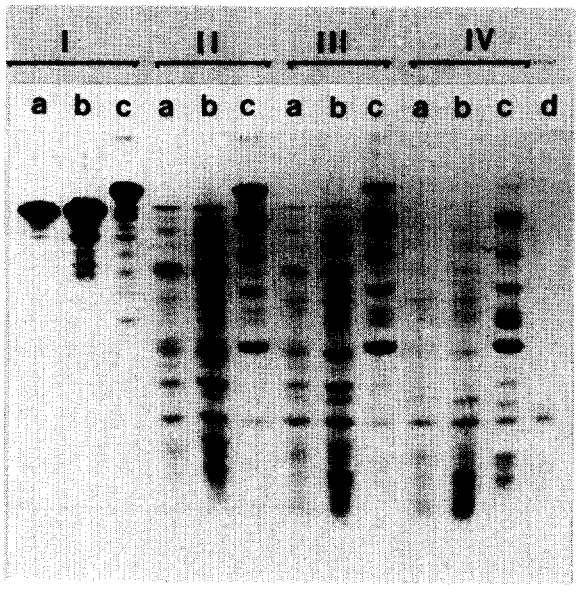

Fig.2. One-dimensional peptide maps of formamide eluate from actin–Sepharose, tubulin and BSA: (a) tubulin; (b) formamide eluate and (c) BSA digested for (I) 0, (II) 3, (III) 5 and (IV) 15 min; (d) chymotrypsin.

even after KCl-elution and could be eluted with formamide, whereas no protein was found in the formamide eluates from BSA–Sepharose and 'empty' Sepharose. Therefore KCl-resistant binding of tubulin to the affinity columns was actin-specific.

It is noteworthy that proteins of M_r greater than that of tubulin were absent from KCl and formamide eluates from the actin–Sepharose column. So actin–tubulin interaction seems to be independent of the high-M_r microtubule-associated proteins.

The results obtained by affinity chromatography of brain extract were confirmed in experiments with isolated brain tubulin. Like tubulin in a brain extract, the purified protein, apparently free of microtubule-associated proteins, was able to bind to actin–Sepharose and could be eluted from it with formamide (not shown).

Further we tested, by analytical and preparative ultracentrifugation, whether G-actin–tubulin interaction is possible in solution. All experiments were carried out in buffer A to maintain actin in a monomeric state. Protein solutions were clarified just before experiments by centrifugation for 40 min at 100 000 × g and 20°C.

In the first set of experiments G-actin was mixed with tubulin at 20°C and the mixture (as well as control samples of actin or tubulin) were analyzed by analytical ultracentrifugation. A significant amount of a fast-sedimenting material was present in the actin–tubulin mixture at the beginning of centrifugation (fig.3A). The control samples of actin and tubulin did not contain such material. After long-term centrifugation, sufficient to separate actin and tubulin (fig.3B), it became clear that the monomeric actin

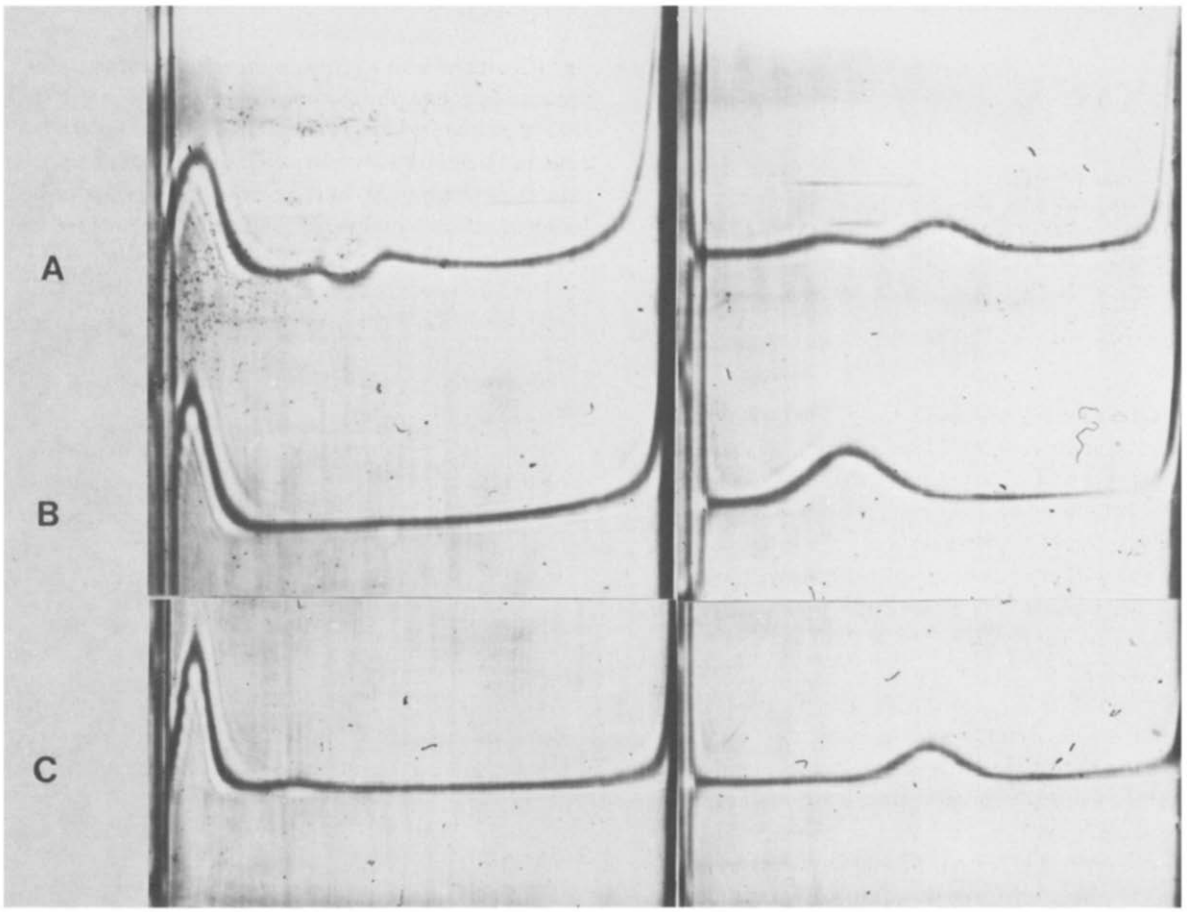

Fig.3. Analytical ultracentrifugation of actin, tubulin and their mixture: (A) mixture of actin and tubulin (final conc. 4.0 and 2.1 mg/ml, respectively); (B) actin (4.0 mg/ml) and (C) tubulin (2.1 mg/ml). Centrifugation times: 5 min (left) and 75 min (right).

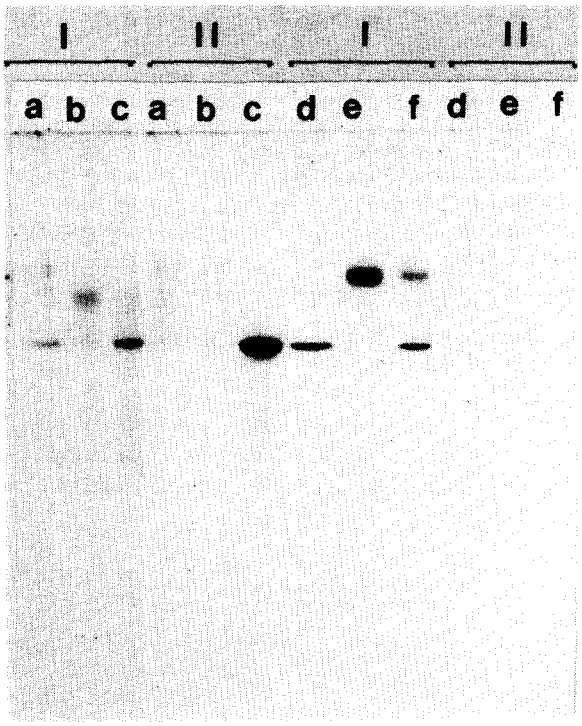

Fig.4. SDS electrophoresis of pellets after centrifugation of actin–tubulin and actin–BSA mixtures and of the individual components: (a) actin (1.1 mg/ml); (b) tubulin (1.2 mg/ml); (c) actin and tubulin (final conc. 1.1 and 1.2 mg/ml); (d) actin (2.2 mg/ml); (e) BSA (3.0 mg/ml); (f) actin and BSA (final conc. 2.2 and 3.0 mg/ml). Pellets after low speed (I) and high speed (II) runs.

peak in the mixture was much smaller than that in the control actin sample. Thus a considerable amount of actin behaved as a fast-sedimenting material in the G-actin–tubulin mixture.

In the second set of experiments the actin–tubulin mixture as well as control samples of individual proteins were incubated for 5 h at 20°C and then centrifuged for 2 min at 45 000 × *g*. Supernatants were layered on 10% sucrose in buffer A and centrifuged for 60 min at 320 000 × *g* and 20°C in SW 60 Ti rotor (Beckman). For comparison actin was mixed, incubated, and centrifuged with BSA instead of tubulin. The pellets formed during low and high speed runs were suspended in equal volumes and analysed by SDS electrophoresis (fig.4). Although protein solutions were cleared before the experiments small quantities of all the proteins sedimented during low speed runs. This suggests that large aggregates of the proteins were formed during long-term incubation at 20°C. The formation of the aggregates sedimentable during low speed runs was slightly enhanced both by tubulin and by BSA.

After the high speed centrifugation, actin was not found in the pellets in the control actin sample and the actin–BSA mixture. A large actin pellet was formed during the high speed centrifugation of the actin–tubulin mixture, the quantity of actin in this pellet being considerably larger than in the pellets after the low speed runs.

So the data of analytical and preparative centrifugation are in good agreement and suggest the formation of fast-sedimenting actin species in the presence of tubulin. It is necessary to underline that this phenomenon occurs under conditions (buffer A) where actin itself is monomeric and does not contain any fast-sedimenting material.

4. Discussion

Microtubules and actin-containing microfilaments are often present in the same cell. One might expect that these two types of cytoskeletal structures would function in cooperation and that some of their proteins would interact. However, evidence for such an interaction are very few. It has been reported [16] that microtubule destruction in human polymorphonuclear leukocytes by colcemide leads to microfilament redistribution. In [17] microtubule-associated proteins interacted with F-actin to increase the viscosity of its solution. In [18], F-actin stimulated microtubule-associated ATPase.

This paper presents for the first time evidence for direct interaction between G-actin and tubulin. We have shown that brain tubulin binds to muscle G-actin immobilized on Sepharose and interacts with G-actin in solution to form a fast-sedimenting actin species.

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