ISOLATION AND CHARACTERIZATION OF TWO FORMS OF A CYTOSKELETON

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ABSTRACT

Isolated petaloid coelomocytes from the sea urchin Strongylocentrotus droebachiensis transform to a filopodial morphology in hypotonic media. Electron micrographs of negatively stained Triton-insoluble cytoskeletons show that the petaloid form consists of a loose net of microfilaments while the filopodial form consists of paracrystalline bundles of microfilaments. Actin is the major protein of both forms of the cytoskeleton. Additional polypeptides have molecular weights of ~220,000, 64,000, 57,000, and 27,000 daltons. Relative to actin the filopodial cytoskeletons have an average of 2.5 times as much 57k polypeptide as the petaloid cytoskeletons. Treatment with 0.25 M NaCl dissociates the filament bundles into individual actin filaments free of the actin-associated polypeptides. Thus, one or more of these actin-associated polypeptides may be responsible for crosslinking the actin filaments into bundles and maintaining the three-dimensional nature of the cytoskeletons.

KEY WORDS cytoskeleton - actin - actin-associated proteins - electron microscopy - gel electrophoresis

Actin-containing microfilament bundles are dynamic structures that can change their form and distribution within a cell, depending on the activity or function of the particular cell type. They exist and function both as axially oriented structural supports for cytoplasmic extensions and as part of the actomyosin-based contractile apparatus found in many motile cells (4, 11, 17, 22). Ultrastructural analysis of these fibrillar structures has shown that the filaments will reversibly bind heavy meromyosin, indicating that they contain actin (1, 13), a fact confirmed and extended by light microscope studies utilizing fluorescent antibodies (12, 15). Actin-associated proteins have been hypothesized to be an integral part of these microfilament complexes; recently, detergent-resistant structures have been prepared for biochemical analysis, and some actin-associated components have been demonstrated (2, 18, 20, 23). The nonmuscle cell actin-associated proteins form a diverse group and include, among others, high molecular weight proteins (21), intermediate-sized polypeptides (14, 16), and relatively small, low molecular weight proteins (6). The role of each of these is currently being investigated.

The present report describes the isolation, from two morphologically distinct forms of the sea urchin coelomocyte, of a detergent-insoluble microfilamentous cytoskeleton. Although these two forms of the coelomocyte cytoskeleton have different structures, they differ in polypeptide composition only in the amount of a 57,000 mol wt polypeptide.

MATERIALS AND METHODS

Collection and Transformation of Coelomocytes

The petaloid coelomocytes from the sea urchin, Strongylocentrotus droebachiensis, are isolated and transformed to the filopodial form as described previously (1, 11). The petaloid form is defined as the state in which the coelomocytes are isolated from the sea urchin, and the filopodial form is defined as the state in which the coelomocytes have been transformed to a filopodial morphology in hypotonic media. The coelomocytes are isolated from the sea urchin and transformed to the filopodial form as described previously (1, 11).
**Egg and Coelomocyte Extracts**

The procedure of Kane (14) was followed for the preparation of egg and coelomocyte extracts. Eggs were collected from *S. droebachiensis* and *S. purpuratus* by KCl injection and washed in an isotonic medium containing 2 mM EGTA before homogenization. The coelomocytes were collected as described above and also washed in the EGTA-containing buffer. Extracts were made using 2 ml of packed, dejellied eggs or coelomocytes. The egg extracts were pelleted by centrifugation at 25,000 g for 15 min, and the coelomocyte extracts were spun at 100,000 g for 3 h. The contents of the coelomocyte extracts were precipitated by addition of cold trichloroacetic acid (see Fig. 7c).
Within each bundle the 65-Å filaments may be packed in one of three different ways: (a) many form a paracrystalline array with filaments spaced 90 Å apart center to center; (b) many are in loose bundles; and (c) rarely there are irregular bundles with a 13-nm longitudinal spacing (8).

Biochemistry of Cytoskeletons

Cytoskeletons washed in a buffer consisting of 0.12 M NaCl, 2 mM MgCl₂, and 5 mM TES, pH 7.5 are composed primarily of actin with four actin-associated polypeptides with molecular weights of ~220,000, 64,000, 57,000, and 27,000 daltons. Repeated washing of the cytoskeletons does not alter their composition but rather results in a diminution of all components. By quantitative densitometry of Fast green-stained sodium dodecyl sulfate (SDS) gels, actin is found to comprise 80–85% of the total cytoskeletal protein. The two types of cytoskeletons have similar compositions except that the filopodial forms are enriched in the 57,000-dalton component (Fig. 3). In the petaloid form, the weight ratio of 57,000 to actin ranged in six preparations from 1:30 to 1:20 and averaged 1:26; in the filopodial form, that ratio varied much less (between 1:9 and 1:11) and averaged 1:10. The other actin-associated proteins did not vary appreciably between the two forms. The weight ratios of those components to actin were: 220k:actin = 1:90, 64k:actin = 1:20, and 27k:actin = 1:5.
FIGURE 2  (a) Sheared filopodia. Note the absence of contaminating nuclei. Bar, 5 µm. × 2,800.  (b) Detergent-lysed filopodia demonstrating the axial core of bundled microfilaments. Bar, 1 µm. × 6,500.  (c) Some bundles exhibit highly ordered, crosslinked arrays of filaments that are spaced 90 Å apart center to center. Other bundles are not so well ordered, cf. Fig. 5a. Bar, 0.1 µm. × 165,000.

FIGURE 3  Preparations of petaloid (P) and filopodial (F) cytoskeletons were electrophoresed, stained with Fast green, and quantitated by scanning densitometrically at 650 nm. This tracing of a pair of scans represents an average preparation although the average values expressed in the text are those calculated from six preparations. Both cytoskeletons have similar components except that the filopodial preparations are enriched in the 57,000 component.

The filopodial cytoskeletons were dissociated into their components by treatment with 0.25 M NaCl for 15 min. at 4°C. By increasing the salt concentration to 0.25 M, the amount of cytoskeletal material that is pelleted during a low-speed spin is reduced (Fig. 4). This is accompanied by the appearance of individual filaments in the supernate (Fig. 5). These filaments comprise the majority (64%) of the actin in the 0.25 M NaCl sample while the actin-associated components, most notably the 57k and 64k proteins, are

FIGURE 4  Tris-glycine-SDS gels (5%) of the low-speed (30,000 g for 10 min) pellets obtained from salt-extracted filopodial cytoskeletons. Note that as the salt concentration approaches 0.25 M NaCl, there is less and less material pelleted.
stripped from the filaments and found in the supernate. In the control sample, more than half (60%) of the actin and most (83%) of the actin-associated proteins are pelleted in the first low-speed spin (see Fig. 6). Thus, the bundles are disrupted into actin filaments and monomer by brief treatment with moderate salt concentrations, and the actin-associated components are solubilized into the supernate.

Comparative Studies

Following the procedures outlined by Kane (14), extracts were made from both sea urchin eggs and petaloid coelomocytes. Extracts of dejellied eggs from either *S. droebachiensis* or *S. purpuratus* would not form a solid gel upon addition of KCl and ATP and/or warming to 40°C for 1 h. Birefringent fibrils were present but sparse in these extracts and could be pelleted at 25,000 g for 15 min and analyzed by SDS-polyacrylamide gel electrophoresis. They contain numerous proteins (Fig. 7b); while coelectrophoresis of cytoskeletons vs. extract-derived fibrils show proteins of similar size in both preparations (Fig. 7a and b), the fibrils were too complex to allow further comparisons. The petaloid coelomocyte extracts also did not form a gel or any birefringent fibrils. A very small amount of actin was pelleted from the extracts (100,000 g for 3 h) indicating that there was little actin polymerization. However, actin and a 57k component were extracted and could be precipitated with cold trichloroacetic acid from the supernate of the high-speed spin (Fig. 7c).

DISCUSSION

The study of the formation, ultrastructure, and composition of cytoskeletons from a variety of cell types is an important step toward understanding how these macromolecular complexes function within cells. Sea urchin coelomocytes are ideal candidates for these studies because they possess the remarkable ability to transform morphologically from a petaloid to filopodial form and provide access to two morphologically unique forms of the same cell. The underlying cytoskeletons of each form are ultrastructurally and biochemically distinct.

Structurally, the isolated cytoskeletons differ in the degree of organization of their constituent microfilaments, and in that regard are indistinguishable from those observed in substrate-attached cells (9) and are therefore suitable for biochemical analysis.

Biochemically, the cytoskeletons contain actin and four actin-associated proteins (Fig. 3). While the greatest differences in amount of 57k protein are observed between the two forms of the cytoskeleton, the wider range of 57k protein to actin ratios observed among the petaloid preparations may be important as well. The 57k protein to actin...
FIGURE 6  The salt-extracted filopodial cytoskeletons are dissociated into filaments that are largely stripped of their actin-associated proteins. Two identical samples of filopodial cytoskeletons were incubated and first centrifuged at low speed (30,000 g for 10 min) and then at high speed (100,000 g for 3 h); the pellets from each spin were collected. Finally, the supernates from the high-speed runs were precipitated and all six samples were electrophoresed. In the control sample on the left which was incubated in 0.12 M NaCl, most (60%) of the actin and associated proteins (83%) are pelleted in the initial low-speed spin (low) as might be expected because the bundles are still intact (see Fig. 5a). After 15 min of 0.25 M NaCl, the bundles are dispersed into filaments which are pelleted in high-speed spin and are largely stripped of their associated components. These pelletable filaments comprise 64% of the actin in this sample. Most of the associated components have been solubilized and were precipitated from the high-speed supernate (0.25, sup).

FIGURE 7  SDS-PAGE showing a comparison of the components in (a) filopodial cytoskeletons, (b) sea urchin egg extracts, and (c) coelomocyte extracts. While neither the egg extract (b) nor the coelomocyte extract (c) formed a solid gel after addition of ATP and warming, the electrophoretic patterns represent the components of each extract which were collected by centrifugation (b) or precipitation (c) (see text).

ratios varied from 30:1 to 20:1 among several petaloid preparations which may reveal a greater degree of cytoskeletal transformation in some populations of coelomocytes. For example, Fig. 1b represents an average petaloid cytoskeleton with a small amount of bundle formation. If the 57k protein is directly involved in bundle formation, then the variations in its amount may be reflected in the variations in extent of bundle formation among cytoskeletons. The 57k protein may be acting as a crosslinking agent in this system, as a protein of similar size and solubility characteristics does in egg extracts (3). Furthermore, some of the filament bundles show periodic striations along their length (8) which are similar to those observed in microvilli of sea urchin eggs (5) and in gelled extracts of eggs (7). The egg actin gels exhibit the most pronounced striations after days of storage (14). The extent of the crosslinking in egg extracts can be altered, as observed in negatively stained preparations, by limiting the amount of 58k protein (3). Interestingly, when the 58k protein to actin weight ratio equals 1:10, i.e., the same as is observed in filopodial coelomocyte preparations, the striations are not so apparent and the bundles are more loosely organized than when the weight ratio is 1:5. Further strength is given to this purported identity by the recent work of Otto et al. (19) in which a fluorescently tagged antibody against the egg 58k protein is presumed to interact specifically with a similar protein in echinoderm coelomocytes and is seen to concentrate, during
the morphological transformation, in the actin filament bundles.

While direct comparisons of the cytoskeletal 57k component and the 58k protein from egg extracts were not feasible, it is worthy of note that the relatively simple, three-component actin gel is not readily prepared in all species of echinoderms, a fact that has been corroborated by other workers (D. Begg, personal communication). The simple cytoskeletal isolation and dissolution technique coupled with the ease of handling and transforming the system as outlined here will allow for the further characterization of these and other cytoskeletal components.

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