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F-actin affinity chromatography: Technique for isolating previously unidentified actin-binding proteins

(actin-associated proteins)

KATHRYN G. MILLER AND BRUCE M. ALBERTS

Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448

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ABSTRACT **We have developed stable and easy to use filamentous actin (F-actin) affinity-chromatography columns that selectively purify proteins that bind to actin filaments from cell extracts. Most traditional assays for actin-associated proteins screen for their effects on actin polymerization or actin filament crosslinking. Because our technique requires only actin-filament binding, it can identify additional types of proteins involved in the function of the actin cytoskeleton. By chromatographing extracts of several types of cells on these columns, we show that known actin-binding proteins are selectively retained as a subset of a larger group of actin-binding proteins that have not been identified previously.**

The functions of cytoskeletal filaments depend on the binding of many different associated proteins to the three major cytoskeletal polymers: actin filaments, microtubules, and intermediate filaments. Detailed studies of these associated proteins are therefore fundamental to our understanding of the cytoskeleton (1, 2).

To provide a general method for identifying and purifying actin-binding proteins (ABPs), we have constructed affinity-chromatography columns containing immobilized actin filaments (F-actin) and used them to chromatograph cell extracts. Our extensive results with extracts of early *Drosophila* embryos suggest that this method allows important cytoskeletal proteins to be isolated that may be difficult to detect by other means (K.G.M., C. M. Field, and B.M.A., unpublished data).

To chromatograph extracts under conditions that release all F-actin-specific proteins in soluble form, we have designed two types of F-actin affinity columns that remain stable under the low-ionic-strength conditions that normally cause depolymerization of F-actin. In one method, we use the mushroom toxin phalloidin to prevent actin monomer dissociation from the filaments (3). For comparison, we have also used actin filaments that have been stabilized by intramolecular crosslinking of the actin monomers with suberimidate (4). Like the phalloidin-treated actin, this modified actin remains polymerized even after extensive dialysis against low-salt buffers.

To work out suitable chromatography procedures, we have used the two types of F-actin columns to fractionate extracts containing known ABPs. As controls, parallel columns are run on which monomeric actin (G-actin) or bovine serum albumin (a protein with a net charge similar to actin) are immobilized. In this report, we present the details of the optimized procedure; in addition, extracts of chicken intestinal brush border, chicken gizzard, and *Acanthamoeba* are analyzed, and the major proteins that bind and dissociate specifically from the F-actin column are shown to include known ABPs. An earlier version of this F-actin affinity-chromatography method was briefly described in a preliminary publication (5).

MATERIALS AND METHODS

Materials. All chemicals used were reagent grade. Phalloidin, leupeptin, pepstatin, aprotinin, suberimidate, and Nonidet P-40 were obtained from Sigma. Rabbit muscle myosin was a gift of Kathy Franke and Roger Cooke (University of California, San Francisco); heavy meromyosin was generated by limited chymotryptic digestion. *Acanthamoeba* α-actinin and antibodies against *Acanthamoeba* α-actinin, spectrin, and 29- and 31-kDa capping proteins were gifts from Tom Pollard (Johns Hopkins School of Medicine). Anti-filamin antibody was obtained from Amersham.

Buffers. The following buffers were used: F-buffer (polymerizing conditions for actin filaments), 50 mM Hepes adjusted to pH 7.5 with KOH (K-Hepes)/0.1 M KCl/0.2 mM $CaCl_2$/0.2 mM ATP/5 mM $MgCl_2$; G-buffer (depolymerizing conditions for actin filaments), 5 mM K-Hepes, pH 7.5/0.2 mM $CaCl_2$/0.2 mM ATP; E-buffer (extract buffer), 5 mM K-Hepes, pH 7.5/0.05% Nonidet P-40/0.5 mM Na_3EDTA/0.5 mM Na_3EGTA/10 μg each of leupeptin, pepstatin, and aprotinin per ml; A-buffer, E-buffer with 50 mM K-Hepes, pH 7.5/2 mM dithiothreitol; and $NaDodSO_4$/polyacrylamide gel sample buffer, 0.0625 M Tris chloride, pH 6.8/3% $NaDodSO_4$/5% 2-mercaptoethanol/10% (vol/vol) glycerol.

Preparation of F-Actin and G-Actin. Actin purified from rabbit skeletal muscle (6) was stored as actin filaments at 4°C at a concentration greater than 5 mg/ml in 5 mM Tris chloride, pH 8.0/0.2 mM $CaCl_2$/0.2 mM ATP/0.1 M KCl/2 mM $MgCl_2$/0.2 mM dithiothreitol/0.01% NaN_3. Immediately before column construction, the required amount of this actin solution was centrifuged at 100,000 × *g* for 2 hr to collect the actin filaments. The pellet was resuspended in G-buffer and dialyzed against this buffer for 15–36 hr at 4°C to depolymerize the actin. After a second centrifugation to remove remaining actin polymers (100,000 × *g* for 2 hr), the supernatant was used directly to prepare G-actin columns (see below). To prepare F-actin, the supernatant was diluted to 2 mg of actin per ml and polymerized in F-buffer by adjusting the salt concentration to 0.1 M KCl, 2 mM $MgCl_2$, and 50 mM K-Hepes (final pH 7.5). After 15 min at 4°C, 10 μg of phalloidin per ml was added to the polymerized actin.

Suberimidate actin was prepared directly from the G-actin by the method of Ohara *et al.* (5). The filamentous protein was collected by centrifugation (100,000 × *g* for 2 hr) after sonification with a Branson model 350 sonifier microtip at maximum power for 30 sec. The pellets obtained were resuspended in G-buffer at an actin concentration of 2 mg/ml.

Preparation of the Affinity Resin. All column beds were packed in sterile plastic syringes (Beckman Dickinson) fitted with polypropylene filter discs (Ace Glass) as bed supports

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Abbreviations: ABP, actin-binding protein; F-actin, filamentous actin; G-actin, monomeric actin.

(6-ml syringes for columns with a bed volume of 3 ml and 60-ml syringes for a bed volume of 25 ml). To preserve flow properties, we have kept the length of the column bed nearly constant (3–5 cm) and increased the cross-sectional area when increasing column size. The outlet of the syringe was fitted with an 18-gauge needle pushed through a rubber stopper mounted on a filter flask. Equal settled volumes of Affi-Gel 10 (Bio-Rad) and Sepharose CL-6B (Pharmacia) were poured into the syringe and washed three times under suction at 4°C with glass-distilled H_2O and once with F-buffer (one or more column volumes each). For each wash the bed was mixed gently with a spatula before suction was applied, and care was taken not to draw air into the bed. (Because the reactive groups on the Affi-Gel begin to decay as soon as it is transferred to aqueous solution, the washes were completed within 10 min.) After removal of the final wash solution from the packed resin, the syringe outlet was closed with a needle plugged with a silicone stopper. The packed resin was immediately mixed with the appropriate protein solution as described below.

Preparation of F-Actin Columns. For coupling F-actin to the column bed, one-half of the resin volume of F-buffer containing F-actin at 2 mg/ml and phalloidin (or suberimidate-crosslinked actin in G-buffer) was added to a syringe containing packed washed resin and gently mixed with a spatula. Coupling was allowed to proceed for 1–15 hr at 4°C in the syringe (no mixing). To terminate the reaction, 3 M ethanolamine (redistilled and neutralized to pH 8) was added to the resin slurry to a final concentration of 50 mM for 1 or more hours. During the termination step, the free liquid in the syringe was recirculated continuously through the column bed by using a peristaltic pump (Gilson) at a flow rate of 1–3 resin volumes per hr (up to a maximum of 25 ml/hr) to pack the resin.

After packing, the column was washed with F-buffer to remove unbound actin. The flow properties of the column were then checked by running a small aliquot of F-buffer containing 5% glycerol and salt through the column with F-buffer. [If the dye channels around the bed rather than moving through it, the resin is gently mixed with a spatula in a minimum volume of F-buffer and allowed to stand undisturbed for several hours; the column bed is then repacked by pumping through F-buffer at 1–3 column volumes per hr as above.] Once a satisfactory column has been prepared, it is washed with 1 M KCl/50 mM K-Hepes, pH 7.5/2 mM $MgCl_2$ (3–5 column volumes) and subsequently with F-buffer. All washes are saved for protein determination (7), and the protein on the column is quantitated by subtracting the total protein eluted from the protein input. Columns are stored in F-buffer containing 10 μg of phalloidin per ml and 0.02% NaN_3 at 4°C and are reusable for a period of at least 3 weeks. Just before use, the column is washed with the buffer that was used to prepare the extract to be chromatographed (see below).

Preparation of G-Actin Columns. G-actin in G-buffer was diluted to 3–4 mg/ml. G-actin reacts strongly with the resin, so to prevent an overcoupling that might denature the actin monomer, the washed Affi-Gel was partially inactivated by incubation for 1.5 hr in G-buffer and then was washed with this buffer before addition of a volume of the G-actin solution equivalent to half of the resin volume. Moreover, the reaction was allowed to proceed for only 20 min before addition of 50 mM ethanolamine (we seek to couple only 70% of the G-actin; the Affi-Gel 10 inactivation times required to attain this level of coupling must be determined for each lot of the resin). In this way, we obtain $\approx$1 mg of G-actin per ml on the bed, about 80% of which is available for binding bovine pancreatic DNase I. Because the actin monomer is relatively unstable, the G-actin columns were prepared on the day of use.

Preparation of Control Columns. Albumin-containing control columns were prepared in a manner similar to that described for the F-actin columns (no preincubation of resin), with bovine serum albumin (Sigma) at a concentration of 4 mg/ml in F-buffer (no phalloidin). Approximately 60% of the albumin is coupled to the resin under these conditions, leaving a final concentration of about 1 mg/ml on the column bed. These columns can be stored at 4°C in F-buffer containing 0.02% NaN_3 and used repeatedly.

Affinity Chromatography of Extracts. F-actin, G-actin, and control (albumin) columns of equal bed size and protein content were equilibrated with A-buffer containing 10% glycerol (or another indicated loading buffer) at 4°C. Equal volumes of the same extract were applied to all of the columns in each experiment (unless otherwise noted) with a flow rate $\leq$ 1 column volume per hr. After the columns were loaded, all were rinsed at 1–2 column volumes per hr with A-buffer containing 10% glycerol until protein in the eluate had reached <10 μg/ml for the F-actin columns. The elution was then carried out step-wise with A-buffer containing 10% glycerol and added salt and/or 1 mM ATP/3 mM $MgCl_2$ as indicated in each experiment. The fractions containing protein in each elution step from F-actin columns (7) were pooled, as were equivalent fractions from control or G-actin columns (which often had no detectable protein peak). After the total protein present in an aliquot of each pool was precipitated with 10% trichloroacetic acid, resuspended in $NaDodSO_4$/polyacrylamide gel sample buffer, and neutralized with 2 M Tris base, an equal proportion of the eluate from each column was electrophoresed in $NaDodSO_4$ through either a 5–15% polyacrylamide gradient gel or an 8.5% polyacrylamide gel (8). Proteins were visualized by Coomassie blue staining of the gels.

RESULTS

F-actin affinity chromatography is complicated by the high viscosity of solutions containing actin filaments. Finding a successful method for F-actin column construction therefore required many empirical trials. The most critical variables turned out to be the concentration of the actin filaments and the flow rate used for packing the column. When actin concentrations exceed 1 mg/ml on the column bed, an uneven flow is produced that can be detected as an uneven penetration of the column bed when a dye solution is passed through the column (see Methods); because of regions of impeded flow in such columns, residual dye remains on the bed even after extensive washing. Equally important for obtaining usable column beds is packing the bed at flow rates of 1–3 resin volumes per hr (up to 25 ml/hr maximum) (see *Materials and Methods*). Too fast a flow rate shears the actin filaments on the resin and causes significant actin loss. Too slow a flow rate produces columns in which flow through the bed is difficult, and channeling occurs around the outside of the bed (as detected by dye flow). When extracts are chromatographed on an F-actin column that does not pass a dye test, actin continues to leach from the bed throughout the experiment, along with a high background of many transiently retained non-ABPs from the extract.

If coupled and packed properly, 90% of the initially added F-actin remains on the bed, producing an F-actin content of about 1 mg per ml of resin. At least 0.5–0.75 mg of actin per ml can be stripped from the bed by washing with distilled water or boiling with $NaDodSO_4$, indicating that most of the actin subunits in a filament are not covalently coupled. Instead, the F-actin remains on the column matrix by covalent coupling of a fraction of the subunits in each filament and by physical trapping within the matrix. Electron microscopy of the beads revealed a dense meshwork of actin filaments throughout the beads (unpublished observations of M. L.

Wong and K.G.M.). When column capacity was quantitated by loading the column with saturating amounts of heavy meromyosin and eluting with 1 M KCl/2 mM sodium pyrophosphate, 50–75% of the actin subunits on the column were found to bind this protein.

Mechanical disruption of the bed (by vigorously stirring, for example) causes actin to wash off. For this reason, the actin filaments are coupled to the matrix in the same column that will be used subsequently for chromatography. If the bed remains undisturbed, the columns are quite stable over the course of an experiment, and very little actin is lost; consequently, they can be reused several times with no loss of capacity for actin-binding proteins. However, storage of F-actin columns for more than 3 weeks is not recommended, as the actin filaments eventually denature and lose their ability to bind ABPs.

When chromatographing extracts from cells rich in actin and myosin, such as 16-hr-old *Drosophila* embryos and *Acanthamoeba*, an actin–myosin coaggregate sometimes will form slowly in the extract as it is being pumped onto the column and will be retained even on control columns because of physical trapping (filtering) in the matrix. Use of dilute extracts (7–10% tissue weight) helps to minimize this problem. Including pyrophosphate or ATP in the extract buffer largely avoids the problem, but it can preclude the detection of the ATP-eluting ABPs in the extract.

Studies on Chicken Gizzard. The gizzard of chickens has been extensively used to characterize ABP organization in smooth muscle cells, and a number of ABPs have been purified and studied from this source (1). When we chromatographed gizzard extracts on our two types of F-actin columns, about 2% of the total protein loaded bound to each F-actin column, compared with <0.2% binding to the G-actin or albumin control columns (Fig. 1). As determined by $NaDodSO_4$/polyacrylamide gel electrophoresis, at least nine major proteins were specifically retained on the F-actin

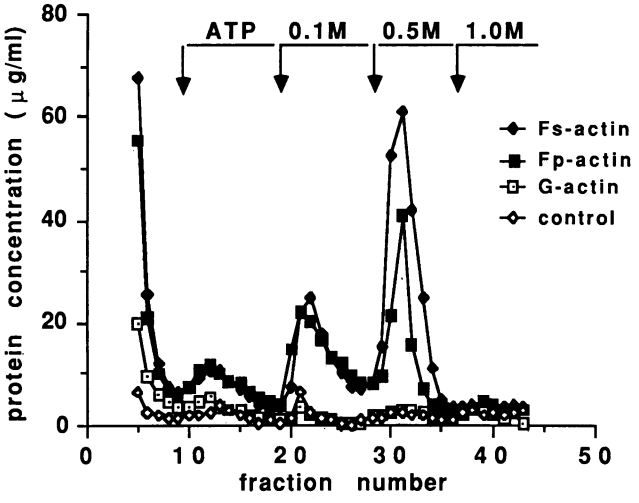

FIG. 1. Elution profile of proteins in chicken gizzard extracts chromatographed on F-actin, G-actin, and control columns. The protein concentration in each fraction that was eluted from the column is plotted versus the fraction number. The matrices used are phalloidin-stabilized F-actin (F_p-actin), suberimidate-crosslinked F-actin (F_s-actin), G-actin, and albumin (control), with each protein linked to an Affi-Gel 10 agarose matrix. To prepare chicken gizzard extract, a single gizzard (Pelco) was thawed, and muscle tissue was dissected away from connective tissue. One gram of minced tissue was suspended in 10 ml of E-buffer, and phenylmethylsulfonyl fluoride was added to 1 mM immediately before homogenization at 4°C with a Polytron (Brinkmann). After centrifugation at 10,000 × *g* for 20 min, the supernatant was adjusted to 50 mM K-Hepes, pH 7.5/2 mM dithiothreitol and clarified by centrifugation at 80,000 × *g* for 1 hr.

columns, whereas no G-actin-specific proteins were detected (Fig. 2*A*). Several minor proteins in the region around 30 kDa and lower were eluted from all columns; therefore, these are nonspecifically bound to the resin.

The most abundant gizzard protein that binds to F-actin columns is filamin (250 kDa), a well-characterized ABP that crosslinks actin filaments (1). Filamin primarily was eluted in the 0.1 M KCl and 0.5 M KCl steps, as identified by immunoblotting with an anti-filamin antibody (Amersham). Essentially all of the filamin in the soluble extract bound to and was eluted from F-actin columns under our conditions (see Fig. 2*B*). Myosin is another well-characterized ABP that was eluted from the two F-actin columns (band at 200 kDa in the ATP elution). Although only a small amount of the total myosin in gizzard was recovered in an ATP elution step, much more was eluted in a combined 1 M KCl and ATP elution (data not shown). Other abundant proteins eluted only from the F-actin columns in Fig. 2 are several of 40–150 kDa (both 0.1 M KCl and 0.5 M KCl) and one of 200 kDa (0.1 M KCl elution); numerous other minor species in the molecular mass range of 80–120 kDa also were found reproducibly. These and other ABPs in Fig. 2*A* were not identified by us, but several previously described proteins that are associated with the actin cytoskeleton have molecular weights similar to these [metavinculin (150 kDa; refs. 9 and 10); talin (215 kDa; ref. 11); and caldesmon (140 kDa; ref. 12)].

In general, identical proteins are bound and eluted from both the suberimidate-crosslinked and the phalloidin-stabilized F-actin columns. However, a prominent protein of ≈32 kDa was bound strongly only by suberimidate-crosslinked actin (0.5 M elution in Fig. 2). Incubation of suberimidate-crosslinked F-actin column with phalloidin did not prevent the binding of this protein (data not shown), which might be either tropomyosin or an actin-polymerization inhibitor (13).

Under the conditions of the experiment in Fig. 2*A*, columns were loaded at well below their capacity for the major protein species detected, since passage of the flow-through fraction through another F-actin column detected only a minor amount of binding (<10% as much of any major protein was recovered). When five times as much protein was loaded onto an identical column (lane 5× in Fig. 2*B*), the filamin and the 150-kDa proteins continued to bind in about the same proportion as they did with the 1× extract. Since 90% of the loaded filamin was still recovered in the elution, there are sufficient binding sites for >500 μg of filamin (plus other proteins) on a column containing 2.5 mg of F-actin. However, when the flow through from the column loaded with 5× extract was reloaded onto another F-actin column, the recovery of several minor ABPs was enhanced (see *Discussion*) (arrows in Fig. 2*B*).

Studies on Intestinal Brush Border. Intestinal epithelial cells have a specialized apical region—called the brush border—composed of a dense network of microvilli that have an actin filament-rich core. The biochemistry of these actin-rich structures has been extensively studied (for review, see ref. 14). Not surprisingly, a large fraction of the protein in an extract prepared from purified brush border is composed of actin-binding proteins and is retained on our F-actin columns (15%), whereas the G-actin and control columns bind much less protein (<2%). Polyacrylamide gel electrophoresis showed that all of the previously characterized ABPs (14) that are solubilized in these extracts bound efficiently to the F-actin columns (Fig. 3). These include a high molecular weight doublet (>220 kDa), called TW 260/240 (a spectrin-like molecule), and its 140-kDa proteolytic fragment (16), villin at 96 kDa, and fimbrin at 68 kDa (actin-bundling proteins). Binding was nearly quantitative, since the ABPs were largely removed from the extract (compare control and F-actin column flow throughs in Fig. 3). Two of the major proteins in the extract (180 kDa and a broad band near 130

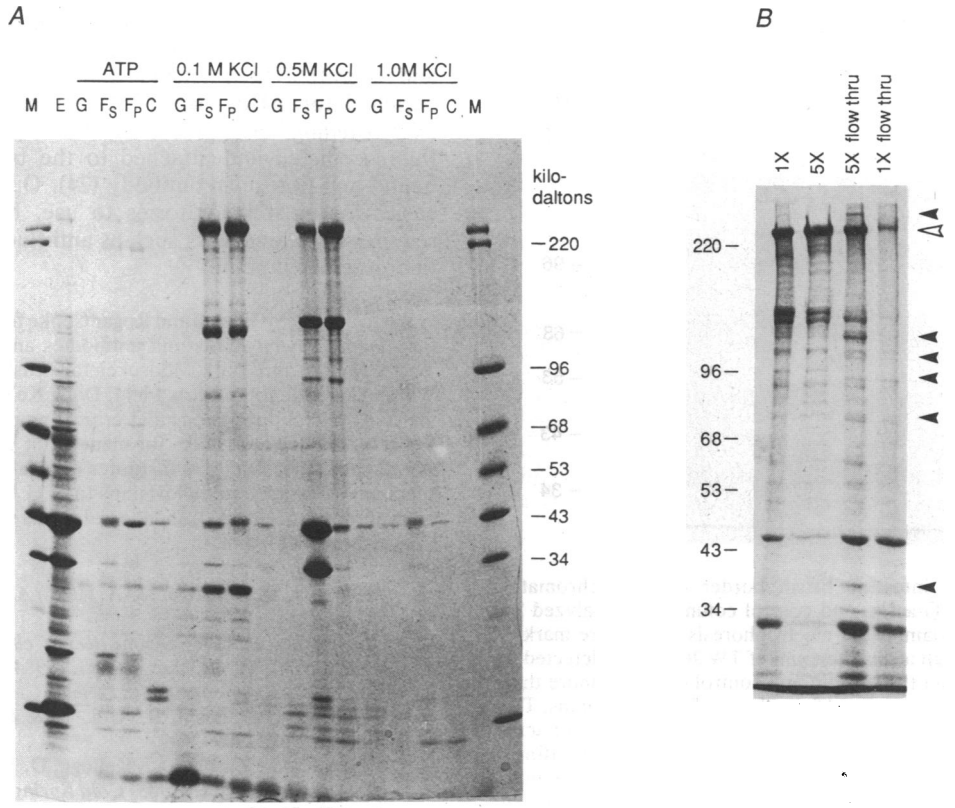

FIG. 2. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis of the proteins eluted from columns loaded with chicken gizzard extracts. (*A*) F-actin, G-actin, and control columns were loaded and eluted in parallel as in Fig. 1. Lanes: E, extract before chromatography; F_s, suberimidate-crosslinked F-actin column; F_p, phalloidin-stabilized F-actin column; G, G-actin column; C, albumin column; M, molecular mass markers. The markers used here and in Fig. 3 are human erythrocyte spectrin, glycogen phosphorylase *b*, bovine serum albumin, tubulin, actin, and T4 bacteriophage gene *32* protein. A total of 5 ml of extract was loaded onto each 3-ml column in this experiment; this extract contained 2 mg of total protein per ml. (*B*) The effect of exposing F-actin columns to very different amounts of chicken gizzard extract. Identical F_p-actin columns were used to chromatograph either the same amount of extract as in *A* (lane 1×), or five times this amount (lane 5×). For lane 5×, only one-fifth as much of the total eluate was loaded onto the polyacrylamide gel for comparison with lane 1×. The unbound fraction that passed through each column was also chromatographed on a second, identical F-actin column; the proteins that bound and were eluted from these columns are shown in the lanes marked "1× flow thru" or "5× flow-thru." To help visualize minor protein species, the proportion of the total eluate loaded in these two lanes is about 10 times that used for the 1× or 5× lanes. The protein bands marked with solid arrows are some of those that appear to be blocked by competition for binding to overloaded columns by filamin (open arrow) and other major proteins.

kDa) did not bind to the F-actin column and were recovered in the flow through; these are not among the characterized ABPs.

Studies on *Acanthamoeba*. *Acanthamoeba* is a motile protozoan with an extensively studied actin cytoskeleton (17, 18). When *Acanthamoeba* extracts (prepared in E-buffer containing 1 mM phenyl methylsulfonyl fluoride and 0.34 M sucrose) were chromatographed on our columns, ≈20 major proteins and many minor ones were eluted from the F-actin columns and were not detected in eluates from either the G-actin or control columns. These proteins range in molecular mass from 250 to ≈20 kDa. By using antibodies to *Acanthamoeba* ABPs to probe protein blots of the eluates from these columns, we determined that spectrin, α-actinin, and the 29- and 31-kDa capping proteins were all present in elutions from F-actin columns but were absent in elutions from the G-actin and control columns (data not shown).

DISCUSSION

We have devised reproducible methods for the chromatography of extracts on two types of F-actin affinity columns. Both types of F-actin columns bind similar sets of proteins; since the same proteins do not bind to G-actin columns, the majority of proteins we detect must be retained through actin filament-specific interactions.

We have observed surprisingly little binding to G-actin columns, where proteins such as profilin (1) should be recovered. Our failure to find monomer-binding proteins may be due to competition for binding with the large amount of G-actin present in the extracts. In addition, nonexchangeable complexes may be formed between some monomer-binding proteins and endogenous actin. Most of the G-actin on our columns can bind DNase I (see *Materials and Methods*), suggesting that the native actin structure is maintained during coupling to the matrix. Others have shown that DNase I-agarose can be used to detect G-ABPs (19).

Our experiments with several different types of cells and tissues have demonstrated that proteins from many of the previously defined classes of ABPs bind to the F-actin columns when crude extracts are chromatographed. These include the contractile protein myosin; the bundling proteins villin and fimbrin; the crosslinking proteins spectrin, TW 260/240, and filamin; and *Acanthamoeba* capping proteins. Many other protein species not previously identified as ABPs are also specifically bound. Our extensive experiments with extracts of *Drosophila* embryos, reported elsewhere (ref. 5 and unpublished data of K.G.M. and C. M. Field), suggest that many of these unidentified proteins are likely to be new ABPs, some with novel activities. The methods described here also have permitted the biochemical identification of new ABPs in yeast (20).

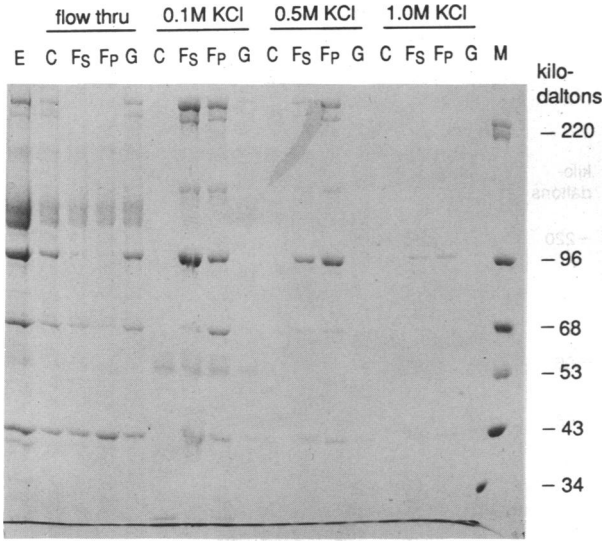

FIG. 3. Chicken intestine brush border extracts chromatographed on F-actin, G-actin, and control columns, as analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lanes are marked as in Fig. 2A. Although a small amount of TW 260/240 is detected in the 0.1 M elution from the G-actin and control columns, more than 10 times this amount is recovered from the two F-actin columns. The background binding may be explained by the small amount of actin binding observed to the control column, combined with a low affinity of TW260/240 for G-actin. Purified brush border prepared by published procedures (15) was generously provided by Tom Coleman and Mark Mooseker. Pellets were suspended in 0.8 M KCl/50 mM K-Hepes, pH 7.5/0.5 mM Na_3EDTA/0.5 mM Na_3EGTA/0.05% Nonidet P-40/10 μg (each) of leupeptin, pepstatin, and aprotinin per ml/1 mM phenylmethylsulfonyl fluoride and incubated on ice for 15 min to extract ABPs. After centrifugation at 100,000 × g for 30 min, the supernatant was dialyzed against A-buffer containing 10% glycerol for 4 hr at 4°C. After centrifugation to remove protein aggregates (80,000 × g for 1 hr), the extract was loaded onto the appropriate columns at 4°C. In this experiment, 1.1 ml of extract containing 0.7 mg of protein per ml was loaded onto each 3-ml column.

Our experiments with known ABPs have revealed some important general features of F-actin affinity chromatography. Unless columns are loaded at well below their maximum capacity for total protein binding, there is a competition between different proteins for mutually exclusive sites, as illustrated by the experiment in Fig. 2B. For large-scale preparations of particular ABPs, F-actin columns should probably be used after ion-exchange chromatography. In this way, the competition between ABPs for binding sites will be reduced or eliminated, so that smaller F-actin columns can be used. However, the F-actin affinity chromatography method described here will also enrich for ABPs when crude extracts are chromatographed, which provides a powerful means to identify new actin binding proteins based solely on their ability to bind to actin.

Other workers have used approaches similar to ours to select proteins that bind specifically to actin filaments. Chromatography on actin filaments stabilized by glutaraldehyde cross-linking (21) or phalloidin (22) has previously been used for the much more limited application of analyzing myosin and its proteolytic fragments. Luna and coworkers (23) have enriched for ABPs in the plasma membrane fraction of *Dictyostelium*, using F-actin-containing beads; the actin is fluoresceinated and attached to the beads via covalently bound anti-florescein antibody (24). Our method should be easier for most laboratories to use, because it does not require special reagents such as anti-fluorescein antibody or fluoresceinated actin.

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