Isolation of a 5-kilodalton actin-sequestering peptide from human blood platelets

(actin polymerization/cytoskeleton/nondenaturing polyacrylamide gel electrophoresis)

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ABSTRACT Resting human platelets contain ≈ 0.3 mM unpolymerized actin. When freshly drawn and washed platelets are treated with saponin, 85–90% of the unpolymerized actin diffuses out. Analysis by polyacrylamide gel electrophoresis under nondenaturing conditions shows that the bulk of this unpolymerized actin migrates with a higher mobility than does pure G-actin, profilactin, or actin–gelsolin complex. When muscle G-actin is added to fresh or boiled saponin extract, the added muscle actin is shifted to the high-mobility form. The saponin extract contains an acidic peptide having a molecular mass in the range of 5 kDa, which has been purified to homogeneity by reverse-phase HPLC. This peptide also shifts muscle actin to the high-mobility form. Addition of either boiled saponin extract or the purified peptide to muscle G-actin also strongly and stoichiometrically inhibits salt-induced polymerization, as assayed by falling-ball viscometry and by sedimentation. We conclude that this peptide binds to the bulk of the unpolymerized actin in platelets and prevents it from polymerizing.

It has been known for >10 years that many cell types contain a pool of unpolymerized actin (1, 2). Much of this actin can polymerize after purification (2, 3). Within the cell, polymerization is also induced by appropriate stimulation (1, 4). The concentration of unpolymerized actin in cells can be up to 2 orders of magnitude greater than the critical concentration for assembly: in platelets, for example, the concentration of unpolymerized actin is estimated at 300–350 μ M (5, 6), whereas the critical concentration is about 1 μ M for the pointed end and about 0.2 μ M for the barbed end (7). Furthermore, since the ion concentrations inside most cells favor actin polymerization, it seems inescapable that some other factor must be present to prevent this actin from polymerizing (8).

The discovery that actin could form a 1:1 complex with either deoxyribonuclease I (9) or profilin (10) showed that actin monomers could be sequestered by other proteins and thereby prevented from polymerizing. Both profilin and profilactin have been isolated from blood platelets (11, 12); however, the role of profilin in maintaining the pool of unpolymerized actin in platelets has been clouded by several discrepancies. First, early estimates of the quantity of profilin in platelets suggested that nearly all of the unpolymerized actin in platelets could be sequestered as profilactin (11, 12), but subsequently Lind *et al.* (13) used the polyproline affinity technique and estimated a profilin concentration that is well below the concentration of unpolymerized actin. Second, the binding constant of profilin for actin appears to be relatively weak, in the range of 1–6 μ M (7, 14). Thus, even if the profilin concentration were equal to the concentration of unpolymerized actin in platelets, the concentration of free G-actin would

be in the range of 20 μ M, which is at least an order of magnitude above the critical concentration for the pointed end. Third, Lind *et al.* (13) have also reported that actin is complexed with profilin only after platelet activation and not in the resting platelet. Fourth, it has been shown (15) that the profilactin complex can be dissociated by phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5) P_2] and that the affinity of profilin for PtdIns(4,5) P_2 is greater than its affinity for actin (16). Calculations suggested that there is sufficient PtdIns(4,5) P_2 in platelets to complex the available profilin. These considerations suggest that profilin may not be available to complex with a significant proportion of the actin in resting platelets.

A soluble actin–gelsolin complex has also been detected in platelets (17); however, results from several laboratories indicate that this complex is formed upon platelet activation and is not present in significant quantities in resting platelets (13, 18).

We recently have investigated the state of unpolymerized actin in platelets by making them permeable with saponin (19) and then analyzing the proteins that diffuse out of the cells by gel electrophoresis under nondenaturing conditions (20). Under our conditions, although soluble proteins including 85–90% of the unpolymerized actin diffuse out of the treated platelets, electron microscopy shows that the platelets retain a relatively intact plasma membrane, many secretory granules, and a cytoskeletal network of F-actin (21). The nondenaturing polyacrylamide gel electrophoresis (PAGE) procedure has been shown to preserve complexes of actin with DNase I, gelsolin, profilin, and vitamin D-binding protein (20) each of which has a different characteristic mobility. Free actin-sequestering proteins are detected by adding *N*-(iodoacetyl)-*N'*-1-sulfo-5-naphthylethylenediamine (Aedans)-labeled actin to the sample; complexes are detected as fluorescent bands. Using this approach, we have identified a new actin-containing complex in resting platelets and have shown that it contains an acidic peptide of about 5 kDa. The purified peptide binds to muscle actin and prevents it from polymerizing.

MATERIALS AND METHODS

Materials. Muscle actin was prepared from an acetone-extracted residue of rabbit skeletal muscle by the method of Spudich and Watt (22). F-actin was fluorescently labeled with Aedans (Sigma) as described by Dos Remedios and Cooke (23) and was depolymerized by dialysis against 1000 vol of buffer containing 10 mM imidazole hydrochloride, 0.2 mM ATP, 0.2 mM CaCl₂, and 0.2 mM NaN₃ (pH 8.0) for 16–24 hr. Platelet G-actin was obtained by dialysis of platelet F-actin bundles, isolated as described (24), in the same buffer. Gelsolin was prepared from frozen chicken serum (GIBCO)

or from outdated human plasma by the method of Cooper *et al.* (25) as adapted (20). Platelet profilactin was isolated by gel filtration as described (11) and further purified by ammonium sulfate fractionation (12).

Monoclonal anti-actin antibody C-4 (26) was the gift of James Lessard (Children's Hospital Medical Center, University of Cincinnati). ATP, apyrase, creatine phosphokinase, chymotrypsin, bovine serum albumin, leupeptin, phosphocreatine, and saponin were obtained from Sigma. Alkaline phosphatase-conjugated antibody and ampholytes were obtained from Bio-Rad. Diaminobenzidine was obtained from Polysciences. Proteinase K was from Boehringer Mannheim and was a gift of Christian Stoeckert (University of Pennsylvania). All other chemicals were of reagent grade.

Preparation of Platelets and Saponin Extracts. Both fresh and outdated human platelets were used. Platelets were prepared as described (19) with the following modifications. Platelets were incubated in the Pipes buffer with 5.5 mM glucose, 0.1 mg of apyrase per ml, and 25 μ M indomethacin at 37°C for 30 min. After centrifugation at $400 \times g$ for 10 min, the pellet was resuspended at 2×10^9 platelets per ml in the high-KCl buffer containing 0.1 mg of leupeptin per ml as described (19). Platelets were then incubated for 10 min with 5 mM EGTA/100 μ g of saponin per ml and centrifuged in a Beckman Microfuge at $8,000 \times g$ for 2 min. The supernatant was used as fresh saponin extract. Boiled saponin extract (BSE) was prepared by incubating fresh saponin extract in a boiling water bath for 3 min; after a brief cooling period, the precipitated material was removed by centrifugation in the cold at $12,000 \times g$ for 10 min. Analysis of the supernatant by sodium dodecyl sulfate (SDS)/PAGE showed only traces of protein and no actin.

Immunoblotting. Actin-containing bands on native gels were detected by immunoblotting (F. Stark, D.S., and V.T.N., unpublished method) with the following modifications. Immobilon-P (Millipore) was used as the transfer membrane. Bovine serum albumin (5%) in 20 mM Tris-HCl/0.5 M NaCl, pH 7.5, was used as the blocking buffer, with the addition of 0.05% Tween for washings. Alkaline phosphatase-conjugated goat anti-mouse IgG was used as the secondary antibody, and the bands with adsorbed antibody were visualized with 5-bromo-4-chloro-3-indolyl phosphate (*p*-toluidine salt) and *p*-nitro-tetrazolium blue (chloride salt) as described by the manufacturer (Bio-Rad).

Gelsolin-containing bands were detected by immunoblotting with an affinity-purified rabbit antibody against human serum gelsolin (F. Stark, D.S., and V.T.N., unpublished data). Bands were detected by using the peroxidase-diaminobenzidine procedure as described (27).

Measurement of Viscosity. Aliquots (70 μ l) were mixed with an equal volume of 7 μ M G-actin in 5 mM Tris-HCl (pH 7.5). After a 10-min incubation, 15 μ l of a $10 \times$ polymerizing buffer (1 M KCl/20 mM $MgCl_2$ /50 mM Tris-HCl, pH 7.5) was added. Viscosity was then measured at intervals by using the falling ball method (28). To test for severing activity, actin was first polymerized at 7 μ M and then mixed with an equal volume of the fraction to be tested.

PAGE. Nondenaturing PAGE was performed as described (20) except that the buffer composition was 25 mM Tris/0.4 M glycine/0.5 mM EGTA/0.2 mM ATP, pH 8.3 at 4°C. SDS/PAGE was performed as described by Laemmli (29). Isoelectric focusing was performed as described (30). Proteins were detected by immunoblotting or by staining with Coomassie brilliant blue R250. To detect bands containing Aedans-labeled muscle actin, gels were first viewed and then photographed under longwave UV light (20). Gels were scanned with a Bio-Rad model 620 densitometer and one-dimensional software package.

High-Performance Liquid Chromatography (HPLC). HPLC was performed by using an ISCO ternary-gradient

system with a variable-wavelength detector and was interfaced to an IBM XT-compatible computer. Two types of reverse-phase column were used: a 10×250 mm preparative column packed with Vydac diphenyl-silica type 219TP2030 (The Separations Group) and a Brownlee Aquapore ODS [octadecyl (C_{18})-silica] column consisting of a 4.6×30 mm and a 4.6×220 mm cartridge in series (ABI, Foster City, CA). To limit contamination of the preparative column, samples of fresh saponin extract or BSE were first centrifuged at $200,000 \times g$ for 30 min and then applied to an open column of diphenyl-silica (0.3- to 0.5-ml bed volume/ml of extract), washed with 20 column volumes of 20% methanol/0.08% trifluoroacetic acid, and eluted with 20 column volumes of 50% methanol/0.05% trifluoroacetic acid. The eluate, which contained all of the actin-sequestering peptide as assayed on native gels, was evaporated, redissolved in water, and injected into the preparative column. For further purification, material recovered from the diphenyl-silica column was rechromatographed on (C_{18})-silica. Fractions from preparative HPLC were brought to dryness, reevaporated twice, and redissolved in deionized water.

Absorbance Spectra. Spectra were recorded on a Hewlett-Packard model 8452A spectrophotometer.

Amino Acid Analysis. Amino acid analysis was performed in duplicate with the Waters PicoTag system by the Protein and Nucleic Acid Chemistry Core Facility, Department of Biochemistry and Biophysics, University of Pennsylvania, under the direction of Dr. Lim Tung.

RESULTS

When a saponin extract from freshly drawn and washed, discoid platelets was analyzed by nondenaturing electrophoresis, the bulk of the actin as identified by immunoblotting migrated with a higher mobility than that of muscle or platelet G-actin [Fig. 1 A (stained gel) and B (immunoblot with anti-actin)]. Platelet profilactin (Fig. 1C, stained gel) and platelet gelsolin-actin complex (Fig. 1D, immunoblot with anti-gelsolin) migrated more slowly than did G-actin. When a native gel of the saponin extract was further analyzed by second-dimension electrophoresis in SDS, the result was ambiguous: trace quantities of other proteins appeared to comigrate with the high-mobility actin, but none appeared to be present in quantities sufficient to form a 1:1 complex with the actin (data not shown). To investigate the possibility that the high electrophoretic mobility of this actin and its failure to polymerize under physiological ionic conditions might result from a covalent modification such as ADP-ribosylation (31), the actin isoform composition of a $200,000 \times g$ supernatant of the saponin extract was compared with that of sedimentable platelet actin by isoelectric focusing (Fig. 2).

FIG. 1. Comparison of platelet saponin extract with muscle G-actin, platelet G-actin, platelet profilactin, and gelsolin-actin complex by nondenaturing PAGE. (A) Lanes: 1, muscle G-actin; 2, platelet G-actin; 3, saponin extract. (Coomassie blue stain.) (B) Lanes: 1, muscle G-actin; 2, saponin extract. (Immunoblot with anti-actin antibody.) (C) Lanes: 1, muscle G-actin; 2, partially purified platelet G-actin; 3, platelet profilactin. (Coomassie blue stain.) (D) Platelet gelsolin-actin complex. (Immunoblot with anti-gelsolin antibody.)

FIG. 2. Analysis of actin isoforms in platelet pellet and supernatant after centrifugation at $200,000 \times g$ for 1 hr. Isoelectric focusing gels were run as described (30) and stained with Coomassie blue. Lanes: 1, muscle actin; 2, platelet actin pellet; 3, platelet actin supernatant; 4, muscle actin plus platelet pellet; 5, muscle actin plus platelet supernatant; 6, platelet pellet plus platelet supernatant.

Both the sedimentable and nonsedimentable actin fractions showed only the β and γ isoforms, with the β isoform predominant.

The failure to detect an additional actin isoform suggested that the high-mobility actin was complexed with an unknown dissociable factor, designated here as "Fx." To determine whether free Fx was also present in the saponin extract, Aedans-labeled G-actin ($3 \mu\text{g}$) was added to a $10\text{-}\mu\text{l}$ aliquot of saponin extract. Fig. 3, lane 2, shows that essentially all of the Aedans-actin was shifted to the high-mobility form. A similar shift in the mobility of Aedans-actin was observed when using BSE (Fig. 3, lane 3), indicating that Fx is relatively heat stable.

To determine whether the shift in mobility correlated with inhibition of polymerization, aliquots of G-actin were mixed with various quantities of BSE, incubated at 24°C for 10 min, and then centrifuged at $100,000 \times g$ for 30 min. The supernatants and pellets were analyzed by SDS/PAGE (Fig. 4). When no salt was added to the G-actin, virtually all of the actin remained in the supernatant (S_1); when $100 \mu\text{l}$ of high-KCl buffer was added to the G-actin, the bulk of the actin was in the pellet (P_2). When $100 \mu\text{l}$ of BSE in high-KCl buffer was added, virtually all of the actin remained in the supernatant (S_3) and only a trace was in the pellet (P_3). When smaller volumes of BSE were added, the proportion of actin remaining in the supernatant varied with the volume of BSE, despite the fact that the salt concentration remained constant (S_4, P_4 to S_6, P_6). Thus, the shift in mobility of muscle actin induced by BSE does correlate with inhibition of polymerization.

To determine whether Fx was susceptible to proteolysis, aliquots of BSE were incubated for 15 min at 37°C with $160 \mu\text{g}$ of chymotrypsin or proteinase K per ml; both proteases abolished the shift in the mobility of actin, indicating that Fx is a protein or peptide. To determine whether Fx was acidic or basic in character, aliquots of boiled saponin extract were applied to microcolumns of DEAE- or CM-cellulose. The unbound material from CM-cellulose, but not from DEAE-cellulose, still shifted muscle actin to the high-mobility form, indicating that Fx binds to DEAE-cellulose and therefore has a net negative charge at neutral pH.

FIG. 3. Effect of saponin extract and BSE on the mobility of Aedans-labeled muscle G-actin in nondenaturing PAGE. Actin ($3 \mu\text{g}$) was added to $10 \mu\text{l}$ of fresh saponin extract or BSE. Fluorescence was photographed under longwave UV illumination. Lanes: 1 and 4, Aedans-actin alone; 2, Aedans-actin plus fresh saponin extract; 3, Aedans-actin plus BSE.

FIG. 4. Inhibition of actin polymerization by BSE. Aliquots ($67 \mu\text{l}$) of $7 \mu\text{M}$ muscle G-actin were mixed with the indicated volumes of high KCl buffer and BSE in high KCl buffer; after a 10-min incubation at room temperature, samples were centrifuged for 30 min at $100,000 \times g$ in a Beckman Airfuge. Samples for SDS/PAGE were prepared from both supernatants (lanes S) and pellets (lanes P); pellet volumes were adjusted to equal supernatant volumes. Despite the fact that the salt concentration remains constant, the proportion of actin remaining in the supernatant increases with increasing BSE. (13% gel stained with Coomassie blue.)

Since BSE is free of endogenous actin and most other proteins, it was used as the starting material for further purification of Fx. Preliminary experiments using open microcolumns showed that Fx could be adsorbed to and recov-

FIG. 5. Isolation of Fx from saponin extract by preparative HPLC. Fractions containing Fx activity were identified by their effect on the mobility of muscle actin in nondenaturing PAGE as shown in the Insets. (A) HPLC on diphenyl-silica: solvent A = 0.1% trifluoroacetic acid and solvent B = methanol. Fx was eluted by a gradient from 20% to 50% solvent B. (Inset) Lanes: 1, fresh saponin extract; 2, BSE plus muscle G-actin; 3, actin alone; 4, actin plus peak I; 5, actin plus peak II; 6, actin plus peak III. (B) Rechromatography of peak I on C_{18} -silica: solvent A = 0.1% trifluoroacetic acid and solvent B = acetonitrile. Fx was eluted by a gradient from 20% to 100% solvent B. (Inset) Lanes: 1, muscle G-actin alone; 2, actin plus peak I; 3, actin plus peak II.

FIG. 6. Stoichiometric shift of muscle actin to the high-mobility complex. Aliquots (3 μ g) of muscle G-actin were mixed with increasing volumes of Fx isolated on diphenyl-silica (peak I) and then analyzed by nondenaturing PAGE. (A) Lanes: 1 and 3, muscle actin alone; 2, saponin extract; 4–7, 3 μ g of actin plus 1.5, 3, 6, and 8 μ l of Fx, respectively (Coomassie blue stain). (B) Densitometry of A, lanes 4–7, showing the increasing proportion of actin migrating as the high-mobility complex.

ered from diphenyl-silica. After initial fractionation on an open column of diphenyl-silica, the material eluted by 50% methanol/0.05% trifluoroacetic acid was applied to a preparative HPLC column packed with the same material and was eluted with a trifluoroacetic acid/methanol gradient (Fig. 5A). Fractions from the diphenyl-silica column were evaporated, redissolved in water, and assayed for their ability to shift G-actin to the high-mobility form in native PAGE. Of three major peaks eluted in the gradient from 20% to 50% methanol, only peak I had activity (Fig. 5A and *Inset*). Upon rechromatography on C_{18} -silica (Fig. 5B), the active fraction from the diphenyl-silica column was resolved into two major peaks. Only peak I was active (Fig. 5B and *Inset*). Fx isolated by HPLC shifted muscle G-actin to the high-mobility form, in proportion to the quantity added (Fig. 6); adding sufficient Fx to shift all of the G-actin to the high-mobility form also completely inhibited the salt-induced polymerization of G-actin as assayed by low-shear viscometry (Fig. 7). When Fx was added to F-actin at the same ratio, no effect on viscosity was observed, indicating that Fx has no detectable severing activity (data not shown).

Fx isolated by HPLC was crosslinked to muscle G-actin by using the water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Fig. 8); the apparent molecular mass of the crosslinked complex is 47.5 kDa, suggesting that Fx has a molecular mass of about 5 kDa. When a fresh saponin extract was fractionated by gel filtration on Sepha-

FIG. 7. Effect of Fx on actin polymerization. Aliquots of muscle G-actin (7 μ M) were mixed with equal volumes of eluate fractions from the diphenyl-silica column; polymerization was initiated by addition of salt, and viscosity (v , in cm/sec) was measured at intervals as described. In the lower curve, the quantity of Fx (peak I) added was sufficient to shift all of the muscle actin to the high-mobility complex on nondenaturing PAGE. Similar results were obtained with Fx further purified on C_{18} -silica (not shown). The upper curve is the control eluate fraction, which did not shift muscle actin to the high-mobility complex.

FIG. 8. Crosslinking of Fx to muscle G-actin. An excess of Fx, purified by HPLC on diphenyl- and C_{18} -silica and redissolved in water was added to muscle G-actin in 3 mM triethanolamine hydrochloride/0.2 mM ATP/0.2 mM $CaCl_2$ /0.2 mM NaH_2PO_4 , pH 7.5. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide was freshly dissolved to 0.1 M in 0.5 M Mes buffer at pH 6.0; 1/10th volume was added to samples of actin alone (lane 2) or actin plus Fx (lane 3). The mixtures were incubated at room temperature for 1 hr and then in the cold overnight. Samples were then prepared for SDS/PAGE and analyzed on a 10% gel. Lane 1, unmodified actin.

dex G-50, Fx was eluted at a volume consistent with a molecular mass in the range of 5 kDa (data not shown). The optical absorbance spectrum of Fx at neutral pH (Fig. 9) is dominated by a peak centered at 198 nm, which effectively masks peptide bond absorbance (220–230-nm range); only a low shoulder is observed in the aromatic range (250–300 nm) even when the absorbance scale is expanded 10-fold. The observed absorbance ratio, $A_{198}/A_{280} = 25.9$, is consistent with the presence of tyrosine and phenylalanine but not tryptophan (32).

Amino acid analysis (Table 1) showed an unusually low proportion of hydrophobic amino acids and a high proportion of charged residues, with acidic residues predominating (amide groups were not determined). Tyrosine and phenylalanine are both present, consistent with the absorbance spectrum. The amino acid composition shows little resemblance to that of platelet profilin or *Acanthamoeba* actobindin. The excess of acidic residues (neglecting amidation) is consistent both with the affinity of Fx for anion- but not cation-exchange cellulose and with the fact that the actin-Fx complex has a higher electrophoretic mobility than that of G-actin alone.

DISCUSSION

Recent findings suggest that profilin and gelsolin occur in quantities too low to complex all of the unpolymerized actin in platelets and that these complexes form only after platelet activation (11–13, 17, 18; F. Stark, D.S., and V.T.N., unpublished data). Other known actin-sequestering proteins (35–38) have not been detected in platelets. We have now isolated and partially characterized a third actin-sequestering protein (or peptide) from platelets, which (i) shifts the mobility of muscle actin in nondenaturing PAGE to match that of unpolymerized platelet actin, (ii) inhibits that salt-induced polymerization of muscle G-actin as measured by sedimentation and by low-shear viscometry, and (iii) can be crosslinked covalently to muscle G-actin with high efficiency. We conclude that it sequesters the bulk of the unpolymerized actin in resting platelets. For convenience, we refer to it as Fx, until primary sequence data are available for comparison with other actin-sequestering proteins.

It is unlikely that Fx is a breakdown product of another actin-binding protein. Analysis of the supernatants and pellets of saponin-treated platelets by SDS/PAGE shows no proteolysis of actin binding protein, talin, or myosin. Fur-

FIG. 9. UV-visible absorbance spectrum of Fx isolated on C_{18} -silica. In the upper curve, the spectrum is replotted on a 10-fold-expanded absorbance scale.

Table 1. Amino acid composition of Fx isolated by reverse-phase HPLC

Amino acid residues	Fx*		Actobindin, [†] mol %	Profilin, [‡] mol %
	No. of residues	mol %		
Aspartate/asparagine	3.3 (3)	7.5	8.0	10.8
Glutamate/glutamine	10.1 (10)	23.2	15.9	5.8
Serine [§]	3.8 (4)	8.7	6.3	7.2
Glycine	1.1 (1)	2.6	6.1	11.5
Histidine	0 (0)	0	4.3	1.4
Arginine	0 (0)	0.6	2.4	3.6
Threonine [§]	3.4 (3)	7.7	3.4	7.9
Alanine	2.6 (3)	6.0	12.9	7.2
Proline	2.9 (3)	6.7	5.9	2.9
Tyrosine	0.8 (1)	1.7	0.2	3.6
Valine	0.1 (0)	0.3	9.9	9.4
Methionine	0.5 (0)	1.1	0.9	2.9
Isoleucine	1.9 (2)	4.3	4.4	3.6
Leucine	2.0 (2)	4.5	5.9	8.6
Phenylalanine	1.9 (2)	4.4	2.2	2.9
Lysine	8.9 (9)	20.4	9.7	7.2
Cysteine	ND ND	ND	0	2.2
Tryptophan [¶]	0 (0)	0	0	1.4
% hydrophobic		27.4	42.1	38.9
% charged		51.1	40.3	28.9
(neglecting amides)				

ND, not determined.

*Calculated for a molecular mass of 5 kDa and a total of 43 residues.

[†]Data for *Acanthamoeba* actobindin were taken from ref. 33.[‡]Data for human platelet profilin were calculated from ref. 34.[§]Serine and threonine residues were corrected for 10% and 5% destruction during hydrolysis.[¶]Determined spectrophotometrically.

thermore, the same change in mobility of Aedans-actin is induced by boiled supernatant from platelets that were lysed with ice-cold 1% Triton X-100 containing 10 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM diisopropyl fluorophosphate, 100 μ g of leupeptin per ml, 0.2 mg of soybean trypsin inhibitor per ml, and 10 mM benzamide in 10 mM imidazole (pH 7.0) and were immediately centrifuged.

To test for specificity of the Fx-actin interaction, we added Fx to tubulin and to actin on the same native gel. In this test Fx increased the mobility of actin but not of tubulin. Therefore, it does not appear to bind unpolymerized tubulin, at least under these conditions. By this technique we cannot determine the effect of Fx on intermediate filament proteins because of their insolubility or on spectrin because of its high molecular mass.

We suggest several reasons why Fx has not been detected previously. First, its low molecular mass and high solubility make it difficult to retain under conditions normally used to fix and stain polyacrylamide gels. Second, its activity is most easily followed on nondenaturing PAGE, a method that has been applied only recently to this problem. Third, the state of actin depends critically on the state of the platelets: our own unpublished observations indicate that the actin-Fx complex disappears and profilactin and actin-gelsolin appear after prolonged storage of the platelets or after lysis by sonication in 0.1 mM CaCl_2 , as in the preparation of platelet profilactin (11).

Our results suggest that the actin-Fx complex is the true storage form of actin in resting platelets. It should be interesting to determine how widely Fx or similar peptides are distributed in other cell types.

Note Added in Proof. In collaboration with Dr. Sally Zigmond (University of Pennsylvania), we confirmed the inhibition of actin polymerization by Fx by using pyrene-labeled muscle G-actin.

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