Platelet Activation Induces the Formation of a Stable Gelsolin-Actin Complex from Monomeric Gelsolin*

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We have studied the interactions between gelsolin and actin in crude extracts from activated and unactivated platelets and in mixtures of purified platelet gelsolin and muscle actin. Extracts were prepared using 10 mM EGTA from human platelets treated either with 100 μM aspirin and 2.5 mM tetracaine to retard activation or with the calcium ionophore A23187 to effect activation. The extracts were fractionated by gel filtration on Sephadex G-150 or by sedimentation on sucrose gradients and then analyzed using anti-gelsolin immunoblots and actin filament nucleation assays. The nucleation activity in both extracts was associated with gelsolin. The activity in the extracts from unactivated platelets sedimented with an S value of 5.2 and had an Mr = 90,000. The activity in the extracts prepared with EGTA from activated platelets sedimented at 6.8 S and had an Mr = 130,000. We have shown previously that the Mr = 130,000 species is an EGTA-stable binary complex of one actin and one gelsolin. Transient exposure of the extracts from unactivated platelets to 100 μM Ca2+ demonstrated higher order complexes including a ternary complex with a sedimentation constant of 8.2 S and an Mr = 165,000. Sedimentation and gel filtration experiments using purified platelet gelsolin and rabbit skeletal muscle actin demonstrated that formation of the EGTA-stable binary complex required Ca2+. Fractionation in the presence of 100 μM Ca2+ demonstrated that, in the presence of EGTA', the formation of the binary complex occurs in the presence of Ca2+. Fractionation in the presence of 100 μM Ca2+ demonstrated higher order complexes including a ternary complex with a sedimentation constant of 8.2 S and an Mr = 165,000. Sedimentation and gel filtration experiments using purified platelet gelsolin and rabbit skeletal muscle actin demonstrated that formation of the EGTA-stable binary complex required Ca2+. At least one additional actin is bound to the binary complex in the presence of Ca2+ but is not sufficiently stable to be purified when EGTA is added. The results suggest that gelsolin exists either as a monomer or perhaps as a weak complex with actin in unactivated platelets but complexes tightly with actin during the transient Ca2+ rise that occurs during activation.

Platelets are an excellent system for studying the regulation of the cytoskeletal proteins involved in contractile events. Unactivated platelets are smooth round discs which contain relatively few actin filaments when studied by the electron microscope or when viewed using biochemical techniques (1–4). Activated platelets, on the other hand, are irregularly shaped cells with many protrusions and spiky filopodia containing numerous actin filaments (5, 6). Preparation of a nonionic, detergent-insoluble "cytoskeletal" network from these activated cells yields a sedimentable aggregate consisting of actin, myosin, actin-binding protein, α-actinin, as well as other minor components (7, 8) thought to be involved in the formation of the filopodia in the early stages of activation and in the process of granule secretion and clot retraction during the later stages of platelet function.

Intracellular calcium fluxes are postulated to play an important role in activating the processes leading to actin polymerization (9) and filopod formation, as well as myosin phosphorylation (10), bipolar filament formation (11), and granule secretion (12). Increases in intracellular free calcium levels preceding shape changes and exocytosis have been documented (13, 14), and drugs that block calcium fluxes, such as local anesthetics, prevent or retard platelet activation (15, 16). Ca2+ ionophores, on the other hand, activate platelets fully (17–19). Drugs that inhibit cyclooxygenase, such as aspirin and indomethacin, also block platelet activation, perhaps by preventing the production of an intracellular Ca2+ ionophore (20). The interactions of actin and actin-binding proteins during platelet activation have been compared by using these drugs to either prevent or stimulate maximal platelet activation.

We have been characterizing one actin-associated protein, platelet gelsolin, a 90-kDa actin filament-capping protein implicated in the regulation of actin filament formation (21, 22). To investigate the role of this molecule in platelet activation, we have compared its behavior in extracts from unactivated and fully activated platelets. In this report, we show that platelet gelsolin behaves as a 90-kDa monomer in extracts from aspirin- and tetracaine-treated human platelets. Activation with the ionophore A23187, ADP, or thrombin resulted in the formation of the 130-kDa, EGTA-stable gelsolin-actin complex that we described previously (22). This aggregation proceeds further in the presence of Ca2+, and we have partially characterized a ternary complex.

The formation of the 130-kDa complex in crude extracts appears to require micromolar levels of calcium, and we have used purified platelet gelsolin and purified rabbit skeletal muscle actin to investigate further the role of calcium in the formation of gelsolin-actin complexes in reconstitution experiments. We have confirmed that Ca2+ is required to form the EGTA-stable gelsolin-actin complex and that, in the presence of micromolar free calcium, this binary complex can bind an additional actin to form a ternary species of gelsolin plus two actin molecules under low ionic strength conditions. The

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results demonstrate that, during the transient rise in cytoplasmic free Ca^2+ that occurs during platelet activation, gel-solin-actin complexes form and may be important in the regulation of actin filament polymerization. Portions of this work have been reported in abstract form (23, 24).

MATERIALS AND METHODS

Platelet Extract Preparation—We will use the term "unactivated" platelets to refer to cells treated with cyclooxygenase inhibitors and/or local anesthetics. These platelets are characterized by the absence of protrusions, filopodia, and platelet clumps when viewed by phase-contrast microscopy. We will use the term "activated" platelets to refer to cells treated with the ionophore A23187, ADP, or thrombin. These preparations are characterized by the presence of large clumps of platelets that have protrusions and filopodia.

Fresh platelet concentrates were used within 48 h after drawing blood from donors. Unactivated platelets were preincubated with 100 μM aspirin and/or 2.5 mM tetracaine in their original plastic bags for at least 1 h at room temperature with gentle rotation. Red and white blood cells were removed by spinning for 5 min at 200 × g in a clinical centrifuge. Platelets were pelleted from the serum by centrifuging at 2,000 × g for 15 min at room temperature and washed once in 125 mM NaCl, 5 mM KCl, 0.1 mM EGTA, 20 mM phosphate, pH 6.5, plus 1 mg/ml of glucose. The washed pellet was resuspended in 100 mM NaCl, 5 mM KCl, 0.5 mM MgCl2, 0.5 mM EGTA, 75 mM PIPES, pH 6.5, and 1 mg/ml of glucose at a final volume of 10 ml/unit of platelets.

The platelets were then incubated for 30-45 min at 37°C. Where appropriate, platelets were activated during the final 20 min of incubation by addition of A23187 to 5 μg/ml. EGTA was added to make the final solution 10 mM, and the platelets were lysed by nitrogen cavitation using a Parr bomb. The platelet suspension was incubated in the bomb at 1000 pounds/square inch of nitrogen pressure for 30 min at room temperature, released, and incubated for another 30 min. Greater than 85% of the platelets were lysed after the second incubation. A crude platelet gelsolin extract was prepared from the lysate by centrifuging at 20,000 × g for 15 min in a Sorvall SS 34 rotor. The final protein concentration of the supernatant was between 2 and 3 mg/ml.

Purification of Platelet Gelsolin—The platelet gelsolin-actin complex was purified using DNase I affinity chromatography as described by Wang and Bryan (27) or by the chromatographic techniques described by Kurth et al. (22). Where necessary, gel filtration on Sephadex G-150 was used to remove breakdown products of gelsolin. DEAE-Sephadex chromatography in 6 M urea, as described in the companion paper by Bryan and Kurth (25), was used to separate platelet gelsolin from platelet actin. The gelsolin pool used for all further experiments was greater than 90% pure, with minor contaminants consisting of breakdown products identified using monospecific antibodies and immunoblots. Contamination with actin was less than 3%, as determined by SDS-polyacrylamide gel electrophoresis.

Actin Purification—Actin was purified from rabbit skeletal muscle by the procedure of Spudich and Watt (26) and then gel-filtered on Sephadex G-150 following the procedures of MacLean-Fletcher and Pollard (27).

Gel Filtration Chromatography and Stokes Radius Determination—Platelet extracts were analyzed by gel filtration on a column (2 × 80 cm) of Sephadex G-150 (40-120-μm size) (Pharmacia, Uppsala, Sweden). The columns were calibrated using dextran blue (void volume), bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease from Pharmacia and aldolase from Boehringer Mannheim. The column was equilibrated with 15 mM Tris-HCl, pH 7.5, containing 125 mM NaCl, 5 mM KCl, 0.5 mM EGTA, and 2 mM EGTA. 3-ml of the platelet extract were applied to the column, and 3-ml fractions were collected at a flow rate of about 25 ml/h.

Reconstituted mixtures were analyzed by gel filtration on a column (1 × 47 cm) of Sephadex G-150 (10-40-μm size). The columns were calibrated with the standards described above as well as lactacystin, maleic hydrazide, and cytochrome c. The columns were calibrated with 15 mM Tris-HCl, pH 7.5, 50 mM NaCl, 100 μM MgCl2, and either 100 μM CaCl2 or 1 mM EGTA. Usually a mixture of actin, gelsolin, lactate dehydrogenase, and maleic hydrazide was eluted at a flow rate of 250 μl/min. The column and 0.5-ml fractions were collected at a flow rate of about 4 ml/h. The fractions were analyzed for actin, gelsolin, and the standard proteins by scanning 10% SDS-polyacrylamide gels stained with Coomassie blue with a Kontes Fiberglas Optic Scanner connected to a Hewlett-Packard 3390A Integrator.

Actin Nucleation Assay—The rate of fluorescence increase of NBD-labeled rabbit skeletal muscle actin was used to measure nucleation of actin filaments as described previously (22). NBD-actin was prepared according to Detmers et al. (28). Column eluates were assayed as follows: 500 μl of each fraction were mixed with an equal volume of 100 mM Tris-HCl, pH 7.5, 2 mM CaCl2, 50 mM KCl, and 4 mM ATP. Polymerization was initiated by addition of 25 μl of a 5-mg/ml solution of NBD-actin in 0.2 mM CaCl2, 0.2 mM ATP, 16 mM Tris-HCl, pH 8.0, and the rate of fluorescence increase was measured in an Aminco SPF-500 spectrofluorometer, temperature regulated at 25°C. The excitation wavelength was 475 nm; the emission wavelength was 530 nm. In order to compare nucleation activity from different batches of NBD-actin, the activities were normalized to "relative units" by the formula:

\[
\text{Relative units} = \frac{\text{experimental rate}}{\text{control rate}} - 1
\]

where the control rate is the rate of NBD-actin polymerization in the presence of the buffer solution alone.

Sedimentation Analysis—5-26% sucrose isokinetic gradient experiments were done using the method of Martin and Ames (29). Briefly, 200 μl of platelet extract were layered over a 4-6.6% sucrose gradient in 15 mM Tris-HCl, 0.5 mM EGTA, 2 mM CaCl2, 5 mM MgCl2, and either 2 mM EGTA or 100 μM CaCl2, Catalase (60 μg), fumarase (50 μg), malate dehydrogenase (50 μg), and cytochrome c (200 μg) were used as internal standards. The gradient was centrifuged in a Beckman SW 50.2 rotor at 100,000 × g for 16 h, and then separated into 24 500-μl fractions. The positions of the internal standards and gelsolin were determined using SDS-polyacrylamide gels scanned at 550 nm, as indicated above.

Preparation of Antiserum against Platelet Gelsolin—Rabbit anti-gelsolin serum was prepared as described previously (22). Briefly, New Zealand White rabbits were injected subcutaneously with 29-50 μg of purified platelet gelsolin that had been cut out of SDS-polyacrylamide gels and homogenized with complete Freund's adjuvant. Antibody titers were determined 2 weeks after each monthly booster by an enzyme-linked immunosorbent assay.

Miscellaneous—SDS-polyacrylamide gels were run according to Laemmli (30). Immunoblots were done according to Towbin et al. (31). The 125I-actin overlay to detect actin-binding proteins in Laemmli SDS gels was done according to Snipes et al. (32). Protein concentrations were determined by a modification of the Bradford (32) method using bovine serum albumin as a standard.

RESULTS

We have previously shown that partially or partially activated platelet extracts have a single major activity that can nucleate the growth of actin filaments. This activity is associated with a one to one complex of actin and platelet gelsolin in unactivated platelets. In order to study the gelsolin further, we have compared crude extracts from aspirin- and tetracaine-treated platelets with crude extracts from A23187-activated platelets using Sephadex G-150 column chromatography in the presence of 2 mM EGTA. Fig. 1 shows the protein elution profile and NBD-actin nucleation activity of equivalent amounts of extract from unactivated (Fig. 1A) and fully activated (Fig. 1B) platelets. The NBD-actin nucleation activity from unactivated platelets eluted at about 90 kDa; the main activity from activated platelets eluted at about 130 kDa. A comparison of the areas under the nucleation activity peaks from activated and unactivated platelet extracts showed that both contained essentially the same amount of activity.

Figs. 2 and 3 show the distribution of proteins seen with SDS-polyacrylamide gel electrophoresis. The Coomassie blue-stained gels appear relatively similar when fractions from unactivated and activated extracts are compared (Fig. 2A versus Fig. 3A). However, the 125I-actin overlay (32) for actin-binding proteins (Fig. 2B versus Fig. 3B) and an immunoblot specific for platelet gelsolin (Fig. 2C versus Fig. 3C) demonstrate a shift in the elution volume of platelet gelsolin. The
apparent molecular weight of the two forms, as determined on a calibrated G-150 column, was approximately 90 kDa for the unactivated form and about 130 kDa for the form in extracts from activated platelets. This shift in elution volume, as detected by nucleation activity, 125I-actin overlays, and gelsolin-specific immunoblots, was a consistent observation in 13 experiments. Two other actin-binding proteins, with molecular weights of 40,000 and 58,000, are detected with 125I-actin. These have been reported on previously, but their origin and function are unknown (33). The behavior of the 130-kDa complex is identical to the material we described previously. The results suggest that platelet gelsolin interacts with actin during platelet activation and that the one to one complex is stable in the EGTA buffers used during fractionation. If complexes exist in the unactivated platelets, they do not appear to be stable in EGTA.

We have used this analysis to study the effects of several pharmacological agents known to affect platelet activation. Table I summarizes these results. In general, treatment of platelets with agents that are known to block platelet activation, such as aspirin, indomethacin, lidocaine, and tetracaine, gave extracts in which the major nucleation activity was associated with a 90-kDa species. Aspirin and tetracaine together seemed to act synergistically since this treatment consistently gave a large 90-kDa peak of nucleation activity associated with gelsolin. Indomethacin could substitute for aspirin in preventing the formation of the 130-kDa complex during routine manipulations. Agents which are known to activate platelets, such as thrombin, ADP, and the Ca2+-ionophore A23187, consistently caused the formation of the 130-kDa gelsolin-actin complex. A23187 promoted complete activation and formation of the 130-kDa complex even after the platelets had been treated with aspirin and tetracaine. The addition of aspirin and tetracaine to activated extracts had no effect on the 130-kDa complex. Transient Ca2+ fluxes have been implicated in platelet activation, and the interaction of actin and gelsolin is Ca2+-sensitive (34, 35), suggesting that elevated Ca2+ levels might be the signal causing the formation of the gelsolin-actin complexes. To test this hypothesis, we raised the free Ca2+ concentration of extracts from aspirin- and tetracaine-treated platelets to 0.2–1.0 mM for approximately 10 min before loading onto a gel filtration column equilibrated with EGTA-containing buffers. A comparable untreated extract was placed on a second column and was run under identical conditions. Fig. 4 illustrates the protein profiles and nucleation activities from this experiment. The majority of the nucleation activity from unactivated platelets not treated with Ca2+ eluted at 90 kDa, while a Ca2+-treated extract showed an equivalent amount of nucleation activity eluting at 130 kDa. In all of these experiments, immunoblots confirmed that gelsolin had shifted from an elution volume at 90 kDa to one at 130 kDa.

Sucrose gradient sedimentation experiments were run to measure the relative sedimentation coefficients of the complexes formed during platelet activation. Fig. 5 shows the results of such an experiment. Extracts from aspirin- and tetracaine-treated platelets were centrifuged through a 5–20% sucrose gradient in the presence of 2 mM EGTA before or after treatment with 100 μM free Ca2+. Alternatively, an aliquot of the extract treated with Ca2+ was centrifuged through an equivalent gradient containing 100 μM free Ca2+. Gelsolin from the unactivated platelet extract sedimented at 5.2 S (Fig. 5, inset, lane A). Treatment of the extract with Ca2+ resulted in the formation of a larger, 6.8 S complex that was stable in EGTA (lane B). A still larger complex, sedimenting at 8.2 S, was observed in the presence of 100 μM Ca2+ (lane C), conditions similar to those used in the NBD-actin nucleation assay. These results confirm the gel filtration data and demonstrate that Ca2+ is required to form the gelsolin-actin complex which is then EGTA-stable. Furthermore, the results indicate that a larger complex or complexes form when Ca2+ is added, but these are not stable in EGTA.
Reconstitution Experiments using Purified Components—In order to investigate these interactions in more detail, we used purified platelet gelsolin and purified rabbit skeletal muscle actin in a series of reconstitution experiments. Gel filtration experiments were done using Sephadex G-150 superfine calibrated with internal standards to determine the Stokes radius of gelsolin in the presence and absence of actin with and without micromolar concentrations of free Ca2+. In addition, mixtures of gelsolin, actin, and standards were centrifuged on 5-20% sucrose gradients containing either 2 mM EGTA or 100 μM calcium for 15 h at 100,000 × g. Gel filtration of fractions of gelsolin alone, but slightly smaller than the 4.55 nm Stokes radius measured for the same mixture chromatographed in 100 μM calcium. Quantitative scans of Coomassie blue-stained SDS-polyacrylamide gels showed the 4.4 nm species was a 1:1 complex of gelsolin and actin equivalent to what we have described previously (22).

Finally, to see if the larger 4.6 nm complex formed in the presence of 100 μM Ca2+ was dissociable with EGTA, we chromatographed the mixture both with and without added EGTA. In the presence of EGTA, the eluting species had a Stokes radius of 4.45 nm; while in the presence of 100 μM EGTA-stable complex with a Stokes radius of 4.4 nm. This was significantly larger than the value of 4.1 nm for gelsolin alone, but slightly smaller than the 4.55 nm Stokes radius measured for the same mixture chromatographed in 100 μM calcium. Quantitative scans of Coomassie blue-stained SDS-polyacrylamide gels showed the 4.4 nm species was a 1:1 complex of gelsolin and actin equivalent to what we have described previously (22).
Ca\textsuperscript{2+}, a slightly larger value of 4.6 nm was found. Upon rechromatography, the purified 1:1 gelsolin-actin complex, obtained by gel filtration in the presence of EGTA, eluted with a Stokes radius of 4.45 nm in either the presence or absence of Ca\textsuperscript{2+}, indicating that the 4.6 nm species was not due to a Ca\textsuperscript{2+}-induced shape change. Scans of the SDS gels showed that the 4.6 nm complex had an actin/gelsolin stoichiometry of about 2:1. Taken together, the gel filtration results showed that Ca\textsuperscript{2+} is required to form the EGTA-stable gelsolin-actin complex of 4.45 nm. This binary complex is identical to the material found in activated platelets, apparently is the most stable species when EGTA buffers are used, and is the species purified from expired platelets (22). If the fractionation is done in the presence of Ca\textsuperscript{2+}, a larger complex of 4.6 nm is formed. The gel scans show that the 4.6 nm species is a ternary complex. This species and any larger complexes are not sufficiently stable in EGTA to be purified by gel filtration.

The sedimentation experiments show that gelsolin in the presence or absence of Ca\textsuperscript{2+} sedimented with an S value of 5.2. This corresponds to the particle with a 4.2 nm Stokes radius. Gelsolin preincubated with actin in the presence of EGTA also sedimented at 5.2 S. The same mixture preincubated in the presence of Ca\textsuperscript{2+} and sedimented in 100 \mu M Ca\textsuperscript{2+} gave evidence of a larger complex at 8.4 S that corresponds to the particle of 4.6 nm Stokes radius. This shift in sedimentation values confirmed the gel filtration results and showed that Ca\textsuperscript{2+} alone did not alter the sedimentation value of gelsolin. When gelsolin was preincubated with an excess of actin in the presence of 100 \mu M calcium and then analyzed on sucrose gradients containing EGTA, a stable gelsolin-actin complex sedimenting at 6.8 S corresponds to the particle with a 4.6-4.5 nm Stokes radius is detected. In the presence of 100 \mu M Ca\textsuperscript{2+}, sedimentation of the same mixture showed the presence of the larger complex at 8.3 S.

Finally, the purified binary complex, obtained by gel filtration, was preincubated with excess actin in the presence of Ca\textsuperscript{2+} to establish the relationship between the complexes identified by gel filtration and the 6.8 and 8.3 S species. In the presence of 100 \mu M Ca\textsuperscript{2+}, conditions where the ternary complex was observed by gel filtration, the 8.3 S species was seen. In the presence of 2 mM EGTA, where the binary complex is stable, the 6.8 S species was seen. SDS-gel scans of the various fractions confirmed that the binary complex has a sedimentation coefficient of 6.8 S and that the ternary complex had a value of 8.3 S. These results show that a stable 8.3 S species can form from actin and the 6.8 S species if Ca\textsuperscript{2+} is present.

Table II summarizes the results of these experiments. Gelsolin, in both the presence and absence of calcium, had an average Stokes radius of 4.08 \pm 0.06 nm and an average sedimentation coefficient of 5.28 \pm 0.15 S. We calculated a molecular mass of 93,000 Da using the equation taken from Siegel and Monty (36).

$$M_r = \frac{6 \pi \eta R_s s_{20,w} N}{1 - \nu p}$$

$M_r$ is the relative molecular mass; $\nu$ is the partial specific volume; $\rho$ is the solvent density; $\eta$ is the solvent viscosity in poise; $N$ is Avogadro’s number; $R_s$ is the measured Stokes radius; and $s_{20,w}$ is the measured coefficient corrected to water at 20 °C. The binary complex has a Stokes radius of 4.44 \pm 0.02 nm, a sedimentation coefficient of 6.72 \pm 0.17 S, and a calculated molecular mass of 129,900 Da. The ternary species has a Stokes radius of 4.55 \pm 0.05 nm, a sedimentation coefficient of 8.28 \pm 0.11 S, and a calculated molecular mass of 164,000 Da. A partial specific volume of 0.73 was assumed for both gelsolin and the gelsolin-actin complexes.

**DISCUSSION**

The results presented in this report show that calcium concentrations in the micromolar range allow platelet gelsolin and actin to form stable complexes. These complexes were identified by their hydrodynamic properties both in crude extracts from activated platelets and in reconstitution experiments using purified gelsolin, actin, and \mu M Ca\textsuperscript{2+}. Unactivated platelets appear to contain the majority of their gelsolin in the uncomplexed form.
with washed, and lysed using the Parr bomb. Three ml of the extract were of gelsolin from stabilized platelet extract.

\[ \text{Gelsolin} \]
\[ \text{Gelsolin/actin} = \frac{0.73}{(1 - \phi)} \]
\[ \phi = 0.73 \text{ from Siegel and Monty (36).} \]

Once the binary complex has formed in the presence of Ca\(^{2+}\), a ternary complex can form that is stable during gel filtration and sucrose sedimentation experiments. Only a small difference in the Stokes radius is observed between the binary and ternary complexes, 4.4 \text{ versus} 4.5 \text{ nm}, although there is a relatively large shift in the sedimentation coefficient from 6.7 to 8.3 S. Control experiments, in which the binary complex sedimented at 6.7 S in the presence and absence of 100 \( \mu \)M Ca\(^{2+}\), as well as titration experiments with fluorescently labeled actin described in the accompanying paper (25), eliminate the possibility that a conformational change of the binary complex is responsible for the increased sedimentation coefficient.

Markey et al. (38) reported that the platelet gelsolin-actin complex gave “anomalous” gel filtration results in the presence of Ca\(^{2+}\). The formation of the ternary complex and larger complexes can explain these observations adequately. Their findings included little or no increase in Stokes radius, but an increased amount of actin co-eluting with gelsolin in the presence of micromolar concentrations of Ca\(^{2+}\). These results are consistent with the formation of the ternary complex of two actin molecules and gelsolin that we have shown has only a minor change in the Stokes radius when compared to binary complex.

The finding that unactivated platelets contain primarily the monomeric form of gelsolin, while activated platelets contain gelsolin tightly complexed to actin, suggests that the formation of gelsolin-actin complexes is an activation-specific event. Our results suggest that the Ca\(^{2+}\) flux during platelet activation is the most likely trigger for the formation of these complexes. The arguments in support of this hypothesis are as follows. 1) Agents that cause platelet activation and the formation of the binary complex are known to elicit increased Ca\(^{2+}\) levels in the platelet cytosol, while drugs that prevent Ca\(^{2+}\) fluxes block platelet activation and complex formation (13-19). 2) A brief exposure of extracts from unactivated platelets to elevated Ca\(^{2+}\) levels generates the binary complex, while the untreated extracts still contain uncomplexed gelsolin. 3) Finally, using purified gelsolin and purified actin in reconstitution experiments demonstrates that Ca\(^{2+}\) exposure is sufficient to mimic the results from the crude platelet extracts. These results argue strongly that the transient increase in Ca\(^{2+}\) levels during platelet activation is the sole trigger required to form gelsolin-actin complexes. The question of whether the binary complex is broken in vivo is unresolved, but the ability to purify this complex in a lengthy procedure using high and low salt concentrations and millimolar concentrations of EGTA suggests that the formation of the complex is irreversible on the time scale of platelet activation unless there are other proteins involved. At this time, no evidence exists that would suggest the presence of factors that dissociate the complexes. It is also unclear whether transient gelsolin-actin complexes form in unactivated platelets in low calcium that are not stable using our isolation techniques. Finally, the precise function of these complexes in platelets remains unknown.
Monomeric Platelet Gelsolin in Unactivated Platelets

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