Human platelets contain gelsolin. A regulator of actin filament length.

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Research Article

Morphologic and biochemical studies suggest that actin in human platelets polymerizes in response to various stimuli and that shortening of actin filaments can be regulated by calcium. We report that human platelets contain gelsolin, a protein of Mr 91,000 that binds reversibly to actin in the presence of calcium. Platelet gelsolin exhibits immunologic crossreactivity with rabbit macrophage gelsolin and shortens actin filaments as demonstrated by viscosity measurements and gel point determinations. Gelsolin is active in micromolar calcium concentrations and its effects upon actin filaments are reversible. Gelsolin may be a dynamic regulator of actin filament length in the human platelet.

RAPID

PUBLICATIONS

Human Platelets Contain Gelsolin

A REGULATOR OF ACTIN FILAMENT LENGTH

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ABSTRACT Morphologic and biochemical studies suggest that actin in human platelets polymerizes in response to various stimuli and that shortening of actin filaments can be regulated by calcium. We report that human platelets contain gelsolin, a protein of M_r 91,000 that binds reversibly to actin in the presence of calcium. Platelet gelsolin exhibits immunologic crossreactivity with rabbit macrophage gelsolin and shortens actin filaments as demonstrated by viscosity measurements and gel point determinations. Gelsolin is active in micromolar calcium concentrations and its effects upon actin filaments are reversible. Gelsolin may be a dynamic regulator of actin filament length in the human platelet.

INTRODUCTION

Actin, one of the most abundant proteins of the human platelet, is a globular monomer that can assemble into filamentous polymers (1). Resting, discoid platelets have very few actin filaments visible in the cytoplasm when examined in the electron microscope. After activation with agents such as ADP or thrombin, platelets contain easily detectable actin microfilaments organized in nets and bundles, implying that the actin has assembled from monomers or oligomers into long filaments (2). There are more long actin filaments in extracts of stimulated platelets than in extracts of unstimulated platelets, provided the calcium concentration is low. Treatment of a stimulated cell extract with calcium reduces the amount of actin in long filaments to levels observed in unstimulated platelet extracts, suggesting that actin polymerization within the platelet is reversible and that calcium is able to mediate a disassembly of actin filaments (3, 4). Several groups have found that platelets contain a calcium-regulated protein with M_r 90–95,000 capable of binding to and shortening actin filaments (5–7).

In this paper we report that human platelets contain gelsolin, a protein first isolated from rabbit macrophages. Platelet gelsolin, M_r 91,000, is capable of binding to and shortening actin filaments in a reversible manner, and is regulated by micromolar concentrations of calcium.

METHODS

Human platelet concentrates were obtained from the blood bank and used within 48–96 h after collection and storage in acid citrate dextrose at 4°C. The platelet suspensions were centrifuged twice at 150 *g* for 10 min at 4°C to remove contaminating erythrocytes and leukocytes. The resulting cell preparation was monitored by phase microscopy and found to contain <0.01% leukocytes. The platelets were collected by centrifugation at 2,200 *g* for 20 min at 4°C and resuspended in a solution containing 0.15 M NaCl, 10 mM potassium phosphate buffer, pH 7.4. 5 mM *N*-ethyl maleimide was added to inhibit the calcium protease of platelets (8). After centrifugation at 2,200 *g* for 20 min at 4°C, the cell pellet was suspended in an equal volume of a solution containing 0.35 M sucrose, 1 mM phenylmethylsulfonyl flouride, 100 μg/ml leupeptin, 5 mM dithiothreitol, 2 mM ATP, 5 mM EGTA, 20 mM imidazole HCl, pH 7.5. The cells were sonicated on ice in 5-s bursts for a total of 30 s and then centrifuged at 10,000 *g* for 15 min at 4°C. The supernatant fluid was designated the platelet extract. The extract

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was made 8 mM with $CaCl_2$ and dialyzed against a solution containing 20 mM imidazole HCl, 1 mM dithiothreitol, 0.5 mM ATP, pH 7.8 (buffer A), with 3 mM $CaCl_2$. DNase-Sepharose column was prepared by the method of Bretscher and Weber (9) and 30 ml of this resin was washed with buffer A containing 3 mM $CaCl_2$. After passing the extract over the column, the column was washed with 400 ml of buffer A containing 0.6 M NaCl and 3 mM $CaCl_2$ to remove protein nonspecifically bound to the actin-gelsolin-DNase complexes.

The column was then washed with buffer A containing 5 mM EGTA and 0.15 M NaCl, and the protein eluted was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis performed by the method of Laemmli (10). Coomassie Blue-stained gels were scanned with a densitometer (EZ Gel Scanner, EZ Em. Co. Inc., Westbury, NY).

Rabbit skeletal muscle actin was prepared by the method of Spudich and Watt (11) and polymerized by the addition of KCl to a final concentration of 0.1 M. Rabbit macrophage actin-binding protein and gelsolin were prepared as described (12). The free calcium concentrations, viscosity, and gel point determinations were made as described (12). The Lowry method was used to determine protein concentrations (13).

The immune gel overlay technique of Towbin (14) was used to identify gelsolin in polyacrylamide gels of platelet extracts and column fractions. An antibody-to-rabbit macrophage gelsolin was made by injecting this protein into goats with Freund's complete adjuvant (15). Polypeptides were transferred to nitrocellulose paper after separation by electrophoresis in SDS-polyacrylamide gels. The nitrocellulose paper was incubated with the goat antigelsolin antibody, followed by a secondary rabbit anti-goat antibody coupled to peroxidase (N. L. Cappel Laboratories Inc., Cochranville, PA). Immunologic crossreactivity was demonstrated by formation of a reaction product at the site of antibody binding upon addition of hydrogen peroxide and o-dianisidine to the washed nitrocellulose paper.

RESULTS

Gelsolin was localized in human platelets by affinity chromatography on a DNase-Sepharose column. The isolation procedure was dependent upon the reversible binding of gelsolin to actin in the presence of calcium. Chelation of calcium with EGTA caused two polypeptides to elute from the column, as assessed by electrophoresis of column fractions in SDS-polyacrylamide gels. As shown in Fig. 1A, a polypeptide with M_r 91,000 was eluted with a second polypeptide, M_r 43,000. The 43,000-dalton polypeptide comigrated with rabbit skeletal muscle actin and is presumed to be endogenous platelet actin.

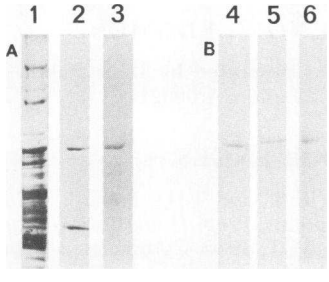

FIGURE 1 Identification of gelsolin in human platelets. (A) A 5–15% SDS-polyacrylamide gel after electrophoresis and staining with Coomassie Blue. Lane 1, platelet extract (0.1 mg); lane 2, a fraction eluted from the DNase-Sepharose column with EGTA-containing buffer (0.004 mg); lane 3, rabbit macrophage gelsolin (0.002 mg). (B) Immune gel replica of the samples shown in A. Immunologic crossreactivity between human platelet and rabbit macrophage gelsolin is demonstrated. Lane 4, platelet extract; lane 5, eluate from the DNase-Sepharose column; lane 6, macrophage gelsolin.

The 91,000-mol wt polypeptide was immunologically crossreactive with rabbit macrophage gelsolin, as shown in Fig. 1B. Antibody to rabbit macrophage gelsolin raised in goats reacted with the 91,000-mol wt polypeptide eluted from the DNase-Sepharose column and with a single polypeptide of similar electrophoretic mobility in the platelet extract. Because the 91,000-mol wt platelet polypeptide binds reversibly to actin in the presence of calcium, comigrates with rabbit macrophage gelsolin on SDS-polyacrylamide gels, and crossreacts with a specific antibody against rabbit gelsolin, we conclude that it is gelsolin.

When the eluate from the DNase-Sepharose column (with a calculated gelsolin concentration of 160 nM) was added to a solution of rabbit skeletal muscle F-actin (final F-actin concentration of 23 μM) in the presence of 0.01 mM $CaCl_2$, the viscosity, as measured in the Ostwald viscometer, was half of that of an identical solution prepared in 0.01 mM EGTA. Gelsolin's action was rapid, the greater part of its activity being apparent as quickly as conditions allowed measurement, and equilibrium was reached 10 min after mixing.

The concentration of a crosslinking agent required to bring about the abrupt transformation of a polymer solution from a sol to a gel state is inversely related to the length of the filaments. It has been shown experimentally that changes in this critical crosslinker concentration reflect directly changes in actin filament length. We found previously that rabbit macrophage gelsolin changes the gel point of crosslinked actin networks, and this change correlates with a change in the length of the actin filaments (10). Rabbit skeletal muscle actin was crosslinked with rabbit macrophage actin-binding protein (ABP)[1], a protein also present in human platelets (4). The critical crosslinker concentration of a 23-μM F-actin solution was 55 nM ABP. When the EGTA eluate from the DNase column was added so that the molar ratio of actin to gelsolin was 468 to 1, the gel point rose to 220 nM ABP, as shown in Fig. 2. The critical crosslinker concentration rose

[1] *Abbreviation used in this paper*: ABP, actin binding protein.

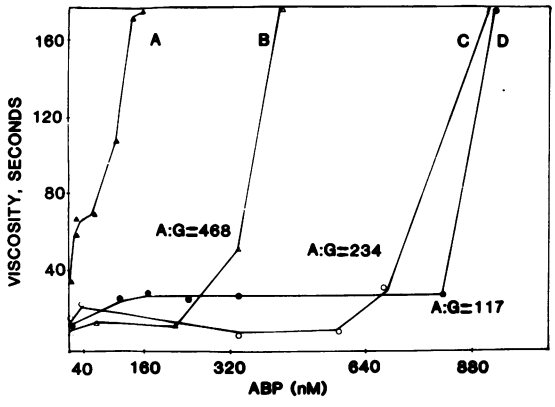

FIGURE 2 Effect of gelsolin on the gelation of actin by actin-binding protein. The apparent viscosity, determined in a low shear falling ball viscometer and expressed in seconds, is shown as a function of the ABP concentration added to a 16-μM actin solution. The molar ratios of actin to gelsolin are shown. (A) No gelsolin. (B) 50 nM gelsolin. (C) 100 nM gelsolin. (D) 200 nM gelsolin. Experiments were performed in a buffer containing 10 mM imidazole HCl, 0.1 M KCl, and 0.01 mM $CaCl_2$, pH 7.4, at 20°C.

in direct proportion to the added gelsolin concentration.

Fig. 3 shows the dependence of gelsolin's activity on the free calcium concentration. Gelsolin is inactive when the free calcium concentration is 10 nM, but is able to shorten actin filaments as demonstrated by a reduction in the viscosity of an actin solution when calcium is present in micromolar concentrations.

To show that gelsolin's effect upon actin filaments is reversible, a solution of gelsolin (0.2 μM) and F-actin (23 μM) was incubated for 15 min in the presence of 0.01 mM $CaCl_2$, and the viscosity was measured with the Ostwald viscometer Cannon-Manning Co., College Park, PA. Concentrated EGTA was added to a final concentration of 1 mM to chelate the calcium. The viscosity of the solution returned to that of control (an identical solution prepared in 0.01 mM EGTA) 90 min after the start of the experiment. A second control prepared in 0.01 mM $CaCl_2$, but not treated with EGTA, had the same viscosity at 15 and 90 min. Thus gelsolin's effect upon actin filaments is reversible, and it is unlikely that gelsolin is simply proteolyzing actin filaments.

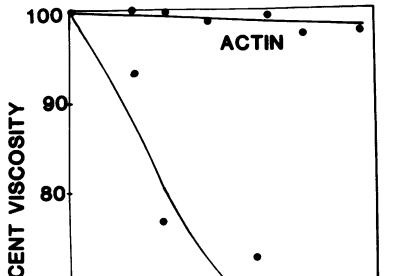

FIGURE 3 Dependence of gelsolin action on the free calcium concentration. The viscosity of solutions containing 16 μM actin and 0.4 μM gelsolin was measured as a function of the free calcium concentration in the low shear falling ball viscometer under the conditions listed under Fig. 2. Viscosity is expressed as a percentage of that of solutions containing 10 nM free Ca^{2+} (38 s).

DISCUSSION

In summary, the current study demonstrates that human platelets contain a protein, gelsolin, that is capable of reversible binding to actin in the presence of calcium. It is a 91,000-mol wt polypeptide that exhibits crossreactivity with an antibody prepared to rabbit macrophage gelsolin and is functionally identical to macrophage gelsolin. We suggest that gelsolin is the 90–95,000-mol wt protein that binds to and shortens actin filaments described (5–7). In addition, we think it is likely that gelsolin is responsible for the calcium-mediated lability of platelet cytoskeletons described by Rosenberg et al. (4) by viture of its ability to shorten actin filaments. Though gelsolin is similar in function to villin [a protein isolated from chicken intestine (9)], we believe that the evidence to date points to it as being the major protein of this class in mammalian tissues (15). The reversibility of its interaction with actin in response to changes in the calcium concentration suggest that gelsolin may be a dynamic regulatory of actin filament length.

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