

Evidence That Activation of Platelet Calpain Is Induced as a Consequence of Binding of Adhesive Ligand to the Integrin, Glycoprotein IIb-IIIa

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Abstract. Calpain (a Ca^{2+} -dependent protease) is present in many cell types. Because it is present in the cytosol, the potential exists that it may regulate critical intracellular events by inducing crucial proteolytic cleavages. However, the concentrations of Ca^{2+} required to activate calpain are higher than those attained in the cytoplasm of most cells. Thus, the physiological importance of calpain and the mechanisms involved in its activation have remained elusive. In this study, we show that calpain rapidly moved to a peripheral location upon the addition of an agonist to suspensions of platelets, but it remained unactivated. We provide three lines of evidence that calpain was subsequently activated by a mechanism that required the binding of an adhesive ligand to the major platelet

integrin, glycoprotein (GP) IIb-IIIa: calpain activation was prevented by RGDS, a tetrapeptide that inhibits the binding of adhesive ligand to GP IIb-IIIa; it was also prevented by monoclonal antibodies that inhibit adhesive ligand binding to GP IIb-IIIa; and its activation was markedly reduced in platelets from patients whose platelets have greatly reduced levels of functional GP IIb-IIIa. Thus, in platelets, binding of the extracellular domain of GP IIb-IIIa to its adhesive ligand can initiate a transmembrane signal that activates intracellular calpain. Because calpain is present in focal contacts of adherent cells, the interaction of integrins with adhesive ligands in the extracellular matrix may regulate activation of calpain in other cell types as well.

CALPAIN is a Ca^{2+} -dependent thiol protease that is active at neutral pH and is present in most animal cells (28). Because it is present in the cytoplasm, it has the potential to play a critical regulatory role, inducing the proteolytic cleavage of key substrates within the cell. If calpain functions in this way, presumably there must be mechanisms that rapidly activate the protease, perhaps at specific locations within the cell. However, little is known about situations in which calpain is activated in cells, the biological functions of calpain, or the mechanisms that regulate its activity within cells.

There are two forms of the enzyme, one that requires $\sim 10 \mu\text{M}$ Ca^{2+} for half-maximal activation, and one that requires millimolar concentrations. Because Ca^{2+} concentrations rise only to 400–1,500 nM within the cytoplasm of most cells (2, 4, 24, 41), it has been suggested that additional mechanisms must exist to induce calpain activation within normal cells. Calpain has been detected in focal contacts of adherent cells, sites where bundles of actin filaments terminate and the plasma membrane is in closest contact with the extracel-

lular matrix (3, 4). Integrins are concentrated at these sites and form a transmembrane linkage between adhesive ligands in the extracellular matrix and the intracellular cytoskeleton (4). Binding of adhesive ligand to integrins at these sites induces intracellular changes, including altered gene expression (39) and cytoskeletal reorganization (4). The intracellular enzymes that induce these changes are not known. Because calpain is found in focal contacts, and because many of the known substrates for this protease are components of the cytoskeleton (7, 12, 13), we have considered the possibility that calpain may be activated as a consequence of ligand binding to integrins.

One cell type in which calpain activation can be induced by the addition of an agonist is the platelet (10). Activation of calpain leads to a remodeling of the platelet cytoskeleton and to an altered association of the cytoskeleton with plasma membrane glycoproteins (11). The major platelet integrin is glycoprotein (GP) IIb-IIIa (29). This integrin is inactive in unstimulated platelets, but upon the addition of agonist it rapidly undergoes a conformational change that enables it to

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1. *Abbreviations used in this paper:* GP, glycoprotein; NIH, National Institutes of Health; RGDS, Arg-Gly-Asp-Ser; RGEs, Arg-Gly-Glu-Ser.

bind the adhesive ligands fibrinogen, fibronectin, and von Willebrand factor (34) and to become incorporated into focal contactlike structures (43). In the present study, we provide evidence that calpain is activated as a consequence of GP IIb-IIIa-ligand interactions. This finding suggests a mechanism by which intracellular calpain could be activated at specific sites within cells. It suggests that proteolytic events may be induced by calpain at sites of integrin-cytoskeletal interaction; such proteolytic events may play a critical role in the normal functioning of cells.

Materials and Methods

Isolation of Platelets

Venous blood was drawn from healthy adult donors or from patients with Glanzmann's thrombasthenia, and platelets isolated from it by centrifugation, as described (10). Platelets were finally resuspended at a concentration of $0.3\text{--}1 \times 10^9$ platelets/ml in a Tyrode's buffer containing 138 mM sodium chloride, 2.9 mM potassium chloride, 12 mM sodium bicarbonate, 0.36 mM sodium phosphate, 5.5 mM glucose, 1.8 mM calcium chloride, 0.4 mM magnesium chloride, pH 7.4.

Incubation of Platelets with Agonist

Platelet suspensions were incubated for 15 min at 37°C in the absence or presence of antibodies or inhibitors. The synthetic peptides consisting of the sequence Arg-Gly-Asp-Ser (RGDS) or Arg-Gly-Glu-Ser (RGES) (Telios Pharmaceuticals, Inc., San Diego, CA) were added in saline. The anti-GP IIb-IIIa antibodies, 10E5 and D9G1, were generous gifts of Dr. Barry Collier (State University of New York, Stony Brook, NY) and Dr. David Phillips (COR Therapeutics, Inc., South San Francisco, CA), respectively. The anti-GP IIb-IX antibody, AK2, was a generous gift of Dr. Michael Berndt (Westmead Hospital, Westmead, New South Wales, Australia). Platelets were subsequently incubated with 0.1 to 1.0 National Institutes of Health (NIH) unit of thrombin/ml (kindly provided by Dr. John W. Fenton II of the New York Department of Health, Albany, NY), 10 μ g of collagen/ml (Hormie, Munich, Germany), or 1.0 μ M ionophore A23187 (Sigma Chemical Company, St. Louis, MO). Unless otherwise indicated, all incubations were performed in the presence of stirring.

Assessment of Platelet Aggregation

Platelet suspensions (0.3×10^9 platelets/ml) were stirred in the presence of an agonist in an aggregometer (Chronolog Corporation, Havertown, PA). The agonist-induced aggregation of platelets was assessed by a change in transmittance of light through the suspension.

Localization of Calpain by Immunofluorescence

Platelet suspensions (1×10^9 platelets/ml) were fixed by the addition of 9 vol of a solution containing 4% paraformaldehyde in a buffer containing 150 mM sodium chloride, 10 mM Tris-HCl, pH 7.4. Approximately 100 μ l of the suspension was placed on poly-L-lysine-coated glass slides. The slides were incubated at ambient temperature for approximately 1 h. Excess suspension was then removed by aspiration and the platelets lysed by the addition of a buffer containing 0.5% Triton X-100, 0.1% Carnation milk, 150 mM ammonium acetate, 150 mM sodium chloride, 10 mM Tris-HCl, pH 7.4. After 15 min, samples were washed three times in a buffer containing 15 mM sodium acetate, 0.1% Carnation milk, 150 mM sodium chloride, 10 mM Tris-HCl, pH 7.4, to which a 1:50 dilution of sheep serum (Sigma Chemical Co.) was added. Samples were then incubated for \sim 16 h at ambient temperature with polyclonal antibodies against calpain. Excess solution was removed by aspiration, samples were washed five times with the buffer containing 15 mM ammonium acetate (but no sheep serum), incubated with biotinylated sheep anti-rabbit immunoglobulin G (Amersham Corporation, Arlington Heights, IL) for 3 h, washed five times with the 15 mM ammonium acetate-containing buffer, incubated with Texas-red-labeled streptavidin (Amersham Corp.) for 30–60 min, washed five times in a buffer containing 150 mM sodium chloride, 50 mM Tris-HCl, pH 7.4, and mounted. The slides were examined with a Zeiss Universal microscope (Carl Zeiss, Inc., Thornwood, NY) and photographed.

Assessment of Ca^{2+} Fluxes

Platelets were loaded with Fura 2 (18). Platelet suspensions (1×10^9 platelets/ml) were stirred with 1.0 NIH U of thrombin/ml in a Perkin-Elmer 650-40 fluorescence spectrophotometer (Perkin-Elmer Corporation, San Jose, CA). The excitation wavelength was 340 nm, and the emission wavelength was 490 nm. Thrombin-induced Ca^{2+} fluxes were assessed by the altered emission at 490 nm.

Analytical Procedures

Platelet suspensions ($0.3\text{--}1 \times 10^9$ platelets/ml) were solubilized in the presence of a reducing agent and analyzed on one-dimensional SDS-polyacrylamide gels by the method of Laemmli (23) using 3% acrylamide in the stacking gel and a 5–20% exponential gradient of acrylamide in the resolving gel. Proteins were stained with Coomassie brilliant blue. Western blotting was performed by the method of Towbin et al. (37). Antibodies to actin binding protein and calpain were raised and characterized as reported previously (12, 22). The concentration of platelets in suspensions of washed platelets was determined with a Coulter counter (Coulter Corporation, Hialeah, FL).

Results

Localization of Calpain in Platelets

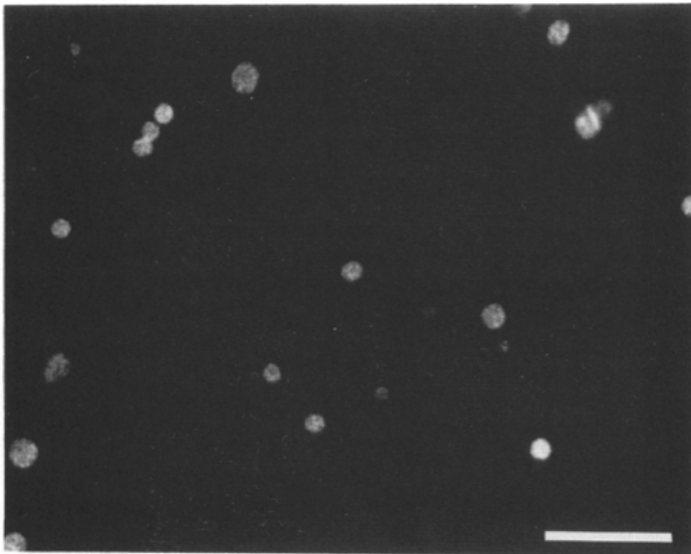
The distribution of calpain was examined in unstimulated and activated platelets. As reported previously (36), calpain had a very diffuse distribution in the unstimulated cell (Fig. 1 A). However, upon the addition of the agonist thrombin, calpain moved to the periphery of the cell (Fig. 1). Similar results were obtained whether the distribution of calpain was assessed by immunofluorescence (Fig. 1 B) or electron microscopy (Fig. 1 C). Calpain redistributed in suspensions that were not agitated (e.g., Fig. 1), but calpain is activated only in suspensions that have been agitated (10, 38). Further, the redistribution occurred extremely rapidly (by 10 s, the earliest time point studied). However, calpain activation was not detected until 30 or 60 s after the addition of thrombin to a stirred platelet suspension. Thus, although the thrombin-induced redistribution of calpain to the periphery of platelets may be necessary for calpain activation, it is not sufficient.

Inhibition of Agonist-induced Calpain Activation by GP IIb-IIIa Antagonist

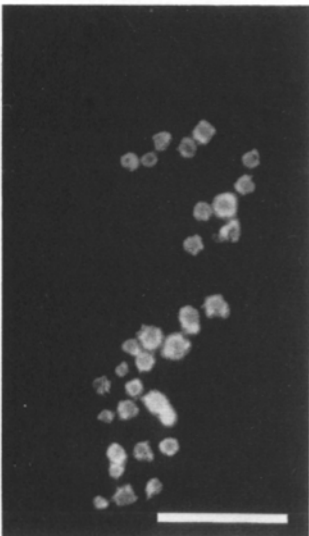
To determine whether the integrin GP IIb-IIIa is involved in the subsequent activation of calpain, platelets were incubated with agents known to inhibit the agonist-induced binding of adhesive ligands to this integrin and subsequently stirred in the presence of the agonists collagen or thrombin. One such agent, the tetrapeptide RGDS, inhibited the subsequent thrombin-induced binding of adhesive ligand to GP IIb-IIIa in a dose-dependent manner (Fig. 2 A). Inhibition of ligand binding was accompanied by inhibition of calpain activation, as detected by hydrolysis of actin-binding protein (Fig. 2 B) or autolysis of calpain (data not shown). Similar inhibitory effects were observed whether platelets were stimulated with thrombin or collagen (data not shown). RGES, a peptide that does not inhibit binding of adhesive ligand (Fig. 2 A), did not inhibit calpain activation (Fig. 2 B).

Agonist-induced binding of adhesive ligand to GP IIb-IIIa is also inhibited by preincubating platelets with 10E5, a monoclonal antibody against the GP IIb-IIIa complex (5). Inhibition of ligand binding was accompanied by inhibition of calpain activation (Fig. 3). Other monoclonal antibodies

A. Unstimulated



B. Activated

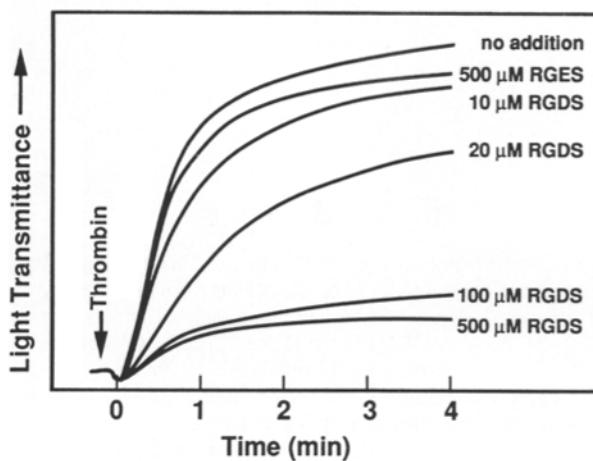


C. Activated



Figure 1. Distribution of calpain in platelets. *A* and *B* immunofluorescence images showing the distribution of calpain in unstimulated platelets (*A*) and in platelets that had been incubated (in the absence of stirring) with 1.0 NIH U of thrombin/ml for 60 s (*B*). *C* is an electron micrograph showing the distribution of calpain in platelets that had been stirred with 1.0 NIH U of thrombin/ml for 15 min. The platelets shown in this panel were found toward the periphery of the dense platelet aggregate that formed. Calpain was detected with polyclonal antibodies raised in rabbits against purified bovine skeletal muscle calpain. (*A* and *B*) Bar, 5 μm ; (*C*) bar, 0.5 μm .

A. Aggregometer Tracing



B. Immunoblot

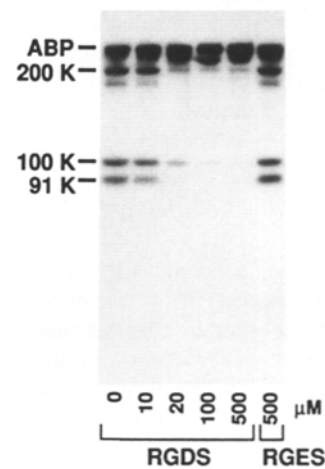


Figure 2. Inhibition of thrombin-induced calpain activation by RGDS. Suspensions of washed platelets were preincubated for 15 min at 37°C in the presence of the indicated concentrations of RGDS or RGES. Platelets were then stirred in an aggregometer with 0.1 NIH U of thrombin/ml for 10 min. Aggregation was recorded as an increase in light transmittance (*A*). Incubations were terminated by the addition of an SDS-containing buffer. Samples were electrophoresed through SDS-polyacrylamide gels. Hydrolysis of actin-binding protein (*ABP*) was detected on immu-

noblots after the transfer of proteins from the gels to nitrocellulose paper (*B*). 200, 100, and 91 K indicate the $M_r = 200,000$, 100,000, and 91,000 calpain-induced degradation products of actin-binding protein (12).

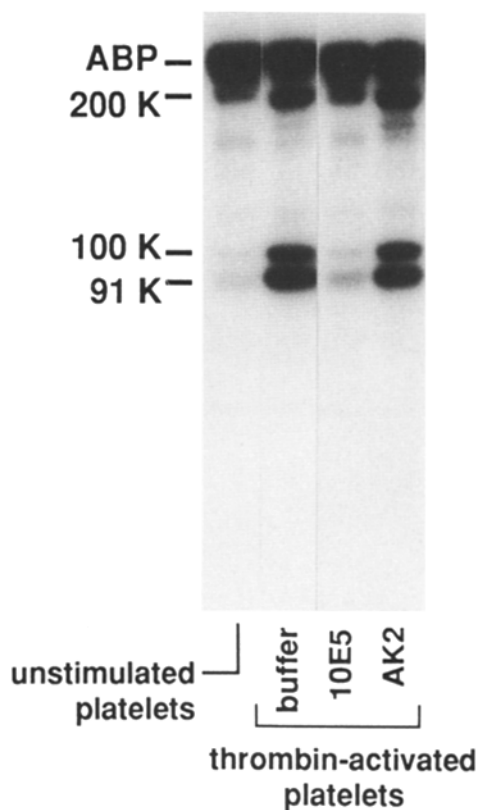


Figure 3. Inhibition of thrombin-induced calpain activation by anti-GP IIb-IIIa. Suspensions of washed platelets were preincubated for 15 min at 37°C in the presence or absence of monoclonal antibodies (1 μ g/ml). 10E5 is an antibody against the GP IIb-IIIa complex; AK2 is an antibody against another platelet membrane glycoprotein, GP Ib-IX. Platelets were then stirred in the aggregometer with 0.1 NIH U of thrombin/ml. After 10 min, the incubations were terminated by the addition of an SDS-containing buffer. Samples were electrophoresed through SDS-polyacrylamide gels. Hydrolysis of actin-binding protein (ABP) was detected on immunoblots after the transfer of proteins from the gel to nitrocellulose paper. 200, 100, and 91 K indicate the $M_r = 200,000$, 100,000, and 91,000 calpain-induced degradation products of actin-binding protein (12).

that inhibit binding of adhesive ligand to GP IIb-IIIa (e.g., D9G1) also inhibited calpain activation (data not shown). We also examined the effect of AK2, a monoclonal antibody against another platelet membrane glycoprotein (GP Ib-IX). AK2, which did not inhibit agonist-induced platelet aggregation, did not inhibit agonist-induced activation of calpain (Fig. 3).

Activation of Calpain in Platelets from Patients with Glanzmann's Thrombasthenia

Platelets from subjects with Glanzmann's thrombasthenia are deficient in functional GP IIb-IIIa complex (15). These platelets change shape and secrete their granule contents normally in response to physiological agonists but show diminished fibrinogen binding and aggregation. Western blotting showed that calpain was present in platelets from patients with Glanzmann's thrombasthenia (Fig. 4). Immunofluorescence images showed that calpain moved to the periphery upon activation of these platelets (Fig. 5). However, there

was extremely little activation of calpain in these platelets in response to the physiological agonists thrombin or collagen, as assessed by autolysis of calpain (Fig. 4) or hydrolysis of actin-binding protein (Fig. 6). Platelets from three different patients all gave similar results. As reported previously (40), activation of calpain occurred normally when the platelets from thrombasthenic patients were stirred in the presence of the Ca^{2+} ionophore A23187 (Fig. 4). Thus, calpain is present in the platelets from patients with Glanzmann's thrombasthenia and can be activated to normal levels if the intracellular Ca^{2+} concentrations are elevated sufficiently (e.g., by use of a Ca^{2+} ionophore). However, the physiological mechanism by which calpain is normally activated is non-functional in these platelets.

Effect of GP IIb-IIIa Antagonists on Ca^{2+} Influx into Platelets

The results presented so far suggest that calpain moves to the periphery of platelets upon platelet activation but that the subsequent activation of calpain requires binding of adhesive ligand to the GP IIb-IIIa complex. The GP IIb-IIIa complex has been implicated in Ca^{2+} influx into platelets (14, 32, 35). Thus, it is conceivable that this ligand-GP IIb-IIIa interaction is required to elevate submembranous Ca^{2+} concentrations sufficiently to induce calpain activation in thrombin-activated cells. However, others have reported that RGDS, which inhibits binding of adhesive ligands to GP IIb-IIIa, does not inhibit agonist-induced increases in cytoplasmic Ca^{2+} concentrations, as measured with the Ca^{2+} indicator Fura 2 (31, 42). Further, D9G1, a monoclonal antibody that inhibited binding of adhesive ligand to GP IIb-IIIa and in-

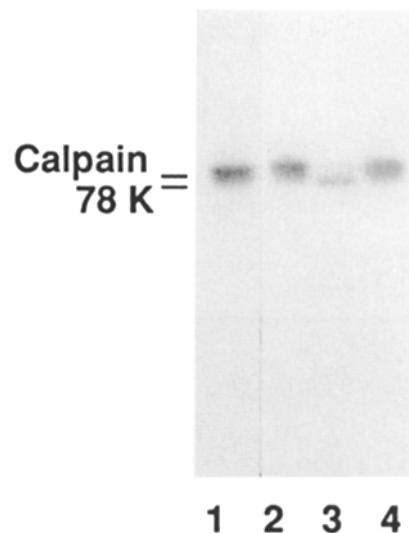
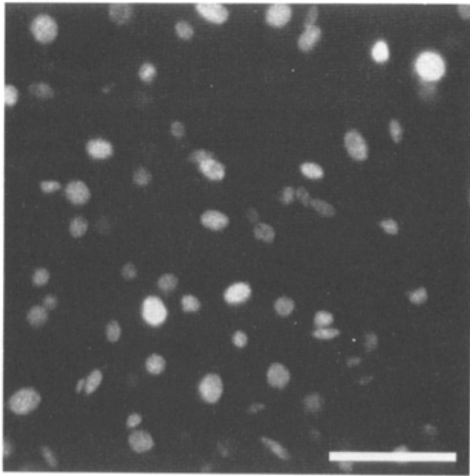


Figure 4. Western blot showing calpain in thrombasthenic platelets. Suspensions of normal (lane 1) or of platelets from thrombasthenic patients (lanes 2-4) were incubated alone (lanes 1 and 2), stirred with 1.0 μ M ionophore A23187 for 15 min (lane 3), or stirred with 1.0 NIH U of thrombin/ml for 15 min (lane 4). Incubations were terminated by the addition of an SDS-containing buffer. Samples were electrophoresed through SDS-polyacrylamide gels. Calpain and its autolytic form (78 K) were detected on immunoblots by using a monoclonal antibody against the $M_r = 80,000$ subunit of bovine skeletal muscle μ -calpain.

A. Unstimulated



B. Activated

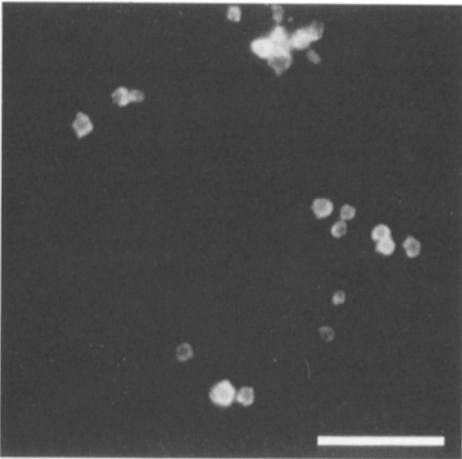


Figure 5. Immunofluorescence images showing the distribution of calpain in unstimulated thrombasthenic platelets (*A*) and in thrombasthenic platelets that have been incubated with 1.0 NIH U of thrombin/ml for 2 min. (*B*). Calpain was detected with polyclonal antibodies raised in rabbits against purified bovine skeletal muscle calpain. Bar, 5 μ m.

hibited calpain activation (data not shown), induced no inhibitory effects on Ca^{2+} influx (Fig. 7).

Discussion

Although calpain is present in many cell types, the mechanisms involved in regulating the functional activity of this protease have remained elusive. It has been suggested that a prerequisite for calpain activation may be its association with the cell membrane (26). Although evidence has been provided that association of calpain with several different phospholipids lowers the concentration of Ca^{2+} required to activate the protease, the conclusion that this would be sufficient to account for activation of calpain within cells has been questioned (6, 17, and Zalawska, T., R. B. Thompson, and D. E. Goll, submitted for publication). The present study shows that calpain did move to the periphery of the cell

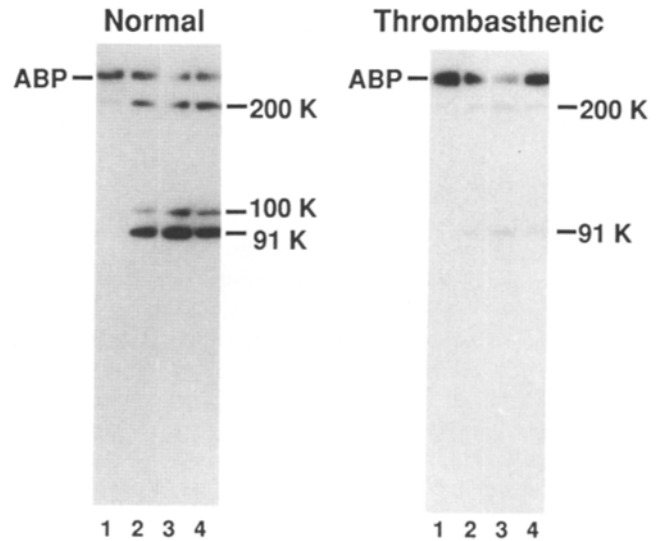


Figure 6. Western blots showing that there is little activation of calpain in platelets from thrombasthenic patients. Suspensions of washed platelets were (lane 1) incubated alone, (lane 2) stirred with 10 μ g of collagen/ml, (lane 3) stirred with 1.0 NIH U of thrombin/ml, or (lane 4) stirred with a combination of 10 μ g of collagen/ml and 1.0 NIH U of thrombin/ml. After 10 min, the incubations were terminated by the addition of an SDS-containing buffer. Samples were electrophoresed through SDS-polyacrylamide gels. Hydrolysis of actin-binding protein (ABP) was detected on immunoblots after the transfer of proteins from the gels to nitrocellulose paper. 200, 100, and 91 K indicate the $M_r = 200,000$, 100,000, and 91,000 degradation products of actin-binding protein. *Left* shows samples obtained from a control donor, whereas the *right* shows samples from a patient with Glanzmann's thrombasthenia.

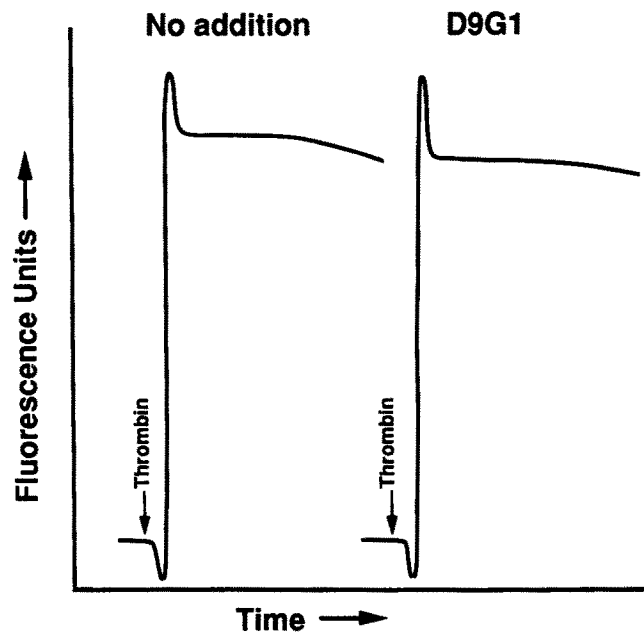


Figure 7. Effects of anti-GP IIb-IIIa antibody on thrombin-induced Ca^{2+} fluxes. Suspensions of platelets were loaded with Fura 2, then incubated for 10 min with no addition or with 30 μ g/ml D9G1, a monoclonal antibody against GP IIb-IIIa. Suspensions were subsequently stirred with 1.0 NIH U of thrombin/ml and the increased intracellular Ca^{2+} was recorded as an increase in fluorescence.

in activated platelets, suggesting that in this cell type it may associate with the plasma membrane. However, an event other than simple association with the plasma membrane was required for calpain activation. Thus, the protease redistributed to the periphery of platelets that had not aggregated, yet calpain activation occurs only in aggregating cells (10, 38). Further, the protease redistributed similarly in platelets from thrombasthenic patients, yet activation of the protease did not occur.

Thus, a signal other than simple translocation of calpain to the periphery of activated cells appears to be required for activation of calpain. In the present study, we provide several lines of evidence that this signal is initiated by binding of adhesive ligand to the GP IIb-IIIa complex on the platelet surface. First, activation of calpain was prevented by RGDS, a tetrapeptide that inhibits the binding of adhesive ligand to GP IIb-IIIa; second, activation of calpain was prevented by monoclonal antibodies that inhibit adhesive ligand binding to GP IIb-IIIa; third, activation of calpain was greatly reduced in platelets from patients with Glanzmann's thrombasthenia, a disorder in which platelets have greatly reduced levels of functional GP IIb-IIIa complex. We conclude that in platelets, binding of the extracellular domain of this integrin to its adhesive ligand can initiate a transmembrane signal that activates intracellular calpain.

The nature of the transmembrane signal is not known. The GP IIb-IIIa complex has been implicated in Ca^{2+} influx into platelets (14, 32, 35). Thus, it is possible that ligand-integrin interactions are required to elevate intracellular Ca^{2+} concentrations sufficiently to induce calpain activation. In the present study, however, the monoclonal antibody D9G1, which inhibits binding of adhesive ligand to GP IIb-IIIa, had no detectable effect on the thrombin-induced increase in cytoplasmic Ca^{2+} concentrations, yet it inhibited the thrombin-induced activation of calpain. It is possible that local Ca^{2+} concentrations adjacent to ligand-occupied integrin become sufficiently high to activate calpain at these sites and that GP IIb-IIIa antagonists inhibit these local increases and thereby prevent calpain activation. Fura 2, which measures the total cytoplasmic Ca^{2+} concentration may not be sensitive enough to detect such changes. Thus, it is possible that inhibitory effects of GP IIb-IIIa antagonists on Ca^{2+} fluxes went undetected in the present study. Alternative mechanisms that may induce activation of calpain are tyrosine phosphorylation reactions (8, 16), cytoskeletal reorganizations (21, 30), or Na^+ - H^+ exchange (1). The reason for suggesting each of these as a potential mechanism is that they are all induced in activated platelets as a consequence of adhesive ligand binding to the GP IIb-IIIa complex. As with calpain activation, these agonist-induced changes only occur in suspensions of platelets that are stirred. Future experiments will be needed to elucidate the mechanism by which each of these intracellular events is initiated and to determine whether any of these events is on the pathway between the initial binding of adhesive ligand and the subsequent activation of calpain.

It is not known why adhesive ligand-induced, integrin-mediated signal transduction requires that platelet suspensions are stirred. When platelets are stirred, adhesive ligand bound to GP IIb-IIIa on one platelet binds to activated GP IIb-IIIa on an adjacent platelet. Thus, in a stirred suspension, GP IIb-IIIa effectively binds to immobilized ligand rather than to ligand in suspension (as occurs in an unstirred suspension). Interestingly, many of the changes that are in-

duced as a consequence of adhesive ligand binding to GP IIb-IIIa in a stirred platelet suspension are the same as those induced in cultured cells, as integrins bind to immobilized adhesive ligands and focal contacts form. For example, as in cultured cells, the binding of adhesive ligand to GP IIb-IIIa in a stirred platelet suspension causes the integrin to associate with cytoplasmic actin filaments (30). In addition, there are many similarities between the focal contacts of cultured cells and the GP IIb-IIIa-cytoskeletal structure that forms in response to stirring. Thus, for example, proteins present in the GP IIb-IIIa-cytoskeleton complex include talin, vinculin, pp60^{c-src}, and protein kinase C (43, Fox, J. E. B., L. Lipfert, E. A. Clark, C. C. Reynolds, C. D. Austin, and J. S. Brugge, manuscript in preparation), proteins known to be concentrated in focal contacts of adherent cells in culture (4). Also, as in cultured cells, incubation of platelets with cytochalasins disrupts actin filaments and inhibits the ability of the integrin to bind adhesive ligand (Fox, J. E. B., D. A. Sanan, and S. J. Shattil, manuscript in preparation), suggesting a role for the cytoskeleton in regulating the adhesive properties of the platelet integrin.

In cultured cells, there is also increasing evidence that ligand-integrin interactions at focal contacts result in intracellular changes such as reorganization of the cytoskeleton and altered gene expression (4, 39). Presumably this transmembrane signaling is essential in regulating the shape, motility, and function of adherent cells, but, as in platelets, it remains to be determined how adhesive ligand-integrin interactions induce these intracellular changes. Interestingly, activation of tyrosine phosphorylation reactions (20) and of the Na^+ - H^+ antiporter (33), events that occur while focal contactlike structures form in aggregating platelets (1, 8, 16), have recently been shown to occur when focal contacts form in cells in culture. In the present study, we show that calpain is another enzyme activated as a consequence of binding of adhesive ligand to an integrin in platelets. Because calpain is concentrated in focal contacts of adherent cells in culture (3), it appears possible that it may be activated as a consequence of ligand-integrin interaction in these cells as well. The major proteins shown to be cleaved by calpain in activated platelets are the cytoskeletal proteins actin-binding protein (filamin) (12), talin (12), and spectrin (13). Other substrates may be protein kinase C (19, 27) and membrane receptors (25). Activation of calpain has been shown to be responsible for cytoskeletal rearrangements in aggregating platelets (9), and many of the proteins known to be substrates for calpain in platelets are also concentrated in focal contacts of other adherent cells (4). Thus, whether in platelets or other adherent cells, it is conceivable that the calpain-induced cleavage of proteins such as these may induce the intracellular changes that occur as a consequence of the binding of integrins to adhesive ligands.

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