Immunocytochemical Study of the Binding of Fibrinogen and Thrombospondin to ADP- and Thrombin-Stimulated Human Platelets

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We have used immunogold staining to locate thrombospondin (TSP) on thrombin-activated human platelets, and have compared its distribution with that of fibrinogen (or fibrin) on thrombin- and ADP-stimulated platelets. To do this, isolated platelets were incubated with monospecific antibodies to TSP or fibrinogen (fib) and the bound IgG located with a second antibody adsorbed to gold particles. Thrombin-induced secretion in Tyrode-Ca^2+ was followed by both anti-TSP and anti-fib binding, with large clusters of gold particles observed on the platelet surface. Little or no labeling was observed on unstimulated platelets with either antibody. When secretion was effected in Tyrode-EDTA, anti-TSP IgG still bound to the activated platelets, but the number of particle clusters was significantly reduced. Little binding of anti-fib IgG now occurred. Platelets activated with ADP in the presence of added fib, and subsequently incubated with anti-fib IgG, showed small particle clusters over the whole platelet surface. Thrombin-stimulated platelets from two patients with thrombasthenia bound anti-TSP IgG similarly to normal platelets activated in Tyrode-EDTA. No anti-fib binding occurred. Our results suggest that fib and TSP bind to specific domains on the stimulated platelet membrane. Such sites may be responsible for the mediation of platelet surface contact interactions.

THROMBOSPONDIN* (TSP) is a high-molecular-weight glycoprotein that is secreted as part of the release reaction of human platelets.1–5 Composed of three mol wt 140,000 polypeptide chains linked by disulfides,3,5 TSP is a major constituent of the α-granules.6,8 Also detected in small amounts in plasma,3 TSP is not exclusive to platelets and has been found in fibroblasts,10,11 muscle cells,10 and endothelial cells.10,12,13

The studies of Gartner et al14–16 showed that platelets stimulated with thrombin and ionophore A23187 expressed a lectin-like agglutinin that was not present on unstimulated platelets. Appearance of the lectin-like activity correlated with the onset of platelet aggregation induced by these agents and was dependent on α-granule secretion.15,16 In 1982, Jaffe et al17 showed that TSP was the endogenous lectin of human platelets. Analysis of the surface-associated proteins of thrombin-activated platelets by 125I-labeling, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography, suggested a calcium-dependent association of TSP and fibrinogen (or fibrin) with platelet receptors.4 Binding studies suggest that other secreted platelet proteins, such as fibronectin,18 von Willebrand factor (vWF), and platelet factor 4 (PF4),19 may also become associated, at least in part, with the platelet membrane after their release. However, although a role for these proteins in platelet surface contact interactions is suggested, the precise function of each protein remains unknown.

In order to better evaluate the role of secreted proteins in platelet aggregation and adhesion mechanisms, more information is required concerning the organization of the bound proteins on the surface of stimulated platelets, and of the nature of the membrane receptors that bind the proteins. In the current study, we have used immunogold staining to locate TSP and fibrinogen (or fibrin) on the surface of thrombin-activated platelets. The experiments were performed in the presence or absence of divalent cations, and the results compared with (a) those obtained for normal platelets stimulated by ADP in the presence of exogenous fibrinogen and (b) thrombasthenic platelets incubated with thrombin. Our results suggest that secreted fibrinogen and TSP bind to focal attachment points on the stimulated platelet membrane and that these may represent domains responsible for the formation of the protein bridges that conclude the platelet aggregation mechanism.

MATERIALS AND METHODS

Materials

Apyrase, N6-0β,4-dibutyryladenosine 3’5’-cyclic monophosphate (db-cAMP), prostaglandin E1 (PGF1), and hirudin were purchased from Sigma Chemical Co (St Louis); (14C) 5-hydroxytryptamine bisoxalate (5-HT) (50.7 mCi/mmol) from New England Nuclear (Dreieich, FRG); glutaraldehyde from Fluka A.G. (Buchs, Switzerland); glutaraldehyde-activated ultragel from Industrie Biologique Francaise (Clichy, France); fibrinogen from Kabi Diagnostics Ltd (Stockholm); human thrombin (fibrinogen) from Ortho Diagnostic Systems (Raritan, NJ); ADP from Laboratoires Stago (Paris); rabbit antiserum against human fibrinogen from Behringwerke Ag (Marburg, FRG); affinity-puriﬁed IgG of a goat anti-rabbit IgG

*Thrombospondin was first known as "thrombin-sensitive protein" and has since also been termed GP Ig or GP-G.

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ADHESIVE PROTEINS ON ACTIVATED PLATELETS

antisera coupled to 5-nm diameter gold particles from Janssen Pharmaceutica (Beerse, Belgium); heparin-Sepharose CL 4B and protein A-Sepharose from Pharmacia (Beerse, Belgium); ADHESIVE PROTEINS ON ACTIVATED PLATELETS 913

Antisera

The anti-fibrinogen IgG were affinity purified before use. Samples (2 mL) of Kabi fibrinogen (5 mg/mL in 0.1 mol/L phosphate buffer, pH 7.4) were further purified by mixing with an equal vol of 8% (wt/vol) polyethylene glycol in the same buffer and incubated for 15 minutes at 4 °C. The fibrinogen precipitate was solubilized in 2 mL phosphate buffer and mixed with 2 mL of glutaraldehyde-activated ultrogel. Cross-linking of the fibrinogen to the ultrogel was performed as described by Kahn et al.21 The fibrinogen-ultrogel was packed into a small column and 3 mL of the rabbit anti-human fibrinogen IgG passed through. After washing, the specific anti-fibrinogen IgG was eluted as described by Kahn et al and previously reported by us.22 Human platelet TSP was prepared from thrombin-released proteins by affinity chromatography on heparin-Sepharose CL 4B according to a modification of the procedure of Lawler et al.23 The protein was at least 95% pure as assessed by SDS-PAGE. A series of rabbits was immunized against the purified protein. Any contaminating antibodies against fibrinogen, fibronectin, or vWF were removed by adsorbing the rabbit serum with cryoprecipitate prepared from normal human plasma isolated in the presence of PGE, and db-CAMP as inhibitors of the platelet release reaction.2124 IgG was isolated by chromatography on protein A-Sepharose CL 4B as previously described.21 The anti-TSP IgG was not further purified. Both the affinity-purified anti-fibrinogen IgG and the adsorbed anti-TSP serum were monospecific. They gave one precipitation line in immunodiffusion against Triton X-100 soluble platelet extracts and reacted specifically with fibrinogen or TSP using a “Western” blotting procedure, following the electrophoretic transfer to nitrocellulose membrane of SDS-solubilized platelet proteins separated by SDS-PAGE. The blotting procedure was performed as described by Kieffer et al.22 Both antibodies reacted identically in the immunoblot procedure with proteins retracted in the presence of calcium or EDTA, respectively.

Washed Platelet Suspensions

Blood (6 vol) from normal adult donors or from two patients (F.L. and J.R.) with Glanzmann's thrombasthenia were collected on acid-citrate-dextrose anticoagulant (ACD, NIH formula A, 1 vol). Platelet-rich plasma (PRP) was prepared by centrifugation at 150 °C for 15 minutes at 120 g. It was immediately acidified to pH 6.5 by addition of a one-tenth volume of ACD-A, PGE, (100 nmol/L) and apyrase (25 mg/mL) were also added. The platelets were washed three times in the washing buffer described by Patscheke and modified by Legrand et al.22 Washed platelets were either resuspended in a modified Tyrode buffer consisting of 137 mmol/L NaCl, 2 mmol/L KCl, 12 mmol/L NaHCO3, 0.3 mmol/L NaH2PO4, 2 mmol/L CaCl2, 1 mmol/L MgCl2, 5.5 mmol/L glucose, 5 mmol/L HEPES, pH 7.4 (Tyrode-Ca2+), or in the same buffer containing 5 mmol/L EDTA and lacking divalent cations (Tyrode-EDTA). Preparation of platelet samples for SDS-PAGE, followed by periodate-Schiff or Coomassie blue R250 staining performed as described by Norden et al,1 revealed severe deficiencies of membrane glycoproteins (GP IIb and IIIa and of fibrinogen. In contrast, platelet TSP levels were normal.

Electron Microscopy

Preparation of platelet samples. Aliquots (0.5 mL) containing 1.5 x 108 platelets in Tyrode-Ca2+ or Tyrode-EDTA were incubated at 37 °C with or without 0.05 U/mL thrombin for periods ranging from one to ten minutes as detailed in the text. Identical incubations were performed in the presence of 10 μmol/L ADP and 50 μg/mL added fibrinogen. No stirring was performed after the initial mixing. The reaction was stopped by the addition of 2 vol 0.1 mol/L phosphate buffer, pH 7.2 (to dilute the proteins present in the medium), followed immediately by 20 vol of 1.25% (vol/vol) glutaraldehyde diluted in 0.1 mol/L phosphate buffer, pH 7.2. Platelet fixation was continued for ten minutes at 37 °C, after which the platelets were washed three times in phosphate-buffered saline. Immunogold staining. Fixed platelets (3 x 108/mL) were incubated for one hour at room temperature with either (a) 200 μg/mL rabbit anti-TSP IgG, (b) 160 μg/mL rabbit anti-fibrinogen IgG, or (c) 200 μg/mL IgG isolated from nonimmune rabbit serum. In all cases, 2 mg/mL purified human IgG was also added to reduce nonspecific binding of the rabbit antibodies to the platelet surface. The platelets were washed in 20 mmol/L TRIS-HCl, 0.15 mol/L NaCl, pH 8.2, containing 0.1% (wt/vol) bovine serum albumin. They were then incubated with goat anti-rabbit IgG coupled with 5-nm gold particles as detailed previously by us.22 Incubation was for four hours at room temperature and overnight at 4 °C. Control experiments revealed little binding of gold particles to stimulated or unstimulated platelets in the absence of incubation of the platelets with the first antibody.

Post-fixation procedures. Platelets were post-fixed in 1% (wt/vol) osmic acid for one hour, dehydrated by graded alcohols and propylene oxide, and finally embedded in Epon.23 Ultrathin sections were cut, stained with uranyl acetate, and observed on a Philips (Eindhoven, Holland) EM 201 electron microscope.

Platelet TSP Measurements. Platelets from both patients were treated with thrombin as described. Platelets from one patient (F.L.) were also incubated with 10 μmol/L ADP in the presence of 50 μg/mL fibrinogen for ten minutes. Samples were fixed and processed exactly as described for stimulated normal platelets. As ADP-activated thrombasthenic platelets fail to express fibrinogen receptors,40,41 this experiment served as an additional control to assess the possible nonspecific attachment of proteins to the platelet surface during the glutaraldehyde-fixation step.

Gold particle density. In some experiments, the number of particle clusters were evaluated on platelet sections at a magnification of x15,000. A cluster was defined arbitrarily as containing more than five particles. Only sections giving the appearance of having crossed the central portion of the platelet (as opposed to a pseudopod) were examined. At least 40 sections were examined in each analysis.

Release Reaction During Thrombin Stimulation

On occasion, PRP was incubated with 1.9 μmol/L (14C) 5-HT for 30 minutes at 37 °C. Washed platelets (3 x 108/mL) in Tyrode-Ca2- or Tyrode-EDTA were stimulated at 37 °C with 0.05 U/mL thrombin for periods ranging from one to ten minutes, at which time 0.5 U/mL hirudin was added. The platelets were sedimented at 12,000 g for two minutes in an Eppendorf centrifuge (GMBH, Hamburg, W Germany) and the 14C radioactivity in the supernatant was measured in an Intertechnique SL.3 liquid scintillation spectrometer (Intertechnique, Plaisir, France).26 Release of α-granule proteins was studied largely as detailed by Norden et al.1 Platelets (108/mL) in Tyrode-Ca2- or Tyrode-EDTA were stimulated with 0.1 U/mL thrombin for five minutes at 37 °C before the addition of hirudin. After cooling in ice, the platelets were sedimented as described. Samples (250 μL) of each supernatant were immediately added to a one-fifth volume of 12% (wt/vol) SDS in 10 mmol/L TRIS-HCl, pH 7.0. Processing of the platelet samples for SDS-PAGE, together with analysis of the samples on 7%–20% gradient acrylamide slab gels, was performed as described by Norden et al.1

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RESULTS
Thrombin-Induced Release of α-Granule Proteins

A prerequisite of our experiments was that platelets were challenged with thrombin at 37 °C in the absence of stirring to prevent aggregate formation in Tyrode-Ca²⁺. Figure 1 confirms that extensive α-granule release occurred under these conditions. In these experiments, most of the platelet β-TG and PF4 was located in the supernatant fraction following incubation of platelets in either Tyrode-Ca²⁺ or Tyrode-EDTA with thrombin. In Tyrode-EDTA, the secreted proteins included TSP and fibrinogen. In contrast, little fibrinogen was recovered in the supernatant fraction after stimulation of platelets in Tyrode-Ca²⁺, and only part of the TSP was found there. Such results are in agreement with a calcium-dependent binding of secreted fibrinogen (or fibrin) and TSP to thrombin-stimulated platelets as reported by Phillips et al.⁴ In another typical experiment, platelets resuspended at 3 × 10⁵/mL in Tyrode-EDTA released 70% of pre-incorporated (¹⁴C)-5-HT within three minutes. A similar result was obtained for platelets in Tyrode-Ca²⁺.

Distribution of TSP on Thrombin-Activated Platelets

The washed platelets used in our study retained their disk shape and appeared to be unactivated (Fig 2A). Platelets lost their discoid form after thrombin stimulation when pseudopods appeared. Granules centralized and became replaced by vacuoles as secretion occurred. A diffuse central mass was often formed (Fig 2B through D).

To visualize TSP expression at the cell surface, platelets were incubated with anti-TSP antibodies that were revealed with goat anti-rabbit IgG coupled to 5-nm gold particles. Few gold particles became bound to unstimulated platelets (Fig 2A). After thrombin stimulation, however, marked staining was observed on the platelet surface (Fig 2B). Initial experiments were performed in Tyrode-Ca²⁺. A feature of all sections was the localization of the particles in clusters. Isolated particles were rarely seen, and between the clusters the platelet membrane was unstained. It was noted that pseudopods were frequently labeled. Increasing the time of incubation with thrombin from three to five minutes did not noticeably modify the particle distribution (not shown). Erythrocytes and white blood cells occasionally present in the platelet preparation bound few particles. Controls performed using nonimmune rabbit IgG resulted in a maximum of two or three single particles per platelet section (data not shown).

Studies were also performed using platelets suspended in Tyrode-EDTA. Although some TSP was detected on the platelet surface after thrombin-induced secretion, visual inspection suggested that the gold particle clusters were now smaller and less numerous (Fig 2C). This was in part confirmed by a semiquantitative analysis, which revealed that the number of clusters on thrombin-stimulated platelets in Tyrode-EDTA was about half that on platelets in Tyrode-Ca²⁺.

Platelets from two patients with Glanzmann’s thrombasthenia behaved differently from normal platelets, although a normal thrombin-induced release of the α-granule proteins occurred in both Tyrode-Ca²⁺ and Tyrode-EDTA (not shown). Immunogold staining of thrombasthenic platelets stimulated in both buffers was identical and resembled that of normal platelets stimulated in Tyrode-EDTA (Fig 2D). A semiquantitative analysis confirmed that the thrombasthenic platelets had about half of the clusters seen on normal platelets incubated in Tyrode-Ca²⁺ (Table 1). Increasing the time of incubation of the thrombasthenic platelets with thrombin (to ten minutes) did not change the number of particle clusters finally observed (data not shown).

Binding of Anti-Fibrinogen Antibodies to Thrombin-Activated Platelets

Only the occasional gold particle or particle cluster was observed on unstimulated platelets incubated with anti-fibrinogen (Fig 3A). However, when the studies were performed on platelets stimulated with thrombin
Fig 2. Localization of TSP on the surface of thrombin-stimulated platelets. Washed platelets (3 x 10^9/mL) were incubated with 0.05 U/mL thrombin for three minutes at 37 °C before glutaraldehyde fixation. The fixed platelets were then incubated with the isolated IgG of a rabbit anti-TSP serum and the bound IgG visualized using the IgG of a goat anti-rabbit IgG antibody coupled to 5-nm gold particles. (A) Washed unstimulated platelets in Tyrode-Ca^2+. (B) Thrombin-stimulated platelets in Tyrode-Ca^2+. Gold particles are distributed in large clusters over the platelet surface. (C) As (B) but with platelets in Tyrode-EDTA. Particle clusters appear fewer and smaller than for platelets in Tyrode-Ca^2+. (D) Thrombin-stimulated thrombasthenic platelets in Tyrode-Ca^2+. Staining resembled that in (C) with the occasional small particle cluster present. Some particle clusters are marked ↓. All sections are magnified × 30,000.
in Tyrode-Ca²⁺, an abundant labeling was observed. Already marked after one minute (Fig 3B), large particle clusters were present on platelets incubated for three minutes with thrombin (Fig 3C). Although some variation was apparent in the degree of labeling of individual platelets in a given sample, visual inspection suggested that on most platelets, particle clusters were thicker and more elongated than was observed with anti-TSP IgG. No additional clusters were located on platelets that had been incubated for five minutes with thrombin. When the studies were repeated with normal platelets resuspended in Tyrode-EDTA, the staining was much reduced. Figure 3D shows that few clusters were now apparent and that those which were detected contained fewer particles.

**Binding of Added Fibrinogen to ADP-Stimulated Platelets**

In these studies, washed platelets were stimulated with ADP in the presence of 0.05 mg/mL fibrinogen. This is slightly in excess of the maximal concentration that can be secreted from platelets suspended at 3 x 10⁶/mL (see Discussion). Binding of fibrinogen, as assessed by immunogold staining, increased with time. Again, the bulk of the particles were present as clusters. However, these appeared smaller and more evenly dispersed than those located on thrombin-activated platelets. Figure 4A shows a typical result as obtained for platelets incubated five minutes with ADP. The platelet α-granules were readily apparent on this section, indicating that secretion had not occurred. Previous studies have indicated that ADP does not induce dense granule secretion from washed platelets incubated under our experimental conditions.²⁸ Platelets from a patient with Glanzmann’s thrombasthenia were devoid of gold particles after incubation with ADP and fibrinogen (Fig 4B). Normal platelets incubated with nonimmune rabbit IgG also exhibited little labeling.

**DISCUSSION**

We have examined the distribution of TSP on thrombin-stimulated platelets and shown that the protein was organized in patches, revealed as particle clusters by the immunogold staining procedure. Previous studies using the same rabbit anti-TSP antibody³³ showed that Fab fragments were inhibitors of thrombin-induced platelet aggregation. Leung et al³² have reported similar findings. Although the precise role of TSP in secretion-dependent platelet aggregation is unclear, one theory is that the protein acts as a lectin or agglutinin. Here, TSP is suggested to play a role in bridge formation between aggregating platelets. If this is so, our results suggest that thrombin-activated platelets have relatively large points of attachment scattered around the outer membrane.

Using a radiolabeling procedure, Phillips et al⁴ concluded that TSP expression on thrombin-activated platelets was mediated by divalent cations. However, George et al,³⁵ using a similar approach but a different labeling technique, clearly located some TSP on the surface of platelets incubated with thrombin in the presence of 4 mmol/L EDTA. Our study has clarified the above findings, because (a) some anti-TSP binding occurred to platelets stimulated in Tyrode-EDTA, but (b) a significantly increased binding occurred in Tyrode-Ca²⁺. In a recent report, Gartner and Dockter,³⁴ using an immunofluorescence technique, also found TSP on platelets stimulated with ionophore A23187 in the presence of EDTA. These authors showed that TSP bound monovalently in the Ca²⁺-free media.

A feature of our study was the comparison of the distribution of secreted fibrinogen and TSP on the surface of thrombin-activated platelets. Anti-fibrinogen antibodies again bound to specific domains on the membrane, but the staining intensity was greater than was observed with anti-TSP IgG. This may be a reflection of the increased amount of fibrinogen in platelets. Ginsberg et al³⁵ found 24.6 μg TSP and 88.3 μg fibrinogen in 10⁹ platelets. Furthermore, close examination of Fig 1 shows that whereas all secreted fibrinogen in Tyrode-Ca²⁺ was retained by the platelets, only part of the secreted TSP was so associated. Any assessment of staining intensities obtained with anti-TSP and anti-fibrinogen IgG must take into account these factors.

The fact that the anti-TSP antibody was an inhibitor of thrombin-induced platelet aggregation (see earlier) suggests that it was efficient at locating TSP bound at the platelet surface. Little is known, however,
Fig 3. Localization of bound anti-fibrinogen antibodies on the surface of thrombin-stimulated platelets. Washed platelets were incubated with thrombin for one or three minutes as described in Fig 2. The fixed platelets were then incubated with affinity-purified IgG of a rabbit anti-fibrinogen serum and the bound IgG visualized by immunogold staining. (A) Washed unstimulated platelets in Tyrode-Ca\(^{2+}\). (B) Platelets stimulated with thrombin for one minute in Tyrode-Ca\(^{2+}\). Numerous clusters of gold particles are present. (C) Platelets stimulated with thrombin for three minutes in Tyrode-Ca\(^{2+}\). Staining intensity appeared increased. (D) Platelets stimulated with thrombin for five minutes in Tyrode-EDTA. Little staining was apparent. Some particle clusters are marked ↓. All sections are magnified ×30,000.
Fig 4. Localization of fibrinogen on the surface of ADP-stimulated platelets. Washed platelets (3 x 10^9/mL) were incubated with 10 µmol/L ADP and 50 µg/mL fibrinogen for five minutes before glutaraldehyde fixation and washing. The fixed platelets were then incubated with affinity-purified anti-fibrinogen IgG and the bound IgG visualized by immunogold staining. (A) Washed normal platelets in Tyrode-Ca^{2+}. (B) Washed thrombasthenic platelets in Tyrode-Ca^{2+}. Some particle clusters are marked. Both sections are magnified x30,000.

of the relationship between the density of the gold particles in a given area and the number of molecules of TSP or fibrinogen present. The size of the clusters seen in Fig 2, for example, makes it highly probable that the antibody was binding to numerous TSP molecules. The fact that we have recently obtained a similar result with a monoclonal antibody to TSP reinforces this view (P.H. and A.T.N., unpublished observations, 1985). In another study, we have used identical methods to examine the distribution of GP Ib-IIIa complexes in the membrane of unstimulated platelets and megakaryocytes. The bound IgG were again detected using a second antibody adsorbed to 5-nm gold particles. Here, most of the gold particles were located singly or in small clusters (less than five particles). Thus the results with the anti-TSP and anti-fibrinogen antibodies provide specific information about the distribution of TSP and fibrinogen (or fibrin) on the surface of thrombin-activated platelets.

It should be emphasized that our studies do not distinguish between proteins that are secreted before their binding to surface receptors, and proteins that remain attached to the α-granule membrane but that are exposed at the platelet surface following the morphological changes which accompany the release reaction. We also do not as yet know if secreted TSP and fibrinogen are co-localized at similar sites on the plasma membrane. In vitro studies suggest that TSP and fibrinogen may form complexes, whereas Gartner et al proposed that fibrinogen was a membrane receptor for the platelet lectin, subsequently identified as TSP. In a parallel study to ours, Stenberg et al have also described how fibrinogen was present as clumps on the surface of thrombin-stimulated platelets. Although TSP was not studied, immunogold staining did show secreted PF4 to be distributed in patches on the activated platelet surface. Others, using immunofluorescent techniques, have suggested a similar organization for PF4 and fibronectin. As several of the secreted α-granule proteins appear able to form complexes with one another, the possibility that secreted proteins bind to the same membrane domains on activated platelets is worthy of investigation.

As the binding of secreted fibrinogen to thrombin-stimulated platelets was studied in the presence of Ca^{2+}, some transformation of fibrinogen to fibrin may
have occurred. That thrombin-stimulated platelets may fix fibrinogen has been shown by Plow and Marguerie,42 but it is not yet known whether fibrinogen and polymerizing fibrin fix to the platelet surface by the same mechanism. It was of interest, therefore, to examine the distribution of exogenous fibrinogen bound to ADP-stimulated platelets. Again, particle clusters were observed to be distributed over the platelet surface. Although the clusters appeared smaller and more discrete than those observed on thrombin-stimulated platelets, a direct comparison is difficult due to our lack of knowledge of the binding mechanisms (in complexes or not?) of the secreted α-granule proteins.

The lack of binding of anti-fibrinogen antibodies to thrombathenic platelets stimulated with ADP in the presence of added fibrinogen is in agreement with the lack of fibrinogen-receptor expression by these platelets9,10 and confirms the specificity of our immunostaining procedure. Of considerable interest was the detection of some TSP on the surface of thrombathenic platelets stimulated with thrombin. As these platelets fail to aggregate on addition of thrombin,43 the presence of this TSP is clearly insufficient in itself to support platelet aggregation. Furthermore, GP IIb-IIIa complexes, which are deficient in thrombathemic platelets,20 cannot be the receptor for TSP bound in the absence of free Ca2+. However, our results suggest that normal platelets may have two mechanisms for binding TSP, one of which is Ca2+-dependent. GP IIb-IIIa complexes may be important in the Ca2+-dependent binding of TSP.

Our results suggest that secreted fibrinogen and TSP bind to specific domains on the stimulated platelet membrane. Evidence is therefore accumulating that newly acquired contact sites may be responsible for the mediation of the later stages of the platelet aggregation mechanism.

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