Thrombospondin (TSP) is a major α-granule protein of platelets and accounts for 3% of the total protein and 25% of the total platelet-secreted protein. Many different cell types (including endothelial cells, fibroblasts, and smooth muscle cells) synthesize, secrete, and incorporate TSP into their extracellular matrix. Like the extracellular matrix protein, fibronectin, TSP has distinct structural domains that interact specifically with macromolecules important in hemostasis, such as fibrinogen, collagen, and heparin. TSP has also been reported to interact with a vast repertoire of macromolecules not believed to be involved in hemostasis, such as extracellular matrix proteins, plasma proteins, and lipids.

The major biologic role of TSP has yet to be elucidated, but there is growing evidence that TSP may function to promote both cell–substratum and cell–cell interactions. For example, TSP agglutinates trypsin-treated erythrocytes (referred to as the endogenous platelet lectin activity) and mediates platelet-monocyte interactions. TSP promotes the cell–substratum adhesion of malaria-parasitized erythrocytes, human squamous carcinoma cells, human melanoma cells, and a variety of cell types independent of species, including muscle, epithelial, and endothelial cells. Although TSP has been postulated to play a major role in platelet aggregation and shown to agglutinate activated formalin-fixed platelets, there has been no direct demonstration that TSP promotes the aggregation of metabolically active platelets. In this report, we demonstrate that TSP promotes the aggregation of both nonstimulated and stimulated platelets and that TSP promotes the aggregation of nonstimulated and stimulated platelets by different mechanisms.

MATERIALS AND METHODS

Materials. All reagents, unless specified otherwise, were purchased from Sigma Chemical (St Louis). The monoclonal antibody, OKM5, to glycoprotein IV (GP IV) was purchased from Ortho Diagnostics (Raritan, NJ). The monoclonal antibody, A9A9, to the GPIIb–IIIa complex was a gift from Dr Joel Bennett (University of Pennsylvania, Philadelphia) and was stored as a 10.9 mg/mL stock solution in phosphate-buffered saline (PBS). vitamin B12 was provided by Dr Sandor Shapiro (Thomas Jefferson University, Philadelphia). TSP was purified by fibrinogen-Sepharose chromatography from the released proteins of ionophore-stimulated platelets essentially as previously described. Purified TSP analyzed on sodium dodecyl sulfate (SDS) gels under reducing conditions runs as a major 180,000-dalton polypeptide. This band was eluted from the gel and used to prepare polyclonal anti-TSP sera.

Platelet isolation. Washed human platelets were gel-filtered from citrated platelet-rich plasma (PRP) in Ca2+-free Hepes-buffered Tyrode’s solution containing 0.3% bovine serum albumin (BSA) (HTB buffer) as previously described.

Platelet aggregation. Platelet aggregation was monitored on either a dual-channel or single-channel aggregometer equipped to measure luminescence (Chrono-Log, Havertown, PA). Platelet ATP secretion was measured using the luciferase-luciferin reagent as described by Chrono-Log. In brief, 250 μL platelet suspension (1 x 108/mL) was aggregated with thrombin or ADP with or without TSP. TSP was dissolved in 20 mmol/L Tris-his-propane buffer, pH 7.35, containing 0.25 mol/L NaCl (TSP buffer). Samples aggregated without TSP contained the appropriate amount of TSP buffer. TSP-dependent aggregation of nonstimulated platelets was performed at 37°C in an aggregation cuvette stirred at 1,200 rpm in a manner analogous to the aggregation of platelets with thrombin or ADP. Aggregate formation was monitored at a 200x magnification with a Nikon inverted-phase microscope (Nikon, Tokyo) equipped with phase-contrast optics.

Monoclonal antibody production. Monoclonal antibodies were prepared as described by Kohler and Milstein. In brief, 1 x 107 nonimmunoglobulin secreting mouse myeloma cells (line SP 2/0 depository no. GM3569B, Institute for Medical Research, Camden, NJ) were fused with spleen cells from a mouse injected with purified TSP at 2-week intervals with a final intravenous (IV) injection prior to fusion. Hybridoma antibodies to TSP were detected by direct enzyme-linked immunos assay using solid-phase immobilized TSP. The hybrid was grown as ascites, and the immunoglobulin was purified with immobilized protein A. The TSP-hybridoma antibody was eluted from the gel and used to prepare polyclonal anti-TSP sera.

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was characterized by immunoprecipitation using purified immunoglobulin coupled to Sepharose (discussed in the Results section).

**Immunoblot analysis.** Protein samples were analyzed on discontinuous SDS slab gels with a 4% polyacrylamide stacking gel and an 8% polyacrylamide resolving gel according to the procedure of Laemmli.\(^\text{24}\) Samples were dissolved in SDS sample buffer containing 4 mol/L urea, reduced by the addition of 5% \(\beta\)-mercaptoethanol and heating in a boiling water bath for five minutes. Gels were immuno-blotted with anti-TSP antibody by methods described previously.\(^\text{8}\)

**Anti-TSP antibody affinity chromatography.** A 0.5-mL column containing anti-TSP hybridoma IgG (700 \(\mu\)g) was prepared by coupling the IgG to CNBr-Sepharose according to the manufacturer’s instructions (Pharmacia, Piscataway, NJ). A 1-mL aliquot of platelet-released proteins prepared as previously described\(^\text{8}\) was passed three times over the anti-TSP column at a flow rate of 0.1 mL/min. The column was washed and equilibrated with TNC (10 mmol/L Tris, pH 8.0, containing 1 mmol/L CaCl\(_2\) and 0.5% NP-40) and eluted with five 1-mL aliquots of 50 mmol/L diethylamine in TNC buffer.

**RESULTS**

**Effect of TSP on aggregation of nonstimulated platelets.** Non-stimulated platelets treated with TSP and stirred at 1,200 rpm in an aggregation cuvette slowly aggregated with time, as revealed by light microscopy (Fig 1). After eight minutes, large platelet aggregates were seen in the presence but not in the absence of TSP. No measurable secretion was induced by TSP even at concentrations of 80 \(\mu\)g/mL, as indicated by the lack of increase in fluorescence measured in the lumiaaggregometer using the lucifenin-luciferase system to detect released AlP. In addition, as a further control to insure that TSP-promoted aggregation of nonstimulated platelets is not mediated by levels of released material undetectable by our assay, formalin-fixed, nonstimulated platelets (obtained from the Helena Ristocetin Cofactor Assay Kit, Helena Laboratories, Beaumont, TX) were stirred with 22 \(\mu\)g/mL TSP. TSP promoted aggregation of these platelets to the same extent as it promoted aggregation of nonstimulated, unfixed platelets; for example, 36.5 \(\pm\) 7 aggregates of fixed platelets were formed after two minutes of stirring with 22 \(\mu\)g/mL TSP, whereas 8.7 \(\pm\) 1 aggregates were formed without added TSP. Together, these results indicate that TSP aggregates nonstimulated platelets.

To investigate the nature of the platelet cell surface receptor that mediates TSP-promoted aggregation of nonstimulated platelets and to establish the specificity of the TSP effect, platelets were treated with TSP in the presence of monoclonal antibodies to either TSP, GPIIb-IIIa, or GPIV. Antibodies to GPIV\(^\text{25}\) and GPIIb-IIIa\(^\text{26}\) have previously been characterized, and the characterization of anti-TSP is shown in Fig 2. Figure 2A shows the SDS-gel analysis of platelet-released proteins applied to and eluted from immobilized anti-TSP IgG; Fig 2B shows the eluted material immunoblotted with a specific polyclonal antiserum to TSP. A single amido-black staining band column (lane 3, Fig 2A) corresponding in molecular weight (mol wt) to purified TSP (lane 2, Fig 2A) was eluted from the column. This band was recognized by anti-TSP polyclonal antibody (lane 2, Fig 2B), as was purified TSP (lane 1, Fig 2B), indicating that our hybridoma antibody specifically binds TSP. With antibodies to either TSP or GPIV, TSP-promoted aggregation was inhibited by 53% to 73% and 86% to 87%, respectively (Fig 3). In addition, anti-GPIV inhibited the TSP-promoted aggregation of formalin-fixed platelets (data not shown). In contrast, anti-GPIIb-IIIa and, in addition, vWF and fibronectin had no effect on TSP-promoted aggregation of nonstimulated platelets (Fig 3). These results indicate that TSP-promoted platelet aggregation is specific and further-

![Fig 1. Platelet aggregates produced with TSP or TSP plus ADP. Nonstimulated platelets were stirred in the presence of 22 \(\mu\)g/mL TSP, and at timed intervals aliquots were taken and examined under phase-contrast microscopy. At 2 minutes in the TSP, a duplicate sample was treated with 10 \(\mu\)mol/L ADP for 1 minute and examined for aggregate formation. Control platelets were treated with TSP buffer and stirred for 8 minutes. Photographs were taken at \(\times\)200 magnification.](image)
Fig 2. Analysis of platelet-released proteins bound to an anti-TSP affinity column. Proteins were analyzed on an 8% polyacrylamide slab gel, transferred to nitrocellulose paper, and either stained with amido black (panel A) or a specific polyclonal antibody to the 180,000-dalton chain of TSP purified from SDS gels (panel B). Panel A: lane 1, mol-wt standards; lane 2, fibrinogen-Sepharose-purified TSP; lane 3, proteins eluted from anti-TSP column; lane 4, platelet-released proteins not adhering to anti-TSP column; lane 5, platelet-released proteins applied to anti-TSP column. Panel B: lane 1, polyclonal anti-TSP immunoblot of fibrinogen-Sepharose purified TSP; lane 2, polyclonal anti-TSP immunoblot of anti-TSP purified TSP.

more suggest that platelet GPIV may serve as a receptor for TSP in the TSP-promoted aggregation of nonstimulated platelets.

Effect of TSP on aggregation of thrombin-stimulated platelets. When purified TSP was preincubated with gel-filtered platelets, aggregation of platelets stimulated with thrombin was potentiated dose dependently as indicated by increases in the aggregometer tracings (Fig 4). Both the rate of aggregation, as measured by the slope of the linear portion of the aggregation curve, and the extent of aggregation, as measured by the height of the curve, were increased by TSP.

Fig 3. Effect of monoclonal antibodies to TSP, GPIb-lla, and GPIV and, in addition, the effect of fibronectin and von Willebrand factor (vWF) on aggregation of nonstimulated platelets. Platelet aggregates in ten fields at a x200 magnification were counted after 2 minutes of stirring at 1,200 rpm, 37°C. Values are the mean of separate experiments performed on different platelets using different preparations of TSP. Within any given experiment, at least a 10% variation was observed with duplicate samples; n value refers to the number of separate experiments; error bar is SEM. TSP, vWF, and fibronectin were added at a final concentration of 22 μg/mL. Anti-TSP, anti-GPIV, and anti-GPIb-IIa were added at a final concentration of 22, 6, and 80 μg/mL, respectively.

The increase in the height of the aggregation curves indicates that TSP promotes formation of very large aggregates of thrombin-stimulated platelets. Examination of the aggregates by light microscopy confirmed this, i.e., the platelet aggregates in the presence of TSP were much larger than those in its absence (data not shown).

Fig 4. Effect of TSP on thrombin-induced aggregation of platelets. Gel-filtered platelets were stimulated with 0.1 U/mL thrombin with varying amounts of TSP added 4 minutes before thrombin addition. Aggregation was monitored optically using a Chrono-Log aggregometer as described in the Materials and Methods section.
The specificity of the TSP-promoted aggregation of thrombin-stimulated platelets was assessed with a monoclonal antibody to TSP (Fig 5). With 100 µg/mL anti-TSP IgG, the effect of exogenously added TSP on aggregation was markedly inhibited. Both the rate and extent of platelet aggregation were affected (curve generated with 165 µg/mL preimmune serum as compared with the curve generated with 100 µg/mL anti-TSP IgG in Fig 5). In addition, 100 µg/mL anti-TSP IgG also inhibited the thrombin-stimulated aggregation of platelets without exogenously added TSP, presumably by inhibiting the endogenously secreted TSP. These results indicate that TSP participates in thrombin-stimulated platelet aggregation.

To investigate the cell surface receptors that mediate the TSP-potentiated aggregation of thrombin-stimulated platelets, platelets were stimulated in the presence of TSP plus a monoclonal antibody to GPIIb-IIIa that was previously shown to inhibit both thrombin-induced platelet aggregation and fibrinogen binding. At concentrations of 10.9 µg/mL anti-GPIIb-IIIa and 40 µg/mL TSP, no TSP-promoted aggregation of thrombin-activated platelets was observed (Fig 6). As expected, concentrations of anti-GPIIb-IIIa in the range of 40 µg/mL inhibited aggregation without exogenously added TSP. In contrast, concentrations of anti-GPIV in the range of 20 µg/mL had no effect on TSP-potentiated aggregation of thrombin-stimulated platelets (data not shown). The results suggest that TSP potentiates the aggregation of thrombin-stimulated platelets by mechanisms involving the GPIIb-IIIa complex, although the participation of GPIV in such a mechanism cannot be excluded.

Effect of TSP on ADP-induced platelet aggregation. TSP also promoted aggregation of ADP-stimulated, gel-filtered platelets (Fig 7). The TSP potentiation of aggregation was dependent on addition of TSP before ADP and did not depend on exogenous fibrinogen (top two curves of Fig 7). In contrast, TSP added after significant ADP-stimulated aggregation had occurred did not cause further aggregation, whereas addition of exogenous fibrinogen stimulated further aggregation (bottom two curves of Fig 7). In addition to showing that TSP promotes ADP-stimulated platelet aggregation, these results suggest that TSP does not directly crosslink platelet-fibrinogen aggregates, since it did not promote aggregation of platelets already stimulated with ADP.

At low ADP concentrations, platelets in plasma undergo two sequential phases of platelet aggregation, characterized by first small and then large aggregates as measured by the aggregometer. The first phase is characterized by a rapid formation of small reversible aggregates with no granule secretion. At slightly higher agonist concentrations, the first phase proceeds to a second phase of aggregation, during which secretion occurs. The platelet aggregates formed during the second wave of aggregation are much larger than those formed in the first and are irreversibly aggregated. TSP promoted the aggregation of both the first and second phases of ADP-induced platelet aggregation in PRP (Fig 8). At 40 µg/mL TSP, there was an increase in the first phase of
platelet aggregation, followed by what appeared to be a small secretion-like second phase of aggregation, although no platelet secretion occurred, as measured by ATP release. At 80 μg/mL TSP, there was a substantial increase in a second-like phase of aggregation characterized by formation of large aggregates. These results indicate that TSP can induce aggregation of ADP-stimulated platelets in plasma that mimics the first and second phases of ADP-induced aggregation even though no secretion was detectable.

**Fig 8.** Effect of TSP on ADP-stimulated platelet aggregation in plasma. Platelets were aggregated with 1 μmol/L ADP either with or without TSP. Aggregation was monitored optically using a Chrono-Log aggregometer as described in the Materials and Methods section.

**DISCUSSION**

The role of TSP in hemostasis is not well understood. It has been postulated that TSP crosslinks platelet-fibrinogen aggregates,21 stabilizes fibrin clot formation,22,28 and modulates fibrinolysis.29 The most convincing evidence suggesting a role for TSP in platelet aggregation has come from studies showing that specific antibodies to TSP inhibit platelet aggregation.21,30,31 However, no direct studies showing that TSP potentiates aggregation of metabolically active platelets, as predicted by the antibody studies, have been reported. On the contrary, Kao et al32 reported that exogenously added TSP inhibited platelet aggregation induced by very low thrombin concentrations and had no effect at thrombin concentrations in the range used in this study. Yet another study suggested that TSP may play a regulatory role in hemostasis by inhibiting platelet adhesion and providing a nonthrombogenic surface.32

In contrast, our results indicate that TSP promotes aggregation of resting and stimulated platelets. The reason for our differing results is not known. TSP preparations purified by fibrinogen-Sepharose in our studies may retain a greater degree of biologic activity than those purified on heparin-Sepharose chromatography, the method of TSP purification used in previous studies. Our preparations show that TSP has an apparent mol wt of 902,000 as measured by gel filtration under nondenaturing conditions, suggesting dimer formation.26 More detailed physical characterization of the biologically active forms of TSP awaits further work and is beyond the scope of the present study. The action of TSP is specific, since it is blocked by a monoclonal antibody to TSP. The TSP-dependent aggregation of resting platelets was also blocked by an antibody to GPIV but not by an antibody to GP Ib-IIIa. This observation is consistent with that of Wolff et al33 who showed that the binding of TSP to nonstimulated platelets was not inhibited by antibodies to GPIb-IIIa, whereas TSP binding to activated platelets was. These data suggest that TSP binds to nonstimulated platelets through receptors other than GPIb-IIIa while binding to activated platelets in a fibrinogen-dependent manner involving GPIb-IIIa. In addition, Aiken34 showed that thrombin-activated thrombasthenic platelets bound twice as much TSP as normal activated platelets, suggesting that platelets can bind TSP by non–GPIb-IIIa receptor-mediated mechanisms. Because our results show that anti-GPIV blocks TSP-mediated aggregation of nonstimulated platelets, GPIV may mediate the binding of TSP to nonstimulated platelets, as described by Wolff et al and Aiken et al. The antibody to GPIV recognizes a 88,000-dalton glycoprotein present on the surface of platelets, endothelial cells, and monocytes.25,35,36

This antibody inhibits the adhesion of malaria parasitized RBCs to endothelial cell monolayers and the human melanoma cell line C32.17 Using this antibody, Ash et al25 recently identified and isolated an 88,000-dalton membrane GP present in platelets, endothelial cells, and monocytes that is the membrane binding site for TSP. Consistent with the findings of Ash et al25 our results also suggest that the platelet TSP-receptor mediating TSP-dependent aggregation of nonstimulated platelets is GPIV. However, steric

Acad Sci USA 80:998, 1986


TSP promotes the aggregation of both thrombin-stimulated and ADP-stimulated platelets. TSP (4 to 40 μg/mL) dramatically increased both the rate and extent of aggregation. The platelets not only aggregated faster, but the size of the final aggregates was larger with TSP than without it. A monoclonal antibody to TSP inhibited the aggregation of thrombin-stimulated platelets both with and without exogenously added TSP, suggesting that endogenous TSP is essential for aggregation. In this system, antibodies to GPIIb-IIIa completely inhibited the TSP potentiation of aggregation, whereas antibodies to GPIb had no effect. These results suggest that TSP-potentiated aggregation is mediated through GPIIb-IIIa, probably through the binding of fibrinogen. However, TSP may also function independently of fibrinogen in the aggregation of stimulated platelets, since Newman et al. showed that platelets treated with monoclonal antibodies to GPIIb and to GPIIIa aggregated in response to ADP but did not bind fibrinogen. In addition, Cattaneo et al. recently described a fibrinogen-independent aggregation of afibrinogenemic platelets that may involve TSP.

When platelets in citrated plasma are aggregated with low levels of ADP, they undergo a secretion-independent reversible first phase of aggregation. Our studies show that exogenously added TSP potentiates the reversible first phase of aggregation. Furthermore, with TSP, the platelets proceed to an irreversible secondlike phase of aggregation that resembles the secretion-dependent second phase of aggregation without exogenously added TSP, even though no secretion occurs. These results suggest that TSP plays an important role in the secretion-dependent phase of platelet aggregation.

TSP has previously been suggested to crosslink platelet aggregates. To test this hypothesis, gel-filtered platelets were partially aggregated with ADP before TSP was added. Under these conditions, the exogenously added TSP did not promote further aggregation, whereas exogenously added fibrinogen did. In contrast, either fibrinogen mixed with TSP or TSP added prior to ADP stimulation promoted aggregation. These results suggest that TSP may not directly crosslink platelet aggregates, but rather may act synergistically with fibrinogen through GPIIb-IIIa on the platelet surface. TSP could either provide additional fibrinogen receptor sites on the platelet surface or increase the binding constant for fibrinogen by interaction with either fibrinogen or the GPIIb-IIIa complex. Finally, TSP could directly interact with GPIIb-IIIa. Further work is needed to differentiate between these possibilities.

REFERENCES


Thrombospondin promotes platelet aggregation

GP Tuszynski, VL Rothman, A Murphy, K Siegler and KA Knudsen