Identification of the cytoskeletal protein α-actinin as a platelet thrombospondin-binding protein

Véronique Dubernard[a,b,*], Didier Faucher[c], Jean-Marie Launay[d], Chantal Legrand[a,b]

[a]*Unité INSERM 353, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010-Paris, France*
[b]*Centre de Recherches sur le Sang et les Vaisseaux de l'Association Claude Bernard, Hôpital Lariboisière, 2 rue Ambroise Paré, 75010-Paris, France*
[c]*Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, Département des Biotechnologies, 13 rue Jules Guesde, 94403-Vitry sur Seine, France*
[d]*Formation de Recherches Associée de Neurochimie des Communications Cellulaires de l'Association Claude Bernard, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010-Paris, France*

Received 9 March 1995

Abstract Binding of the α-granular thrombospondin (TSP) to the plasma membrane of activated platelets has long been documented, yet the molecular mechanism involved in its secretion and surface expression have not been elucidated. Using a ligand blot binding assay where electrophoretically separated platelet proteins were incubated with purified ^{125}I-labeled TSP, we observed a strong interaction of [^{125}I]TSP with a 100 kDa single chain protein. On performing a platelet subfractionation, the 100 kDa protein was predominantly localized in the cytosol from which it was purified by preparative electrophoresis and was identified by amino acid sequencing to the cytoskeletal protein, α-actinin. We further demonstrated that [^{125}I]TSP interacts with α-actinin in a specific manner and with a high affinity (K_d = 6.6 nM) in a solid-phase binding assay.

Key words: Thrombospondin; α-Actinin; Molecular interaction; Human platelet

1. Introduction

Thrombospondin-1 (TSP) is a 420 kDa adhesive multifunctional glycoprotein which is synthesized and secreted by a large variety of cells and may interact with many molecules, such as heparin, heparan sulfate proteoglycans, sulfated glycolipids, collagens, fibronectin, laminin, fibrinogen and plasminogen [1–3]. Depending on the cellular types, potential receptors for TSP include proteoglycans [4], CD36 (also named glycoprotein (GP)IV or GPIIIb, in platelets) [5,6] and the integrins of the β_3 family: $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ [7,8]. Three distinct receptors for TSP have also been identified on different tumor cell lines [9–11]. Due to these multiple interactions, the expression of TSP on cell surfaces has been related to a number of processes such as cell adhesion, migration and proliferation as well as cell–cell interactions [1–3]. In addition, TSP has been shown to play a role in platelet aggregation and fibrin clot formation [1–3] and is considered as a potential regulator of fibrinolysis [12] and angiogenesis [3]. However, the precise molecular mechanisms underlying the various biological functions of TSP are not clearly defined. An additional level of complexity was recently introduced with the identification of five structurally and genetically distinct forms of TSPs exhibiting different tissue distribution and potentially different functions [13]. To date, most studies concerned with biological functions have been conducted with platelet TSP, now referred to as TSP-1.

In platelets, TSP is secreted from α-granules and plays a major role in platelet aggregation by promoting the formation of irreversible platelet macroaggregates [14–16]. This effect is thought to be mediated by its binding to cell surface-associated fibrinogen and subsequent stabilization of the interaction of fibrinogen with its platelet receptor, the GPIIb–IIIa complex or $\alpha_{IIb}\beta_3$ integrin [14–17]. Integral membrane glycoproteins, including $\alpha_{IIb}\beta_3$ itself, $\alpha_V\beta_3$ and GPIV, are also considered as potential receptors for secreted TSP on activated platelets [5–8]. However, none of these molecules appear to be essential, as platelets from thrombasthenic patients that lack $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ or those from individuals lacking GPIV bind TSP normally [18–21]. Other studies have suggested that binding of α-granular proteins on the surface of activated platelets might arise from the secretion of these proteins already bound to endogenous receptors [22,23]. Thus, intracellular TSP binding molecules, to date unidentified, might also contribute to the surface expression of endogenous TSP on activated platelets.

In this study, we have used a ligand blot binding assay combined with a platelet subfractionation to identify platelet components able to interact with purified ^{125}I-labeled TSP. We report on the identification of the cytoskeletal actin binding protein, α-actinin, as a specific TSP binding protein.

2. Materials and methods

2.1. Platelet isolation and subfractionation

Fresh human platelets were isolated from acid-citrate-anticoagulated blood, as described elsewhere [20]. Subcellular fractions were prepared according to previous procedures [24,25], with some modifications. Washed platelets were resuspended at 2×10^9 cells/ml in 15 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 250 mM sucrose, 0.2 mM leupeptin, 1 mM benzamidine, 100 nM prostaglandin E_1 (Sigma Chemical Co., St. Louis, MO) and 25 μg/ml apyrase (grade I; Sigma), pH 7.4. The cells were disrupted on ice by controlled sonication: 3 cycles of 5 s at 40 W with 1 min intervals (Vibra cell Sonifier; Bioblock Scientific, Illkirch, France) and centrifuged for 15 min at $1,200 \times g$ and 4°C. The pellet of unlysed platelets was resuspended and resonicated. After centrifugation, the two supernatants were combined, which comprised more than 90% of the platelet proteins, and centrifuged for 30 min at $19,000 \times g$ and 4°C to isolate a crude organelle fraction. The supernatant was further centrifuged for 60 min at $100,000 \times g$ and 4°C to separate a crude membrane fraction from the cytosolic fraction. Organelle and membrane fractions were washed once by centrifugation as described for their isolation. The purity of each platelet subfraction was assessed by measurements of 5-hydroxytryptamine [26]. β-thromboglobulin (Radioimmunoassay kit;

*Corresponding author. Fax: (33) (1) 42 49 07 33.

SSDI 0014-5793(95)00362-2

Amersham, Les Ulis, France), β-glucuronidase [27], lactate dehydrogenase [28] and adenylate cyclase activities [29], the respective markers of platelet dense bodies, α-granules, lysosomes, cytosol and plasma membrane.

2.2. Electrophoresis procedures

Discontinuous SDS-PAGE was carried out on polyacrylamide slab gels, as described elsewhere [20], and two-dimensional electrophoresis was performed according to the procedure of O'Farrell, modified as described [30]. All reagents for electrophoresis were purchased from Bio-Rad (Richmond, CA).

2.3. Demonstration of a TSP binding protein

A TSP binding protein of 100 kDa was demonstrated in platelets by a ligand blot binding assay. Platelet proteins were separated by SDS-PAGE and electrotransferred to nitrocellulose sheets as previously described [20], except that electrotransfer was carried out using a Semi-Phor semi-dry Transfer Unit (Hoefer Scientific Instruments, San Francisco, CA). The nitrocellulose sheet was probed with [^{125}I]TSP (≈0.3 μCi/ml, ≈2–3 μg/ml) in 15 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.04% (w/v) Ficoll 400 and 0.1% (v/v) Tween 20, pH 7.4, containing 1.5% (w/v) bovine serum albumin (BSA, fraction V; Boehringer-Mannheim, Germany) for 2 h at 20°C.

2.4. Purification and identification of the TSP binding protein

The TSP binding protein found to be enriched in platelet cytosol was purified by preparative electrophoresis from this fraction. Platelet proteins (5 mg) from the platelet cytosolic fraction were separated by SDS-PAGE on a 6.5% polyacrylamide 3 mm-thick slab gel. The gel was immersed in a 4 M solution of sodium acetate for a rapid visualization of the protein bands [31] and the band at 100 kDa corresponding to the electrophoretic migration of the TSP binding protein was marked with a scalpel. The gel was extensively washed with 2.5% (w/v) Triton X-100 (BDH Chemicals Ltd., Poole, England) to remove sodium acetate and SDS, and equilibrated in 10 mM Tris-HCl, pH 6.8. The 100 kDa protein band was cut out from the gel, crushed and left overnight at 4°C into 10 mM Tris-HCl, pH 6.8. The eluted protein (≈100 μg) was recovered after centrifugation of the sample for 15 min at 12,000 × *g* and 4°C, and lyophilized.

For identification of the TSP binding protein by amino acid sequencing, peptides were generated from the purified protein by overnight digestion with trypsin (1:60, w/w; Boehringer-Mannheim) at 37°C. The material was lyophilized, solubilized in 200 μl 6 M guanidinium chloride and injected onto a 218TP52 Vydac (Hesperia, CA) HPLC reverse-phase C_{18} column. The peptides were eluted with 0.1% trifluoroacetic acid in water then with a 0–35% linear gradient of 0.1% trifluoroacetic acid in acetonitrile at a flow rate of 200 μl/ml. Fractions were sequenced using an ABI 477 A sequencer with an on-line 120 A analyser (Applied Biosystems, Foster City, CA) and compared to sequences of the proteins contained in the Swiss-Prot protein data bank, using the Intelligenetics Inc. computer software.

2.5. Solid-phase binding assays

Solid-phase binding assays were performed as previously described [15] in microtiter wells coated in duplicate with 10 μg/ml purified α-actinin, diluted in 20 mM Tris-HCl, 150 mM NaCl, 2 mM $CaCl_2$, pH 7.2. BSA-coated wells were used as controls. Increasing concentrations of purified [^{125}I]TSP were incubated with α-actinin or BSA-coated wells for 60 min at 37°C. Non-specific binding of [^{125}I]TSP was determined as the residual binding measured in the presence of a 60-fold excess of unlabeled TSP. Purified fibrinogen (IMCO Corporation, Stockholm, Sweden) and fibronectin (Boehringer-Mannheim) were radiolabeled similarly to TSP and used in separate samples.

3. Results

3.1. Evidence for a specific association of ^{125}I-TSP with a 100 kDa platelet protein

When incubated with electrophoretically separated proteins from a platelet lysate in a blot binding assay (Fig. 1), [^{125}I]TSP exhibited a strong reactivity with a component of an apparent molecular mass of 100 kDa (Fig. 1A), the mobility of which did

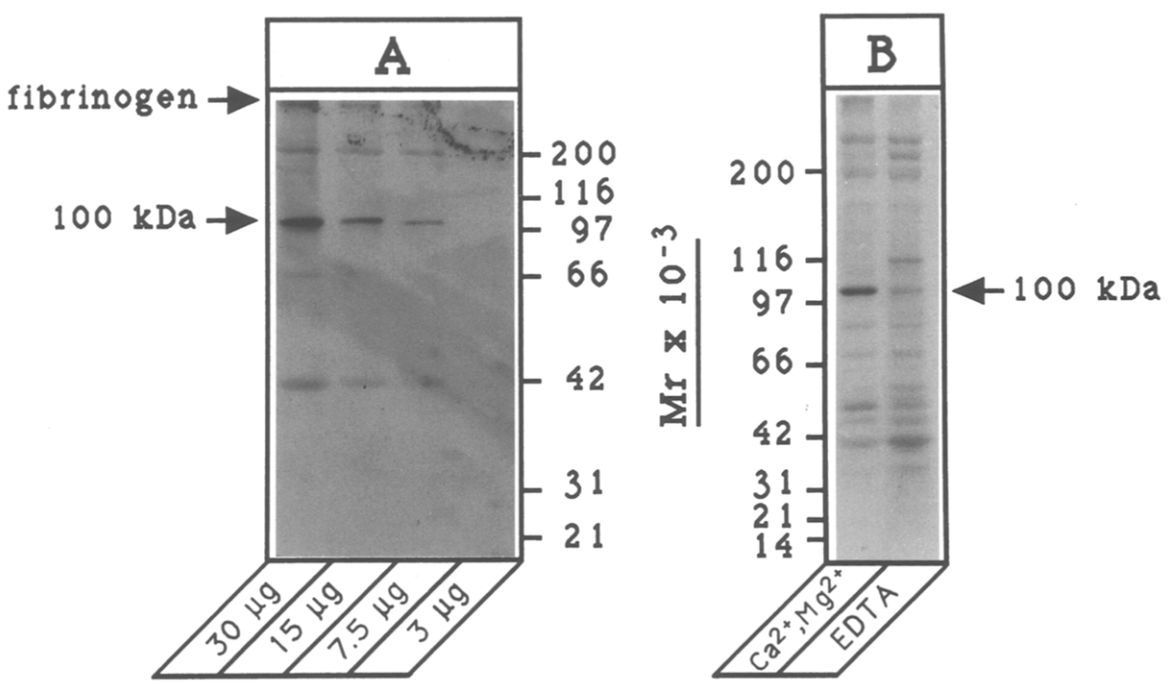

Fig. 1. Specific interaction of [^{125}I]TSP with a 100 kDa platelet protein by a blot binding assay. Proteins from total platelet lysates were separated by SDS-PAGE, under non-reduced conditions, using an 8% polyacrylamide gel (A) or a 5–12% gradient polyacrylamide gel (B), electrotransferred to nitrocellulose sheets and incubated with 3 μg/ml [^{125}I]TSP, as described in section 2. Bound radioactivity was visualized by autoradiography. (A) Decreasing amounts of platelet proteins were probed with [^{125}I]TSP. (B) Platelet proteins (30 μg) were probed with [^{125}I]TSP in the presence of 2 mM $CaCl_2$ and 1 mM $MgCl_2$, as in A, or 2 mM EDTA, as indicated in the figure.

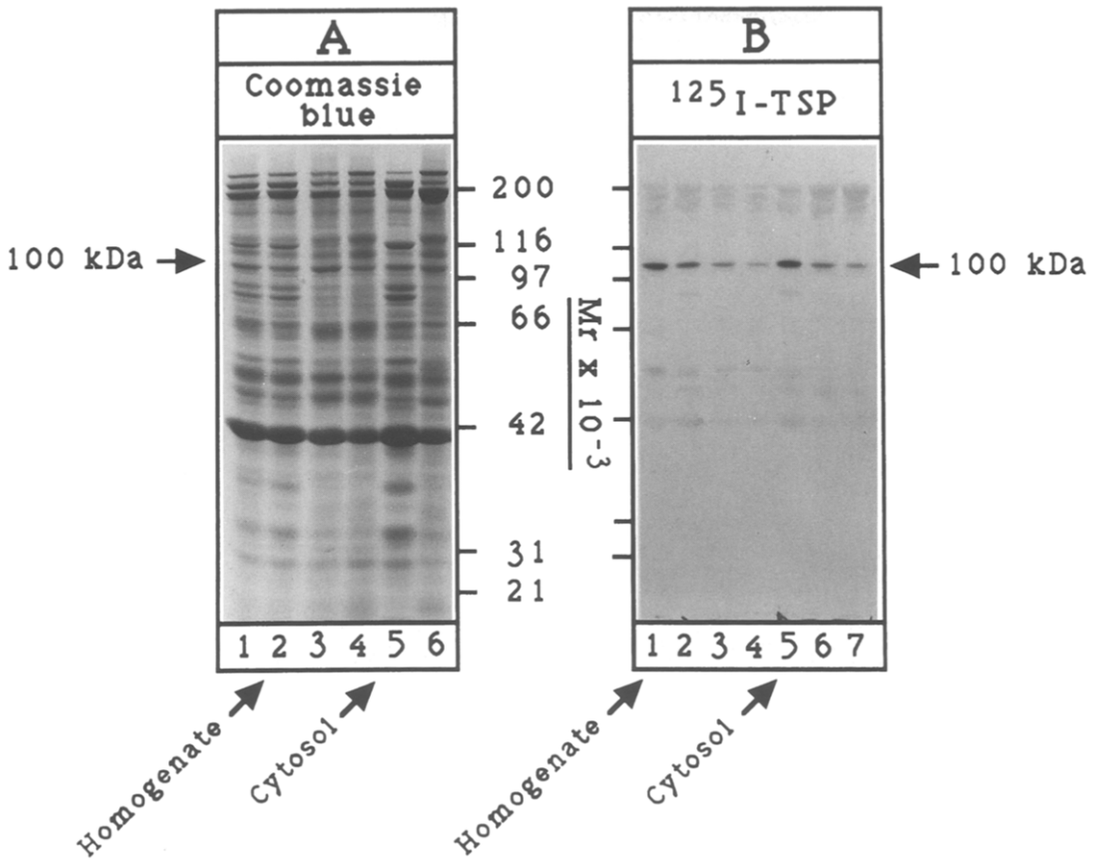

Fig. 2. Subcellular fractionation showing the localization of the TSP binding protein in the platelet cytosol. Proteins (30 μg) from total platelet lysates or the different subfractions were electrophoresed on a 8% polyacrylamide gel under reduced conditions. (A) Proteins were stained by Coomassie blue: intact platelets (lane 1), platelet homogenate (lane 2), residual pellet from sonicated platelets (lane 3), granular fraction (19,000 × *g* pellet) (lane 4), cytosolic fraction (100,000 × *g* supernatant) (lane 5), membrane fraction (100,000 × *g* pellet) (lane 6). (B) Proteins were electrotransferred and incubated with 2 μg/ml [^{125}I]TSP: platelet homogenate (lane 1), supernatant (lane 2) and granular fraction (lane 3) from the 19,000 × *g* centrifugation, washed granular fraction (lane 4), cytosolic fraction (lane 5), membrane fraction (lane 6), washed membrane fraction (lane 7).

not change upon disulfide reduction (see Fig. 2B). This protein did not correspond in size to any of the proteins so far identified as TSP binding molecules in platelets. By comparison, fibrinogen, which is one of the most abundant proteins in platelets, exhibited a lower reactivity with [^{125}I]TSP. Similar results were obtained when the blots were incubated with unlabeled TSP followed by a radiolabeled anti-TSP antibody (not shown), clearly demonstrating that TSP, and not a possible minor contaminant in the purified preparation, was actually interacting with the 100 kDa protein. The interaction of [^{125}I]TSP with the 100 kDa protein appeared to be very strong as it was detected even when as little as 7.5 μg of platelet proteins was probed (Fig. 1A) and was divalent cation-dependent as no significant binding occurred in the presence of 2 mM EDTA (Fig. 1B).

3.2. Subcellular localization of the TSP binding protein

To investigate the cellular localization of the 100 kDa protein, a platelet subfractionation procedure was performed. The organelle fraction obtained, containing α-granules, dense bodies and lysosomes, was enriched 2–3 fold in β-thromboglobulin, 5-hydroxytryptamine and β-glucuronidase relative to the homogenate. The cytosolic and membrane fractions were enriched 2- and 10 fold, respectively, in lactate dehydrogenase and adenylate cyclase. When equal amounts of protein from these subfractions (Fig. 2A) were probed with [^{125}I]TSP in the blot binding assay (Fig. 2B), the 100 kDa component was predominantly found in the cytosolic fraction (lane 5) comprising approximately 50% of the total platelet proteins. A residual labeling was associated with the washed organelle and membrane fractions (lanes 4 and 7) which contained approximately 20% and 10% of the platelet proteins, respectively. By counting the radioactivity associated with the 100 kDa band, we estimated that about 68%, 10% and 7% of the 100 kDa protein was recovered in the cytosolic, organelle and membrane fractions, respectively.

3.3. Identification of the TSP binding protein to α-actinin

The 100 kDa protein was purified from the cytosolic fraction by preparative electrophoresis. Analysis by the O'Farrell two-dimensional electrophoresis procedure indicated the presence of a single protein with a series of charged isoforms ($5.7 < pI < 6.2$) (Fig. 3A and B), all of which reacted with [^{125}I]TSP (Fig. 3C). When submitted to the Edman degradation procedure for amino acid sequencing, no amino acid was released from the 100 kDa protein, indicating that the NH_2-terminus was blocked. Therefore, a tryptic digestion of the

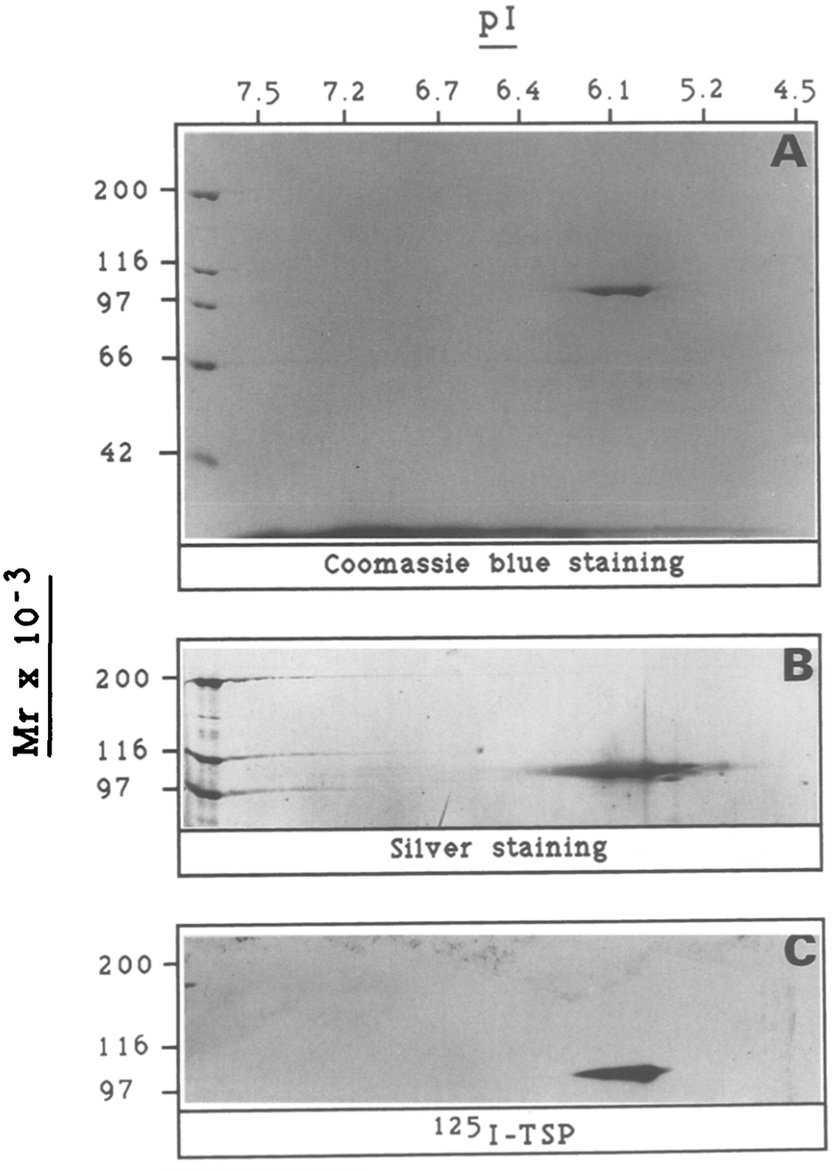

Fig. 3. Two-dimensional analysis of the purified TSP binding protein. The 100 kDa TSP binding component was isolated from platelet cytosol by preparative electrophoresis, as described in section 2, and analyzed by two-dimensional O'Farrell electrophoresis. The purified protein (20 μg) was subjected to isoelectrofocusing in the first dimension followed by electrophoresis on a SDS-6.5% polyacrylamide gel under reducing conditions in the second dimension. The protein was either stained with Coomassie blue (A) and subsequently with silver (B) or probed with 3 μg/ml [^{125}I]TSP (C).

protein was performed and two fractions collected from the HPLC chromatography of the hydrolysate were sequenced. Four short sequences were identified as part of the α-actinin molecule: T(727)INEVENQILTR(738), E(851)LPPDQAEYX-IAR(863), L(566)AILGIHNEVSK(578) and I(835)LAGDKN-YITMDELR(849) [32].

3.4. *[^{125}I]TSP binding to solid-phase adsorbed purified α-actinin*

A solid-phase binding assay was used to measure the affinity of the interaction of [^{125}I]TSP with purified α-actinin. When incubated with [^{125}I]TSP over the range of 1.1–22 nM, immobilized α-actinin was found to bind increasing amounts of [^{125}I]TSP (Fig. 4A). By comparison, no binding of [^{125}I]fibrinogen or [^{125}I]fibronectin was measured. Non-specific binding of [^{125}I]TSP to α-actinin amounted to 20–30% of the total binding; the specific binding was calculated and plotted following Scatchard analysis (Fig. 4B). A mean dissociation constant (K_d) of 6.6 nM was calculated from the data of two reproducible experiments. Finally, the binding of [^{125}I]TSP to solid-phase adsorbed α-actinin was found to require the presence of divalent cations and to be inhibited by a monoclonal antibody to α-actinin used at 10 μg/ml (data not shown).

4. Discussion

The present study demonstrates for the first time a specific interaction of TSP with the cytoskeletal actin binding protein, α-actinin. The specificity of the interaction was demonstrated

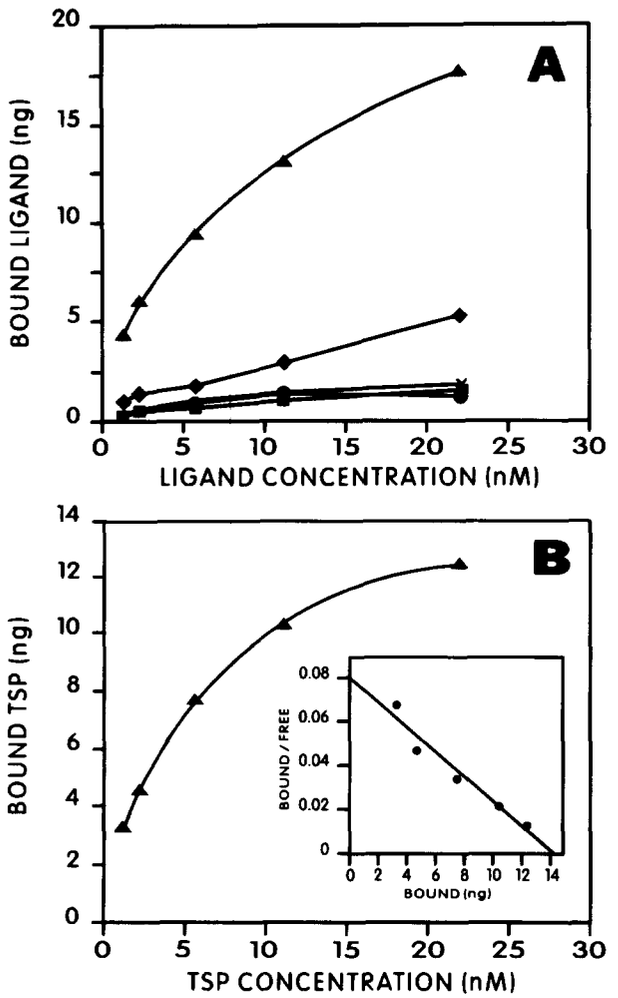

Fig. 4. Specific interaction of [^{125}I]TSP with α-actinin in a solid-phase binding assay. (A) Microtiter wells were coated in duplicate with 10 μg/ml of purified α-actinin and incubated with 1.1–22 nM [^{125}I]TSP (▲), [^{125}I]fibrinogen (■) or [^{125}I]fibronectin (●). In parallel samples, [^{125}I]TSP binding was measured in microtiter wells coated with BSA alone (×). Non-specific binding of [^{125}I]TSP to α-actinin was measured in the presence of excess (60×) unlabeled TSP (◆). (B) Specific binding of [^{125}I]TSP to α-actinin was calculated by subtracting non-specific binding from the total binding; the Scatchard plot is shown in the inset.

in a solid-phase binding assay: (i) binding of [^{125}I]TSP to α-actinin was concentration-dependent and saturable; (ii) excess of unlabeled TSP inhibited this binding by 70–80%; (iii) the binding was specifically inhibited by a monoclonal antibody to α-actinin; and (iv) two other α-granular adhesive proteins, namely [^{125}I]fibrinogen and [^{125}I]fibronectin, failed to interact with α-actinin by this technique.

Using a blot binding assay performed with whole platelet proteins, [^{125}I]TSP was bound almost exclusively to α-actinin. Other platelet proteins, including fibronectin, histidine-rich glycoprotein, plasminogen and GPIV were undetectable although they were previously shown, in the purified form, to interact with TSP by the blot binding technique [5,33]. Our failure to detect these proteins may be due to their low concentrations in platelets. The weak reactivity observed with fibrinogen, which is abundant in platelets, as compared to α-actinin, demonstrates a higher affinity of TSP for α-actinin. Indeed, from the results obtained in the solid-phase binding assay, we measured a dissociation constant of 6.6 nM for the TSP–α-actinin interaction in comparison to 40 nM for the interaction of TSP with fibrinogen under similar conditions (personal results).

An association between secreted adhesive α-granular proteins and the cytoskeleton is currently thought to be mediated by transmembrane proteins, such as integrins, acting as receptors for the adhesive proteins [34]. The present demonstration of a direct association of the α-granular protein TSP with the cytoskeletal protein α-actinin raises the question of when do these two proteins have an opportunity to interact with each other in a cellular context. The presence of α-actinin in the α-granules of resting platelets was already evidenced by an immunoelectron microscopic study [35]. In addition, upon platelet activation by thrombin, α-actinin was found to be redistributed towards the plasma membrane [36,37]. We have also addressed this point by comparing the localization of α-actinin and TSP in platelets. By performing indirect immunofluorescence studies on resting platelets we confirmed the cytosolic localization of α-actinin shown by others [36,37]. However, additional staining of α-actinin with a distribution similar to that of TSP was also observed, suggesting a possible co-localization of these two proteins in the α-granules (manuscript in preparation). This is compatible with the results obtained by platelet subfractionation (Fig. 2) showing a fraction of α-actinin in the granular-enriched subfraction. Further studies are needed to investigate the physiological significance of the interaction between TSP and α-actinin during the platelet activation process.

Acknowledgements: We are most grateful to Dr. Suzanne Menashi for advice on the preparation of platelet subfractions and critical review of our manuscript, Dr. Marie-Louise Giron for help in O'Farrell two-dimensional electrophoresis, and Françoise Cuiné for technical assistance in amino acid sequencing. We also thank Jacqueline Boisse, Robin Nancel and Elizabeth Savariau for the photographic art work.

References

[1] Lawler, J.W. (1986) Blood 67, 1197–1209.
[2] Murphy-Ullrich, J.E. (1991) J. Lab. Clin. Med. 118, 418–420.
[3] Frazier, W.A. (1991) Curr. Opin. Cell Biol. 3, 792–799.
[4] Sun, X., Kaesberg, P.R., Choay, J., Harenberg, J., Ershler, W.B. and Mosher, D.F. (1992) Sem. Thromb. Hemostasis 18, 243–251.
[5] Asch, A.S., Barnwell, J., Silverstein, R.L. and Nachman, R.L. (1987) J. Clin. Invest. 79, 1054–1061.
[6] Asch, A.S., Liu, I., Briccetti, F.M., Barnwell, J.W., Kwakye-Berko, F., Dokun, A., Goldberger, J. and Pernambuco, M. (1993) Science 262, 1436–1440.
[7] Karczewski, J., Knudsen, K.A., Smith, L., Murphy, A., Rothman, V.L. and Tuszynski, G.P. (1989) J. Biol. Chem. 264, 21322–21326.
[8] Lawler, J. and Hynes, R.O. (1989) Blood 74, 2022–2027.
[9] Yabkowitz, R. and Dixit, V.M. (1991) Cancer Res. 51, 3648–3656.
[10] Tuszynski, G.P., Rothman, V.L., Papale, M., Hamilton, B.K. and Eyal, J. (1993) J. Cell Biol. 120, 513–521.
[11] Gao, A.G. and Frazier, W.A. (1994) J. Biol. Chem. 269, 29650–29657.
[12] Mosher, D.F., Misenheimer, T.M., Stenflo, J. and Hogg, P.J. (1992) Ann. NY Acad. Sci. 667, 64–69.
[13] Adams, J. and Lawler, J. (1993) Curr. Biol. 3, 188–190.
[14] Leung, L.L.K. (1984) J. Clin. Invest. 74, 1764–1772.
[15] Legrand, C., Thibert, V., Dubernard, V., Begault, B. and Lawler, J. (1992) Blood 79, 1995–2003.

[16] Legrand, C., Morandi, V., Mendelovitz, S., Shaked, H., Hartman, J.R. and Panet, A. (1994) Arterioscler. Thromb. 14, 1784–1791.
[17] Asch, A.S., Leung, L.L.K., Polley, M.J. and Nachman, R.L. (1985) Blood 66, 926–934.
[18] Aiken, M.L., Ginsberg, M.H. and Plow, E.F. (1987) Sem. Thromb. Hemostasis 13, 307–316.
[19] Boukerche, H. and McGregor, J.L. (1988) Eur. J. Biochem. 171, 383–392.
[20] Dubernard, V. and Legrand, C. (1991) J. Lab. Clin. Med. 118, 446–457.
[21] Kehrel, B., Kronenberg, A., Schwippert, B., Niesing-Bresch, D., Niehues, U., Tschöpe, D., Van de Loo, J. and Clemetson, K.J. (1991) Biochem. Biophys. Res. Commun. 179, 985–991.
[22] Ginsberg, M.H. and Plow, E.F. (1981) J. Supramol. Struct. Cell. Biochem. 17, 91–98.
[23] Legrand, C., Dubernard, V. and Nurden, A.T. (1989) Blood 73, 1226–1234.
[24] Enouf, J. and Levy-Toledano, S. (1984) Br. J. Pharmac. 81, 509–518.
[25] Menashi, S., Hornebeck, W., Robert, L. and Legrand, Y. (1989) Thromb. Res. 53, 11–18.
[26] Walker, R.F., Friedman, D.W. and Jimenez, A. (1983) Life Sci. 33, 1915–1924.
[27] Dangelmaier, C.A. and Holmsen, H. (1980) Anal. Biochem. 104, 182–189.
[28] Launay, J.M., Geoffroy, C., Costa, J.L. and Alouf, J.E. (1984) Thromb. Res. 33, 189–196.
[29] Vittet, D., Mathieu, M.N., Cantau, B. and Chevillard, C. (1988) Eur. J. Pharmacol. 150, 367–372.
[30] Mishell, B.B. and Shiigi, S.M. (1980) in: Selected Methods in Cellular Immunology (Mishell, B.B. and Shiigi, S.M., eds.) pp. 411–435, Freeman, San Francisco.
[31] Higgins, R.C. and Dahmus, M.E. (1979) Anal. Biochem. 93, 257–260.
[32] Millake, D.B., Blanchard, A.D., Patel, B. and Critchley, D.R. (1989) Nucleic Acids Res. 17, 6725.
[33] Walz, D.A., Bacon-Baguley, T., Kendra-Franczak, S. and DePoli, P. (1987) Sem. Thromb. Hemostasis 13, 317–325.
[34] Fox, J.E.B. (1993) Thromb. Haemostas. 70, 884–893.
[35] Puszkin, E.G., Jenkins, C.S.P., Ores-Carton, C. and Zucker, M.B. (1985) J. Lab. Clin. Med. 105, 52–62.
[36] Debus, E., Weber, K. and Osborn, M. (1981) Eur. J. Cell Biol. 24, 45–52.
[37] Sixma, J.J., van den Berg, A., Jockusch, B.M. and Hartwig, J. (1989) Eur. J. Cell Biol. 48, 271–281.