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STRUCTURAL AND FUNCTIONAL COMPARISON OF THROMBOSPONDIN FROM PLATELETS, ENDOTHELIAL CELLS AND FIBROBLASTS.

P. Clezardin and J.L. McGregor. INSERM U 63 - Laboratoire d'Hémobiologie, Faculté de Médecine A. Carrel, Lyon, France.

Thrombospondin (TBSP) is a 450 kDa glycoprotein secreted by a wide range of cells including platelets, endothelial cells and fibroblasts. Using non-denaturating conditions, we recently reported that platelet TBSP was structurally different from endothelial and fibroblast TBSP (P. Clezardin et al., Eur. J. Biochem., 1986, 159, 569-579). The aim of this study was to compare the structure of TBSP purified from platelets, endothelial cells and fibroblasts using denaturating conditions. Moreover, the interaction of fibrinogen with these three forms of TBSP was also investigated. TBSPs were first purified by heparin-Sepharose and immunoaffinity chromatography followed by Mono Q anion-exchange chromatography on a FPLC system. Thermolysin digests of purified TBSPs were analysed by SDS-polyacrylamide gel electrophoresis under reducing conditions and the subsequent electrophoresed proteolytic fragments identified by Coomassie and silver staining. The interaction of undigested and digested TBSPs with solid-phase adsorbed fibrinogen was investigated by enzyme-linked immunosorbent assay using an anti-TBSP monoclonal antibody (P10). When using Coomassie staining, a 70 kDa proteolytic fragment of thermolysin-treated platelet TBSP was absent from the endothelial and fibroblast TBSP digests. Moreover, a 18 kDa fragment from thermolysin-treated endothelial and fibroblast TBSP was undetectable in the platelet TBSP digest when using silver staining on SDS-polyacrylamide gels. The binding of undigested TBSPs to solid-phase adsorbed fibrinogen was different from that obtained with digested TBSPs. These results indicate that the observed structural differences might induce functional differences between platelet and the two other forms of TBSP.

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Thrombospondin Promotes Cell-and Platelet-Substratum Adhesion.

George P. Tuszynski, Vicki L. Rothman, Andrew Murphy, Katherine Siegler, Linda Smith, Sena Smith, Jerzy Karczewski, and Karen A. Knudsen. Lankenau Medical Research Center, Lancaster Avenue west of City Line Avenue, Philadelphia, Pa. 19151, U.S.A.

Thrombospondin (TSP), isolated from human platelets, promotes the in vitro, calcium-specific adhesion of a variety of cells, including platelets, melanoma cells, muscle cells, endothelial cells, fibroblasts, and epithelial cells. The cell adhesion-promoting activity of TSP is species independent since human, bovine, pig, rat and mouse cells all adhered to TSP. Furthermore, the cell adhesion-promoting activity of TSP is specific and not due to a nonspecific protein effect or to contamination by fibronectin, vitronectin, or laminin. That is, neither bovine serum albumin nor TSP preparations treated with a monospecific anti-TSP antibody support cell adhesion. As analyzed by polyacrylamide-gel electrophoresis and specific antibody binding assays, the TSP preparations used in these studies contained no detectable fibronectin or laminin and less than 0.04% vitronectin. The cell surface receptor for TSP appears distinct from that of fibronectin since an antiserum that blocks cell adhesion to fibronectin has no effect on adhesion to TSP. In addition, The platelet cell surface receptor for TSP appears distinct from that of fibrinogen since thrombasthenic platelets adhere to TSP as well as control platelets. Antibodies to the GPIIb-GPIIIa complex block platelet adhesion to fibrinogen but have no effect on adhesion to TSP. Initial characterization of the cell surface receptor for TSP shows it to be protein in nature since cells treated with trypsin fail to adhere to TSP. In summary, our results provide the first clear evidence that TSP specifically promotes cell-substratum adhesion of a variety of cell types independent of the animal species. Our preliminary evidence suggests that the cell-surface receptor(s) for TSP is protein and that it is distinct for the receptor for fibronectin and fibrinogen. Our data suggest that TSP may play a central role in normal adhesive events mediated by platelets and other cells, such as those involved in hemostasis and wound healing. In addition, TSP may be involved in pathological adhesive events mediated by platelets and tumor cells, such as those involved in cardiovascular disease and tumor cell metastasis.

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USE OF A MONOCLONAL ANTIBODY TO MEASURE THE SURFACE EXPRESSION OF THROMBOSPONDIN FOLLOWING PLATELET ACTIVATION.

C. Legrand (1), V. Dubernard (1), N. Kieffer (2) and A.T. Nurden (1). U-150 INSERM, UA 334 CNRS, Hôpital Lariboisière, Paris, France (1) and U-91 INSERM, Hôpital Henri-Mondor, Créteil, France.

A radiolabelled monoclonal antibody (mAb) against native thrombospondin (TSP) has been used to quantitatively assess the surface exposure of intracellular TSP following platelet stimulation. This mAb, designated 5G11, was purified from ascitic fluid by ammonium sulfate precipitation followed by chromatography on DEAE Trisacryl. The isolated IgG were labelled with ^{125}I by the chloramine T method (sp.act. 200-500 cpm/ng). The specificity of the mAb was established by immunoblotting and crossed immunoelectrophoresis using platelet protein extracts. When the labelled IgG (20 µg/ml) were incubated with resting platelets in Tyrode's buffer binding was of the order of 2,000 molecules per platelet. Binding was increased 2 fold and 5-7 fold respectively upon ADP- and thrombin-(or ionophore A23187) stimulation. Unactivated platelets from 2 patients with the Gray Platelet Syndrome bound baseline levels of 5G11, but binding did not increase after platelet activation. In the presence of saturating concentrations of mAb 5G11, an average of 30,000 molecules of IgG were bound by normal platelets stimulated by thrombin. This binding was strongly reduced in the presence of EDTA. It was not significantly affected by AP-2, an anti-GP IIb-IIIa monoclonal antibody which inhibited by more than 85% the binding of plasma fibrinogen but which did not inhibit the surface expression of platelet fibrinogen. It was decreased but not prevented by the presence of an excess of rabbit anti-fibrinogen Fab fragments during the stimulation, while binding at the lower end of the normal range was observed on two different occasions using platelets isolated from an afibrinogenemic patient lacking platelet fibrinogen. These results suggest that while platelet fibrinogen may contribute to the surface organization of TSP other component(s) are required for the full expression of TSP on the platelet surface.

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THROMBOSPONDIN SPECIFICALLY INTERACTS WITH AMINO ACID SEQUENCES WITHIN THE A α- AND B β- CHAINS OF FIBRINOGEN.

Theresa Bacon-Baguley, Suzanne Kendra-Franczak and Daniel A. Walz. Wayne State University, Department of Physiology, Detroit, MI, U.S.A.

Thrombospondin (TSP) is responsible for the secretion-dependent phase of platelet aggregation. The mechanism of this action is believed to be through the binding of TSP to fibrinogen, resulting in the stabilization of the platelet aggregate. It has been established that the binding of fibrinogen to the platelet surface is dependent upon peptide sequences present, respectively, in the Aα- and γ-chains. We have hypothesized that the binding of TSP to fibrinogen is also dependent upon unique fibrinogen peptide sequences. To test this hypothesis we have examined the interaction of TSP and fibrinogen using a blot-binding assay of reduced fibrinogen, the separated fibrinogen chains, selected fibrinogen domains or peptides generated from cyanogen bromide cleaved chains. Iodinated TSP bound specifically to the Aα - and Bβ - chains. Binding to these chains was calcium independent, mutually exclusive and could be blocked either by preincubation of TSP with 9.4 µM fibrinogen or by preincubation of fibrinogen with 1.1 nM thrombospondin. TSP bound to the D and DD plasmin fragment of fibrinogen; TSP interacted exclusively with the β-chain component of the DD fragment. The cyanogen bromide fragments of the separated Aα - and Bβ -chains were resolved through a combination of gel filtration and reverse-phase chromatography. TSP was found to bind to a single peptide within these fibrinogen chains. These studies domonstrate that thrombospondin interacts with at least two distinct sites on fibrinogen, and these sites differ from those already described for fibrinogen binding to platelets.