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ISOLATION AND CHARACTERIZATION OF HUMAN PLATELET MEMBRANE GLYCOPROTEINS Ia AND IIa.

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Glycoproteins (GP) Ia and IIa are relatively minor components of the platelet surface with similar molecular properties. Nieuwenhuis et al. (Nature 319, 470-72, 1985) described a patient whose platelets show no response to collagen. The correlating lack of GPIa in the platelets of this patient suggests this glycoprotein being the receptor for collagen. Santoro (Cell, 46, 913-20, 1986) described a 160 kDa glycoprotein that binds to collagen in the presence of MG2+ and is possibly identical with GPIa. The role of GPIIa is still unknown but a similar molecule has also been found on endothelial cells. It has been suggested that GPIa and GPIIa are complexed with a further membrane component GPIc. The two glycoproteins show only slight difference in molecular weight, isoelectric point and in their affinity for various lectins. As a result they coisolate using most separation techniques.
GPIa is usually associated with the cytoskeleton while GPIIa is mostly found in the soluble phase. GPIa is dissociated from the cytoskeleton by addition of 2% SDS (final conc.) and sonication. Performing Triton X-114 phase partition, GPIIa is found in the detergent phase. After the detergents of the GPIa and GPIIa enriched protein solutions are exchanged with the non-ionic octanoyl-N-methyl glucamide, the glycoproteins are further purified by affinity chromatography on wheat germ agglutinin-Sepharose followed by Lens culinaris lectin-Sepharose both of which bind GPIa and GPIIa. A major contaminant during the purification is GPIb. Final purification of GPIa and GPIIa was obtained by preparative SDS-PAGE using electroelution into a membrane trap. Latest results show an enrichment of GPIa and a lack of GPIb in pseudopodes, isolated by the method of Rotman et al. (Proc. Natl. Acad. Sci. USA, 4357-61, 1982).

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HUMAN PLATELET GLYCOPROTEIN Ic-IIa IS AN ACTIVATION-INDEPENDENT FIBRONECTIN RECEPTOR.

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Human platelets express the membrane glycoprotein (GP) heterodimer GPIIb-IIIa, which functions as an activation-dependent fibronectin (Fn) receptor. We have immunopurified the components of an activation-independent Fn receptor (FR) from human platelets employing a well-characterized rabbit polyclonal antibody raised against the beta chain of the chicken embryo fibroblast (CEF) FR (anti-band 3). This antibody crossreacts with antigen(s) expressed on both chicken thrombocytes and human platelets and inhibits the binding of both normal and thrombasthenic platelets (lacking GPIIb-IIIa) to Fn-coated surfaces in the absence of platelet activation. A monoclonal antibody directed against GPIIb-IIIa (AP2) partially inhibits the adhesion of normal platelets to Fn, but the combination of AP2 and anti-band 3 results in a level of inhibition greater than that obtained with either antibody alone. Thus, the presence of the FR alone is sufficient for the observed normal to enhanced binding of thrombasthenic platelets to Fn, whereas adhesion of normal platelets involves the synergistic action of the FR and GPIIb-IIIa. The adhesion of platelets to Fn mediated by the FR is inhibited by the tetrapeptide RGDS.

Immunopurified FR appears to be a complex of two proteins: an alpha chain with an apparent molecular weight of 155/130 KD (nonreduced/reduced) and a beta chain with an apparent molecular weight of 125/147 KD. The alpha chain is composed of two subunits, dissociated by reduction, with electrophoretic mobilities identical to platelet glycoproteins previously designated Icα and Icβ. The beta chain comigrates with that platelet glycoprotein known as GPIIa. In an immunoblot assay, anti-band 3 binds to GPIIa but not to GPIc. The fact that anti-band 3 immunoprecipitates both GP therefore suggests that they exist in a complex.

Our findings establish GPIc-IIa as yet another platelet glycoprotein receptor complex and pave the way for future studies of the relative role of GPIIb-IIIa and GPIc-IIa in the adhesion of platelets to physiologic surfaces.

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PLACENTAL ANTICOAGULANT PROTEIN

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An anticoagulant protein was purified from the soluble fraction of human placenta by ammonium sulfate precipitation and column chromatography on DEAE-Sepharose, Sephadex G-75, and mono S (Pharmacia). Approximately 30 mg of the protein was purified from one placenta. The purified protein gave a single band on SDS polyacrylamide gel and had a molecular weight of 36,500. This protein inhibited both kaolin and thromboplastin induced clotting times of normal human plasma. It also inhibited the clotting time of platelet-rich plasma induced by factor Xa, but did not affect thrombin activity of fibrinogen-fibrin conversion. The protein neither bound factor Xa nor inhibited the amidase activity of factor Xa. This protein specifically bound to phospholipid vesicles prepared from a mixture of phosphatidylserine and phosphatidylcholine (20 to 80 weight ratio) in the presence of calcium ions. The purified protein was digested with cyanogen bromide and the resulting fragments were separated by FPLC. Partial amino acid sequences of the cyanogen bromide fragments showed that this protein was composed of at least three repeats that were homologous to the four repeats found in lipocortin I and II. Lipocortins are known to inhibit the phospholipase A_2 activity, probably by binding to the phospholipid substrate. These results indicate that the placental anticoagulant protein is a member of the family of lipocortins and probably inhibits coagulation by binding to phospholipid vesicles.
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IMMUNOLOGICAL AND CHEMICAL DETECTION OF VAC IN CULTURED ENDOTHELIAL CELLS.

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Recently we reported the presence in human umbilical cord vessels of an anticoagulatory protein (VAC, M_r = 32,000) which inhibits phospholipid dependent procoagulant reactions through a high affinity binding, in the presence of calcium, to the phospholipid surface. The mechanism of anticoagulation differs fundamentally from those of the well-known physiological anticoagulants.
Polyclonal antibodies, raised in rabbits against purified VAC, bind in cultured endothelial cell lysates to an antigen with M_r = 32,000, as was revealed with immunoblotting techniques. It is demonstrated with chemical techniques, that this antigen is identical to VAC.

VAC appears to be an intracellular protein, attached to fine granular structures, which are located outside the nuclear area. Quiescent endothelial cells do neither secrete VAC in detectable amounts, nor contain detectable VAC on the extracellular side of their plasma membrane. In the presence of 1 mM calcium, endothelial VAC binds reversibly, as was indicated with EDTA, to the subcellular structures of the endothelial cell. Once VAC is bound to the subcellular structures, their apparent procoagulant activities are diminished, as was shown in a one-stage coagulation assay.

Based on these findings, we propose that the presence of VAC in endothelial cells supplies the endothelium with an additional anticoagulatory mechanism, which can be activated after cell injury, when intracellular structures become exposed to plasma constituents. VAC then controls the formation of procoagulant complexes, localized to the subcellular structures.

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