

ISOLATION OF SOME PLATELET MEMBRANE GLYCOPROTEINS; K.J.Clemetson, S.L.Pfueller, A.Sturk, E.F.Lüscher and C.S.P.Jenkins. Theodor Kocher Institut, University of Berne, Switzerland and Department of Haematology, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands.

The platelet is surrounded by a pronounced glycocalix formed by carbohydrate moieties of the membrane glycoproteins. The number of glycoproteins of the outer platelet membrane is greater in number than had previously been reported: when solubilized membranes are analyzed by SDS-polyacrylamide gel electrophoresis the number of separated carbohydrate entities was found not only to be dependant on the concentration of acrylamide and of bisacrylamide used but also on the buffer system employed.

The major platelet membrane glycoproteins have been solubilized and subjected to affinity chromatography on the lectins from *Lens culinaris*, wheat germ and *Abrus precatorius*. SDS-polyacrylamide gel electrophoresis in the presence and absence of a reducing agent together with the differential binding of the lectins to the glycoproteins permitted the distinction of at least seven glycoprotein entities. Using combinations of lectin columns, two platelet membrane glycoproteins have been isolated and others have been greatly purified.

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STUDIES ON PLATELET GLYCOPROTEINS BY SURFACE LABELING AND SDS POLYACRYLAMIDE GEL ELECTROPHORESIS. Ey I.Hagen, N.O.Solum and M.Peterka, Institute for Thrombosis Research, University of Oslo, Rikshospitalet, Oslo, Norway.

Platelet surface (glyco)proteins, and alterations in these in connection with the thrombin-induced release reaction has been studied. Platelets were labeled by lactoperoxidase-catalyzed iodination, and examined by SDS gel electrophoresis in two different gel systems, one conventional (J.Biol.Chem.1969 244 4406), and the other containing urea and EDTA in the gels. In the conventional system the bulk of radioactivity coincided with a PAS band (GP III) of MW about 100,000. In the other system, the main radioactive peak appeared in the GP II area (MW 120,000), and a shift in the PAS stain intensity from GP III to GP II was seen. Thrombasthenic platelets showed only traces of the GP II band in both systems. The bulk of radioactivity was associated with the surface glycopoly-peptide GPS, which is present, but not labeled in normal platelets. In thrombin-released platelets, GPS in its unreduced state has an altered electrophoretic mobility compared to control platelets and platelets which have been incubated with metabolic inhibitors to prevent secretion. The findings indicate that the GP III band consists of two different polypeptides, one of which appears in the GP II area in gels containing urea and EDTA. Further, that thrombasthenic platelet membrane exists in a conformational state different from that of normal platelets. And finally, GPS is in some way involved in, or influenced by, the thrombin-induced release reaction.

IDENTIFICATION OF THE THROMBIN-BINDING PORTION OF PLATELET GLYCOCALICIN.

G.A. Jamieson and T. Okumura, The American National Red Cross, Blood Research Laboratory, Bethesda, Maryland 20014 USA.

Glycocalicin is a high molecular weight ( $M_r$  150,000) glycoprotein of the platelet glycocalyx which is obtained in soluble form following platelet homogenization. Under carefully controlled conditions, glycocalicin can be cleaved by endogenous or exogenous proteases (trypsin 1:100, 2 min) to yield a macroglycopeptide ( $M_r$  120,000) and a (nonglyco)peptide ( $M_r$  45,000). These two fragments and intact glycocalicin have been compared with respect to their ability to prolong the thrombin clotting time of fibrinogen and to inhibit the binding of thrombin to intact platelets. The platelet macroglycopeptide was without effect in either system. The clotting time of fibrinogen (2mg/ml) by thrombin (2 u/ml) was prolonged from a control value of 16 sec to a value of 28 sec with glycocalicin (45 ug) and to 46 sec with the (nonglyco)peptide (6.5 ug). The binding of thrombin to intact platelets, as assayed by the Majerus technique, showed competitive inhibition with both glycocalicin and the (nonglyco)peptide, similar  $K_i$  values of 20-30 nanomolar being obtained in each case. These results indicate that the thrombin-binding site of platelet glycocalicin is located in the (nonglyco)peptide "tail" portion of the molecule.