

LOW AFFINITY PLATELET FACTOR 4 AND HIGH AFFINITY PLATELET FACTOR 4 - TWO ANTIHEPARIN PROTEINS SECRETED BY HUMAN PLATELETS. S. Niewiarowski, B. Rucinski, A.Z. Budzynski and K. Subbarao. Specialized Center for Thrombosis Research, Temple Univ. Hlth. Sci. Ctr., Philadelphia PA, USA.

A material with antiheparin activity released by thrombin from human washed platelets was precipitated by 0.04 M ZnSO_4 and applied on heparin-agarose column. Fractions eluted at 0.5 M and 1.5 M NaCl contained antiheparin activities and were denoted as low affinity (LA-PF₄) and high affinity (HA-PF₄) platelet factor 4, respectively. In Factor Xa-heparin inhibition assay 1 mg of each LA-PF₄, HA-PF₄ and protamine sulfate neutralized 0.86, 7.6 and 11.7 units of heparin, respectively. LA-PF₄ and HA-PF₄ were homogenous in SDS electrophoresis and had apparent MW of 13,700 and 10,300, respectively. Treatment with reducing agents had no effect on the migration of HA-PF₄ but it decreased apparent MW of LA-PF₄ by 50%. It has been shown by immunodiffusion and by radioimmunoassay that LA-PF₄ and β -thromboglobulin (β TG) share common antigenic determinants. LA-PF₄ and HA-PF₄ were antigenically different. LA-PF₄ and human PF₄ prepared previously in our laboratory by DEAE cellulose column chromatography appeared to be identical proteins. β TG and LA-PF₄ had similar patterns of migration on SDS electrophoresis in reduced and non-reduced systems and similar amino acid composition. However, on cellulose acetate electrophoresis, LA-PF₄ appeared as one homogenous band both at pH 8.4 and 9.9 which migrated in the γ -globulin region in contrast to β TG migrating with the β -globulin mobility. Migration of LA-PF₄ was not affected by Zn ions. Secretion of LA-PF₄ from stimulated platelets occurred in parallel with ADP and serotonin and preceded secretion of HA-PF₄. In conclusion, thrombin releases from human platelets two distinct proteins with different antiheparin activities.

PLATELET PLASMA MEMBRANE GLYCOPROTEINS IN NORMAL AND GENETICALLY ABNORMAL PLATELETS, by David R. Phillips, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee

One approach to determine what specialized structures on the platelet membrane surface perform platelet specific functions is to compare the surface of normal to genetically abnormal platelets. We have used three techniques; lactoperoxidase-catalyzed iodination, neuraminidase/galactose oxidase/ ^3H -NaBH₄ labeling, and periodate/ ^3H -NaBH₄ labeling, to investigate the molecular organization of the proteins in these membranes. Several generalizations can be made about the normal membrane: (i) all of the major proteins exposed on the surface (12 have been identified thus far), are glycosylated, (ii) some of these glycoproteins are trans-membrane (i.e., they span the thickness of the membrane), (iii) the glycosylated segments are exposed to the outside of the cell, (iv) many disulfides (both inter- and intramolecular) are present in the platelet membrane glycoproteins, and (v) most of these glycoproteins contain sialic acid.

Analysis of the glycoprotein composition of the platelets from 16 individuals with Glanzmann's thrombasthenia (characterized by a lack of aggregation) showed a decreased concentration of glycoproteins IIb and III. In contrast, platelets from an individual with Bernard-Soulier syndrome (characterized by absent adhesion) had primarily a decrease in glycoprotein Ib. The data demonstrate that both genetic abnormalities are caused by different defects in platelet membrane glycoproteins and suggest molecular entities which may be involved in specific platelet functions. Supported by NIH Career Development Award HL-0080 and Grant HL-15616.

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 glycoprotein (Mr 150,000) present on the outer surface of platelets and obtained in soluble form following platelet homogenization. Glycocalicin has been purified and shown to bind to thrombin and to Factor VIII and to inhibit platelet aggregation caused by these reagents. When solubilized from membranes, the thrombin-binding ability of glycoprotein I is identical with that of glycocalicin and the amount of thrombin bound is proportional to the amount of glycocalicin/glycoprotein I present on platelets. The thrombin-binding site has been located in the (nonglyco)peptide "tail" portion (Mr 40,000) of glycocalicin. These results suggest that glycocalicin plays a central role in platelet function.

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