LOW AFFINITY PLATELET FACTOR 4 AND HIGH AFFINITY PLATELET FACTOR 4—TWO ANTIHEPARIN PROTEINS SECRETED BY HUMAN PLATELETS. S. N. Gregorowicz, B. Buchaki, A. F. Ruddy and K. Sobhasen.

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A material with antiheparin activity released by thrombin from human washed platelets was precipitated by 0.06 M ZnSO4 and applied on heparin agarose column. Fractions eluted at 0.5 M and 1.5 M NaCl contained antiheparin activities and were designated low (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 0.46, 7.6 and 11.7 units of heparin respectively. La-PF4 and Ha-PF4 were removed from reduced and non-reduced systems and similar amino acid composition. However, on cellulose acetate electrophoresis, La-PF4 appeared as one homogeneous band both at pH 8.4 and 9.9 which migrated in the β-globulin region in contrast to SD migration 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 K. 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/NaBH4 labeling, and periodic acid/SnCl2 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 except those exposed on the surface (i.e., have been identified thus far), are glycosylated, (ii) some of these glycoproteins are transmembrane, (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 glycoprotein 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 GLYCOGALACIN. G. A. Yameian and T. Okumura, The American National Red Cross, Blood Research Laboratory, Bethesda, Maryland 20014 USA.

Glycocalcin is a high molecular weight glycoprotein (Mr 150,000) present on the outer surface of platelets and obtained in soluble form by following platelet homogenization. Glycocalcin 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 glycocalcin I is identical with that of glycocalcin and the amount of thrombin bound is proportional to the amount of glycocalcin/glycoprotein I present on platelets. The thrombin-binding site has been located in the (non)glycopeptide "tail" portion (Mr, 40,000) of glycocalcin. These results suggest that glycocalcin plays a central role in platelet function.

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