PURIFICATION OF MEMBRANE-BOUND GLYCOPROTEIN I. M. Hasitz, T. Okumura, and G. A. Jamieson, The American National Red Cross, Blood Research Laboratory, Bethesda, Md. 20014 USA.

Glycocalcin (M_r 150,000) is a component of the platelet glycocalyx and is released in soluble form on platelet homogenization. Glycocalcin appears to play a central role in platelet aggregation induced by thrombin and ristocetin. In addition, the membrane fraction can be resolved into bands by one-dimensional gel SDS electrophoresis (M_r 150,000, 120,000, 100,000, and 85,000). Since glycoprotein I and glycocalcin are similar electrophoretically and in the nature of their macroglycopeptides, we have endeavored to solubilize and characterize membrane-bound glycoprotein I. Extraction of isolated platelet plasma membranes with 1 M sodium chloride or other salts (KBr, KI, KCNS) consistently solubilized 40-60% of glycoprotein I together with thrombosthenin and other components. Homogeneous glycoprotein I was obtained following chromatography on hydroxylapatite. Glycoprotein I purified in this way and glycocalcin, itself, had identical thrombin-binding ability as measured by the Majerus technique. These results suggest that the two types of molecule are functionally identical but that the molecule can present itself in the platelet in three different forms -- glycocalcin, saline-soluble glycoprotein I and detergent soluble glycoprotein I.

COMPLEX FORMATION OF COVALENTLY CROSSLINKED FIBRIN OLIGOMERS WITH AGAROSE COUPLED FIBRINOGEN AND FIBRIN. R. von Hugo, R. Hafter, A. Stemberger and H. Graeff, I. Frauenklinik d. Universität, München, and Max-Planck-Institut für Biochemie, Martinsried, Germany.

Crosslinked high molecular weight derivatives of fibrin (fibrin oligomers) were observed during intravascular coagulation. It was the purpose of this study to investigate the complex formation of fibrin oligomers with fibrinogen and fibrin monomer. Fibrinogen coupled to agarose (Fg-ag) as well as fibrin monomer coupled to agarose (Fm-ag) was used as substrate. Soluble oligomers of fibrin were produced by incubating fibrinogen with thrombin, calcium-chloride, cysteine and F XIII. They were separated from fibrinogen by gel filtration. γ -dimers were demonstrated in fractions from the void volume and the shoulder prior to the fibrinogen peak. These fractions were subjected to affinity chromatography. Crosslinked oligomers of fibrin were not adsorbed on Fg-ag, yet adsorption occurred on Fm-ag. This indicates that fibrin oligomers have no affinity to fibrinogen, yet readily form complexes with fibrin. This could mean that in vivo they compete with fibrinogen for the fibrin monomer part of soluble fibrin monomer complexes, and hence have a tendency to increase in size.

PLASMIN INHIBITORS IN HUMAN URINE. H. Sumi, Y. Takada and A. Takada, Hamamatsu University, School of Medicine, Hamamatsu, Shizuoka, Japan.

By the gel filtration of urinary protein on Sephadex G-200, two peaks of activity to hydrolyze N^{α} -acetylglycyl-L-lysine methyl ester (AGLMe) were detected. One was the native form of urokinase, and the molecular weight was about 54,000. The other was of high molecular weight, and eluted in void fraction. The high molecular form was thought to be a complex of urokinase and urinary plasmin inhibitor (UPI). By using Arg-Sepharose, membrane filter (M.W. 10,000), and pevikon block electrophoresis, we could isolate four types of UPI from normal human urine. One UPI was positively charged at pH 8.6, and of high molecular weight. Other types were negatively charged, and the molecular weights by gel filtration on Sephadex G-200 were about 67,000 (UPI_{6.7}), 45,000 (UPI_{4.5}), and 22,000 (UPI_{2.2}), respectively. In acrylamide disc gel electrophoresis, UPI_{6.7} migrated to serum prealbumin fraction, and UPI_{4.5} and UPI_{2.2} were less anionic. Negative-charged UPIs could be adsorbed on trypsin-Sepharose, and were thought to be identical to urinary trypsin inhibitors. Purified UPIs showed strong inhibition on caseinolytic- and esterolytic-activities of plasmin, and the total activity was about 16 UPI U (inhibited 16 casein U of plasmin)/liter of urine.