

RECONSTITUTION OF LIPOSOMES BEARING PLATELET MEMBRANE GLYCOPROTEINS. A FUNCTIONAL STUDY WITH VON WILLEBRAND FACTOR. P. Sié<sup>1</sup>, M. Gillois<sup>2</sup>, H.J. Chap<sup>3</sup>, B. Boneu<sup>1</sup>, R. Bierné<sup>1</sup>, L. Douste-Blazy<sup>3</sup>. 1 Lab. Hémostase CRTS ; 2 CRBG Univ. Paul Sabatier and 3 INSERM U 101, Hôpital Purpan, 31059 Toulouse cedex France.

Functional studies on isolated membrane glycoproteins (GP) are impaired by their poor solubility in the absence of detergent. The major glycoprotein of the platelet plasma membrane (GP Ib) does not escape to the rule since its soluble derivative (glycocalicin or GP Is) does not exactly share the properties of the integral GP.

This study aimed to develop a membrane model where the GP Ib was solubilized from fresh human platelet membranes by desoxycholate, isolated using wheat-germ-agglutinin (WGA)-affinity chromatography and finally reincorporated into phospholipid vesicles by removal of the detergent. As shown by electron microscopy, the method yielded individual, large-sized, single bilayered vesicles: WGA reversibly agglutinated these vesicles arguing for the incorporation of the GP in the wall of the vesicles and the accessibility of the oligosaccharidic moiety. Additionally, a rapid and convenient method for assessing agglutination was developed using thin-layer chromatography on agarose gel.

Since the GP Ib is the putative receptor for von Willebrand factor (vWF) we studied the effect of vWF on these vesicles. vWF was purified from cryoprecipitate by gel exclusion chromatography followed by an ion-exchange chromatography on DEAE-Sephacel. vWF, vWF plus ristocetin but not ristocetin alone induced a strong specific agglutination of the vesicles. This ristocetin-independent effect markedly differs from the natural behaviour of intact platelets and we speculate that the present model might help to analyse the factors which, at the platelet surface, refrain the plasmat vWF from directly interacting with the exposed GP Ib.

## 0300

10:30 h

FACTOR VIII/VON WILLEBRAND FACTOR IS AN INTEGRAL COMPONENT OF THE PLATELET SURFACE. Z.M. Ruggeri, R. Bader, F.I. Pareti, L. Mannucci and P.M. Mannucci. Hemophilia and Thrombosis Centre "Angelo Bianchi Bonomi" Milano, Italy

Platelets contain Factor VIII/von Willebrand Factor (FVIII/vWF) but its localization on the platelet surface is still controversial. We now demonstrate that anti-FVIII/vWF antibodies induce serotonin release and aggregation when added to suspensions of washed intact platelets from normal individuals (n=8) and patients with autosomal dominant von Willebrand's disease (VWD) (n=6), but not from patients with severe, homozygous-like VWD (n=4). One monospecific rabbit anti-human FVIII/vWF antiserum, adsorbed with severe VWD plasma, and four unadsorbed homologous antisera, obtained from multitransfused patients with severe VWD, gave identical results. Serotonin release and aggregation were dependent on the anti-FVIII/vWF titer and were completely blocked when purified human FVIII/vWF was added to the platelet suspension. Pre-incubation with 6-deoxy-glucose and antimitycin rendered the platelet suspension unresponsive, suggesting that aggregation rather than agglutination was occurring. There was no evidence of cell lysis as shown by lack of release of the cytoplasmic marker adenylate kinase. Fab fragments prepared from anti-FVIII/vWF IgG failed to induce aggregation but completely blocked that by whole IgG. Radio-labeled Fab fragments bound to normal (n=6) and autosomal dominant VWD (n=4) platelets to a similar extent (mean binding  $20 \pm 0.3$  pg/ $10^5$  platelets) but no binding was observed with severe VWD platelets (n=2). In one patient with severe VWD platelets remained unresponsive during and after prophylactic replacement therapy with FVIII/vWF concentrates for one month. Our studies suggest that FVIII/vWF is accessible on the surface of washed platelets except in severe VWD.

EFFECT OF THE MULTIMERIC STRUCTURE OF THE FACTOR VIII/VON WILLEBRAND FACTOR PROTEIN ON BINDING TO HUMAN PLATELETS. H. Gralnick, S. Williams and D. Morisato. National Institutes of Health, Bethesda, Maryland USA.

We have studied the characteristics of binding of intact factor VIII/von Willebrand factor (f.VIII/vWF) protein and 2-mercaptoethanol (2ME) treated f.VIII/vWF protein to human platelets. The purified f.VIII/vWF was radiolabelled with tritiated H potassium borohydride;  $4.5 \times 10^3$  molecules of the intact radiolabelled material bound per platelet. Of these some molecules bound with a high affinity/low capacity (Kd 0.21 nM and  $1.5 \times 10^3$  molecules) and another with a low affinity/high capacity (Kd 2.5 nM and  $3 \times 10^3$  molecules). When the material was reduced with 2ME at 0.01%, it bound with an intermediate affinity of 1.6 nM with a capacity of  $4.0 \times 10^3$  and a low affinity binding of 12.5 nM and a capacity of  $4.0 \times 10^3$ . The 0.1% 2ME-treated material revealed only low affinity binding with a Kd of 15 nM and the number of molecules bound  $13 \times 10^3$ . Studies of competitive inhibition of the intact f.VIII/vWF binding to human platelets by the reduced materials revealed that the smallest f.VIII/vWF protein (i.e., 0.1% 2ME) was the least effective while the 0.01% 2ME material was intermediate between the 0.1% and the intact material. The differences noted in the ability to displace the intact material as well as in binding to the human platelet were paralleled by decreases in the vWF activity of these proteins.

These studies aid in our understanding of the binding of the f.VIII/vWF to platelets in that the binding sites on platelets may be homogeneous while the ligand is heterogeneous. These studies reinforce the structure/function relationships of f.VIII/vWF proteins which have been defined using ristocetin-induced platelet aggregation (i.e., the minimum molecular size of the f.VIII/vWF protein and the penultimate galactose residues on the carbohydrate side chain). We conclude that these defects of the f.VIII/vWF protein also interfere with the protein binding to its platelet receptor and that f.VIII/vWF binding to platelets is an important primary step in hemostasis.

## 0301

10:45 h

THE EFFECT OF SERRATIA MARCESCENS PROTEASE ON THE AGGREGATION OF HUMAN PLATELETS BY VON WILLEBRAND FACTOR AND THROMBIN. H. Cooper, W. Bennett, G. White and R. Wagner. Center for Thrombosis & Hemostasis and Dept. Path., Univ. of N.C., Chapel Hill, U.S.A.

*Serratia marcescens* produces an extracellular metalloprotease (SP) that selectively attacks the surface of human fixed washed platelets and renders them unresponsive to human or bovine von Willebrand factor (vWF). SP treated fresh washed human platelets were studied for [ $^{14}$ C]serotonin release, alterations in surface glycoproteins, and aggregation by bovine vWF and thrombin. Platelet membrane glycoproteins were identified by conventional discontinuous gel electrophoresis techniques using periodic acid Schiff reagent (PAS) to stain for carbohydrate. With SP concentrations above 0.6 µg/ml, there was loss of a single PAS band of ~180,000 MW, corresponding to GPIb. With an even more sensitive technique employing [ $^3$ H]labelled platelets and autoradiography no significant loss of any of the other membrane glycoproteins was found, even at SP concentrations of 20 µg/ml. Supernatants from these treated platelets showed only a single glycoprotein of ~125,000 MW. The use of SP alone with fresh platelets did not cause aggregation or release. SP treatment did cause a complete loss of both serotonin release and aggregation by bovine vWF, but had only a minimal effect on the release and aggregation caused by thrombin. These studies show that SP selectively cleaves GPIb from the platelet surface. The cleaved fragment is smaller than glycocalicin (145,000 MW), suggesting that SP cleaves at a different site than the  $\text{Ca}^{2+}$  dependent protease(s) normally present on the platelet surface. These studies suggest that the 125,000 MW fragment cleaved from GPIb is essential for vWF to cause normal platelet release and aggregation. On the other hand, it appears that cleavage of the majority of GPIb is of only minor importance in the release and aggregation mechanism mediated by thrombin.