

DIFFERENCES IN THE APPEARANCE OF IMMUNOPRECIPITATE 16 (GP IIB/GP IIIa) BY CROSSED IMMUNOELECTROPHORESIS OF TRITON SOLUBILIZED PLATELET PROTEINS DEPENDING ON THE SOLUBILIZATION CONDITIONS. I. Hagen, R. Korsmo, O.J. Bjerrum, G. Gogstad and N.O. Solum Research Institute for Internal Medicine, University of Oslo, Oslo, Norway, and The Protein Laboratory, University of Copenhagen, Copenhagen, Denmark.

Crossed immunoelectrophoresis (CIE) of Triton solubilized platelet proteins has demonstrated that the major immunoprecipitate (16) represents GP IIB and GP IIIa. CIE of proteins solubilized from isolated membranes has often showed patterns with reduced area or absence of immunoprecipitate 16 concomitant with the appearance of new arcs. The aim of this study was to establish conditions to control this phenomenon. By CIE of proteins solubilized from platelet membranes isolated in the presence of EDTA, the area of immunoprecipitate 16 was reduced, and two new arcs, one at the cathodic side (16a) and one at a similar position (16b) as immunoprecipitate 16 appeared. In contrast, CIE of extracts from corresponding membranes which had been washed once in an EDTA-free medium revealed one major 16 peak. Despite the major differences between these patterns, SDS PAGE of these extracts showed no differences in the glycoprotein patterns. The anodic leg of immunoprecipitate 16a fused with 16, and a specific antibody obtained by immunization with immunoprecipitate 16 also reacted with 16a. Partial or total disappearance of immunoprecipitate 16 was also seen after CIE of proteins solubilized from whole platelets in the presence of EDTA, whereas leupeptin had no such effect. The different patterns may reflect biochemical alterations related to platelet activation.

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FIBRINOGEN INDUCES CHANGES IN PLATELET MEMBRANE POTENTIAL. H. Vainer and A. Caprani. Institut de Recherches sur les Maladies du Sang, Hôpital Saint Louis, 75010 Paris and Université P. et M. Curie, CR4-CNRS, 75230 Paris, France.

Changes in platelet membrane potential (MP) reflecting ion transmembrane transport accompany the platelet activation by various stimuli. We have studied the platelet MP and the effect of fibrinogen on its expression, using an electrochemical method. The MP determination is based on the evaluation of the transmembrane partition coefficient (PC) (Ci/Ce) of an electroactive tracer, such as the ferrocyanid anion. Washed human platelets are incubated with the tracer solution; the amount of tracer in the supernatant (Ce) and in the acellular lysate of SDS-treated platelet pellet (Ci) is measured as the diffusion limiting current corresponding to the tracer oxidation at the surface of a rotating disc electrode. The kinetics for the PC evaluation have been studied in order to standardize the experimental conditions which allow to express the MP by the Nernst equation: $Vi - Ve = RT/4F \cdot \log Ci/Ce$.

The mean value \pm SD (N = 9) of the platelet MP in saline is -5.5 ± 0.5 mV (PC = 0.445 ± 0.020). When fibrinogen replaces the saline, a MP of -8.83 mV (PC = 0.257) is observed. While it contrasts sharply with the MP of platelets in saline, it is comparable to the MP of platelets in plasma, which is: -8.63 mV (PC=0.266). We have also measured the effect of human albumin and gamma globulins on the MP; both these plasmatic proteins cause a much smaller decrease of MP, as compared to fibrinogen (or plasma), all used at physiological concentrations in saline and without the platelet pretreatment with any agent.

The results suggest that a relationship might exist between the marked decrease of platelet MP caused by fibrinogen - possibly via mediators of the transmembrane transport - and/or some of the platelet membrane glycoproteins which appear to function as binding sites for fibrinogen and must undergo (conformational) changes that allow fibrinogen to bind.

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ANTIBODIES AGAINST MEMBRANE GLYCOPROTEINS: AGGREGATION, CROSS IMMUNOELECTROPHORESIS STUDIES, AND RELEVANCE TO GLANZMANN'S THROMBASTHENIA. C.S.P. Jenkins, E.F. Ali-Briggs and K.J. Clemetson. Departments of Hematology, Wilhelmina Gasthuis, Amsterdam, and Montefiore Hospital, Bronx, NYC, and Theodor Kocher Institute, Berne, Switzerland.

In Glanzmann's thrombasthenia, glycoproteins (GPs) IIB and IIIa are missing or strongly reduced in concentration and aggregation to ADP, collagen and thrombin is impaired. Antibodies against GPs IIB and IIIa did not entirely induce a thrombasthenia-like state in normal platelets; however they did strongly inhibit collagen-induced aggregation, inhibited the second wave of aggregation induced by ADP, inhibited to some extent thrombin-induced aggregation and clot retraction, and were totally without effect on ristocetin-human VIII:WF.

Crossed immunoelectrophoresis studies using Triton X-100 extracts of whole platelets and platelet membranes with these antibodies gave a single immunoprecipitate. This immunoprecipitate was absent when similar studies were carried out with thrombasthenic platelets. Anti-whole platelets antibodies gave a number of immunoprecipitates with normal platelets and differences were observed with thrombasthenic platelets, the most notable of which was a marked reduction in one of the major immunoprecipitates.

These results provide further evidence that GPs IIB and IIIa are involved in the later stages of platelet aggregation.

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COMPARISON OF FIBRINOGEN BINDING TO PIG INTACT PLATELETS AND THEIR MEMBRANES. C.S. Cierniewski, T. Krajewski* and A. Janiak*. *Department of Biophysics, Medical School of Łódź, **Department of Biochemistry, University of Łódź, Łódź, Poland.

In order to explain the precise role of fibrinogen in ADP dependent aggregation of platelets, purification of the presumed platelet fibrinogen receptors is required. We, therefore, compared binding of fibrinogen to the intact platelets and their membranes. Fibrinogen, free of plasminogen and vitamin K-dependent factors, was radiolabelled and used as the probe for a receptor in the platelets membranes. Platelet membranes isolated according to Barber and Jamieson /1970/ were used in the binding assay as a suspension in 0.025 M Tris-HCl buffer, pH 7.4, containing 0.14 M NaCl and 0.66 % polyethylene glycol. The binding specificity was determined by test in which cold fibrinogen completely inhibited binding of the iodinated fibrinogen, whereas other proteins did not. The pattern of fibrinogen binding was characteristic for a saturable receptor. Scatchard analysis of the binding indicated a single class of receptors, with an estimated association constant of $5 \times 10^8 \text{ M}^{-1}$, comparable to that estimated for fibrinogen binding to the intact pig platelets. In contrast to the intact platelets, binding of fibrinogen was not dependent upon the ADP stimulation. We conclude that a specific receptor for fibrinogen is exposed on the surface as a result of cell damage which is the first step of platelet membrane isolation procedure. The isolated membranes retain binding properties of the intact platelets and may serve as a source for the isolation of fibrinogen receptors.