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CA^{2+}-INDUCED STRUCTURAL TRANSITIONS OF THE PLATELET GP IIb-IIIa COMPLEX. B. Steiner (1) and D.R. Phillips (2). Gladstone Foundation Laboratories, Univ. of California, San Francisco, USA (2) and F. Hoffmann-La Roche & Co., CH-4002 Basle, Switzerland (1)

Previous studies have shown that the membrane glycoprotein (GP) IIb-IIIa complex can be reversibly dissociated by incubating platelets for 5 min at 37°C in an EDTA-containing buffer. Prolonged incubations (30 min) with EDTA, however, result in the formation of high molecular weight aggregates of GP IIb and GP IIIa. These aggregates of individual GP's neither bind fibrinogen nor support platelet aggregation, indicating that chelation of Ca^{2+} can affect the functional activity of GP IIb-IIIa. The present study was designed to identify conditions for the generation of functionally active GP IIb and GP IIIa. Functionally active subunits were defined as those which reformed GP IIb-IIIa complexes. The complexes were quantified by sucrose gradient sedimentation (complexed, dissociated and aggregated GP's have different sedimentation coefficients) and thrombin hydrolysis (dissociated and aggregated GP IIb are susceptible to hydrolysis by thrombin while GP IIb in the GP IIb-IIIa complex is thrombin resistant). Purified GP IIb-IIIa could be dissociated by a 5 min incubation at 37°C with $\leq 10^{-5}$ M Ca^{2+}. When the complexes were dissociated in the presence of Ca^{2+} concentrations below 10^{-6} M, the monomeric GP IIIa was converted to a slower sedimenting form; this change in structure caused it to become functionally inactive. In the presence of very low Ca^{2+} concentrations ($\leq 10^{-8}$ M) both dissociated subunits subsequently formed high molecular weight aggregates. However, these changes in structure and loss in function could be prevented by dissociating the complexes in 10^{-6} M Ca^{2+} and immediately readding mM Ca^{2+} at 4°C. When this solution was warmed to 20°C, almost 70% of the dissociated subunits reformed heterodimeric complexes. Storage at 4°C for as long as 6 h did not alter the functional activity of these subunits. Octylglucoside, but not Triton X-100, completely inhibited reassociation. Experiments performed in the presence of various H^+ and salt concentrations showed that the interactive forces between GP IIb and GP IIIa are both electrostatic and hydrophobic. Thus, conditions have been obtained for the preparation of functionally active GP IIb and GP IIIa which can reform the native heterodimeric complex. Various Ca^{2+} concentrations can have multiple effects on the structure of the dissociated subunits.

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STIMULUS-DEPENDENT CHANGES IN THE SURFACE EXPRESSION OF GPIIb/IIIa AND FIBRINOGEN RECEPTORS. RELATIONSHIP TO PLATELET AGGREGATION. K. Niiya , E. Hodson , R. Bader , V. Byers-Ward, E. F. Plow , and Z. M. Ruggeri . Scripps Clinic and Research Foundation, La Jolla, CA, U.S.A.

Platelet stimulation altered the binding of three monoclonal antibodies (monovalent Fab' fragment) directed against the glycoprotein (GP)IIb/IIIa complex. We found that 47,600-60,300 molecules of antibody bound per platelet before stimulation, as compared to 89,200-146,500 molecules per platelet after thrombin stimulation. These changes were observed in parallel with a small but significant increase in the dissociation constant (Kd) of two antibodies. In contrast, no statistically significant changes were observed with ADP-stimulated platelets. The increased binding of LJ-CP3, but not of the other two antibodies, to activated platelets decreased by 30-40% in the presence of EDTA at 22-25°C, suggesting the occurrence of divalent-cation mediated, activation-dependent changes in the corresponding GPIIb/IIIa epitope. Platelets stimulated by thrombin bound more fibrinogen than those stimulated by ADP, and significant differences in the extent but not in the affinity of fibrinogen binding were observed with different platelet agonists. When the pool of GPIIb/IIIa molecules exposed on the surface of unstimulated platelets was reacted with monoclonal antibody LJ-CP3 to block ADP-induced fibrinogen binding and platelet aggregation, thrombin stimulation still induced substantial binding and aggregation. This effect of thrombin required exposure of platelets to the active agonist and was not mediated by molecules released by thrombin into the medium. Therefore, platelets activated with "strong" agonists exhibit increased number of surface-oriented epitopes associated with GPIIb/IIIa. The GPIIb/IIIa molecules bearing these newly exposed epitopes are functional in that they bind fibrinogen and mediate platelet aggregation.

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BIOSYNTHESIS AND ASSEMBLY OF PLATELET GPIIbIIIa. A. Duperray, A. Troesch, R. Berthier, E. Chagnon and G. Marguerie. DRF/Laboratoire d'Hématologie/INSERM U217, CEN G, 38041 Grenoble

Platelet GPIIbIIIa is a calcium-dependent heterodimer which is constituted of two proteins subunits GPIIb and GPIIIa. The GPIIb is itself made of two disulfide-linked subunits IIbα and IIbβ. GPIIIa is a single chain protein. GPIIbIIIa serves as a receptor for fibrinogen, fibronectin and von Willebrand factor and is implicated in platelet adhesive reactions. This protein is a member of an adhesion receptor protein family for which the name "cytoadhesins" has been proposed. As a preliminary step in the study of the genetic diversity of the members of this family, we have analysed the biosynthesis and assembly of GPIIbIIIa in human megakaryocytes and in a human megakaryocytic cell line : LAMA-84. Megakaryocytes were isolated from liquid cultures of cryopreserved blood cell concentrates from patients in the chronic phase of chronic myeloid leukemia. Using these cell preparations, we have shown that the α and β subunits of GPIIb derive from a common precursor, the pro-GPIIb, which associated in an early step with GPIIIa. In a second set of experiments, we have analysed the expression of the GPIIbIIIa complex in LAMA-84. Only a minority of the native cells were reacting with the anti GPIIbIIIa antibodies as tested by immunofluorescent labeling. In contrast, the expression of GPIIbIIIa in these cells was amplified in the presence of the phorbol ester TPA. Metabolic labeling experiments indicated that a large quantity of pro-GPIIb was synthesized in the native cells, while very little of the mature forms of GPIIb and IIIa were detected. After TPA induction, the expression of GPIIIa was greatly enhanced with a simultaneous increase in mature GPIIb. These data indicate that a deficit in GPIIIa results in the biosynthesis of a non-associated pro-GPIIb which cannot be further processed, suggesting that the GPIIIa subunit is a regulatory component in the biosynthesis of the GPIIbIIIa complex.

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LOCALIZATION AND CHEMICAL SYNTHESIS OF A DIVALENT CATION REGULATED EPITOPE IN PLATELET MEMBRANE GPIIb. J.C. LOFTUS, E.F. Plow, A.L. Frelinger III, M.A. Smith, S. D'Souza, and M.H. Ginsberg, Research Institute of Scripps Clinic, La Jolla, CA, U.S.A.

Platelet membrane glycoprotein (GP)IIb-IIIa is a component of a common adhesive protein receptor for fibrinogen, fibronectin, and von Willebrand factor. A monoclonal antibody, PMI-1, defines a divalent cation dependent regulation of the surface orientation of the heavy chain of GPIIb. Exposure of the PMI-1 epitope inversely correlates with the capacity of platelets to bind fibrinogen and aggregate. We have now localized and chemically synthesized this epitope. A 1.1 Kb cDNA clone which directs the synthesis of a fusion protein which bears the PMI-1 epitope was isolated from a lambda gt 11 expression library constructed from mRNA from the human erythroleukemia (HEL) cell line. The position of the N-terminal sequence of the light chain of GPIIb in the deduced amino acid sequence of the clone defined the orientation of the light and heavy chains of GPIIb. Analysis of the amino acid sequence corresponding to the heavy chain of GPIIb identified a single region with a high likelihood of containing a continuous epitope. A synthetic 17 residue peptide, corresponding to the predicted antigenic site, inhibited the binding of PMI-1 to platelets. Two uM peptide was required to inhibit binding 50% in the presence of 1 uM PMI-1, indicating an approximate dissociation constant of 1.5 uM for the peptide-antibody complex. This figure should be compared to a Kd of 0.95 uM (JCI 78:1103, 1986) for PMI-1 binding to GPIIb. A second peptide, corresponding to the region immediately adjacent to the predicted antigenic site, failed to inhibit PMI-1 binding. Neither peptide inhibited the binding of two other monoclonal anti GPIIb-IIIa's to platelets. The peptides had similar effects on PMI-1 interaction with purified GPIIb-IIIa in detergent solution. These data localize the PMI-1 epitope to a 17 amino acid region located near the carboxyl terminal of the heavy chain of GPIIb. Thus, they chemically define a region of GPIIb whose surface expression reflects the competence of GPIIb-IIIa as a component of a platelet receptor for adhesive proteins.