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INTERACTION OF MEMBRANE GLYCOPROTEIN GPIIb AND IIIa WITH CYTOSKELETAL PROTEINS DURING PLATELET ACTIVATION.
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Experiments were designed and performed to analyse the cytoskeleton assembly and the interaction of glycoprotein (GP)IIb, IIIa and cytoskeletal proteins during platelet activation. A23187 stimulated ^{125}I labeled platelets were solubilised with Triton X-100 solution and centrifuged. The insoluble fraction were analysed by two dimensional electrophoresis and the soluble fraction were fractionated with 5-25% sucrose gradient centrifugation and analysed by SDS PAGE. In Triton X-100 insoluble fraction, high molecular weight protein fraction(MW > 10^6) was present after stimulation which were consisted of actin binding protein(ABP), myosin heavy chain(MHC), actin and GPIIb and IIIa. And some of the ABP and MHC formed dimer. ABP and actin in this fraction were decreased with 1 mM $CaCl_2$ treatment but the reduction of ABP was inhibited by leupeptin. In Triton X-100 soluble fraction after stimulation, some of the ABP, MHC, P235 protein, actin and small amount of GPIIb, IIIa were sedimented in the same high density fraction but most proteins were sedimented as a monomer form or GPIIb-IIIa complex form. The GPIIb, IIIa incorporation in high molecular weight protein fraction or high density fraction was absent in Ca^{++} chelating condition or the presence of competitive fibrinogen binding inhibitor which blocked the platelet aggregation. It is concluded that cytoskeletal proteins and GPIIb, IIIa are assembled each other and formed high molecular weight protein fraction or dimer formation during activation. In stimulated platelets these assembled cytoskeletal proteins containing GPIIb, IIIa were also found in Triton X-100 soluble fraction as a precursor of high molecular weight fraction in Triton X-100 insoluble fraction. The binding of fibrinogen to GPIIb-IIIa complex induce the linkage of GPIIb, IIIa to assembled cytoskeletal proteins.

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THE ROLE OF GPIIb-IIIa IN MODULATION OF ADHESION REACTIONS.
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We have previously reported that patients with Glanzmann's Thrombasthenia (GT) fail to adhere to a carbon-formvar surface and undergo contact-induced shape change in a non-flow system. The ability of ADP to reverse this adhesion defect suggested that it may be secondary to defective dense granule release rather that a direct requirement for GPIIb-IIIa. To further assess the role of GPIIb-IIIa in adhesion, we examined the effect of two mouse monoclonal antibodies to the GPIIb-IIIa complex, AP2 (IgG, kappa) from T. Kunicki, Milwaukee Blood Center and MAb36 (IgM, lambda) from D. Peterson, Rice University. AP2 (1:50 dil) and MAb36 (1:200 dil) both completely abolished aggregation by ADP, collagen and epinephrine and prevented clot retraction. In a transmission EM (TEM) whole mount assay of adhesion and contact-induced shape change, both antibodies inhibited platelet attachment to the substrate and impaired spreading in those few platelets that did attach. This antibody-induced adhesion defect was reversed by the addition of $2x10^{-6}$ M ADP just prior to exposure of platelets to the activating surface. In parallel studies, antibody treated platelets demonstrated a dose-related defect in ATP release as measured in a Lumiaggregometer with total absence of release at antibody dilutions that abolished aggregation. Using a colloidal gold-fibrinogen probe, virtual absence of binding of exogenous fibrinogen was demonstrated in antibody treated platelets induced to spread by ADP stimulation. These studies suggest that while GPIIb-IIIa may play a role in adhesion in non-flow systems, as suggested by the altered adhesion seen in GT platelets, adhesion and adhesion-induced shape change can be supported by ADP stimulation in the absence of fibrinogen binding to GPIIb-IIIa.

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CONTACT ACTIVATED PLATELETS BIND VON WILLEBRAND FACTOR TO GPIIb-IIIa.
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Using a rabbit polyclonal anti-von Willebrand factor (vWF) antibody, normal human adherent platelets extensively bind vWF in a diffuse pattern as detected by immunogold electron microscopy. This pattern differed significantly from the zonal pattern observed for direct fibrinogen-gold labelling in contact activated platelets. In order to determine if contact activated platelets bind vWF to GPIIb-IIIa or GPIb, the extent and pattern of bound vWF in platelets from patients with Glanzmann's thrombasthenia (GT) and Bernard Soulier Syndrome (BSS) was determined. Virtually no bound VWF was detected by immunogold labeling in GT platelets previously characterized as being deficient in GPIIb-IIIa. On the other hand, BSS platelets, lacking GPIb, demonstrated extensive labeling of vWF in a pattern identical to that seen in normal platelets. This data is consistant with vWF binding to GPIIb-IIIa in contact induced adhesion and spreading.

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THE ROLE OF THE GP IIb/IIIa COMPLEX AND vWF IN PLATELET - COLLAGEN INTERACTION
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Our recent studies showed that collagen fibrils (CF) are internalized by platelets in citrated plasm. This phenomenon was not observed in EDTA-PRP. In order to investigate whether collagen internalization is mediated by a receptor we studied the effect of monoclonal antibodies against the receptor molecules GP IIb/IIIa (Gi5) and GP Ib (AN51, Dakopatts GmbH, Hamburg). Washed human platelets from healthy donors were incubated with 10µg of Gi5 or 4µg of AN51 per ml of platelet suspension (2 x 10^8 platelets per ml). After 10 min. at 37° C we added 40µg collagen (Hormonchemie, München) per ml of platelet suspension. After an additional incubation period of 10 min. the reaction was stopped by glutaraldehyde fixation (40µl of a 5% glutaraldehyde and of a 0.2 % tannin solution in phosphate buffer). To investigate the role of vWF in the internalization process we added 10µg/ml ferritin-labeled anti-vWF Fab-fragments to the platelet suspension two min. in advance of collagen stimulation (40µg/ml; 10 min). The values in percent of the inhibition of the internalization phenomena were obtained by statistical evaluation of these phenomena observed on ultrathin serial sections. Washed platelets interacted with CF in the same manner as platelets in citrated plasm, but aggregates were only formed after stirring. Washed platelets which had internalized CF were found to be completely degranulated in contrast to platelets which had only contacted CF without internalization. These platelets were hardly degranulated. The membrane system containing the internalized CF surrounded the contractile sphere of the platelets and always displayed an opening to the platelet surface.We found that Gi5 inhibited collagen internalization up to 95% and anti-vWF to approximately 50%, whereas AN51 showed no effect on this process. Futhermore, Gi5 inhibited the platelet function aggregation and the release reaction which was enhanced by CF internalization. These findings show that the GP IIb/IIIa complex is strongly involved in the internalization of CF by platelets and suggest an involvement of vWF released from the stimulated platelets in this process by binding to the GP IIb/IIIa complex.
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