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FIBRINOGEN SEQUENCES INTERACTING WITH PLATELET GPIIbIIIa.
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Fibrinogen (Fg), fibronectin (Fn) and von Willebrand factor (vWF), interact with GPIIbIIIa on ADP stimulated platelets, and a common mechanism has been postulated for the binding of these adhesive proteins. Fg, Fn and vWF contain the tripeptide Arg-Gly-Asp and synthetic analogues to this sequence inhibit their interaction with platelet and their concomitant adhesive reactions. On the other hand, sequences corresponding to the Fg γ chain inhibit the binding of Fg, Fn and vWF to platelet and may also represent a potential recognition site. This raises the possibility that the γ chain sequence and Arg-Gly-Asp interact with the same site or represent primary and secondary sites for the Fg molecule. Within this context, the capacity of these sequences to interact with GPIIbIIIa and to block fibrinogen binding were compared. The smallest γ chain sequence that was active in inhibiting this reaction was the hexamer Lys-Gln-Ala-Gly-Asp-Val corresponding to the last six amino acid residues at the C-terminus of the γ chain. In parallel, peptides with the structure Arg-Gly-Asp-X were synthesized and tested in vitro. The activity of these peptides was dependent upon the hydrophobicity of the amino acid residue at position X. Arg-GLy-Asp-Phe corresponding to the sequence at position 95-98 in the Fg Aα chain was 5 to 10 times more active than Arg-GLy-Asp-Ser, present at position 572-575 in the Aα chain, and was 10 to 20 times more active than the γ chain hexamer. Both the Aα chain and γ chain sequences however, inhibited Fg binding by greater than 90%. When the γ chain sequence and the Arg-Gly-Asp-X sequence were coupled to Sepharose, GPIIbIIIa interacted with these sequences and was eluted from each column by either of the peptides. Finally direct binding experiments indicated that Arg-Gly-Asp-X and γ chain sequences are competitive antagonists. These results suggest that both sequences interact with the same site on GPIIbIIIa and comparison of the hydrophilicity of these peptides suggests that the binding domain on GPIIbIIIa exhibits hydrophobic properties.

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FIBRIN PROTOFIBRIL AND FIBRINOGEN BINDING TO ADP-STIMULATED PLATELETS: IS THERE A COMMON MECHANISM?
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We have investigated the molecular basis of platelet:fibrin binding by studying interactions between platelets and protofibrils, soluble two-stranded polymers of fibrin which are intermediates on the fibrin assembly pathway. We have taken advantage of the well-characterized anticoagulant properties of the fibrinogen degradation product, fragment D, to prepare solutions of short protofibrils, composed of fewer than twenty fibrin monomer molecules per polymer. Fibrin protofibrils bound to ADP-activated platelets in a time- and concentration-dependent process which was effectively blocked by excess unlabelled fibrinogen, i.e. the binding was specific and appeared to involve a common receptor. ADP-stimulated cells bound approximately 3 micrograms of fibrin protofibrils/10^8 platelets, compared to 4 micrograms of fibrinogen/10^8 cells, following a 30 min incubation period at room temperature. The apparent first order rate constant for fibrin protofibril binding was found to be five-fold slower than that measured for fibrinogen. Two monoclonal antibodies directed against the glycoprotein IIb:IIIa complex inhibited the binding of fibrin protofibrils and fibrinogen in a similar, concentration-dependent manner, providing strong evidence for a common receptor. Neither fibrin protofibrils nor fibrinogen bound to Glanzmann's thrombasthenic platelets, further supporting the hypothesis that fibrinogen and fibrin bind to a common platelet receptor present on the glycoprotein IIb:IIIa complex. The specificity of these interactions was examined with transmission electron microscopy, which clearly showed thin fibers of lengths up to 150 nm attached to the surface of normal, stimulated platelets. Immunogold electron microscopy using rabbit anthihuman fibrinogen as the first stage antibody verified identity of the surface bound molecules and the immunogold distribution paralleled that observed with the fibrin/fibrinogen molecules alone. Contacts between the ends of the fibers and the platelets were frequently observed, but lateral contacts were also evident.

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DIFFERENT SITES FOR FIBRINOGEN AND FIBRIN RECEPTORS ON PLATELETS.
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Platelet stickiness and aggregation depend on availability of surface membrane glycoprotein IIb-IIIa complex to bind fibrinogen. The development of isometric tension in platelet-rich clots is a manifestation of fibrin binding to the cells as well as platelet contractile activity. The possibility that fibrinogen and fibrin may bind to different portions of the GPIIb-IIIa complex has apparently not been considered. In order to determine whether the receptors for the fibrinogen and fibrin on the GPIIb-IIIa complex are identical, we investigated the effect of various monoclonal antibodies to the IIb-IIIa complex and the tetrapeptide Arg-Gly-Asp-Ser (RGDS), a recognition site on fibrinogen for IIb-IIIa, on the development of isometric tension and ultrastructure of platelet-fibrin clots. Monoclonal antibodies A2A6 and 7E3 decreased the maximal tension, as well as the rate of tension development. Platelets and fibrin were oriented longitudinally in antibody treated clots, but the concentrations of fibrin and platelet aggregates determined by morphometry were significantly reduced. T10 and 10E5 increased tension, while AP2 and PAC1 had no substantial effect. Increasing concentrations of RGDS from 62.5 μM to 500 μM resulted in greater maximal tension and rate of tension development, reaching a five-fold increase when 500 μM RGDS was used. RGDS did not affect the Mg-stimulated platelet actomyosin ATPase activity. Morphometry revealed increased concentrations of oriented fibrin and platelet aggregates in RGDS-treated clots. Results of this study confirm the different topography of the epitopes on the IIb-IIIa complex and provide evidence for different receptor sites for fibrinogen and fibrin on the IIb-IIIa complex.

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REVERSIBLE EXPOSURE OF BINDING SITES FOR FIBRINOGEN ON PLATELET MEMBRANE GLYCOPROTEIN IIB-IIIA.
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When human, gel-filtered platelets are stimulated with Platelet Activating factor (PAF, 500 nM) in the presence of 1 μM ^{125}I-fibrinogen (*F) 15.360 ± 4.850 (n = 5) mol*F bind per platelet after 10 min incubation at 22°C. Under similar conditions 10 μM adrenaline and 10 μM ADP induce the binding of 17.020 ± 4.550 and 20.500 ± 3.910 mol*F/platelet, respectively. Stimulation with PAF in the absence of fibrinogen followed by addition of *F at different intervals (Δt) thereafter, reveals an exponential decrease in bound *F reaching 29 ± 8% of initial binding at Δt = 30 min. Similar patterns are seen with ADP and adrenaline. This decrease is enhanced by raising cyclic AMP (5 μM forskolin) or reducing the cell's energy supply (30 mM deoxyglucose - 1 mM CN^-). Thus, exposed binding sites disappear in the absence of fibrinogen.

Following a first stimulation (with PAF; *F absent), a second stimulation (ADP or adrenaline; *F present) restores the binding to the range found with the second stimulator under optimal conditions. Thus, following a first disappearance of accessible binding sites a second type of binding can be induced.

Repeated stimulation with different agonists (PAF-Δ t-adrenaline-Δ t-ADP-Δ t-collagen, 5 μg/ml) induces repeated exposure and disappearance of binding sites without exhaustion of accessible binding sites. Re-exposure of binding sites is complete with adrenaline and ADP as second and third stimulator, whereas collagen induces 60-70% of the first binding induced by PAF. When the 10 min incubation with *F is increased to 60 min, re-exposure results in binding of 40.000-50.000 mol*F/platelet, which is close to the number per platelet of glycoprotein IIb-IIIa complexes, the binding sites for fibrinogen.

These data support the concept that exposure and disappearance of fibrinogen binding sites is a reversible process and that the maintenance of the exposed state is under tight metabolic control.