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IDENTIFICATION OF GLYCOPROTEIN Ib_β AS THE M_r = 24,000 PLATELET POLYPEPTIDE PHOSPHORYLATED BY AGENTS THAT ELEVATE CYCLIC AMP. J. E. B. Fox (1), C. C. Reynolds (1), J. K. Boyles (1), R. A. Abel (2), and M. M. Johnson (2). Gladstone Foundation Laboratories, University of California, San Francisco, CA, U.S.A. (1), and Christiana Hospital, Newark, DE, U.S.A. (2).

Platelet function is inhibited by agents that elevate intracellular cyclic AMP concentrations, presumably as a result of the cyclic AMP-stimulated phosphorylation of intracellular proteins. Polypeptides that become phosphorylated are of M_r = 250,000, M_r = 51,000 (P51), M_r = 36,000 (P36), M_r = 24,000 (P24), and M_r = 22,000 (P22). The M_r = 250,000 polypeptide is actin-binding protein, but the identity of the other polypeptides is unknown. In the present study, we identified the P24 polypeptide. Platelets were radiolabeled with [^{32}P]P_i and then incubated for 2-5 min in the presence or absence of 5 μM prostaglandin E_1 (PGE_1). The PGE_1-induced phosphorylation of P24 was detected on autoradiograms of SDS-gels. Since P24 has been shown to be membrane-associated, its molecular weight was compared with those of known membrane proteins. P24 comigrated with the β-chain of purified GP Ib on reduced gels (M_r = 24,000) and also on nonreduced gels (when GP Ib_β is disulfide-linked to GP Ib_α and migrates with M_r = 170,000). Like GP Ib_β, P24 was associated with actin filaments in Triton X-100 lysates. Both GP Ib_β and P24 were selectively associated with filaments of the membrane skeleton and were released from filaments when the Ca^{2+}-dependent protease was active. Antibodies against GP Ib immunoprecipitated P24 from platelet lysates. Finally, exposure of Bernard-Soulier platelets (that lacked GP Ib) to PGE_1 resulted in phosphorylation of actin-binding protein, P51, P36, and P22, but not P24. We conclude that P24 is GP Ib_β. To determine whether phosphorylation of GP Ib_β is responsible for the inhibitory effects of PGE_1 on platelets, we compared the action of PGE_1 on control platelets with that on Bernard-Soulier platelets. One of the ways in which PGE_1 inhibits platelet activation is by inhibiting the polymerization of actin. While PGE_1 inhibited actin polymerization in control platelets, it did not in Bernard-Soulier platelets. We conclude that GP Ib_β is phosphorylated by agents that elevate cyclic AMP and that phosphorylation of this glycoprotein results in inhibition of platelet function.

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MOLECULAR CLONING OF HUMAN PLATELET GLYCOPROTEIN Ib. J.A. Lopez (1,2,3), D.W. Chung (3), K. Fujikawa (3), F.S. Hagen (4), T. Papayannopoulou (2), and G.J. Roth (1). Veterans Administration Medical Center (1), Department of Medicine (2), and Biochemistry (3), University of Washington, and ZymoGenetics Inc. (4), Seattle, WA, U.S.A.

Glycoprotein Ib (GPIb) mediates von Willebrand factor-dependent platelet adhesion and participates in the resulting platelet activation process. In the present investigation, the primary structure of human platelet GPIb was studied. GPIb and its proteolytic fragment glycocalicin were purified to near homogeneity from human platelets by affinity chromatography using wheat germ agglutinin and anti-GPIb monoclonal antibody (D. Nugent, University of Washington) coupled to Sepharose. GPIbα chain, β chain, and glycocalicin were isolated, reduced and carboxymethylated, and then fragmented by trypsin and S. aureus V8 protease. Peptides were isolated by HPLC and subjected to amino acid sequence analysis. Approximately 200 amino acid residues were identified. Affinity purified rabbit antibodies directed against the α chain, the β chain, and glycocalicin were prepared and shown to be monospecific by Western blot analysis. Total RNA was prepared from human erythroleukemia cells grown in the presence of phorbol acetate. Poly(A)$^+$ RNA was selected and used to prepare a cDNA library in λgt11. The library was screened with [125]I-labeled polyclonal antibody to glycocalicin. The clone with the largest cDNA insert was sequenced and shown to code for amino acid sequences corresponding to those determined by Edman degradation of glycocalicin. The predicted amino acid sequence contains at least six tandem repeats of 24 amino acids that are highly homologous with 13 repeats present in leucine rich α2 glycoprotein of human plasma. Another region in the protein contains a second repeat rich in threonine and serine, which shows some homology to a 9 amino acid repeat in the connecting region of human factor V. This region is probably the major site of attachment of clusters of O-linked carbohydrate in GPIbα. These results indicate that human platelet glycoprotein Ibα has a multi-domain structure composed of a number of repetitive sequences. Supported in part by grants from the American Heart Association, Robert Wood Johnson Foundation, Veterans Administration, and National Institutes of Health.

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A DEFINITION OF HEPARIN ANTICOAGULANT POTENCY APPLICABLE TO ALL HEPARINS AND HEPARIN-LIKE SUBSTANCES AND ITS PRACTICAL APPLICATION IN ASSAYING HEPARIN. Craig M. Jackson, American Red Cross Blood Services, S.E. Michigan Region, Detroit, MI, USA

Heparins increase the rate of inactivation of proteinases by antithrombin without being consumed in the inactivation reaction. The anticoagulant activity of any heparin or heparin preparation is thus determined by the increase in the inactivaton rate which it produces. This rate increase is dependent on the concentration of the heparin in the sample and on some now well known structural properties of the individual heparin molecules that produce high affinity for antithrombin . All proteinases are not inactivated by antithrombin equally rapidly in the absence of heparin, nor are heparins and heparin derivatives of different molecular weight equally effective in the inactivation of the same proteinase. Under appropriate conditions, the observed rate constant (k_{obs}) for the heparin catalyzed proteinase inactivation reaction is simply related to the intrinsic potencies and concentrations of the individual high affinity heparin molecules in the sample. The intrinsic potency of a high affinity heparin molecule is the efficiency with which it catalyzes the inactivation of the particular proteinase, e.g. Factor Xa or thrombin, i.e., it is a second order rate constant, (designated k^*). After k^* has been determined from k_{obs} for a known heparin or heparin preparation and a particular proteinase, the concentration of heparin in an unknown sample can be calculated from the equation

$$[H] = [HAT] = k_{obs}/k^*$$

In general terms, the appropriate conditions, i.e.,the antithrombin and proteinase concentrations, the pH, and ionic strength, required for this equation to be used are those conditions for which all of the high affinity heparin is bound to the antithrombin and pseudo first order kinetic behavior occurs. At very low heparin concentrations, a correction for the inactivation of the proteinase by antithrombin alone is necessary, but is easily made.

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RELATIONSHIP BETWEEN HAEMORRHAGIC AND LIPASE-RELEASING PROPERTIES OF HEPARIN AND LMW HEPARIN. A. Maggi (1), T.W. Barrowcliffe (2), E. Gray (2), M.B. Donati (1), R.E. Merton (2) and I. Pangrazzi (1). Mario Negri Institute, Milan, Italy (1) and National Institute for Biological Standards and Control, Hampstead, London U.K. (2).

In a preliminary study, a good correlation (r = 0.97) was noted between the relative abilities of an unfractionated heparin, a LMW heparin, pentosan polysulphate and dermatan sulphate to prolong the template bleeding time in rabbits and their lipase-releasing potencies. In the present study, we have measured the prolongation of both the template and transection bleeding times in groups of 5 rats given i.v. injections of 0.75 mg/kg of two different unfractionated heparins (UFH), A and B, three different LMW heparins, X, Y and Z, and a heparan sulphate, HS. Lipase release was measured in plasma samples from different groups of 5 rats, using a tritiated triolein method.

UFH A had the most haemorrhagic effect, with an approximate doubling of both template and transection bleeding times and was also the most potent lipase-releaser, giving an average lipase level of 1126 mu/ml. UFH B had no significant effect on the template bleeding time, but did prolong the transection time; its lipase releasing potency was 70% of UFH A. LMW heparin X had no effect on template or transection bleeding time and released only 40% lipase compared with UFH A. LMW heparins Y and Z did not affect the template bleeding time but did prolong the transection time; they released more lipase (60%) than LMW heparin X. Correlation coefficients with lipase release were 0.97 for the template bleeding time and 0.69 for the transection bleeding time. HS released only 7% lipase but gave significant prolongations of both bleeding times.

These results confirm a strong positive correlation between the haemorrhagic and lipase releasing properties of heparin and LMW heparin, suggesting very similar structural requirements for the two biological activities. This correlation exists also for dermatan sulphate and pentosan polysulphate, but not for the heparan sulphate sample tested.