PLATELET GLYCOPROTEIN Ib (1)

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IDENTIFICATION OF GLYCOPROTEIN Ib DAs THE M, = 24,000 PLATELET POLYPEPTIDE PHOSPHORYLATED BY AGENTS THAT ELEVATE CYCLIC AMP. H. B. Fox (1), C. C. Reynolds (1), J. E. Boyle (1), A. A. Abel (2), and M. N. 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 cyclic AMP. This reaction is probably mediated by the cyclic AMP-stimulated phosphorylation of intracellular proteins. Polypeptides that become phosphorylated are of Mr = 250,000, Mr = 170,000, Mr = 22,000 (P22) and Mr = 24,000 (P24).

The Mr = 250,000 polypeptide is actin-binding protein, but the identity of the other polypeptides is unknown. In the present study, the Mr = 24,000 polypeptide (P24) was radiolabeled with [32P]P, and then incubated for 2-5 min in the presence or absence of 5 µm prostaglandin E (PGE).

The PGE-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 co-migrated with the a-chain of purified GP Ib on reduced gels (Mr = 24,000) and also on nonreduced gels (when GP Ib is disulfide-linked to GP IIb, and migrates with Mr = 170,000).

As GP Ib, P24 was associated with filamentous actin, and that on Bernard-Soulier platelets, we compared the action of PGE on control and 5000 U/ml heparin-treated platelets.

To determine whether phosphorylation of GP Ib is responsible for the inhibitory effects of PGE, on platelets, we compared the action of PGE on control platelets with that on Bernard-Soulier platelets. One of the ways in which PGE inhibits platelet activation is by inhibiting the polymerization of actin. While PGE inhibited actin polymerization in control platelets, it did not in Bernard-Soulier platelets.

Conclusion: P24 is phosphorylated by agents that elevate cyclic AMP, and that phosphorylation of this glycoprotein results in inhibition of platelet function.

MOLAR CLONING OF HUMAN PLATELET GLYCOPROTEIN Ibl. J.A. Lopez (1), D.K. Chung (1), K. J. Treadwell (2), J.S. Komm (3), R.S. Hynes (3), B. G. Mann (4), G. J. Roth (1). Veterans Administration Medical Center (1), Department of Medicine (2), and Biotechnology (3), University of Washington, and Zymogenetics Inc. (4), Seattle, WA, U.S.A.

Glycoprotein Ib (GP Ib) 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 GP Ib was studied. GP Ib is a proteolytic fragment of an antigenically heterogeneous polypeptide which can be purified from human platelets by affinity chromatography using wheat germ agglutinin and anti-GP Ib monoclonal antibody (D. Nagent, University of Washington) coupled to Sepharose.

GP Ib chain, D chain, and glycoparcin were isolated, reduced and carbamylated, 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 a chain, the D chain, and glycoparcin were prepared and shown to be monospecific by Western blot analysis. Total RNA was prepared from human erythroleukaemia cells grown in the presence of phorbol acetate. Poly(A)+ RNA was selected and used to prepare a cDNA library in acgll. The library was screened with [125]labeled polyclonal antibody to glycoparcin. The clone with the largest cDNA insert was sequenced and shown to code for amino acid sequence corresponding to those determined by Edman degradation of glycoparcin. 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 proteoglycan. Another clone contains a second repeat rich in threonine and serine, which shows some homology to a 9 amino acid repeat present in the connecting region of human factor VIII. These clones also enable the major site of attachment of clusters of O-linked carbohydrate in GP Ib. These results indicate that human platelet glycoprotein Ib 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.

DECLARATION OF INTEREST

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PLATELET GLYCOPROTEIN Ib (2)

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A DEFINITION OF HEPARIN ANTICOAGULANT POTENCY APPLICABLE TO ALL HEPARINS AND HEPARIN-LIKE SUBSTANCES AND ITS PRACTICAL APPLICATION IN ASSESSING HEPARIN. Craig M. Jackson, American Red Cross Blood Services, S.1. Michigan Region, Detroit, MI, USA.

Heparins increase the rate of inactivation of proteinases by antithrombin similarly by inhibiting the inactivation reaction. The anticoagulant activity of any heparin or heparin preparation is thus determined by the increase in the inactivation 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 fast. The rate of inactivation of proteinase, e.g. Factor Xa or thrombin, i.e., it is a second order rate constant, (designated k). After k has been determined from kobs 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:

\[ k = \frac{k_{obs}}{[H]} \]

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 proteinase concentrations, the inactivation of the proteinase by antithrombin alone is necessary, but is easily made.

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RELATIONSHIP BETWEEN HEPARINIC AND LIPASE-RELEASE PROPERTIES OF HEPARIN AND LMW HEPARIN. A. Moggi (1), T.W. Narcomilaise (1), E. Gray (2), M.B. Donati (1), R.E. Morton (2) and A. Maggi (1), Mario Negri Institute, Milan, Italy (1), and National Institute for Biological Standards and Control, Hampstead, London, UK. (2).

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

UHM A had the most heparinomic effect, with an approximate doubling of both template and transaction bleeding times and was also the most potent lipase-releaser, giving an average lipase level of 1126 µU/ml. UHM B had no significant effect on the template bleeding time, but did prolong the transaction time; its lipase releasing potency was 70% of UHM A. LMW heparin Y had no effect on template or transaction bleeding time and released only 40% lipase compared with UHM A. LMW heparin Y and Z did not affect the template bleeding time, but prolonged the transaction 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 transaction bleeding time. HS released only 7% lipase but gave significant prolongations of both bleeding times.

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