834

S PROTEIN/VITRONECTIN NEUTRALIZES THE ANTICOAGULANT ACTIVITY OF GLYCOSAMINOGLYCANS IN THE INHIBITION OF THROMBIN BY HEPARIN COFACTOR II. K.T. Preissner (1) and P. Sie (2). Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen, F.R.G. (1) and Hemostasis Laboratory, Hospital Purpan, F-31052 Toulouse, France (2).

The complement inhibitor S protein, which is identical to the adhesive protein vitronectin, functions as heparin-neutralizing factor by protecting thrombin against fast inactivation by antithrombin III. The interference of S protein with glycosaminoglycan-catalyzed inhibition of thrombin by heparin cofactor II was investigated in a purified system. In the presence of 0.3 µg/ml heparin, or 0.5 µg/ml pentosan polyphosphate (SP 54), or 2 µg/ml dermatan sulfate, S protein induced a concentration-dependent reduction of the inhibition rate of thrombin by heparin cofactor II. This resulted in a decrease of the apparent pseudo-first order rate constants by about 17-fold (heparin), or about 7-fold (SP 54), but only by about 2-fold for dermatan sulfate at a physiological ratio of S protein to heparin cofactor II. Likewise, S protein significantly counteracted the anticoagulant activity of heparin and SP 54 bot not of dermatan sulfate when tested in an inhibition assay using various concentrations of glycosaminoglycans. For heparin, the activity of S protein at the point of 50% inhibition of thrombin was expressed in the range 0.06-0.6 µg/ml (0.01-0.1 U/ml) and for SP 54 in the range 0.3-2 µg/ml. Exposure of the glycosaminoglycan-binding region of S protein by reduction and carboxymethylation of the protein even increased the neutralizing activity of S protein towards heparin and SP 54. S protein not only was found together with thrombin in a binary complex. S protein also became incorporated into a ternary complex with thrombin and heparin cofactor II as judged by crossed immunoelectrophoresis, regardless whether complex formation was initiated by heparin or dermatan sulfate. These findings underline the role of S protein as potent glycosaminoglycan-neutralizing protein in plasma and as scavenger protein which may bind to enzyme-inhibitor complexes of the coagulation system.

835

PREFERENTIAL RECOGNITION OF VITRONECTIN (S-PROTEIN) BY A MONOCLONAL ANTIBODY UPON INTERACTION WITH THROMBIN, ANTITHROMBIN AND GLYCOSAMINOGLYCANS. B.R. Tomasini and D.F. Mosher. Departments of Physiological Chemistry and Medicine, University of Wisconsin, Madison, WI, U.S.A.

Vitronectin/S-Protein (VN/SP) is a glycoprotein present at a concentration of 200-400 ug/ml in plasma and serum. It has been shown to promote cell-substratum adhesion and to act as an inhibitor of the membrane attack complex of complement and of the inactivation of thrombin by antithrombin III in the presence of low levels of heparin. We have previously shown that VN/SP binds more avidly to heparin-agarose and to a monoclonal antibody (MaVN/SP)-Sepharose column when present in serum rather than in plasma. In order to examine the possibility of a serum-induced conformational change, we utilized, in this study, an indirect enzyme-linked immunosorbent system to test for the exposure of new antigenic determinants. When MaVN/SP was incubated with plasma or serum, recognition of VN/SP in serum was approximately 50 fold greater than recognition of VN/SP in plasma. Since VN/SP has been shown to interact strongly with the thrombin-antithrombin complex, we examined the antigenicity of VN/SP when incubated with thrombin and antithrombin in the presence and absence of heparin. Incubation of VN/SP with heparin promoted a 2.5-fold increase in recognition by MaVN/SP. When MaVN/SP was incubated with thrombin-antithrombin but not thrombin or antithrombin alone, recognition was increased by 7-fold in the absence of heparin and by 32-fold in the presence of heparin. This differential recognition of VN/SP was not observed with a second monoclonal antibody raised originally against S-Protein. Treatment of VN/SP with various glycosaminoglycans and polysaccharides demonstrated the following relative potencies for induction of the partial antigenic change: dextran sulfate>fucoidan>heparin>dermatan sulfate>hyaluronic acid. No effect was detected upon incubation of VN/SP with keratan sulfate, heparan sulfate or chondroitin sulfate. These data suggest a conformational change induced by thrombin-antithrombin which may allow VN/SP to interact more avidly with other molecules such as heparin. The physiological role of this putative conformational change is under investigation.

836

INTERACTION OF S PROTEIN/VITRONECTIN WITH CULTURED ENDOTHELIAL CELLS: PROMOTION OF ATTACHMENT AND SPECIFIC BINDING. K.T. Preissner, E. Anders and G. Müller-Berghaus. Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen, West-Germany.

The interaction of the complement inhibitor S protein, which is identical to the serum spreading factor, vitronectin, with cultured human endothelial cells of macro- and microvascular origin was investigated. Purified S protein, coated for 2 h on polystyrene petri dishes, induced concentration- and time-dependent attachment and spreading of human umbilical vein endothelial cells (HUVEC) as well as human omental tissue microvasular endothelial cells (HOTMEC) at 37°C. With 3 x 10^5 cells/ml (final concentration) more than 50% of the cells attached within 2 h incubation at 0.3 - 3 µg/ml S protein. The effect of S protein was specific, since only monospecific antibodies against S protein prevented attachment of cells, while antibodies against fibronectin, fibrinogen or von Willebrand factor were uneffective. The pentapeptide Gly-Arg-Gly-Asp-Ser, which contains the cell-attachment site of these adhesive proteins including S protein, inhibited the activity of S protein to promote attachment of endothelial cells in a concentration-dependent fashion; at 200 µM peptide, less than 10% of the cells became attached. Direct binding of S protein to HUVEC and HOTMEC was studied with cells in suspension at a concentration of 1 x 10^6 cells/ml in the presence of 1% (w/v) human serum albumin and 1 mM $CaCl_2$ and was maximal after 120 min. Both cell types bound S protein in a concentration-dependent fashion with an estimated dissociation constant K_D=0.2µM. More than 80% of bound radiolabelled S protein was displaced by unlabelled S protein, whereas binding was reduced to about 50% by the addition in excess of either fibronectin, fibrinogen, von Willebrand factor or the pentapeptide. These findings provide evidence for the specific association of S protein with endothelial cells, ultimately leading to attachment and spreading of cells. Although the promotion of attachment was highly specific for S protein, other adhesive proteins than S protein, also known to associate with endothelial cells, may in part compete with direct S protein binding.

837

NEUTRALIZATION OF HEPARIN-LIKE SACCHARIDES BY COMPLEMENT S PROTEIN/VITRONECTIN. DA Lane, AM Flynn, G Pejler, U Lindahl and K Preissner. Department of Haematology, Charing Cross and Westminster Medical School, London W6, England, the Department of Veterinary Medical Chemistry, Swedish University of Agricultural Science,The Biomedical Centre, S-751 23 Uppsala, Sweden and the Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen, FRG.

S protein, a major inhibitor of the assembly of the membrane attack complex of complement, has recently been shown to be identical to the serum spreading factor vitronectin. It has also been demonstrated to have anti-heparin properties. We have studied the heparin neutralizing properties of S protein/vitronectin using heparin, heparan sulfate and heparin oligosaccharides with high affinity for antithrombin. The ability of heparin fractions, Mr 7800-18800, and of the International Heparin Standard, to accelerate the inactivation of thrombin and Factor Xa by antithrombin was readily neutralized by S protein/vitronectin. Addition of the protein to the various saccharide fractions at a molar ratio 1-3/1 produced 50% neutralization, while complete neutralization was achieved at a molar ratio of <10/1. Moreover, S protein/vitronectin efficiently neutralized oligosaccharides of Mr 2400-7200, unlike the two other physiologically occuring heparin neutralizing proteins histidine-rich glycoprotein (HRG) and platelet factor 4 (PF4) (Lane et al (1986) J. Biol. Chem.261, 3980; Lane et al (1984) Biochem. J. 218, 725). Like PF4, but unlike HRG, S protein/vitronectin readily neutralized the anticoagulant activities of heparan sulfate of Mr ~20000. These findings indicate that S protein/vitronectin requires little more than the antithrombin-binding pentasaccharide with which to interact in order to express its anti-heparin activity. Furthermore, the results suggest that S protein/vitronectin may be a physiologically important modulator of the anticoagulant activity of heparin-like material on or near the vascular endothelium.