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SULFATIDES AND GLYCOLIPIDS IN PLATELETS AND ENDOTHELIAL CELLS
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Sulfatides are sulfated glycolipids which are negatively charged and thought to influence receptor mediated activities. Sulfatides have the capacity to provide a surface for the initiation of in vitro coagulation tests and these acidic lipids represent the potential biological surface for the initiation of the contact and intrinsic systems in vivo. Several sulfatides have been demonstrated in blood platelets. We have investigated sulfatides and other glycolipids in endothelial cells and platelets in order to define the cellular sources for sulfatides that would be available for influencing hemostasis. Endothelial cells were derived from primary cultures of human umbilical veins and human platelets were obtained from freshly-collected blood. Cellular lipids were extracted by the Folch method. Sulfatides and glycolipids were purified by silicic acid chromatography, separated by thin-layer chromatography, and quantitated by the assay of sphingosine. Glycolipids were also analyzed by HPLC. Globoside was found to be the predominant glycolipid in endothelial cells while lactosyl ceramide was the predominant glycolipid in platelets. Sulfatides were detected by two approaches: 1) Sulfatide synthesis by the incorporation of [35S]-Sulfate; 2) The specific binding of [125I]-thrombospondin and [125I]-von Willebrand's factor (vWF) to sulfatides separated by thin-layer chromatography (TLC). Several sulfatides were identified in endothelial cells and platelets by virtue of the incorporation of [35S]-sulfate into glycolipids separated by TLC. [125I]-TSP and [125I]-vWF bound to the glycolipids that had incorporated [35S]-sulfate. [35S]-sulfate was primarily incorporated into sulfated galactosyl ceramide but both cells also synthesized complex glycolipids. TSP and vWF were shown to bind to sulfated galactosyl ceramide, a band that comigrated with glycosyl ceramide as well as with two more complex sulfatides in both cells. However, differences in sulfatide synthesis and binding of TSP to sulfatides were observed in endothelial cells from that in platelets. The study indicates that endothelial cells and platelets contain several sulfatides and thus are potential sources for sulfatides for the initiation of coagulation.

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PROTEOGLYCANS AND SULFATED PROTEINS OF PLATELETS: CHARACTERIZATION AND RESPONSE TO THROMBIN AND ADP.
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The proteoglycans (PG) and sulfated proteins of guinea pig platelets were labeled in vivo by intraperitoneal injection of (35S)sulfate. At 3 days after injection, platelets contained 3 distinct populations of chondroitin-6-sulfate proteoglycans which together constitute about 65% of the cellular (35S) label. Most PG elute from a DEAE-Sephacel column with 4M Gdn HCl (PG-1, 87%), and elute at K_{av} 0.12 on Sepharose CL-6B. The PG-1 can be resolved by SDS-PAGE into two fractions. The remainder (PG-2, 13%) elutes from the DEAE-Sephacel column with 4M Gdn HCl/2% Triton X-100 or 2% CHAPS, and has a K_{av} of 0.07 on Sepharose CL-6B. About 20-25% of the cell (35S) label elutes from DEAE-Sephacel in the wash-through or with 0.23M NaCl, and can be resolved by SDS-PAGE into at least 8 distinct bands which we have tentatively characterized as sulfated glycoproteins. The remainder of the (35S) is in low molecular weight (LMW) material which does not adhere to DEAE-Sephacel and has not been further characterized.

Platelets were treated with either thrombin or ADP, and the cells were then separated from the supernatant by centrifugation. The radiolabeled molecules in the supernatant and the cells were analyzed by DEAE-Sephacel and Sepharose CL-6B column chromatography. About 65% of the total cell (35S) was released from the cells by thrombin. Most of this radiolabel adhered to the DEAE-Sephacel column, and was found to be PG-1. The remainder of the released (35S) was about half the LMW material. In contrast, only 10-15% of the (35S)labeled material retained by the cells adhered to DEAE-Sephacel and was found to be PG-2. The remainder of the (35S)-labeled material retained by the platelets was the sulfated proteins and the LMW material. ADP caused release of about 15% of the (35S), and this was found to be in part PG-1 and in part the LMW material, but not PG-2. None of the (35S)-labeled molecules appeared to be degraded during platelet activation. We suggest that the PG-1 represent the α-granule and PG-2 the membrane proteoglycans. The sulfated proteins have not been described previously. Their role is not known, but we hypothesize that they may form part of the negative charge of the glycocalix and thus be part of the reactive surface of the platelet.

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PROTEIN C, PROTEIN S AND THE FIBRINOLYTIC SYSTEM IN PATIENTS WITH A HISTORY OF THROMBOSIS.
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Consecutive patients with a history of thrombo-embolic disease (n = 241, 109 males, 132 females, mean age 46 y), referred to the Coagulation Laboratory during an 18 month period, were analysed for defects in their coagulation and fibrinolytic systems. The diagnosis of thrombosis had been verified with phlebography and that of pulmonary embolus with scintigraphy or angiography. Retinal venous thrombosis was found in 15 of the patients. In 15 cases the thrombotic episodes occurred postoperatively, in 15 during pregnancy, in 12 during the postpartum period and in 20 during use of oral contraceptives. In the remaining cases no clinical riskfactors were identified.

The concentration of protein C zymogen was measured with an immunoradiometric assay. Functional protein C was determined with a clotting inhibition assay. Protein C deficiency was found in 8 cases. Two of these had a functional protein C deficiency with normal zymogen levels. The concentration of total, as well as free (not in complex with C4b-binding protein), protein S was determined with a radioimmunoassay. Two cases of protein S deficiency were detected. Three patients with antithrombin III deficiency and two with plasminogen deficiency were found.

The fibrinolytic activity after venous occlusion was analysed in 216 patients. Decreased levels were found in 32 %. The concentration of tissue plasminogen activator inhibitor (PAI) was measured in 110 patients and found to be increased in 65 % of the cases. In 99 patients both the fibrinolytic activity and the PAI concentration were measured. A combination of decreased fibrinolytic activity and increased levels of PAI was found in 44 cases. The concentration of tissue plasminogen activator antigen was decreased in 22 % of 105 cases analysed.

Thus, in this material of patients with thrombo-embolic disease, abnormalities were found in 47 %. Defects in the fibrinolytic system were the most common findings. Protein C or protein S deficiency was diagnosed in less than 5 % of the cases.

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MONOCLONAL ANTIBODIES THAT RECOGNIZE Ca^{2+}-INDUCED CONFORMER OF PROTEIN C, INDEPENDENT OF GLA RESIDUES.
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Monoclonal antibodies (MCA's) were prepared against human protein C (PC) according to Köhler & Milstein, and those that recognize the Ca^{2+}-dependent PC conformers were screened by direct ELISA in the presence of 2 mM either $CaCl_2$ or EDTA. Out of nine MCA's thus screened, five MCA's designated as HPC-1~5, respectively, were found to react with PC in the presence of Ca^{2+} but not EDTA. By SDS-PAGE coupled with Western Blotting performed in the presence of 2 mM $CaCl_2$, we found that two MCA's HPC-1 and 2, recognized the light chain, and two others, HPC-3 and 4, recognized the heavy chain of PC. But another MCA, HPC-5 was found to react with only non-reduced antigens. Further study showed that HPC-1 and 5 failed to react with the Gla-domainless PC, i.e. PC from which the N-terminal Gla-domain of the light chain had been cleaved off by α-chymotrypsin. However, all the other three MCA's retained the reactivity with the antigen in the presence of Ca^{2+} even after the Gla-domain had been removed. The binding of these MCA's to PC in the presence of Ca^{2+} was found to be saturable with respect to the Ca^{2+} concentration and the half maximal binding for each MCA was calculated to be about 0.5 mM. Moreover, many other divalent cations such as Mg^{2+}, Mn^{2+}, Ba^{2+}, Zn^{2+}, Co^{2+}, Sr^{2+}, were found to substitute for Ca^{2+} in inducing the metal ion-dependent but Gla-domain-independent conformer of PC.

Cross-reactivity to other vitamin K-dependent plasma proteins was examined by direct ELISA; HPC-2 and 3 reacted solely to PC, but HPC-1 and 4 also reacted with prothrombin and HPC-5 with both prothrombin and factor X.

These findings indicated that there are two or more metal binding sites besides the Gla-domain, possibly one in the light chain and the other(s) in the heavy chain. The presence of these metal binding sites may contribute to the unique conformer of vitamin K-dependent plasma proteins including protein C.