
To evaluate the role of membrane sialyltransferase in the initiation of platelet aggregation, we studied the stimulatory effect of epinephrine and adenosine diphosphate in the inhibition effect of aspirin on the platelet surface sialyltransferase activity. The enzyme activity was assayed under optimal conditions as determined previously. The assay mixture consisted of intact washed human platelets, UDP-[3H]-sialic acid, desalted fucoid, Hepes, and buffer to a final volume of 1 ml. The enzyme activity was enhanced to 175% of control by 1 µM, 150% by 5 µM, and 165% by 10 µM epinephrine. Adenosine diphosphate enhanced the enzyme activity to a lesser extent: 103% at 1 µM and 113% at 5 µM. In contrast, aspirin inhibited the enzyme activity to 46% of control when 10 µg/ml of aspirin was used. Higher concentrations of aspirin failed to cause further inhibition. In the in-vivo experiment, 600 mg aspirin was given to normal subjects and the surface enzyme activity was determined 12 hours later. The enzyme activity reduced to 42% following aspirin administration. Furthermore, we studied the enzyme activity in a patient with 'aspirin-like' release disorder. While the mean surface enzyme activity of 10 normal subjects was 1.56 ± 0.2 (S.D.) pmol/hr-1 per 10^8 platelets, the enzyme activity of the patient was only 0.01 pmol/hr-1. The results strongly suggest that the membrane sialyltransferase plays an important part in the initiation of platelet release reaction.

Ca^[2+]: REGULATION OF BOVINE THROMBOPOEINTIN. L. Muszkat, J. Kudlinski and W. Drabikowski. Department of Pharmacology, University School of Medicine, Debrecen, Hungary, and Department of Biochemistry of Nervous System and Muscle, Nencki Institute of Experimental Biology, Warsaw, Poland.

In order to reveal the type of its Ca^[2+]-regulation bovine thrombostatin - natural platelet actomyosin - was investigated by competitive actin and myosin binding assay and by urea gel electrophoresis. The Ca^[2+]-resistance low ionic strength NaCl/KCl activity of platelet actomyosin was not influenced by the addition of excess skeletal muscle actin free of regulatory proteins. In contrast, the replacement of platelet myosin by skeletal muscle myosin resulted in a hybrid actomyosin insensitive to Ca^[2+]. If actomyosin was reconstituted from crude platelet actin and skeletal muscle myosin again no regulatory effect of Ca^[2+] could be observed. In the presence of RBA a fast moving band with the mobility corresponding to muscle tropalin C (TH-C) was detected by alkaline urea polyacrylamide gel electrophoresis. However, if Ca^[2+] was added this protein, unlike TH-C, neither changed its mobility nor formed a complex even if muscle tropalin I was included into the system. The experimental results indicate that our Ca^[2+]-sensitive bovine thrombostatin preparation does not contain TH-C like component and is not regulated by an actin - , but rather by a myosin-connected system.

BIOCHEMICAL PROPERTIES OF HUMAN PLATELET FACTOR 4. D.A. Mals, L.R. McCoy, and V.Y. Wu. Wayne State University School of Medicine, Detroit, Michigan, U.S.A.

Human platelet factor 4 (PF4) was isolated from outdated platelet concentrates to evaluate its biochemical properties. Platelets were washed in saline, lysed by freeze-thawing, and centrifuged to remove particulates from the lysate. PF4 was adsorbed on heparin immobilized on Sepharose. The yield of PF4, eluted in molar saline, was 9.3 µg/ml platelets. Polyacrylamide electrophoresis indicated a single-chain protein with an apparent molecular weight of 9,600. Amino acid composition indicated a 92 residue chain having 2 disulfide bridges and free of Met, Phe, and Thr. Electrophoresis of equal molar ratios of heparin and PF4 in the presence of 0.1% SDS resulted in the appearance of a slow migrating band having an apparent molecular weight of 20,000. The isolated PF4 was poorly soluble in the absence of high concentrations of urea, and PF4 was resistant to the heparin PPACK. Polyacrylamide gel electrophoresis and membrane filtration studies were indicative of molecular aggregation. PF4 appeared to be totally resistant to trypsin and chymotryptic hydrolysis but not to pepsin. However, PF4 bound to heparin but lost its resistance to trypsin or chymotryptic hydrolysis. Heparin appeared to be totally resistant to trypsin or chymotryptic hydrolysis of reduced-alkylated PF4 while the peptide pattern after chymotryptic hydrolysis of reduced-alkylated PF4 had no apparent affinity for heparin unless heparin was present. These peptides after chymotryptic hydrolysis of heparin-bound-reduced-alkylated PF4 may serve as models for the design of small, synthetic heparin antagonists. (Supported by USFNS MH grant #MH-20317-01 and the Charles F. Kiling Foundation.)