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PLENARY III

Fibrinolysis

ROLE OF SIALIC ACID IN THE DYSFIBRINOGENEMIA ASSOCIATED WITH LIVER DISEASE. J. Martinez, J. Palascak, and D. Kwasniak (intr. by S. S. Shapiro). Cardeza Foundation, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

We have described an abnormal fibrinogen in 6 patients with liver disease who had prolonged plasma thrombin times due to impaired fibrin monomer aggregation. To investigate the role of sialic acid in this functional abnormality, fibrinogen was purified from normal and patient plasmas by the glycine precipitation method. Sialic acid content of the fibrinogens was measured by the thriobarbituric acid assay after acid hydrolysis. Normal fibrinogen had 6.1 ± 0.5 residues per molecule of fibrinogen, whereas patient fibrinogen sialic acid content ranged between 7.5 and 10 residues per molecule. The reduced fibrinogen demonstrated normal mobility of Aα, Bβ and γ chains on SDS polyacrylamide gel electrophoresis when stained for protein and, similar to normal fibrinogen, only the Bβ and γ chains stained with PAS. The degree of prolongation of the thrombin times of the purified patient fibrinogens appeared to correlate with the increase in the fibrinogen sialic acid. The effect on fibrin monomer aggregation of decreasing patient fibrinogen sialic acid content was studied. Partially desialated patient fibrinogen was prepared by treating the protein with Vibrio cholerae neuraminidase for varying periods of time. Partial removal of sialic acid from patient fibrinogen resulted in normalization of the thrombin time and improvement in fibrin monomer aggregation. Thrombin times ranged from 31.5 to 49.5 seconds prior to removal of excess sialic acid compared to 20.5 to 25.5 seconds post removal. These findings indicate that the dysfibrinogenemia associated with liver disease is biochemically characterized by increased sialic acid content and removal of this sialic acid results in a functional normalization of the protein.

THE FUNCTIONAL ACTIVITIES OF THE HUMAN PLASMIN-DERIVED LIGHT(B) CHAIN. K. C. Robbins, L. Summaria and R. C. Wohl. Michael Reese Research Foundation, Chicago, Illinois, U.S.A.

A human plasmin-derived light(B) chain has been isolated and found to be functionally active. It is both a proteolytic and an esterolytic enzyme, but it has no plasminogen activator activity. It reacts with streptokinase to form an equimolar complex which has both human and bovine plasminogen activator activities. The binding site for streptokinase is located on the light(B) chain of plasmin. The light(B) chain, with N^{α}-Cbz-L-lysine-p-nitrophenyl ester as the substrate, had a K_m of 83.0 μM and a k_{cat} of 2.08/sec whereas the light(B) chain·streptokinase complex had a K_m of 71.4 μM and a k_{cat} of 9.00/sec, indicating an increased catalytic efficiency of the active site in the activator complex. The human Lys-plasminogen activator activity of the complex, when measured by the esterase activity of the plasmin being generated, showed a K_m value of 1.65 μM and a k_{cat} of 43.2/sec, a two-fold increase in catalytic rate over streptokinase. Fragmentation of streptokinase beyond SK1, in the complex, results in decreased activator catalytic rates. The light(B) chain·streptokinase complex was an effective activator in in-vitro clot lysis systems using cross-linked fibrin clots. The light(B) chain adsorbs to fibrin clots resulting in accelerated lysis of these clots by activators.