

FREE COMMUNICATIONS XII

Platelets: Molecular Structure and Interactions.

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IDENTITY AND KINETIC PROPERTIES OF A PROTEIN (ACTIN) WHICH BINDS METABOLIC PLATELET ADP IN AN ETHANOL-INSOLUBLE FORM. J.L. Daniel, L. Robkin, and H. Holmsen, Specialized Center for Thrombosis Research, Temple University, Philadelphia, Pennsylvania, U.S.A.

Thirty to fifty percent of the metabolic ADP in platelets is bound to protein and the complex is insoluble in ethanol, from which it can be isolated from platelets and subsequently extracted by perchloric acid. We have tentatively identified the protein as actin based on the following: 1) comparison of the amount of ethanol insoluble ADP and the actin content of the platelets and other nucleotide-binding proteins are not present in sufficient amounts; 2) its behavior on a gel filtration column; 3) its solubility properties; 4) the exchangeability of the bound ADP in platelet lysates. The kinetic behavior of the "actin"-bound nucleotide in the intact platelet was studied in cells that had their (ethanol-soluble and -insoluble) metabolic pool preequilibrated with ^{14}C -adenine. The change with time of the $^3\text{H}/^{14}\text{C}$ ratio in the pools after addition of ^3H -adenine was measured. In the resting platelet the $^3\text{H}/^{14}\text{C}$ ratio increased at the same rate in the ethanol soluble and -insoluble pool, indicating rapid equilibrium between the pools. However, when ^3H -adenine was added to ^{14}C -labelled platelets that had been incubated with antimycin and deoxyglucose, the $^3\text{H}/^{14}\text{C}$ ratio in the protein bound pool clearly lagged behind that of the soluble pool, the $^3\text{H}/^{14}\text{C}$ in the individual nucleotides suggested that soluble ATP was a precursor for protein-bound ADP. These results indicate that ATP is consumed to maintain a certain equilibrium between G- and F-actin in resting platelets. This technique $^3\text{H}/^{14}\text{C}$ ratio of studying the kinetic platelets parameters of "actin"-bound ADP may be of use for elucidating the role of contractile proteins in platelet functions.

THROMBOCYTIN, A NOVEL PLATELET ACTIVATING ENZYME FROM BOTHROPS ATROX (MARAJOENSIS) VENOM. S. Niewiarowski, E.P. Kirby, G.J. Stewart, R. Tuma, M. Wiedeman, M. Millman and K. Stocker, Temple Univ. Health Sci. Ctr., Philadelphia, PA and Pentaparm Laboratories, Basel, Switzerland.

Thrombocytin (TCN) was purified from Bothrops atrox (BA) venom by precipitation with 1.2% Na-salicylate and chromatography on heparin-agarose column using increasing concentrations of lysine as eluent. It was homogeneous on SDS electrophoresis and had an apparent MW of 36,000. Immunoelectrophoresis with polyvalent anti-BA venom serum gave one cathodic arc indicating an isoelectric point higher than pH 8.6. TCN at a concentration of 1 $\mu\text{g}/\text{ml}$ caused aggregation of human platelets, release of low affinity platelet factor 4 and serotonin, and stimulated platelets to retract fibrin. TCN was essentially free of fibrinogen clotting and fibrinolytic activities. TCN action on platelets was not mediated by the formation of thrombin since TCN did not activate Factor X or prothrombin and its action was not inhibited by hirudin. TCN is a serine protease since it was inhibited by DFP and it hydrolyzed a synthetic peptide, chromozyme UK (BZ-Val-Gly-Arg-pNA·HCl). TCN-induced aggregation of human platelets was completely inhibited by soy bean trypsin inhibitor, heparin, prostaglandin E_1 and apyrase. Washed human platelets were 2-4 times less sensitive to TCN as compared to platelets in freshly prepared platelet rich plasma (PRP); their sensitivity to TCN gradually deteriorated during incubation of PRP at room temperature for 3 hours. Electron microscopic observations revealed formation of platelet aggregates characterized by pseudopod formation, centralization and partial loss of platelet granules. Infusion of TCN (3 μg) into the main artery of bat wing resulted in the formation of platelet aggregates seen on arterial and venous side which occasionally occluded small vessels.