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0879 INTERACTION OF THROMBOCYTIN WITH PLATELETS AND PLASMA CLOTTING FACTORS

Edward P. Kirby*, Stefan Niewiarowski, and Kurt Stocker, Temple University, Philadelphia, Pennsylvania, U.S.A. and Pentapharm Laboratories, Basel, Switzerland.

Thrombocytin (Tcn), a thrombin-like enzyme from Bothops atrox marajoensis venom, has been purified to homogeneity by precipitation with sodium salicylate and chromatography on heparin-agarose. It is a single chain glycoprotein (mw = 36,000) which is a typical serine protease and is readily inhibited by DFP and soybean trypsin inhibitor. It is also inhibited by anti-thrombin III in the presence of heparin, and the formation of a covalent complex can be demonstrated. Tcn aggregates platelets, induces the release reaction, and stimulates clot retraction in a manner similar to thrombin, but has only 0.1% as much fibrinogen-clotting activity as thrombin. Preincubation of platelets with prostaglandin E_1, or with metabolic inhibitors inhibits aggregation by Tcn. Like thrombin, Tcn cleaves prothrombin to form Intermediate 1 and Fragment 1, and it activates Factor XIII by cleaving the a chain at approximately the same position as does thrombin. Tcn can also degrade the α-chain of fibrinogen. It activates Factor VIII and Factor X, but these activities are very weak. In summary, Tcn possesses many of the activities of thrombin, especially in regard to platelet aggregation and release, but has only a very slight fibrinogen-clotting activity. This may make it very useful for studying thrombin-mediated platelet activation in plasma-containing systems without the complications associated with fibrin generation.

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0880 INTERACTIONS BETWEEN ACROSIN AND THE COAGULATION SYSTEM

N.U. Bang*, L.E. Mattler, P.J. Burck, C.A. Marks and R.E. Zimmerman, Lilly Research Labs, Indiana Univ. School of Medicine, Dept. of Medicine, Indianapolis, Indiana, U.S.A.

Highly purified acrosin (A), the serine protease of the acrosome, which facilitates penetration of sperm through the zona pellucida, rapidly hydrolyzes tri- and tetrapeptide synthetic substrates relatively specific for thrombin (T) (S2238, chromozym TH, S2160) and factor Xa (Xa) (S2222) but only minimally hydrolyzes plasmin and plasma kallikrein substrates. A shares other biological properties with T and Xa. A slowly converts highly purified fibrinogen to fibrin and rapidly converts purified bovine prothrombin into T in a factor V or phospholipid-independent fashion. A, like T and Xa, is inhibited by antithrombin III; the inhibition is enhanced by heparin. A, like T, is inhibited by the pancreatic secretory trypsin inhibitor of Kazal but not by the Kunitz-Northrop trypsin inhibitor. A, unlike T, is not inhibited by hirudin and does not aggregate platelets. As has been reported for T and other serine proteases, A stimulates the growth of cultured fibroblasts in a concentration-dependent fashion. In T3T mouse fibroblasts only A stimulated DNA synthesis; T, plasmin and trypsin had no effect. Although the evolutionary and physiologic implications of similarities between A and clotting serine proteases remain unclear, the observed effect of A on cell proliferation may assign a new role for this enzyme in early embryogenesis.

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0881 PURIFICATION OF STAPHYLOCOAGULASE BY A BOVINE PROTHROMBIN-SEPHAROSE 4B COLUMN AND ITS PHYSICOCHEMICAL PROPERTIES

H. Igarashi[1]*, T. Morita[2] and S. Iwanaga[3], [1]Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, [2]Dept. of Biol. Chem., Washington Univ. School Med., St. Louis, Mo., U.S.A., [3]Dept. of Biology, Faculty of Science, Kyushu Univ. Fukuoka, Japan.

Staphylocoagulase is known to coagulate human plasma, but not bovine's, by forming an active molecular complex with prothrombin. However, there is no enough knowledges in respect to the molecular mechanism of prothrombin activation with staphylocoasulase. A new simplified method was developed for the large-scale purification of coagulase from culture filtrates of Staphylococcus aureus, strain st-213, using Sepharose 4B covalently linked with bovine prothrombin. This affinity column adsorbed strongly the coagulase, which was eluted with 1.0 M NaSCN. The yield of coagulase activity was in the range of 75 to 85%. The purified coagulase showed a single symmetrical peak by ultracentrifugal analysis ($S_{20,w}$=6.47), and it gave a single precipitin line against anti-purified staphylocoagulase serum, as revealed by the immunodiffusion test. However, the preparation was shown to contain three active components by the isoelectric foccusing method, suggesting some microheterogeneity. The molecular weight estimated by SDS-gel electrophoresis was 71,000 (major material). No cystine residue was found in the purified material and its NH_2-terminal sequence was Ile-Ile-. It is of interest that staphylocoagulase interacts strongly with bovine prothrombin-Sepharose 4B, whereas it does not form any active complex with the prothrombin, unlike human prothrombin.