Wednesday, July 15, 1981

Oral Presentations

Coagulation – XII

Thrombin, Prothrombin

08:00–09:30 h

Vessel Wall – III

Prostaglandins

09:45–11:00 h

Grand Ballroom East

0547

08:00 h

THE ASSOCIATION OF THROMBIN WITH FIBRIN AND ITS SOLUBILIZATION DURING PLASMIC DIGESTION. Charles W. Francis, Victor J. Marder, Grant H. Barlow and S. Eric Martin. Hematology Unit, Department of Medicine, University of Rochester School of Medicine & Dentistry, Rochester, New York, USA.

It has previously been demonstrated that thrombin binds to fibrin during clot formation. We have studied the nature of this association and the fate of bound thrombin during fibrin degradation by plasmin. Approximately 10% of human thrombin used to clot human fibrinogen bound to the fibrin and could not be removed by washing in buffers of physiologic pH and ionic strength. Plasmic digestion released thrombin into the lysate where it retained enzymatic activity, as measured by chromogenic and clotting assays. The fibrin was degraded to a group of unique degradation complexes of molecular weight between 230,000 and greater than 800,000. The active thrombin was present in the lysate both as free enzyme and bound to certain of these complexes, primarily those larger than 230,000 daltons. At low initial thrombin concentrations, most of the active enzyme released during plasmic digestion was bound to fibrin derivatives, while at higher initial thrombin concentrations, most was released as free enzyme. This suggests that there are at least two types and/or sites of association between thrombin and fibrin. The presence of thrombin on fibrin clots and bound to soluble fibrin derivatives may have pathophysiologic importance in the propagation of thrombi and in the development of hypercoagulable states.

0548

08:15 h

THE BINDING OF THROMBIN BY CLOTS FORMED FROM FRAGMENT X. C.Y. Liu, H.L. Nossel, K.L. Kaplan. Department of Medicine Columbia University College of Physicians & Surgeons, New York, NY, U.S.A.

Previous studies showed that: 1. thrombin was specifically and reversibly bound by fibrin, 2. Scatchard analysis of the data suggested high and low affinity binding sites, and 3. the bound thrombin was quantitatively released following proteolysis of the fibrin by plasmin. In the present study thrombin binding to clots formed from fragment X was studied. The binding of thrombin to fibrin decreased progressively in relation to the original fibrinogen concentration as the fibrin was formed from fibrinogen progressively degraded with plasmin and thus progressively less clottable. Fragment X was then isolated by Sephadex G-200 filtration of partially proteolysed fibrinogen. The fragment X preparation exhibited 76% clottability with thrombin, was heterogeneous with an average molecular weight of 292,000 $\pm$ 36,000, and contained 2 moles fibrinopeptide A and 0.25 moles fibrinopeptide B per mole. Fibrin formed from clottable fragment X bound thrombin with a molar binding ratio of 0.32 compared to 0.35 for fibrin formed from intact fibrinogen and a binding constant of $7.5 \times 10^5\ M^{-1}$ compared to $6.6 \times 10^5\ M^{-1}$ for the high affinity site on fibrin from intact fibrinogen. The data indicate that the NH_2-terminal end of the Bβ chain and the COOH-terminal portion of the Aα chain are not required for high affinity thrombin binding. Because the demonstrated binding is to clottable plasmin degradation products and the molar binding ratio is less than one, it is suggested that the higher affinity thrombin binding site is not present in the fibrinogen molecule but is formed by two or more fibrin molecules present in a polymer.

0549

08:30 h

THROMBIN NONCOVALENT-PROTEIN BINDING AND FIBRIN(OGEN) RECOGNITION. J. W. Fenton II, M. P. Zabinski, K. Hsieh, and G. D. Wilner. Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201, U.S.A., and Department of Pathology, The Jewish Hospital and Washington University School of Medicine, St. Louis, MO 63110, U.S.A.

Procoagulant α-thrombin is a highly selective proteolytic enzyme with central bioregulatory functions in hemostasis. Unlike with plasma protease inhibitors, human α-thrombin forms a noncovalent, yet high-affinity complex with hirudin ($K_i \sim 6.3$ pM), whereas noncoagulant γ-thrombin has $\sim$ 200-fold reduced affinity. Like ^{125}I-α-thrombin alone, ^{125}I-DIP- or ^{125}I-PMS-α-thrombin in the presence of unlabeled α-thrombin incorporates into fibrin clots, but ^{125}I-γ-thrombin or the hirudin complex of ^{125}I-α-thrombin does not. Similarly, α-thrombin and its catalytic-site conjugated forms (e.g., DIP-α-thrombin) bind to nonpolymerized fibrin agarose and elute at higher ionic strengths (I $\sim$ 0.2 M) than do noncoagulant nitro-α- or γ-thrombin. Hirudin prevents this binding of α-, DIP-α-, or γ-thrombin, demonstrating a common site. The NDSK fragment of fibrinogen is a moderate inhibitor of the esterolytic activity of α-thrombin ($K_i \sim 1$ µM), while the residue-17 through -27 synthetic homologue of the fibrinogen Aα chain is not an inhibitor. These results suggest contiguous exosites in thrombin for noncovalent-protein binding and fibrin(ogen) recognition, where these sites are removed from the enzyme catalytic site and correspond to structural regions on the fibrin side of fibrinogen other than those of the α-chain NH_2-terminus required for fibrin polymerization. Such binding/recognition sites may explain the physiological proteolytic and receptor specificity of α-thrombin.