INVITED SYMPOSIUM XI

Factor X Activities.

KINETICS OF FACTOR X ACTIVATION CATALYZED BY FACTOR VII

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Factor X activation is the point at which the intrinsic and extrinsic pathways of coagulation converge. Accordingly, events which control the rate of activation of Factor X might be central to the overall control of coagulation. We have therefore attempted to extract the kinetic parameters which govern this reaction. We have also devised and utilized a new assay for this reaction based on the release of radiolabelled activation peptide from Factor X. Utilizing Factor VII (2-chain VII) in the presence of saturating amounts of tissue factor, the $K_M$ was found to be 0.34 $\mu$M and the $k_{cat}$ was 1.0 sec$^{-1}$. The value for the $K_M$ is almost twice the concentration of Factor X in plasma. This means that only small variations in Factor X concentration, such as those encountered in the normal population, would have significant effects on the rate at which Factor X is activated. We have also found that the effect of tissue factor on this reaction is mainly on the catalytic activity of Factor VII although in the absence of tissue factor there is also a 10-fold increase in the $K_M$. Factor VII is predominantly present in plasma as a single polypeptide chain. Utilizing similar techniques we have also examined the reaction catalyzed by this form of Factor VII. All progress curves showed a prolonged lag followed by a sharp upward deflection. The mechanism underlying this phenomenon is now being investigated and will be discussed.

THE ROLE OF FACTOR IXa AND FACTOR VIII IN THE ACTIVATION OF FACTOR X

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Factor IXa and Factor VIII participate in the middle phase of blood coagulation. These two proteins convert Factor X to Factor Xa in the presence of calcium ions and phospholipid. The coagulant activity of factor VIII is increased 50-100 fold by the addition of thrombin, and this activity is stabilized in the presence of CaCl$_2$. The activated product (tentatively identified as activated factor VIII) was readily inhibited by diisopropyl phosphorofluoridate or antithrombin III, suggesting that it is a serine enzyme. The exact role of this enzyme in the conversion of Factor X to Factor Xa, however, is not known. When factor X (bovine or human) is converted to factor Xa, an activation peptide is cleaved from the amino-terminal end of the heavy chain. This gives rise to a new amino-terminal sequence of Ile-$\alpha$-Val-$\alpha$-Gly-$\alpha$-Gly-$\beta$ in the heavy chain. No change occurs in the light chain during the activation reaction. These data indicate that the basic mechanism involved in the conversion of human and bovine factor X to factor Xa appears to be essentially identical and probably involves the formation of a charge relay system characteristic of the pancreatic serine proteases.