STUDIES ON THE INTERACTION BETWEEN FACTOR Va AND FACTOR Xa.

M. Lindhout, J. Goverse-Biemlag, J. Roos and H. Henkens.
Department of Biochemistry, Limburg University, Maastricht, the Netherlands.

Thrombin activated bovine factor Va is composed of two polypeptide chains with molecular weights 94,000 and 80,000. The two polypeptide chains are complexed via Ca⁺⁺ ions.

Factor Va enhances the rate of thrombin formation by drastically increasing the Vmax of the prothrombin activation. We have undertaken a study of the interactions of factor Va with the different components of the prothrombinase complex (e.g. factor Xa and prothrombin), in order to get more insight in the mode of action of factor Va.

Our kinetic experiments in solution show that the functional enzyme in the prothrombinase complex is an equimolar complex of factor Va and factor Xa. The dissociation constant, as determined over a wide range of prothrombin concentrations, has a value of 3×10⁻¹⁰ M⁻¹.

For the stimulating effect of factor Va on the prothrombin activation by factor Xa in solution, the presence of Ca⁺⁺ ions is required. The dissociation constant of the Va-Xa complex was found to be independent of the Ca⁺⁺ concentration. In order to reveal whether an interaction between Ca⁺⁺ and γ-carboxyglutamic acid residues is responsible for the observed Ca⁺⁺ requirement, identical experiments were carried out with decarboxyfactor Va and factor Xa.

The isolated polyepptide chains of factor Va have, in the presence or absence of factor Xa, no effect on the kinetic parameters of the prothrombin activation. This let us conclude that there is no interaction between factor Va and the separate polyepptide chains of factor Va.

The affinity of factor Xa for negatively charged phospholipid or activated bloodplatelets is greatly enhanced by the presence of factor Va. Our Kd value measured for the Xa-Va complex in combination with reported dissociation constants of factor Xa-phospholipid and Factor Va-phospholipid complexes gives a quantitative expression for the above mentioned effect of factor Va on the binding of factor Xa to phospholipid membranes.

INHIBITION OF HUMAN FACTOR Xa BY VARIOUS PLASMA PROTEASE INHIBITORS.

M.P. Scully, V. Ellis, I.R. MacGregor, and V.V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London, England.


Activation of human factor VII by activated factors IX and X in purified systems. D.R. Mays, S.P. Bajaj and S.I. Rapaport. Department of Medicine, University of California, San Diego, La Jolla, California and Naval Regional Medical Center, San Diego, California.

Factor VII activity, as measured in a one-stage clotting assay, increases when whole blood is clotted in glass. Prior studies in this laboratory using factor-deficient plasmas suggested that this factor VII activation was due to activated factor IX (IXa). We therefore studied activation of VII by IXa and by activated factor X (Xa) in purified systems. Human factors II, VII, IX, and X were each purified to homogeneity as judged by SDS-polyacrylamide gel electrophoresis. Reaction mixtures of VII, IXa or Xa, and other cofactors and enzymes were made, and subsampled for VII activity. The activation state of VII was judged by comparison of one-stage clotting assay to a coupled amidolytic assay using a synthetic substrate. In the presence of phospholipid (PL) and calcium (Ca), both IXa and Xa activated VII 25 fold; however, Xa was roughly 800 times more effective than IXa. In the absence of PL, Xa was roughly 20 times more effective than IXa, in Ca-containing solutions. Only slight activation of VII by either enzyme occurred in the absence of Ca. The addition of prothrombin (II) markedly slowed activation of VII by both Xa and IXa; however, this effect did not occur if fully-decarboxylated II was used. The addition of antithrombin III and thrombin-modified factor VII at physiologic concentrations did not change rates of VII activation by IXa or Xa.

These results confirm the ability of IXa and Xa to activate factor VII at physiologic concentrations in purified systems. The higher relative efficiency of Xa over IXa under all conditions studied contrasts strikingly with observations in whole plasma systems where the VII activation measurable after clotting is greater in Xa-deficient than in IX-deficient plasma. The activation of VII by Xa and IXa may serve as an amplification loop in the generation of clotting by either "intrinsic" or "extrinsic" cascades.


In the intrinsic pathway, factor X is activated by a complex consisting of activated factor IX, factor VIII, phospholipid and calcium ions. The contribution of each of this components has been estimated by recompression of purified human coagulation factors, and measurement of the initial rate of factor Xa formation using a spectroscopic assay.

In the absence of phospholipid and factor VIII, factor X is activated at a level 10⁻⁴ fold lower than that in normal factor VIII deficient plasma. The activation of either phospholipid or activated factor VIII stimulates the initial rate of this reaction 250 and 500 fold, respectively. The activation of factor VIII by factor Xa war by thrombin is essential for the stimulating effect of factor VIII. Factor VIII complex, containing both VIIIIR:MF and VIIIIC, and purified factor VIIIIC behave identically in this system, purified factor VIIIIR:MF does not influence the rate of Factor Xa formation.

The time kinetic approach was used for the characterization of preparations of abnormal factor VIII, obtained from plasma of haemophilia A patients.