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Thrombin activated bovine factor V 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 IX 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 3x10^-6 M.

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 ion concentration. In order to reveal whether an interaction between Ca ions and γ-carboxyglutamic acid residues is responsible for the observed Ca ion requirement, identical experiments were carried out with decarboxyfactor Va and decarboxyprothrombin.

The isolated polypeptide chains of factor Va have, in the presence or absence of factor Va, 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 polypeptide chains of factor Va.

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


In the intrinsic pathway, factor X is activated by a complex consisting of factor IXa and factor VIII. This activation is necessary for the further activation of factor X. The activation of factor X is dependent on the presence of Ca ions and phospholipid. The contribution of factor VIII to the activation of factor X is not clear.

The activation of factor X is measured as the change in the rate of factor Xa formation. The rate of factor Xa formation is dependent on the concentration of factor X, factor IXa, factor VIII, and Ca ions. The activation of factor X is a second order reaction.

The second order rate constant for the interaction of human factor Xa with antithrombin III (in the absence of heparin) α2-antitrypsin and α2-macroglobulin have been determined at pH 7.4 (50mM Tris HC1, 0.1M NaCl) in the presence of 3mM CaCl2. Factor Xa (free of activation peptide) and α2-antitrypsin were purified from outdated human plasma. Pure human preparations of antithrombin III and α2-macroglobulin was used. The affinity of factor Xa for negatively charged phospholipid or stimulated bloodplatelets is greatly enhanced by the presence of factor Va. Our Kd value measured for the Xa-Xa complex in combination with reported dissociation constants of factor Va-phospholipid and Factor Va-phospholipid complexes give a quantitative value for the above mentioned effect of factor Va on the binding of factor Xa to phospholipid membranes.

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