Factor X and its Activation

Waterloo Room

Tim
14.00

0392 KINETICS OF FACTOR X ACTIVATION BY FACTOR IX_a. THE EFFECTS OF PHOSPHOLIPID AND FACTOR $VIII_a$.

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The kinetics of factor X activation by activated clotting factor IX_a was measured either in the presence or absence of phospholipid, Ca-ions and factor $VIII_a$. This study was carried out with purified bovine clotting factors. The rate of factor X_a formation was measured using the chromogenic substrate S 2222. Both the K_m for factor X and the V_{max} of factor X_a formation were determined for different compositions of the factor X activating complex in order to get more insight in the role of phospholipid, Ca^{2+} and factor $VIII_a$ in factor X activation. With factor IX_a alone, the activation of factor X is a very inefficient process. The presence of phospholipids predominantly lowers the K_m for factor X while in contrast factor $VIII_a$ mainly increases the V_{max} of factor X_a formation. The implications of these findings for the mechanism of factor X activation in the intrinsic pathway will be discussed.

14.15

0393 KINETICS OF THE INHIBITORY EFFECT OF FACTOR IX_{Bm} IN THE ACTIVATION OF FACTOR X BY FACTOR VII AND BOVINE TISSUE FACTOR.

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$F.IX_{Bm}$ is giving a prolonged prothrombin time with bovine tissue factor (T.F.). In the present study the inhibitory effect of purified $F.IX_{Bm}$ was tested in a F.VII assay that measures the ability of F.VII to activate F.X in the presence of bovine T.F. and Ca^{++}, followed by the quantitation of formed F.Xa by the synthetic substrate benzoyl -L-Ile-Glu-Gly-L-Arg-p-nitroanilide. Experiments were carried out with different concentrations of $F.IX_{Bm}$ and F.X, keeping the concentrations of F.VII and bovine T.F. constant. Lineweaver-Burk plot of the data showed that all the curves crossed the 1/V axis in one point - no inhibition by $F.IX_{Bm}$ at infinite concentrations of F.X. Thus, $F.IX_{Bm}$ acts as competitive inhibitor to F.X in the activation of F.X by F.VII and bovine T.F. By plotting the 1/V against the inhibitor concentrations ($F.IX_{Bm}$ conc), a Dixon plot, the K_i for $F.IX_{Bm}$ was determined to be 0.017 µM.
Normal F.IX was also found to be a competitive inhibitor to F.X but 4-5 times higher concentrations of F.IX were needed to give the same inhibition as $F.IX_{Bm}$. Utilizing human T.F. instead of bovine T.F. in this assay resulted in no significant inhibition by either F.IX of $F.IX_{Bm}$.