FACTOR X ACTIVATION IN SODIUM CITRATE. D.L. Aronson, Bureau of Biologics, Bethesda, Md. U.S.A. Early studies showed that partially purified prothrombin preparations generated thrombin in solutions of $(\text{NH}_4)_2\text{SO}_4$ or sodium citrate. Milstone established that the prothrombin activating enzyme Thrombokinase (X_a) would activate in $(\text{NH}_4)_2\text{SO}_4$. More recent studies of the physiologic activation of Factor X have defined a role for both the intrinsic and extrinsic independent of Factor VII or IX and results from the direct action of either thrombin or X_a on Factor X.

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When Factor X is incubated in sodium citrate there is a lag phase followed by the rapid generation of Factor X_a . This is followed by the degradation of X_a to a form which cannot interact with the cofactors of prothrombin conversion. If DFP is added during the phase of rapid X generation the reaction is inhibited. The addition of DFP prior to the activation has no effect. SBTI totally inhibits the activation of Factor X. Removal of residual Factor VII from the Factor X preparations prevents citrate activation and readdition of Factor VII restores this capability. Preincubation of the Factor VII in citrate does not effect the lag phase and the addition of Factor X_a to VII deficient Factor X has minimal effect.

It is proposed that citrate activation of Factor X is Factor VII dependent but that the Factor VII must be activated by Factor X_a (or possibly thrombin). The inability of the degraded form of X_a to react with the cofactors may be due to the loss of the γ -carboxy glutamate containing portions of the molecule.

THE ROLE OF γ -COOH GLUTAMIC ACIDS IN THE ENZYMIC FUNCTIONS OF FACTOR X. Lindhout, M.J., Kop-Klaassen, B.H.M., Hemker, H.C. Department of Biochemistry, State University Limburg, Maastricht, The Netherlands.

In order to study the function of γ -carboxyglutamic acid residues (gla) in factor X (X_a) we compared the properties of factor X (X_a) and abnormal factor X (X_a) from anticoagulated cows (PIVKA-X_a). We studied Ca^{++} and phospholipid binding, spectral changes upon Ca^{++} -binding and the activation by a specific fraction from Russell's Viper venom (RVV). In factor X but not in PIVKA-X a cooperative binding of about 5 Ca^{++} ions as well as a cooperative effect of Ca^{++} ions in spectral shift was observed. PIVKA-X can be converted into an active esterase by RVV. This reaction is Ca^{++} dependent. The Hill coefficient of this dependence is 2.8 for factor X and 1.0 for PIVKA-X. Factor X_a binds to micelles of an equimolar mixture of (dioleoyl) phosphatidylserine and dioleoyl phosphatidylcholine in the presence of Ca^{++} ions. Consistent with the inability of PIVKA-X to bind Ca^{++} ions cooperatively, PIVKA-X does not bind to phospholipid vesicles. Kinetic experiments on the activity of factor X_a and PIVKA-X_a, in the absence of Ca^{++} ions and phospholipids towards amides shows that both enzymes have identical K_m values for each of the substrates but different k_{cat} values. The results indicate a positive effect of gla residues on the rate of hydrolysis of amides. These studies suggest that the gla residues not only function to anchor factor X (X_a) on phospholipid via Ca^{++} ions, but that they also assist in obtaining conformational changes in the protein molecule as distant as to influence the active site. Indeed even the "anchor function" may depend upon such changes.

A BYPASS MECHANISM FOR ACTIVATING HUMAN FACTOR IX WITH HUMAN FACTOR VII AND THROMBOPLASTIN. B. Østerud and S.I. Rapaport. University of California and V.A. Hospital, San Diego, California, U.S.A.

The data reported herein were obtained in a search for mechanisms triggering intrinsic clotting which bypass the contact activation factors. An assay system was designed which permitted us to examine the effect of thromboplastin and Factor VII (F.VII) upon F.IX under conditions which produced minimal direct effect of thromboplastin and F.VII on F.X. Purified F.IX was incubated with purified F.VII, brain thromboplastin and Ca ions. Then, a mixture of purified bovine F.VIII (activated with thrombin), phospholipid, Ca ions and F.X was added. The reaction was stopped at 3 min with EDTA, and a subsample assayed for F.X_a with the chromogenic substrate S-2222. When 10 μl of F.IX (15.4 U/ml) was incubated with 10 μl of F.VII (0.2 U/ml), 5 μl brain thromboplastin, and 3 μl 50 mM CaCl_2 for 10 min at 37°, F.IX_a was formed as shown by generation of 0.830 U/ml of F.X_a. When 10 μl of F.XI_a (0.24 U/ml) was substituted for the F.VII and thromboplastin, 1.040 U/ml of F.X_a was generated. Both the thromboplastin-F.VII generation of F.IX_a and the F.XI_a generation of F.IX_a was time-dependent. Only minimal F.X_a was generated in this assay system when the first incubation mixture contained thromboplastin and F.VII without F.IX (0.088 U/ml), thromboplastin and F.IX without F.VII (0.002 U/ml), or F.VII and F.IX without thromboplastin (0.000 U/ml). Only minimal F.X_a was generated when F.VIII was left out of the second incubation mixture. These data establish that thromboplastin and F.VII can activate F.IX in a reaction bypassing F.XI_a.