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Blood-clotting enzymology. The activation of factor X by factor IXa; the effect of phospholipid and of antihaemophilia factor VIII (F VIII) on the activation reaction.

The activation of bovine clotting factor X is mediated by the enzyme factor IXa. Activation of factor X, i.e. the formation of the serine-protease Xa, is the result of the specific cleavage of a peptide bond in factor X. The rate of activation is strongly enhanced by the cofactor VIII, and by a phospholipid (PL) surface, onto which the protein adsorbs and where the reaction is thought to occur.

In order to get more insight into the mechanism of the activation reaction and the role of PL, Ca²⁺ and factor VIII, we have determined the kinetic constants of factor X activation and the effects of PL and factor VIII on these constants. Without PL and factor VIII, Kₘ for factor X is 272 ± 2, and V is 6 mmol Xₐ mol⁻¹ IXₐ min⁻¹. With PL (10 µM) the Kₘ is lowered to 0.06 µM and V is unchanged. In the presence of PL and factor VIII, Kₘ is 0.02 µM and V is 111 mol Xₐ mol⁻¹ IXₐ min⁻¹. The factor X concentration in plasma is 0.16 pM, so we conclude that PL in plasma serves to lower the K to 0.06 µM, and that factor VIII stimulates factor X activation because it increases V of Xₐ formation at least 10 000 times.

We further investigated the effect of PL concentration on the kinetic parameters with factor VIII left out. V for factor Xₐ formation does not change very much, but Kₘ for factor X increases from 0.06 µM at 10 µM PL to 1.35 µM at 260 µM PL. Earlier Rosing et al. (1980), studying the prothrombinase complex, found a similar relationship between PL concentration and the Kₘ for prothrombin.

This led to the hypothesis that it is the local substrate concentration at the phospholipid surface that determine the kinetics of its activation. An increased local concentration explains the dramatic decrease of Kₘ in the presence of PL. Further addition of PL lowers the substrate density at the PL surface with as consequence an increase of Kₘ.

The validity of this hypothesis can be tested if the binding parameters of substrate to PL are known. Recently, we found a new method to determine parameters of factor X binding to PL. This method is based on the observation that RVV-X (i.e., the factor-X-activating enzyme from Russell's viper venom) activates free factor X, but not factor X bound to PL. We found that PL vesicles consisting of equal amounts of phosphatidyserine and phosphatidylcholine (also used in our kinetic experiments) bind factor X with a dissociation constant (Kₐ) of 3.7 x 10⁻⁸ M. The concentration of factor-X-binding sites on these vesicles is 0.7 µM sites per 100 µM PL.

These data were used to convert Kₐ values from the kinetic experiments to Kₘ values expressed as factor X bound per PL surface area. The difference in Kₘ disap-
peared and one $K_m$ value was found of $0.010 \pm 0.003 \mu M$ factor X/μM PL for all PL concentrations. These results further support our hypothesis that the kinetics for X activation are governed by the density of the reactants bound to the PL surface.

Reference