ISOLATION AND PROPERTIES OF THE ABNORMAL FACTOR IX MOLECULE OF HEMOPHILIA B. B. Astrup, K. Levinson, D. E. Rasker, and J. Rapport. University of California, and V.A. Hospital, San Diego, California, and Orthopedic Hospital, Los Angeles, California, U.S.A.

Abnormal factor IX from a hemophilia B patient (F.IX-B) has been isolated and purified from cryoprecipitate by a two-stage affinity chromatography. The purified F.IX-B was found to have no measurable procoagulant activity when incubated with F.IXa in a two-stage F. IX assay (normal F. IX, 65 sec; F.IX-B, >30 min). F.IX-B inhibited the activation of F.X by F.VII and activated thromboplastin as measured in an antithrombic assay for factor X.

Normal F.IX also inhibited this reaction but to a five times lesser degree. F.IX-B has the same molecular weight and SDS gel electrophoresis as normal F.IX (25,000) and does not differ from normal F.IX in its amino acid composition. F.IX cleaves F. IX-B in the presence of Ca ions at the same site as it cleaves normal F. IX, yielding a heavy chain of 27,000 molecular weight and a light chain of 16,000 molecular weight. However, the cleavage does not give rise to procoagulant activity. Like normal F.IX, the cleaved forms of F.IX-B appear to bind phospholipid since F.IX-B protein was precipitated with phospholipid in the presence of Ca ions. These data support an hypothesis that the abnormality in the F.IX-B molecule stems from a defect at the active site.


We have shown that the pentapeptide Pro-Leu-Glu-Glu-Val serves as a substrate for a vitamin K-dependent liver microsomal carboxylase that converts peptide-bond-glutamyl residues to y-carboxyglutamyl residues. Requirements of the carboxylated microsomal system for peptide carboxylation have been determined and are similar to requirements for prothrombin precursor carboxylation. Additional peptides have now been synthesized, and the available data suggest that specificity of the vitamin K-dependent carboxylase involves both macromolecular and amino sequence factors. Inhibition of the in vitro carboxylation system by the anticoagulant Warfarin has now been studied in detail. Dithiothreitol stimulates carboxylation activity and replaces the requirement for NADH as a reductant to form the hydroquinone. When driven by carboxylation reaction in intact microsomes, but with a much poorer inhibitor when NADH is used as a reductant. In a microsomal system with DTT (but not NADH) as a reductant, vitamin K epoxide is an active form of the vitamin. In a Triton X-100 solubilized system, NADH is required, Warfarin is ineffective as an inhibitor, and the epoxide is not an active form of the vitamin, and the conversion of vitamin K epoxide to the vitamin cannot be demonstrated. These data suggest that vitamin K activity through its action on vitamin K epoxide and suggests that the vitamin K enzyme is driven by a nonglycine nucleotide pathway which, in vitro, can use DTT instead of an unidentified physiologically active reducing agent.


Bennett and Rastoff [1977] reported that in haemophilia the coagulant activity of Factor VIII (F VIII:C) disappeared faster than the factor VIII-related antigen (F VIII:R:Ag). We decided to investigate this phenomenon with radio-labelled factor VIII. Using this technique, we allowed us to study the behaviour of various factor VIII fractions after infusion. Human factor VIII was purified from cryoprecipitate, labelled with 125 I by the lactoperoxidase-glucose oxidase method, mixed with a human albumin solution and sterilized by filtration through a Millipore filter. F VIII:C, F VIII:R:Ag, F VIII:R:Ba, and a cross-reaction with Factor VIII purified by PAGE. Among 14 patients, 13 were free of radiolabel in 125 I was present, yield and survival were studied in 6 normal volunteers. The yield was somewhat lower (7%) than expected after turnover. The survival curve was biphasic showing half lives of 24 hours and 40 hours. In 6 haemophiliacs the yield and disappearance were essentially similar. The F VIII:C in these patients who also received cryoprecipitate of 20 donors disappeared with the same kinetics as the radiolabel. Evidence was obtained indicating that the highest molecular weight forms of Factor VIII disappeared more rapidly, while losing the F VIII:R:Ag, than the lower molecular weight forms. Radiolabel in cryoprecipitate showed a biphasic, more rapid disappearance; label in cryoprecipitate plasma disappeared slower.