ISOLATION AND PROPERTIES OF THE ABNORMAL FACTOR IX MOLECULE OF HEMOPHILIA B. B. Asterud, K. Lavrin, C. E. Rudder, and L. 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 to homogeneity on the ultracentrifuge gel electrophoresis by the same technique utilized for purifying F.IX-A. F.IX-B, generated no measurable procoagulant activity when incubated with F.IX-A in a two-stage F.IX assay (normal F.IX: 56 sec; F.IX-B, >30 min). F.IX-B inhibited the activation of F.IX by F.VII and ex blood thromboplastin as measured in an antihemolytic assay for factor Xa. Normal F.IX also inhibited this reaction but to a five times lesser degree. F.IX-B had the same molecular weight on SDS gel electrophoresis as normal F.IX (55,000) and does not differ from normal F.IX in its amino acid composition. F.IX-B cleaves F.IX in the presence of Ca ions at the same rate 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-bound glutamyl residues to γ-carboxyglutamyl residues. Requirements of the solubilized 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 peptide sequence recognition. Inhibition of the in vitro carboxylation system by the anticoagulant Warfarin has now been studied in detail. Ethylmalonate stimulates carboxylase activity and replaces the requirement for NADH as a reductant needed to form the hydroquinone. DTT driven carboxylation reaction in intact microsomes, but 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, 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 support the hypothesis that Warfarin inhibits vitamin K activity through an effect on epoxide reduction and suggests that this reaction is driven by a non-pyridine nucleotide pathway which, in vivo, can use DTT instead of an unidentified physiologically active reducing agent.


Bennett and Batoff (1977) reported that in hemophilliae the coagulant activity of Factor VIII (F.VIII:C) disappeared faster than the factor VIII-related antigen (F.VIII:R:A). We decided to investigate this phenomenon with radiolabelled factor VIII. This also allowed us to study the behaviour of various factor VIII fractions after infusion. Human factor VIII was purified from cryo precipitate, 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:A, F.VIII:Ag, F.VIII:Hep assay, gel isoelectric focusing, crossed immunoelectrophoresis and PAGE studies were carried out. No significant changes due to the procedure were observed. The radiolabel in the plasma after in vivo administration was for more than 98% bound to F.VIII:C as demonstrated with immunoeabsorption with an antiserum prepared against a subunit of Factor VIII purified by PAGE. About 1.5-6% of free 125 I was also present. Yield and survival were studied in 6 normal volunteers. The yield was somewhat lower (7%) than expected after transfusion. The survival curve was biphasic showing half lives of 48 hours and 20 hours. In 6 hemophilliae 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:A, than the lower molecular weight forms. Radiolabel in cryo precipitate showed a bimodal, more rapid disappearance; label in cryoprecipitate plasma disappeared slower.