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The activated form of factor VIII (FVIIIa) acts as cofactor for the serine protease factor IXa (FIXa) in the Ca^{2+} -dependent conversion of the zymogen factor X (FX) to its active form FXa. FVIIIa increases the rate of cleavage of FX by FIXa by several orders of magnitude, dramatically increasing the catalytic rate constant (k_{cat}) of FIXa.

Insights into basic aspects of FVIII function can be gained by biochemical and biophysical study of interactions of mutated FVIII with components of the intrinsic FXase complex. Numerous missense mutations have been described as causing hemophilia A, however there are few studies in which the biochemical effects of the mutations have been examined.

Aim 1: Effects of hemophilia A-associated mutations in the A3 domain on FIXa binding. FVIIIa interacts with factor IXa (FIXa) via the A2 and A3-C1-C2 subunits. The A3 domain of the A3-C1-C2 subunit is considered to contain the high affinity-

Determination of the effect of hemophilia A mutations in the A3 and C1 domains of factor VIII

binding site of FVIIIa with FIXa. Initially, the study will focus on the effects of hemophilia A-associated mutations in the putative FIXa binding region contained within residues 1778-1840 of the FVIII A3 domain. There are nine different mutations associated with moderate to mild hemophilia A contained within this region as described by the hemophilia mutation and structure database (<http://europium.csc.mrc.ac.uk>). The effects of the mutations on FVIIIa-FIXa affinity will be assessed and kinetic rate constants determined. We predict some of the mutations will alter affinity of FVIIIa for FIXa, however the mutations may also have an independent effect on the catalytic efficiency of the FXase complex by alteration of other aspects of FVIIIa function. This will be determined by our investigations.

Aim 2: Effects of mutations on the surface of the C1 domain on FVIII(a) interactions. The role of the C1 domain in FVIII(a) function has not been well defined. By determining the effect of specific clusters of hemophilia A-associated mutations and targeted residue to Ala on the surface of the C1 domain, we hope to identify ligand interactions between the C1 domain and components of the FXase complex and also examine the interaction between FVIII mutant forms and von Willebrand factor.

Plan of Investigation: FVIII incorporating either hemophilia A-associated mutations or targeted residue-to-Ala mutations, will be stably expressed in BHK cells using a B-domainless FVIII expression vector and purified from

conditioned media by ion-exchange chromatography. Investigations on the highly purified FVIII will include clotting assays, and assessment of the FXase complex by FXa generation in a purified component system, allowing determination of kinetic rate and affinity constants. Biophysical determinations will also be performed such as surface plasmon resonance allowing direct measurement of inter-protein interaction. Additionally the effects of the mutations on thrombin generation will be assessed by determination of the endogenous thrombin potential.

The studies outlined will contribute both to the basic understanding of FVIII function and pathology of hemophilia A.

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