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A novel one-step purification of mouse factor IX

Vijaya S. Pilli¹,

UNC School of Medicine, Department of Biochemistry, Chapel Hill, NC, United States; LSU Health Science Center, Department of Biochemistry and Molecular Biology, New Orleans, LA-70112, United States

William E. Plautz¹,

UNC School of Medicine, Department of Biochemistry, Chapel Hill, NC, United States; LSU Health Science Center, Department of Biochemistry and Molecular Biology, New Orleans, LA-70112, United States

Dougald M. Monroe III, and

UNC School of Medicine, Department of Biochemistry, Chapel Hill, NC, United States

Rinku Majumder

UNC School of Medicine, Department of Biochemistry, Chapel Hill, NC, United States; LSU Health Science Center, Department of Biochemistry and Molecular Biology, New Orleans, LA-70112, United States

Dear Editors

Factor IX (FIX) is a vitamin K-dependent plasma protein that functions as a key regulator in hemostasis and blood clotting. Mutations within FIX can cause potentially fatal conditions such as hemophilia B and venous thromboembolism [1,2]. The development of new treatments for FIX-associated diseases requires technology that enables FIX to be easily purified, in order to study the protein's diverse set of interactions. Importantly, the technology should be applicable to the isolation of FIX from non-human sources, so that potential new therapies could be tested in animals. To that end, we investigated the applicability of the *GE IXSelect* Column, designed specifically for affinity purification of human FIX, in its ability to purify mouse FIX from mouse plasma. With this column, we isolated 80 µg of mouse FIX from 100 mL of mouse plasma. This result demonstrates not only a novel use for *IXSelect* in the purification of a FIX homolog, but also suggests the existence of a structure conserved between mouse and human FIX that interacts with the *IXSelect* binding ligand.

Corresponding author at: LSU Health Science Center, Department of Biochemistry and Molecular Biology, New Orleans, LA-70112, United States. rmajum@lsuhsc.edu.

¹Both authors contributed equally to this work.

Authorship: V. Pilli and W. Plautz contributed equally to the study.

V. Pilli performed the purification. V. Pilli, W. Plautz, D. Monroe, and R. Majumder analyzed the data. W. Plautz wrote the manuscript. R. Majumder conceived of the idea for the project and revised the final manuscript.

Conflict of interest: None.

100 mL of frozen Mouse Plasma (Innovative Research Innovative grade US Origin, CD-1 mouse Plasma Na Citrate-18090) was thawed at 37 °C. A *GE IXSelect* column was equilibrated with 6 column volumes (CV) of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4. The thawed plasma was then loaded by a peristaltic pump at 1 mL/min into a *IXSelect* column at RT. Following loading, the column was washed, first with 10 CV of the equilibration buffer, then with 10 CV of 20mMTris-HCl, 500 mM NaCl, 0.01% Tween™80, pH of 7.4, and finally the column was washed with 3 CV of the equilibration buffer. Mouse FIX was eluted with a buffer containing 20mMTris-HCl and 2.0 M MgCl₂, pH of 7.4. The column could be regenerated by washing with 0.1 M glycine containing 0.1 M NaCl at a pH of 2.0. The eluted fractions were analyzed for protein concentration and dialyzed at 4 °C against 5 L of 20 mM Tris-HCl, 150 mM NaCl, pH of 7.4. Total mouse FIX yield was 80 µg.

This procedure was repeated with a human plasma control. From 100 mL of human plasma, 200 µg of FIX was recovered, suggesting that the *IXSelect* ligand binding site has a weaker affinity for the mouse homolog.

Gel analysis confirmed the purification of mouse FIX; the eluted fractions exhibited a band at approximately 72kD (Fig. 1A). Furthermore, a western blot of the fractions confirmed that this band was mouse FIX (Fig. 1B); similar results were observed in the purification of human FIX from human plasma (Fig. 1C, D).

The activity of the purified mouse FIX was assessed by means of a modified aPTT assay [3], which utilizes FIX-deficient plasma. With the addition of 90 nM purified mouse FIX, the clotting time decreased from 90 s to 37.5±4 s; with the addition of 90 nM human FIX, a clotting time of 32.5 ± 5.3 s was measured. These results demonstrated that the isolated proteins were functional.

In conclusion, it is intriguing that active mouse FIX, which shares a mere 80% amino acid sequence identity with its human homolog, could be isolated from mouse plasma in a single-step purification, by utilizing the highly specific human *IXSelect* column. We have followed the same protocol here that we have used in making FIX from cell lysates without any modification. Because mouse proteins are crucial for *in vivo* experiments, the ability to isolate mouse FIX, quickly and easily, is an invaluable advancement for future studies.

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Fig. 1. Purification of FIX from plasma. A) Ponceau S- [4] stained blot of mouse FIX purification. B) Immunoblot probed for mouse FIX with anti-mouse FIX antibody. C) Ponceau S-stained blot of human FIX purification. D) Immunoblot probed for FIX with anti-human FIX antibody. Lane 1: Plasma, Lane 2: Flow through, Lane 3: Wash1, Lane 4: Wash 2, Lane 5: Elute.