

Purification of Factor VIII From Cryoprecipitation Using MacroCap™ Q Column

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Abstract. This article reported a method to prepare high quality of factor VIII using MacroCap™ Q. Results indicated that the specific activity of the FVIII eluate was varying between 35 and 80 IU/mg of protein. This means that this procedure could provide at least 95- to 216-fold purer FVIII compared with the original cryoprecipitation with a recovery rate approximately 60.5 %. The procedure is easily applicable to large-scale production of FVIII with acceptable recovery.

Introduction

The most common inherited bleeding disorders are associated with deficiencies or defects in factor VIII (FVIII) or von Willebrand protein. Bleeding occurs due to defective synthesis of coagulation FVIII in patients with “Haemophilia A” and FVIII rich source prepared by cryoprecipitation of fresh frozen plasma is used to replace FVIII to control bleeding. Since the late 1960s, patients suffering from hemophilia A have been treated by administration of plasma concentrates [1].

Many methods have been proposed for the purification of FVIII from human plasma or cryoprecipitation. The purification achieved is approximately 350- to 800-fold compared with the plasma from which the fractions are separated by precipitation with concanavalin A [2]. The purification of FVIII, averaged 67 units/mg of protein, prepared from chylomicron-poor human plasma is 6300-fold compared to the plasma from which they are separated by utilizing agarose gel filtration [3]. In the presence of high concentration of salt FVIII could be separated into two components. A high-molecular-weight (HMW) component excluded by the gel contained the bulk of protein, but possesses little or no procoagulant activity. Another,

low-molecular-weight (LMW) component retarded by the gel and possesses the bulk of procoagulant activity, but is relatively poor in proteins [4]. Controlled pore glass (500 Å pore diameter) [5], compact porous tubes [6] as well as size-exclusion columns [7] are used for large-scale purification of FVIII. In latest studies, anion exchange chromatography is found to be good at large-scale purification of FVIII, and the specific activity is greater than 300 IU/mg protein.

Materials and Methods

Materials

MacroCapTM Q gel was purchased from GE healthcare Bio-Sciences AB (Uppsala, Sweden). The cryoprecipitation was the product of Shandong Taibang Biologic Products, Inc. (Shandong, China). Deficient plasma for the assay of factor VIII:C with a clotting assay was from Hyphen BioMed. (Neuville Sur Oise, France). Bicinchoninic acid (BCA) protein quantitative kits were the products of Solarbio (Beijing, China). All reagents used in this study were of analytical grade.

Preparation of Cryoprecipitation

10.0 g cryoprecipitation was dissolved in 30 mL of buffer A (25 mM citrate, 5 mM CaCl₂, 8.5 mM NaCl, 0.1 M glycine, and 2 IU/mL heparin sodium, pH 7.0) at 4 °C, and centrifuged at 2000 × g for 15 min. The freshly prepared FVIII containing supernatant was kept at 4 °C before purification.

Purification of FVIII

The chromatographic steps were performed on a MacroCapTM Q column (GE healthcare, Sweden, 26 × 300 mm) coupled to an ÄKTA FPLC system. This separation was similar to our previous method for isolation of polysaccharide [8,9]. For linear washing: the column was pre-equilibrated with buffer A, and then 5.0 mL FVIII containing supernatant was loaded onto the column; the flow rate was 5.0 mL/min with the following elution gradient, 2.0 CV of buffer A, 2.0 CV of 0 % -100 % buffer B (25 mM citrate, 5 mM CaCl₂, 1 M NaCl, 0.1 M glycine, and 2 IU/mL heparin sodium, pH 7.0), 2.0 CV of buffer B, and then re-equilibrated with 2.0 CV of buffer A. For step washing: the column was pre-equilibrated with buffer A, and then 15.0 mL FVIII containing supernatant was loaded onto the column; the flow rate was 5.0 mL/min with the following elution gradient, 2.0 CV of buffer A, 2.0 CV of 25 % buffer B, 2.0 CV of buffer B, and then re-equilibrated with 2.0 CV of buffer A. The eluent was monitored by absorbance at 280 nm by UV monitor (Pharmacia, Sweden) and collected using fraction collector 5.0 mL/tub. Column CIP was performed as following: 2.0 CV of 2.0 M NaCl, 10.0 CV of distilled water, 2.0 CV of 1.0 M NaOH and 20.0 CV of distilled water.

FVIII Activity Assay

The FVIII procoagulant activity of each fraction was measured by a one-stage activated

thromboplastin time test, using commercial FVIII-deficient human plasma as a substrate. This experiment was carried out according to the manufacturer's instructions.

Protein Determination

Protein concentration was determined spectrophotometrically according to the Bradford methods using BCA protein assay kit. Bovine serum albumin was used as standard.

Results and Discussion

The elution profile of procoagulant protein, with the conditions described above, is shown in Fig 1A and B. The largest part of proteins, such as γ -globulins, fibrinogen, fibronectin, von Willebrand factor and other contaminants are eliminated during washing with buffer A. FVIII containing fractions were obtained with approximately 25 % buffer B (Fig 1 A and B), the specific activity of FVIII were about 9.0 IU/mL in the peak fractions. The specific activity of the FVIII eluate was varying between 35 and 80 IU/mg of protein. The wide range of variability can be ascribed in part to the quality of the raw material, and the sensibility of the analysis methods, both biological activity and protein determination. The specific activity of the cryoprecipitation before purification was approximately 0.37 IU/mg protein. Therefore, this procedure could provide at least 95- to 216-fold purer FVIII compared with the original cryoprecipitation with a recovery rate approximately 60.5 %.

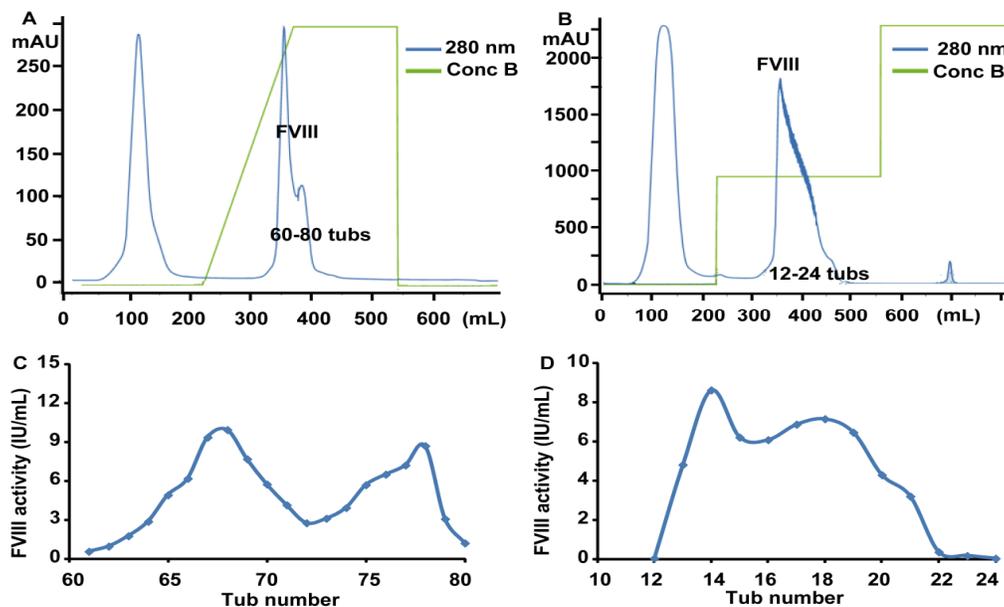


Figure 1. Anion exchange chromatography and FVIII activity assay. A, linear elution curve for FVIII; B, step-wise elution curve for FVIII; C, FVIII activity determination of the linear elution peak containing FVIII; D, FVIII activity assay of the step-wise elution peak containing FVIII.

This method reported yields a high quality FVIII using MacroCapTM Q. The procedure is

easily applicable to large-scale production of FVIII with acceptable recovery. Additionally, the high specific activity obtained should be ascribed mainly to the reduction of high molecular weight proteins, such as fibrinogen, fibronectin and immunoglobulins. The results of this study were consistent with several domestic reports, but the specific activity was lower than the previous publications [6,7].

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