Purification of Factor VIII From Plasma by Q Sepharose™
Fast Flow Column

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Abstract. This article reported a method to prepare high quality of factor VIII by Q Sepharose™ Fast Flow. Results indicated that the specific activity of the FVIII obtained was varying between 55 and 103 IU/mg of protein. This means that this procedure could provide at least 458- to 858-fold purer FVIII compared with the original plasma with a recovery rate approximately 71.2 %. 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 [1,2].

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 [3]. 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 [4]. 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 [5]. Controlled pore glass (500 Å pore diameter) [6], compact porous tubes [7] as well as size-exclusion columns [8] 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. Additionally, the addition of synthetic amino acids as a stabilizer leads to an increase of yield and improves solubility of the freeze-dried FVIII [9].

Materials and Methods

Materials

Q Sepharose™ Fast Flow gel was bought from GE healthcare Bio-Sciences AB (Uppsala, Sweden). B type Plasma (number: 370415078911) was the product of Xiajin Antai Blood Plasma Collection Co., LTD. (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

The citrate phosphate dextrose plasma was thawed at 4 °C, and centrifuged at 2000 × g for 15 min. The freshly prepared supernatant and cryoprecipitation was kept at 4 °C before purification. The supernatant was used for purification of FVIII in this study.

Purification of FVIII

The chromatographic steps were performed on a Q Sepharose™ Fast Flow 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 [10,11]. For linear washing: the column was pre-equilibrated with buffer A (20 mM Tris, 5 mM CaCl$_2$, 8.5 mM NaCl, 0.1 M glycine, and 2 IU/mL heparin sodium, pH 7.0), 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 (20 mM Tris, 5 mM CaCl$_2$, 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. 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. Most of the unwanted 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 1B), the specific
activity of FVIII were about 15.0 IU/mL in the peak fractions. The specific activity of the FVIII eluate was varying between 55 and 103 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 supernatant plasma before purification was approximately 0.12 IU/mg protein, that is to say cryoprecipitation kept most of the FVIII. Therefore, this procedure could provide at least 458- to 858-fold purer FVIII compared with the original cryoprecipitation with a recovery rate approximately 71.2%.

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

This method reported yields of a high quality FVIII using Q Sepharose™ Fast Flow. The procedure is easily applicable to large-scale production of FVIII with high 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 [7,12].

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References


