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COST EFFECTIVE METAL AFFINITY CHROMATOGRAPHY FOR PROTEIN C SPECIFIC, MINI-ANTIBODY PURIFICATION

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1. INTRODUCTION

Protein C (PC) is a vitamin K dependent (VKD) anticoagulant, antithrombotic, and anti-inflammatory protein in blood plasma[1]. PC deficiency causes abnormal blood clot formation that hinders oxygen transport to vitals organs. Therefore, inexpensive PC supply is important for patients with various thromboembolic disorders, including the PC deficient patients. All VKD proteins are synthesized in the liver. Molecular weights and structures of some VKD proteins are very similar to PC but most of them are clotting factors[1]. Therefore, only highly specific purification methods such as immuno-affinity chromatography can separate PC from these homologues. Since monoclonal antibodies (Mabs) used in affinity chromatography are expensive to produce, recombinant *E. coli* strains producing single chain variable fragments (ScFv, mini-Mabs) against PC has been developed[2]. Currently, the mini-Mab production level using the protocol developed by our research group is approximately 450 mg/L per day.

Because the purification yield for our mini-Mab using protein A was relatively low[3]and protein A is rather costly, immobilized metal affinity chromatography (IMAC) was investigated as an alternative to protein A chromatography. Histidine in the protein is a common target amino acid for IMAC[4, 5].

This paper reports the effect of pH on the adsorption of the mini-Mabs against PC, for the IMAC purification using Cu^{+2} and IDA chelator combination. Utilizing the purified mini-Mab, PC purification performances of four commercially available matrices were investigated. After the selection of the best performing matrix, purification of PC from the mixture of PC and four PC homologues, factors II, VII, IX, and X, was also performed.

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2. MATERIALS AND METHODS

2.1. Mini-Antibody Purification Using IMAC

Materials and methods for the *E. coli* growth, the mini-Mab production, and its purification using Protein A (Sigma, St. Louis, MO) are as described by Korah, et al[3]. IMAC purification of the mini-Mab was done using HiTrap™ Chelating Sepharose (Amersham Biosciences; Piscataway, NJ), following the manufacturer's instructions. For this feasibility study, pure PC mini-Mab was loaded to the column (binding capacity, 12 mg of histidine tagged proteins) and the non-adsorbed material was washed with the equilibrium/washing (E/W) buffer (0.02 M phosphate buffer). Five column volumes (CV) of 0.5 M imidazole in 0.02 M phosphate buffer was used to elute the bound protein. Five tenths (0.5) M NaCl was added to all buffers to minimize non-specific electrostatic binding. In the experiment to study the efficacy of a lower concentration of imidazole for removing the weakly retained impurities, 5 mM imidazole was employed in E/W buffer. After the purification process, Cu^{+2} was stripped off the resin using 50 mM EDTA (5 CV) and then the resin was washed with DI water (5 CV). The mini-Mab quantification was performed by the ELISA, using anti-c-myc antibody, 9E10[3] (Santa Cruz Biotechnology; Santa Cruz, CA).

2.2. PC Purification

The four affinity chromatography resins tested for the PC purification performance are CNBr-activated Sepharose™ 4B (CNBr; Amersham Biosciences), NHS-activated Sepharose™ 4 Fast Flow (NHS; Amersham Biosciences), Actigel ALD™ (Actigel; Sterogene; Carlsbad, CA), and Epoxy Activated Ultraflow 4™ (Epoxy; Sterogene). PC was provided by the American Red Cross (Rockville, MD) and factors II, VII, IX, and X were purchased from Innovative Research, Inc. (Southfield, MI). Rabbit polyclonal factor II antibody was from Biomeda Corporation (Foster city, CA) and mouse monoclonal factor II antibody, from Enzyme Research Laboratories (South Bend, IN). Rabbit polyclonal factor VII antibody was obtained from Novus Biologicals, Inc. (Littleton, CO), and goat polyclonal factor X antibody, from US biological (Swampscott, MA). Rabbit polyclonal antibodies for PC and factor IX, mouse monoclonal antibodies against PC, and factors VII, IX, and X were purchased from Sigma (St. Louis, Mo).

For PC purification, purified PC mini-Mab was immobilized on the selected matrix. The PC purification was performed by the method described by Kang, et al[6]. The amounts of the mini-Mab and PC were quantified by ELISA.

3. RESULTS AND DISCUSSION

3.1. Mini-Antibody Purification Using IMAC

IMAC has two main components: a chelator and an immobilized metal ion. The chelator is linked to a matrix via a spacer arm by covalent bonds and the metal ion is immobilized to the chelator by coordinate bonds[7]. In IMAC, the binding force between the metal ion and the chelator, and the force between the protein to be purified and the metal ions are strongly dependent on pH[8]. With IDA chelator, the force between Cu^{+2}