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THERMODYNAMIC APPROACH TO OPTIMIZE IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY PURIFICATION OF PROTEIN C

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1. INTRODUCTION

Deficiency of protein C (PC) reduces the body's natural ability to prevent coagulation and thrombosis and increase the possibility of blood clot formation, preventing the transport of oxygen, nutrient, and metabolic byproduct to tissue. If large quantities of PC can be produced at a low cost, it could be used as a safer alternative to coumadin and heparin for treatment, which can result in bleeding and skin necrosis from long-term use. Currently, PC is purified using immunoaffinity chromatography (IAC), which is a very expensive process, due to the difficulty of separating PC among other homologous vitamin K-dependent (VKD) proteins by other method. However, the relative high specificity of Immobilized Metal Affinity Chromatography (IMAC) has shown a high potential of separating PC from the PC homologues at a lower cost.[1, 2]

The aim of this study is to optimize IMAC/PC purification process for high yield, purity, and bioactivity by utilizing thermodynamic principles involved in the adsorption and elution processes of PC and other VKD proteins in IMAC. Thermodynamic parameters, such as, adsorption equilibrium constant (K_a) and the Gibb's free energy (ΔG_r^o) of the adsorption/elution are being analyzed to study how various chromatographic operating conditions affect PC purification. This paper presents the scheme to obtain K_a and ΔG_r^o of the IMAC process for purifying PC.

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2. BACKGROUND

2.1. Protein C

Protein C has been chosen as the model molecule for this study for its importance as a therapeutic protein, our long-term interest, and availability of PC. PC is an anticoagulant, antithrombotic, and anti-inflammatory,[3, 4] and is produced in the liver.[5, 6] The current clinical method for treating PC deficiency is by coumadin and heparin. However, long-term applications of these drugs may cause complications, such as, bleeding or skin necrosis. PC has promising clinical potential for patients diagnosed with genetic deficiency of PC, septic shock, coumadin-induced necrosis, and heparin-induced thrombocytopenia, pregnant women who are PC deficient, patients undergoing hip and knee replacement, and patients undergoing angioplasty. PC has no known clinical side effects, and should be an ideal therapeutic for PC deficiency. PC is one of several VKD proteins that have a 60-71% homology in the amino acid sequence. These VKD proteins include coagulants, Factors II (prothrombin), VII, IX, and X.[5, 6] PC has fifteen surface histidine residues, which are the largest number, and therefore, possibly higher affinity in IMAC among VKD proteins.[7, 8]

2.2. IMAC for PC Purification

Purification of PC from human plasma is not possible by traditional ion exchange chromatography because of the many homologues in the plasma as previously stated. Immunoaffinity chromatography (IAC) yields a high affinity and specificity, but the high cost of monoclonal antibodies (MAbs) that are used as ligand for IAC is a significant disadvantage. The cost of MAb is approximately $1,200/mg, while the cost of Cu^{2+} used for IMAC is only $0.16/g. Therefore, it can be estimated that IMAC is roughly 100,000 times less expensive than IAC based on ligand cost. Results from our research group demonstrated the effectiveness of the IMAC with the combination of Cu^{2+} and iminodiacetic acid (IDA) for PC separation from prothrombin, a thrombotic VKD protein and known to be the most difficult to separate.[1] The PC purification by IMAC has also shown 83% PC recovery from inexpensive Cohn Fraction IV-1derived from blood plasma.[2]

In IMAC the adsorption affinity of PC and other homologous VKDs depends largely on the number of surface histidine residues.[7] It is also contributed by the surrounding hydrophobic residues which aid/hinder the protein histidine affinity to the metal ion.[8, 9] Therefore, the operational conditions of IMAC need to be designed with the consideration of these various binding forces for favoring PC adsorption.[8, 10] Some operation conditions influencing the binding forces include salt concentration, pH, buffer concentration, and temperature.[11, 12]

2.3. Thermodynamics of PC Adsorption

The energy required for protein adsorption are dependent on intra- and inter-molecular interactions between the protein molecules, the environment the proteins are in, and the site of protein adsorption. The equilibrium binding constant (K_a) and standard Gibb's free energy of a reaction (ΔG_r^o) are two thermodynamic parameters that could