523c Protein C Purification Using Pc Specific Mini-Mab from the Inexpensive Source, Cohn Blood Plasma Fraction VI-1

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Protein C (PC) is an important, vitamin K-dependent (VKD) component in the blood coagulation pathway. It is synthesized in liver and circulated in blood plasma as inactive form. In addition to its anticoagulation function, it acts as antithrombotic and anti-inflammatory agent in blood plasma. PC can be used as a therapeutic product to treat the hypercoagulable state or PC deficiency associated with patients undergoing surgery or trauma, sepsis patients, complications during pregnancy, and patients undergoing organ transplantation, severely burned patients. Therefore, inexpensive PC may be effectively used for the treatment of these patients with little side effect, unlike the currently used anti-coagulants (heparin and coumarin). Because of the similar structures of other VKD proteins in plasma, immunoaffinity chromatography, an expensive process, has been used for PC purification from blood plasma. To lower the cost of PC purification process, PC specific single chain variable fragments (mini-Mab) that can be produced by E. coli were developed.

As an economic method for separating PC mini-Mab from cell culture broth, metal affinity chromatography (IMAC) was investigated. Since the binding forces between the metal and the chelator and binding forces between the metal and the protein are strongly dependent on pH, an investigation of the optimum pH for selective adsorption of PC mini-Mab was performed. Cohn Fraction IV-1, a byproduct of human serum albumin fractionation from blood plasma, is a very inexpensive source with large amount of PC. PC purification from the Cohn Fraction IV-1 was investigated. The amounts of PC, human serum albumin, and other VKD PC homologues in the Cohn Fraction IV-1 were determined. Preliminary study of PC purification from the Cohn Fraction IV-1 using the mini-Mab showed approximately 15 % of PC purification yield. The process of PC purification from the Cohn Fraction IV-1 now is being optimized. The cost of PC purification using mini-Mab showed tens of times cheaper than that of PC purification using monoclonal antibody in the economic analysis of this process.

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