**Purification of recombinant human erythropoietin by multimodal and hydrophobic chromatography**

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**Highlights**

* Properties of Capto MMC and Capto Phenyl for rhEPO purification were investigated
* Presence of ionic and hydrophobic interactions at Capto MMC was confirmed
* Non-conventional elution methods must be used
* Strong hydrophobic character of the rhEPO was observed

**1. Introduction**

Erythropoietin (EPO) is a glycoprotein hormone regulating the production of red blood cells in the organism. EPO is an important and expensive medicinal product. Therefore many purification procedures were developed to obtain pure recombinant human erythropoietin (rhEPO) suitable for clinical use. Conventional methods include the combination of common chromatography purification techniques as ion-exchange, hydrophobic, reverse-phase and size-exclusion chromatography. Some researchers purified EPO by affinity and immunoaffinity chromatography [1, 2, 3]. In this work we compared the properties of a multimodal (Capto MMC) and a strong hydrophobic (Capto Phenyl) chromatography resin during the purification of rhEPO from biological material.

**2. Methods**

The multimodal adsorbent Capto MMC and the hydrophobic adsorbent Capto Phenyl were obtained from GE Healthcare. rhEPO was produced by human embryonic kidney cell 293 line. The post-culture medium was directly used without any further treatment for the purification experiments. Batch experiments were performed to facilitate the optimization of separation conditions. Flow experiments were carried out using the FPLC system ÄKTA Purifier (GE Healthcare, Uppsala, Sweden). The separation was carried out under the optimized conditions according to batch experiments. The concentration of rhEPO was determined by indirect ELISA assay [4]. The protein concentration was measured according to a standard BCA protein assay protocol [5].

**3. Results and discussion**

For both tested chromatography resins batch experiments were performed to find the best adsorption and elution conditions. In case of Capto MMC, a larger portion of rhEPO was adsorbed using 50 mM citrate-phosphate buffer with pH 6 supplemented with 300 mM NaCl. Under these conditions, the binding of contaminant proteins was reduced. Multimodal resins have both ion-exchange and hydrophobic functional groups and this makes the elution of the adsorbed proteins more difficult. Practically no rhEPO was eluted, when the ionic strength of the buffer was increased. 56 % of rhEPO was recovered by simultaneous change of pH and ionic strength of the buffer. The yield of rhEPO was significantly improved by chaotropic agent, arginine. This behavior of the rhEPO confirmed that at multimodal resins, both ionic and hydrophobic interactions can participate in the binding of proteins.

Flow experiments were carried out under the optimized conditions. The adsorption of rhEPO was good, only 3 % of rhEPO was not adsorbed on the resin. The elution was carried out using 50 mM Tris-HCl buffer at pH 7 supplemented with 1 M arginine. During the purification, more than 50 % of contaminating proteins was removed and 80 % of rhEPO was recovered. In a single bind-elute step, the concentration of rhEPO increased nineteenfold compared to that in the feed.

In case of Capto Phenyl, the best rhEPO adsorption was observed at 1.5 M NaCl concentration. The pH of the post-culture medium was adjusted to 6. The adsorption of rhEPO was also enhanced by (NH4)2SO4 but due to a possible precipitation of proteins caused by high concentration of (NH4)2SO4, the further purification was carried out at the presence of NaCl. Due to the strong hydrophobicity of the resin, commonly used elution procedures during hydrophobic interaction chromatography were not effective. Practically no rhEPO was recovered by reducing the ionic strength of the buffer and only 25 % of the adsorbed rhEPO was eluted by increasing the pH of the elution buffer to 9 and addition of 6 M urea. Good recovery was achieved when organic solvents as ethanol and isopropanol were used.

Flow experiments provided similar results as at Capto MMC. Many of the contaminating proteins left the column during the adsorption and washing phase, while only 5 % of the rhEPO was lost during this stage. Most rhEPO was recovered when 50 % ethanol or 30 % isopropanol in 20 mM Tris-HCl buffer with pH 9 was used for the elution.

**4. Conclusions**

In this work was investigated the possible application of a multimodal resin Capto MMC and a hydrophobic resin Capto Phenyl for rhEPO purification. In both cases many contaminating proteins were removed in a single bind-elute mode and highly concentrated rhEPO was obtained. According to the results Capto MMC is more suitable for rhEPO purification. Organic solvents, used to recover rhEPO at Capto Phenyl can cause denaturation of the target protein and loss of activity.

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