Purification of Murine IgG_1 Using UNOsphere™ S and CHT™ Ceramic Hydroxyapatite Chromatography

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Introduction

Immunoglobulins (IgGs) from a variety of sources have been purified using the conventional process composed of a protein A chromatography step along with ion exchange chromatography and hydrophobic interaction chromatography steps (Jiskoot et al. 1989, Godfrey et al. 1993, Shadle et al. 1995, Ford et al. 2001). Protein A chromatography is normally applied as the first step in the process, yielding antibodies with very high purity in a single step. However, the disadvantages of protein A chromatography are: 1) antibody isomers are not well separated from different species; 2) protein A leakage requires additional chromatographic steps for protein A removal; and 3) protein A resins are substantially more expensive than ion exchange and ceramic hydroxyapatite supports.

Here we present an alternative process for antibody purification consisting of UNOsphere S chromatography and CHT ceramic hydroxyapatite chromatography. This process is simpler than the process utilizing protein A and avoids the leakage of a contaminating ligand (Jiskoot et al. 1989).

UNOsphere S support is a strong cation exchanger made from acrylamido and vinylic copolymers. It exhibits high protein binding capacity and low column backpressure at high linear velocity. Thus, it is a suitable capture resin in the downstream purification process. CHT ceramic hydroxyapatite support, with Ca^{2+} ions and PO_4^{3-} ions in its spherical and ceramic structure, provides excellent resolution and selectivity for protein isolation.

Methods and Results

The BioLogic DuoFlow™ workstation was used to develop the processes. An analytical column (0.7 x 5 cm) was loaded with UNOsphere S support for small-scale process development. A preparative column (2.2 x 20 cm) was used in process scale-up. All SDS-PAGE was performed using Ready Gel® precast gels, the Criterion™ cell, and the PowerPac™ 3000 power supply. Ceramic hydroxyapatite (Type I, 10 µm and 20 µm) media were used for this study.

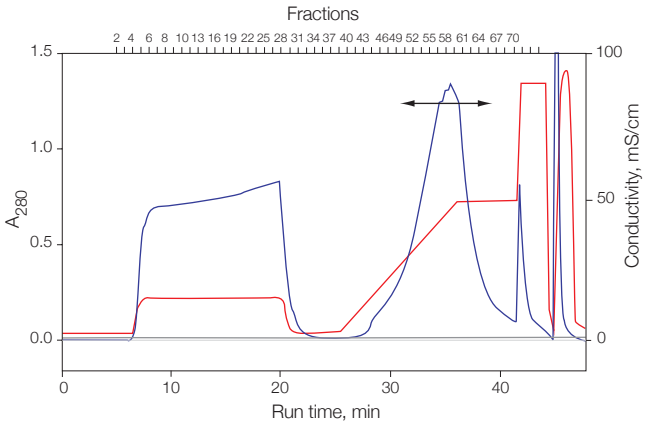

Fig. 1. Chromatogram of murine IgG_1 purification on the UNOsphere S column during small-scale process development. Diluted cell culture (20 ml) was loaded onto an UNOsphere S 0.7 x 5 cm column at a flow rate of 600 cm/hr in 20 mM phosphate-citrate buffer, pH 4.0. The sample was eluted in 10 column volumes (CV) of a 0–0.5 M NaCl gradient, followed by 5 CV of 1 M NaCl in the same buffer. The column was then cleaned in 1 M NaOH. The double-headed arrow indicates the fractions (1 ml each) containing IgG_1 (see Figure 2). Blue trace, A_{280}; red trace, conductivity profile.

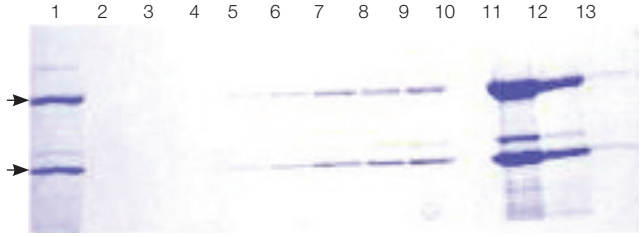

Fig. 2. SDS-PAGE analysis of murine IgG_1 purification by UNOsphere S chromatography during small-scale process development. Lane 1, sample load; lanes 2–9, flow-through fractions 4, 6, 8, 11, 12, 13, 21, 29; lane 10, wash fraction 40; lanes 11, 12, elution fractions 56, 61; lane 13, fraction after cleaning column with NaOH. Arrows indicate IgG_1 bands.

Small-Scale Process Development

UNOsphere S chromatography was used as the capture step for murine IgG_1 isolation. The binding capacity of UNOsphere S support for murine IgG_1 was optimized at pH values from 7.0 to 4.0. Decreasing the pH in the cell culture increased the binding capacity of UNOsphere S support for murine IgG_1 (data not shown). The murine IgG_1 was stable for at least 6 hr in buffer at pH 4.0 (data not shown), so the cell culture was adjusted to pH 4.0. The cell culture was then loaded onto the UNOsphere S column as described in Figure 1. The murine IgG_1 was eluted in the NaCl gradient. The fractions were analyzed by SDS-PAGE (Figure 2). It was found that Phenol Red and some contaminating proteins were removed in the flow-through pool by UNOsphere S chromatography.

For ceramic hydroxyapatite chromatography, the murine IgG_1 fractions were pooled and diluted 5-fold with 1 mM sodium phosphate buffer, pH 6.8. The murine IgG_1 elution pool from the UNOsphere S column could not be loaded directly on the CHT supports because the ionic strength of the pooled solution was too high to allow murine IgG_1 to bind. The diluted sample was loaded onto the CHT column at 300 cm/hr. Some contaminants were removed by a NaCl gradient. The murine IgG_1 was eluted by a phosphate gradient (Figure 3).

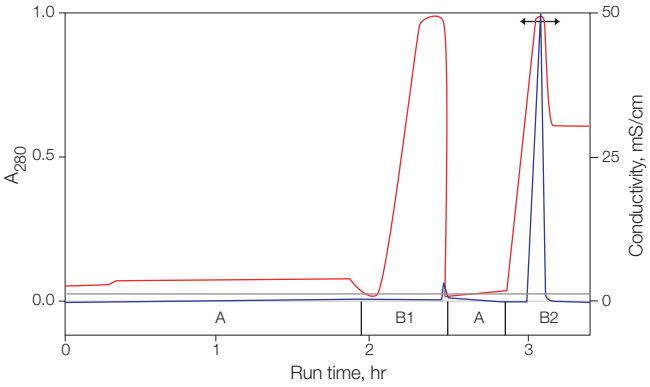

Fig. 3. Chromatogram of murine IgG_1 purification by CHT chromatography during small-scale process development. The murine IgG_1 pool (100 ml) from the UNOsphere S column (fractions indicated with arrow in Figure 1) was loaded onto a CHT-I 10 µm, 0.7 x 5 cm column at a flow rate of 300 cm/hr in 1 mM sodium phosphate, pH 6.8 (buffer A). The sample was then eluted with a gradient of 0–0.5 M NaCl in buffer A over 10 CV followed by 5 CV of 0.5 M NaCl in buffer A (step B1), and 10 CV of buffer A. Finally, the column was eluted with 10 CV of a gradient of 1 mM–0.4 M sodium phosphate buffer, pH 6.8 (step B2). The arrow indicates the pooled fractions containing IgG_1. Blue trace, A_{280}; red trace, conductivity.

The fraction pool was analyzed by SDS-PAGE (Figure 4). We estimated that >95% purity of murine IgG_1 was achieved in this purification process.

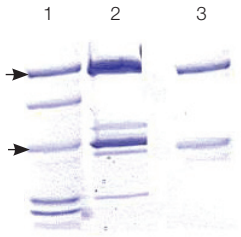

Fig. 4. SDS-PAGE analysis of the murine IgG_1 elution pool from the CHT ceramic hydroxyapatite column during small-scale process development. Lane 1, protein molecular weight standards (250, 150, 100, 75, 50, 37, 25, 15, and 10 kD); lane 2, UNOsphere S elution pool; lane 3, CHT elution pool. Arrows indicate IgG_1 bands.

Process Scale-Up

A 2.2 x 20 cm column was packed with 60 ml of UNOsphere S to a bed height of 16 cm and run as described (Figure 5). Some contaminants were removed, and the murine IgG_1 could be isolated in the process at this scale. The results indicated that the UNOsphere S purification process could be scaled up at least 30 times.

The murine IgG_1 pool from the UNOsphere S column was further purified by CHT chromatography as described in Figure 6. Murine IgG_1 was eluted in a phosphate gradient applied after some contaminants were removed by a NaCl gradient.

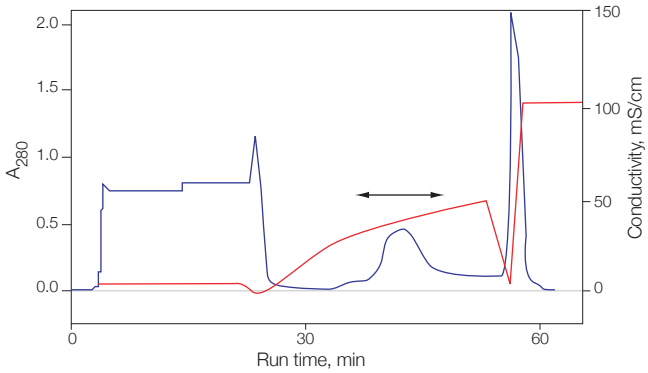

Fig. 5. Chromatogram of murine IgG_1 purification by UNOsphere S chromatography during scale-up. The column was equilibrated to pH 4.0 with 1 mM sodium acetate, pH 4.0 (buffer A). The pH of the cell culture sample was adjusted to 4.0 with concentrated acetic acid, then loaded onto the column at 300 cm/hr, the same linear velocity used in the small-scale purification process. Once 600 ml of pH-adjusted sample had been loaded, the column was washed with buffer A. The murine IgG_1 was eluted with 20 CV of a 0–0.5 M NaCl gradient in buffer A. The column was then cleaned with 10 CV of 1 M NaCl in buffer A followed by 5 CV of 0.5 M NaOH. The arrow indicates the pooled fractions containing IgG_1. Blue trace, A_{280}; red trace, conductivity.

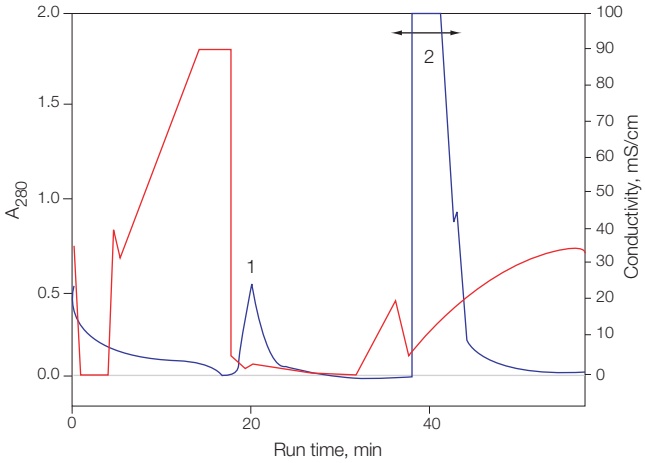

Fig. 6. Chromatogram of murine IgG_1 purification by CHT chromatography during scale-up. Murine IgG_1 fractions from the UNOsphere S column (indicated by arrow in Figure 5) were pooled, diluted 5 times to 1.85 L with 1 mM sodium phosphate buffer, pH 6.8 (buffer A), and loaded onto a 20 ml CHT Type I column (20 µm, 1.1 x 10 cm) at 300 cm/hr. The column was washed with buffer A, then eluted with a 0–1 M NaCl gradient in buffer A for 10 CV followed by 1 CV of 1 M NaCl. The column was then washed again with buffer A for 5 CV. A 1 mM–0.4 M sodium phosphate gradient (pH 6.8) was run for 20 CV to elute the murine IgG_1. The arrow indicates the pooled fractions containing IgG_1. Blue trace, A_{280}; red trace, conductivity.

SDS-PAGE analysis showed that the scaled-up CHT purification process yields murine IgG_1 of similar purity to that in small-scale purification (Figure 7). The murine IgG_1 was isolated by phosphate gradient; however, some minor bands were observed in lane 3 when the gel was overloaded with 40 µg of a murine IgG_1 elution pool. These bands suggest that there are still some degradation products or contaminants in the purified murine IgG_1. Average recovery in this scale-up purification process was about 80%. Data from flow cytometry analyses suggest that the murine IgG_1 solution from our purification process yields very similar immunospecificity to processes using protein A chromatography (data not shown).

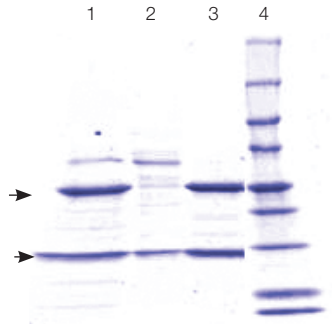

Fig. 7. SDS-PAGE analyses of CHT column fractions during scale-up. Lane 1, load; lane 2, peak 1 elution pool; lane 3, peak 2 elution pool; lane 4, molecular weight standards. Arrows indicate IgG_1 bands.

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References

Ford CH et al., Affinity purification of novel bispecific antibodies recognising carcinoembryonic antigen and doxorubicin, J Chromatogr B Biomed Sci and Appl 754, 427–435 (2001)

Godfrey MAJ et al., Assessment of the suitability of commercially available SpA affinity solid phases for the purification of murine monoclonal antibodies at process scale, J Immunol Methods 160, 97–105 (1993)

Jiskoot W et al., Two-step purification of a murine monoclonal antibody intended for therapeutic application in man. Optimisation of purification conditions and scaling up, J Immunol Methods 124, 143–156 (1989)

Shadle PJ et al., Antibody purification, US patent 5,429,746 (1995)

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