Purification of Transgenic Antibody from Corn Seed Using UNOsphere™ S and CHT™ Ceramic Hydroxyapatite Supports

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Introduction

Production of protein biopharmaceuticals in transgenic plants offers several advantages over traditional sources. For example, correct posttranslational processing has been demonstrated for proteins expressed in plants (Kusnadi et al. 1998), which does not occur in bacteria or yeast. Plant systems also offer low production cost, provide high storage stability, lack animal and human pathogens, and have the potential for virtually unlimited scale-up either in suspension culture or at the greenhouse or field scale (Kusnadi et al. 1998, Fischer et al. 2000).

Recombinant antibodies are among the key biopharmaceutical agents that have been expressed in plants. Although few details on isolation of functional proteins from plant sources have been published, affinity chromatography using protein A or protein G has been used in the process (Fischer et al. 2000). Disadvantages of this method include high cost and ligand leaching (Gagnon 1996). We report here on a method for the purification of a recombinant antibody from corn seed using UNOsphere S support in the capture step and CHT ceramic hydroxyapatite in the polishing step. This process is efficient and scalable, and it avoids the use of protein A or G.

Methods and Results

A model system of crude corn extract spiked with transgenic IgG purified from corn was used in these studies due to limited sample availability. The sample was loaded onto a UNOsphere S support column at pH 4.0 and eluted by the salt and pH gradients described in Figure 1. The fractions were analyzed by SDS-PAGE (Figure 2). It was shown that some contaminants were removed by a NaCl gradient. The transgenic IgG was eluted by a subsequent pH gradient. Some low molecular weight contaminants remained in this fraction.

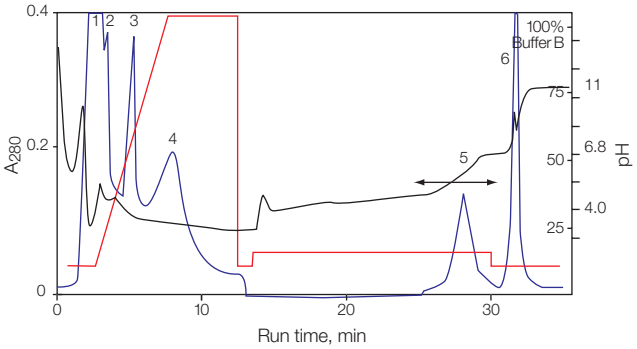

Fig.1. Chromatogram of corn transgenic IgG separation by UNOsphere S chromatography. Column size, 1.1 x 10 cm (10 ml); sample, 1.8 ml of corn crude extract + 0.2 ml (1.22 mg) partially purified transgenic IgG. The column was equilibrated with 1 mM acetate buffer, pH 4.0, until the pH value in the effluent was approximately 4.0. The sample was loaded, and the column was washed with 5 column volumes (CV) of 10 mM phosphate buffer, pH 6.8, followed by 10 CV of a 0–1 M NaCl gradient (buffer B). The column was then washed with 10 CV of 1 M NaCl in 10 mM phosphate buffer, pH 6.8 (producing a pH gradient from 4.0 to 6.8). The flow rate was 600 cm/hr. Between runs, the column was cleaned with 10 CV of 0.5 N NaOH. Blue trace, A_{280}; black trace, pH; red trace, buffer B %. Peaks are numbered for reference. The double-headed arrow indicates IgG-containing fractions (see Figure 2).

Fig. 2. SDS-PAGE analyses of fractions in UNOsphere S chromatography. Lane 1, standards (top to bottom: 50, 37, 25, 15, and 10 kD); lane 2, crude extract with spiked IgG; lane 3, elution pool from peak 1 of Figure 1; lane 4, elution pool from peak 2; lane 5, elution pool from peak 3; lane 6, elution pool from peak 4; lane 7, elution pool from peak 5; lane 8, elution pool from peak 6. The Criterion™ Tris-HCl gel was stained with Coomassie Brilliant Blue R-250 under reducing conditions. The locations of IgG light (L) and heavy (H) chains are indicated with arrowheads.

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Fractions containing the corn transgenic IgG elution (peak 5 in Figure 1) were pooled and then loaded onto a CHT column. The column was eluted with a NaCl gradient and then a phosphate gradient as described in Figure 3. Minor contaminants were eluted by the NaCl gradient. Essentially pure transgenic IgG was eluted from the CHT in the phosphate gradient.

Samples from the CHT elution pool, corn crude extract, and partially purified IgG were loaded on a 4–20% SDS-PAGE Ready Gel® precast gel. The proteins in the SDS-PAGE gel were transferred to a nitrocellulose membrane in a Trans-Blot® SD semi-dry transfer cell. Transgenic IgG was detected with an anti-human IgG-alkaline phosphatase conjugate from the Immun-Blot® kit (catalog #170-6462), indicating that the IgG purified from corn seed was transgenic human IgG.

The CHT eluate exhibited a single peak on a Bio-Silect® SEC 250-5 column with a molecular weight slightly less than 150 kD (data not shown), indicating that there were no aggregates in the purified IgG fractions.

The quantity of IgG in each step was determined by radial immunodiffusion. Protein concentration was determined by the BCA assay. A summary of the transgenic IgG purification and recovery is shown in the table.

Table. Summary of transgenic IgG purification.

Purification Step	Total IgG (mg)	Total Protein (mg)	Recovery (%)	Purity* (%)
Crude corn extract + IgG	1.1	6.1	100	22
UNOsphere S	1.07	1.47	97	80
CHT	0.88	0.91	82	95

*Protein purity was determined by gel scanning using a GS-710 densitometer.

Discussion

A process for purifying transgenic human IgG from corn seeds was developed using UNOsphere S and CHT chromatography. The process avoids the ligand leaching associated with protein A or G affinity chromatography. The purity of the IgG prepared by this process was 95%, and recovery was at least 80%.

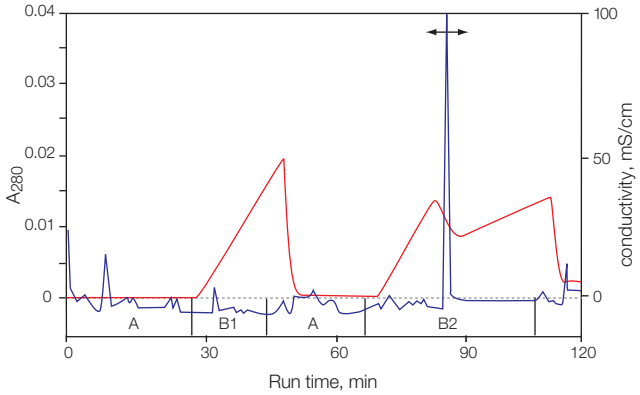

Fig. 3. Chromatogram of corn IgG purification by CHT chromatography. Column size, 0.5 x 10 cm (2 ml); support, CHT Type I, 20 µm; sample, pool from peak 5 of UNOsphere S column, 500 µl; flow rate, 300 cm/hr. The column was equilibrated with buffer A (1 mM phosphate buffer, pH 6.8). The sample was loaded and then washed with 5 CV of buffer A, followed by 10 CV of a 0–0.5 M NaCl gradient (step B1). The column was then washed with 5 CV of buffer A followed by 10 CV of a 0–0.4 M phosphate gradient (step B2). Between runs, the column was cleaned with 5 CV of 0.5 N NaOH. The double-headed arrow indicates the IgG peak. Blue trace, A_{280}; red trace, conductivity.

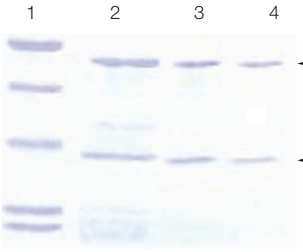

Fig. 4. SDS-PAGE analyses of corn transgenic IgG elution pool from the CHT column. Lane 1, molecular weight standards; lane 2, cell culture; lane 3, IgG pool from the UNOsphere S column; lane 4, IgG pool from the CHT column. The locations of IgG light and heavy chains are indicated with arrowheads.

Fig. 5. Detection by immunoblotting of transgenic human IgG purified from corn seeds. Lane 1, positive control (partially purified transgenic IgG); lane 2, crude corn extract (negative control); lane 3, IgG elution pool from the CHT column. The locations of IgG light (L) and heavy (H) chains are indicated with arrowheads.

References

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