Purification of Recombinant Human Alpha1-Antitrypsin From Transgenic Milk Using CHT™ Ceramic Hydroxyapatite

Samuel G Franklin, PhD, Bio-Rad Laboratories, Hercules, CA 94547 USA

Introduction

Alpha1-antitrypsin (α1-AT) is the principal serum inhibitor of proteases such as elastase. Deficiency of α1-AT causes chronic emphysema due to uncontrolled elastase activity released from lung neutrophils. Elastase degrades elastin and collagen, producing the fibrosis and stiffening of tissues that are characteristic of the disease. An estimated 75,000 to 150,000 individuals in the US have hereditary α1-AT deficiency, 20,000 to 40,000 of whom develop emphysema. The dose requirement for α1-AT protein replacement therapy is about 120 g/patient/yr and, although plasma-derived products exist, manufacture of enough of these products is not possible. The advent of transgenic technology has made it possible to express α1-AT in sheep and goat's milk at 1–35 g/L, and 3 L/day/goat is obtained (Wright et al. 1991). Thus, assuming 20 g/L are expressed, some 200 goats could supply US requirements of about 4,000 kg/yr.

Human α1-AT is a 52 kD glycoprotein consisting of 394 residues. It is relatively fragile, being susceptible to heat denaturation and methionine oxidation, and aggregates progressively at pHs above and below 7.5 (Vemuri et al. 1993). Various techniques including ion exchange, hydrophobic interaction, and immobilized metal affinity chromatography (IMAC) have been used to purify α1-AT, but the number of steps and relatively harsh elution conditions have led to destabilization of the bulk protein. Purification from milk is additionally challenging because some goat α1-AT (about 1%) is present, and two major milk proteins, α-lactalbumin and β-lactoglobulin, have isoelectric points and titration curves very similar to α1-AT. In this note, we describe the development of a simple, gentle process for the purification of α1-AT from clarified goat's milk, using CHT™ ceramic hydroxyapatite.

Methods and Results

Goat's milk clarifed by ultrafiltration was equilibrated with 10 mM sodium phosphate buffer, pH 7.0 (buffer A) by dialysis or desalting column. It was applied directly to a 2.0 ml Bio-Scale™ ceramic hydroxyapatite Type I column (CHT-I, catalog #751-0021) equilibrated with buffer A. Elution was accomplished by a shallow linear gradient from 10 to 400 mM sodium phosphate buffer, pH 7.0 (buffer B). Figure 1 shows the elution profile from this column. SDS-PAGE analysis (Figure 2) indicated that the α-lactalbumin

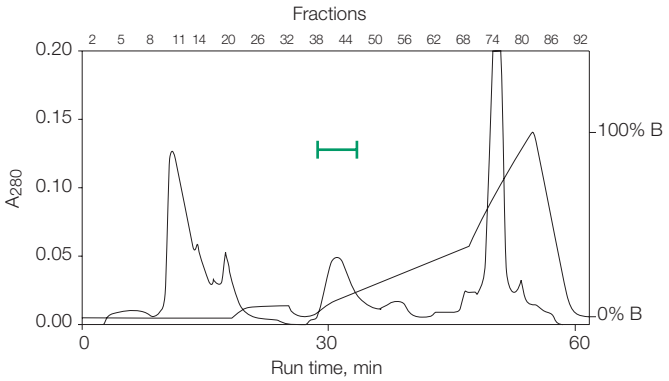

Fig. 1. Purification of α1-AT on CHT-I. Column, 7 x 52 mm; load, 5.0 ml. Buffer A, 10 mM sodium phosphate, pH 7.0; buffer B: 400 mM sodium phosphate, pH 7.0. Gradient, 0–5% B and 5–30% B in 5 column volumes (CV) each, 30–100% B in 10 CV. Flow rate, 2.5 ml/min. Fractions were 1.5 ml, pooled as follows: pooled fraction I = run fractions 12–17, II = 23–24, III = 42–49, IV = 49–50, V = 51–53, VI = 54–58, VII = 69–71, VIII = 74–77. Green bar indicates where α1-AT eluted.

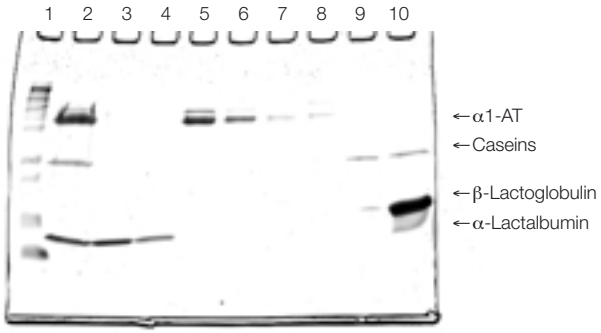

Fig. 2. SDS-PAGE analysis of pooled fractions from Figure 1. Lane 1, MW markers (203, 118, 86, 51.6, 34.1, 29, 19.2, 7.5 kD); lane 2, load; lanes 3–10, pooled fractions I–VIII. Protein migration positions were identified by running standards (not shown). Gel was 15% acrylamide and stain was Coomassie Brilliant Blue R-250.

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emerged in the unbound fraction, while the caseins and β-lactoglobulin were tightly bound to the column and emerged at the end of the gradient. The central peak (Figure 1, green bar) contained the α1-AT. The minor component above the α1-AT (Figure 2, lanes 5 and 6) may be due to microheterogeneity or may be the goat species. Because of the presence of this trace component, a two-step procedure was evaluated.

A capture step using immobilized copper affinity chromatography provided approximately 50% pure α1-AT (not shown). The product from this column was equilibrated with Buffer A and applied to the CHT-I column under conditions identical to those described above. The profile from this column (Figure 3) was similar to that generated by clarified milk (Figure 1) except that the large peak near the end of the gradient, shown previously to contain the β-lactoglobulin, was absent. This was confirmed by SDS-PAGE (Figure 4), which also showed that the α1-AT peak (Figure 3, green bar) was now homogeneous. Reversed phase HPLC analysis of the fractions indicated that pooled fractions III–V were 83–98% pure.

Discussion

This two-step process was shown to provide highly purified α1-AT. Although an additional polishing step may be required for viral clearance and pharmaceutical requirements, CHT-I has been shown to complement IMAC by providing a simple, gentle alternative to conventional chromatography. The rather large difference in retention times for α1-AT, goat β-lactalbumin, and goat β-lactoglobulin, which have very similar isoelectric points (5.27, 5.16, and 4.77, respectively), is a good illustration of the unique separation capabilities afforded by CHT-I.

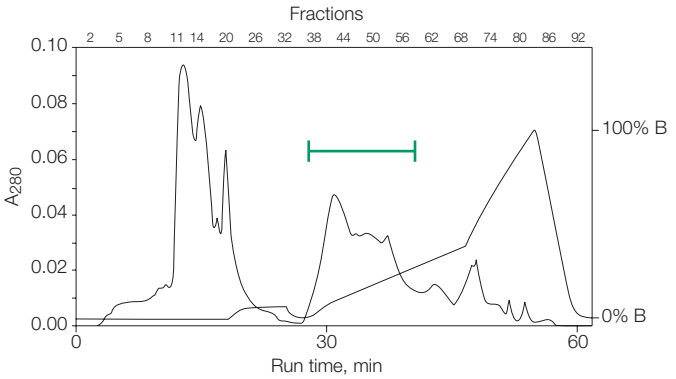

Fig. 3. Purification of copper chelate product on CHT-I. Load, 5.0 ml. Conditions were as in Figure 1. Pooled fraction I = run fractions 13–17, II = 20–21, III = 23–24, IV = 40–46, V = 49–58, VI = 63–65, VII = 68–71. Green bar indicates where α1-AT eluted.

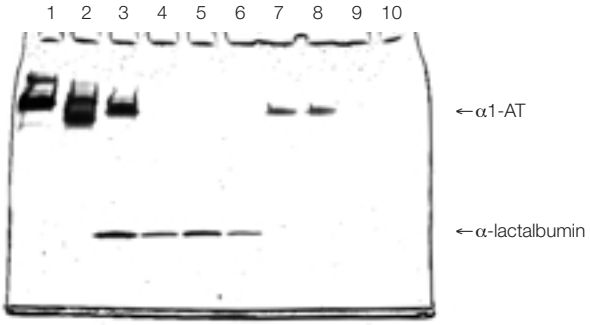

Fig. 4. SDS-PAGE analysis of pooled fractions from Figure 3. Lane 1, HSA; lane 2, α1-AT (Sigma); lane 3, load; lanes 4–10, pooled fractions I–VII. Conditions were as in Figure 2.

References

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Wright T et al., High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep, Biotechnology 9, 830-834 (1991)

Acknowledgement

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