Workflow for Protein Purification from Whey Using Nuvia™ Ion Exchange Media

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Introduction

Whey proteins play a vital role in the formulation of food supplements because of numerous desirable nutritional and functional properties. Here we present a process for whey purification using Nuvia S and Nuvia Q ion exchange media. The major whey proteins are α-lactalbumin (ALA), β-lactoglobulin (BLG), bovine serum albumin (BSA), and bovine immunoglobulins (Hahn et al. 1998) (Table 1). Minor proteins such as lactoperoxidase, lactoferrin, and proteose-peptone account for the other protein components in whey.

Table 1. Protein composition of bovine whey.

Protein	Average Concentration, g/L	Proportion in Whey, %	Molecular Weight	Isoelectric Point
ALA	1.5	22	14,200	4.7–5.1
BLG	3–4	51	18,400	5.2
BSA	0.3–0.6	6.6	65,000	4.9
IgG, IgA, IgM	0.6–0.9	11	150,000–900,000	5.8–7.3
Lactoperoxidase	0.06	<1	78,000	9.6
Lactoferrin	0.05	<1	78,000	8.0
Proteose-peptone	0.5	7	4,000–20,000	—

The objective of this study was to develop a separation process to fractionate usable proteins from whey (Figure 1). First, we purified a total whey protein isolate (WPI) because of its high-end functional advantages, as evidenced by a demand from nutrition and health segments. Secondly, ALA and BLG were purified because of their value in infant formula and confections, respectively.

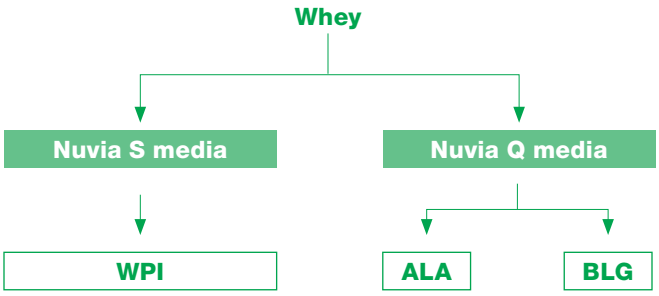

Fig. 1. Workflow for protein purification from whey with Nuvia S and Nuvia Q media.

Materials and Methods

Whey

Whey was obtained as a concentrate from Hilmar Ingredients. The solution was aliquoted, stored frozen at –20°C, and thawed before the experiments. The whey concentrate was diluted tenfold in equilibration buffer and filtered through a 1.2 micron filter (Sartorius Group) to remove particulates prior to chromatography.

Purified Proteins and Reagents

Purified bovine proteins, including ALA, BLG, and BSA, were purchased from Sigma-Aldrich. The proteins were solubilized in the appropriate column equilibration buffer at a concentration of 2 mg/ml on the day of the experiments. All chemicals used for the preparation of buffers were of analytical grade. The buffers were sterile filtered prior to use.

Electrophoresis

For SDS-PAGE, the sample was first mixed with an equal volume of loading buffer, then heated at 95°C for 5 min. Up to 30 µl of the mixture was loaded into the wells of a 4–20% Criterion™ or Criterion™ TGX™ precast gel (Bio-Rad Laboratories, Inc.) and separated at 200 V or 300 V, respectively. The gel was stained with Bio-Safe™ Coomassie stain and scanned with a GS-800™ USB calibrated densitometer (both from Bio-Rad).

Column Chromatography

All chromatography columns and instruments were from Bio-Rad. Experiments were automated and executed using a BioLogic DuoFlow™ system and software. Nuvia S or Nuvia Q media was packed in a Bio-Scale™ MT2 column. For WPI preparation, the system was programmed to repeatedly run the following chromatography cycle (60 column volumes (CV)/hr) when using Nuvia S media:

- Equilibration with 3 CV of 0.04 M Na lactate, pH 4.0
- Loading with 20 CV of 1/10 diluted whey
- Washing with 10 CV of 0.04 M Na lactate, pH 4.0
- Elution with 20 CV of 0.04 M Na phosphate, pH 8.0
- Washing with 3 CV of 1 M NaCl
- Washing with 3 CV of 1 M NaOH
- Pre-equilibration with 3 CV of 0.2 M Na lactate, pH 4.0
- Equilibration with 5 CV of 0.04 M Na lactate, pH 4.0

To determine the elution profile of individual proteins or mixtures of proteins, chromatography was carried out with a gradient at the target pH. For example, the column was equilibrated in 0.020 M Na phosphate, pH 6.0. After injecting a protein sample of 100 µl, the column was washed with five CV of equilibration buffer. The adsorbed protein was eluted using 40 CV 0–0.3 M linear gradient of NaCl (buffer B). The column was cleaned with five CV of 1 M NaCl and 1 M NaOH, and stored in 0.1 M NaOH.

To purify ALA and BLG from whey, a segmented gradient method using Nuvia Q media was developed:

- Equilibration with 3 CV of 0.02 M Na phosphate, pH 6.0
- Loading 5 ml of 10x diluted whey
- Washing with 4 CV of 0.02 M Na phosphate, pH 6.0
- Gradient elution with 10 CV 0–0.1 M NaCl
- Step elution with 5 CV in 0.1 M NaCl
- Gradient elution with 10 CV 0.1–0.3 M NaCl
- Step elution with 5 CV in 0.3 M NaCl
- Gradient elution with 10 CV 0.3–0.9 M NaCl
- Step elution with 10 CV in 0.9 M NaCl

pH and A_{280} Measurements

The pH and A_{280} of the chromatography fractions were monitored using the BioLogic DuoFlow system instrumentation. Total protein was determined by the Quick Start™ Bradford protein assay (Bio-Rad).

Results and Discussion

Preparation of Whey Protein Isolate

As defined by the Whey Protein Institute (www.wheyoflife.org), WPI is the most pure and concentrated form of whey protein with little or no lipids and lactose. Since the majority of the proteins have isoelectric points above 4.7, loading of whey at pH 4.0 onto Nuvia S media resulted in the capture of these proteins (Etzel 2004, Hahn et al. 1998). Lipids, lactose, and unbound proteins remain in the flowthrough and the wash. As shown in Figure 2, a single peak was collected upon elution with pH 8.0 elution buffer. Capacity of the Nuvia S column, based on the measurement of the protein content in the eluate pool, was 60 ± 2 g/L (60 CV/hr, n = 4). As shown in Figure 3, proteins in WPI and in whey are similar. Interestingly, densitometry of the major species, including ALA, BLG, and BSA in whey and WPI, showed nearly identical distributions.

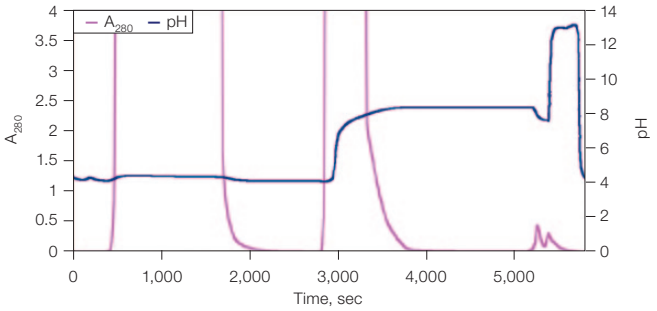

Fig. 2. Cation exchange chromatography of 80 mg of crude whey on a Nuvia S column. Column: 7 x 27 mm; 2 ml fractions were collected; flow rate: 60 CV/hr; buffer A: 0.04 M Na lactate, pH 4.0; buffer B: 0.04 M Na phosphate, pH 8.0.

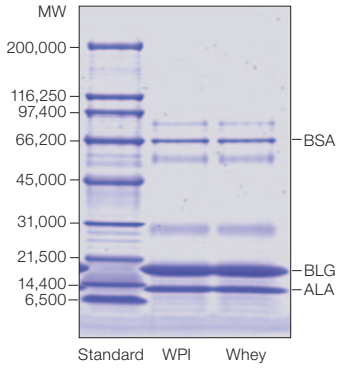

Fig. 3. SDS-PAGE analysis of WPI and whey. Ten mg of whey protein and WPI along with Bio-Rad SDS-PAGE standards were run on a 4–20% Criterion TGX precast gel. Proteins were stained using BioSafe Coomassie stain. MW, molecular weight.

Anion Exchange Media Selection and Optimization

ALA and BLG account for about 75% of the proteins in whey. These two model proteins were therefore selected for further experimentation. In an initial screen, Nuvia Q media was compared to two media with high binding capacity (Media A and Media B). The media were tested using a small mixture of ALA and BLG (10% CV) that was eluted using a shallow NaCl gradient (0–0.3 M). This permits rapid and uniform evaluation of process parameters but further optimization will be needed for the selected media. The resulting chromatograms from this screening experiment were compared (Figure 4). All three media produced two equally well-resolved peaks. Peaks of Media A eluted sooner, suggesting weaker anion exchange properties than Nuvia Q media and Media B. The bead size of Media A is the smallest of the three media, which may lead to higher column pressure upon scale-up. Additionally, rigidity, cleanability, capacity, and cost will have to be factored in when choosing the appropriate media for large-scale purification.

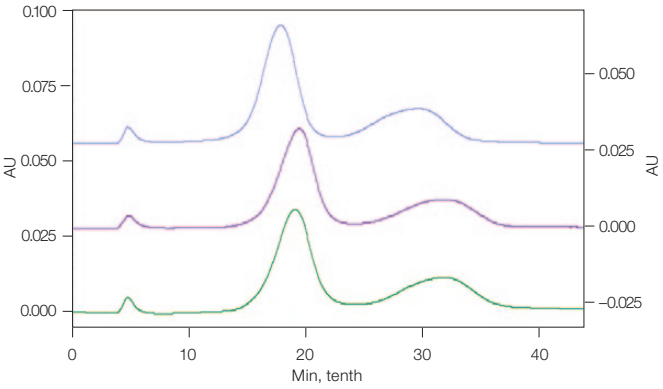

Fig. 4. ALA and BLG purification chromatograms on three anion exchangers. Comparison of Nuvia Q media (—), Media A (—), and Media B (—) performance. AU, absorbance units.

Purification of ALA and BLG from Whey

To minimize processing costs, a purification scheme for ALA and BLG from whey ideally should require a single chromatographic step to purify both proteins. Initial studies (Kim et al. 2003, Gerberding and Byers 1998) demonstrated the removal of the major whey proteins (ALA, BSA, and BLG) in a segmented gradient (data not shown). Further work was performed to optimize these conditions. The purification profile of each protein was first identified by injecting the purified protein alone and then a mixture containing the three purified proteins. As expected, separation of the mixture paralleled that of the individual proteins. When whey was used as the feed stock, two major pools were resolved upon adsorption and elution with the segmented gradient (Figure 5).

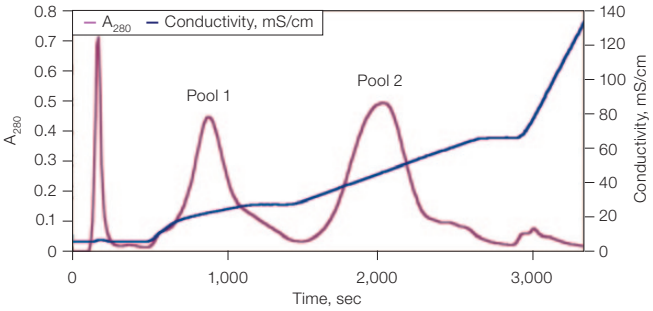

Fig. 5. Anion exchange chromatography of 8 mg of crude whey on a 7 x 27 mm Nuvia Q media column. Fractions of 2 ml were collected; flow rate: 60 CV/hr; buffer: 0.02 M Na phosphate, pH 6.0; segmented gradient: 0–0.1 M NaCl, 0.1–0.3 M NaCl, and 0.3–0.9 M NaCl.

SDS-PAGE analysis (Figure 6) of the fractions showed two peaks, one containing predominantly ALA (Pool 1) and the other containing predominantly BLG (Pool 2). The corresponding ALA and BLG standards are also shown in the gel scan. Purities of the two whey-derived proteins in each peak were estimated by densitometry to be at least 85%.

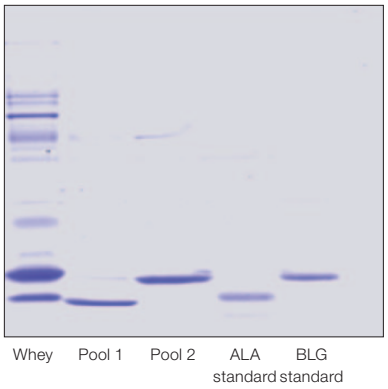

Fig. 6. SDS-PAGE analysis of whey, purified ALA (Pool 1), and purified BLG (Pool 2) against protein standards.

Conclusions

Nuvia S and Nuvia Q media, two novel high-capacity media for ion exchange chromatography, allow efficient enrichment of proteins from crude mixtures. The feasibility to prepare WPI, purified ALA, and purified BLG has been demonstrated. Because of the high-throughput properties of Nuvia media, a rapid single-step purification of proteins from whey is possible.

References

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