Fractionation of whey proteins with a hexapeptide ligand affinity resin

Patrick V. Gurgel¹, Ruben G. Carbonell² & Harold E. Swaisgood¹∗

¹Department of Food Science, Box 7624, North Carolina State University, Raleigh, NC, 27695-7624, USA
²Department of Chemical Engineering, Box 7905, North Carolina State University, Raleigh, NC, 27695-7905, USA
∗Corresponding author

Received 14 November 2000. Accepted in revised form 26 February 2001

Key words: α-lactalbumin, bioselective adsorption, peptide ligands, whey protein isolate

Abstract

The isolation of individual proteins from whey would allow production of more consistent and reliable products by the food industry and possibly would also increase their use in the pharmaceutical industry. α-Lactalbumin is the second most prevalent protein in bovine milk whey and has many uses including serving as an excellent protein source in infant formulas, power drinks and other beverages that require soluble, nutritional protein. In this study, we describe two methods for production of α-lactalbumin from whey protein isolate using bioselective adsorption. The use of a peptide ligand (WHWRKR) attached to a resin allowed production of an α-lactalbumin-rich fraction with a purity of 90.6% and a recovery of 47.9%, while also producing other fractions of commercial interest. The combined use of an amino resin followed by the WHWRKR resin produce a highly purified α-lactalbumin (100%) with a yield of 35.2%.

Introduction

During the past 10 – 15 years, whey has evolved from waste product to a value-added ingredient for the food industry. Its potential as nutritional protein source has been recognized and its functional properties have become more utilized. It has been proposed that the use of individual whey proteins could be more valuable than their use as a mixture (Huffman and Harper, 1999). The fractionation of whey and isolation of individual proteins would lead to better-controlled products, with fewer variations in composition. Another advantage would be the use of individual proteins with unique or desirable characteristics in specialized products.

The most prevalent whey proteins are β-lactoglobulin, α-lactalbumin, bovine serum albumin (BSA), immunoglobulins, lactoferrin and lactoperoxidase. β-Lactoglobulin is a good source of essential amino acids, with good gelling properties and potential use in power drinks due to its solubility, BSA and immunoglobulins have been reported to have anticancer properties and to enhance immunity, lactoferrin is used as an antibacterial agent in infant formulas and lactoperoxidase has demonstrated anticarial activity in toothpaste (Horton, 1996). α-Lactalbumin is the preferred protein source for infant formulas due to its high nutritional value, high digestibility, and lower potential for causing allergies, when compared to β-lactoglobulin (Heine et al., 1996). Alternative uses of α-lactalbumin may include its use as a morphinomimetic and antitumor agent (Muller et al., 1999), as well as a contraceptive agent (Maubois and Olivier, 1997).

Several methods for whey fractionation have been proposed, including membrane filtration (Zydney, 1998), ion-exchange chromatography (Gerberding and Byers, 1998, Girardet et al.; 1989, Hahn et al., 1998) and thermal (Hill, 1988; Bramaud et al., 1995)
or chemical precipitation (Igarashi, 1995). Although valid and useful for producing fractions enriched in a particular protein, most of these methods lack specificity, generating fractions that have purity levels lower than desirable for some purposes, or are not practical for scale-up. To obtain high purities, the individual proteins have to be isolated using their unique characteristics, requiring more refined methods.

α-Lactalbumin has been isolated using several methods that provide a wide variation of yields and purities. Ortin et al. (1992) combined a precipitation step (using PEG) with hydrophobic partitioning in an aqueous two-phase system to isolate α-lactalbumin and β-lactoglobulin. The final yield of α-lactalbumin was 28.7%, with a purity of 88.3%. Outinen et al. (1996a) compared different fractionation procedures and found it difficult to produce α-lactalbumin efficiently using such methods.

A centrifugal separation of α-lactalbumin and β-lactoglobulin was performed by Tupasela et al. (1997). The method allowed a recovery of 23.3% of the α-lactalbumin, under optimal conditions.

Outinen et al. (1996b) reported a chromatographic fractionation of α-lactalbumin and β-lactoglobulin using polystyrenic strongly basic anion-exchange resins. Eleven different resins were tested. Using the best under optimal conditions gave a recovery of 75–80%, and purity of about 66% for α-lactalbumin. Yoshida (1990) reported the isolation of α-lactalbumin and β-lactoglobulin by gel filtration using Sephacryl S-200 followed by diethylaminoethyl ion-exchange chromatography to further purify the α-lactalbumin-rich fraction. A 73% pure fraction of α-lactalbumin was obtained but no data on recovery yield was given.

Blomkalns and Gomez (1997) used immobilized metal ion affinity chromatography to purify α-lactalbumin from whey protein isolate (WPI). Using this method, a purity of 90% and a recovery of 80% were achieved.

This paper describes a method of purification of α-lactalbumin from whey protein isolate using bioselective adsorption. The resin used consists of a hexapeptide (WHWRKR) covalently bound through its carboxyl terminal to a polymethacrylate matrix. The hexapeptide, with demonstrated affinity for α-lactalbumin, was isolated from a combinatorial solid-phase peptide library (Gurgel et al., 2001). Although the peptide displays affinity for α-lactalbumin, the resin also binds other proteins, probably because the amino groups in the peptide serve as ion-exchangers. Because α-lactalbumin does not bind strongly to amino groups, the peptide was acetylated in an attempt to block these groups. A two-step method was also examined, taking advantage of the non-specific binding of whey proteins to amino groups.

### Materials and methods

All chemicals were obtained from Sigma (St. Louis, MO, USA). Whey protein isolate (WPI) was obtained from Davisco (Minneapolis, MN, USA). The amino resin used was originally a polyhydroxylated methacrylate polymer (TosoHaas AF Chelate 650, TosoHaas, Montgomeryville, PA, USA) modified to generate free amino groups. The peptide resin was produced by synthesizing the peptide H2N-WHWRKRA-COOH (WHWRKR resin) onto the amino resin as solid support, using a Gilson AMS Multiple Peptide Synthesizer, following the procedure described by Buettner et al. (1996). A modification of the peptide WHWRKR, designated AcWHWRKR resin, with an acetylated terminal amino group was produced by Peptides International.

Chromatographic runs were conducted by injecting a 500 µl sample of 5.55 mg ml⁻¹ WPI in 50 mM phosphate buffer, pH 7.0, in a 0.6-ml HPLC column packed with the desired resin. The flow rate was 0.15 ml min⁻¹ and elution was accomplished using a step gradient consisting of 20 min of phosphate buffer, followed by 20-min washes with each of the following solutions: 0.1 M NaCl in phosphate buffer, 0.25 M NaCl in phosphate buffer, 0.5 M NaCl in phosphate buffer, and 2% acetic acid in water. The protein composition of the WPI sample injected is given in Table 1.

For the two-step process, the unbound fraction from the amino resin was collected and concentrated to 500 µl using a Centricon cartridge (Millipore, Bedford, MA, USA) with a molecular weight cut-off of 5000. The concentrated sample was then injected into the WHWRKR resin.

### Table 1. Protein composition of the original sample.

<table>
<thead>
<tr>
<th>Protein</th>
<th>% of total protein</th>
<th>Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td>3.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>6.0</td>
<td>0.17</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>11.7</td>
<td>0.32</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>45.3</td>
<td>1.26</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>25.5</td>
<td>0.71</td>
</tr>
</tbody>
</table>