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Co-purification of chicken egg white proteins using polyethylene glycol precipitation and anion-exchange chromatography

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ABSTRACT

Obtaining several proteins from chicken egg whites from a single extraction in a continuous process is imperative because there are potential applications for egg white proteins in the pharmaceutical and food industries. In this study, chicken egg whites were divided into four components using polyethylene glycol precipitation. All precipitates, except for ovomucin (Precipitate A), were further purified through Q Sepharose Fast Flow anion-exchange chromatography. Lysozyme, ovotransferrin, ovalbumin and ovoflavoprotein were purified from Precipitate B, Precipitate C and Supernatant D, respectively, which were determined to be 91.84%, 94.55%, 96.45% and 88.16% pure, respectively, by HPLC. The results showed that this method is feasible for the co-purification of major egg white proteins with high purity. This method is scientifically practical and easy to use and thus will suitable for industrial-scale production.

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1. Introduction

Chicken egg whites are one of the most ideal sources of active proteins, and most of the identified chicken egg white proteins possess significant biological activities. An increasing number of studies have focused on the biological activities of egg white proteins, including iron-chelation, antimicrobial activity, protease inhibition, immunoregulation, and antioxidant activity [1]. The purity of the proteins from the egg white matrix is therefore very important when studying their bioactivities. A method to highly purify egg white proteins is urgently needed for applications involving egg white proteins.

Different methods, such as salt precipitation, isoelectric precipitation, ultrafiltration and liquid chromatography, have been applied for separating and purifying egg white proteins. Separating proteins via salt precipitation is widely used, but the proteins must be desalted before ion-exchange chromatography. Isoelectric precipitation is not effective for separating egg white proteins because the isoelectric points of most egg white proteins are similar. Ultrafiltration is also a technique that has usually been used for separating egg white proteins, but membrane fouling is a pervasive problem, especially for proteins with ovomucin [2–4]. Purifying proteins through liquid chromatography allows high selectivity without protein denaturation. The well-known egg proteins, such as lysozyme, ovalbumin, ovotransferrin and ovoflavoprotein, are mainly obtained through anion-exchange chromatography [5–8].

However, until now, most of the separation and purification methods had been developed for only one or two individual types of egg white proteins rather than a co-purification process. A method to fractionate and purify the major egg white proteins is needed for laboratory-scale preparation and industrial production. Wu et al. obtained ovomucin by using a two-step precipitation method with NaCl solutions [9], and the suspensions were further separated into several proteins by two-step ion exchange chromatography [10]. Another co-extraction process for ovalbumin, ovotransferrin, ovomucoid and lysozyme from egg whites was described by Tankrathok et al. [11]. But the purity of proteins obtained by these methods [10,11] was dissatisfactory; most components were mixed with ovalbumin, perhaps because the high concentration of ovalbumin impaired selectivity.

In this study, we attempted to separate ovomucin and most of the ovalbumin from the other egg white proteins using polyethylene glycol (PEG) precipitation. Ovomucin was then isolated from Precipitate A, and anion-exchange chromatography of Precipitate B, Precipitate C and Supernatant D with Q Sepharose Fast Flow yielded four other proteins: lysozyme (LYZ), ovalbumin (OVA), ovotransferrin (OVT) and ovoflavoprotein (OVF). Unlike salt precipitation, desalination was not required before anion-exchange chromatography, and PEG was conducive to maintaining the bioactivity of proteins. Moreover, Q Sepharose Fast Flow is an industrial-scale support, which makes this method suitable for scaled up production.

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2. Experiment

2.1. Materials

Fresh eggs laid within 24 h from Hy-Line Brown were collected from a local hennery (Jiufeng farm, Wuhan, China) and used within the same day for the experiment. Polyethylene glycol 8000 (PEG-8000) was made by Merck Chemicals Co., Ltd. (Shanghai China). Q Sepharose Fast Flow was supplied by RuiDaHengHui S&T Co., Ltd. (Beijing, China). The reagents used in SDS–PAGE were purchased from Guge Biotechnology Co., Ltd. (Wuhan, China). Prestained protein molecular weight markers were supplied by Fermentas China Co., Ltd. (Shenzhan, China). The standard lysozyme, ovalbumin, ovotransferrin and ovoflavoprotein proteins were obtained from Sigma (St. Louis, MO, USA). All other chemicals used in the experiment were produced by SINOPHARM Chemical Reagent Co., Ltd (Shanghai, China).

2.2. PEG precipitation

All separation procedures were carried out in an ice water bath (2–5 °C). Egg whites (270 g) were stirred for 2 h with 3 volumes of 50 mM NaCl solution to uniformity. The pH of the solution was adjusted to 6.0 with 2 M HCl, and PEG-8000 was added while stirring (final w/w = 3%). The dispersion was allowed to sit for 2 h, and the homogenate was centrifuged at 15,000 × g (Sigma, Laborzentrifugen, 3–30 K, Germany) at 4 °C for 10 min. The precipitate (Precipitate A) was collected, and the supernatant was adjusted to 9% (w/w) by adding PEG-8000. Precipitate B (the precipitate of 3–9%) was obtained by centrifugation at 12,000 × g for 10 min at 4 °C. By increasing the PEG-8000 concentration in the supernatant to 15%, Precipitate C (the precipitate of 9–15% PEG-8000) and Supernatant D (the supernatant of 15% PEG-8000) were separated after centrifugation at 12,000 × g for 10 min at 4 °C. Precipitates A, B and C were lyophilized and stored at −20 °C, and Supernatant D was stored at 4 °C. The components of these four samples were identified by SDS–PAGE analysis.

2.3. Purification of ovomucin from Precipitate A

The purification of ovomucin was modified from Omana's [9] method. We obtained an ovomucin-rice fraction (Precipitate A) with a lower concentration of NaCl solution and with the adoption of 3% PEG-8000. Precipitate A was suspended in 500 mM NaCl solution with stirring for 4 h at 4 °C. After centrifugation at 15,000 × g for 10 min at 4 °C, the precipitate was collected and then washed three times by suspending it in distilled water and centrifuging (15,000 × g at 4 °C for 10 min). The final precipitation was lyophilized and stored at −20 °C. Protein concentration of ovomucin was determined according to BCA method, and the purity was analyzed with SDS–PAGE by Gel-Pro Analyzer Version 4.0.

2.4. Purification of proteins from Precipitate B, Precipitate C and Supernatant D

The Precipitate B (106 mg) was suspended in Tris–HCl buffer (pH 8.0, 20 mM) for 2 h and then centrifuged at 12,000 × g at 4 °C for 10 min to remove any insoluble material. The sample was applied to a Q Sepharose Fast Flow column (50 × 1.6 cm, Automatic low pressure liquid chromatography system, JiaPeng Technology Co., Ltd., Shanghai, China), which was equilibrated with the same buffer. The flow-through fraction was eluted using Tris–HCl buffer (pH 8.0, 20 mM), followed by isocratic elution using 20 mM Tris–HCl buffer (pH 8.0) successively containing 0.08, 0.18 and 0.30 M NaCl, at a flow rate of 2 mL/min. Each peak was pooled and dialyzed against distilled water four times and then lyophilized and stored at −20 °C. Protein concentrations of each peak were determined according to Bradford's method with bovine serum albumin as a standard (Microplate Reader, Bio-Rad), and the components of these peaks were identified by SDS–PAGE analysis.

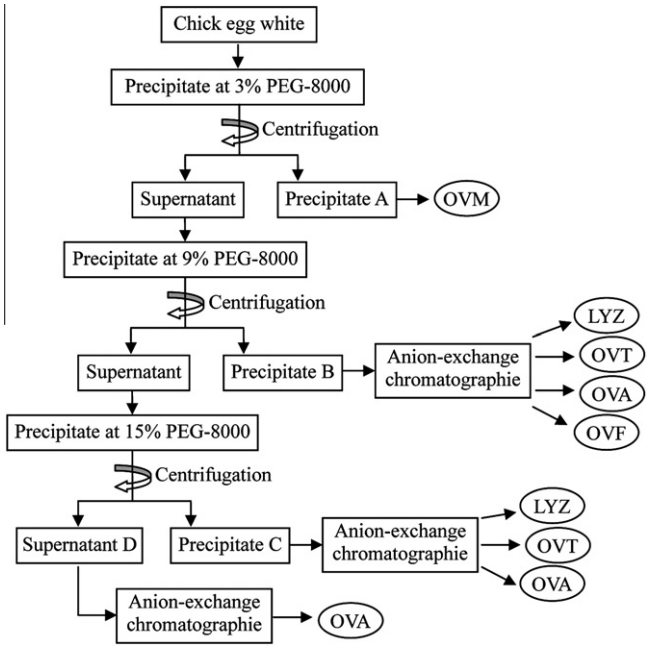

Fig. 1. The scheme of the entire purification process. OVM: ovomucin; LYZ: lysozyme, OVT: ovotransferrin; OVA: ovalbumin; OVF: ovoflavoprotein.

Proteins from Precipitate C (104 mg) were also separated using the same method. In spite of the high concentration of PEG-8000, Supernatant D (10.5 mL) was injected into chromatographic column directly because PEG-8000 neither influences separation process nor interacts with the filler.

The scheme of the entire purification scheme is shown in Fig. 1.

2.5. SDS–PAGE

A 12% polyacrylamide gel was employed for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) (DYY-12, Beijing Liuyi Instrument Factory). The amount of protein loaded was approximately 4–8 μg of sample. PageRuler™ Plus Prestained Protein Ladder (#SM1811, Fermentas Instrument Inc.) with high-range molecular weight markers was loaded for comparison of the molecular weights. The protein bands were stained with Coomassie Brilliant Blue R-250 (0.1% in the staining solution, containing 25% ethanol and 8% acetic acid) for 30 min at 45 °C and destained by diffusion in destaining solution (25% ethanol and 8% acetic acid) [12].

2.6. Purity determination by using HPLC

Reverse phase (RP) chromatography was performed on a Grace VydacC4 (214TP, 5 μm, 250 × 4.6 mm) column with a Waters 2695 Separations Module. A linear gradient of acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA) was used for elution, at a flow-rate of 1.0 mL/min. The linear gradient elution increased from 5 to 80% ACN in 15 min, decreased to 5% ACN in 1 min, then remained at a constant concentration for 4 min. The temperature of the column was maintained at 35 °C. Detection was carried out at 280 nm with a Waters 2696 photodiode array detector.

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