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Short communication

One-step purification of proteins from chicken egg white using counter-current chromatography

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Abstract

Proteins present in chicken egg white are separated by counter-current chromatography (CCC) in one step using a cross-axis coil planet centrifuge (X-axis CPC). The separation was performed with an aqueous polymer two-phase system composed of 16% (w/w) poly(ethylene glycol) 1000 and 12.5% (w/w) dibasic potassium phosphate by eluting the lower phase at a flow-rate of 1.0 ml/min. From about 20 g of the crude egg white solution, lysozyme, ovalbumin, and ovotransferrin were resolved within 5.5 h. Each component was identified by 12% SDS gel electrophoresis with Coomassie brilliant blue staining.

Keywords: Counter-current chromatography; Aqueous polymer two-phase system; Proteins

1. Introduction

Recently, several types of cross-axis coil planet centrifuges (X-axis CPCs) have been designed for performing counter-current chromatography (CCC) using highly viscous polymer phase systems [1]. The apparatus equipped with column holders mounted in the off-center position on the rotary shaft such as types XL, XLL and L allows satisfactory retention of the stationary phase of aqueous–aqueous polymer phase systems including poly(ethylene glycol) (PEG) 1000–potassium phosphate buffer and PEG 8000–dextran T500 systems. This beneficial effect may be attributed to the strong centrifugal force field acting across the diameter of the column. Using aqueous polymer phase systems, these X-axis CPCs have been applied to the separation and purification of a variety of protein samples including a mixture of cytochrome *c*, myoglobin, ovalbumin and hemoglobin [2], histones and serum proteins [3], recombinant uridine phosphorylase from *Escherichia coli* lysate [4], human lipoproteins from serum [5–7], lactic acid dehydrogenase from a crude bovine heart extract [8] and profilin–actin complex from *Acanthamoeba* extract [9].

This paper describes the separation of three protein components from chicken egg white by the type-XL X-axis CPC using polymer two-phase sys-

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tems composed of 16% (w/w) PEG 1000–12.5% (w/w) potassium phosphate buffers at optimized pH. The nature of the proteins in the eluted fractions was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2. Experimental

2.1. Apparatus

The CCC purification of proteins from chicken egg white was performed using type-XL X-axis CPC. The photograph and the horizontal cross-sectional view of the apparatus are shown in Fig. 1A and B. The apparatus holds a pair of horizontal rotary shafts symmetrically, one on each side of the rotary frame, at a distance of 10 cm from the central axis of the centrifuge. A spool-shaped column holder is mounted on each rotary shaft at a lateral location 10 cm away from the midpoint. Each multilayer coil was prepared from 2.6 mm I.D. polytetrafluoroethylene (PTFE) tubing (Zeus Industrial Products, Raritan, NJ, USA) by winding it onto a 5 cm diameter holder hub making three layers of left-handed coils between a pair of flanges spaced 5 cm. A pair of columns was serially connected on the rotary frame using a flow tube (PTFE, 0.85 mm I.D.) to give a total column capacity of 165 ml. The rotation speed of the apparatus is maintained at 500 rpm with a speed control unit (Bodine Electric, Chicago, IL, USA).

2.2. Reagents

Poly(ethylene glycol) 1000 and anhydrous potassium phosphate were purchased from Kanto (Tokyo, Japan). The protein standards including ovotransferrin, ovalbumin and lysozyme, were all obtained from Sigma (St. Louis, MO, USA).

2.3. Procedures

The aqueous two-phase polymer system was prepared by dissolving 192 g of PEG 1000 and 150 g of anhydrous potassium phosphate in 858 g of distilled water. The pH of the solvent system was adjusted from 6.8 to 9.2 by choosing the appropriate ratios between monobasic and dibasic potassium phosphates. These solvent systems provide approximately equal volumes of upper and lower phases. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

The partition coefficients of three protein components, ovotransferrin, ovalbumin and lysozyme, were determined spectrophotometrically by the following procedure using the above two-phase solvent system at various pH values: about 1.5 ml of each phase was delivered to a test tube to which 1 mg of the standard protein was added. The contents were thoroughly mixed and allowed to settle at room temperature. After two clear layers were formed, an aliquot (usually 1 ml) of each phase was removed and diluted with 1 ml of distilled water to determine the absorbance at 280 nm using a Shimadzu UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). The partition coefficients ($K=C_U/C_L$, where C_U and C_L are the solute concentrations in the upper and the lower phases, respectively) were calculated by dividing the absorbance value in the upper phase with that of the lower phase.

The sample solution was prepared from fresh chicken egg white according to the method described by Awade et al. [10] as follows: one volume of fresh egg white was mixed with two volumes of 0.05 *M* Tris–HCl (pH 9.0) containing 0.4 *M* NaCl and 10 m*M* β-mercaptoethanol and the mixture was gently stirred overnight. The sample solution for CCC was then prepared by adding 4.5 g of PEG 1000 and 3.5 g of anhydrous dibasic potassium phosphate to 20 g of the egg white solution to meet the composition of the solvent system used for the separation.

The separation was initiated by filling the entire column with the PEG-rich upper stationary phase. This was followed by injection of the sample solution (28 g) through the sample port. Then the apparatus was rotated at 500 rpm while the phosphate-rich lower phase was pumped into the column at a flow-rate of 1.0 ml/min in the proper elution mode [11]. The effluent from the outlet of the column was continuously monitored with an Eyela UV-9000 absorbance monitor (Tokyo Rikakikai, Tokyo, Japan) at 280 nm and fractionated using a

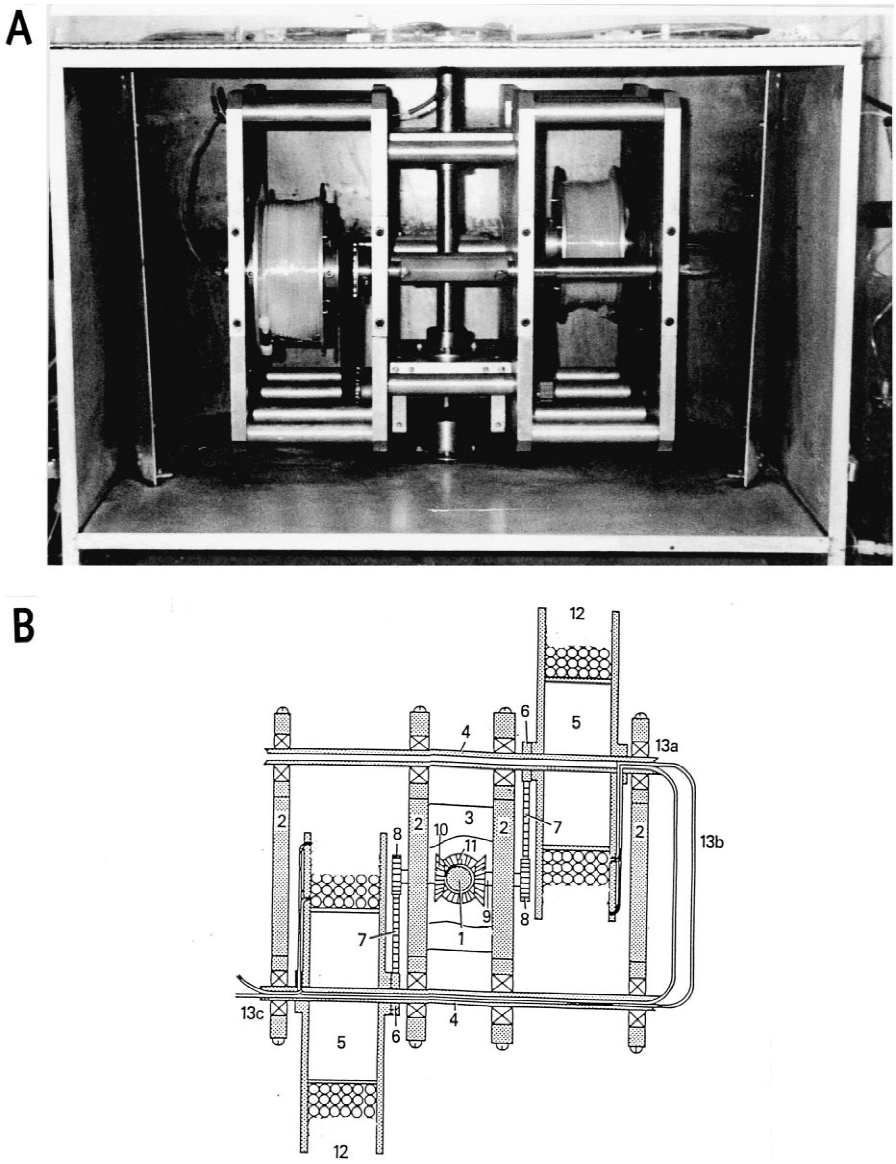

Fig. 1. Photograph (A) and horizontal cross-sectional view (B) of type-XL cross-axis coil planet centrifuge. 1= Central axis; 2=side plates; 3=bottom plate; 4=column holder shafts; 5=column holders; 6, 8=toothed pulleys; 7=toothed belts; 9=countershafts; 10=planetary miter gears; 11=stationary miter gear; 12=multilayer coils; 13a to c=flow tubes.

Bio-Rad Model 2110 fraction collector (Bio-Rad, Richmond, CA, USA). Fractionated proteins were confirmed by SDS-PAGE with Coomassie brilliant blue staining according to the method of Laemmli [12].

3. Results and discussion

In Fig. 2 the partition coefficients (K) of three standard proteins, i.e., ovotransferrin, ovalbumin and lysozyme, are plotted against the pH of the two-

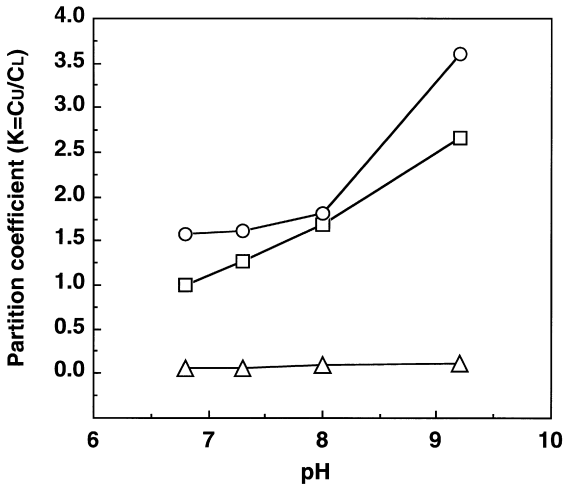

Fig. 2. Partition coefficients (K) of ovotransferrin (△), ovalbumin (□) and lysozyme (○) in the 16% (w/w) PEG 1000 and 12.5% (w/w) potassium phosphate at various pH values.

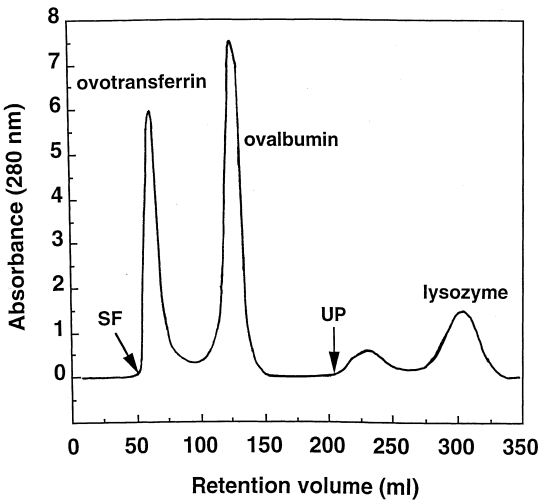

Fig. 3. Purification of chicken egg white proteins using the PEG 1000–potassium phosphate system. Experimental conditions: apparatus: the XL cross-axis coil planet centrifuge with a pair of multilayer coil columns of 2.6 mm I.D. and a 165-ml total capacity; solvent system: 16% (w/w) PEG 1000–12.5% (w/w) potassium phosphate (pH 9.2); stationary phase: PEG-rich upper phase; mobile phase: phosphate-rich lower phase; sample: 28 g of chicken egg white solution as described in Section 2.3); flow-rate: 1.0 ml/min; revolution: 500 rpm; SF=solvent front; UP=upper phase eluted in the reversed direction.

phase polymer system. The K values for ovalbumin and lysozyme show a steady decrease with the decrease in pH. On the other hand, the ovotransferrin always distributed to the potassium phosphate-rich lower phase. An evenly scattered ideal distribution of K values for these three proteins is found at pH 9.2.

The capability of the X-axis CPC was demonstrated on the separation of these proteins from fresh chicken egg white by eluting the 28 g of the sample solution with the lower phase at a flow-rate of 1.0 ml/min. As shown in Fig. 3, ovotransferrin and ovalbumin were eluted from the column in the reverse order of their K values as expected (because of the lower mobile phase). After the elution of these two protein peaks, the upper phase which was initially used as the stationary phase was eluted through the column in the reversed direction to facilitate a rapid elutions of the ovomucin and the lysozyme still retained in the column. The upper stationary phase retained in the column was estimated as 65% of the total column capacity (165 ml) prior to the application of the reversed elution with upper phase. The separation was completed within 5.5 h.

Fig. 4 shows the SDS-PAGE patterns of CCC fractions corresponding from each peak. Fractions 20 and 40, collected from the center of the first and second peaks in the chromatogram (Fig. 3), contained ovotransferrin and ovalbumin which migrated to the respective positions in lanes 2 and 3. The third peak must be ovomucin containing ~1.5% of the egg white proteins. It is impossible to detect the ovomucin in the CCC fractions by 12% SDS-PAGE

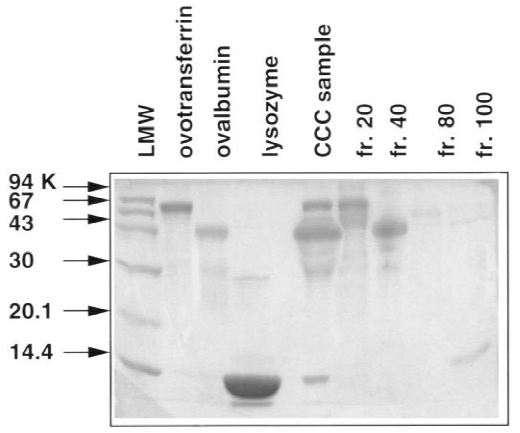

Fig. 4. 12% SDS-polyacrylamide gel electrophoretic profile of the CCC fractions.

because of the high molecular mass of this protein. The fourth peak in the chromatogram is lysozyme which was detected at the spot corresponding to the standard sample in lane 4.

Since CCC performs separations without the solid support matrix, adsorptive loss and denaturation of protein at the liquid–solid interface are minimized compared with the column chromatographic methods. These results indicate that the present method is capable of purifying proteins from the crude sample solution prepared from fresh egg white in a one-step operation in a relatively short elution time. We think that the method may also be effectively used for separation and purification of other proteins.

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