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Purification of single-strand DNA binding protein from an *Escherichia coli* lysate using counter-current chromatography, partition and precipitation

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Abstract

Single-strand DNA binding protein (SSB) from *Escherichia coli* lysate was purified by counter-current chromatography (CCC) using the ammonium sulfate precipitation method in a coiled column. About 5 ml of *E. coli* lysate was separated by CCC using a polymer phase system composed of 16% (w/w) polyethylene glycol (PEG) 1000 and 17% (w/w) ammonium sulfate aqueous polymer two-phase solvent system. The precipitation of proteins in the lysate took place in the CCC column, and the SSB protein was eluted in the fraction 51–56. Many other impurities were either eluted immediately after the solvent front or precipitated in the column. The identities of the proteins in the fractions and in the precipitate were confirmed by SDS–polyacrylamide gel electrophoresis with Coomassie Brilliant Blue staining.

Keywords: Purification; Proteins, single-strand DNA binding; Single-strand DNA

1. Introduction

In general, single-strand DNA binding protein (SSB) from *E. coli* is purified using repeated precipitation with ammonium sulfate and affinity and/or gel filtration chromatography methods [1]. However, CCC is able to carry out such separation without a solid matrix so that adsorptive loss and denaturation of proteins are minimized [2,3].

This paper describes preliminary results of purification of the SSB protein using the cross-axis synchronous flow-through coil planet centrifuge (cross-axis CPC) [4,5]. Recently, this system has been improved in terms of retention of the stationary phase. The apparatus is designed to accommodate a pair of small column holders each at a lateral location 10 cm from the center of the holder shaft. As reported elsewhere [6], this type or cross-axis

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CPC has the unique capability of retaining large amounts of stationary phase even with the low-interfacial tension, viscous solvent systems used for separation of proteins.

2. Experimental

2.1. Apparatus

The detailed design of the cross-axis coil planet centrifuge (cross-axis CPC) has been described earlier [4,5] and a photograph of the apparatus is shown in Fig. 1. 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 position 10 cm 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 15.2-cm diameter holder forming multiple layers of left-handed coils between a pair of flanges spaced 5 cm apart. The columns consist of four layers of the coil with a 170-ml capacity. A pair of columns mounted on the rotary frame was connected in series to make up a total capacity of 340 ml. The total column length is 68 m calculated from the internal diameter of the coil and column capacity. Both inflow and outflow tubes exit together at the center of the top plate of the centrifuge case where they are tightly supported with a silicon-rubber-padded clamp. The rotatory speed of the apparatus is regulated at 500 rpm with a speed control unit (Bodine Electric Company, Chicago, IL, USA).

2.2. Preparation of solvent system and sample solution

The aqueous polymer phase system was prepared by dissolving 160 g of PEG 1000 (Kanto Chemicals, Tokyo, Japan) and 170 g of ammonium sulfate (Kanto Chemicals) in 670 g of distilled water. This composition yielded approximately equal volumes of upper and lower phases. The solvent mixture was throughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

The cell lysate containing SSB protein was pre-

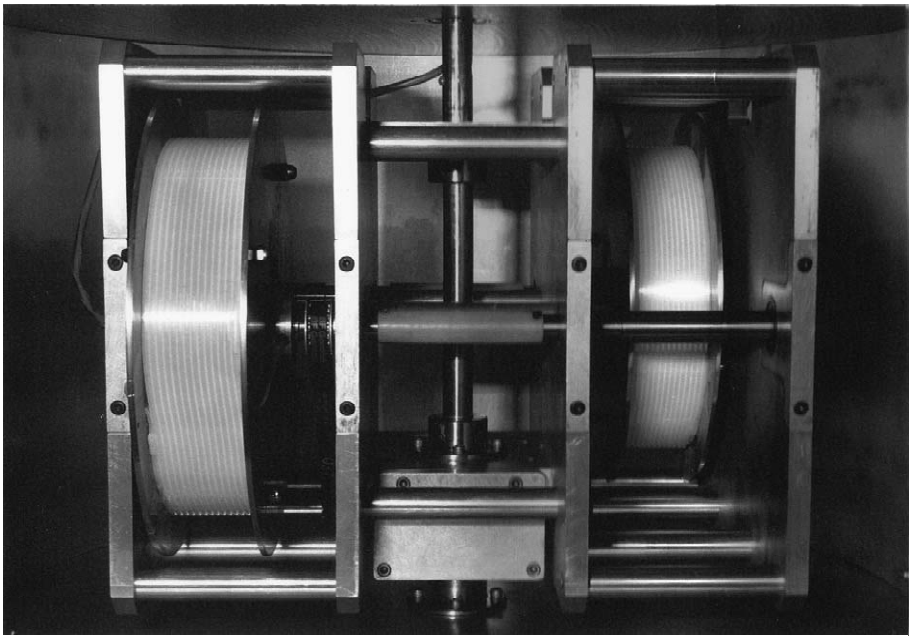

Fig. 1. Photograph of type-XL cross-axis coil planet centrifuge.

pared according to the method of Kinebuchi et al. [7]. The crude extract was used as the CCC sample. The sample solution was prepared by mixing 5 ml of crude extract and 2 ml each of the upper and the lower phase to which proper amounts of PEG and ammonium sulfate were added to adjust the two-phase composition.

2.3. CCC separation procedure

The separation column was first entirely filled with the ammonium sulfate-rich lower phase followed by sample injection using an EYELA type SV-6000 sample injector (Tokyo Rikakikai, Tokyo, Japan). Then the PEG 1000-rich upper phase was pumped into the column at 0.5 ml/min while the apparatus was rotated at 500 rpm in the proper elution mode [4]. The effluent from the outlet of the column was continuously monitored with an ISCO UA-5 absorbance monitor (Instrumentation Specialties, Lincoln, NE, USA) at 280 nm and fractionated into a fraction collector (Instrumentation Specialties). Each peak fraction contained a white precipitate.

2.4. Analysis of CCC fractions

The fractions were centrifuged at 1000 rpm for 10 min. Then the supernatant from each fraction was diluted with distilled water and the absorbance was determined at 280 nm with a Shimadzu UV-1200 spectrophotometer. The fractions were placed into dialysis tubing (Spectro/Por, molecular mass cut-off 3500 Da; Spectrum Medical Industries, Los Angeles, CA, USA) and immersed in an aqueous 30% (w/w) PEG 8000 solution. After 5–6 h of dialysis, the fraction was concentrated to 0.5 ml by evaporation in vacuum. The SBB proteins in each fraction were confirmed by 12.0% SDS–polyacrylamide gel electrophoresis (PAGE) [8]. The precipitate in each fraction was dissolved in a small amount of distilled water and was also confirmed by SDS–PAGE.

3. Results and discussion

SSB protein, which binds DNA, has become of interest in terms of maintaining DNA in a single-stranded form during replication, recombination and repair. Currently, SSB protein is produced on a large scale by recombinant DNA technology. The repeated precipitation method and liquid chromatographic techniques using phosphocellulose and Sephacryl S-200 column packings, have been applied for purification of SSB protein from *E. coli* cell lysate [7].

In this study, the use of counter-current chromatography (CCC) with an aqueous two-phase solvent system eliminates all complications arising from the solid support materials. The use of the cross-axis coil planet centrifuge was demonstrated by purification of the SSB protein by eluting 5 g of the lysate with PEG-rich upper phase of the polymer phase system composed of 16% (w/w) PEG 1000 and 17% (w/w) ammonium sulfate. As shown in Fig. 2, the SSB protein was eluted at fractions 51–55 together with the other proteins as a peak shoulder. The separation was completed within 12 h and the volume of the lower stationary phase retained in the column was about 30% of the total column capacity (340 ml). Fig. 3 shows the SDS–polyacrylamide gel electro-

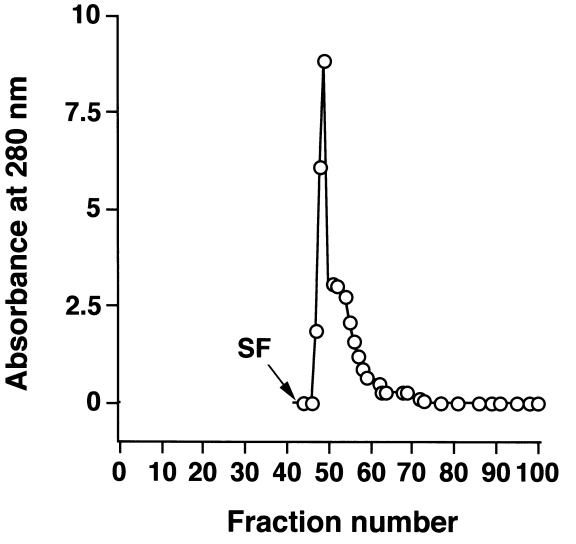

Fig. 2. Counter-current chromatographic purification of SSB proteins from *E. coli* lysate. Experimental conditions: apparatus: type-XL cross-axis coil planet centrifuge with a pair of multilayer coil columns of 2.6 mm I.D. and 340 ml total capacity; solvent system: 16% (w/w) PEG 1000–17% ammonium sulfate; stationary phase: ammonium sulfate-rich lower phase; mobile phase: PEG 1000-rich upper phase; sample: 5 g of *E. coli* lysate; flow-rate: 0.5 ml/min; revolution speed: 500 rpm; SF=solvent front.

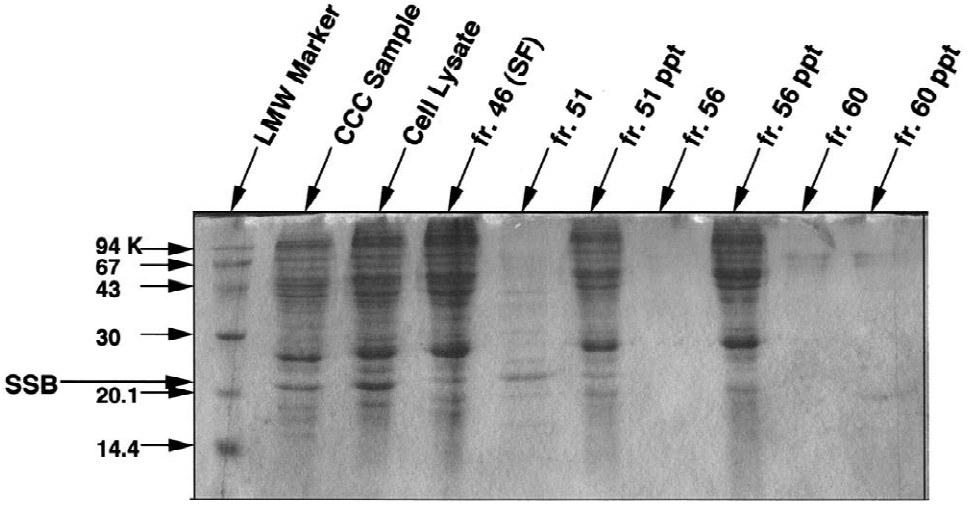

Fig. 3. 12% SDS–polyacrylamide gel electrophoretic profile of the CCC fractions.

phoretic patterns of each peak. The fractions 51–56, corresponding to center cuts of the second shoulder peak in the chromatogram (Fig. 2), contained SSB protein. The precipitate produced in fractions 51–56 was dissolved in a small amount of distilled water and analyzed by SDS–PAGE. The precipitate in fraction 51 had a small amount of SSB protein band and it consisted of other types of proteins present in the cell lysate.

The mechanism of the present separation may be interpreted as follows. Both PEG and ammonium sulfate are well-known precipitants for proteins [9]. The ammonium sulfate is considered the more effective precipitant than PEG in the solvent system used in this experiment. Among many proteins present in the cell lysate, the target protein, SSB, has relatively high solubility against both precipitants and therefore is retained in the column longer as a solute. Many other proteins less soluble in ammonium sulfate (ca. 35% or 50% saturation) are partitioned into the upper PEG-rich phase and eluted immediately after the solvent front. Other proteins with poor solubility in both precipitants are precipitated and retained longer in the column. Although these proteins contaminate the SSB fractions, they are easily removed by centrifugation after collecting the fractions.

Prior to CCC separation of SSB protein from lysate using an aqueous two-phase system containing ammonium sulfate, we tried the purification using a solvent system composed of 16% PEG 1000–12.5% potassium phosphate which has conventionally been used for the separation of several proteins. However, SSB protein was not separated from other proteins in the cell lysate (data not shown). It seems that the combined use of partitioning and precipitation in the present method is important in the CCC purification of the SSB protein.

One important issue related to the present purification method of SSB protein is how to remove the PEG 1000 from the purified SSB fraction. One effective procedure to remove PEG is to pass the CCC fractions through the Sephacryl S-200 column and recover the SSB protein subsequently.

Aqueous polymer two-phase systems consisting of PEG and specific salts of phosphate or sulfate offer a number of advantages in purification of proteins. In conclusion, the cross-axis CPC is capable of providing adequate retention of aqueous polymer two-phase systems. Its use in purifying of SSB protein was demonstrated in this study.

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