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Aqueous–aqueous two-phase systems composed of low molecular weight of polyethylene glycols and dextrans for counter-current chromatographic purification of proteins

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Abstract

New aqueous–aqueous two-phase systems composed of relatively low molecular weight polymers such as polyethylene glycol (PEG) (Mr: 1000–4000) and dextran (Mr: 10,000 and 40,000) were evaluated for purification of proteins by counter-current chromatography (CCC). The compositions of aqueous two-phase systems were optimized by measuring parameters such as viscosity and volume ratio between the two phases. CCC purification of a glucosyltransferase (GTF) from *Streptococcus mutans* (SM) cell-lysate was successfully demonstrated with a 7.5% PEG 3350–10% dextran T40 system containing 10 mM potassium phosphate buffer at pH 9.0. After CCC purification, both PEG and dextran contained in the CCC fractions were easily removed by ultrafiltration in a short period of time. The fractionated column contents containing GTF were analyzed by enzymatic activity as well as sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The recovery of the enzyme from CCC fraction was over 95% as estimated by enzymatic activities.

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1. Introduction

Aqueous–aqueous polymer phase systems for the partitioning of biological macromolecules were first established by Albertsson in the 1950s [1]. Among many types of aqueous–aqueous polymer phase systems available, polyethylene glycol (PEG)–dextran and PEG–potassium phosphate systems have been most commonly used for the partition of biological samples. Using these solvent systems, several different approaches have been made for performing purification of biological samples, such as single step partitioning, repetitive batch extraction, counter-current distribution (CCD) [2,3] and counter-current chromatography (CCC) [4]. This method has been termed after two classic partition techniques, that is, the CCD and liquid chromatography. The flow-through centrifuge systems [5,6] have been developed for performing CCC and provide various advantages for continuous elution through a rotating column. Among these techniques, CCC is considered to be most effective in terms of partition efficiency and separation times.

CCC is a form of liquid–liquid partition chromatography. The unique feature of CCC among other chromatographic systems is derived from the fact that the method uses no solid support and the stationary phase is retained in the column by an Archimedes screw effect and the centrifugal force. Consequently, the system eliminates various complications arising from the use of solid supports. The cross-axis coil planet centrifuge (CPC) allows satisfactory levels of stationary phase retention for the aqueous–aqueous polymer phase systems so that it can be effectively used for the preparative separation of proteins [7,8]. With PEG–potassium phosphate systems, the cross-axis CPC has

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been successfully used for the separations of a variety of protein samples, including a mixture of cytochrome *c*, myoglobin, ovalbumin and hemoglobin [9], human plasma lipoproteins (HDL, LDL and VLDL) [10–12], cholinesterase from human serum [13], lactic acid dehydrogenase from bovine heart crude extract [14,15], alcohol dehydrogenase from bovine liver crude extract [16], various recombinant enzymes [17,18] from *E. coli* lysate. Single-strand DNA binding protein was also purified by one step operation of CCC from an *E. coli* lysate with a combined use of partition and precipitation method [19]. Because of the high salts concentration of the PEG–potassium phosphate and PEG–ammonium sulfate systems, however, these aqueous two-phase systems are not suitable for the separation of proteins which are easily precipitating out by these salts. In this case, we have to use other types of aqueous–aqueous polymer two-phase systems composed of PEG and dextran which form two phases without an addition of salts. In the past the CCC separations of basic histones, serum proteins [20], and profilin-actin complex from crude Acanthamoeba extract [18] were performed using PEG–dextran two-phase systems. Recently, we have demonstrated the purification of glucosyltransferase (GTF) from *S. mutans* cell-lysate and *S. sobrinus* culture medium [21,22] using a 7.7% PEG 8000–4.0% dextran T500 solvent system. After CCC purification, these polymers were removed from CCC fractions by hydroxyapatite chromatography. However, due to irreversible adsorption onto the hydroxyapatite (HA) column packings GTF was lost about 10% from *S. mutans* samples and 54% from *S. sobrinus* samples.

In this study, we have developed new aqueous–aqueous two-phase systems composed of relatively low molecular weight of PEGs and dextrans, which are easily removed from the CCC fractions by ultrafiltration in a short time. The purification of GTF which tends to be precipitated out from high salt concentration two-phase system, such as 16% PEG 1000–12.5% potassium phosphate system, was performed using the solvent system composed of relatively low molecular weight of PEG and dextran by CCC without loss of activity and high recovery.

2. Experimental

2.1. Apparatus

The CCC purification of GTF was performed using the type-XL cross axis CPC fabricated at the machine shop of National Institutes of Health, Bethesda, MD, USA. 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 spood-shaped column holder is mounted on each rotary shaft at a lateral location 10 cm away from the midpoint. The separation column was prepared from 2.6 mm I.D. and 10.4 m long polytetrafluoroethylene (PTFE) tubing (Tokyo Rikakikai Co. Ltd., Tokyo, Japan) by winding it onto a 5 cm diameter holder hub, making two layers of left-handed coils between a pair of flanges spaced 5 cm apart. A pair of columns mounted on the rotary frame was connected in series to provide a total capacity of 110 ml. The revolution speed of the apparatus is regulated at 400 rpm with a speed control unit (Bodine Electric, Chicago, IL, USA).

2.2. Reagents and bacterial strain

Polyethylene glycol 1000, 1540, 2000, 4000 and 8000 (average molecular mass, Mr: 1000, 1300–1600, 1850–2150, 2700–3400 and 8000, respectively), and monobasic and dibasic potassium phosphate were purchased from Kanto Chemicals (Tokyo, Japan). Dextran T10 (weight average molecular mass, Mr: 10,000) and T40 (Mr: 40,000) were obtained from Amasham Biosciences AB (Uppsala, Sweden) and PEG 3350 (Mr = 3000–7000) from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were of analytical reagent grade.

S. mutans MT8148 (sero type c) was kindly provided by Dr. I. Nasu from Nihon University, School of Dentistry at Matsudo, Japan.

2.3. Preparation of S. mutans cell-lysate

S. mutans MT8148 (SM) was grown for 24 h at 37 °C in 3 l of TTY broth [23] composed of 1.5% (w/v) trypticase soy broth (BD, MD, USA), 0.4% (w/v) bacto tryptose (BD), 0.4% (w/v) yeast extract (Sigma, MO, USA), 0.2% dibasic potassium phosphate, 0.4% (w/v) monobasic potassium phosphate, 0.2% (w/v) sodium carbonate, 0.2% (w/v) sodium chloride and 1.0% (w/v) glucose. The cultured SM cells were concentrated by centrifugation at 3500 × *g* and rinsed three times with 10 mM phosphate buffered saline (PBS; pH 7.4). After a sonication of 1 g (wet weight) of SM cells with 10 ml of PBS, the solution was centrifuged at 25,000 × *g*, and the supernatant was filtered through a DISMIC 13HP filter cartridge (Advantec Toyo, Tokyo, Japan). The filtrate was used as the SM cell-lysate for the subsequent studies.

2.4. Measurement of GTF enzymatic activity

Enzymatic activity of GTF was estimated from the amount of water-insoluble glucan (WIG) produced from sucrose by GTF [24]. For a rapid assay of crude GTF solution such as the SM cell-lysate and its chromatographic fractions, 100 μl of sample solution was incubated in 2 ml of 0.5 M potassium phosphate buffer (pH 6.0) containing 1% (w/v) sucrose and 0.05% (w/v) sodium azide in the presence of primer dextran T10 (20 μl) for 18 h at 37 °C. After incubation, the amount of WIG in the mixture was subjected to nephelometry for determination of the increased absorbance at 550 nm using a V-530 UV–vis spectrophotometer (JASCO, Tokyo, Japan). In addition, for the determination of specific activity of the purified GTF, an aliquot of WIG was measured by the phenol-sulfate method [25]. A 10 μl amount of the purified GTF solution was incubated in 2 ml of 0.1 M potassium phosphate buffer (pH 6.0) containing 1% (w/v) sucrose and 0.05% (w/v) sodium azide for 18 h at 37 °C. After incubation, the mixture was centrifuged at 19,000 × *g* for 10 min at 4 °C. The precipitated WIG was rinsed with 50% (v/v) ethanol containing 0.1 M potassium phosphate buffer (pH 6.0) three times and sonicated with 0.5 ml of 1 M NaOH solution for

20 min. The solution was incubated with 0.5 ml of 5% (w/v) phenol solution and 2.5 ml of concentrated sulfuric acid for 30 min at room temperature. After incubation, the absorbance of the solution was measured at 490 nm. Finally, using a calibration curve made from the standard glucose solutions, the amount of WIG produced by GTF was expressed as the concentration of glucose where one unit (1 U) of GTF was defined as the amount of enzyme required to convert 1.0 μmol of glucose residue of the sucrose molecule into WIG per minute.

2.5. Preparation of aqueous–aqueous two-phase systems composed of PEG and dextran

Counter-current chromatography utilizes a pair of immiscible solvent phases preequilibrated in a separatory funnel: one phase is used as the stationary phase and the other as the mobile phase. The following aqueous two-phase solvent systems were prepared by modifying the standard polymer phase systems used for the protein separation: in order to facilitate removal of the polymers by ultrafiltration after CCC fractionation, low molecular weight of polyethylene glycols (PEGs) and dextrans were used for the two-phase systems as shown in Table 1. For example, 17.5% PEG 1000–20.0% dextran T10 system is prepared by dissolving 175 g of PEG 1000 and 200 g of dextran T10 in 625 g of 10 mM potassium phosphate buffer solution. All these PEG–dextran systems form PEG-rich upper phase and dextran-rich lower phases, which provide high solubility for various proteins due to their low salt concentration.

2.6. Determination of kinematic viscosity of upper and lower phases of aqueous two-phase systems

In the CCC experiments with aqueous two-phase systems, the volume of the stationary phase retained in a column greatly depends on the viscosity of both upper and lower phases. In general the lower the viscosity, the higher retention is obtained. For the selection of suitable solvent systems which provide satisfactory retention of the stationary phase, the kinematic viscosity of upper and lower phases of aqueous two-phase systems was determined using a capillary viscometer [26]: sample solution (upper or lower phase of an aqueous two-phase system) was placed into an Ubbelohde viscometer to measure the time (in second) required for the unit volume of the sample solution to fall down through the capillary. For each polymer system, the kinematic viscosity (mm^2/s) of the upper and the lower phases was calculated.

Table 1
Volume ratio of upper and lower phases of several polyethylene glycol (PEG) dextran T10, 40 aqueous two-phase systems

Aqueous–aqueous two-phase systems[a]	Volume ratio (UP/LP)[b]	Kinematic viscosity (mm^2/s)	
		UP[b]	LP[b]
17.5% PEG 1000–20.0% dextran T10	1.6	6.8	High viscosity
15.0% PEG 1540–17.5% dextran T10	1.7	6.8	High viscosity
12.5% PEG 2000–15.0% dextran T10	1.7	6.3	35
12.5% PEG 3350–15.0% dextran T10	1.7	7.9	40
12.5% PEG 4000–15.0% dextran T10	1.7	7.6	33
10.0% PEG 8000–12.5% dextran T10	1.9	12.0	18
15.0% PEG 1000–15.0% dextran T40	1.6	4.4	High viscosity
10.0% PEG 1540–15.0% dextran T40	0.9	4.5	High viscosity
10.0% PEG 2000–15.0% dextran T40	1.1	4.7	High viscosity
7.5% PEG 3350–10.0% dextran T40	1.1	5.5	20
7.5% PEG 4000–10.0% dextran T40	1.3	5.0	22
5.0% PEG 8000–12.5% dextran T40	1.0	6.7	24

[a] pH at 9.0.
[b] UP: upper phase, LP: lower phase.

2.7. Determination of partition coefficient of SM cell-lysate and GTF

Optimization of the solvent composition to adjust the partition coefficient (K_{GTF}) of the GTF is essential for successful separation. This can be done spectrophotometrically by a simple test tube experiment as described elsewhere [21]. For the determination of partition coefficient of SM cell-lysate (K_{lysate}), the total UV at 220 nm of the proteins in both upper and lower phases were measured with a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). On the other hand, the partition coefficient of GTF in the lysate was determined from the enzyme activity in each phase.

2.8. Determination of dextran T40 concentration by measurement of optical rotation

Dextran T40, the highest molecular weight of the constituent of an aqueous two-phase systems used for this studies, was filtered through an ultrafiltrate filter and the concentration of dextran T40 in the retained and filtered solution are determined. A 1 ml volume of 10% dextran T40 solution was diluted with 10 ml of water and filtered with an ultrafiltration membrane YM 30 (Amicon Co. Ltd.) to 1 ml (residual solution). The optical rotations [α_D] of a 10% dextran T40 solution, a residual solution and a filtrate were measured at 20 °C according to the method described in the Japanese Pharmacopoeia Fifteenth Edition [27].

2.9. Counter-current chromatography of GTF

In each experiment, the CCC column of cross-axis CPC was first entirely filled with the dextran-rich lower stationary phase and the sample solution was injected into the column using an EYELA type SV 6000 sample injector (Tokyo Rikakikai, Tokyo, Japan). The PEG-rich upper phase was eluted through the column at a flow rate of 0.5 ml/min by an EYELA LP-1100 pump (Tokyo Rikakikai), while the apparatus was rotated at 400 rpm. The effluent from the outlet of the column was continuously monitored with an EYELA UV 9000 absorbance monitor (Tokyo Rikakikai) at 280 nm, and fractionated into test tubes using Advantec CHF 100AA fraction collector (Toyo Seisakusho, Chiba, Japan). Most of the proteins in the SM cell-lysate were eluted from the column with the upper PEG-rich upper phase, and GTF still retained in the lower liquid stationary phase was collected by empting the column contents using an EYELA SMP-23 cassette tube pump (Tokyo Rikakikai). Finally,

an aliquot of each fraction was diluted with distilled water and the absorbance measured at 280 nm with a Shimadzu UV-1200 spectrophotometer. The GTF enzyme activity of each fraction was also measured by enzymatic assay described above.

2.10. Analysis of CCC fractions by SDS gel electrophoresis

CCC fractions were diluted with five times volume of 20 mM potassium phosphate buffer solution at pH 7.4 containing 200 mM sodium chloride, 1 mM zinc chloride and 10 mM β-mercaptoethanol. The diluted CCC fractions were filtered through an ultrafiltration membrane to remove PEG and dextran. These fractions were analyzed by 10% sodium dodecyl sulfate slab gel electrophoresis (SDS–PAGE), according to the method of Laemmli [28]: gels containing 3% (w/v) (stacking gel) and 10% (w/v) (separation gel) acrylamide were prepared from a stock solution of 30% (w/v) acrylamide and 0.8% (w/v) *N*,*N*′-methylene-bis acryl amide. Both 5.5 cm × 10 cm separation gel and a 1.0 cm × 10 cm stacking gel, each 0.75 mm thick, were prepared between glass plates. A 5 μl volume of eluate was mixed with 95 μl of sample solution composed of a mixture of 0.025 M tris(hydroxymethyl)aminomethane, 2% (w/v) sodium dodecyl sulfate, 5% (w/v) 2 mercaptoethanol, 4% (w/v) glycerol, and 0.01% (w/v) bromophenol blue (BPB). An aliquot of 10–20 μl of the solution was loaded over the stacking gel. Electrophoresis was proceeded at 10 mA until the BPB marker reached the separation gel. Thereafter, the current was increased to 20 mA and the electrophoresis continued until the BPB marker reached the bottom of the separation gel. The migrated proteins were stained for 5 min at room temperature with a staining solution composed of 0.25% (w/v) Coomassie Brilliant Blue, 50% (v/v) methanol and 10% (v/v) acetic acid. The gel was distained by washing in a mixture of 7.5% (v/v) acetic acid and 2.5% (v/v) methanol.

3. Results and discussion

3.1. Selection of aqueous two-phase system for a separation of GTF

The aqueous two-phase system composed of PEG and dextran has a unique partition behavior in that it tends to distribute small molecules rather evenly between the two phases whereas macromolecules such as DNA and large proteins are mostly partitioned unilaterally in either phase depending upon the pH of the solvent system. Consequently, the system can be effectively applied to the separation of macromolecules using a pH gradient elution or affinity CCC with a suitable ligand in the stationary phase.

Since the PEG–dextran system forms two layers without an addition of salt; it provides high solubility for various protein samples, which would be precipitated out in the standard PEG–phosphate systems due to their high salt concentrations. On the other hand, the PEG–dextran system has a serious setback in its CCC application. A high concentration of dextran and PEG in the system increases the viscosity and a close polarity between the two phases results in extremely low interfacial tension between the two phases, both of which often seriously affect the retention of the stationary phase. In this study, we prepared 30 kinds of PEG–dextran two-phase systems composed of PEGs and dextrans with relatively small molecular weights. Among these solvent systems, twelve PEG–dextran systems with the volume ratio of upper and lower phases ranging from 0.9 to 1.9 were listed in Table 1 together with the kinematic viscosity of each phase. These two-phase systems are prepared at high concentration of PEGs and dextrans compared with the aqueous–aqueous polymer phase systems composed of high molecular weight of polymers, such as PEG 8000 and dextran T500. It seems that the two-phase systems prepared in these studies are high viscosities than PEG 8000–dextran T500 systems.

CCC is a support-free liquid–liquid partition chromatographic technique where the liquid stationary phase is retained in the column by the aid of a centrifugal force field. Therefore, good retention of the stationary phase is critical, and it is greatly affected by the viscocities of both the upper PEG-rich phase and the lower dextran-rich phase. As mentioned earlier, in all these solvent systems the upper phase is rich in PEG and the lower phase is rich in dextran, and the kinematic viscocities (mm^2/s) of the dextran-rich lower phases are always greater than those of the PEG-rich upper phases. High viscositie of the lower phases with over 15% of T40 or over 17.5% of dextran T10 could not be determined by the present method. Relatively low viscosity two-phase solvent systems were obtained from dextran T40 below 12.5% mixed with PEG 3350, 4000 and 8000 below 7.5%. The volume ratio of the upper and the lower phases of 7.5% PEG 3350–10.0% dextran T40, 7.5% PEG 4000–10.0% dextran T40 and 5.0% PEG 8000–12.5% dextran T40 were 1.1, 1.3 and 1.0, respectively. Therefore, we selected these three solvent systems for CCC purification of the GTF from the cell-lysate.

3.2. Measurement of partition coefficient of GTF in PEG–dextran T40 aqueous–aqueous two-phase systems

For achieving efficient purification of GTF, it is essential to optimize the partition coefficient of GTF in two-phase solvent systems by selecting a proper molecular weight of PEGs of the systems. Table 2 lists the partition coefficient of SM cell-lysate and GTF in the three kinds of two-phase systems composed of PEG 3350, 4000 and PEG 8000 with dextran T40 each buffered with 10 mM dipotassium phosphate at pH 9.0. The partition

Table 2
Partition coefficient of SM cell-lysate and GTF in PEG–dextran two-phase systems

Aqueous–aqueous two-phase systems[a]	K_{lysate}[b]	K_{GTF}[c]
5.0% PEG 8000–12.5% dextran T40 (pH 9.0)	0.34	0.10
7.5% PEG 4000–10.0% dextran T40 (pH 9.0)	0.55	0.10
7.5% PEG 3350–10.0% dextran T40 (pH 9.0)	0.56	0.11
7.5% PEG 3350–10.0% dextran T40 (pH 8.0)	0.45	0.11
7.5% PEG 3350–10.0% dextran T40 (pH 7.0)	0.35	0.05
7.5% PEG 3350–10.0% dextran T40 (pH 6.0)	0.30	0.05

[a] PEG 3350, 4000, 8000–dextran T40–10 mM potassium phosphate buffers.
[b] K_{lysate}: partition coefficient of SM cell-lysate.
[c] K_{GTF}: partition coefficient of GTF.

coefficient values of SM cell-lysate (K_{lysate}) obtained in these aqueous two-phase systems containing PEG 3350 and PEG 4000 are almost identical. Increasing the molecular weight of PEG from 4000 to 8000 resulted in decrease of K_{lysate}. On the other hand, K_{GTF} values obtained from three different aqueous two-phase systems, i.e., 7.5% PEG 3350–10.0% dextran T40, 7.5% PEG 4000–10.0% dextran T40 and 5.0% PEG 8000–12.5% dextran T40 are 0.11, 0.10 and 0.10, respectively. Since the retention of the stationary phase greatly depends on the viscosity of the stationary phase, we selected the solvent systems composed of 7.5% PEG 3350–10.0% dextran T40–10 mM dipotassium phosphate, which has lower viscosity.

K_{lysate} and the K_{GTF} are also greatly affected by the pH of the solvent systems. To obtain a large difference of K_{lysate} and K_{GTF}, these values are measured in the 7.5% PEG 3350–10.0% dextran T40–10 mM potassium phosphate buffers at pH 6.0, 7.0, 8.0 and 9.0 (Fig. 1). Both the K_{lysate} and the K_{GTF} values increases as the solvent pH increases. The largest difference between the K_{lysate} and K_{GTF} is obtained at pH 9.0. Then the GTF purification was performed with the aqueous two-phase solvent systems composed of 7.5% PEG 3350–10.0% dextran T40–10 mM dipotassium phosphate at pH 9.0 by the cross-axis CPC.

3.3. Determination of dextran T40 in the residue and in the filtrate after ultrafiltration

As mentioned in Section 2.9, dextran T40 was filtered through an ultrafiltration membrane and the concentrations of dextran T40 in the retained and filtered solution were determined: a 1 ml volume of 10% dextran T40 solution was diluted with 10 ml distilled water and filtered with ultrafiltrate membrane up to 1 ml (residual solution). The filtrate was also collected for the determination of optical rotation.

3.4. Purification of GTF from SM cell-lysate by CCC

Fig. 2 shows a CCC chromatogram of GTF from SM cell-lysate obtained by the cross-axis CPC using 7.5% (w/w) PEG 3350–10.0% (w/w) dextran T40–10 mM potassium phosphate buffer (pH 9.0). The CCC column was filled with the dextran-rich lower phase and the sample solution was injected into the column. The PEG-rich upper phase was eluted through the column at 0.5 ml/min during the column rotated at 400 rpm. The solid circles indicate the absorbance at 220 nm in the eluted upper phase. The solvent front emerged at 41 fraction and two peaks eluted from the column with upper phase (frs. 41–90). These peaks show no GTF enzyme activity, indicating that the first peak (frs. 41–48) contain almost all protein component other than GTF and second peak (frs. 50–90) contain impurities in the cell-lysate (Fig. 3). After CCC run stop at frs. 90, the column contents was emptied by air pressure and fractionated from frs. 91–155. The solid triangles indicate the absorbance at 220 nm in the stationary lower phase of the column contents and the bar graphs indicate the GTF enzyme activities in the column contents. As expected from the K value, the GTF activities in the

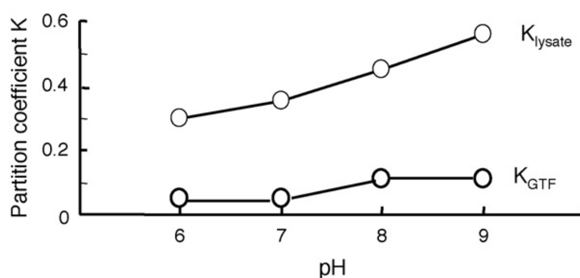

Fig. 1. Effect of pH of two-phase systems on partition coefficient of cell-lysate and GTF.

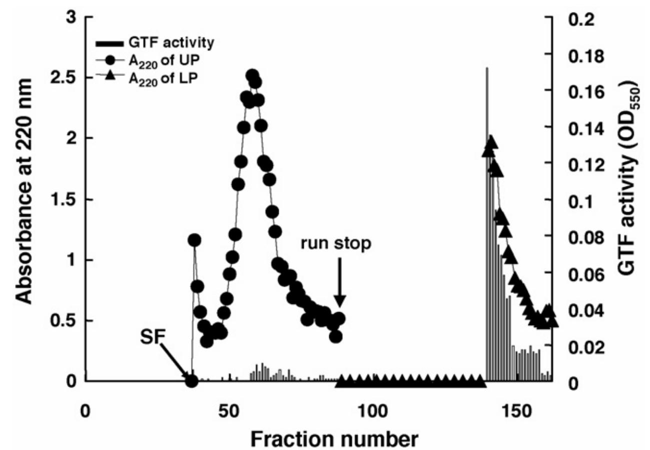

Fig. 2. Separation profile of *S. mutans* (SM) cell-lysate by counter-current chromatography using cross-axis coil planet centrifuge. Experimental conditions: apparatus: type-XL cross-axis coil planet centrifuge with a pair of multilayer coil columns of 2.6 mm I.D. and 110 ml total capacity; solvent system: 7.5% PEG 3350–10% dextran T40–10 mM potassium phosphate at pH 9.0; stationary phase: dextran-rich lower phase; mobile phase: PEG 3350-rich upper phase; 5 g of SM cell-lysate containing 0.45 g PEG 3350, 0.61 g dextran T40; flow rate: 0.5 ml/min; revolution speed: 400 rpm; fractionation: 3 ml per tube; SF, solvent front.

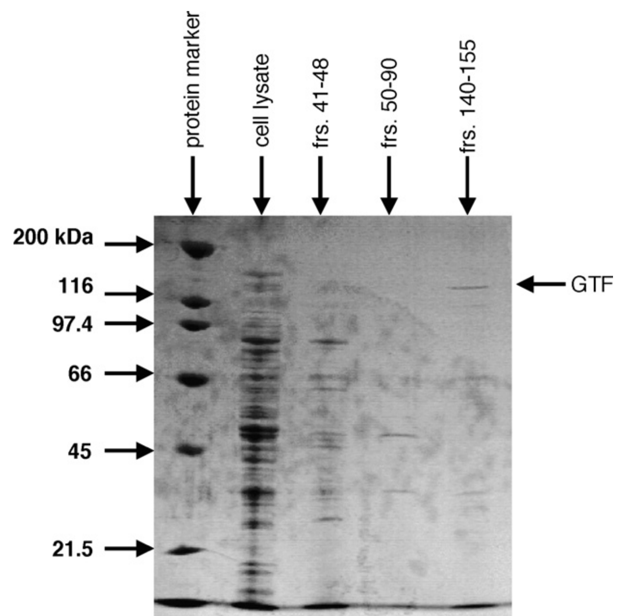

Fig. 3. 10% SDS polyacrylamide gel electrophoresis profile of CCC fractions.

CCC fractions are solely found in frs. 140–155 collected from the column contents.

3.5. Removal of PEG and dextran from CCC fractions by ultrafiltration

The GTF fractions obtained by CCC contained a large quantity of PEG 3350 and dextran T40. In this situation it is very difficult to analyze the CCC fraction by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), because the purified GTF band is smeared by PEG or dextran present in the fraction. Therefore, it is necessary to remove both PEG 3350 and dextran T40 before application to the gel. In our previous papers [21,22] dealing with GTF purification by CCC using aqueous polymer two-phase systems, we have used high molecular weight polymers of PEG 8000 (Mr: 6000–7500) and dextran T500 (Mr: 500,000). After the purification of GTF by CCC, the CCC fractions were diluted with 10-fold volume of potassium phosphate buffer and passed through a hydroxylapatite column to eliminated these polymers. However, the recovery of the enzyme from a hydroxyapatite column was only about 70% of the loaded GTF due to irreversible adsorption and/or deactivation by the solid support.

In our studies, PEG and dextran were removed by ultrafiltration as described above. For this purpose we used relatively low molecular weight polymers of PEG 3350 and dextran T40 instead. After CCC purification of GTF, CCC fractions 41–48, 50–90 and 140–155 were diluted with 5-fold volume of 10 mM potassium phosphate buffer at 7.0. About 120–225 ml volume of diluted sample solution was ultrafiltered with a large diameter (63.5 mm) ultrafiltration membrane. Fig. 3 shows the SDS–PAGE profile of the sample solutions obtained by ultrafiltration. The concentrated fractions from 140 to 155 contained GTF corresponding to the molecular weight of 150 kDa. The specific activity of GTF after concentrated by ultrafiltration was about 100 times high activity compared with that in the cell-lysate and the recovery of the enzyme from CCC fraction was over 95% as estimated by enzymatic activities.

The overall results of our studies indicate that the aqueous two-phase system composed of 7.5% PEG 3350 and 10.0% dextran T40 is very useful for the purification of GTF from SM cell-lysate by CCC, since these polymers are easily removed from the CCC fractions by ultrafiltration. Consequently, the method eliminates the hydroxylapatite chromatography procedure and yields high recovery of GTF for a short time. The present polymer phase system may be useful for purificatioin of various other proteins.

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