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Note

Fractionation of tRNA on siliconized porous glass coated with trialkylmethylammonium chloride

TAKAHIKO NARIHARA, YUKO FUJITA and TAKAHARU MIZUTANI*
Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-Ku, Nagoya 467 (Japan)
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In the course of a study on the recognition mechanism between tRNA and aminoacyl-tRNA synthetase[1], we carried out some studies on tRNA[2–4], but the mechanism was not resolved. Pure tRNA is necessary for investigating the mechanism. For the separation of tRNA, Pearson *et al.*[5] reported a reversed-phase chromatographic technique at a low flow-rate, named RPC-5. Subsequently we developed separation methods using porous glass[6,7]. We report here a method involving high flow-rates for separating tRNA on siliconized porous glass[8] coated with trialkylmethylammonium chloride.

EXPERIMENTAL

Preparation of adsorbent

The porous glass used was CPG-10, 350 Å (100-μm particles) (Electro-Nucleonics, Fairfield, NJ, U.S.A.). After being washed and dried, the glass (1 g) was tightly coated with 200 μl of silicone oil (dimethylpolysiloxane, KF 96; Shinetsu Chemicals, Tokyo, Japan) according to previous work[8]. The siliconized glass (10 g) was shaken for 2 h according to the literature[5] with 20 ml of chloroform containing 0.4 ml of Adogen 464 (a trialkylmethylammonium chloride with the predominant chain length of the alkyl groups being C_8–C_{10}) (Ashland Chemical Co., Columbus, OH, U.S.A.). The slurry of siliconized glass in chloroform was dried in a glass tray. The glass treated with Adogen 464 immediately sank in an aqueous medium even though siliconized porous glass floated on the surface of water[8].

The siliconized glass coated with Adogen 464 was suspended in a solution composed of 0.45 *M* sodium chloride, 0.01 *M* magnesium chloride, 0.01 *M* Tris–hydrochloric acid buffer (pH 7.6) and 1 m*M* β-mercaptoethanol. After being washed with the solution, the glass was packed in a column having a water jacket maintained at 37°C. A sample of tRNA was dissolved at a concentration of 10 mg/ml in the above solution and applied on to the column. After the column had been washed with one column volume of the solution, elution was carried out with a linear gradient of sodium chloride at a flow-rate of 0.6 ml/min · cm^2.

Transfer RNA

Transfer RNA was prepared from bovine liver as follows. Bovine liver (1 kg)

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was minced and mixed in 5 l of 1 *M* socium chloride–0.02 *M* Tris–hydrochloric acid buffer (pH 7.5) containing 0.5 m*M* EDTA, 0.01 *M* magnesium chloride, 1 % sodium dodecylsulphate and 20 % phenol. The supernatant obtained from the above solution after standing overnight in a cold room was mixed with 2 volumes of ethanol. The precipitate was collected and dissolved in 0.3 *M* sodium acetate solution. The high-molecular-weight nucleic acid, such as rRNA and DNA, was precipitated with 0.4 part of isopropanol[9]. After the DNA precipitate had been removed, the solution was mixed with 0.6 part of isopropanol relative to the first supernatant and the precipitate was collected by centrifugation at 600 *g*. Glycogen in the precipitate was removed by treatment with ethylene glycol monomethyl ether[10] and the extract containing tRNA was dialysed. After dialysis, tRNA was incubated in 1 *M* Tris–hydrochloric acid buffer (pH 9.0) to remove amino acid bound on tRNA and then precipitated by addition of ethanol to 70 %. This tRNA preparation was further purified on Sepharose 6B and fractions eluting at K_{av} 0.25 were collected; 0.98 g of tRNA was obtained from 1.0 kg of bovine liver.

The tRNA (1.1 g) obtained on Sepharose 6B was then dissolved in 100 ml of buffer (pH 4.6) consisting of 0.4 *M* sodium chloride, 0.01 *M* magnesium chloride and 0.01 *M* potassium acetate and chromatographed on a benzoylated DEAE-cellulose column (22.6 × 5.2 cm I.D.) according to the method of Gillam *et al.*[11]. Elution was carried out at 23–26°C with a linear gradient (total volume 4 l) from 0.4 to 1 *M* sodium chloride in 0.01 *M* potassium acetate (pH 4.6) and 0.01 *M* magnesium chloride. The fraction volumes were 50 ml. The chromatographic pattern is shown in Fig. 1.

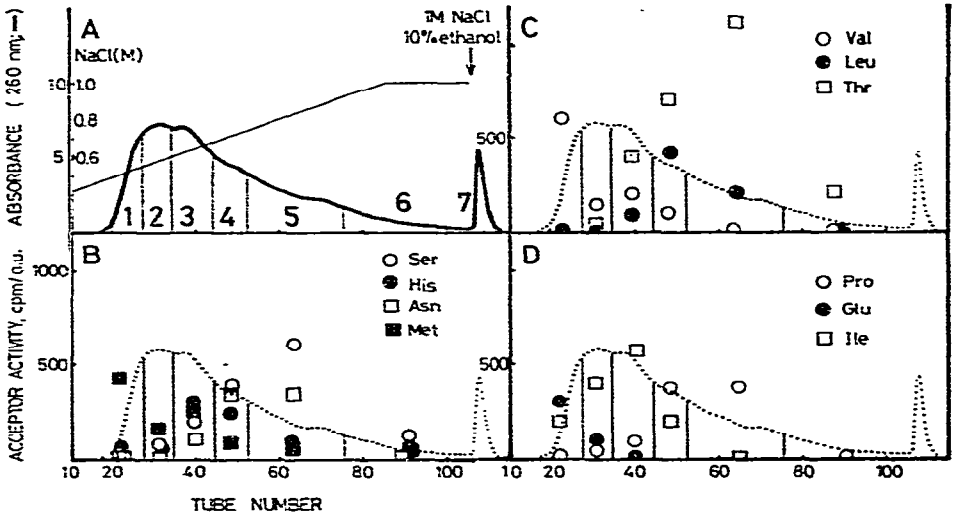

Fig. 1. Elution pattern of tRNA on BD-cellulose. The sample (1.1 g, 16,500 absorbance units) was applied on a column (22.6 × 5.2 cm I.D.) and eluted with a linear gradient (total volume 4 l) from 0.4 to 1.0 *M* sodium chloride at 23–26°C. (A) Result of measurement of absorbance at 260 nm; identical patterns are shown in (B)–(D) with broken lines. Fraction volumes were 50 ml. (B)–(D), Amino acid acceptor activities of each fraction. The cpm/absorbance unit values indicate the amounts of amino acids bound on tRNA.

Preparation of aminoacyl-tRNA synthetase

The synthetase was prepared from Donryu strain rat liver in a cold room[12]. The supernatant (300 ml) from centrifugation at 105,000 g for 60 min of the homogenate of liver (38 g) in 0.25 *M* sucrose–0.02 *M* Tris–hydrochloric acid buffer (pH 7.4) was dialysed against a solution composed of 0.01 *M* Tris–hydrochloric acid buffer (pH 7.5), 0.01 *M* magnesium chloride, 0.01 *M* β-mercaptoethanol and 5% glycerin, and then loaded on a DEAE-cellulose column (20 × 5 cm I.D.) which had previously been equilibrated with the above buffer. After the column had been washed with the buffer, proteins were eluted with 0.3 *M* potassium chloride in the buffer. The protein peak measured at 280 nm was dialysed against a solution composed of 0.01 *M* Tris–hydrochloric acid buffer (pH 7.5), 0.01 *M* β-mercaptoethanol, 2 m*M* EDTA and 50% glycerin. The protein concentration of the dialysate was 28.5 mg/ml by the Lowry method[13]. The dialysate was kept in a freezer at −20°C. This dialysate was used as aminoacyl-tRNA synthetase and the activity was generally stable for several months.

Assay of aminoacylation of tRNA was carried out as follows. tRNA was dissolved in a solution composed of 0.2 *M* Tris–hydrochloric acid buffer (pH 7.6), 0.02 *M* potassium chloride and 0.02 *M* magnesium chloride. The tRNA solution (40 μl) was mixed with 50 μl of the same solution described above but containing 5 m*M* ATP and 40 μ*M* [^{14}C]amino acid (10 μCi per 2 μmol; Radiochemical Centre, Amersham, Great Britain). The aminoacylation reaction was started at 37°C by the addition of 10 μl of enzyme solution to the above tRNA–amino acid solution (90 μl). The incubation was allowed to proceed for 30 min. Before the reaction was stopped, the reaction mixture was layered on to a filter-paper and the paper immersed in 0.2 *M* hydrochloric acid. After being washed for 20 min with three portions of 0.2 *M* hydrochloric acid the filter-paper was placed in a solution of diethyl ether–ethanol (1:1) and then dried. The dried filter-paper was counted in common toluene–PPO–POPOP solution with a Searle Analytical liquid scintilation counter. The assay was carried out duplicate. A blank and a control (crude tRNA, 2.4 absorbance units) were usually measured at the same time as the sample. The counting efficiency for [^{14}C]amino acids was about 60%.

RESULTS AND DISCUSSION

Fig. 2 shows the results of chromatography at 37°C of bovine liver tRNA (the fraction obtained on Sepharose 6B) on a 45 × 0.8 cm I.D. column of siliconized glass coated with Adogen 464. The activity of tRNAs accepting methionine, valine and serine is also shown in Fig. 2. Fraction 1 in Fig. 2 did not contain $tRNA^{Val}$ and $tRNA^{Ser}$, but contained $tRNA^{Met}$. $tRNA^{Val}$ and $tRNA^{Ser}$ were contained in fractions 2 and 3, respectively. Thus, tRNA species were separated on the siliconized glass coated with Adogen 464. The order of elution of tRNA species from the glass column is methionine, valine and serine, which is similar to that on an RPC-5 column[5]. However, the concentration of sodium chloride necessary for eluting tRNA on the glass column is higher than the system on an RPC-5 column.

Fig. 3 shows the results of further purification of the partially purified $tRNA^{Ser}$ (fraction 5 in Fig. 1) on a siliconized glass column coated with Adogen 464 (45 × 0.8 cm I.D.). Major $tRNA^{Ser}$ was eluted in fraction 4 in Fig. 3 and minor $tRNA^{Ser}$ in fraction 1. Thus, iso-accepting $tRNA^{Ser}$ species were separated on the siliconized

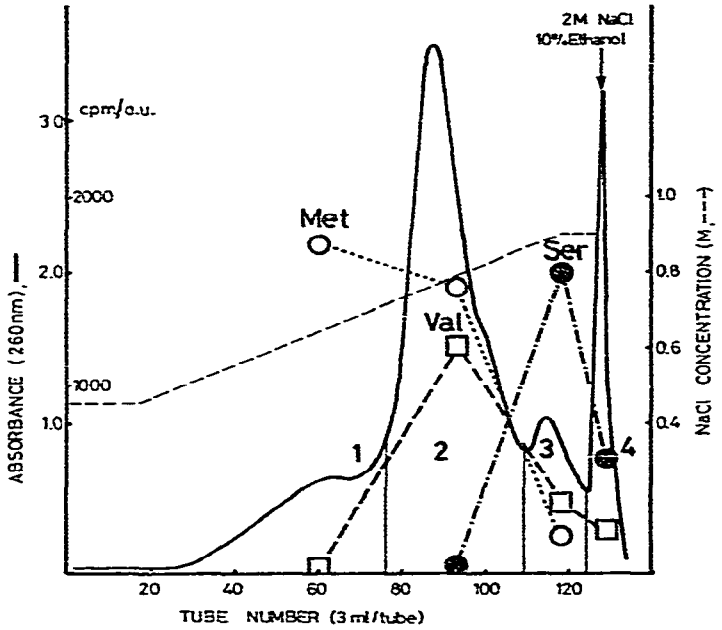

Fig. 2. Elution profile of crude tRNA (20 mg) on siliconized porous glass coated with Adogen 464. Column, 45 × 0.8 cm I.D. Elution was carried out at 37°C with a linear gradient (total volume 300 ml) from 0.45 to 0.9 *M* sodium chloride in 0.01 *M* magnesium chloride, 0.01 *M* Tris-hydrochloric acid buffer (pH 7.6) and 1 m*M* β-mercaptoethanol at a flow-rate of 0.6 ml/cm^2 · min. Amino acid acceptor activity of tRNA is shown by cpm/absorbance unit as follows; O---O, Met; □---□, Val; ●-.-.-●, Ser.

Adogen 464-coated glass and tRNA^{Ser} was separated from other impurities. It is possible that major tRNA^{Ser} corresponds to serine codons UCX and minor tRNA^{Ser} to AGC and AGU, from the results on RPC-5[14].

Partially purified tRNA^{Ser} (1430 absorbance units, 72 mg; fraction 5 in Fig. 1) was phenoxyacetylated according to the literature[15] and chromatographed on BD-cellulose. Phenoxyacetylated tRNA^{Ser} had an affinity to BD-cellulose and eluted later than other inpurities, as shown in Fig. 4A. The tRNA^{Ser}-rich fraction in Fig. 4A was

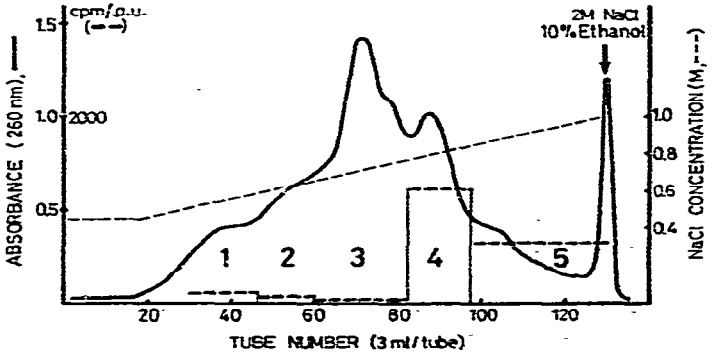

Fig. 3. Purification of tRNA^{Ser} on siliconized porous glass column coated with Adogen 464. The partially purified tRNA^{Ser} (10 mg) used was fraction 5 in Fig. 1. Other conditions identical with those in Fig. 2.

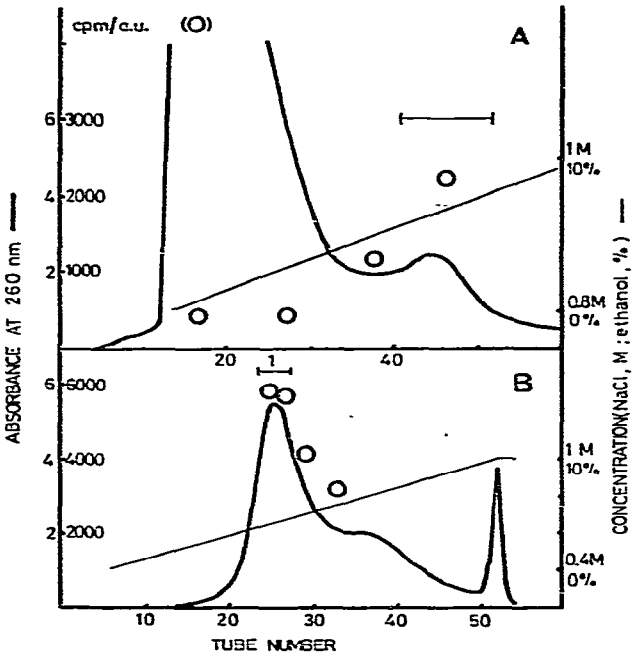

Fig. 4. Purification of $tRNA^{Ser}$ on BD-cellulose. (A) tRNA (1430 absorbance units) was applied on a BD-cellulose column (15 × 1.6 cm I.D.). Total elution volume of a linear gradient from 0.8 to 1.2 *M* sodium chloride–20% ethanol was 400 ml. Fraction volumes were 4 ml. (B) Results of re-chromatography of a fraction (tubes 41–51) rich in $tRNA^{Ser}$ in (A) on a BD-cellulose column (10 × 0.4 cm I.D.). Fraction volumes were 1 ml. Fractions in tubes 24–27 in (B) were pooled and used in Fig. 5.

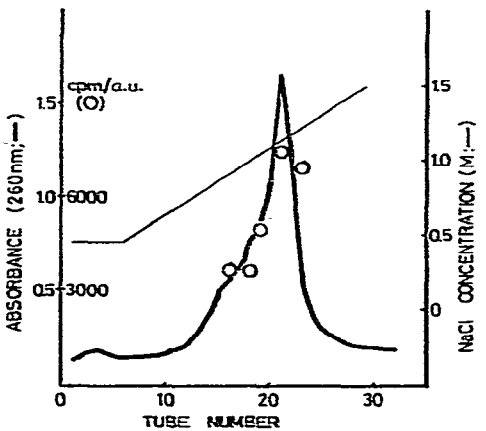

Fig. 5. Isolation of $tRNA^{Ser}$ on the siliconized and Adogen 464-coated porous glass. Sample (62 absorbance units, fraction 1 in Fig. 4B) was dissolved in 0.5 ml of 0.01 *M* Tris–hydrochloric acid buffer (pH 7.6), 0.45 *M* sodium chloride, 0.01 *M* magnesium chloride and β-mercaptoethanol and applied on a 20 × 0.8 cm I.D. column. Elution was carried out at a constant temperature of 37°C with an elution buffer (100 ml) composed of a linear gradient from 0.45 to 1.5 *M* sodium chloride. Fraction volumes were 4 ml.

deaminoacylated and re-chromatographed on a small BD-cellulose column as shown in Fig. 4B. The fraction in tubes 24–27 in Fig. 4B was further purified on the siliconized Adogen 464-coated porous glass and the chromatographic pattern is shown in Fig. 5. Serine tRNA was eluted in tubes 21–23 and impurities in tubes 12–20 in Fig. 5. Purified $tRNA^{Ser}$ accepts 1.4 nmole of serine per absorbance unit of tRNA.

The recoveries of tRNA in Figs. 2, 3 and 5 were better, averaging 90%, than that obtained with the Plaskon–Adogen 464 (RPC-5) system. In the work with the RPC-5 column[5], a linear gradient was pumped through the column, because of the small size of the particles of Plaskon. Using the siliconized porous glass coated with Adogen 464, a high flow-rate was obtained under no pressure. The glass was used repeatedly several times and the reproducibility of the chromatographic patterns remained constant. However, the glass floated on an aqueous medium after repeated use, because Adogen 464 was removed from the surface of the siliconized glass. Therefore, it is advisable to re-coat the glass with Adogen 464 after use several times.

CONCLUSION

Chromatography of bovine liver tRNA with use of a sodium chloride gradient and a siliconized porous glass column treated with Adogen 464, eluted tRNA from the column in the order $tRNA^{Met}_f$, $tRNA^{Val}$ and $tRNA^{Ser}$, and the recovery of tRNA from the glass column averaged 90%. $tRNA^{Ser}$, partially purified on benzoylated DEAE-cellulose, was isolated on the siliconized Adogen-coated porous glass.

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