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Note

Rapid fractionation of tRNA on benzoylated DEAE-porous glass

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We have previously reported on the adsorption of different proteins on porous glass surfaces[1] and have developed an adsorption chromatographic method[2,3]. We found that protein adsorption on silicone-coated porous glass[4] and siliconized porous glass occurred in adsorption chromatography[5]. Ribonucleic acids of high molecular weight were adsorbed at high salt concentrations on porous glass or siliconized glass[6,7], and the resulting materials were used as supports for the adsorption chromatography of nucleic acids, such as tRNA, mRNA and rRNA[8,9].

tRNA has usually been fractionated by RPC-5 chromatography[10] and using benzoylated DEAE(BD)-cellulose[11]. We have previously developed an RPC-5-type system on siliconized porous glass by coating with Adogen 464[12,13]. On the other hand, reversed-phase high-performance liquid chromatography (HPLC) of nucleic acids, which is not based on ionic bonding, has been developed on Kel-F[14] and C_{18}[15,16], but this method is not suitable for preparing RNAs. BD-cellulose chromatography is a valuable method for the separation of tRNA[11], but it is impossible to achieve a high flow-rate on BD-cellulose. BD-cellulose shrinks in the column after loading of tRNA and the chromatographic pattern is disturbed. In order to improve these properties of BD-cellulose, we attempted to separate tRNA species on BD-porous glass (BD-PG) and found that BD-PG is a better support than BD-cellulose for the separation of tRNA.

EXPERIMENTAL

BD-PG was generously supplied by Central Glass (Tokyo, Japan) with a mean pore diameter of 670 Å and a particle size of 80–200 mesh. The contents of benzoyl and DEAE groups in BD-PG were 51 and 51 μmol/ml (2.5 ml/g), respectively. The content of DEAE groups (29 μmol/ml) in BD-PG was about one-fifth of that in BD-cellulose (150 μmol/ml), and the content of benzoyl groups in BD-PG was also less than that in BD-cellulose[11]. BD-PG (1 g) bound approximately 30 mg of tRNA in 0.01 *M* acetate buffer–0.01 *M* magnesium chloride at pH 4.6, estimated by the depletion technique[17]. Regeneration of BD-PG was achieved by elution in sequence with 2 *M* sodium chloride–15% ethanol, water, 0.05 *N* sodium hydroxide, water, 1% sodium dodecyl sulphate and finally 0.01 *M* acetate buffer –0.01 *M* magnesium chloride.

Crude tRNA and aminoacyl-tRNA synthetase were prepared from bovine liver

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as described previously[12,13]. Assay of the extent of aminoacylation of tRNA in the eluate from BD-PG columns was also carried out according to the literature[12].

RESULTS AND DISCUSSION

Fig. 1 shows the results of chromatography of tRNA (50 mg) on a column (50 × 1.1 cm I.D.; 20 g) at 18°C and at a flow-rate of 40 ml/cm² · h. In Fig. 1A, $tRNA^{Ile}$ was eluted in tubes 81–85 and $tRNA^{Ser}$ in tubes 103–119. In Fig. 1B, $tRNA^{Val}$ was eluted in fraction 3 and $tRNA^{Arg}$ in fractions 9–12. Isoacceptor species of $tRNA^{Thr}$ and $tRNA^{Pro}$ were separated as shown in Fig. 1B and C, respectively. These tRNAs in each fraction were purified 3–8-fold over those in crude tRNA. Finally, the order of elution of tRNA species from the column was $tRNA^{Val}$, $tRNA^{Gly}$, $tRNA^{Ile}$, $tRNA^{Thr}$, $tRNA^{Pro}$, $tRNA^{Ser}$, $tRNA^{Arg}$ and $tRNA^{Phe}$. The order of elution of tRNA species from the BD-PG column was compared with that on BD-cellulose[12], and was found to be identical. However, tRNA was eluted from BD-PG at a higher concentration of sodium chloride than that from BD-cellulose. For example, $tRNA^{Val}$ was eluted at 0.83 *M* sodium chloride from BD-PG and at 0.53 *M* sodium chloride from

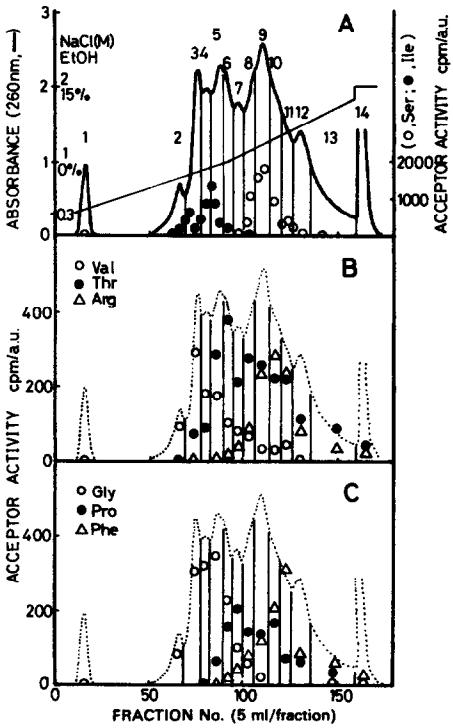

Fig. 1. Elution pattern of bovine liver tRNA (50 mg) on BD-PG. The first linear gradient (total volume 400 ml) from 0.3 to 1 *M* sodium chloride in 0.01 *M* acetate buffer and 0.01 *M* magnesium chloride at pH 4.6 was used for elution (fractions 1 to 92). The second linear gradient consisted of 1 *M* to 2 *M* sodium chloride and 15% ethanol (EtOH) in the same buffer (fractions 93 to 160). (A) Results of measurement of absorbance at 260 nm; identical patterns are shown in B and C with broken lines. Amino acid acceptor activities of tRNA in each fraction are shown in A–C. The cpm/absorbance values indicate the amount of amino acids bound on tRNA.

BD-cellulose. The eluting order on BD-PG was also similar to that on siliconized porous glass[9].

Fig. 2 shows the results of chromatography of tRNA (25 mg) on the same column at 37°C and at a flow-rate of 150 ml/cm²/h. This flow-rate was obtained with a peristaltic pump by joining at the end of the column. In Fig. 2, $tRNA^{Ile}$ and major $tRNA^{Ser}$ were eluted similarly to that in Fig. 1. The width of each peak was also identical with that in Fig. 1, and therefore rapid fractionation at a high flow-rate did not influence the separation of tRNA. However, the overall elution pattern in Fig. 2 was slightly different from that in Fig. 1, in addition to the salt concentration for tRNA to be eluted. This is caused by the temperature (18°C in Fig. 1 and 37°C in Fig. 2). The secondary structure of tRNA became looser at higher temperature and the elution points changed. For example, in Fig. 2 an isoaccepting $tRNA^{Ser}$ species was separated and a peak of minor $tRNA^{Ser}_{GCU}$ was eluted between the peaks of $tRNA^{Ile}$ and major $tRNA^{Ser}_{IGA}$. tRNA was eluted at a lower salt concentration in Fig. 2 than that in Fig. 1. Recoveries of tRNA were about 85% in both Figs. 1 and 2 and the remaining fraction was eluted with 0.05 *N* sodium hydroxide solution. BD-PG was stable in 0.05 *N* sodium hydroxide solution, although porous glass decomposed. However, one should avoid soaking BD-PG in 0.05 *N* sodium hydroxide solution for a long time(1 h) when washing.

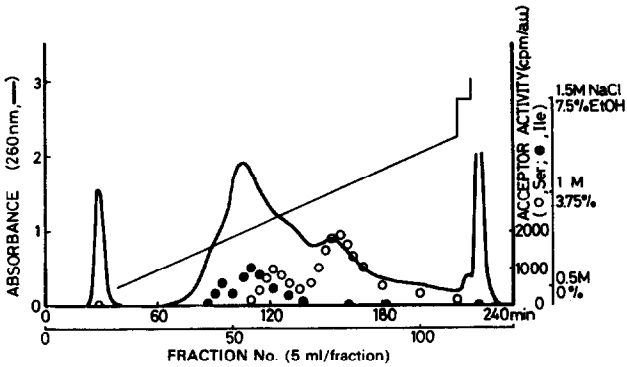

Fig. 2. Elution pattern of tRNA (25 mg) on BD-PG at 37°C and at a high flow-rate (150 ml/cm²/h) with a linear gradient (total volume 600 ml) from 0.5 to 1.5 *M* sodium chloride–7.5% ethanol (EtOH) in the buffer. After elution with the gradient, the column was eluted with 2 *M* sodium chloride–15% ethanol in the buffer (at fraction 115). Other conditions are identical with those in Fig. 1.

CONCLUSION

Bovine liver tRNA was rapidly fractionated on BD-PG. The order of elution of tRNA species from the column was $tRNA^{Val}$, $tRNA^{Gly}$, $tRNA^{Ile}$, $tRNA^{Thr}$, $tRNA^{Pro}$, $tRNA^{Ser}$, $tRNA^{Arg}$ and $tRNA^{Phe}$, which is identical with that on BD-cellulose. BD-PG is better than BD-cellulose, because the former gives a high flow-rate and easy packing and it does not shrink in the column after loading of tRNA.

REFERENCES

1 T. Mizutani and A. Mizutani, *J. Chromatogr.*, 111 (1975) 214.
2 T. Mizutani and A. Mizutani, *J. Chromatogr.*, 120 (1976) 206.

3 T. Mizutani and A. Mizutani, *J. Chromatogr.*, 168 (1979) 143.
4 T. Mizutani, *J. Colloid Interface Sci.*, 82 (1981) 162.
5 T. Mizutani and T. Narihara, *J. Chromatogr.*, 239 (1982) 755.
6 T. Mizutani, *J. Non-Cryst. Solids*, 55 (1983) 173.
7 T. Mizutani, *J. Colloid Interface Sci.*, 93 (1983) 270.
8 T. Mizutani, *J. Chromatogr.*, 262 (1983) 441.
9 T. Mizutani, *J. Biochem*, 94 (1983) 163.
10 R. L. Pearson, J. F. Weiss and A. D. Kelmers, *Biochim. Biophys. Acta*, 228 (1971) 770.
11 I. Gillam, S. Millward, D. Blew, M. von Tigerstrom, E. Winner and G. M. Tener, *Biochemistry*, 6 (1967) 3043.
12 T. Narihara, Y. Fujita and T. Mizutani, *J. Chromatogr.*, 236 (1982) 513.
13 T. Mizutani, T. Narihara and A. Hashimoto, *Eur. J. Biochem.*, 143 (1984) 9.
14 D. A. Usher, *Nucl. Acids Res.*, 6 (1979) 2289.
15 P. N. Nguyen, J. L. Bradley and P. M. McGuire, *J. Chromatogr.*, 236 (1982) 508.
16 S. Garcia and J. P. Liautard, *J. Chromatogr.*, 296 (1984) 355.
17 T. Mizutani and A. Mizutani, *J. Non-Cryst. Solids*, 30 (1978) 23.