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Note

Oligo(dT)-glyceryl porous glass, a better support for the preparation of mRNA

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We have previously reported data pertaining to the adsorption of different proteins on porous glass surfaces[1]. We have also developed an adsorption chromatographic method for biopolymers, such as proteins and nucleic acids[2]. Recently, we have published data concerning chromatographic methods for tRNA on modified porous glass, such as an RPC-5 type support and benzoylated DEAE-porous glass[3].

The preparation of animal mRNA is important for the preparation of complemental DNA, which is integrated in plasmids and used to produce proteins in bacteria. Generally, animal mRNA has been purified by affinity chromatography on oligo(dT)-cellulose[4] and by sucrose-gradient centrifugation[5]. Oligo(dT)-cellulose adsorbs mRNA which has poly(A)-rich regions. Chromatography on oligo(dT)-cellulose is better since more mRNA is obtained when compared to the centrifugation method. However, crude mRNA preparations sometimes contain milky, turbid particles, which causes the flow-rate from the oligo(dT)-cellulose column to decrease. In order to get a high flow-rate when turbid mRNA solutions are used, we attempted to separate mRNA on oligo(dT)-glyceryl porous glass [oligo(dT)-GPG]. It was found that mRNA prepared on oligo(dT)-GPG is more active in a rabbit reticulocyte protein synthesizing system than that on oligo(dT)-cellulose. A better support for purifying mRNA will contribute to the development of genetic engineering.

EXPERIMENTAL

The method of preparing oligo(dT)-GPG followed the procedure of Gilham[6] except that cellulose was replaced by GPG (350 Å, 120–200 mesh; Electro-Nucleonics, Fairfield, NJ, U.S.A.). Anhydrous thymidine 5′-phosphate (0.7 mmol), from Yamasa (Chiba, Japan), was dissolved in dry pyridine (3 ml) and dicyclohexylcarbodiimide (1.4 mmol), from Wako Pure Chemicals (Tokyo, Japan). Glass beads were added and the flask was shaken vigorously for 5 days. The total product was mixed in the absence of moisture with dicyclohexylcarbodiimide (1.5 g) and GPG (50 ml, about 6 mmol alcoholic OH residues) which had been dried *in vacuo* over phosphorous pentoxide. Dry pyridine (50 ml) was added and the mixture was shaken for another 5 days. The porous glass was collected by filtration, washed with pyridine and allowed to stand overnight in 50% aqueous pyridine. The product was placed in a glass column (20 cm × 3 cm), washed extensively with warm ethanol to remove

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dicyclohexylurea, then with 2 *M* sodium chloride–0.01 *M* Tris–hydrochloride solution at pH 7.2 until the optical density at 260 nm of the eluent was less than 0.03 and finally with 0.01 *M* Tris–hydrochloride buffer at pH 7.2. One ml of oligo(dT)-GPG prepared in this manner was saturated with *ca.* 80 μg of poly(A) (Yamasa) in 0.5 *M* sodium chloride–0.01 *M* Tris–hydrochloride. The oligo(dT)-GPG can be regenerated for further use by elution with 0.1 *M* potassium hydroxide for 10 min. Oligo(dT)-cellulose, used as a control adsorbent, was obtained from Sigma Chemicals. Other materials were standard reagent grade.

High-molecular-weight polysomal RNA containing globin-mRNA was prepared from rabbit reticulocyte polysomes which were obtained from reticulocyte lysates (60 ml) by ultracentrifugation at 150 000 *g* for 30 min[7]. The polysomes were solubilized in 20 ml of 4 *M* guanidine thiocyanate–0.01 *M* Tris–hydrochloride at pH 7.2 and 0.1 *M* β-mercaptoethanol in a glass homogenizer. The solution was acidified to pH 5 by addition of 0.8 ml of 1 *M* acetic acid. The RNA was precipitated by the addition of 10 ml of ethanol. After mixing, the solution was kept at −20°C for 2 h. The precipitated RNA was collected by centrifugation and the resulting pellet was dissolved in 10 ml of 6 *M* guanidine hydrochloride, 10 m*M* EDTA at pH 7 and 0.01 *M* β-mercaptoethanol. The solution was heated at 70°C for 5 min and rapidly cooled in ice–water. The RNA was again acidified to pH 5 by the addition of 0.8 ml of 1 *M* acetic acid and precipitated by 10 ml of cold ethanol. It was collected by centrifugation at 600 *g*. The final pellet was dissolved in 0.24 *M* sodium acetate (pH 5.5). The RNA was precipitated by the addition of 2.5 volumes of ethanol. The total RNA was stored as the ethanol precipitate at −20°C until required.

Mouse liver polysomal RNA was obtained by precipitation at 150 000 *g* according to the above method. Before chromatography, the RNA precipitate was collected by centrifugation and was dissolved to give 50 A_{260} units per ml of 0.5 *M* sodium chloride–10 m*M* Tris–hydrochloride at pH 7.2. All chromatography operations were done at room temperature.

Chromatography on oligo(dT)-GPG and oligo(dT)-cellulose was carried out by first packing these adsorbents in columns (15 cm × 0.6 cm; 4 ml). The columns were then washed and equilibrated with 0.5 *M* sodium chloride–10 m*M* Tris–hydrochloride at pH 7.2. Crude RNA solution, obtained from rabbit reticulocyte lysates or mouse liver, was applied on the columns at a flow-rate of 20 ml/h. The columns were then washed with the same buffer solution until the absorbance of the eluate at 260 nm decreased to 0.05. The RNA bound on these columns should be composed of mRNA and was eluted with 10 m*M* Tris–hydrochloride at pH 7.2 with a flow-rate of 20 ml/h. The RNA concentration in the eluate was determined by measurement of the absorbance at 260 nm. Fractions of volume 0.5 ml were collected: the fractions passed through the columns were pooled, as were the fractions eluted with 10 m*M* Tris–hydrochloride. Both fractions were used in the measurement of mRNA activity.

The assay of mRNA activity in RNA samples obtained from the columns was carried out using rabbit reticulocytes according to the literature method[8]. The reticulocyte lysate mixture was treated with micrococcal nuclease (PL Biochemicals, U.S.A.) to hydrolyze endogenous mRNA. The nuclease was then inactivated by the addition of ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) solution. The lysate (25 μl) was mixed with 1 μl of [^{14}C]leucine (13 GBq/mmol,

740 kBq/ml) and used for the measurement of the mRNA activity of each preparation (0.01 A_{260} unit per 5 μl). After incubation at 30°C for 30 min, the mixture was spotted on a filter-paper. The filter-paper was then placed in cold 5% trichloroacetic acid solution, then in hot 5% TCA solution for 10 min and finally washed with 0.2 *M* hydrochloric acid. The radioactivity of this paper was counted using a previously published method[9].

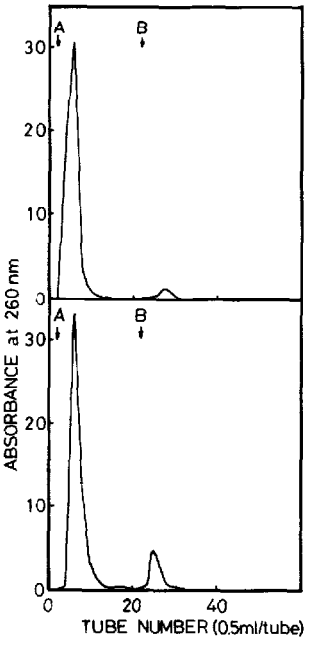
RESULTS AND DISCUSSION

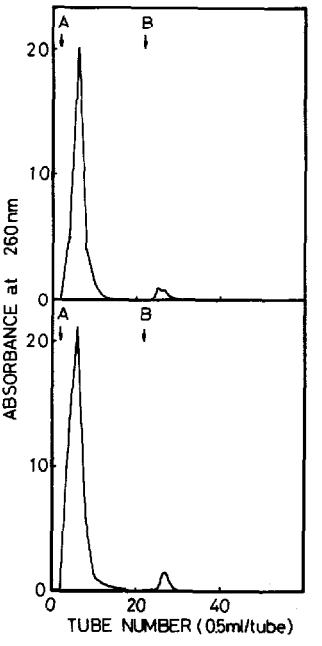
Fig. 1 shows the results of the chromatography of reticulocyte polysomal RNA on columns of oligo(dT)-GPG (upper) and oligo(dT)-cellulose (lower). Polysomal RNA solution (1 ml, 50 A_{260} units) was applied on these columns in 0.5 *M* sodium chloride–10 m*M* Tris–hydrochloride at pH 7.2. The RNA fractions that passed through the columns were mainly composed of rRNA. After the columns were washed with the same buffer, bound RNA was eluted as mRNA with 10 m*M* Tris–hydrochloride. The recovery of the mRNA fraction from oligo(dT)-GPG was 2.9% of the total RNA applied on the column, while the recovery from oligo(dT)-cellulose was 7.5%. The mRNA activities were 2350 cpm per 0.02 A_{260} unit of the mRNA fractions obtained from oligo(dT)-GPG and 1030 cpm per 0.02 A_{260} unit of that from oligo(dT)-cellulose. Thus, the recovery of the mRNA fraction from oligo(dT)-GPG was lower than that from oligo(dT)-cellulose but the specific activity was higher. The mRNA activities of the passed-through fractions in Fig. 1 were 86 cpm per 0.02 A_{260} unit of the RNA obtained from oligo(dT)-GPG and 175 cpm per 0.02 A_{260} unit of the RNA obtained from oligo(dT)-cellulose. As a result, oligo(dT)-GPG is a more specific adsorbent for mRNA than oligo(dT)-cellulose. These results were confirmed with mouse liver polysomal RNA.

Fig. 2 shows the chromatographic pattern of mouse liver polysomal RNA on oligo(dT)-GPG (upper) and oligo(dT)-cellulose (lower). The mRNA activity of the fraction eluted with 10 m*M* Tris–hydrochloride from oligo(dT)-GPG was 608 cpm per 0.02 A_{260} unit, while the fraction from oligo(dT)-cellulose showed lower activity (12 cpm per 0.02 A_{260} unit). The recovery of the mRNA fraction from oligo(dT)-GPG was 1.6% of the total RNA applied on the column, which was lower than that obtained from oligo(dT)-cellulose (2.3%).

The rabbit reticulocyte mRNA fraction obtained from oligo(dT)-cellulose was rechromatographed on oligo(dT)-GPG. The result is shown in Fig. 3. Only half of the RNA passed through the column while the remainder was bound and later eluted with 10 m*M* Tris–hydrochloride. The mRNA activities of these two fractions were 122 cpm per 0.02 A_{260} and 1388 cpm per 0.02 A_{260} unit respectively. Thus the mRNA preparation from oligo(dT)-cellulose was further purified on oligo(dT)-GPG. When the same sample was loaded on oligo(dT)-cellulose, a small amount of RNA was found in the passed-through fraction as shown in the lower part of Fig. 3.

Oligo(dT)-GPG is mechanically strong and it is easy to obtain a rapid flow-rate. Porous glass is unstable in alkaline solution, but oligo(dT)-GPG is stable because the reactive silanol groups are blocked with glyceryl residues. Therefore, oligo(dT)-GPG can be treated with 0.1 *M* potassium hydroxide to regenerate it for further use. Both oligo(dT)-cellulose and oligo(dT)-GPG are better from this point of view than poly(U)-Sepharose, from which poly(U) is lost in alkaline solution. The poly(A) binding capacity of oligo(dT)-GPG (80 μg/ml) is lower than that of oligo(dT)-cellulose (200 μg/ml), because the amount of hydroxy residues on GPG (0.12





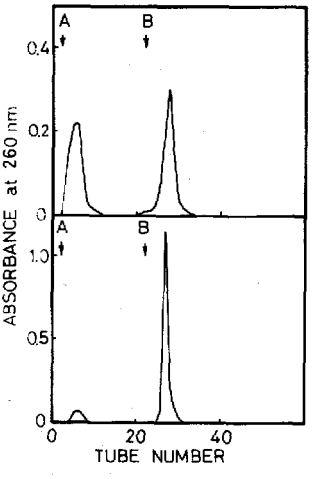


Fig. 1. Chromatographic patterns for rabbit reticulocyte polysomal RNA on an oligo(dT)-GPG (upper; 15 cm × 0.6 cm) and an oligo(dT)-cellulose column (lower; 15 cm × 0.6 cm). Arrow A indicates the elution of sample solution (1 ml, 50 A_{260} units) with 0.5 *M* sodium chloride–10 m*M* Tris–hydrochloride at pH 7.2. Arrow B is the point of elution with 10 m*M* Tris–hydrochloride. Column operations were done at room temperature. The assay of the mRNA activity in the eluate is described in the text.

Fig. 2. Chromatographic patterns for mouse liver polysomal RNA on oligo(dT)-GPG (upper) and oligo(dT)-cellulose (lower). Amounts of RNA applied: 50 A_{260} units. Other conditions as in Fig. 1.

Fig. 3. Rechromatography of the mRNA fraction on oligo(dT)-GPG (upper) and oligo(dT)-cellulose (lower). Sample: crude globin mRNA (0.8 A_{260} units) obtained from oligo(dT)-cellulose. Columns and other conditions as in Fig. 1.

mmol/g) is lower than that on cellulose (18 mmol/g). However, the difference in their capacities is negligible. Oligo(dT)-GPG is a better support for separation of mRNA than is oligo(dT)-cellulose. This must be a function of the fundamental characters of porous glass and cellulose. Porous glass, itself, is a good support for adsorption chromatography of high-molecular-weight RNAs, such as rRNA and mRNA[10]. Sepharose and cellulose are adsorbents for low-molecular-weight RNAs, such as tRNA, at high salt concentrations[11].

REFERENCES

1 T. Mizutani and A. Mizutani, *J. Chromatogr.*, 111 (1975) 214.
2 T. Mizutani, *J. Liq. Chromatogr.*, 8 (1985) 925.
3 T. Mizutani and Y. Tachibana, *J. Chromatogr.*, 324 (1985) 480.
4 H. Aviv and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.*, 69 (1972) 1408.
5 M. J. Evans and J. B. Lingrel, *Biochemistry*, 8 (1969) 3000.
6 P. T. Gilham, *J. Am. Chem. Soc.*, 86 (1964) 4982.
7 R. McCandliss, A. Sloma and S. Pestka, *Methods Enzymol.*, 79 (1981) 51.
8 H. Suzuki, *FEBS Lett.*, 72 (1976) 309.
9 T. Narihara, Y. Fujita and T. Mizutani, *J. Chromatogr.*, 236 (1982) 513.
10 T. Mizutani, *J. Chromatogr.*, 262 (1983) 441.
11 W. H. Holmes, R. E. Hurd, B. R. Roid, R. A. Rimerman and G. W. Hatfield, *Proc. Natl. Acad. Sci. U.S.A.*, 72 (1975) 1068.