Journal of Chromatography, 262 (1983) 441–445
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,742

Note

Adsorption chromatography of ribonucleic acids on controlled-pore glass

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(First received November 12th, 1982; revised manuscript received February 1st, 1983)

The preparation of nucleic acids has been carried out by ion-exchange chromatography on a polystyrene ion exchanger[1], a DEAE exchanger[2] or a RPC-5 system[3]. However chromatography of high molecular ribonucleic acids (RNAs), such as rRNA and mRNA, is impossible on those adsorbents, as RNAs are not eluted from these columns. Recently, adsorption chromatography of nucleic acids, a technique which is not based on ionic bonding, has been developed on Kel-F[4] and μBondapak C_{18}[5], although the recovery by these methods was not good.

The principle of adsorption chromatography of RNAs on controlled-pore glass (CPG) is based on the reports previously published on the separation of proteins by affinity chromatography on phenyl-Sepharose[6] and hydrogen bond adsorption chromatography on cellulose[7]; these separations were performed by lowering the salt concentration after adsorption in the presence of high salt concentrations. The behaviour of RNAs to salts in a water medium is similar to that of proteins[8], and RNAs of high molecular weight have an aggregating property in a highly concentrated solution[9]. It was previously reported that RNA is adsorbed onto CPG in highly concentrated salt solutions[10]. Globin mRNA was also adsorbed on oligo(dT)-cellulose by hydrogen bonding in 0.5 *M* KCl–10 m*M* Tris–HCl (pH 7.5) and was eluted by lowering the ionic concentration[11]. In this paper, a new high-recovery method of adsorption chromatography of RNA on CPG by lowering the concentration of salt is reported.

EXPERIMENTAL

The pore glass used was CPG-10, 240 Å (Electro-Nucleonics, Fairfield, NJ, U.S.A.); it had a 96% silica content, a surface area of 97 m^2/g and a particle size of 100 μm. The glass, suspended in a water medium, was packed in a column [9 × 0.6 cm I.D.; 2.4 ml (1.2 g) of CPG] and the column was washed with 1% sodium dodecyl sulphate (SDS), a chromic acid mixture, and thoroughly with distilled water[12]. After the column had been neutralized with 1 *M* Tris–HCl at pH 7.6, the column was equilibrated with 5 *M* NaCl–10 m*M* Tris–HCl at pH 6.3. Preliminary experiments showed that porous glass (1 g; 2 ml) adsorbed 10.3 mg (206 A_{260} units; estimated from the value 1 mg = 20 A_{260} units) of tRNA in 5 *M* NaCl at pH 4.1 (ref. 10).

The RNA used was *Escherichia coli* rRNA, prepared from the precipitate at

0021-9673/83/$03.00

105,000 *g* of the extract by the SDS–phenol method. The rRNA contained a minor tRNA as an impurity. Standard bovine liver tRNA was prepared according to a previous report[13]. The rRNA was dissolved in 0.1 ml of 1 *M* Tris HCl at pH 7.6 at room temperature and mixed with 1.9 ml of 5 *M* NaCl–10 m*M* Tris–HCl at pH 6.3. The mixture was centrifuged at 600 *g* and the supernatant (14.9 A_{260} units; 0.75 mg; this amount was approximately one-fifteenth of the maximum amount of RNA adsorbed on the CPG) was applied on to the CPG column. The column was washed with five column volumes of 5 *M* NaCl–Tris–HCl buffer and elution was carried out at 28°C and a flow-rate of 1.5 ml/cm² min with a linear gradient (total volume 60 ml) from 5 *M* NaCl–10 m*M* Tris–HCl at pH 6.3 to 10 m*M* Tris–HCl at pH 7.6. The concentration of RNA in the eluates was determined by measuring the absorbance at 260 nm from the value of $E^{1\%}_{1cm}$ at 260 nm = 200. The fractions eluted from the column were dialysed against distilled water at room temperature for 2 h and RNA in the dialysate was precipitated by addition of two volumes of ethanol.

Rechromatography of the fractions obtained from the first chromatography of RNA was performed in order to confirm the reproducibility. The fractions obtained were dialysed against 5 *M* NaCl–10 m*M* Tris HCl at pH 6.3 and applied onto the second CPG column, which had been previously equilibrated with 5 *M* NaCl–10 m*M* Tris–HCl. The second column was then operated according to the above methods.

Electrophoresis was performed on 8% polyacrylamide gel in 0.05 *M* Tris–borate–0.01 *M* EDTA buffer at pH 8.3 as reported[14]. Electrophoresis was carried out at 700 V and 6 mA for 2 h. After the xylene cyanol of the electrophoresis marker dye had moved 5 cm, the electrophoresis was stopped and the gel was removed for staining. This was carried out with 0.04% methylene blue in 0.4 *M* sodium acetate at pH 4.6 for 20 min. Destaining was carried out in water. The stained gels were traced by a densitometer (Fuji–Riken FD-AIV).

RESULTS AND DISCUSSION

Fig. 1 shows the results of the adsorption chromatography of rRNA on CPG.

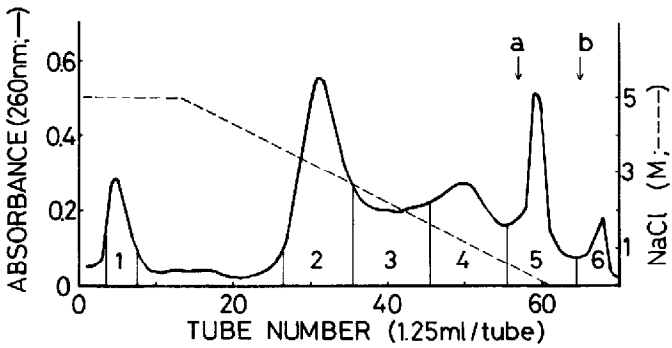

Fig. 1. Chromatographic pattern of crude rRNA (14.9 A_{260} units) on CPG column (9 cm × 0.6 cm I.D.). Elution was carried out at 28°C with a linear gradient (total volume 60 ml) from 5 *M* NaCl in 10 m*M* Tris–HCl at pH 6.3 to 10 m*M* Tris–HCl at pH 7.6 at a flow-rate of 1.5 ml/cm² min. At points a and b, 10 m*M* Tris–HCl at pH 7.6 and 1% SDS in the buffer, respectively, were eluted on the column.

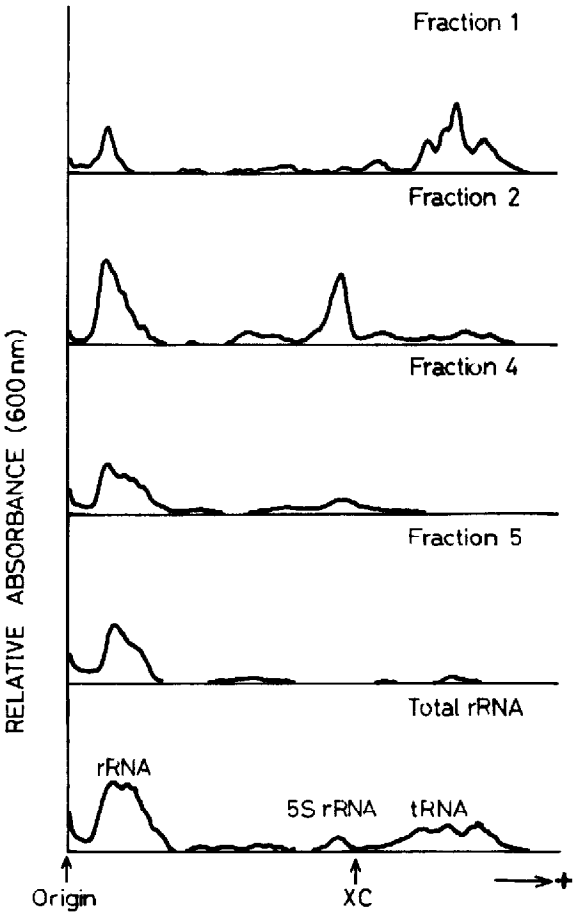

Fig. 2. Densitometric tracings of the stained gels of the fractions shown in Fig. 1. Electrophoresis on 8% polyacrylamide gel was carried out at 700 V and at 6 mA for 2 h in a cold room at 4°C. The numbers coincide with the fraction number in Fig. 1; XC = xylene cyanol of the electrophoresis marker dye. The position of tRNA and 5S rRNA were determined from the results of the electrophoresis of a standard bovine liver tRNA preparation.

Fig. 2 shows the results of the densitometric tracing of the stained gels of the fractions shown in Fig. 1. The major RNA in fraction 1 of Fig. 1, eluted from the column with 5 *M* NaCl–10 m*M* Tris–HCl at pH 6.3, was not rRNA but tRNA as shown in Fig. 2. Fraction 2 contained 5S rRNA, which moved slightly slower than the marker dye (XC), and rRNA of high molecular weight. The electrophoretic patterns of fraction 4 showed that the fraction contained high-molecular-weight rRNA. RNA, which was not eluted with the gradient, was eluted with 10 m*M* Tris–HCl buffer (pH 7.6) as fraction 5 and with 1% SDS as fraction 6. The overall recovery of RNA from the column was 91%.

Fig. 3 shows the results of the rechromatography. The RNAs in fractions 1, 2 and 3 were eluted at identical points on the rechromatograms as in the first chromatography (top chromatogram in Fig. 3). However, some parts of fraction 4 were

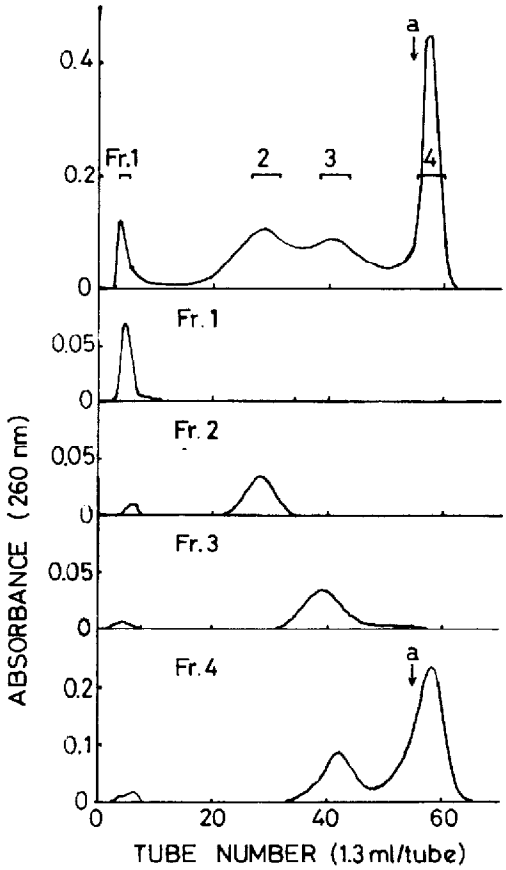

Fig. 3. Patterns of rechromatography of crude rRNA (6.0 A_{260} units). Chromatographic conditions were identical with those in Fig. 1. The top most figure is the pattern of the first chromatography. Fractions 1–4 in the top most figure were rechromatographed and the results are show in the lower columns. Fractions 1, 2, 3 and 4 correspond to fractions 1, 2, 4 and 5 in Figs. 1 and 2.

eluted at the higher salt concentration and the rechromatogram shows two peaks. The electrophoretic patterns in Fig. 2 show that fractions 4 and 5 in Fig. 1 (both coincident with fractions 3 and 4 in Fig. 3) have similar constituents. Therefore, it is possible that some parts of the high molecular weight rRNA have differently ordered structures which influence the pattern of chromatography, and also have different affinities to CPG. As expected, the major parts of fraction 4 in Fig. 3 were eluted at the same positions as in the first chromatography.

Stability of the pH values of the mobile phase was essential in order to achieve good reproducibility of the chromatographic patterns and had to be determined not by pH test paper but by a pH meter. In 5 *M* NaCl at pH 4.0, tRNA was well adsorbed on CPG and was eluted along with rRNA in one peak on the chromatogram. At pH 8.0, some parts of rRNA passed through the column with tRNA. Therefore, adsorption chromatography of RNA on CPG must be carried out at neutral pH (6.3–7.6). It was reported that the effect of pH was important for the separation of

tRNA on Sepharose[15]. The adsorption chromatography of DNA using a system identical with that used for RNA did not give good separation. This might be due to the behaviour of DNA in highly concentrated solutions[16], in which DNA was soluble, even though DNA fragments were adsorbed onto glass beads in highly chaiotropic, NAI solutions[17]. This method may be useful for the purification of tRNA, viral RNA, phage RNA and mRNA.

CONCLUSION

Adsorption chromatography of rRNA on CPG was performed by lowering the concentration of NaCl from 5 *M* in 10 m*M* Tris–HCl at pH 6.3 to zero in the Tris–HCl buffer at pH 7.6. The separation of the RNA was shown by the gel electrophoretic patterns, the overall recovery of RNA from the column being 91%. This is the first report of separation of RNA on porous glass by adsorption chromatography.

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