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Note

Separation of ribonucleic acids by chromatography on spermine–agarose columns

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The separation of RNA species of different molecular weights poses problems if large amounts of RNA are to be processed. Separation on agarose–acrylamide composite gels[1], or on acrylamide gels[2,3] is only suitable for analytical work, and even centrifugation in sucrose gradients[4] is not easy with milligram amounts of RNA. Recently, a method[5] involving lysine covalently coupled to agarose has been used for separating RNA. In our hands, this method gave good results in the separation of *Escherichia coli* 5S and 4S RNA from the high-molecular-weight ribosomal RNA species. However, we could not completely separate 4S from 5S RNA or 16S from 23S RNA; the former separation was particularly poor when large amounts of 4S RNA were to be separated from small amounts of 5S RNA (as, for example, with yeasts).

Moreover, chromatography on lysine–agarose is not feasible at temperatures below 10°, as the salt concentration required for RNA elution is much greater at cold-room temperature, and this makes it more difficult to avoid contamination of the RNA preparations with traces of ribonucleases. We therefore sought a better separation medium for RNA, which would allow us to use temperatures of *ca.* 4°. This paper describes such a separation on columns with spermine covalently bound by a six-carbon "spacer" to agarose.

MATERIALS AND METHODS

Chemicals

Activated CH-Sepharose 4B was purchased from Deutsche Pharmacia (Freiburg, G.F.R.), spermine from Serva Feinbiochemica (Heidelberg, G.F.R.) and deoxyribonuclease I (analytical grade) from Boehringer (Mannheim, G.F.R.); all other chemicals were of analytical grade (E. Merck, Darmstadt, G.F.R.).

Preparation of spermine–agarose

A 3-g portion of activated CH-Sepharose 4B (*ca.* 9 ml of swollen gel), together with 1 mg of spermine per ml, was suspended in 20 ml of 0.1 *M* phosphate buffer of pH 6.6. The suspension was mixed with a rolling motion for 2 h at 22° and then rinsed with an excess of 0.05 *M* Tris–HCl (pH 8) that was 0.5 *M* in NaCl, followed by

0.05 *M* formic acid buffer (pH 4), also 0.5 *M* in NaCl. The unbound spermine was determined (as the dansylated product) by high-pressure liquid chromatography (HPLC).

RNA preparation

RNA was prepared from whole cells by the method of Aviv and Leder[6]. It was freed from DNA by incubation with DNase I, followed by phenol extraction. Finally, RNA was precipitated from the aqueous layer by addition of sodium acetate to a concentration of 2% and 2 volumes of ethanol. The precipitate was allowed to settle overnight at −20°.

RESULTS

Under the conditions described, 2–3 μmoles of spermine bind to 1 ml of agarose gel. Although, at pH values above 6.5, more polyamine binds to the gel, the separation efficiency of the product decreases. A 1.2 cm × 8 cm column containing 9 ml of gel can bind 5 mg of RNA in adsorption buffer (0.04 *M* Tris–HCl, pH 7.2, that is 0.05 *M* in NaCl); the RNA can then be eluted with a gradient from 0.05–0.3 *M* in NaCl at room temperature or from 0.05–0.4 *M* in NaCl at 4°. Fig. 1 shows the separation of total *E. coli* RNA, with use of a photoelectronic gradient former (Mixograd gradient former, Gilson, France), which automatically stops the gradient when a peak is eluted. Thus, the different RNA species are well separated.

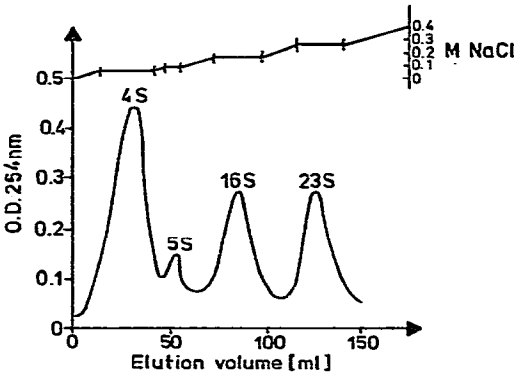

Fig. 1. Separation of *E. coli* RNA species by spermine–agarose chromatography. The salt gradient was kept constant during elution of each species. Column size, 12 mm × 80 mm; flow-rate, 1.5 ml/min; temperature, 8°.

When we checked the separation efficiency by electrophoresis of the RNA bands on polyacrylamide–agarose slab gels, we found that the purity of the separated species ranged from 75% (for 5S) to 95% (for 4S, 16S and 23S). Repetition of the chromatographic procedure increased the purity of each fraction to more than 95%. Individual classes of total RNA from the yeast *Trigonopsis variabilis* could be separated as shown in Fig. 2. Even in the presence of very large amounts of 4S RNA, 5S RNA could be obtained more than 70% pure by a single chromatographic run. Cutting fractions around the elution peaks always gave pure fractions, without detectable cross-contamination.

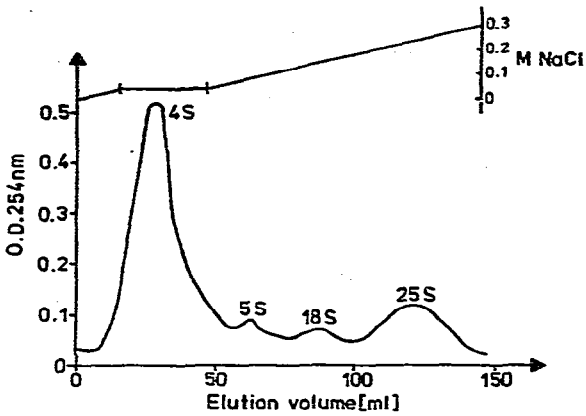

Fig. 2. Separation of RNA species from the yeast *Trigonopsis variabilis*.

DISCUSSION

These results demonstrate that chromatography on spermine–agarose offers a convenient and efficient method for separating RNA species from eukaryotic as well as from procaryotic cells according to size. As the method is simple, cheap and easy to perform, it should be especially useful in the separation of large amounts of RNA. Since, in contrast to chromatography on lysine–agarose[5], the temperature dependence of the binding of RNA to the gel is only slight, the method is also practicable at 4°, at which temperature degradation of RNA by ribonucleases is less. Spermine–agarose chromatography yields RNA fractions of high purity; this quality can be improved by cutting the fractions through the peaks on the elution curve.

Owing to the high capacity of the spermine–agarose, the separation of 5 to 7 mg of RNA in a 10-ml column is feasible, the nucleic acids being eluted at concentrations sufficiently high to enable direct precipitation of RNA by addition of ethanol; this minimises losses and circumvents concentration steps. Spermine–agarose chromatography is a simple, rapid and highly efficient method for the separation and purification of RNA species.

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REFERENCES

1 C. W. Digman and A. C. Peacock, *Biochemistry*, 7 (1968) 659.
2 U. E. Loening, *Biochem. J.*, 102 (1967) 251.
3 R. de Wachter and W. Fiers, *Methods Enzymol.*, 21 (1971) 167.
4 E. H. McConcey, *Methods Enzymol.*, 12 (1967) 620.
5 D. S. Jones, H. K. Lundgren and F. T. Jay, *Nucleic Acid Res.*, 3 (1967) 1569.
6 H. Aviv and P. Leder, *Proc. Nat. Acad. Sci., U.S.* 69 (1972) 1408.