MAK CHROMATOGRAPHY AT 35° CAUSES DENATURATION OF 5 S RNA FROM *SALMONELLA TYPHIMURIUM*

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1. Introduction

MAK chromatography* has been widely used for the separation of different species of RNA, i.a. for the separation of tRNAs. Different conformations of a single species, viz. 5 S RNA from *E. coli*, have also been separated by this method [1, 2]. In the course of other experiments we found that 5 S RNA from *S. typhimurium*, which was homogeneous upon gel filtration, was eluted in two distinct peaks from a MAK column at 35°. We could show that the first of these peaks is native 5 S RNA while the other is the result of denaturation of part of the 5 S RNA by the material of the column. Since the MAK column itself can apparently cause a conformational change the greatest caution in interpreting elution profiles form this column should be exercised.

2. Materials and methods

Wild type *Salmonella typhimurium* was grown with aeration at 37° on the mineral medium described by Schaechter et al. [3] supplemented with 0.2% glycerol. Growth was followed by measuring the absorbance of the culture at 450 nm. Ribosomes were isolated by suspending 80 g of cells (harvested in mid-log phase) in cold TM-buffer to a volume of 160 ml. To this suspension 1 mg/ml of egg-white lysozyme (Armour Pharmaceutical Co. Eastbourne, England) and 0.01 mg/ml of desoxyribonuclease (Nutritional Biochem. Co. Cleveland, Ohio) were added. The suspension was frozen in liquid N_2, thawed at room temperature and incubated for 10 min at 0°. Cells were then broken by sonication at 18,000–20,000 cps for 1 min in an MSE ultrasonic disintegrator. After a second incubation at 0° for 5 min, the suspension was made 1% in SDS (British Drug Houses Poole, England); special ly pure) and cell debris was removed by centrifugation in a Spinco rotor Ti60 at 17,500 rpm for 5 min. The pellet was washed once with cold TM-buffer containing 1% SDS. The supernatants were combined and ribosomes were collected by centrifugation for 60 min at 60,000 rpm in rotor Ti60. The ribosomal pellet was washed twice with cold TM-buffer and resuspended in the same buffer.

Low-molecular weight rRNA was isolated by extracting the ribosomes with an equal volume of 80% (v/v) redistilled phenol in TM-buffer at 4° for 30 min in the presence of 0.5% SDS. The layers were separated by centrifugation and the phenol layer was washed twice with half a volume of fresh cold TM-buffer. The combined water layers were made 1 M in NaCl and RNA was precipitated by adding two volumes of 96% ethanol cooled to −20°. The precipitate was collected by centrifugation, washed twice with ice-cold 70% ethanol and dissolved in distilled water to a volume of 100 ml. High-molecular weight RNA was precipitated by adding ammonium sulphate to 50% saturation at 0° [4]. The precipitate was collected by high-speed centrifugation and washed once with 25 ml of 50% saturated ammonium sulphate solution at 0°. The supernatants were combined, dialysed overnight against 200 volumes of distilled water at 4° and lyophilized. The dry material was

* *Abbreviations:*
MAK, methylated albumin kieselguhr;
SDS, sodium dodecylsulphate;
TM-buffer, 0.01 M tris-HCl + 0.01 M $MgCl_2$ pH 7.5;
tRNA, transfer RNA; rRNA, ribosomal RNA.

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dissolved in distilled water. Contaminating transfer and high molecular weight RNA was removed from these preparations by gel filtration on a column of Sephadex G-100 in 0.1 M NaCl + 0.01 M CH_3COOK + 0.01 M $MgCl_2$ pH 7.0. Fractions of 2.24 ml were collected at a rate of 10 ml/hr. The void volume of the column was determined with the aid of dextran blue. The fractions which contained 5 S RNA were pooled, dialysed against 100 volumes of distilled water for 4 hr at 4° and lyophilized. MAK chromatography was carried out on columns of 11.5 X 2.1 or 12.0 X 0.9 cm according to the method of Mandell and Hershey [5]; the column consisted of only one layer of coated kieselguhr. Before adsorbing the RNA, the column had been equilibrated at 35°. A maximum amount of 5 mg of RNA in 100 ml respectively 1 mg in 20 ml of 0.05 M phosphate buffer with 0.3 M NaCl pH 6.7 was adsorbed on the column. Elution was carried out with a linear gradient of 0.3 M NaCl (250 ml) to 1.3 M NaCl (250 ml) in 0.05 M phosphate buffer pH 6.7. Fractions of 5 ml were collected at a rate of 50 ml/hr. Recovery of UV-absorbing material was 95% or better. Methylated serum albumin was prepared according to Mandell and Hershey [5]. Kieselguhr was Celite-Hyflo-Supercel (Union Chimique Belgique Brussels, Belgium). Renaturation was carried out by the method of Lindahl et al. [6] and urea denaturation by that of Aubert et al. [1].

3. Results and discussion

Fig. 1A shows that upon MAK chromatography at 35° of RNA isolated from *S. typhimurium* ribosomes, three separate peaks elute at low salt concentrations. Peak I is tRNA, peaks II and III elute at positions similar to those of the A and B forms of *E. coli* 5 S RNA [1]. As both II and III appear to be absent from the elution pattern of RNA isolated from a ribosome-free supernatant (fig. 1B), it is highly probable that they contain 5 S RNA, albeit in different conformations. Low-molecular weight RNA was isolated from ribosomes, freed from tRNA by gel filtration and chromatographed on a MAK column.

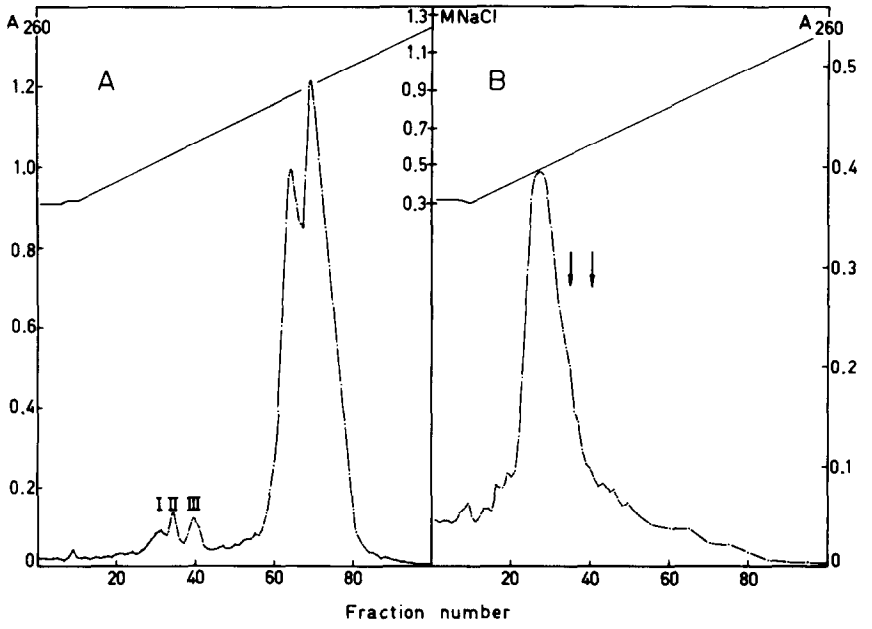
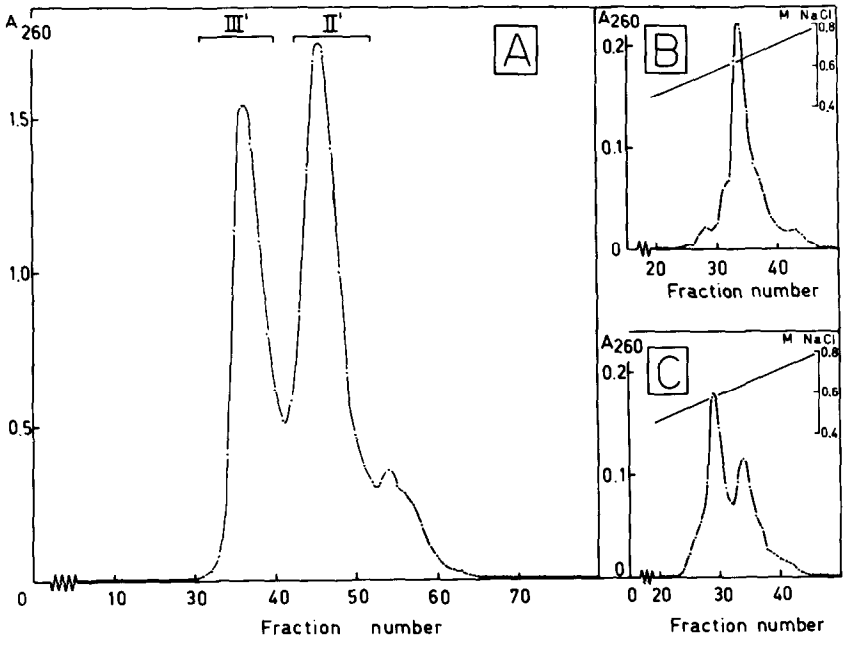


Fig. 1. MAK chromatography of RNA extracted from ribosomes (A) and ribosome-free supernatant (B) of exponentially growing *S. typhimurium*. Ribosomes were isolated as described in Materials and methods. Ribosome-free supernatant was obtained by centrifuging the supernatant of the first high-speed centrifugation for an additional three hours at 105,000 *g* and collecting the supernatant. RNA extraction and chromatography were carried out as described in Materials and methods.
Arrows in B indicate the positions at which components II and III would have eluted.

All fractions eluting up to 0.6 M NaCl were pooled, dialysed and lyophilized. This material, which was homogeneous upon gel filtration before MAK chromatography, was separated on Sephadex into about equal amounts of two components II′ and III′ (fig. 2A). MAK chromatography of the material in the separate peaks showed that peak III′ consisted of almost pure "peak III material" (fig. 2B), whereas peak II′ again separated into two peaks, which eluted at positions II and III, respectively (fig. 2C). This indicates that under the conditions used for gel filtration and MAK chromatography, component III is stable. Component II′, however, either is contaminated by component III′ or is partly converted to III′ during MAK chromatography. The first possibility was ruled out by the following experiment. Component II was purified separately by gel filtration. This purified material was subjected to MAK chromatography, which again resulted in the appearance of a peak at position III. These results increase the probability that components I and II and thus II′ and III′ are different conformations of the same species of RNA. Definite proof of this idea was obtained by the experiment given in fig. 3. Here purified component III was dissolved in 0.01 M tris-HCl + 0.01 M $MgCl_2$ pH 6.9 and heated at 60° for 5 min [6]. By this treatment it was converted to component II.

We conclude that component II (II′) is native 5 S RNA and component III (III′), which only appears after MAK chromatography at 35°, is a denatured form. In agreement with our conclusions component III′ eluted from a Sephadex column at a position simi-



F ig. 2A. Sephadex G-100 gel filtration of low-molecular weight rRNA after MAK chromatography. RNA was prepared as described in Materials and methods and then chromatographed on a MAK column. All fractions eluting up to 0.6 M NaCl were pooled, dialysed for 4 hr against 100 volumes of distilled water at 4° and lyophilized. The residue was dissolved in distilled water. Gel filtration was carried out on a column of 135 X 1.2 cm as described in Materials and methods. Void volume was at 30 ml. Pooled material is indicated by brackets.

Fig. 2B and 2C. Rechromatography of component II′ and III′ on a MAK column. Part of the pooled, dialyzed and lyophilized material of peak II′ and III′ (fig. 2A) was subjected to MAK chromatography on a column of 12.0 X 0.9 cm. Only the first part of the elution pattern is shown. There were not other peaks of UV-absorbing material. Fig. 2B: component III′; Fig. 2C: component II′.

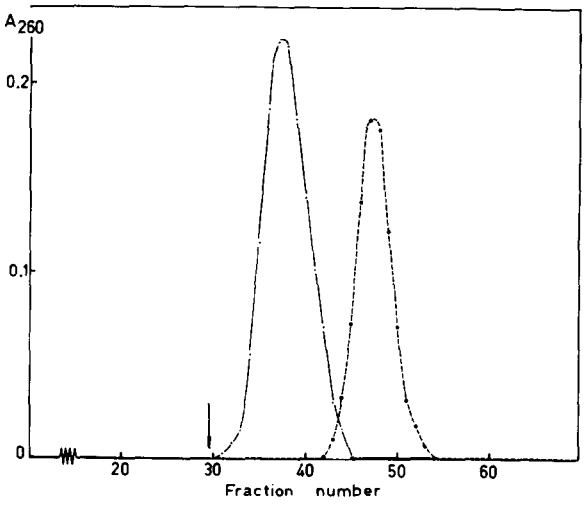


Fig. 3. Effect of renaturation treatment on component III. 1 mg of the pooled, dialysed and lyophilized material from peak III′ of fig. 2 was dissolved in 0.01 M tris-HCl + 0.01 M $MgCl_2$ pH 6.9. Half of the solution was heated for 5 min at 60°, the other half was kept at room temperature. Both preparations were chromatographed on the same Sephadex G-100 column in different runs. —●—● unheated; - - ● - - ● - - heated. The arrow indicates the void volume of the column. The same column as in fig. 2 was used.

lar to that of urea-denatured 5 S RNA (unpublished observations). We cannot decide whether the urea-denatured form is identical to component III, since no cochromatography was carried out. Anyhow MAK chromatography under the conditions described causes denaturation of 5 S RNA. Incubation of 5 S RNA, purified by gel filtration, for 3.5 hr at 35° in 0.05 M phosphate buffer + 0.5 M NaCl pH 6.7 did not result in the appearance of component III upon Sephadex chromatography. Therefore, the column material itself seems to be essential for the denaturation. We did not make a detailed study of the denaturation phenomenon under other conditions. Nontheless, we feel that separation of an otherwise homogeneous RNA fraction, e.g. a specific tRNA species, into different peaks by MAK chromatography should be considered with the utmost reserve. This separation might well be an artifact due to denaturation during chromatography.

References

[1] M.Aubert, J.F.Scott, M.Reynier and R.Monier, Proc. Natl. Acad. Sci. U.S. 61 (1968) 292.
[2] N.Sarkar and D.G.Comb, J. Mol. Biol. 39 (1969) 31.
[3] M.Schaechter, O.Maaløe and N.O.Kjeldgaard, J. Gen. Microbiol. 19 (1958) 592.
[4] S.Avital and D.Elson, Biochim. Biophys. Acta 179 (1969) 297.
[5] J.D.Mandell and A.D.Hershey, Anal. Biochem. 1 (1960) 66.
[6] T.Lindahl, A.Adams and J.R.Fresco, Proc. Natl. Acad. Sci. U.S. 55 (1966) 941.