CHROM. 5185

Simplified methylalbumin–kieselguhr column chromatography of aminoacyl-tRNA

The identification and fractionation of nucleic acids on a methylalbumin–kieselguhr column (MAK column) according to the method described by MANDELL AND HERSHEY[1] was carried out, and improvements were made by various coworkers for tRNA studies. The resolution efficiency of MAK columns allows the separation of isoacceptor tRNA's arising from different tissues, or analyses the evolution of these tRNA species during a biological process such as virus infection, sporulation, secretion or cell differentiation.

Chromatography on an MAK column utilizes acylated tRNA, extracted by cold phenol and precipitated several times by ethanol. The tRNA is eluted by a linear NaCl gradient in the 0.35–0.55 *M* range. The time between acylation *in vitro* and chromatographic elution must be very short, in order to limit the hydrolysis of the aminoacyl linkage. The half-life of aminoacyl-tRNA depends greatly on the pH and temperature of the solution, from a few minutes to several hours[2,3] when the pH of the medium is changed from 8.6 to 7, and the temperature from 37 to 30°.

The use of a Mg^{2+} ion-chelating agent here described, which facilitates the dissociation of the aminoacyl-tRNA–ligase complex without breaking the aminoacyl linkage reduces the risk of hydrolysis of the ester bond and simplifies the chromatographic routine work. Time taken for this procedure is reduced from about 24 h to 3 h.

Our acylation conditions described elsewhere[4] have been adapted for larger volumes: Tris–HCl buffer 100 μmoles, $MgCl_2$ 100 μmoles, ATP 50 μmoles, tRNA 100–200 μg, [^{14}C]- and [^{3}H]amino acid from C.E.A., France (10 μCi/1.7 μM for [^{14}C]alanine, 100 μCi/1 μM for [^{3}H]alanine, 100 μCi/5 mμM for [^{3}H]phenylalanine), crude enzymatic solution 10–15 mg, total volume 2.5 ml; the final pH was 7.2 and the incubation temperature 37°, the incubation time being 15–20 min.

After the incubation period 2.5 ml of a 0.1 *M* sodium ethylenediamine tetraacetate (EDTA) solution were added with stirring for 5 min in a water bath at 37°. The solution was cooled to about 4° and adjusted to 0.1 *M* with NaCl and when necessary to pH 7.0. The solution was immediately applied to the column in a cold chamber at 4° under normal pressure. It was washed with about 50 ml of a standard buffer solution (0.01 *M* Tris–HCl pH 7.0, 0.10 *M* NaCl). Elution is carried out with a total volume of 200 ml of a linear gradient from 0.20 to 0.65 *M* NaCl. Wash and elution procedures were performed with a peristaltic pump adjusted for a 2 ml/min flow. After spectrophotometric recording at 260 and 280 nm (Elugraphe EPL, Seive Paris) and measurement of the refractive index, fractions of 4 ml were precipitated by the addition of 1 ml of 20% trichloroacetic acid and 0.2 ml of a 1% bovine serum albumin solution. The precipitate was collected on a Whatman GF/C glass filter or directly evaporated into the counting flask and the radioactivity measured using a liquid scintillation spectrometer (Intertechnique ABAC-SL 40, France).

MAK columns were prepared as follows: for two columns of 2.0 cm diameter and 20 cm high, 10 g of commercial kieselguhr (Celite 545, Prolabo, Paris) were added to 100 ml of the standard buffer. The suspension was boiled for a few minutes with stirring using a glass rod and then cooled in an ice bath while being agitated with

a magnetic stirrer. 5 ml of a 1% methylated serum albumin solution were added dropwise to the cooled suspension. After 10 min of stirring the suspension was divided into two fractions. After pouring it into the column tube, a few ml of untreated kieselguhr solution was then added to the top, forming a 0.5 cm high protective phase. The column was then washed with a large volume of standard buffer until the optical density reached zero. These packed columns were used within 48 h.

The extraction of radioactive acylated tRNA with cold phenol was performed according to BERG *et al.*[5].

Enzymes, amino acids and ATP are not retained by an MAK column in 0.1 *M* salt solution whereas RNA and aminoacyl-tRNA are adsorbed. The charge capacity of our MAK columns did not exceed 30 absorbance units at 260 nm. The average yield, that is the ratio between the amount of tRNA deposited and the amount eluted, measured by absorbance or radioactivity is 90 ± 10%. The ratio between the c.p.m. of the acid-insoluble fraction of the effluent, and the c.p.m. of the acid-insoluble fraction of the material varies from 1 to 3; this signifies that 50 to 75% of the acylated tRNA is actually adsorbed to the methylalbumin–kieselguhr, the rest is associated with the soluble proteins and is washed from the column with the effluent. Nevertheless good co-chromatograms were obtained with less than 5000 c.p.m. of radioactive material applied to the column.

Fig. 1 shows the elution profile of a chromatogram of a mixture of [^{3}H]Ala-tRNA from baker's yeast (S-RNS Boehringer, Mannheim, G.F.R.) and [^{14}C]Ala-tRNA from calf liver. The elution profiles of these two tRNA's are clearly different, which implies clear structural differences. Ala-tRNA from yeast is in fact a mixture of two isoacceptor species[6]; its elution peak is twice as broad as that of the liver Ala-tRNA.

Fig. 2 shows the elution profile of a Phe-tRNA preparation treated with cold phenol and Fig. 3 corresponds to the same calf liver Phe-tRNA separated from its ligase by EDTA. Quantitative differences are noticeable between the three fractions A, B and C characterized in Table I.

The complexing of Mg^{2+} ions by the EDTA solution (final molarity 0.05 *M*)

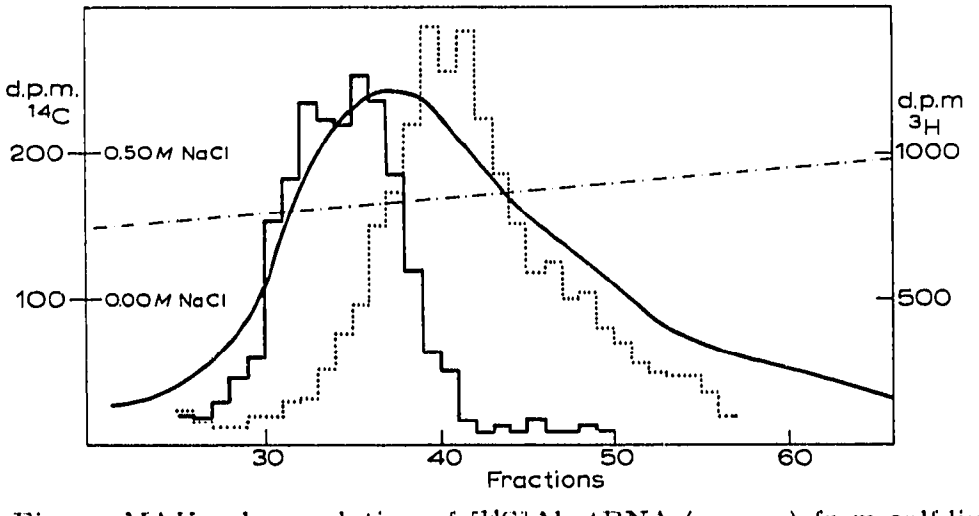

Fig. 1. MAK column elution of [^{14}C]Ala-tRNA (———) from calf liver and [^{3}H]Ala-tRNA from baker's yeast (· · · · · ·). Optical density at 260 nm ———. NaCl gradient (– · – · –) from 0.10 to 0.50 *M* in a Tris–HCl buffer 0.01 *M* pH 7.0 Fractions of 5 ml. For column preparation and acylation conditions, see text.

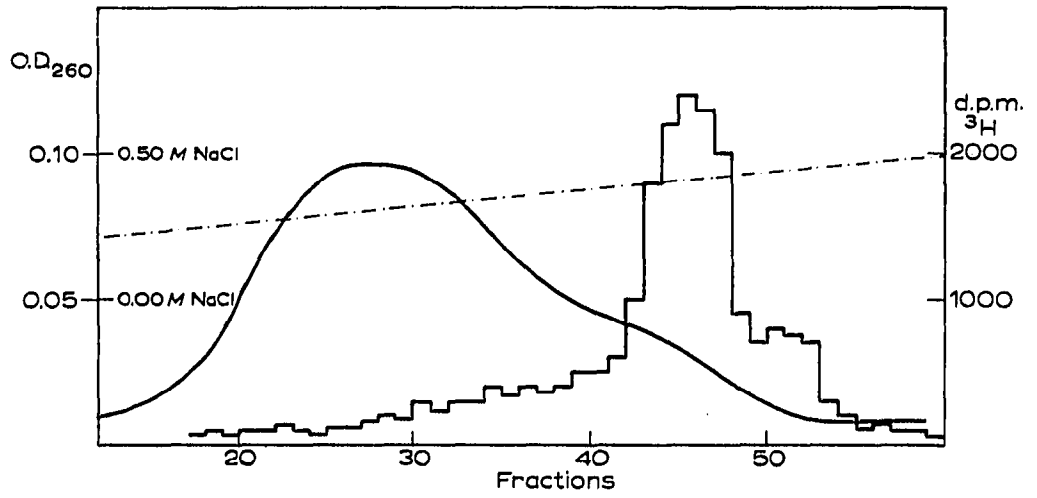

Fig. 2. MAK column elution of [^{3}H]Phe-tRNA from calf liver, extracted by the phenol method according to BERG *et al.*[5].

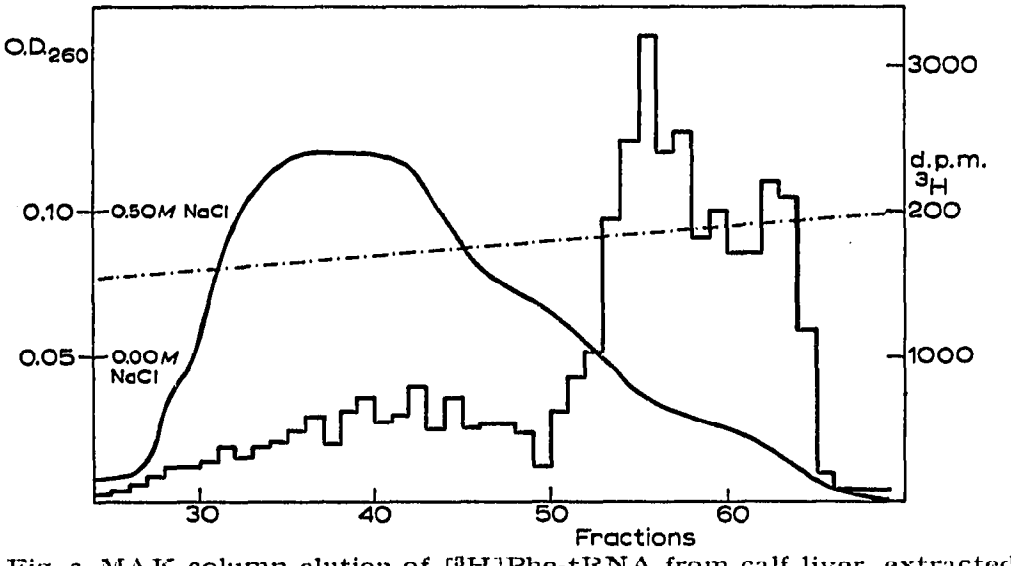

Fig. 3. MAK column elution of [^{3}H]Phe-tRNA from calf liver, extracted by the EDTA method.

leads to an increase in the two minor species eluted before and after the major peak of Phe-tRNA. Doubtless, the complete removal of Mg^{2+} from one or several divalent cation sites on the tRNA molecule causes a less compact conformation[7] which is eluted at a higher ionic strength, as GARTLAND AND SUEOKA[8] have been observing with *E. coli* Trp-tRNA treated during phenol extraction with a 0.004 *M* EDTA solution. Consequently the increased amount of species C corresponds to a conformational modification of Phe–tRNA. It is possible that the small amount (12%) of this form recovered after classical phenol extraction in a medium without Mg^{2+} ions can be attributed to a partial change of the tertiary structure. The polymorphism of liver Phe–tRNA has been demonstrated by TAYLOR *et al.*[9] using normal extraction conditions and MAK column chromatography. Species C is found as a shoulder in Phe-tRNA from rabbit liver, acylated *in vitro*.

The minor species A, corresponding probably to the UUC codon, has been

TABLE I

PHE-tRNA ISOACCEPTORS FROM CALF LIVER

Species	*Phenol method*			*EDTA treatment*		
	Fractions	*d.p.m.*[a]	%	*Fractions*	*d.p.m.*[a]	%
A	28–38	2 300	12.5	27–48	7 720	22
B	39–49	13 800	75.5	50–59	18 980	56
C	50–54	2 150	12.0	60–66	8 500	24

[a] Significant values for the radioactivity were calculated allowing for a background of 100 d.p.m.

observed with *in vivo* acylated Phe-tRNA from rabbit liver and also with Phe-tRNA from mouse embryonic cells which were acylated using heterologous ligases from Ehrlich ascites tumor or rabbit kidney[9]. AXEL *et al.*[10] have directly chromatographed the aqueous phase, which contains about 20% phenol, on MAK columns and observed a slightly asymmetric profile for Phe-tRNA from rat liver. Species A has a structure certainly very close to that of the major Phe-tRNA which recognizes the UUU codon and has a chromatographic behavior highly sensitive to variations in the ionic environment.

This simplified MAK column chromatography has been successfully applied to the study of structural differences between calf lens and liver tRNA's[11], between tRNA's extracted from the posterior part of the silk gland of *Bombyx mori* L during two different physiological phases at the Vth instar[12].

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1 J. D. MANDELL AND A. D. HERSHEY, *Anal. Biochem.*, 1 (1960) 66.
2 S. CHOUSTERMAN, G. HERVE AND F. CHAPEVILLE, *Bull. Soc. Chim. Biol.*, 48 (1966) 1295.
3 J. R. MENNINGER, *J. Mol. Biol.*, 16 (1966) 556.
4 J. P. GAREL AND P. MANDEL, *Eur. J. Biochem.*, 5 (1968) 474.
5 P. BERG, F. H. BERGMANN, E. J. OFENGAND AND M. DIECKMANN, *J. Biol. Chem.*, 236 (1961) 1726.
6 R. H. REEVES, N. IMURA, H. SCHWAM, G. B. WEISS, L. H. SCHULMAN AND R. W. CHAMBERS, *Proc. Nat. Acad. Sci.*, 60 (1968) 1450.
7 T. LINDAHL, A. ADAMS AND J. R. FRESCO, *Proc. Nat. Acad. Sci.*, 55 (1966) 941.
8 W. T. GARTLAND AND N. SUEOKA, *Proc. Nat. Acad. Sci.*, 55 (1966) 948.
9 M. W. TAYLOR, G. A. GRANGER, C. A. BUCK AND J. J. HOLLAND, *Proc. Nat. Acad. Sci.*, 57 (1967) 1712.
10 R. AXEL, B. WEINSTEIN AND E. FARBER, *Proc. Nat. Acad. Sci.*, 58 (1967) 1255.
11 J. P. GAREL, N. VIRMAUX AND P. MANDEL, *Biochimie*, in press.
12 J. P. GAREL, G. CHAVANCY, J. DAILLIE AND P. MANDEL, *Fed. Eur. Biochem. Soc., Letters*, 12 (1970) 249.

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