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The separation of nucleic acids on basic polyamino acid–kieselguhr columns

A rapid, one-step procedure for the isolation of 5 S rRNA, in a highly purified form, from total nucleic acids using columns of basic polyamino acids adsorbed onto kieselguhr has been recently described[1]. These columns (BPAK) are easy to prepare and use, and are stable at room temperature. They can be rapidly regenerated after use and can be used repeatedly without loss of resolution or decrease in flow rate. A high flow rate (3–4 ml/min) can be used without loss of resolution, thus making the analytical procedure very rapid. The resolution obtained is easily reproducible and the columns are very stable. The columns are short, longer ones made of the same material having shown no advantages, and, in conjunction with a recording photometer, provide a fast and convenient analytical method. In view of these practical advantages, we wish to report the usefulness of these columns for the resolution of other species of nucleic acids.

Poly-L-arginine sulfate (mol. wt. 21,900), poly-L-lysine HBr (mol. wt. 20,000) and poly-L-ornithine HBr (mol. wt. 15,800) were purchased from Yeda-Miles Laboratories Inc., Rehovoth, Israel, and Hyflo Supercel (particle size 5–25 μ) from Serva, Heidelberg, G.F.R. Uniformly ^{14}C-labeled amino acids were products of the Department of Biology, C.E.A., Saclay. *Escherichia coli* K12 tRNA for the experiments with aminoacyl tRNA was obtained from Schwarz Bioresearch, Orangeburg N.Y., U.S.A., and from General Biochemicals, Chargrin Falls, Ohio, U.S.A.; amino acids from Calbiochem; membrane filters (MF 50) from Sartorius Membranfilter-Gesellschaft, Göttingen G.F.R.; and alumina (bacteriological grade) from Alcoa Company of America.

The *E. coli* K12 strain used in this work was grown under vigorous aeration in a New Brunswick Microferm fermentor in a medium which contained per l: 10 g Bacto Yeast, 10 g glucose, 17 g KH_2PO_4 and 21.8 g K_2HPO_4. The pH was adjusted to 7.4. The nucleic acids were extracted from exponentially growing cells by grinding the cells with alumina and subsequently treating them with phenol[2]. Suspensions of kieselguhr (8 g) in the starting buffer (60 ml) of the elution gradient, which were sufficient to give a column of 2 × 6.5 cm, were heated to boiling, with manual stirring, to remove air bubbles and then cooled in an ice–water mixture. Polyamino acids, at a concentration of 2 mg/ml, were dissolved in water, with the exception of polyarginine which was dissolved in 0.01 *M* HCl. Each column was made by adding the required volume of the polyamino acid solution to the cooled suspension of kieselguhr and the mixture was stirred well. The slurry was poured into a glass column (2 × 15 cm), fitted with a fritted disc which had been covered with a thin layer of Whatman Standard cellulose powder, and, for reasons of speed, packed under pressure. A layer consisting of kieselguhr (2 g) in buffer (10 ml) was added and a light plug of glass wool was placed on top of the column. The packed column was washed with starting buffer (150 ml) before the sample was applied. Chromatography was carried out using a linear elution gradient at room temperature, the elution buffers being supported on a platform 200 cm above the head of the column. The elution was monitored by an LKB Uvicord absorptiometer. Subsequently, the optical density of each of the fractions was measured at 260 nm in a Zeiss spectrophotometer PMQ II.

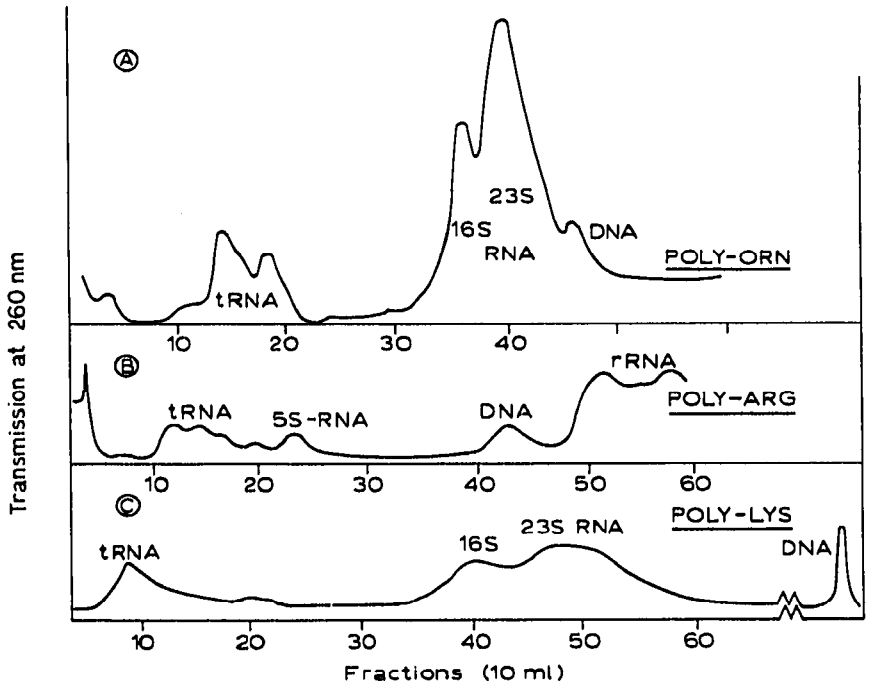

Fig. 1. Elution patterns of a nucleic acid mixture from basic polyamino acid–kieselguhr columns. The columns consisted of 6 mg polyamino acid and 8 g kieselguhr as described in the text and chromatography of the nucleic acid mixture (4–5 mg) was carried out at room temperature. The linear elution gradient, which was formed from 300 ml of starting buffer and a 300 ml reservoir of buffer and which was found to give optimal resolution of the nucleic acids, is shown for each column A = polyornithine, 0.5–3.0 *M* NaCl in 0.15 *M* phosphate (pH 6.7); B = polyarginine, 1.0 *M* NaCl in 0.15 *M* phosphate (pH 6.7)–5.0 *M* NaCl in 0.1 *M* Tris-HCl (pH 8.9); C = polylysine, 0.1 *M* NaCl in 0.15 *M* phosphate (pH 5.3)–1.6 *M* NaCl in 0.15 *M* phosphate (pH 6.7); DNA was then eluted with 3.0 *M* NaCl in 0.15 *M* phosphate (pH 6.7). The flow rate of the effluent through the cell of an LKB Uvicord was 3–4 ml/min and the transmission was plotted automatically every 20 sec. The peaks were identified as described in the text.

It was found that 6 mg of polyamino acid added to 8 g kieselguhr suffice for optimal fractionation of 5 mg nucleic acid. The elution gradients used for optimal resolution of the nucleic acid species on the three different columns are shown in Fig. 1. The identity of the peaks of the elution sequence was established by a comparative method. When applied to an MAK column, nucleic acids are resolved into a series of peaks whose nature and sequence is well known[3]. In our experiments, each of the known peaks eluted from an MAK column was chromatographed separately on each of the BPAK columns and the elution position of the various nucleic acids so found served to establish the elution sequence. It can be seen that the degree of resolution obtained with BPAK columns is different, in several respects, from that obtained with MAK columns, particularly in that 5S rRNA is well separated from 4S RNA. This latter separation was the subject of a recent communication[1]. The conditions of elution in all cases indicate that the nucleic acids are more tightly bound to BPAK columns than to MAK columns, presumably owing to the higher basicity of the former.

Under the conditions used, chromatography on polyarginine columns leads to considerable purification of DNA since rRNA is very tenaciously bound and can only

be partially eluted under conditions which do not lead to its degradation. On the other hand, the two rRNA species are eluted from columns of polylysine and polyornithine earlier than DNA: the reason for this reversal of elution sequence is not known, but a different mode of chromatography is indicated. In the case of polylysine columns DNA is very well separated from the rRNA and can only be displaced from the column by 3.0 *M* NaCl. However, in order to achieve this resolution a mildly acidic pH gradient was found to be essential; a salt gradient at a constant pH close to neutral did not lead to fractionation. Polylysine columns have already been used for the chromatography of DNA[4].

On polyarginine columns the tRNA peak is partially resolved. In order to investigate more closely the degree of resolution attained, ^{14}C-labeled aminoacyl tRNAs, the label being uniformly distributed in the amino acid moiety, were chro-

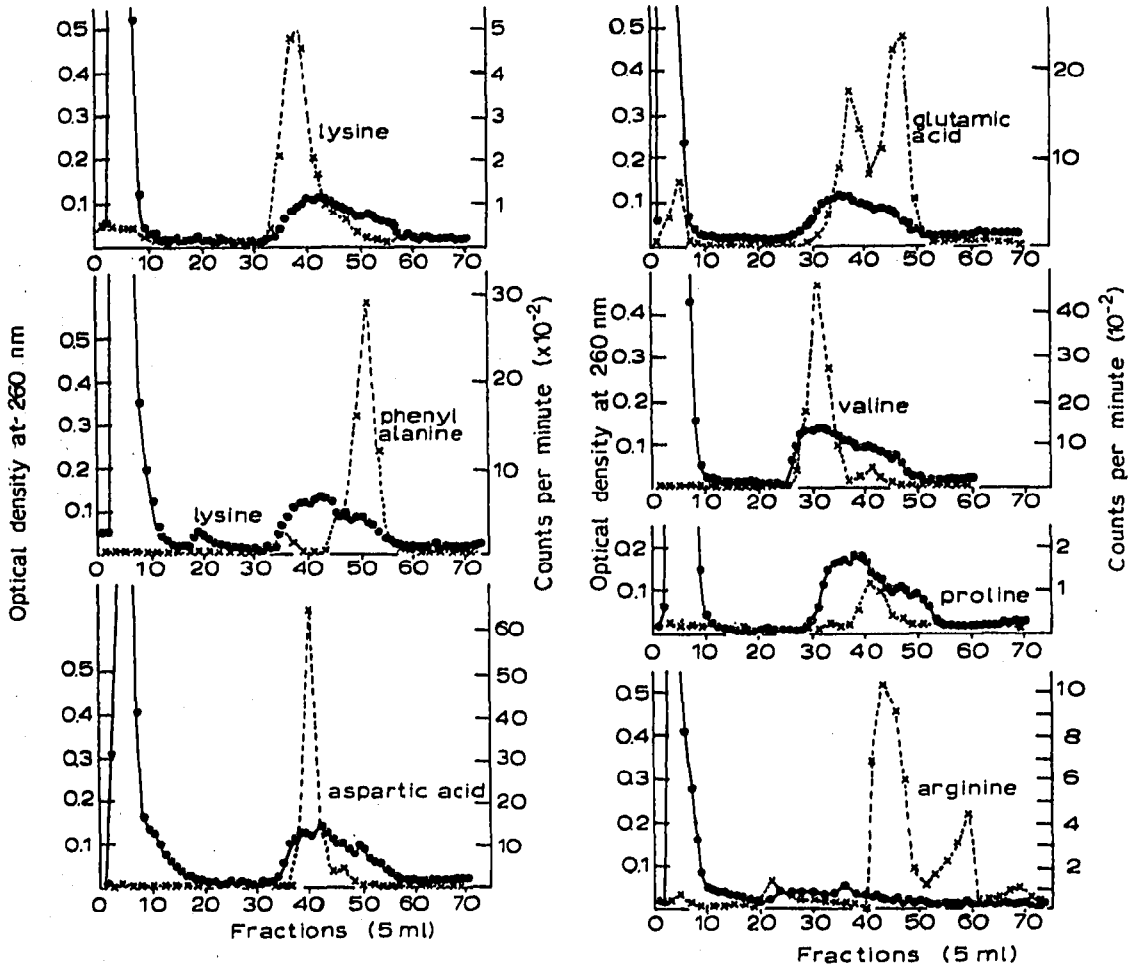

Fig. 2. Sequence of elution of ^{14}C-aminoacyl tRNA species from polyarginine columns. Stripped tRNA (5 mg) was aminoacylated as described[6], one of the twenty amino acids being uniformly labeled with ^{14}C. After phenol extraction and ethanol precipitation, the aminoacyl tRNAs were applied to a column, which was prepared from 6 mg polyarginine and 8 g kieselguhr, in 2 ml 0.4 *M* NaCl in 0.15 *M* phosphate, pH 6.0 (starting buffer). The column was eluted with a linear gradient formed from 250 ml starting buffer and 250 ml 2.5 *M* NaCl in 0.15 *M* phosphate, pH 6.0, the flow rate being 3–4 ml/min. The optical density (○—○) of each fraction was measured in a Zeiss spectrophotometer at 260 nm. Alternate fractions were adjusted to 5% with trichloroacetic acid, filtered and the filters were counted in a Packard scintillation counter (×– – – ×).

matographed. Elution patterns for seven amino acids are shown in Fig. 2. Six other amino acids whose elution profiles are not shown, were also found to be fractionated. A buffer at pH 6 was employed in order to minimize hydrolysis of the aminoacyl substituent. The resolution obtained was found to be at least as good as that found with MAK columns[5]. However, a surprising finding was that, in every case, 90% of the optical density was excluded whereas 100% of the counts/min were retained on the column, except in the case of glutamic acid. The potentially interesting implications of this finding are under further investigation.

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