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# HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF tRNA

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## SUMMARY

A rapid separation of tRNA isoacceptor species is described using high-pressure liquid chromatography of tRNA samples on RPC-5 resin. The technique is shown to be highly reproducible. A minimum of 8000 counts in the sample is necessary for analysis in a flow cell counting device, although less radioactive samples can be counted in a liquid scintillation counter. The technique is proposed for the analysis of changes in tRNA complements from differentiating cells or from cells that have been grown under different conditions.

## INTRODUCTION

The proliferation of structural work on transfer RNAs has been due largely to the introduction of the reversed-phase chromatography (RPC) resins developed by Pearson *et al.*[1]. Subsequent modification of this system permitted Kelmers and Heatherly[2] to achieve excellent separations of tRNA isoacceptors on short columns operating at moderate pressure. We report here an extension of these efforts using high pressure which, while maintaining the high resolution of the RPC resin, reduces the time of analysis to 20 min.

## MATERIALS AND METHODS

### *Equipment and chemicals*

The chromatograph illustrated in Fig. 1 was constructed from the following components: the pump used was the Model 396-89 Milton Roy minipump having a flow capacity of 46–460 ml/h. The column was made from a 35 cm length of precision bore stainless steel of 6.2 mm outside diameter and 4.0 mm inside diameter. The injection port was made from a Swagelok union tee containing in the injection branch a silicon rubber septum which was sealed with a cap. All other stainless-steel tubing and Swagelok fittings were obtained from Laurentian Valve and Fitting of Montréal. The system was fitted with a Model D Penny and Giles pressure transducer. Radioactivity was measured with a Nuclear Chicago scintillation counter equipped with a 2.4-ml anthracene flow cell. Alternatively, samples from fraction-collecting experiments were counted in a Packard Tricarb using a Triton–toluene cocktail. Salt

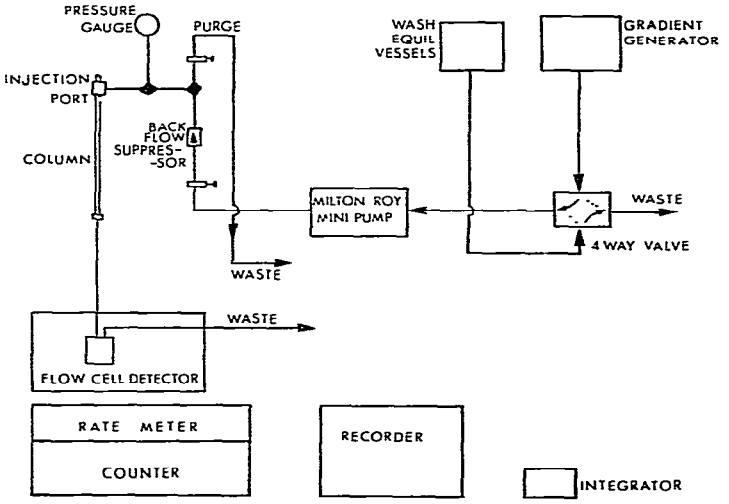


Fig. 1. Representation of the chromatograph.

gradients were generated by a Buchler Varigrad. Output from the flow cell counter was recorded on a recorder equipped with an electronic integrator of laboratory design.

Radioactive amino acids were obtained from New England Nuclear (Boston, Mass., U.S.A.). Bacterial cells used for tRNA and aminoacyl-tRNA synthetase extractions were grown in this laboratory. Beef liver was obtained from a local abattoir and placenta was provided by a local hospital.

*Extraction of tRNA*

tRNA was extracted according to the method of Robison and Zimmerman[3]. With bacterial material the intermediary isopropanol extraction could be eliminated.

*Preparation of aminoacyl-tRNA synthetases*

Aminoacyl-tRNA synthetases were extracted by a modification of the method of Hoskinson and Khorana[4]. The ammonium sulfate precipitate was dissolved in a 0.05 *M* Tris–$H_2SO_4$ buffer at pH 7.5 containing 0.005 *M* ethylenediamine tetraacetic acid and chromatographed on a 100 × 2 cm Sephadex G-200 column which was eluted with the same buffer. Enzymatically active fractions were dialyzed against 80% Carbowax in the same Tris–$H_2SO_4$ buffer. Preparations from mammalian tissue were also dialyzed against 80% Carbowax before the Sephadex G-200 chromatography. The enzymes were conserved at −20° in 50% glycerol and were stable for at least 6 months. Synthetases from placenta lost 20% of their activity in one year.

*Aminoacylated tRNA*

Aminoacylated tRNA was prepared by the method of Yang and Novelli[5] using up to 5 $A_{260\,nm}$ units of tRNA in a total volume of 1 ml. The level of aminoacylation was determined by acid precipitation of aliquots. After completion of the reaction, the remaining solution was acidified to pH 4.5 with 200 $\mu$l of 2 *M* acetate buffer and extracted with buffer-saturated phenol. Three volumes of cold ethanol

were added to the aqueous phase, and the precipitated RNA was recovered. Approximately 75% of the radioactive aminoacyl-tRNA was isolated in the precipitate.

*Preparation of the column*

The RPC-5 resin which consists of inert polychlorotrifluoroethylene beads of 10 $\mu$m diameter to which has been absorbed a pellicular layer of trioctylammonium chloride (Adogen 464), was prepared by method B of Pearson *et al.*[1]. The column was packed by either a dry or a wet method. Dry packing was accomplished by the addition of a small amount of resin followed by a light tapping of the column. The column could also be packed by running small amounts of a concentrated resin slurry on to the column under pressure. After filling, the column was washed with a 0.01 *M* sodium acetate buffer at pH 4.5 containing 0.4 *M* NaCl and 0.01 *M* $MgCl_2$. Prior to use the resin was equilibrated with the same acetate buffer containing the molarity of NaCl used to start the salt gradient.

*Chromatography*

The aminoacyl-tRNA sample prepared as above was dissolved in 200 $\mu$l of starting buffer and injected through the septum, after removal of the threaded plug, directly on to the top of the column. The port was closed and the resin was washed with starting buffer under pressure from the pump running at 4 ml/min. At this speed the pressure at the top of the column is between 1000 and 3000 p.s.i. depending on the age of the column. After washing the linear salt gradient (generally between 60 and 120 ml total) in the acetate magnesium buffer was started. The actual gradient depends on the sample and is indicated in each figure. In general the flow cell counter was used. The efficiency of $^{14}C$-counting in this system is 50%, and the minimum radioactivity in a sample to be analyzed is of the order of 8000 dpm. Samples of less radioactivity were counted in a scintillation counter.

## RESULTS

The resolving capability of the chromatographic system can be seen in Fig. 2a which represents a separation of seryl-tRNA isoacceptors from *Escherichia coli*. In a second chromatography, the eluting buffer volume was trebled (Fig. 2b), but little improvement in the separation of the 4 seryl-tRNA isoacceptors can be seen. A summary of these two experiments is found in Table I.

The separation of leucyl-tRNA isoacceptors from *Klebsiella aerogenes* is shown in Fig. 3. The position of each peak corresponds very well with positions of a comparable leucyl-tRNA preparation from *E. coli*[1]. This similarity is undoubtedly a mirror of the large structural homology between macromolecules of these two enterobacteria[6]. We note, however, large differences in the relative quantity of individual tRNA species (Table I). Although it is possible that these different levels may be dependent on growth conditions[7], it would seem more likely that these results mirror intrinsic differences between these two closely related bacteria. It is thought that different tRNA isoacceptor levels are related to the regulation of genetic expression[8].

We have carried out a statistical study of the reproducibility of the technique using tyrosyl-tRNA from *K. aerogenes* (Fig. 4). The chromatography of this material

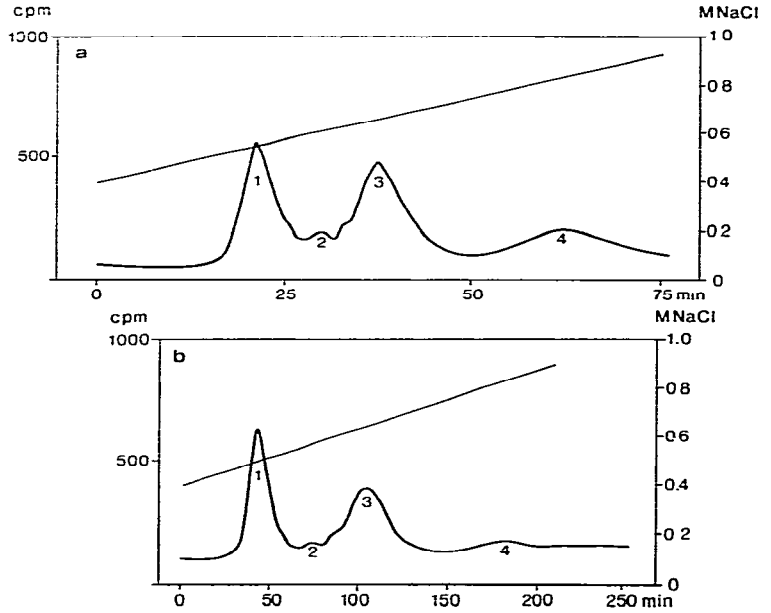


Fig. 2. A 5 $A_{260\ nm}$ sample of tRNA from *E. coli* was aminoacylated with 10 nmole of [$^{14}$C]serine of 50 mCi/mmole. The sample was dissolved in 200 $\mu$l of 0.01 *M* sodium acetate buffer at pH 4.5 containing 0.01 *M* $MgCl_2$ and 0.4 *M* NaCl. (a) The salt gradient was composed of 150 ml of a 0.4–1.0 *M* NaCl gradient in 0.01 *M* sodium acetate and 0.01 *M* $MgCl_2$ at pH 4.5. The pump was run at 2 ml/min (pressure 900 p.s.i.). Total radioactivity placed on the column was 12,000 cpm, and radioactivity in the effluent was counted in the flow cell counter. (b) The salt gradient was composed of 500 ml of a 0.4–0.9 *M* NaCl gradient in 0.01 *M* sodium acetate and 0.01 *M* $MgCl_2$ at pH 4.5. The pump was run at 2 ml/min. Total radioactivity placed on the column was 12,000 cpm and radioactivity in the effluent was counted in the flow cell counter.

shows two isoacceptors which are eluted at 0.67 $\pm$ 0.01 *M* and 0.85 $\pm$ 0.01 *M* NaCl at 95% probability. The relative area of the first peak is 50.6 $\pm$ 6.1% at 95% probability. These values are based on 10 independent experiments.

One application of this technique is the rapid screening of tRNA complements from cells grown under different conditions. The chromatography of phenylalanyl-

TABLE I

ELUTION SALT CONCENTRATION AND RELATIVE QUANTITY OF tRNA ISOACCEPTORS

| *Figure* | *tRNA* | *Isoacceptor species** | | | | |
|---|---|---|---|---|---|---|
| | | *1* | *2* | *3* | *4* | *5* |
| 2a | Serine | 0.54 *M* (37%) | 0.60 *M* (10%) | 0.66 *M* (39%) | 0.84 *M* (14%) | — |
| 2b | Serine | 0.56 *M* (37%) | 0.60 *M* (10%) | 0.64 *M* (36%) | 0.82 *M* (17%) | — |
| 3 | Leucine | 0.55 *M* (29%) | 0.58 *M* (26%) | 0.61 *M* (20%) | 0.72 *M* (12%) | 0.75 *M* (13%) |
| | Leucine** | 0.57 *M* (50%) | 0.60 *M* ( 8%) | 0.62 *M* (14%) | 0.70 *M* (12%) | 0.74 *M* (12%) |

* Salt concentration is given first and relative quantity is given in parenthesis.

** Calculated from Kelmers and Heatherly[2].

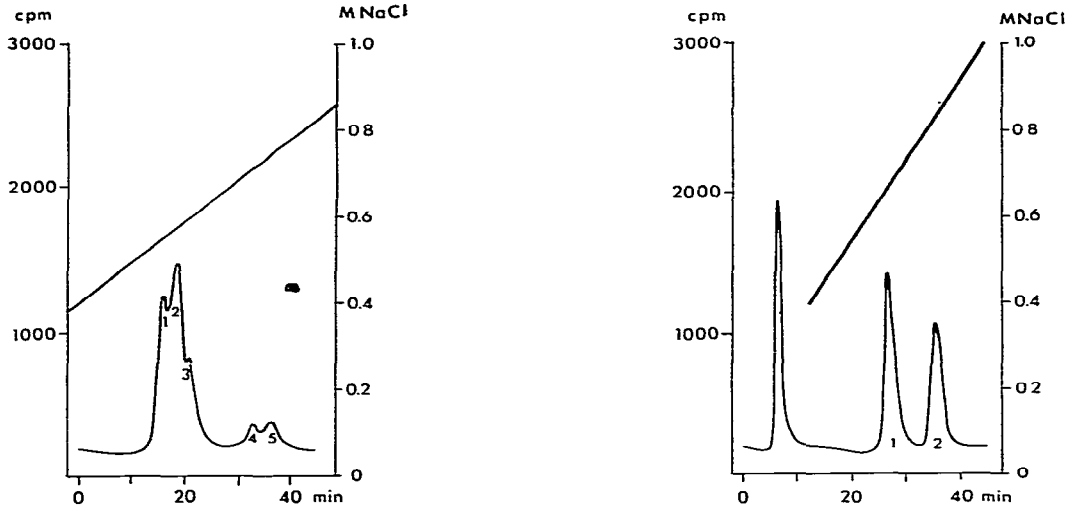


Fig. 3. A 5.0 $A_{260\ nm}$ specimen of tRNA from *Klebsiella aerogenes* was aminoacylated with 10 nmole of [$^{14}$C]leucine of 50 mCi/mmole. The sample was dissolved in 200 $\mu$l of starting buffer. The salt gradient was composed of 200 ml of 0.4–0.9 *M* NaCl in 0.01 *M* sodium acetate and 0.01 *M* $MgCl_2$ at pH 4.5. The pump was run at 4 ml/min (pressure 1400 p.s.i.). Total radioactivity placed on the column was 50,000 cpm, and radioactivity of the effluent was counted in the flow cell counter.

Fig. 4. A 4.0 $A_{260\ nm}$ sample of tRNA from *K. aerogenes* was aminoacylated with 10 nmole of [$^{14}$C]tyrosine of 420 mCi/mmole. The sample was dissolved in 200 $\mu$l of starting buffer. The salt gradient was 120 ml of 0.4–1.2 *M* NaCl in 0.01 *M* sodium acetate and 0.01 *M* $MgCl_2$ at pH 4.5. The pump was run at 4 ml/min (pressure 1400 p.s.i.). Total radioactivity placed on the column was 32,000 cpm, and radioactivity in the effluent was counted in the flow cell counter.

tRNA isoacceptors isolated from *Rhodospirillum rubrum* cells grown either anaerobically in the presence of light or aerobically in darkness is shown in Fig. 5. The two major species are easily detected in mixtures of tRNA from the two sources.

## DISCUSSION

We present here a rapid, highly reproducible technique for the separation of tRNA isoacceptors. The apparatus permits pressures up to 5000 p.s.i., thereby effecting separations in as little as 20 min with a turnover time of 35–40 min. In addition due to the high pressure capability columns can be used for longer periods; we have produced up to 40 chromatograms on a single column. Finally, the apparatus is easily assembled from readily available components.

Statistical analysis of our data indicates that the elution molarity of salt of a given tRNA species is virtually constant and therefore we propose that tRNA isoacceptors be denoted by their elution salt molarity rather than by the confusing numbering system in present use.

We feel that this technique is particularly useful for the rapid screening of tRNA complements isolated from cells grown under different conditions as in *R. rubrum* or from eukaryotic cells that are in the process of differentiation. Along this latter line the chromatograph is currently being applied to the analysis of lysine-tRNA

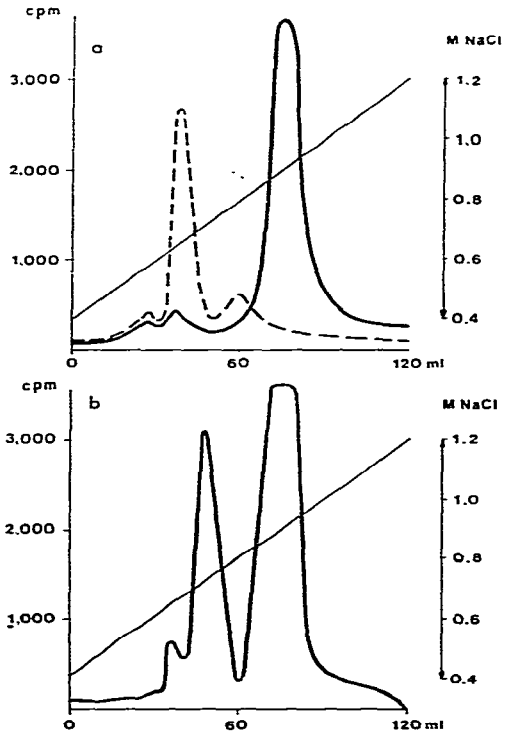


Fig. 5. A 2 $A_{260\ nm}$ sample of tRNA from aerobically or photosynthetically grown *Rhodospirillum rubrum* was aminoacylated with 1.25 nmole of [$^{14}$C]phenylalanine of 450 mCi/mmole. The sample was dissolved in starting buffer containing 0.4 *M* of NaCl. The salt gradient was 120 ml of 0.4–1.2 *M* NaCl in 0.01 *M* sodium acetate and 0.01 *M* $MgCl_2$ at pH 4.5. The pump was run at 4 ml/min (pressure 1400 p.s.i.), and radioactivity of the effluent was counted in the flow cell counter. (a) Photosynthetic tRNA (———); aerobic tRNA (– – –). (b) A mixture of the two preparations of tRNA.

isoacceptor species isolated from Friend's murine cells and differentiating induced cells. In the course of this work tRNA isoacceptor patterns were obtained as well from placenta and beef-liver sources.

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