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Note

Separation of tRNA by high-performance liquid chromatography at ambient temperature*

SHEN-BEI ZHANG**, PATRICIA M. BRONSKILL, QI-SONG WANG*** and J. TZE-FEI WONG*
Department of Biochemistry, University of Toronto, Toronto M5S 1A8 (Canada)
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All cells require a large array of tRNA species to mediate codon distribution to twenty protein amino acids. Separation of the individual species is therefore a prerequisite to many biophysical and biochemical investigations on tRNA. Fractionations based on the capacity of tRNA to accept amino acids can bring about a rapid group separation between the twenty classes of isoacceptor tRNA[1–3]. However, separations between isoacceptors for the same amino acid, or between tRNA species more generally have to rely on chromatographic or electrophoretic procedures. HPLC methods, by virture of their potential speed and high resolution, promise to be particularly advantageous in this regard. Following an early application of HPLC on RPC-5 by Dion and Cedergren[4] more highly resolving systems have been developed. Pearson *et al.*[5] *n*-alkylated silicas with short-chain (C_1, C_2 and C_4) chlorosilanes and employed gradients of decreasing ammonium sulfate at 55°C and also at 24°C; the higher temperature increased resolution and decreased retention time. Bischoff *et al.*[6] coated ODS-Hypersil with trioctylmethylammonium chloride and employed a gradient of increasing ammonium acetate at 35°C; more recently[7] they also *n*-alkylated APS-Hypersil WP with short-chain acids (C_2, C_4, C_6 and C_8) and employed a gradient of decreasing ammonium sulfate at 35°C. Wang *et al.*[8] employed simultaneous gradients of decreasing sodium formate–magnesium chloride and increasing methanol on a prepacked Waters Protein I-60 column. Since the short-chain alkyl systems and simultaneous gradients are both based on reversed-phase partition, we have sought to combine features of the two aproaches. Thus, by applying the simultaneous salt and methanol gradients to a Vydac-C_4 column, it has been found possible to reduce thermal degradation of tRNA, and the prepacked column eliminates the need for coating or chemical modification of packings.

EXPERIMENTAL

The Vydac C_4-derivatized silica column, 10 μm particle size, 250 × 4.6 mm I.D., was obtained from The Separations Group (Hesperia, CA, U.S.A.); HPLC-

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** Present address: Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China.
*** Present address: Institute of Genetics, Fudan University, Shanghai, China.

grade methanol from BDH (Toronto, Canada) [^{3}H]valine, [^{3}H]phenylalanine and [^{3}H]tryptophan from Amersham (Arlington Heights, IL, U.S.A.) and *Escherichia coli* B tRNA from Gibco Laboratories (Grand Island, NY, U.S.A.).

Chromatography was performed on a Waters (Toronto, Canada) 720 liquid chromatography system with variable-wavelength detector. Aminoacyl-tRNA synthetases were prepared and used for tRNA as described[9].

Except where specified otherwise, chromatography was carried out at 22°C with a gradient formed from Buffer A (10 m*M* sodium phosphate (pH 7.0), 1 *M* sodium formate, 8 m*M* magnesium chloride and Buffer B (10 m*M* sodium phosphate (pH 7.0), 10% methanol). The shape of the gradient conformed to Curve 7 of the Waters 720 system. Sample was loaded in 100 μl of Buffer A. In the case of some tRNA preparations, the concentration of sodium formate in the load was increased to 3 *M* to ensure complete adsorption. The flow-rate was 1 ml/min. A 60-min gradient was followed by 20 min of isocratic elution with Buffer B.

RESULTS

When *E. coli* tRNA was chromatographed on Vydac C_4 by simultaneous gradients of decreasing sodium formate–magnesium chloride and increasing methanol, over 20 peaks were obtained at room temperature in an 80-min run (Fig. 1). The shape of the gradient is an important factor. Fig. 2 describes the gradient curves obtainable on the Waters 720 instrument. Resolution was retained when Curve 7 was replaced by Curve 8, although the peaks were not as evenly spread, but was reduced when Curve 7 was replaced by Curve 6 or Curve 5. Change of pH from 7.0 to 5.0 led to some loss of resolution.

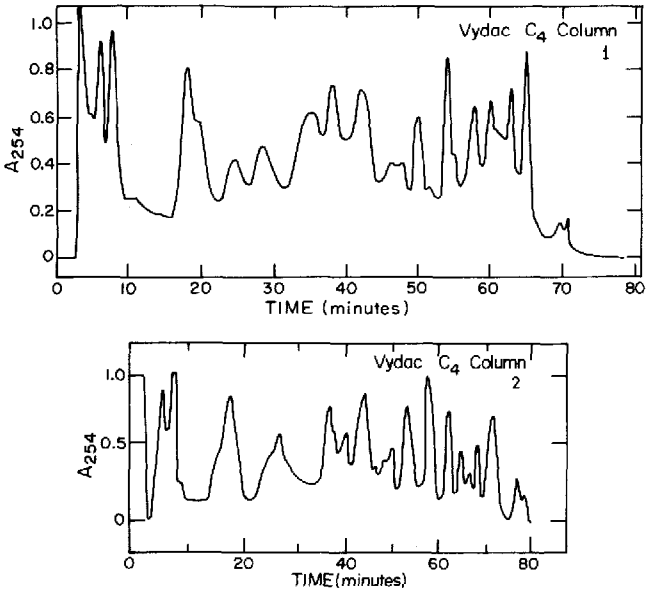

Fig. 1. Chromatography of tRNA on two different Vydac C_4 columns.

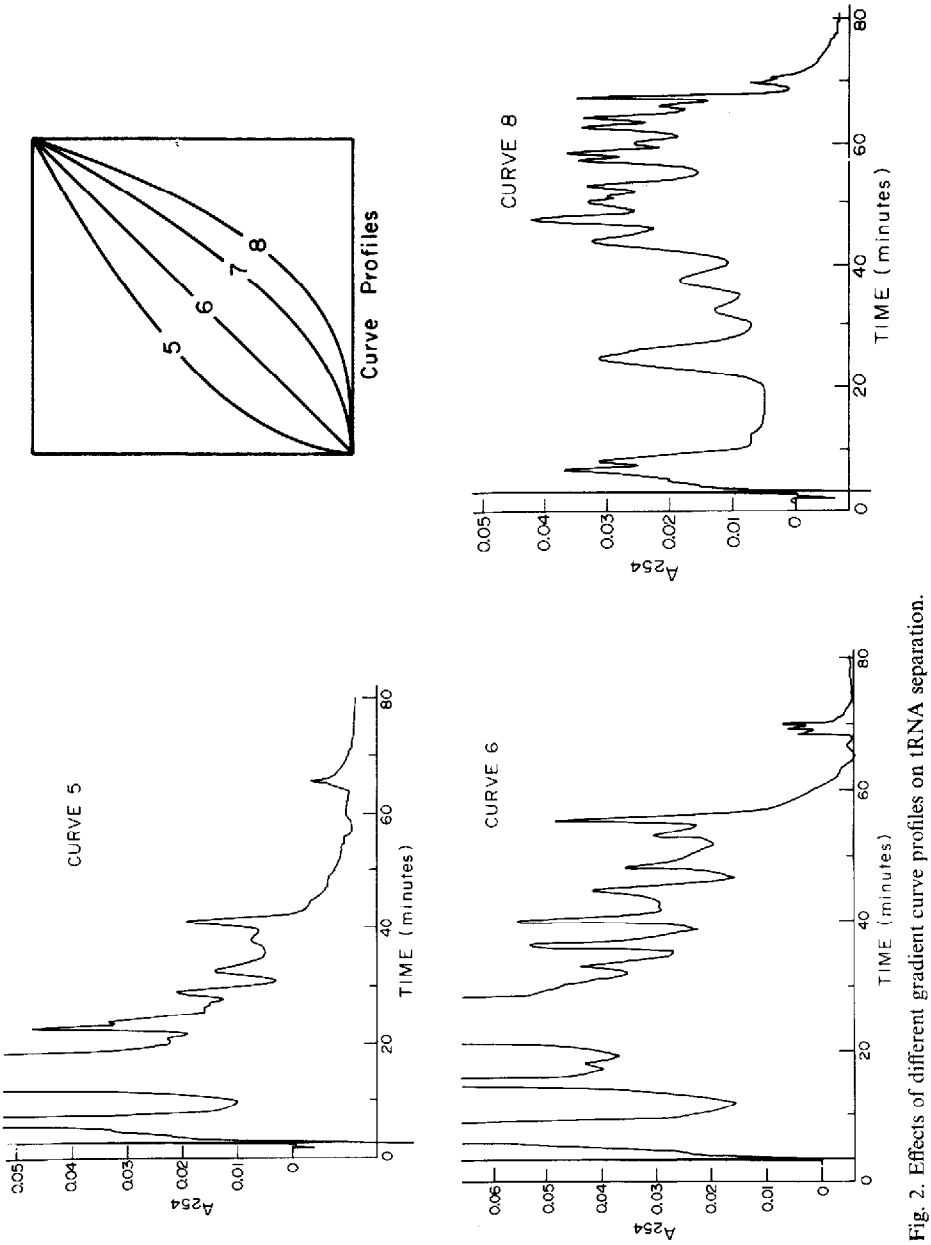

Fig. 2. Effects of different gradient curve profiles on tRNA separation.

Decreasing gradients of Mg^{2+} concentration from 8 m*M* to 0 m*M* and from 4 m*M* to 0 m*M* gave comparable results (Fig. 3). A constant Mg^{2+} concentration of 8 m*M* was consistent with high resolution, but a constant concentration of 0 m*M* or an increasing gradient from 0 m*M* to 8 m*M* resulted in some loss in resolution.

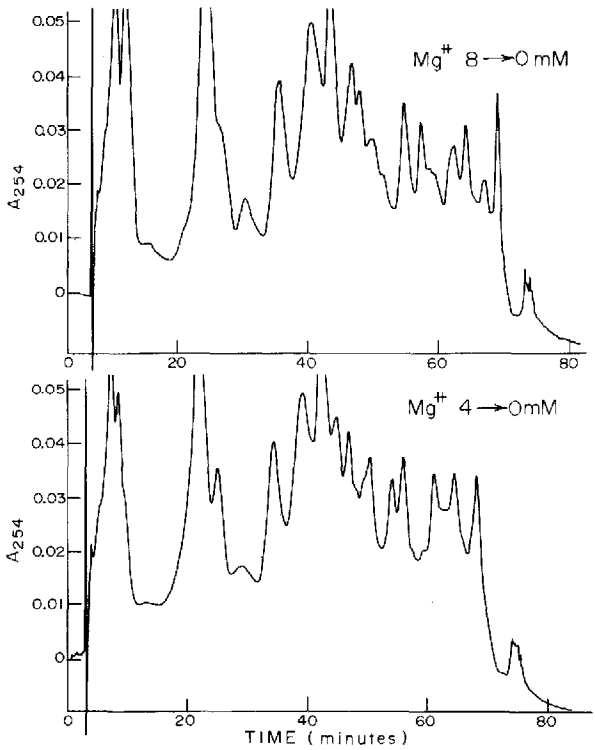

Fig. 3. Effects of different gradients of decreasing Mg^{2+}.

Lowering the temperature did not grossly retard tRNA elution, but some loss of resolution was observed at 10°C. Cooling to 0°C did not bring about further deterioration (Fig. 4). When the tRNA, resolved at room temperature, was assayed for amino acid acceptance, $tRNA^{Phe}$ yielded a single peak, $tRNA^{Trp}$ a major peak with at least one minor component and $tRNA^{Val}$ multiple peaks (Fig. 5).

DISCUSSION

In this study, simultaneous salt and methanol gradients were found to provide extensive resolution of *E. coli* tRNA into over 20 peaks. Previously, it has been pointed out that high temperatures would prevent retardation by interfacial precipitation and enhance hydrophobic interactions, factors that facilitate a rapid chromatographic resolution[5]. However, high temperatures favor chemical degradation of

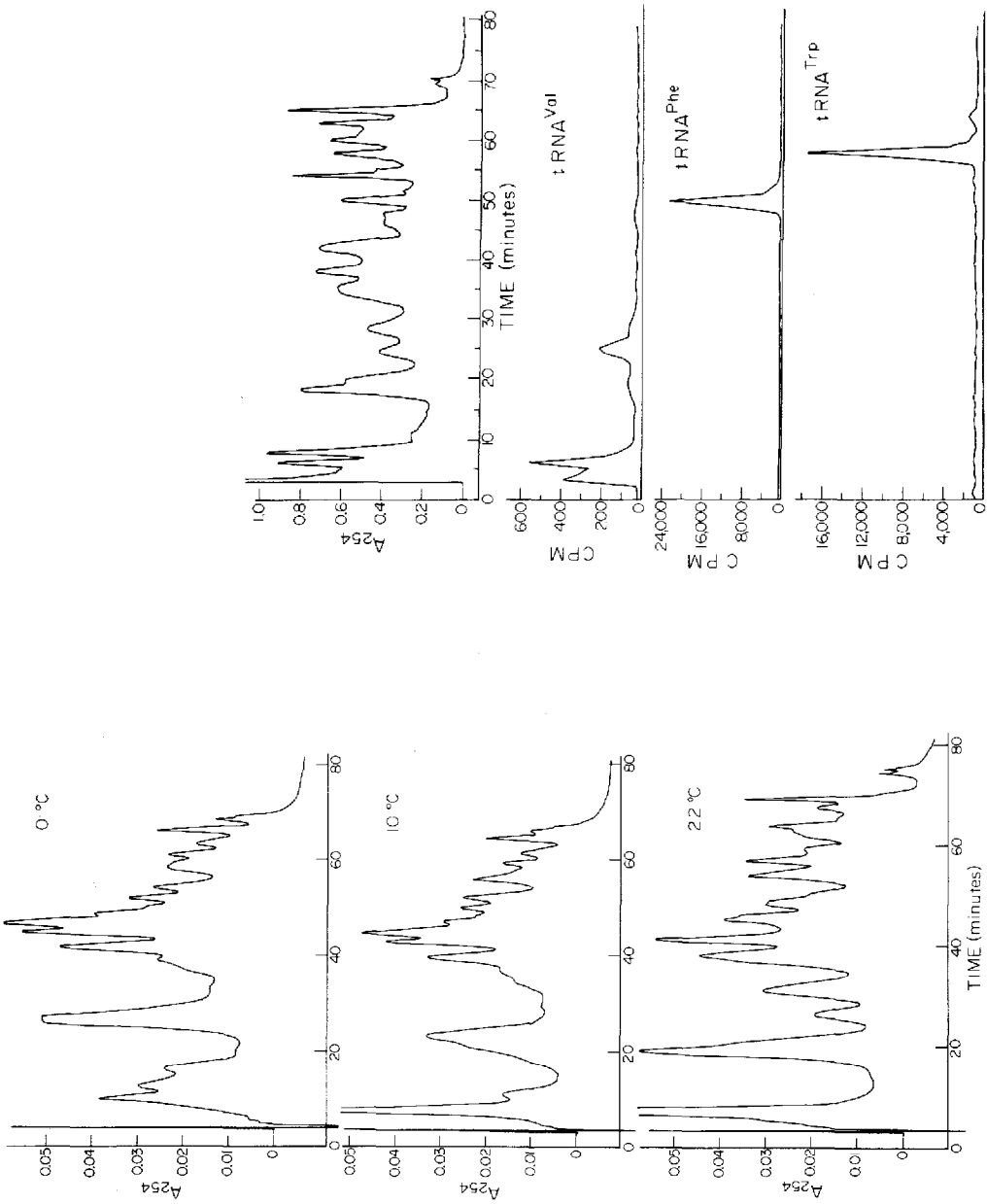

Fig. 4. Effects of temperature on separation.
Fig. 5. Distribution of acceptance activities for different amino acids.

tRNA and even more so aminoacyl-tRNA complexes. With the simultaneous-gradients system, retardation by low temperatures appears not to be important. Average elution times were not significantly increased by temperature reduction from 22°C to 10°C or even 0°C. Thus, the system readily lends itself to effective tRNA fractionation at ambient temperature.

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