PREPARATION OF A HIGHLY PURIFIED LEUCINE TRANSFER RIBONUCLEIC ACID*

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Received November 9, 1966

A previous paper (Kelmers, 1966) described the preparation of a highly purified phenylalanine transfer ribonucleic acid** from E. coli B by reversed-phase and gel filtration chromatographic techniques. During the course of those experiments, other reversed-phase chromatographic fractions containing a partially purified leucine tRNA were obtained. This paper describes the preparation of a highly purified leucine tRNA from those fractions. It is identified as $tRNA_4^{Leu}$ since it was the fourth, and last, leucine tRNA eluted from the reversed-phase column.

Other chromatographic methods have been used to obtain concentrated leucine tRNA. Some of the early work involved Ecteola-cellulose chromatography (Offengand, Dieckmann, and Berg, 1961). Individual leucine tRNAs have been obtained by hydroxylapatite chromatography (Muench and Berg, 1966 a) and chromatography on Sephadex columns (Muench and Berg, 1966 b). Fractionation methods involving the chemical binding of large molecules to the leucine moiety of leucyl-tRNA have also been used to obtain concentrated samples of leucine tRNAs (Zubay, 1962; Mehler and Bank, 1963). However, such methods have the disadvantage of concentrating all the different leucine tRNAs into the same sample.

*Research sponsored by the U.S. Atomic Energy Commission under contract with the National Institutes of Health and Union Carbide Nuclear Corporation.

**Abbreviations: tRNA, transfer ribonucleic acid; $tRNA_4^{Leu}$, the fourth leucine tRNA; leucyl-$tRNA_4^{Leu}$, the fourth leucine tRNA with a leucyl residue attached.

EXPERIMENTAL PROCEDURE

The source of materials, chromatographic procedures, and analytical methods were described in the previous paper (Kelmers, 1966).

The conditions of the amino acid acceptor activity assay were optimized with regard to pH, buffer, Mg^{++}, ATP, tRNA, L-leucine and synthetase concentrations, and reaction time in order to obtain maximal [^{14}C] leucyl-tRNA formation (Rubin and Kelmers, 1966). The reaction mixture contained: 50 μmoles of sodium cacodylate buffer at pH 8.2, 10 μmoles of magnesium acetate, 1 μmole of ATP, 2.5 μmoles of KCl, 5 μmoles of β-mercaptoethanol, 0.75 μmole of [^{14}C] L-leucine, approximately 0.1 absorbancy unit* of $tRNA_4^{Leu}$ and approximately 0.1 mg of crude aminoacyl-RNA synthetase, in a final volume of 0.5 ml. The mixture was incubated at 37°C for 15 minutes and 50 μl aliquots were pipetted onto paper discs, washed and counted as described (Kelmers, 1966).

RESULTS AND DISCUSSION

Reversed-Phase Chromatography – Twenty grams of crude *E. coli* B tRNA (General Biochemicals Lot 651068) were fractionated in a total of nine experiments. The final portion of the chromatogram from one of these experiments was shown in the previous paper (Kelmers, 1966). The $tRNA_4^{Leu}$ peak was identified by leucine acceptance and ultraviolet absorbance. The $tRNA_4^{Leu}$ peaks from the reversed-phase chromatographic experiments were pooled to yield Sample 1, partially purified $tRNA_4^{Leu}$, containing a total of 4400 absorbancy units. The sample accepted only leucine (470 μμmoles/absorbancy unit) and thus was free of contamination by $tRNA^{Phe}$ or other active tRNAs.

Gel Filtration Chromatography – Final purification of the $tRNA_4^{Leu}$ was achieved by gel filtration chromatography. The partially purified $tRNA_4^{Leu}$ (Sample 1) was desalted using Bio-Gel P-2 columns and then concentrated by

*One absorbancy unit is a total absorbancy of one optical density unit in one ml of solution measured at 260 mμ in a cell of 1 cm light path with distilled water as the solvent.

flash evaporation. A 2.4 x 240 cm column of Bio-Gel P-100 was then used to remove inert, higher molecular weight RNA. The first and second chromatographic peaks (Figure 1) did not accept appreciable amounts of leucine and were discarded. The third peak contained the $tRNA_4^{Leu}$, as shown by the leucine acceptance curve. In this experiment fractions 126 through 160 were pooled. These were combined with the pooled fractions from a second P-100 run to yield the final sample of highly purified $tRNA_4^{Leu}$ (Sample 2). This sample contained a total of approximately 1675 absorbancy units.

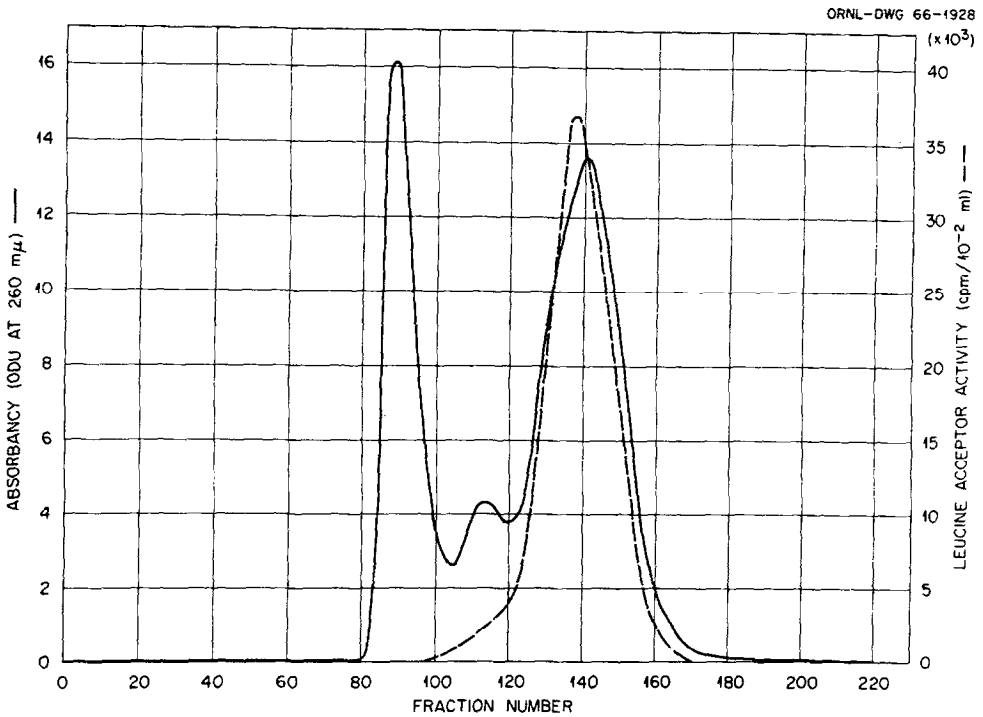

Figure 1. Final Purification of $tRNA_4^{Leu}$ by Gel Filtration Chromatography. The sample, applied in a 4 ml volume, was rechromatogrammed in 0.4 M NaCl, 0.01 M $MgCl_2$ and 0.05 M Tris-HCl buffer at pH 7.0 on a 2.4 x 240 cm column of Bio-Gel P-100 at approximately 5°C at a flow rate of 0.5 ml/min.

Properties, Concentration and Purity of $tRNA_4^{Leu}$ – The purity of the $tRNA_4^{Leu}$ (Sample 2) was established by [^{14}C] leucyl-tRNA formation and analyses for terminal nucleoside content (Table I). A value of 1000 μμmoles of

leucine accepted per absorbancy unit was obtained. No other amino acids were accepted. An alkaline hydrolysate of Sample 2 was assayed by Dr. Mayo Uziel for its free nucleotide content by the method of Cohn and Uziel (Cohn and Uziel, 1965). The sample contained 1070 μμmoles of adenosine per absorbancy unit and 70 μμmoles of cytidine per absorbancy unit. Guanosine was below the detection level. These data indicate that Sample 2 contained at least 88% active $tRNA_4^{Leu}$. The presence of approximately 6% cytidine suggests contamination by $tRNA_4^{Leu}$ molecules lacking the terminal adenosine or by other inert RNAs.

The data in Table I cannot be used to calculate recovery since the initial assay of the crude tRNA for leucine acceptance gives a total value for all four leucine tRNAs. The purified $tRNA_4^{Leu}$, Sample 2, had approximately 17 times the leucine accepting activity per absorbancy unit of the crude tRNA.

The apparent molecular weight of the $tRNA_4^{Leu}$ was estimated by gel filtration chromatography on calibrated columns of Bio-Gel P-150 (Egan, 1966).

Table I
Analysis of Purified $tRNA_4^{Leu}$

Sample	Leucine Acceptance Activity	Terminal Adenosine Content
	μμmoles/ absorbance unit	μμmoles/ absorbance unit
Initial mixed tRNA	48[a]	
After reversed-phase chromatography (Sample 1)	470	
Final purified sample (Sample 2)	1,000 ± 25	1,070 ± 50[b]

[a]Total value for all leucine tRNAs present.
[b]After alkaline hydrolysis.

A value of 29,500 was obtained. When compared to the molecular weight of

26,000 previously obtained by this method for $tRNA_4^{Phe}$ (Kelmers, 1966), the higher molecular weight for $tRNA_4^{Leu}$ is in agreement with the lower values of leucine acceptance and terminal nucleoside content per absorbancy unit.

In earlier reversed-phase chromatography experiments with E. coli B tRNA at pH 7.4, a total of five leucine tRNAs were observed (Kelmers, Novelli, and Stulberg, 1965), while in these experiments at pH 4.5, only four leucine tRNAs were detected. The single $tRNA_4^{Leu}$ peak obtained at pH 4.5 might consist of two leucine tRNAs chromatogramming identically under these conditions, therefore to see if Sample 2 consisted of more than one leucine tRNA, an aliquot was rechromatogrammed on a 1 x 240 cm reversed-phase column at pH 7.4, as previously described (Kelmers, Novelli, and Stulberg, 1965). A single ultraviolet absorbance peak was eluted and leucine acceptance coincided with the absorbance peak. Rechromatography of aliquots of Sample 2 on Bio-Gel P-100 or P-150 columns also yielded only a single absorbance peak. Since Sample 2 showed no tendency to split into two peaks on rechromatography, it was concluded that it contained only a single component, $tRNA_4^{Leu}$.

ACKNOWLEDGMENTS

The author wishes to acknowledge the contributions of the following people: Dr. G. David Novelli and Dr. Waldo E. Cohn for advice and consultation; Dr. Mayo Uziel for terminal nucleoside data; Dr. B. Zane Egan for molecular weight data; and Mr. I. B. Rubin for amino acid acceptance assays.

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