Two Additional Reversed-Phase Chromatographic Systems for the Separation of Transfer Ribonucleic Acids and Their Application to the Preparation of Two Formylmethionine and a Valine Transfer Ribonucleic Acid from *Escherichia coli* B*

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ABSTRACT: Two new reversed-phase chromatographic systems for the separation of *Escherichia coli* transfer ribonucleic acids are described. These systems employ the water-insoluble quarternary ammonium salts, trioctylpropylammonium bromide or dimethyldilaurylammonium chloride, immobilized on a hydrophobic diatomaceous earth support, as the active extractants. Sodium chloride gradient elution is used to develop the chromatograms. Multiple isoaccepting peaks (heterogeneity) were observed for 11 of 17 transfer ribonucleic acids determined. These systems proved particularly useful for the resolution of transfer ribonucleic acids near the front of the chromatograms. Two formylmethionine transfer ribonucleic acids were identified by formylation following aminoacylation. The codon responses for these two formylmethionine transfer ribonucleic acids were identical, both responding to AUG and GUG and, at a lower level, to UUG. The preparation of purified samples of these two formylmethionine and a valine transfer ribonucleic acid are described. The activity of these samples, calculated from amino acid acceptance per terminal adenosine, was $tRNA_1^{fMet}$ 100%, $tRNA_2^{fMet}$ 87%, and $tRNA^{Val}$ 88%.

We have previously described two reversed-phase chromatographic systems for the separation of tRNAs. For convenience, we now refer to these as reversed-phase chromatography 1 (Kelmers *et al.*, 1965) and reversed-phase chromatography 2 (Weiss and Kelmers, 1967). The reversed-phase chromatography system 1 was employed in the preparation of a phenylalanine tRNA (Kelmers, 1966a) and a leucine tRNA (Kelmers, 1966b), while the reversed-phase chromatography system 2, which displays a more even distribution of tRNAs throughout the chromatogram, may be better suited for analytical investigations. In each case a quaternary ammonium chloride dissolved in an organic solvent was supported, in the form of a film, on hydrophobic diatomaceous earth. Different tRNAs display different distribution coefficients between the bound organic phase and the mobile aqueous phase; thus, NaCl gradient elution techniques lead to sequential elution of tRNAs from the columns. If a sufficient number of stages are obtained by optimum selection of column geometry and gradient conditions, appreciable separation of adjacent tRNAs can be achieved.

The two new systems described in this paper (reversed-phase chromatography 3 and 4) are in principle similar to those previously reported. However, in each system the order of elution is somewhat different, so that by selection of fractions containing a partially purified tRNA from one column and then further purifying it by rechromatography with a different reversed-phase chromatography system, it should be possible to prepare a larger number of purified tRNAs. In particular, the reversed-phase chromatography system 3 yields better chromatographic resolution of the tRNAs that elute near the front of a chromatogram than any of the other systems. This characteristic enabled us to prepare samples of methionine and valine tRNAs and to demonstrate the existence of two formylmethionine tRNAs.

Materials and Methods

Materials. Crude tRNA from *Escherichia coli* B was prepared by the method previously described (Kelmers *et al.*, 1965), although on a much larger scale. The crude tRNA thus obtained was purified by adsorption onto, followed by batch elution from, a Whatman DEAE-52 column and was then precipitated with ethanol. This precipitate was the tRNA used in these chromatographic experiments and is designated feed tRNA. Trioctylpropylammonium bromide was obtained from Eastman Organic Chemicals and dimethyldilaurylammonium chloride (Aliquat 204) from General Mills, Chemical Division. Chromosorb W (acid washed, dimethyldichlorosilane treated, 100–120 mesh size) was obtained from Johns-Manville Products, Corp. All other chemicals were of reagent grade.

Preparation of Column Packing for Reversed-Phase Chromatography 3. Trioctylpropylammonium bromide

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(5 g) was dissolved in 500 ml of chloroform. The chloroform solution was then poured onto 100 g of Chromosorb W in a shallow glass tray and the mixture was stirred continuously with a ceramic or plastic paddle while the chloroform was allowed to evaporate. When most, but not all, of the chloroform had evaporated (the mixture had the consistency of loose brown sugar), it was placed in a 1-l. plastic bottle. To ensure even distribution of the trioctylpropylammonium bromide in the remaining chloroform on the surface of the Chromosorb W, the bottle was tightly capped and the packing was tumbled for 2 hr. It is very important to achieve even distribution in order to subsequently obtain sharp chromatographic peaks. After being rotated for 2 hr, the bottle was uncapped and the remaining chloroform slowly and carefully evaporated from the packing while still in the plastic bottle, with continuous rotation and mixing by hand. The prepared packing was then stored dry until needed. Other mechanical methods of preparing packing can be employed; however, it is critical that even distribution of the trioctylpropylammonium bromide on the Chromosorb W be obtained.

Preparation of Column Packing for Reversed-Phase Chromatography 4. The technique was the same as that used for reversed-phase chromatography 3: 2.3 g of dimethyldilaurylammonium chloride was dissolved in 500 ml of chloroform, this was poured onto 100 g of Chromosorb W, and the chloroform was evaporated as described.

Column Preparation. Jacketed-glass columns, maintained at 25, 37, or 50° in different experiments, were filled with the initial column equilibration solution. For reversed-phase chromatography 3 columns, this was 0.15 M NaCl, 0.01 M buffer at the operating pH, and frequently, 0.01 M $MgCl_2$. For reversed-phase chromatography 4 columns the solution was 0.45 M NaCl, 0.01 M buffer, and 0.01 M $MgCl_2$. The dry prepared packing was first mixed with the appropriate equilibration solution in a beaker, poured into the column as a thick slurry, and allowed to settle while maintaining the maximum possible aqueous flow of equilibration solution through the column. The bed was then subsequently compacted by pressurization under flow to about 60 psi to obtain a denser bed and sharper chromatographic peaks. Columns were equilibrated prior to use by pumping two to three column volumes of equilibration solution at 15–20 psi. The columns were normally operated at a positive pressure of 15–20 psi to prevent formation of air pockets in the column from dissolved air in the elution solutions.

Chromatographic Operation. In a typical reversed-phase chromatography 3 experiment using a 1 × 240 cm column at 37°, about 1000 A_{260} units of feed tRNA was dissolved in 10 ml of initial equilibration solution, heated to 37° for 1 hr, and then diluted to 80–100-ml total volume with additional equilibration solution. This feed was pumped onto the column (Beckman Accu-Flow or Milton Roy Mini Pump, positive displacement piston pumps) at flow rates of up to 5 ml/min. Elution of the tRNAs was brought about by NaCl gradient elution at 1.5 ml/min. In addition to NaCl, the eluent contained 0.01 M Tris buffer at pH 7.0 or sodium acetate buffer at pH 4.5 and, at times, 0.01 M $MgCl_2$. A 3-l., nine-chamber gradient generator was employed to produce the linear or concave gradients. The form of the concave gradient was described previously (Weiss and Kelmers, 1967). The column eluate was collected in approximately 15-ml fractions and the absorbance was linearly recorded with an on-line Beckman DB-G spectrophotometer and log scale recorder. The reversed-phase chromatography 4 experiments were carried out in a similar manner; however, different sodium chloride concentrations were used in the buffer solution. The columns were equilibrated with 0.45 M NaCl solution, which also contained 0.01 M $MgCl_2$ and 0.01 M Tris buffer at pH 7.0 or sodium acetate buffer at pH 4.5 and elution gradients were run linearly from 0.45 to 0.70 or 0.75 M NaCl.

The reversed-phase chromatography 3 columns could be used for three to five experiments without regeneration. Any residual RNA was discharged between runs by passing approximately 300 ml of 1.2 M NaCl and 0.01 M buffer solution through the column. The trioctylpropylammonium bromide has a low aqueous solubility and is slowly washed from the column. After three to five runs, the chromatographic resolution began to decay due to loss of trioctylpropylammonium bromide. The packing was then removed, washed successively with water, ethanol, and acetone, dried, and then recoated with trioctylpropylammonium bromide. Alternately, it is possible to extend the life of a column by passing 1 l. of equilibration solution saturated with trioctylpropylammonium bromide through the column (and then equilibrating in the usual manner) before attempting another run. When treated in this manner after the fourth or fifth run, the life of the column may be extended. The dimethyldilaurylammonium chloride, used in the reversed-phase chromatography 4 columns, had an extremely low aqueous solubility above 0.4 M NaCl; thus these columns could be used repeatedly following cleanup with 1.2 M NaCl solution between runs.

Amino Acid Acceptance Assay. The acceptance activity of selected chromatographic fractions was determined as previously described (Kelmers *et al.* 1965; Rubin *et al.*, 1967), except for methionine and valine. For these, the assay conditions were optimized to obtain maximum formation of the aminoacyl-tRNAs as is shown in Table I.[1]

Assay for Transformylation. The formyl-accepting activity of selected $tRNA^{fMet}$ samples[2] was assayed by Dr. Lee Shugart.[3] The $N^{5,10}$-[^{14}C]methenyltetrahydrofolate was added directly to the aminoacylation mixture

[1] D. W. Hatcher and G. Goldstein, Analytical Chemistry Division, Oak Ridge National Laboratory, personal communication.

[2] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: $tRNA^{fMet}$, the methionine tRNA that can subsequently be formylated; $tRNA^{Met}$, the normal methionine tRNA.

[3] L. Shugart, Biology Division, Oak Ridge National Laboratory, personal communication.

(with [^{12}C]methionine) since the crude synthetase preparation was known to contain transformylase activity. Analysis for incorporation of [^{14}C]formyl group into tRNA was performed by the disk method (Mans and Novelli, 1961).

Results and Discussion

A number of preliminary chromatographic experiments were carried out with reversed-phase chromatography 3 and 4 columns to investigate the effect of operational variables, to compare these systems with the previous reversed-phase chromatography 1 and 2 systems, and to define the optimum conditions for tRNA separation. Flow rate had no significant effect on the elution position (NaCl molarity) or on the chromatographic resolution (peak shape) with reversed-phase chromatography 1, 3, and 4, while with the reversed-phase chromatography 2 increased flow rates led to decreased resolution. With reversed-phase chromatography 2 a slow exchange step, possibly transfer of the tRNA between the aqueous and organic phase, must exist. Surprisingly, column length had almost no effect on the elution position with reversed-phase chromatography 1, 3, and 4 while with reversed-phase chromatography 2 increased column length caused the tRNA to be eluted at higher sodium chloride concentrations. An effect due to column length would be anticipated since longer columns should contain a greater number of effective stages and, therefore, more exchange sites. In all systems, columns longer than optimum gave decreased resolution (broader peaks) possibly due to band spreading or diffusion within the column. The load of tRNA applied to a given column had little effect with reversed-phase chromatography 1 and 4, up to the point where the column was saturated with tRNA, while the reversed-phase chromatography 2 and 3 systems were quite sensitive to tRNA load, increased loads leading to elution at decreased sodium chloride molarities and a gradual loss of resolution.

The operational characteristics of the reversed-phase chromatography 2 and 3 columns seem to be closely related to liquid ion-exchange phenomena. In both the reversed-phase chromatography 2 and 3 systems a distinct and immobile liquid phase exists on the surface of the Chromosorb. In reversed-phase chromatography 2 it is due to the presence of the water-insoluble Freon solvent and in reversed-phase chromatography 3 to the extraction of water and chloride ion from the equilibrating eluent by the trioctylpropylammonium bromide. Test-tube experiments in which trioctylpropylammonium bromide was shaken up with solutions of various salts indicated that the unique and distinct liquid phase forms only in the presence of chloride ion and does not form in solutions containing only bromide salts. A column equilibrated only with bromide salts cannot be used to separate tRNAs. In reversed-phase chromatography 1 and 4, however, a true liquid phase probably does not form; the dimethyldilaurylammonium chloride in contact with aqueous sodium chloride solutions forms an emulsion. Thus, reversed-phase chromatography 1 and 4 may act partially through precipitation or salting-in phenomena, which have been applied to the separation of polyanions by quarternary ammonium salts (Scott, 1961, 1962).

TABLE I

	Methionine	Valine
Buffer (μmoles)	HEPES,[a] pH 8.0 (50)	Bicine,[b] pH 7.5 (50)
Magnesium acetate (μmoles)	5	5
ATP (μmoles)	2	0.5
KCl (μmoles)	5	5
β-Mercaptoethanol (μmoles)	5	5
[^{14}C]Amino acid (1 mμmole)	L-Methionine	L-Valine
tRNA (A_{260} unit)	~0.03	~0.05
Crude synthetase (mg)	~0.1	~0.1
Final volume (ml)	0.5	0.5
Incubation (deg, min)	30, 30	37, 20

[a] *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. [b] *N*,*N*-Bis(2-hydroxyethyl)glycine.

Reversed-phase chromatography 3 experiments were run to evaluate the effect of 0.01 M magnesium chloride and temperature. At 37° and with 0.01 M $MgCl_2$ present at pH 7.0 (Figure 1), the tRNA elution range was 0.25–0.70 M NaCl. In the absence of $MgCl_2$ this range increased to 0.35–0.80 M NaCl. Similar magnesium ion effects have previously been observed (Kelmers *et al.*, 1965; Weiss and Kelmers, 1967) and presumably result from specific interactions between the magnesium ion and the tRNA that reduce the binding of the tRNA to the quarternary ammonium compound. At 25° the sodium chloride elution range was shifted to slightly lower concentrations and some loss of chromatographic resolution was noted. At 50°, very sharp chromatographic peaks were obtained; however, aminoacylation was considerably reduced.

Chromatograms were run at pH 4.5 and 7.0 with the reversed-phase chromatography 3 system under optimum conditions to investigate the distribution of 17 tRNAs (Figures 1 and 2). At least 11 tRNAs were clearly heterogeneous (exhibited multiple isoaccepting peaks) and in other cases unevenly shaped peaks suggested the presence of additional unresolved isoaccepting tRNAs. For example, there are three or four peaks for methionine, three for valine, two for lysine and alanine, and three isoleucine accepting peaks. In the chromatograms shown in Figures 1 and 2 between 80 and 100% of the tRNA applied to the column was recovered in the eluate fractions, as determined by 260-mμ absorbance, and 100% of the methionine acceptance was recovered as determined by assay.

A chromatogram with a reversed-phase chromatography 4 column at pH 4.5 and 37° is shown in Figure 3.

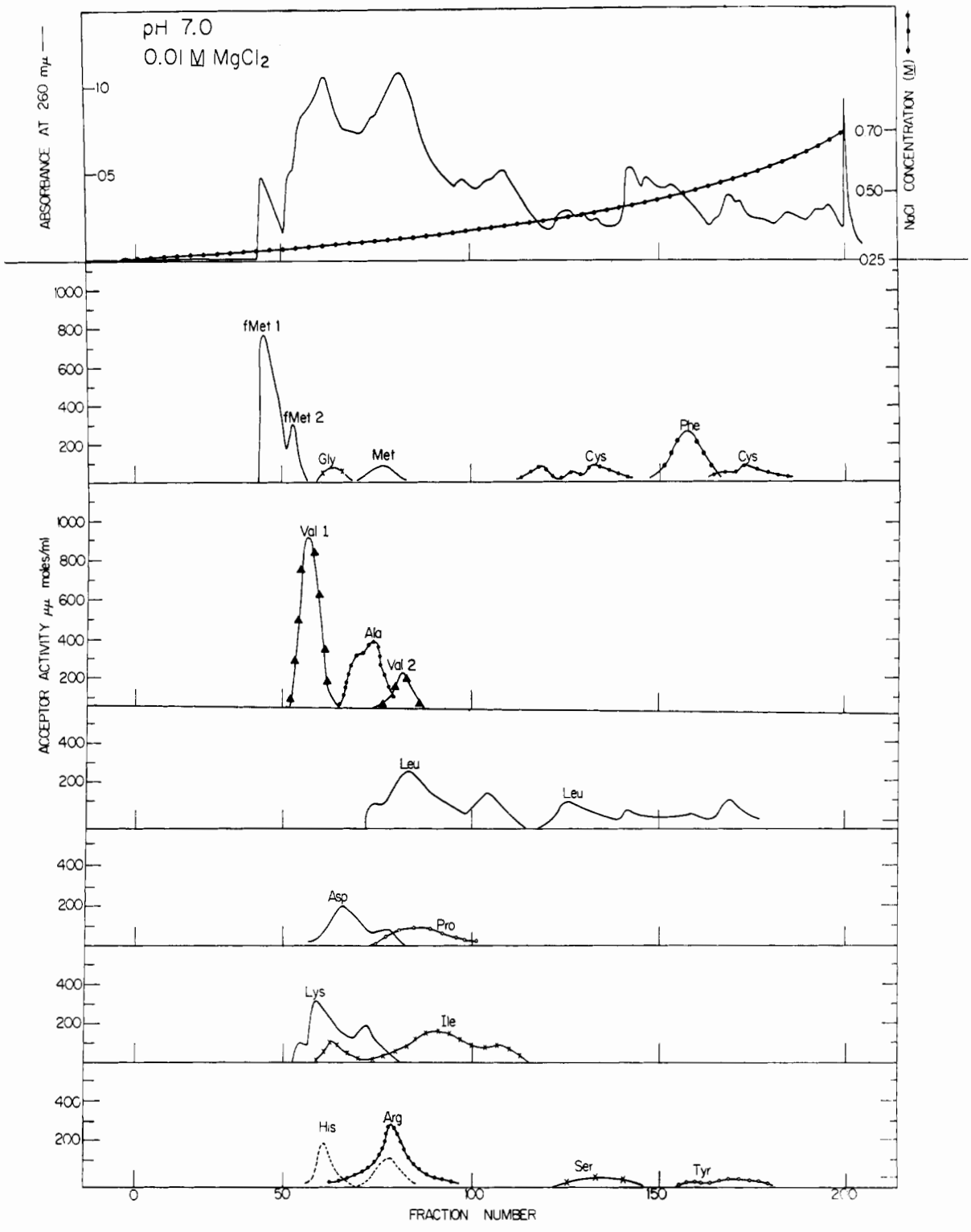

FIGURE 1: Reversed-phase chromatography 3 of *E. coli* B tRNA at pH 7.0. The column was 1 × 240 cm maintained at 37° and equilibrated with solution containing 0.15 M NaCl, 0.01 M $MgCl_2$, and 0.01 M Tris buffer at pH 7.0. Approximately 1000 A_{260} units of feed tRNA was dissolved in 80 ml of equilibration solution, preheated to 37° for 1 hr, and pumped on the column at 2 ml/min. A 3-l. concave elution gradint from 0.25 to 0.70 M NaCl, plus $MgCl_2$ and buffer, was used at a flow rate of 1.5 ml/min. Approximately 15-ml fractions were collected. The 260-mμ absorbance was monitored by an on-line Beckman DB-G spectrophotometer. Selected fractions were assayed for amino acid acceptance as described in the Methods section.

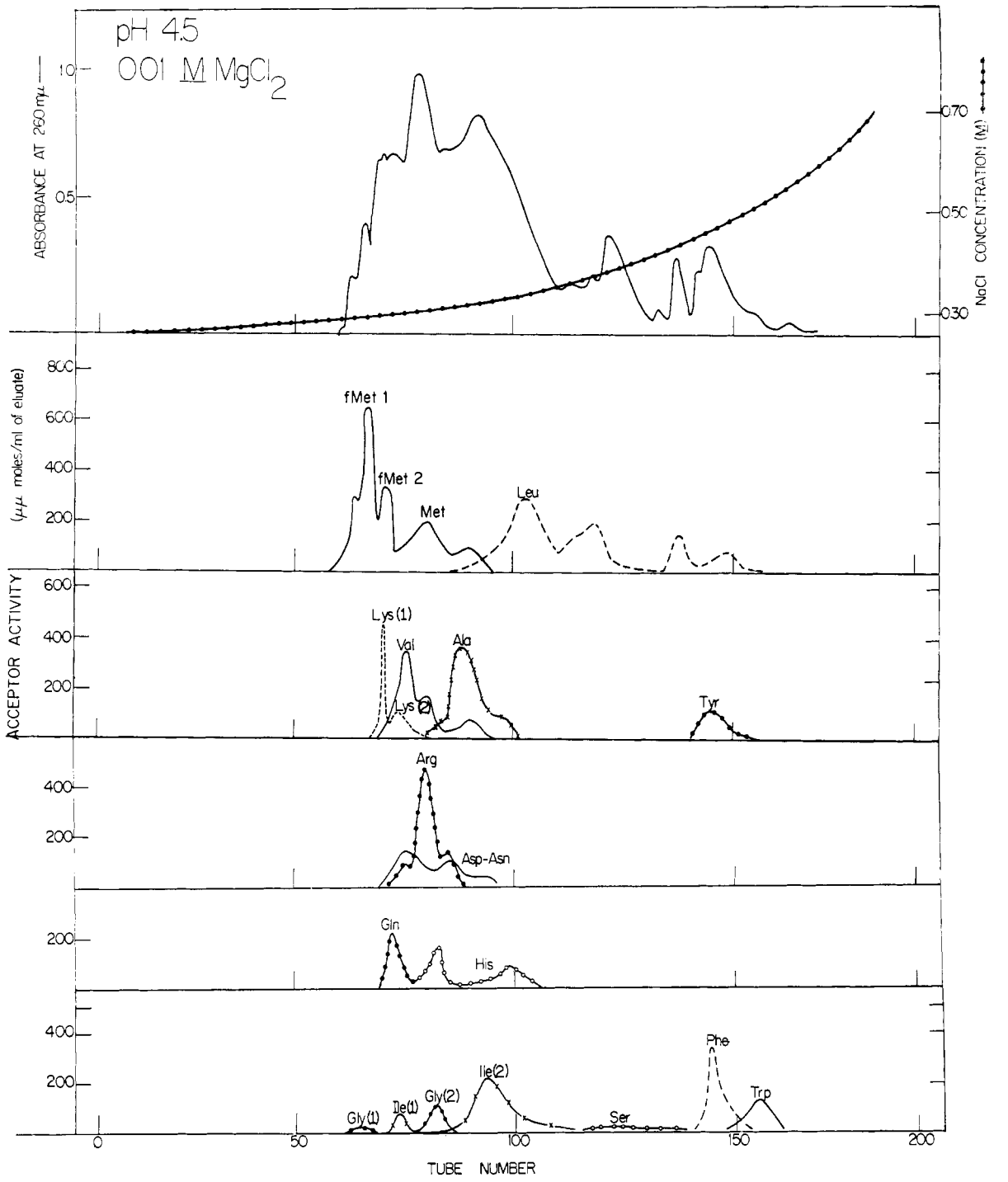

FIGURE 2: Reversed-phase chromatography 3 of *E. coli* B tRNA at pH 4.5. Experimental conditions were the same as those described for Figure 1 except changes in the buffer to 0.01 M sodium acetate at pH 4.5 and concave 3-l. elution gradient from 0.26 to 0.70 M NaCl.

Both the 260-mμ absorbance trace and the amino acid acceptance data show sharp chromatographic peaks. In some cases, the degree of heterogeneity shown is the same as that in Figure 1 for reversed-phase chromatography 3 at pH 4.5; there are three peaks for valine and two for histidine acceptance. However, other tRNAs were not as well resolved; only one methionine acceptance peak and one alanine were detected. The elution sequence for many tRNAs on reversed-phase chromatography 4 is somewhat different from reversed-phase chromatography 3, so that by combination of these column techniques it should be possible to find conditions permitting the purification of a number of tRNAs.

A sample of *E. coli* B crude tRNA was fractionated on a 1 × 240 cm reversed-phase chromatography 3 column with an extended sodium chloride gradient so that greater separation of the tRNAs in the front

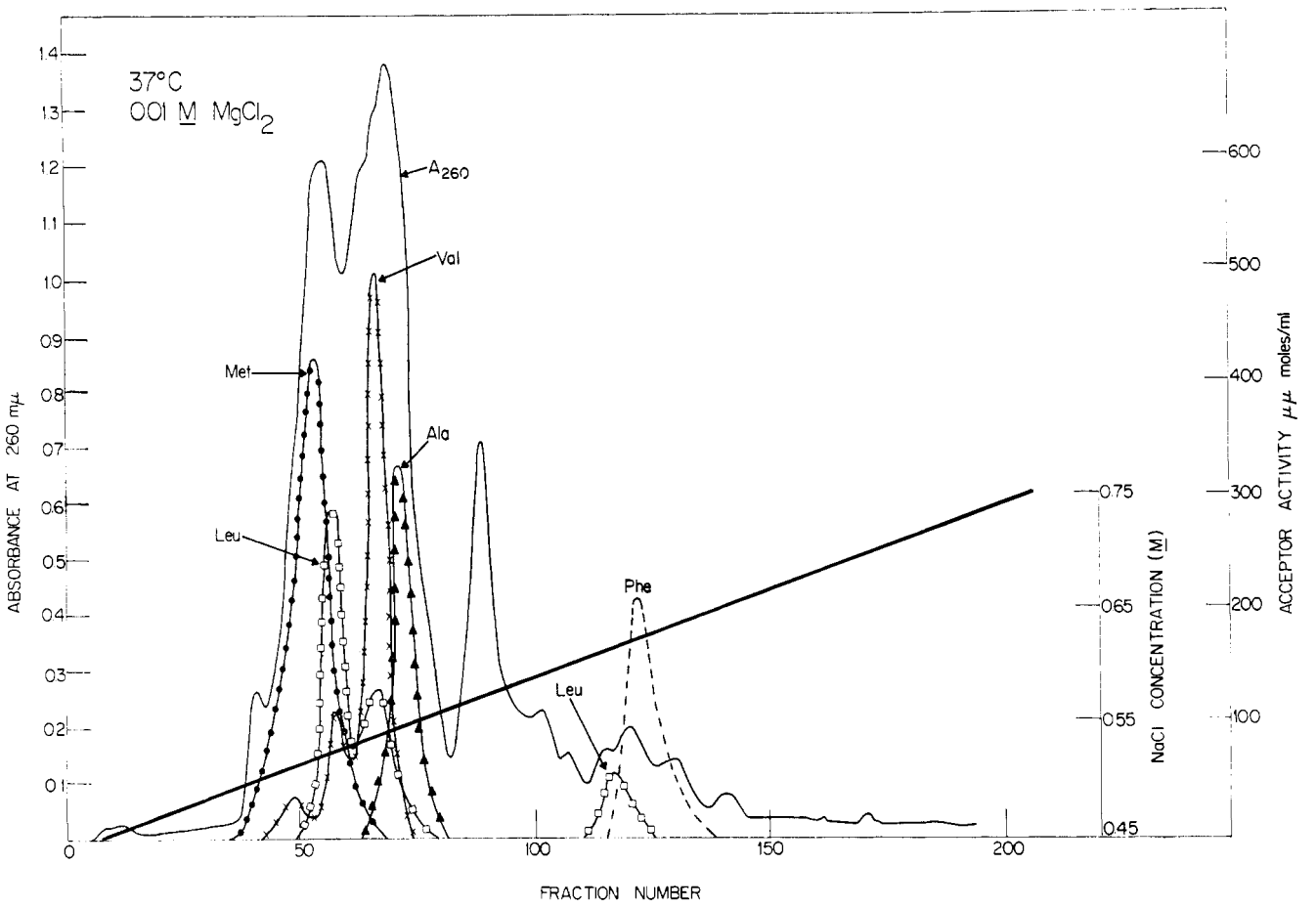

FIGURE 3: Reversed-phase chromatography 4 of *E. coli* B tRNA at pH 4.5: 1000 A_{260} units of feed tRNA was dissolved in 80 ml of equilibration solution, 0.45 M NaCl, 0.01 M $MgCl_2$, and 0.01 M sodium acetate buffer at pH 4.5 preheated to 37° for 1 hr, and pumped on a 1 × 240 cm reversed-phase chromatography 4 column, maintained at 37°, at 2 ml/min. A 3-l. linear elution gradient from 0.45 to 0.75 M NaCl, plus magnesium and buffer, was used at a flow rate of 1.5 ml/min. The column effluent was monitored at 260 mμ and approximately 15-ml fractions were collected. Selected fractions were assayed for amino acid acceptance as described under Methods.

of the chromatogram would be achieved. The resulting chromatogram is shown in Figure 4. Amino acid acceptance peaks coincided with 260-mμ absorbance peaks. Three methionine tRNAs were defined. Two of these ($tRNA_1^{fMet}$ and $tRNA_2^{fMet}$) were subsequently shown to accept [^{14}C]formyl groups following aminoacylation equivalent to the degree of methionine acceptance and were thus identified as formylmethionine tRNAs.[3] The third methionine tRNA, $tRNA^{Met}$, accepted no formyl groups and is the normal methionine tRNA. The only other tRNAs present in this region of the chromatogram were two lysine tRNAs and a valine tRNA.

In order to prepare larger samples of the two formylmethionine tRNAs, a 5 × 90 cm reversed-phase chromatography system 3 was used to fractionate 20,000 A_{260} units of feed tRNA, as shown in Figure 5. The $tRNA_1^{fMet}$ peak was sufficiently separated to permit pooling selected fractions to yield a product sample. The $tRNA_2^{fMet}$ and $tRNA^{Val}$ eluted together and these fractions were pooled. Essentially 100% of the formylmethionine tRNA activity applied to the column (60% of the total methionine-accepting activity) was accounted for in the chromatographic fractions. The $tRNA_1^{fMet}$ product pool, shown in Figure 5, contained 78% of the $tRNA_1^{fMet}$ and the $tRNA_2^{fMet}$–$tRNA^{Val}$ pool contained 86% of the $tRNA_2^{fMet}$. The fractions pooled were selected to optimize $tRNA_2^{fMet}$ recovery and, thus, the $tRNA^{Val}$ recovery in this experiment was only about 60%. The $tRNA_2^{fMet}$ –$tRNA^{Val}$ solution from reversed-phase chromatography 3 was adjusted to 0.45 M NaCl and then pumped directly onto a reversed-phase chromatography 4 column and these tRNAs were separated by rechromatography on a 2.5 × 100 cm reversed-phase chromatography 4 as shown in Figure 6. Following rechromatography on reversed-phase chromatography 4, the $tRNA_2^{fMet}$ and $tRNA^{Val}$ were separately pooled to yield purified samples of these tRNAs. Approximately 65% of the $tRNA_2^{fMet}$ and 100% of the $tRNA^{Val}$ applied to the reversed-phase chromatography 4 column were recovered in the chromatographic fractions; 49% of the $tRNA_2^{fMet}$ was contained in the $tRNA_2^{fMet}$ product pool and 68% of the $tRNA^{Val}$ in the corresponding $tRNA^{Val}$ product pool.

Analyses of purified samples of $tRNA_1^{fMet}$, $tRNA_2^{fMet}$,

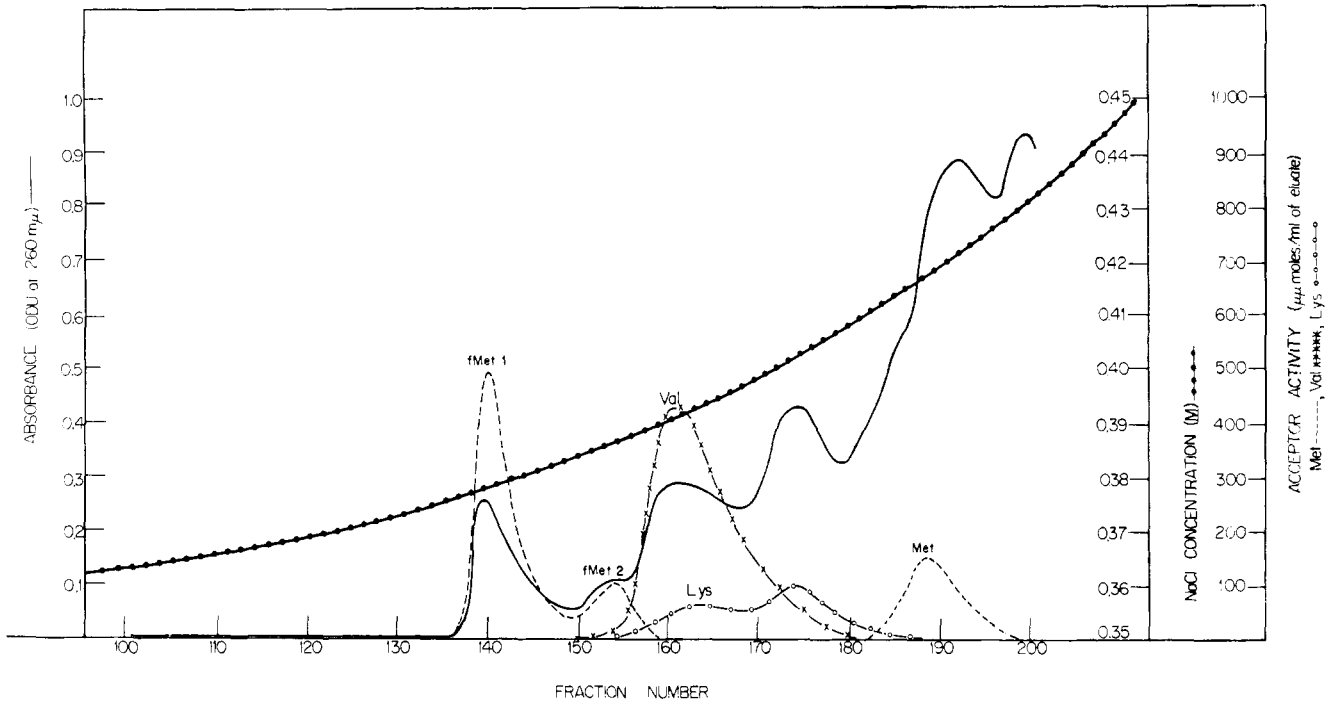

FIGURE 4: Expanded separation of tRNAs in front region of a reversed-phase chromatography 3 chromatogram at pH 7.0. Approximately 1000 A_{260} units of feed tRNA was dissolved in 80 ml of equilibration solution, 0.15 M NaCl, and 0.01 M Tris (pH 7.0), and preheated to 37°. The reversed-phase chromatography 3 column was 1 × 240 cm maintained at 37°. The feed was pumped onto the column at 2 ml/min. A concave 3-l. elution gradient from 0.35 to 0.45 M NaCl, plus buffer, was used at 1.5 ml/min. Approximately 15-ml fractions were collected. The column effluent was monitored and assayed as described under Methods. The remainder of the tRNAs were removed from the column with approximately 500 ml of 1.2 M NaCl solution.

TABLE II: Activity of Purified tRNA Samples.

tRNA	Terminal Adenosine ($\mu\mu$moles/A_{260})[a]	Methionine Acceptance ($\mu\mu$moles/A_{260})	Formylation ($\mu\mu$moles/A_{260})	Act.[b] (%)
fMet 1	1208 ± 91	1207 ± 99	1207	100
fMet 2	1330 ± 60	1151 ± 87	1086	87
		Valine Acceptance ($\mu\mu$moles/A_{260})		
Val	1370 ± 47	1210 ± 19		88

[a] A_{260} absorbance measured in the presence of 0.01 M $MgCl_2$. [b] Amino acid acceptance per terminal adenosine.

and $tRNA^{Val}$ from a series of reversed-phase chromatography 3 and 4 chromatographic experiments are shown in Table II. The terminal adenosine content was determined by alkaline hydrolysis followed by Chelex ion-exchange chromatography (Burtis and Goldstein, 1968). The methionine acceptance and formylation assays are described in the Methods section. For the $tRNA_1^{fMet}$ all three of these values were essentially identical at approximately 1200 $\mu\mu$moles/A_{260} unit. Thus, for this sample an activity (amino acid acceptance per terminal adenosine) of 100% was obtained. Similarly, the $tRNA_2^{fMet}$ was 87% active and the $tRNA^{Val}$ was 88% active. For these three samples, no terminal cytidine groups were detected and acceptance of other amino acids was also not detected. Thus, from these data, it was concluded that these samples, obtained from repeated runs on reversed-phase chromatography 3 and 4 columns, were highly purified.

Three codons for formylmethionine tRNA have been reported, AUG, GUG, and UUG (Clark and Marcker, 1965, 1966a,b; Kellogg *et al.*, 1966; Ghosh *et al.*, 1967). The codon response of the $tRNA_1^{fMet}$ and

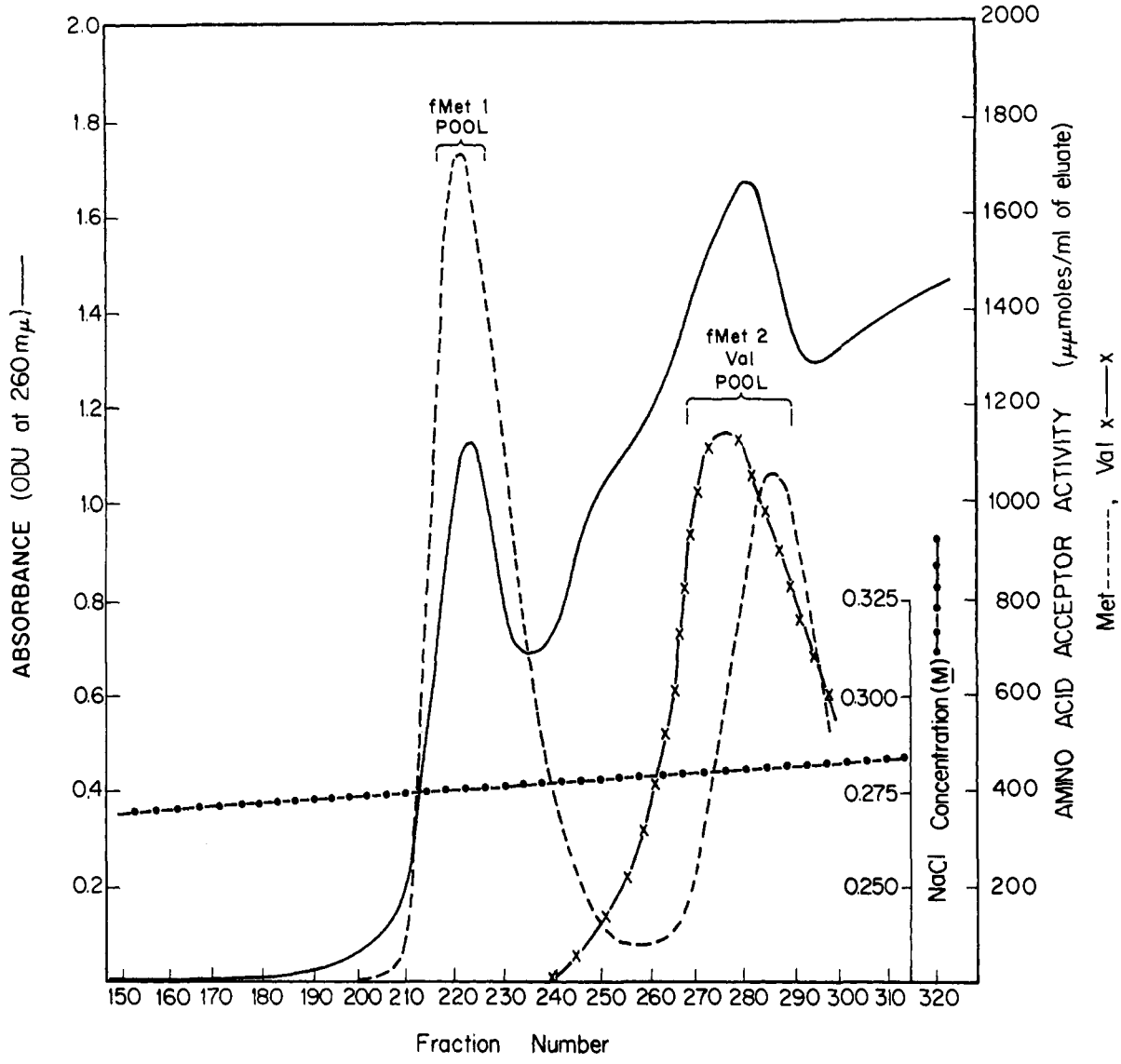

FIGURE 5: Larger scale reversed-phase chromatography 3 separation of $tRNA_1^{fMet}$ and $tRNA_2^{fMet}$ at pH 7.0. Approximately 20,000 A_{260} units of feed tRNA was dissolved in 1500 ml of equilibration solution, 0.20 M NaCl, 0.01 M $MgCl_2$, and 0.01 M Tris buffer pH 7.0, and pumped onto a 5 × 90 cm reversed-phase chromatography 3 column (Pharmacia, up-flow adapters) at 4 ml/min. The column packing consisted of 2 wt % trioctylpropylammonium bromide on Chromosorb G, AW-DMCS, 100–120 mesh size. An 8.0-l. linear elution gradient from 0.26 to 0.30 M NaCl, plus magnesium and buffer, was used at a flow rate of 4 ml/min. The column effluent was monitored at 260 mμ and approximately 24-ml fractions were collected. Selected fractions were assayed for methionine and valine acceptance.

$tRNA_2^{fMet}$ was investigated by binding to ribosomes in the presence of triplets.[4] The results (Table III) show no difference in the binding response of the two formylmethionine tRNAs. Maximum binding was achieved in the presence of AUG, but significant binding also occurred with GUG. A smaller degree of binding also was stimulated by UUG. These experiments indicate no differences in the anticodon region in the two formylmethionine tRNAs.

Other differences that have not been investigated may exist between the two formylmethionine tRNAs, such as nucleotide composition differences in regions other than the anticodon or possible conformational differences. Changes in the reversed-phase chromatographic position of an *E. coli* tryptophan tRNA with changes in secondary structure have been recently reported (Ishida and Sueoka, 1967). Experiments are under way to attempt to define differences between these formylmethionine tRNAs and to investigate their biological significance.

Acknowledgments

The authors wish to acknowledge the advice and help of Dr. G. David Novelli, to acknowledge the

[4] J. C. Brown, Chemistry Department, Harvard University, personal communication.

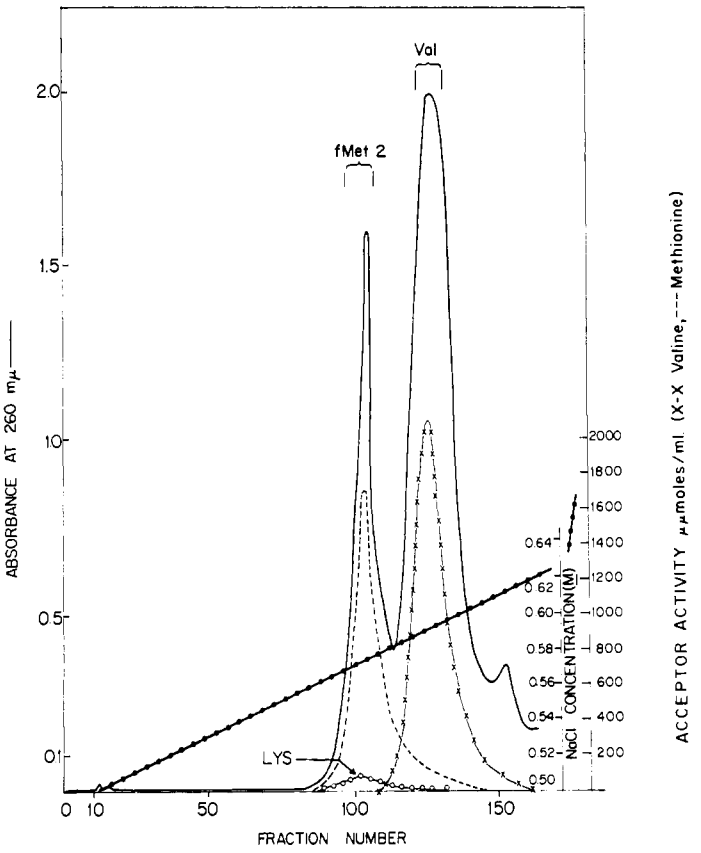

FIGURE 6: Separation of $tRNA_2^{fMet}$ and $tRNA^{Val}$ on reversed-phase chromatography 4 at pH 7.0: 1000 A_{260} units of pooled material (see Figure 5) (800 ml) was adjusted to 0.45 M NaCl and pumped onto an 1 × 240 cm reversed-phase chromatography 4 column for rechromatography at 37°. The column was equilibrated with 0.45 M NaCl and 0.01 M Tris (pH 7) before loading. A 3-l. linear elution gradient from 0.500 to 0.625 M NaCl plus 0.01 M Tris buffer (pH 7) was used at a flow rate of 1.5 ml/min. The run was monitored and selected fractions were assayed as described.

TABLE III: Binding of Formylmethionine tRNAs to Ribosomes in the Presence of Triplets.[a]

Codon	μμmoles of tRNA Bound	
	$tRNA_1^{fMet}$	$tRNA_2^{fMet}$
AUG	6.70	7.63
GUG	2.24	3.38
UUG	1.36	1.74
CUG	0.49	0.76
None	0.29	0.64

[a] Assay conditions: 0.05 M Tris acetate (pH 7.5), 0.10 M NH_4Cl, 0.005 M magnesium acetate, 1 × 10^{-4} M GTP, 0.2 mM codon, ribosomes, 50 μg of initiation factors, ~40 μμmoles of methionyl-tRNA, final volume 50 μl, incubated 20 min at room temperature, and filtered on a Millipore filter.

contributions of Dr. Lee Shugart for formyl acceptance assays, Mr. Jay C. Brown for codon binding assays, and Dr. Gerald Goldstein for terminal adenosine assays, and to acknowledge the excellent technical assistance of Mr. J. P. Eubanks.

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