SEPARATION AND COMPARISON OF PRIMARY STRUCTURES OF THREE FORMYLMETHIONINE tRNAs FROM E. coli K-12 MO*

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Three chromatographically distinct $tRNAs^{fMet}$ from E. coli K-12 MO were separated by reversed-phase chromatography and designated $tRNA_A^{fMet}$, $tRNA_B^{fMet}$, and $tRNA_3^{fMet}$. The $tRNA_A^{fMet}$ corresponds to the published sequence for $tRNA^{fMet}$ (E. coli). The $tRNA_B^{fMet}$ differs from $tRNA_A^{fMet}$ in that the 4-thiouridine in nucleotide position 8 has interacted with cytidine in position 13 to form a cross-linked product. The $tRNA_3^{fMet}$ differs from $tRNA_A^{fMet}$ in that 7-methylguanosine (in position 47) has been replaced by adenosine.

INTRODUCTION

The primary structure of the protein initiator tRNA, $tRNA^{fMet}$ (E. coli), has been reported (1,2), and existence of a second minor species has been suggested. Effects of various modifications of $tRNA^{fMet}$ have been interpreted in terms of this structure (3-8). Recently, the importance of photochemically induced cross-linking between 4-thiouridine and cytidine has been discussed (9-16), and the separation of an intramolecular cross-linked form of $tRNA^{fMet}$ from E. coli has been reported (17).

The separation of three chromatographically distinct $tRNAs^{fMet}$ from E. coli K-12 MO was achieved previously by reversed-phase chromatography (18,19), and samples of $tRNA_1^{fMet}$ and $tRNA_3^{fMet}$ were made available for distribution to other investigators (20). By further rechromatography on an improved reversed-phase chromatography system (RPC-5) (21), $tRNA_1^{fMet}$ was separated into two species, designated $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$. Due to the widespread use of the distributed samples and the implications of multiple

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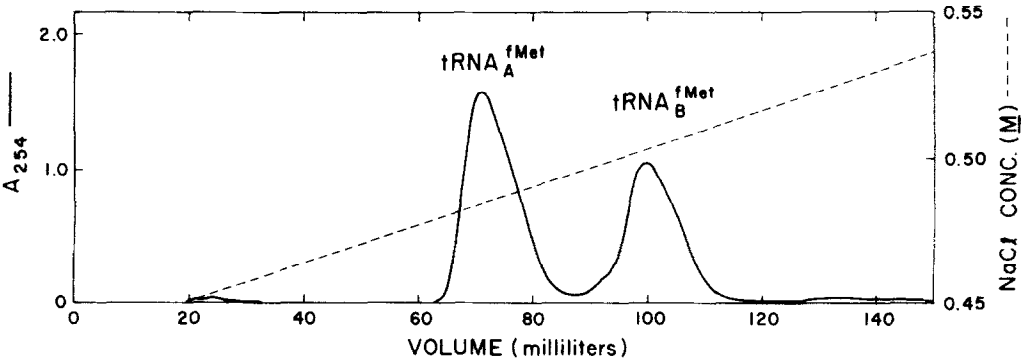

Fig. 1. Separation of $tRNA_1^{fMet}$ into Two Species, $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$, by Reversed-Phase Chromatography. Column, 0.63 x 93 cm, RPC-5; eluent, 0.05 *M* Tris-HCl, pH 7.3, 0.01 *M* $MgCl_2$, NaCl gradient as shown; temperature, 37°C; flow rate, 1.0 ml/min.

chain initiator tRNAs, it was deemed important to determine the structural relationships among these tRNAs in terms of the published structure.

MATERIALS AND METHODS

Chromatographic methods and materials for reversed-phase chromatography have been described previously (19,21,22).

Ribonuclease T_1 was obtained from Calbiochem. Pancreatic ribonuclease A, bacterial alkaline phosphatase, and venom phosphodiesterase were from Worthington Biochemical Corporation. Ribonuclease T_1 and pancreatic ribonuclease stock solutions contained 10,000 units/ml and 5.0 mg/ml, respectively, in 0.05 *M* Tris-HCl, pH 7.4. In a typical digestion, 50 to 100 µl of the T_1 or pancreatic ribonuclease solution was added to 0.5 to 1.0 ml of solution containing ~100 A_{260} units* of $tRNA^{fMet}$ per milliliter and incubated for 4 to 5 hr at 37°C. The resulting digests were applied directly to the RPC-5 column.

The oligonucleotides were separated by reversed-phase chromatography as described previously (22).

Base composition of the oligonucleotides was determined by ion exchange chromatography (23,24) of the constituent nucleosides. The nucleosides were

*One A_{260} unit is the quantity of RNA dissolved in 1 ml of solution which gives an absorbance of 1 at 260 nm in a 1-cm cell.

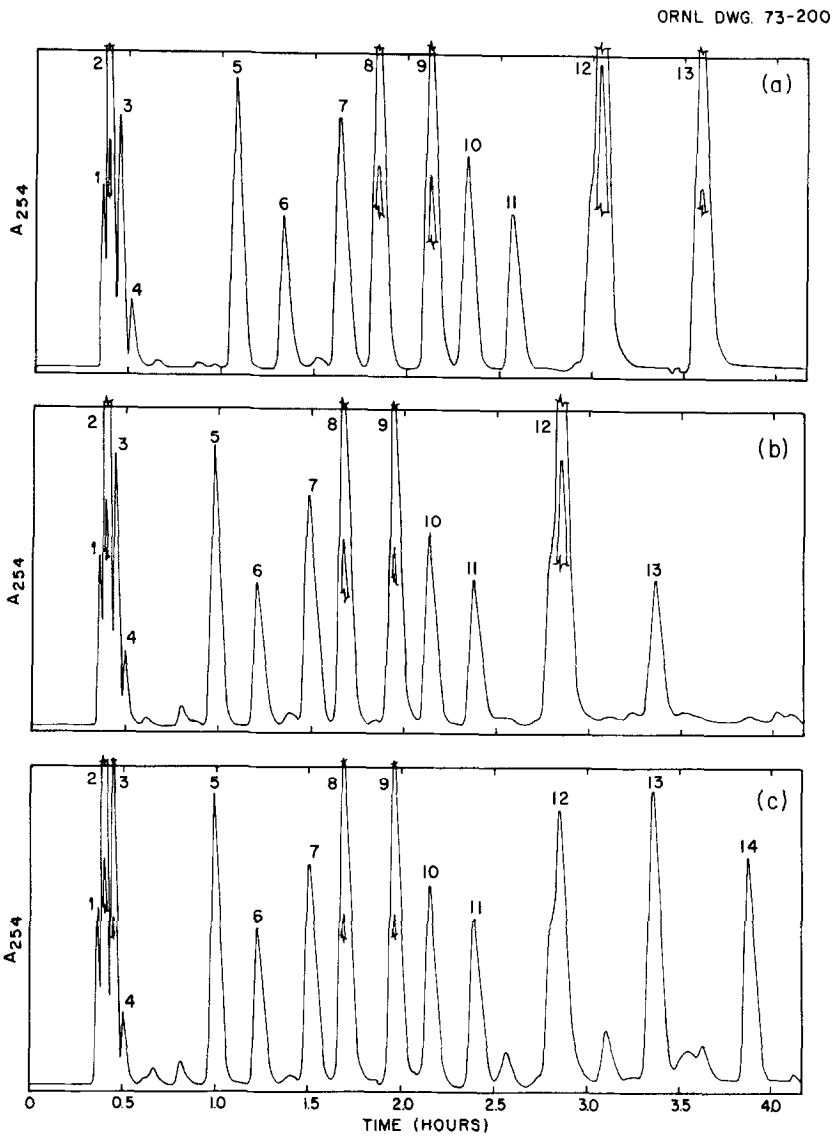

Fig. 2. Comparison of Reversed-Phase Chromatograms of Pancreatic Ribonuclease Digests of (a) 49 A_{260} Units of $tRNA_A^{fMet}$, (b) 46 A_{260} Units of $tRNA_B^{fMet}$, and (c) 50 A_{260} Units of $tRNA_3^{fMet}$. Column, 0.63 x 98 cm, RPC-5; eluent, 250 ml of 0.5-2.0 *M* (linear) ammonium acetate-acetic acid, pH 4.4; temperature, 37°C; flow rate, 1.0 ml/min.

obtained by digestion of the oligonucleotides with venom phosphodiesterase and alkaline phosphatase (25).

RESULTS

Separation of $tRNAs^{fMet}$

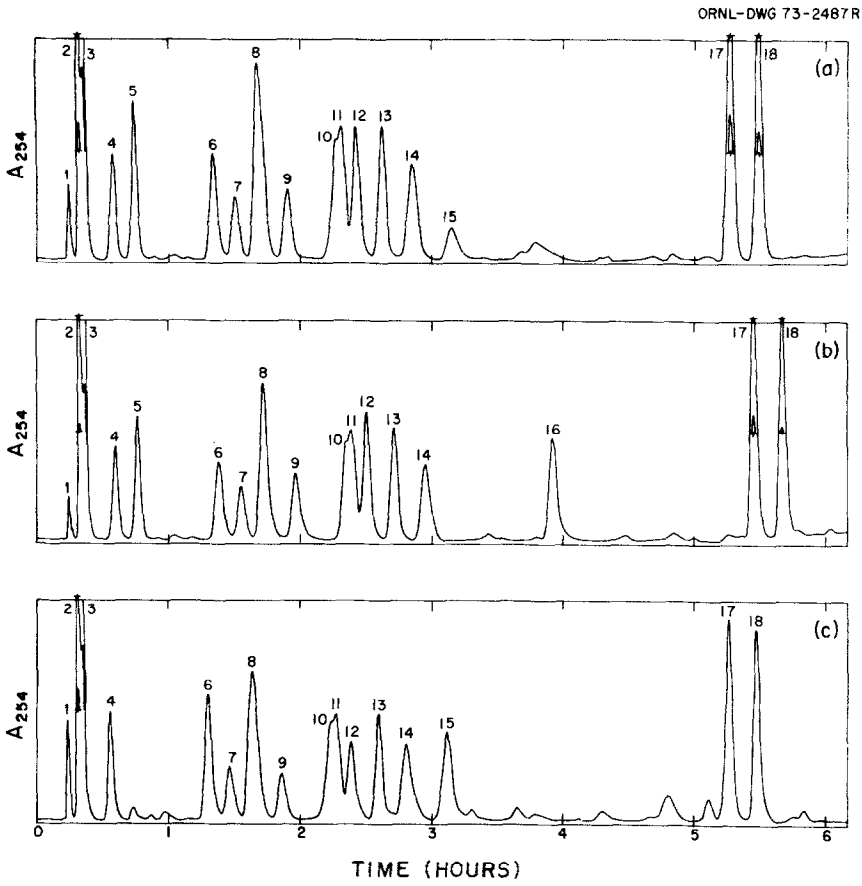

Fig. 3. Comparison of Ribonuclease T_1 Digests of (a) $tRNA_A^{fMet}$, (b) $tRNA_B^{fMet}$, and (c) $tRNA_3^{fMet}$. Column, 0.63 x 97 cm, RPC-5; eluent, 600 ml of 0.6-1.9 *M* ammonium acetate-acetic acid, pH 4.4; temperature, 37°C; flow rate, 1.5 ml/min. Each sample contained approximately 6 A_{260} units.

Samples of $tRNA_1^{fMet}$ and $tRNA_3^{fMet}$ were obtained by reversed-phase chromatography on an RPC-3 system (18). Rechromatography of the $tRNA_1^{fMet}$ using RPC-5 columns yielded two peaks, designated $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$ (Fig. 1). A preparative column (1.0 cm diam. by 116 cm long) was used to resolve a sample containing 500 A_{260} units of $tRNA_1^{fMet}$. A 2.5- by 19-cm DEAE-cellulose column was used to concentrate the pooled fractions. The two fractions, $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$, gave amino acid acceptances (19) of 1780 and 1700 picomoles/A_{260} unit, respectively, and were approximately 93% and 87% formylatable. The $tRNA_3^{fMet}$ could not be further resolved; it gave an amino acid acceptance of 1560 picomoles/A_{260} unit and was 88% formylatable.

TABLE I. Identification and Comparison of Oligonucleotides from Pancreatic Ribonuclease Digests of Three $tRNAs^{fMet}$

Peak (Fig. 2)	Fragment		
	$tRNA_A^{fMet}$	$tRNA_B^{fMet}$	$tRNA_3^{fMet}$
1	A_{OH}	A_{OH}	A_{OH}
2	Cp, Ψp	Cp, Ψp	Cp, Ψp
3	Up	Up	Up
4	pCp	pCp	pCp
5	GCp	GCp	GCp
6	AUp	AUp	AUp
7	GUp	GUp	GUp
8	AACp	AACp	AACp
9	AGCp	AGCp	AGCp
10	GGCp	GGCp	GGCp
11	GGhUp	GGhUp	GGhUp
12	GGTp	GGTp	GGTp
	AAAUp	AAAUp	AAAUp
	$GAAGm^7GUp$	$GAAGm^7GUp$	–
13	GGGCmUp	GGGCmUp	GGGCmUp
	GGAGCp	–	GGAGCp
14	–	–	GAAGAUp

Comparison of Pancreatic Ribonuclease Digests of the Three $tRNAs^{fMet}$

Chromatograms of pancreatic ribonuclease digests of the three $tRNAs^{fMet}$ are compared in Fig. 2. The peaks are identified in Table I. Each sample was digested and chromatographed under identical conditions, as described in Methods. The chromatograms are essentially the same from peaks 1 through 11. However, peak 12 contains three fragments in $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$, one of which is missing in $tRNA_3^{fMet}$. Peak 13 contains two fragments in $tRNA_A^{fMet}$ and $tRNA_3^{fMet}$, but only one in $tRNA_B^{fMet}$. The $tRNA_3^{fMet}$ contains an additional peak 14 which is not present in $tRNA_A^{fMet}$ and $tRNA_B^{fMet}$.

Peaks 12, 13, and 14 were digested with ribonuclease T_1, and the resulting fragments were separated by reversed-phase chromatography and identified by

TABLE II. Identification and Comparison of Oligonucleotides from Ribonuclease T_1 Digests of Three $tRNAs^{fMet}$

Peak (Fig. 3)	Fragment		
	$tRNA_A^{fMet}$	$tRNA_B^{fMet}$	$tRNA_3^{fMet}$
2	Gp	Gp	Gp
4	CGp	CGp	CGp
5	m^7GUCGp	m^7GUCGp	–
6	AGp	AGp	AGp
7	pCGp	pCGp	pCGp
8	UCGp	UCGp	UCGp
	CAGp	–	CAGp
9	hUAGp	hUAGp	hUAGp
10, 11	CUCGp	CUCGp	CUCGp
	CCUGp	CCUGp	CCUGp
12	$CAACCA_{OH}$	$CAACCA_{OH}$	$CAACCA_{OH}$
13	CCCCCGp	CCCCCGp	CCCCCGp
14	AAGp	AAGp	AAGp
15	s^4UGp	–	s^4UGp
	–	–	AUCGp
16	–	CAGp U*Gp	–
17	CmUCAUAACCCGp	CmUCAUAACCCGp	CmUCAUAACCCGp
18	TψCAAAUCCGp	TψCAAAUCCGp	TψCAAAUCCGp

base composition and spectra. The missing fragment in peak 12 of $tRNA_3^{fMet}$ was found to be GAAGm^7GUp; the missing fragment in peak 13 of $tRNA_B^{fMet}$ was identified as GGAGCp; and the extra peak 14 in $tRNA_3^{fMet}$ corresponded to GAAGAUp. The fragment, GGGGs^4Up, expected from the published sequence of $tRNA^{fMet}$, was not eluted under the chromatographic conditions used.

Comparison of Ribonuclease T1 Digests of the Three $tRNAs^{fMet}$

Chromatograms of the ribonuclease T_1 digests of the three $tRNAs^{fMet}$ are compared in Fig. 3 and summarized in Table II. Again, there are significant differences in terms of the presence or absence of certain peaks.

The fragment m^7GUCGp is missing in $tRNA_3^{fMet}$. The trinucleotides, UCGp and

CAGp, were not separated under the chromatographic conditions used, but base analysis of peak 8 (Fig. 3) indicated that CAGp was missing in $tRNA_E^{fMet}$.

Peak 15, s^4UGp, was also missing in $tRNA_B^{fMet}$; peak 15 in $tRNA_3^{fMet}$ contained AUCGp in addition to s^4UGp.

Peak 16 occurred in significant amounts only in $tRNA_B^{fMet}$ and was identified by nucleoside composition and spectra (9,11,15) as the cross-linked fragment, $\overline{CAGp\ U^*Gp}$.

DISCUSSION

The results can be summarized as follows: (i) $tRNA_A^{fMet}$ appears to correspond to the published sequence (1) for $tRNA^{fMet}$; (ii) $tRNA_B^{fMet}$ differs from $tRNA_A^{fMet}$ in that the 4-thiouridine in nucleotide position 8 (from the 5'-terminus) has interacted with the cytidine in position 13 to form a cross-linked product (14,17); (iii) $tRNA_3^{fMet}$ differs from $tRNA_A^{fMet}$ in that 7-methylguanosine in position 47 has been replaced by adenosine, corresponding to the minor species suggested by Dube *et al.* (1).

It is interesting that no cross-linked form of $tRNA_3^{fMet}$ was observed. This result suggests that the tertiary structure of these tRNAs allows close proximity of nucleotide 47 to the cross-linking area.

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