A CHROMATOGRAPHICALLY DIFFERENT FORM OF THE FORMYL-ACCEPTING METHIONINE TRANSFER RNA FROM ESCHERICHIA COLI[1]

Lee Shugart, Barbara Chastain, and G. David Novelli

Biology Division, Oak Ridge National Laboratory,

Oak Ridge, Tennessee 37830

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A study of the origin of two chromatographically distinct formyl-accepting species of methionine transfer ribonucleic acid from Escherichia coli shows that one arises from the other after isolation of the tRNA from the microorganism. No characteristic differences are noted in their physical or biological properties. A nucleoside analysis indicates a decreased 4-thiouridine content for the modified species.

Kelmers et al. (1) and Weiss et al. (2) reported the isolation and purification of two formyl-accepting species of $tRNA_f^{Met}$ from Escherichia coli strain B by reversed-phase column chromatography.[2] One of the two species was made available for distribution to the scientific community (3) and was subsequently crystallized (4). This report details our attempt to characterize these tRNA's (designated $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$), and shows that the $tRNA_{f2}^{Met}$ material is an artifact that arises from $tRNA_{f1}^{Met}$, and that the latter species is apparently the major naturally occurring $tRNA_f^{Met}$ in E. coli B.

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[2]Abbreviations: tRNA, transfer ribonucleic acid; $tRNA_f^{Met}$, the formyl-accepting species of methionine tRNA; Met-$tRNA_f$, $tRNA_f^{Met}$ aminoacylated with methionine; fMet-tRNA, formylmethionyl-tRNA; absorbance unit, that amount of tRNA in 1 ml which possesses an absorbance of 1.0 when measured with a 1-cm optical path at the wavelength designated; RPC-3, reversed-phase chromatographic system No. 3; -C-C-A terminus, 3'-OH terminal nucleotide sequence of tRNA.

Results and Discussion. We were unable to detect dimerization in either species, although we tried all three of the following procedures: Sephadex G-100 column chromatography (5), gel electrophoresis (6,7), and analytical ultracentrifugation (8,9). In addition, melting curve analyses at 260 and 280 nm show no significant difference, and the data obtained compare favorably with

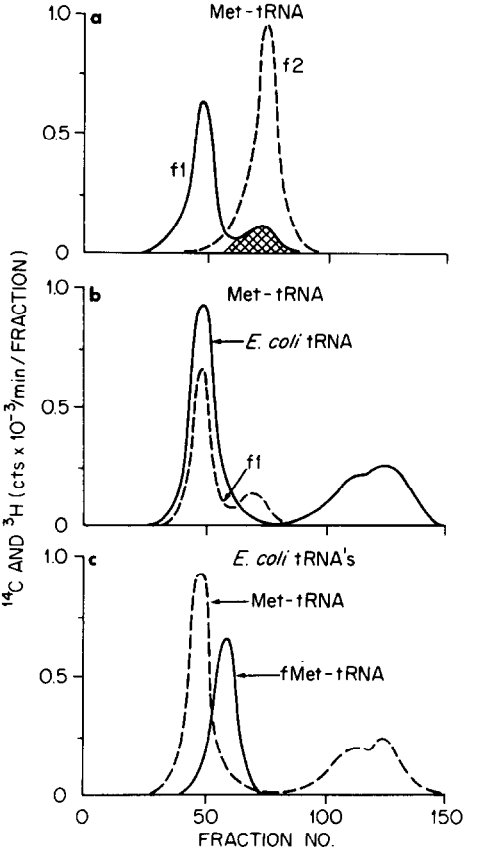

Figure 1. Elution profiles of various $tRNA^{Met}$ species on reversed-phase column (RPC-3 system). The purified $tRNA^{Met}_{f1}$ and $tRNA^{Met}_{f2}$ species from Escherichia coli B and unfractionated tRNA's from the same microorganism were isolated as outlined by Weiss et al. (2) and by Kelmers et al. (18). Cochromatography was as detailed by Shugart et al. (14). (a) Solid line, [^{14}C]methionyl-$tRNA_{f1}$; dashed line, [^{3}H]methionyl-$tRNA_{f2}$. (b) Solid line, [^{14}C]methionyl-tRNA (E. coli unfractionated tRNA's); dashed line, [^{3}H]methionyl-$tRNA_{f1}$. (c) Solid line, [^{14}C]formyl[^{12}C]methionyl-tRNA (E. coli unfractionated tRNA's); dashed line, [^{3}H]methionyl-tRNA (E. coli unfractionated tRNA's).

those previously reported for $tRNA_f^{Met}$ (10). Since neither species incorporates methyl groups when assayed with a homologous methylase preparation (11) and since both contain 7-methyl guanosine as determined by thin-layer chromatography after acid hydrolysis of each (12), the possibility that either is the minor $tRNA_f^{Met}$ (in which 7-methyl guanosine is replaced by an adenosine) reported by Dube et al. (13) was eliminated.

After aminoacylation, Met-$tRNA_{f1}$ and Met-$tRNA_{f2}$ were cochromatographed on an analytical RPC-3 column (14); Fig. 1a gives the chromatographic profiles obtained. The difference between the two tRNA's cannot be due to an incomplete -C-C-A terminus (15), since during aminoacylation this terminus would have been repaired as a result of the presence of tRNA-adenylyltransferase activity in our aminoacyl-tRNA synthetase preparation. Neither the treatment outlined by Lindahl et al. (16) for the renaturation of tRNA nor the conditions specified by Gartland and Sueoka (17) for the interconversion of two (E. coli) tryptophan tRNA's affect the chromatographic profiles shown in Fig. 1a.

To determine whether or not a specific growth condition of the microorganism was responsible for the appearance of an additional $tRNA_f^{Met}$, we screened tRNA samples obtained from numerous cultures of E. coli. Our screening technique consisted of isolating unfractionated tRNA's from E. coli (18) and comparing Met-tRNA of these samples with Met-$tRNA_{f1}$ by cochromatography on an RPC-3 column (14). The $tRNA_{f1}^{Met}$ species contains some (about 10%) $tRNA_{f2}^{Met}$ material (cf., Fig. 1a, hatched area), and thus in the screening experiments it is a chromatographic marker for both $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$ material.

No peak corresponding to Met-$tRNA_{f2}$ could be found in unfractionated tRNA from E. coli strain B taken in either the log or the stationary phase at cell densities from 4 to 50 g/liter (wet weight), under aerobic growth conditions in a synthetic culture medium, with or without pH control, and containing glucose as an energy source. Neither could Met-$tRNA_{f2}$ be demonstrated in unfractionated tRNA from E. coli strains K_{12}, W, and $K_{12}W_6$ (an RC^{rel} mutant), or in strain B growth under anaerobic growth conditions or infected with T_2

phage. It was concluded from these experiments that neither the growth conditions normally employed to culture E. coli in this laboratory nor the strain of microorganism used is responsible for the occurrence of an additional $tRNA_f^{Met}$ species. Further, the single species that is found corresponds (chromatographically) to $tRNA_{f1}^{Met}$ material. Figure 1b shows a typical chromatogram of unfractionated (E. coli) tRNA precharged with [^{14}C]methionine and cochromatographed on an RPC-3 column with $tRNA_{f1}^{Met}$ charged with [^{3}H]methionine. It is evident that $tRNA_{f2}^{Met}$ material is absent in the unfractionated (E. coli) tRNA preparation. The [^{14}C]Met-tRNA material occurring between fractions 100 and 150 was identified as the methionine tRNA that is not formyl-accepting and is also present in unfractionated tRNA's (cf. Fig. 1c).

Several observations indicate that the $tRNA_{f2}^{Met}$ species originated from

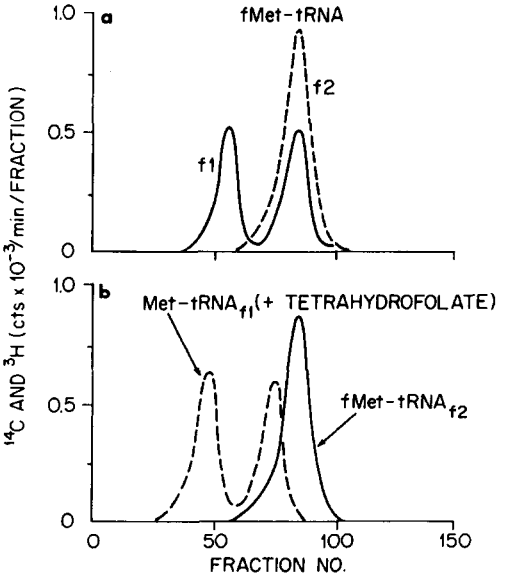

Figure 2. Elution profiles of $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$ on reversed-phase column (RPC-3 system) after various treatments. See Fig. 1 for details concerning the isolation of the tRNA's and methods on chromatography procedures. (a) Solid line, [^{14}C]formyl[^{12}C]methionyl-$tRNA_{f1}$; dashed line, [^{12}C]formyl[^{3}H]methionyl-$tRNA_{f2}$. (b) Solid line, [^{14}C]formyl[^{12}C]methionyl-$tRNA_{f2}$; dashed line, [^{3}H]methionyl-$tRNA_{f1}$ (charged in presence of tetrahydrofolate as outlined in text).

the $tRNA_{f1}^{Met}$ material. First, in screening an old (2-year) preparation of unfractionated tRNA's from E. coli $K_{12}W_6$, we found Met-$tRNA_{f2}$ material. This material must have originated after the isolation of the tRNA because we were unable to demonstrate it earlier in freshly prepared unfractionated tRNA's from the same microorganism. Second, the chromatographic profile of Met-$tRNA_{f1}$ is altered by exposure to those conditions employed for formylation (Fig. 2a) and results in the appearance of new fMet-$tRNA_{f2}$ (compare Figs. 1a and 2a). The Met-$tRNA_{f2}$ species is not affected by this treatment. Formylation per se of Met-$tRNA_{f1}$ is not responsible for the appearance of new fMet-$tRNA_{f2}$, because altering the usual conditions for formylation by replacing the formyl donor N^{10}-formyltetrahydrofolate with tetrahydrofolate results in the appearance of Met-$tRNA_{f2}$, which can be clearly distinguished from fMet-$tRNA_{f2}$ (Fig. 2b). The $tRNA_f^{Met}$ in unfractionated (E. coli) tRNA cannot be converted to $tRNA_{f2}^{Met}$ material by exposure to conditions for formylation (cf. Fig. 1c). These results indicate that $tRNA_{f2}^{Met}$ arises from $tRNA_{f1}^{Met}$ because of some prior modification(s) of the latter. The following findings suggest an explanation as to the type of modification that occurred: We analyzed the two tRNA's and found that $tRNA_{f2}^{Met}$ has one more ureido group than $tRNA_{f1}^{Met}$, hence an additional dihydrouridine (19), and that $tRNA_{f2}^{Met}$ has fewer sites available for reduction by $NaBH_4$ than $tRNA_{f1}^{Met}$ (20,21,22). The 4-thiouridine content, as measured at 335 nm, is equivalent for both species. Since 4-thiouridine and dihydrouridine both are reduced by $NaBH_4$, the preceding analyses are inconsistent. Therefore direct analysis for these minor bases was performed. The tRNA's were hydrolyzed enzymatically to the nucleoside level and the nucleoside content was determined by ion-exchange chromatography (23). The pertinent data show that the amount of minor nucleosides (except 4-thiouridine) is equivalent to the extent of methionylation (Table 1). This result is in agreement with the published sequence of $tRNA_f^{Met}$ (10,13).

The identity of nucleoside X is unknown, but it is believed to be a modified product of 4-thiouridine for two reasons: (1) the decrease in 4-thiouridine content of $tRNA_{f2}^{Met}$ correlates with the concomitant increase of

Table 1. Minor nucleoside content of purified $tRNA_f^{Met}$ species from Escherichia coli B

Nucleoside	Nucleoside content of tRNA (nmoles/A_{260} unit tRNA)	
	$tRNA_{f1}^{Met}$	$tRNA_{f2}^{Met}$
7-methylguanosine	1.32	1.23
2'-O methylcytosine	1.73	1.47
dihydrouridine	1.22	1.30
4-thiouridine	1.06	0.39
X	(1.00)*	(3.67)*

Ten A_{260} units of each tRNA were enzymatically hydrolyzed to nucleosides with snake venom phosphodiesterase and E. coli alkaline phosphatase. The nucleoside content was determined quantitatively by ion-exchange chromatography (23). Methionylation was 1.49 ($tRNA_{f1}^{Met}$) and 1.30 ($tRNA_{f2}^{Met}$ nmoles/A_{260} unit tRNA, as determined by the procedure of Shugart et al. (14).

*Expressed as relative A_{310} units.

nucleoside X as measured by its absorption at 310 nm; and (2) nucleoside X chromatographs in a position similar to unknown nucleoside(s) from unfractionated (E. coli) tRNA's that contain sulfur (as determined by ^{35}S measurements). Clark (24) reported that 4-thiouridine can form a photoproduct in $tRNA_f^{Met}$. Nucleoside X is definitely not the disulfide of 4-thiouridine, and we are currently attempting to establish its identity.

Figure 3 is the absorption spectra of $tRNA_{f1}^{Met}$ and $tRNA_{f2}^{Met}$ between 300 and 400 nm. The spectra are not superimposable; however, they are essentially identical from 330 to 345 nm--the spectral region usually measured to determine 4-thiouridine content of tRNA (25,26). The spectral data for these species (Fig. 3) probably are not an accurate measure of the 4-thiouridine content, since these data are not in agreement with the data obtained by direct measurement (Table 1).

We conclude that $tRNA_{f2}^{Met}$ arises from $tRNA_{f1}^{Met}$ as a result of a chemical

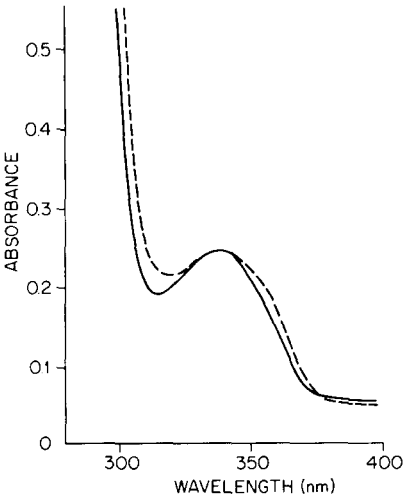

Figure 3. Absorption spectra of purified $tRNA_{f1}^{Met}$ (solid line) and $tRNA_{f2}^{Met}$ (dash line). The tRNA's were suspended in 0.001 M $MgCl_2$ at a concentration of 10 A_{260} units of tRNA per ml, and their absorption spectra were determined at room temperature in a Cary model 14 recording spectrophotometer.

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modification to the 4-thiouridine moiety. Furthermore, we emphasize the need for caution in accepting the resolution of tRNA into chromatographically distinct species as a criterion for distinguishing new isoaccepting tRNA's.

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