Transfer RNA (5-Methylaminomethyl-2-thiouridine)-Methyltransferase from *Escherichia coli* K-12 Has Two Enzymatic Activities*

(Received for publication, December 31, 1986)

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The tRNA (5-methylaminomethyl-2-thiouridine)-methyltransferase, which is involved in the biosynthesis of the modified nucleoside 5-methylaminomethyl-2-thiouridine (mm5s2U) present in the wobble position of some tRNAs, was purified close to homogeneity (95% purity). The molecular mass of the enzyme activity has a pH optimum of 8.0-8.5, is inhibited by magnesium ions, and stimulated by ammonium ions. Two different intermediates in the biosynthesis of mm5s2U34 are present in tRNA from the mutants *trmC1* and *trmC2*. Unexpectedly, the product present in tRNA from *trmC1* cells was identified by mass spectrometric and chromatographic analyses as 5-carboxymethylaminomethyl-2-thiouridine (cmnm5s2U), *i.e.* a more complex derivative than the final product mm5s2U. The product present in tRNA from *trmC2* cells was identified as 5-aminomethyl-2-thiouridine (nm5s2U). In the absence of S-adenosylmethionine the most purified enzyme fraction converts both cmnm5s2U34 and nm5s2U34 into mm5s2U34. In the absence of S-adenosylmethionine, however, cmnm5s2U34 is converted into nm5s2U by this enzyme fraction. We conclude that the primary polypeptide has two enzymatic activities; one actually demodifies cmnm5s2U to nm5s2U and the other catalyzes the transfer of a methyl group from S-adenosylmethionine to nm5s2U, thus forming mm5s2U. The sequential order of the biosynthesis of mm5s2U34 is suggested to be: U34 → s2U34 → cmnm5s2U34 → trmC1 nm5s2U34 → trmC2 mm5s2U34. The molecular activity of the methyltransferase activity (nm5s2U34 → mm5s2U34) is 74 min⁻¹, and the steady state concentration of the enzyme is only 78 molecules/genome equivalent in cells growing at a specific growth rate of 1.0/h.

Transfer RNA from eubacteria contains about 10% modified nucleosides. The biosynthesis of these modified nucleosides constitutes an integral step in the maturation process of tRNA. Most modification reactions occur at the polynucleotide level, *i.e.* after the primary transcript has been synthesized and concomitant with exo- and endonucleases which are operating in the maturation process. The biosynthesis of modified nucleosides is catalyzed by highly specific enzymes. At least one enzyme for each type of modified nucleoside is required, and it has been calculated that at least 40 different modifying enzymes are needed in eubacteria to modify tRNA. A coding capacity of 1% of the total genome is therefore needed to make the necessary tRNA-modifying enzymes. The presence of some of these modified nucleosides has been shown to influence the decoding capacity of the tRNA (reviewed by Björk et al., 1987).

Some of the modified nucleosides are simple derivatives of the ordinary nucleosides like 5-methyluridine (m5U) and 1-methylguanosine (m1G). However, some modified nucleosides, like 5-methylaminomethyl-2-thiouridine (mm5s2U), are complex and it is likely that more than one enzyme is involved in the biosynthesis of such modified nucleosides. In addition modification reactions generally occur in a sequential order. The mm5s2U is present in the wobble position of tRNA-reading codons of the type NA(N) where N can be C, A, or G.

This nucleoside has been shown to be important in the decoding step (Hagervall and Björk, 1984b; Elsevier et al., 1984; Sullivan et al., 1985, reviewed in Björk et al., 1987). As a first step to elucidate the biosynthetic pathway of mm5s2U, two mutants defective in the biosynthesis of this modified nucleoside were isolated (Marinus et al., 1975; Björk and Kjellin-Sträby, 1978). Transfer RNA from both these mutants accepts methyl groups in *vitro* using S-adenosylmethionine as methyl group donor. The product formed in both cases was shown to be mm5s2U. Thus, it was suggested that these mutants were defective in the same gene (trmC) and the mutations were denoted *trmC1* and *trmC2*, respectively. However, recently we have shown that tRNA from these mutants contain two different derivatives of mm5s2U, suggesting lesions in two different genes (Hagervall and Björk, 1984b). We have further shown that a plasmid harboring a 5.2-kb chromosomal segment including the *trmC* gene complemented both the *trmC1* and *trmC2* mutations (Hagervall and Björk, 1984a). The 5.2-kb chromosomal fragment has a coding capacity for at least two polypeptides consistent with the concept that the *trmC1* and *trmC2* mutations affect two different loci. However, we have subconcloned this area of the chromosome

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* This work was supported by Swedish Cancer Society Project 680, National Science Foundation Project B-BU 2890, National Institute of General Medical Sciences Grant GM 21584, and by Swedish Board of Technical Development Grant 3384. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Abbreviations used:** m5U, 5-methyluridine; m1G, 1-methylguanosine; mm5s2U, modified 5-methylaminomethyl-2-thiouridine; cmnm5s2U, 5-carboxymethylaminomethyl-2-thiouridine; nm5s2U, 5-aminomethyl-2-thiouridine; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; AdoMet, S-adenosylmethionine; LC/MS, directly combined liquid chromatography-mass spectrometry; B, base fragment of nucleoside; BH₄, protonated base; MH⁺, protonated molecule; HPLC, high pressure liquid chromatography; kb, kilobase pairs.
and found that a smaller plasmid carrying only a 2.4-kb chromosomal fragment is able to complement both mutations. tRNA(mnm5s2U) methyltransferase has a molecular weight of 80,000 (Taya and Nishimura, 1973; 1977). Therefore, our results suggest that only one polypeptide of \( M_r = 80,000 \) with two enzymatic activities, giving the potential of using tRNA from either a trmC1 or trmC2 mutant as substrate, is coded for by the cloned DNA. However, such genetic results do not exclude the possibility of two closely linked genes encoding two polypeptides with molecular weights of about 80,000 and 20,000, respectively. One way to resolve the question whether trmC1 and trmC2 are allelic or not, would be to purify both potential polypeptides using tRNA from both mutants as substrates. This paper demonstrates that tRNA from trmC1 cells contains 5-carboxymethylaminomethyl-2-thioruridine (cmnm5s2U) and the tRNA from trmC2 cells contains 5-aminomethyl-2-thioruridine (nm5s2U). Unexpectedly, the enzyme is able to demodify cmnm5s2U to nm5s2U and also to act as methyltransferase to catalyze the formation of mm5s2U from nm5s2U using S-adenosylmethionine as methyl donor.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Purification of tRNA(mnm5s2U)Methyltransferase**—As source of enzyme for purification, cells from *E. coli* strain 1100 harboring plasmid pTH32 were used. Construction of this plasmid is shown in Fig. 1 (Miniprint). Due to the presence of plasmid pTH32 both enzymatic activities, as defined by using tRNA from trmC1 and trmC2 mutants as substrates and measuring methyl group incorporation, are overproduced about 17-fold. The cells were disrupted using a French press and by sonication. Ribosomes were removed by high speed centrifugation in the presence of 0.6 M \( (NH_4)_2SO_4 \), and the recovery was about 50–60% for both activities, indicating that the enzyme(s) has a tendency to bind to ribosomes even at high salt concentration. Following precipitation with \( (NH_4)_2SO_4 \) (50% saturation), the enzyme was subjected to molecular sieve chromatography using an Ultrogel AcA34 column (Fig. 2A, Miniprint). The majority of the RNA was removed in this step, but the two enzymatic activities were not separated from each other. Both activities eluted as two chromatographically different compounds; one did not penetrate the gel and the other was retarded (Fig. 2A). The fractions containing the compound not penetrating the gel were pooled and rechromatographed on an identical column. The elution profile was the same as earlier (data not shown). Therefore, the enzyme probably forms an unstable complex, which separates from the unaggregated form using such a column. The compound which was retarded on the column was subjected to ion-exchange chromatography on a DEAE-Sephadex column. The two enzymatic activities co-chromatographed as one component at about 0.22 M NaCl with a recovery of 85% for the trmC1 activity and 80% for the trmC2 activity. The purification in both cases was about 4 times (Fig. 2B, Table I). The enzyme(s) was further purified using chromatography on heparin-Sepharose CL-6B (Fig. 2C). The two enzymatic activities eluted very late in the gradient (0.4 M NaCl) but again as a single component. This step was very efficient and a 105- and 50-fold purification of the trmC2 and trmC1 activities, respectively, was obtained (Table I).

**Enzyme Purity and Molecular Weight**—After heparin-Sepharose CL-6B chromatography, both enzyme activities migrated as one single major peak (\( M_r = 79,000 \)) in sodium dodecyl sulfate-polyacrylamide electrophoresis (Fig. 3 lane g), which coincides with one of the polypeptides expressed from pTH32 in minicells (data not shown). Some additional polypeptides were present but, judging from the intensities of these bands, the enzyme is at least 95% pure. Furthermore, all impurities appeared to have an \( M_r \) above 40,000 (see “Discussion”). In conclusion, these results suggest that the two enzymatic activities are expressed by one polypeptide of \( M_r = 79,000 \).

**Optimal Assay Conditions**—The purified tRNA(mnm5s2U)-methyltransferase had the highest specific activities using either substrates in the pH range of 8–8.5. Both enzymatic activities were stimulated by NH4Cl, with an optimum for both around 20 mM, and they were inhibited when the concentration was increased to 80 mM (Fig. 4). The enzymatic activities were strongly inhibited by Mg2+, where a concentration of 5 mM MgCl2 reduced the trmC1 activity to 10% and the trmC2 activity to 25%, respectively. The trmC1 activity was inhibited by addition of 100 mM NaCl while a concentration of 200 mM was needed to significantly reduce the trmC2 activity. The enzymatic activities were also inhibited by p-chloromercuribenzoate (2 mM) (data not shown). Presence of putrescine (6 mM) or spermidine (2 mM) had no significant effect on the activities.

**Structures of Precursors S1 and S2 Present in tRNA from trmC2 and trmC1 Cells, Respectively**—To understand on a molecular level what the nature of the two activities possessed by the trmC1 polypeptide is, it was necessary to identify the precursors (compounds S1 and S2) to mm5s2U present in tRNA from trmC2 and trmC1 cells, respectively. Since the thiolated nucleoside S1 is present in general methyl deficient tRNA and its conversion to mm5s2U requires AdoMet, this compound is suggested to be nm5s2U (see below). Buck et al. (1982) have shown that tRNA from *Salmonella typhimurium* contains cmnm5s2U. It migrates in their HPLC system as our compound S2. Furthermore, cmnm5s2U migrates in thinline chromatography using the solvent system described by Hogg et al. (1975) in a similar position as S2 (cf. Yamada et al., 1981 and Fig. 5). These results suggest that compounds S1 and S2 might be nm5s2U and cmnm5s2U, respectively. To firmly establish the identities of intermediates S1 and S2, enzymatic hydrolysates of trmC2 and trmC1 tRNA were examined by directly combined HPLC-mass spectroscopy. In addition, tRNA from *E. coli* strain TH10 was analyzed as a control for presence of the fully modified product mm5s2U. All three tRNAs were separately analyzed using decylacylano and octadecyl HPLC columns (Systems A and B) which possess differing selectivity for the three nucleosides of interest. The m/z values and structure assignments for ions monitored are shown in Fig. 6. Authentic cmnm5s2U and mm5s2U were used to establish the suitability of ions chosen for monitoring, as established from their thermospray mass spectra recorded using HPLC System B. The authentic nucleosides were also used as chromatographic markers in separate LC/MS experiments (Systems A and B) to establish their elution times under conditions identical to those used for the tRNA analyses. The ions used for detection of mm5s2U (Fig. 6), for which no authentic material is available, represent the

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2 Portions of this paper (including "Experimental Procedures," Table I, and Figs. 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86 M-4522, cite the authors, and include a check or money order for $2.80 per set of photocopies. Full size photocopies are included in the microfilm edition of the Journal which is available from Research Document.  

8489  

T. G. Hagervall and G. R. Björk, unpublished observations.
tRNA(mmm^5s^2U)Methyltransferase from E. coli

### TABLE I

| Enzyme fraction                        | Volume | Total protein | Specific activity | Recovery | Purification |
|----------------------------------------|--------|---------------|-------------------|----------|--------------|
|                                        | ml     | mg            | units/mg          | %        |              |
| I. Crude extract, pTH32/1100           | 125    | 5,387         | 0.87 2.26         | 100      | 1.0 1.0      |
| II. Crude extract—ribosomes            | 86     | 2,955         | 1.01 2.62         | 64       | 1.2 1.2      |
| III. AmSO_4-fraction 0–50%             | 36     | 988           | 1.21 3.44         | 25       | 1.4 1.5      |
| IV. Ultrogel Aca34                     | 100    | 727           | 0.85 2.40         | 13       | 1.0 1.1      |
| V. DEAE-Sephalocel                    | 57     | 154           | 3.19 9.08         | 11       | 3.7 4.0      |
| VI. Heparin-Sepharose CL-6B            | 2.4    | 0.71          | 168.6 925.2       | 2.6 5.4  | 194 409      |

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples from different stages in the purification of the tRNA(mmm^5s^2U)methyltransferase. Lanes: a, molecular weight standards; b, fraction I, crude extract, 86 µg of protein; c, fraction II, crude extract—ribosomes, 37 µg of protein; d, fraction III, (NH_4)_2SO_4 fraction 0–50%, 53 µg of protein; e, fraction IV, Ultrogel Aca34 pool, 108 g of protein; f, fraction V, DEAE-Sephalocel pool, 41 µg of protein; g, fraction VI, heparin-Sepharose CL-6B pool, 0.51 µg of protein; h, heparin-Sepharose CL-6B pool, 5.1 µg of protein; i, molecular weight standards. Standard proteins are 94.0, 67.0, 43.0, 30.0, 20.1, and 14.4 kDa, respectively.

FIG. 4. Methyl group incorporation as a function MgCl_2 and NH_4Cl concentrations. Open symbols represent MgCl_2 concentrations, and filled symbols represent NH_4Cl concentrations.

FIG. 5. Schematic illustration of the distribution of nucleosides found in tRNA from trmC^*, trmC1, and trmC2 cells. The illustration indicates the positions of some nucleosides including the different thiolated nucleosides S1, S2, and mmm^5s^2U after two-dimensional chromatography and their relative distribution is shown in Table II. In this paper we show that compound S2 is cmnm^5s^2U and compound S1 is nm^5s^2U (see Fig. 7).
hydrolysates of tRNA.

...permitted in the other hydrolysates. Under conditions prevalent for chromatographic System A, the molecular species (MH⁺) for nm5s²U and mnm5s²U are observed but not in the case of System B. In both cases for which authentic nucleosides are available, the elution positions are appropriately shifted when tested in the second chromatographic system in which the relative elution positions have been shifted.

Analysis and Specificity of the Enzyme Activities—Transfer RNA from trmC2 and trmC1 mutant strains which contain nm5s²U (compound S1) and mnm5s²U (compound S2), respectively (Fig. 5 and Table II), were used to analyze the two enzymatic activities. In addition, tRNA from methionine-starved E. coli W6 (relA, metB1) cells, which is generally methyl-deficient, was used as substrate. This tRNA contains most probably nm5s²U instead of mnm5s²U in position 34 (Table II). These tRNAs were methylated using ³²C-labeled AdoMet as methyl group donor and were digested to nucleosides which were separated by two dimensional thin-layer chromatography. The chromatograms were autoradiographed to visualize radioactive nucleosides. The results of these analyses were identical for the various tRNA preparations since the majority of the radioactivity (95%) was found in a compound comigrating with the nucleoside marker mnm5s²U. One additional minor radioactive compound was also found, indicating that the pure enzyme fraction contains another methyltransferase activity. Alternatively, this compound is an intermediate or secondary product of the normal biosynthesis of mnm5s²U (data not shown). Since the pure enzyme is able to convert cmnm5s²U and nm5s²U into mnm5s²U the 79,000

\begin{table}[h]
\begin{center}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{tRNA from strain} & \textbf{Addition} & \textbf{Relative distribution of nucleosides} \\
& & & & \\
\hline
TH48 (trmC²) & – & – & – & 79 \% \\
TH49 (trmC²) & – & – & – & 0 \% \\
TH49 (trmC2) & + & + & – & 75 \% \\
TH49 (trmC1) & + & + & – & 76 \% \\
TH49 (trmC2) & + & + & + & 73 \% \\
TH49 (trmC2) & + & + & + & 70 \% \\
TH69 (trmC1) & + & + & – & 58 \% \\
TH69 (trmC2) & + & + & – & 61 \% \\
TH69 (trmC2) & + & + & + & 65 \% \\
TH69 (trmC2) & + & + & + & 53 \% \\
TH69 (trmC2) & + & + & + & 56 \% \\
TH69 (trmC2) & + & + & + & 77 \% \\
TH69 (trmC2) & + & + & + & 75 \% \\
TH69 (trmC2) & + & + & + & 70 \% \\
TH69 (trmC2) & + & + & + & 78 \% \\
\hline
\end{tabular}
\end{center}
\end{table}

In \textit{vivo} ³²S⁸O₄-labeled tRNA from trmC², trmC1, and trmC2 cells were used as substrates. Following incubation under indicated conditions, the tRNA was digested to nucleosides and the distribution of ³²S-labeled compounds was determined as shown in Fig. 5. Compound S1 in Fig. 5 is nm5s²U and compound S2 is cmnm5s²U. Transfer RNA from strain W6 in \textit{vivo} labeled under methionine starvation in the presence of ³²SO₄. Thus, this ³²S-labeled tRNA is generally methyl-deficient.

...tRNA(mnm5s²U)Methyltransferase from E. coli 8491

...in trmC1 and wild-type tRNAs. These data further support the identity of compound S1 as nm5s²U. The fully modified nucleoside mnm5s²U is clearly observed in the wild-type strain TH10 (Fig. 7, E and F) and is essentially absent in the other hydrolysates. Under conditions prevalent for chromatographic System A, the molecular species (MH⁺) for nm5s²U and mnm5s²U are observed but not in the case of System B. In both cases for which authentic nucleosides are available, the elution positions are appropriately shifted when tested in the second chromatographic system in which the relative elution positions have been shifted.
polypeptide is likely to possess two different enzymatic activities (Fig. 5, Table II).

Reaction Order—Since cmnm5s2U and nm5s2U are both thiolated precursors to mnm5s2U, it is possible to use 35S-labeled tRNA as a substrate and monitor the conversion of nm5s2U and cmnm5s2U to mnm5s2U. Thus, the different enzymatic activities might be assayed for, even in the absence of the methyl group donor AdoMet. Strains TH49 (trmC2) and TH69 (trmC1) were labeled with 35SO4, and tRNA was prepared. The tRNA was incubated together with purified enzyme, with or without AdoMet and in the absence or presence of NH4+. The conditions employed allowed the modification reaction to go to completion. The tRNA was next digested to nucleosides and analyzed by thin-layer chromatography. In all cases where AdoMet was added both cmnm5s2U and nm5s2U were converted into mnm5s2U and presence of NH4+ had no effect (Table II). However, in the absence of AdoMet, nm5s2U was not converted into mnm5s2U, while cmnm5s2U was converted into mnm5s2U. Therefore, the reaction cmnm5s2U → mnm5s2U must involve the addition of a methyl group which is consistent with the observation that methyl-deficient tRNA contains nm5s2U (Table II). The conversion of cmnm5s2U to nm5s2U does not require AdoMet. However, the reaction is enzymatic since it requires the presence of the purified enzyme (Table II). Thus, the sequential order of the enzymatic reaction is: cmnm5s2U → nm5s2U → mnm5s2U where the first step is AdoMet independent, while the second requires the presence of AdoMet. Other intermediates cannot be excluded.

cmnm5s2U May Be Present in tRNA from Wild-type Cells—A low level of what appears to be cmnm5s2U is also found in wild-type cells (Hagervall and Björk, 1984b; Table II). To determine if the radioactive compound S2 found in wild-type tRNA in fact is identical to cmnm5s2U found in tRNA from a trmC1 mutant, 35S-labeled tRNA from wild-type and trmC1 mutant cells was digested to nucleosides and separated on HPLC according to Buck et al. (1983). Fractions were collected and those containing compound S2 were further analyzed in five different chromatographic systems. No difference in mobility was observed between compounds S2 from wild-type and cmnm5s2U from trmC1 cells consistent with the observation that methyl-deficient tRNA contains nm5s2U (Table II). The conversion of cmnm5s2U to nm5s2U does not require AdoMet. However, the reaction is enzymatic since it requires the presence of the purified enzyme (Table II). Thus, the sequential order of the enzymatic reaction is: cmnm5s2U → nm5s2U → mnm5s2U where the first step is AdoMet independent, while the second requires the presence of AdoMet. Other intermediates cannot be excluded.

**TABLE III**

| Compound                  | Rf-values in different chromatographic systems |
|---------------------------|-----------------------------------------------|
|                           | A  | B  | C  | D  | E  |
| mnm5s2U (wt)*              | 0.85 | 0.98 | 0.47 | 0.68 | 0.29 |
| S2 (wt)*                   | 0.39 | 0.86 | 0.35 | 0.55 | 0.93 |
| cmnm5s2U (trmC1)*          | 0.39 | 0.86 | 0.35 | 0.55 | 0.93 |

* From strain TH48 (trmC1).

**TABLE IV**

| Strain | mmnm5s2U/mnm5s2A | sU/mnm5s2A | S2/mm5s2A |
|--------|------------------|-------------|------------|
| DEV1 (trmC2) | 0.59            | 0.01        | 0.13       |
| DEV16 (trmC2) | 0.01            | 0.47        | 0.03       |

**DISCUSSION**

This paper describes a 400-fold purification and the characterization of the tRNA (mmnm5s2U)methyltransferase from *E. coli* If the enrichment of the enzyme, due to the presence of a trmC2 plasmid is included, the enzyme has been purified 7,100-fold. The enzyme has an apparent *M₀* of 79,000 on sodium dodecyl sulfate-gel chromatography and was judged to be at least 95% pure (Fig. 3, lanes g and f). The enzymatic activities have a pH-optimum of 8.0–8.5 and are stimulated by NH4+ but severely inhibited by Mg2+ (Fig. 4). This enzyme has been purified before using methyl-deficient tRNA 35S as substrate (Taya and Nishimura, 1973; 1977). They reported that the enzyme had an *M₀* of 80,000 and a methyltransferase activity since it transfers methyl groups from AdoMet to tRNA 35S from general methyl-deficient cells. By using tRNA from the trmC1 and trmC2 mutant as substrates, we have demonstrated that the 79,000 polypeptide has two different enzymatic activities (Table II). We also demonstrate that transfer RNA from these mutants contains cmnm5s2U and nm5s2U, respectively (Fig. 7). Both mutations are complemented by plasmid pTH32, which carries a chromosomal insert of 2.4 kb (data not shown). A polypeptide of 79,000 requires a coding capacity of 2 kb of DNA. Thus, about 0.4 kb of additional coding capacity, corresponding to a 17,000 polypeptide is present on plasmid pTH32. It was therefore not possible from the genetic data to rule out that the two mutations affected two separate but closely located genes, one coding for the 79,000 polypeptide and the other for a 17,000 polypeptide. Here we show that the most purified enzyme fraction is able to convert both cmnm5s2U34 and nm5s2U34 to mnm5s2U in the presence of AdoMet. Furthermore, the few impurities observed have an *M₀* well above 17,000. Thus, it is likely that the 79,000 polypeptide harbors two enzymatic activities. The tRNA (mmnm5s2U)methyltransferase is unusually large, since most tRNA-modifying enzymes have a molecular weight of 60,000 or less (Björk, 1984). This large size may be explained by the presence of two enzymatic activities in the same polypeptide. However, our results do not rule out the existence of overlapping genes contributing to two large polypeptides but which are synthesized in vastly different amounts. We find this unlikely since the 79,000 polypeptide is in itself produced in very small amounts in the cell (see below). At present we favor the hypothesis that the 79,000 polypeptide harbors two separate enzymatic activities, but the final answer has to await the complete DNA sequence of this region of the chromosome.

The methylation reaction was inhibited by magnesium ions irrespective of which substrate was used. Most tRNA-modifying enzymes are stimulated or do not respond to this ion. The conversion of nm5s2U34 to mmnm5s2U34 is unique in its sensitivity toward Mg2+ ions. The conversion of cmnm5s2U34 to nm5s2U34 does not require AdoMet. Therefore, the similarities in the response to different conditions by trmC1 and trmC2 tRNA measured as methyl group incorporation might
only reflect the response in the methylation reaction, i.e. the conversion of \( \text{nm}^{m} \text{s}^{2} \text{U} \) to \( \text{mnm}^{m} \text{s}^{2} \text{U} \). Taya and Nishimura (1973; 1977) also noted the unusual inhibition by \( \text{Mg}^{2+} \) in the methylaing reaction. The specific activity of tRNA-(\( \text{mnm}^{m} \text{s}^{2} \text{U} \))methyltransferase is 0.13 millinits/mg (tmrC2) in E. coli K-12 strain 1100, grown in glucose minimal medium. Assuming that no factors present in crude extract influence the specificity of the enzyme, the fraction of total protein which is tRNA-(\( \text{mnm}^{m} \text{s}^{2} \text{U} \))methyltransferase is \( 1.4 \times 10^{-11} \). This corresponds to about 78 molecules/genome equivalent and is about 78 molecules/genome for \( \text{trmC}^{+} \) cells, but its level in \( \text{trmC}^{+} \) cells, but its level in \( \text{trmC}^{+} \) cells is significantly reduced (Table IV). A minor compound suggested to be \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) was also found upon nucleotide analysis of purified tRNA from S. typhimurium which is consistent with this idea (Sullivan et al., 1985). tRNA from S. typhimurium contains \( \text{cmnm}^{m} \text{s}^{2} \text{U} \), and although this compound was not observed in tRNA from a \( \text{trmC}^{+} \) E. coli strain, its presence in small amounts was not excluded (Buck et al., 1982). However, \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) in strain TH10 (\( \text{trmC}^{+} \)) is absent or below 10% of the level of \( \text{mm}^{m} \text{s}^{2} \text{U} \) (Fig. 7). Therefore, the level of \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) in the tRNA may be influenced by the genetic background, the composition of the growth medium or the growth phase of the cells. If so, \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) is an intermediate in the biosynthesis of \( \text{mm}^{m} \text{s}^{2} \text{U} \) in all tRNA molecules which contains this nucleoside in the wobble position.

Alternatively, one or more tRNA species could normally contain \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) and will not be a substrate for the \( \text{trmC}^{+} \) activity of the \( \text{trmC}^{+} \) polypeptide. One such candidate would be tRNA that is not present in the tRNA mixture and contains the wobble nucleoside mnm5s2U.

The side chain at the position of \( \text{mm}^{m} \text{s}^{2} \text{U} \) contains two carbon atoms. Preliminary results from \( \text{methyl}-^{14} \text{C}-\text{L-methionine labeling in vitro} \) experiments suggest that only one of the two carbon atoms originates from \( \text{AdoMet} \). Taya and Nishimura (1977) also noted that formyltetrahydrofolate might be a donor for the first carbon atom in the side chain. Generally methyl-deficient transfer RNA from methionine-starved cell contains the undermodified derivative \( \text{nm}^{m} \text{s}^{2} \text{U} \) in addition to \( \text{cmnm}^{m} \text{s}^{2} \text{U} \), which suggests that the \( \text{mm}^{m} \text{s}^{2} \text{U} \) conversion requires \( \text{AdoMet} \) as methyl group donor.

Table II shows that strain TH48 (\( \text{trmC}^{+} \)) contains a significant amount of \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) in its tRNA from cells grown in rich medium containing \( \text{SO}_{4} \). The presence of \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) was shown by thin-layer chromatography using several chromatographic systems (Table III). Furthermore, \( \text{cmnm}^{m} \text{s}^{2} \text{U} \) is also present in strain DEV1 (\( \text{trmE}^{+} \)) cells, but its level in tRNA from strain DEV16 (\( \text{trmE}^{+} \)) cells is significantly reduced.
Acknowledgments—We are greatly indebted to the skillful technical assistance by Kristina Palm. We also acknowledge the generous gift of the trmE mutant by Dr. D. Elseviers, Valhalla, NY, and gifts of authentic nucleoside samples by Dr. K. Murao, Jichi Medical School, and Dr. S. Nishimura, National Cancer Center Research Institute, Tokyo. We are grateful to Dr. D. Gisch, Supelco, Inc. for helpful discussions and gift of prototype cyanodecyl HPLC columns.

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Preparation of tRNA

Transfer RNA was prepared essentially according to metal and explan. except that the guanosine diphosphate was used.

Detection of tRNA by TLC analysis

Quantities of total RNA were determined by spectrophotometry. RNA samples were solubilized in 0.1 N NaOH (1 mg/ml) and precipitated with 2 vol. of ethanol. The precipitates were then dissolved in 10 mM sodium acetate (pH 5.5) and 1 M sodium chloride, and the precipitated RNA was isolated by the usual procedure.

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tRNA(mnm5s2U) Methyltransferase from E. coli

8495

Protein concentration determination:
Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Special conditions and labeling to date:
E. coli 100 harboring plasmid pODZ was grown in 50 liters medium containing 1% nutrient broth (Oxoid), 0.5% tryptone (Difco), 0.2% glucose and sodium 8 (Fygar) and thymin (Beynon), 15% sucrose. The cells were harvested at an optical density of 0.5 at 600 nm and resuspended in 50 mM Tris- HCl buffer (pH 7.4) with 10 mM MgCl2. The cells were added to a 10 ml mixture containing 0.5 M KOAc and 50 mg/ml of tRNA and incubated at 37°C for 24 hours. The reaction was stopped by the addition of 10 ml of 50 mM Tris- HCl buffer (pH 7.4) and 10 mM EDTA. The mixture was centrifuged at 13,000 rpm for 20 minutes. The supernatant was assayed for tRNA(mnm5s2U) methyltransferase activity.

Figure 2a. Chromatographic profile of tRNA(mnm5s2U).

Figure 2b. Chromatographic profile of E. coli.

Figure 2c. Chromatographic profile of tRNA(mnm5s2U).

Figure 2d. Chromatographic profile of E. coli.

Figure 2e. Chromatographic profile of tRNA(mnm5s2U).

Figure 2f. Chromatographic profile of E. coli.

Figure 2g. Chromatographic profile of tRNA(mnm5s2U).

Figure 2h. Chromatographic profile of E. coli.

Figure 2i. Chromatographic profile of tRNA(mnm5s2U).

Figure 2j. Chromatographic profile of E. coli.

Figure 2k. Chromatographic profile of tRNA(mnm5s2U).

Figure 2l. Chromatographic profile of E. coli.

Figure 2m. Chromatographic profile of tRNA(mnm5s2U).

Figure 2n. Chromatographic profile of E. coli.

Figure 2o. Chromatographic profile of tRNA(mnm5s2U).

Figure 2p. Chromatographic profile of E. coli.

Figure 2q. Chromatographic profile of tRNA(mnm5s2U).

Figure 2r. Chromatographic profile of E. coli.

Figure 2s. Chromatographic profile of tRNA(mnm5s2U).

Figure 2t. Chromatographic profile of E. coli.

Figure 2u. Chromatographic profile of tRNA(mnm5s2U).

Figure 2v. Chromatographic profile of E. coli.

Figure 2w. Chromatographic profile of tRNA(mnm5s2U).

Figure 2x. Chromatographic profile of E. coli.

Figure 2y. Chromatographic profile of tRNA(mnm5s2U).

Figure 2z. Chromatographic profile of E. coli.

Figure 3a. Chromatographic profile of E. coli.

Figure 3b. Chromatographic profile of E. coli.

Figure 3c. Chromatographic profile of E. coli.

Figure 3d. Chromatographic profile of E. coli.

Figure 3e. Chromatographic profile of E. coli.

Figure 3f. Chromatographic profile of E. coli.

Figure 3g. Chromatographic profile of E. coli.

Figure 3h. Chromatographic profile of E. coli.

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