RECOGNITION OF ALTERED E. COLI FORMYLMETHIONINE TRANSFER RNA BY BACTERIAL T FACTOR*

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SUMMARY: Treatment of E. coli formylmethionine tRNA with sodium bisulfite produces six C -> U base changes in the tRNA structure. Four of these modifications have no effect on the ability of $tRNA_f^{Met}$ to be aminoacylated or formylated. Prior to bisulfite treatment, $Met\text{-}tRNA_f^{Met}$ is not able to form a ternary complex with bacterial T factor and GTP, as measured by Sephadex G-50 gel filtration. After bisulfite treatment, a large portion of the modified tRNA is bound as $T\text{-}GTP\text{-}Met\text{-}tRNA_f^{Met}$. Formylation of bisulfite-modified $Met\text{-}tRNA_f^{Met}$ completely eliminates T factor binding. Unmodified $tRNA_f^{Met}$ is unique among the tRNAs sequenced to date in having a non-hydrogen-bonded base at the 5' terminus. Bisulfite-catalyzed conversion of this unpaired C_1 to U_1 results in formation of a normal $U_1\text{-}A_{73}$ base pair at the end of the acceptor stem. It is likely that this structural alteration is responsible for the recognition of bisulfite-modified $Met\text{-}tRNA_f^{Met}$ by T factor.

In recent years, work from several laboratories has clarified the mechanism of action of elongation factors T and G in bacterial protein synthesis (1). T factor, which consists of two components, Tu and Ts, serves to bind aminoacyl-tRNAs to the acceptor site on the ribosome during polypeptide chain elongation. T factor-dependent binding has been shown to occur through formation of an intermediate Tu-GTP-AA-tRNA[1] complex (2-7). This ternary complex is not formed with deacylated or N-acyl aminoacyl-tRNAs (2-5), thus preventing unwanted ribosome binding of these species during translation. In addition, no detectable ternary complex is formed with the E. coli initiator tRNA (8). Since all other AA-tRNAs, including $Met\text{-}tRNA_m^{Met}$, are recognized

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[1]Abbreviations used are: AA-tRNA, aminoacyl-tRNA; $tRNA_f^{Met}$, the methionine accepting tRNA from E. coli which can be enzymatically formylated; $Met\text{-}tRNA_f^{Met}$, methionyl-$tRNA_f^{Met}$; $fMet\text{-}tRNA_f^{Met}$, N-formylmethionyl-$tRNA_f^{Met}$; $tRNA_m^{Met}$, the methionine accepting tRNA from E. coli which cannot be enzymatically formylated; $tRNA^{Phe}$, phenylalanine accepting tRNA.

by T factor, the discrimination against Met-$tRNA_f^{Met}$ must be due to the presence of some unique structural feature in the tRNA itself. We now report that introduction of specific C→ U base changes in the primary sequence of E. coli $tRNA_f^{Met}$ by treatment with sodium bisulfite produces a modified tRNA species which forms a ternary complex with T factor and GTP.

MATERIALS AND METHODS: Purified E. coli $tRNA_f^{Met}$ was isolated from crude E. coli K12 tRNA as described previously (9) and accepted 1.8 nmoles methionine and formate per A_{260} of tRNA. The sample of $tRNA_f^{Met}$ used in these studies was the isomer having an A residue rather than a 7Me G residue at position 47 from the 5' terminus (10). The $tRNA_m^{Met}$ used here was a partially purified fraction obtained free of $tRNA_f^{Met}$ by chromatography on benzoylated DEAE cellulose (11) and accepted 0.3 nmoles methionine per A_{260}. Phe-$tRNA^{Phe}$ was prepared from a partially purified fraction of tRNA accepting 0.14 nmoles phenylalanine per A_{260}. Crude E. coli synthetase was prepared from E. coli strain Q13 as described previously (9).

Uniformly labeled [^{14}C]methionine (71 cts/min/pmole) was obtained from Amersham/Searle and [^{14}C]phenylalanine (63 cts/min/pmole) from Schwarz Mann. Folinic acid was obtained from General Biochemicals, converted to 5,10-methenyltetrahydrofolate as described by Dubnoff and Maitra (12) and stored in 50mM mercaptoethanol, 0.1N HCl at -20°. This compound was converted to the formyl donor, 10-formyltetrahydrofolate, by neutralization of the solution just before use. Met-$tRNA_f^{Met}$ and Met-$tRNA_m^{Met}$ were prepared as described previously (9). fMet-$tRNA_f^{Met}$ was prepared in a reaction mixture containing 100 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM KCl, 10 mM NH_4Cl, 2 mM reduced glutathione, 2 mM ATP, 0.15 mM [^{14}C]methionine, 1 mM formyl donor, 36 μM $tRNA_f^{Met}$ and 2.9 mg/ml crude E. coli synthetase. After incubation at 37° for 25 min, protein was removed by passage of the solution through a small DEAE cellulose column (9). The reaction mixture for preparation of Phe-$tRNA^{Phe}$ was the same as that described above except that $tRNA_f^{Met}$, methionine, and formyl

donor were omitted and 0.16 mM [^{14}C] phenylalanine and 58 μM $tRNA^{Phe}$ were added.

Bisulfite modification of $tRNA_f^{Met}$ was carried out as described previously (13, 14).

γ-^{32}P-GTP and purified T factor (Tu, Ts) were generous gifts of Dr. Umadas Maitra and were prepared as described elsewhere (15). The T factor used in these experiments had been stored for 4 months in 20 mM Tris-HCl pH 7.8, 1 mM dithiothreitol, 30% glycerol at -20°. One unit of T factor is defined as the amount of factor which binds 1 pmole of GDP in 10 min at 0° as measured by retention of [^{3}H] GDP on Millipore filters (15).

Formation of T-GTP-AA-tRNA complexes was assayed by the procedure of Gordon (3). The reaction buffer consisted of 50 mM Tris-HCl pH 7.5, 150 mM NH_4Cl, 10 mM $MgCl_2$ and 1 mM dithiothreitol. The reaction mixture for complex formation contained reaction buffer, 400 pmoles of γ-^{32}P-GTP (specific activity 1500 cpm per pmole), 14 units of T factor and 2 to 48 pmoles of AA-tRNA in a total volume of 0.125 ml. After incubation at 25° for 10 min, the mixture was cooled to 0° and a 0.1 ml aliquot was applied to a Sephadex G-50 column (0.5 x 20 cm) which had been equilibrated with reaction buffer at 0°. The sample was eluted with the same buffer. Fractions (0.15 ml) were collected and counted in Bray's scintillation fluid. Formation of the ternary complex was assayed as the amount of γ-^{32}P-GTP in the excluded volume of the column. This fraction was completely separated from free GTP and P_i under the conditions used.

RESULTS AND DISCUSSION: As part of our studies on structure-function relationships in E. coli formylmethionine tRNA (9, 14, 16, 17) we have recently investigated the effects of sodium bisulfite-catalyzed deamination of cytosine residues in this tRNA on its ability to be enzymatically aminoacylated and formylated. Treatment of $tRNA_f^{Met}$ with 3M $NaHSO_3$ at 25° results in a first-order loss of these biological activities as a function of time and produces

C ——> U base changes at six different sites in the tRNA structure (13, 14). The modifications at C_{35} in the anticodon and C_{76} in the CCA-OH sequence have been shown to be responsible for the loss of biological activity while the four remaining modifications at C_1, C_{16}, C_{17}, and C_{75} have no effect on the ability of $tRNA_f^{Met}$ to be acylated or formylated (14). The positions of these modifications are indicated on the clover leaf structure of $tRNA_f^{Met}$ shown in Fig. 1.

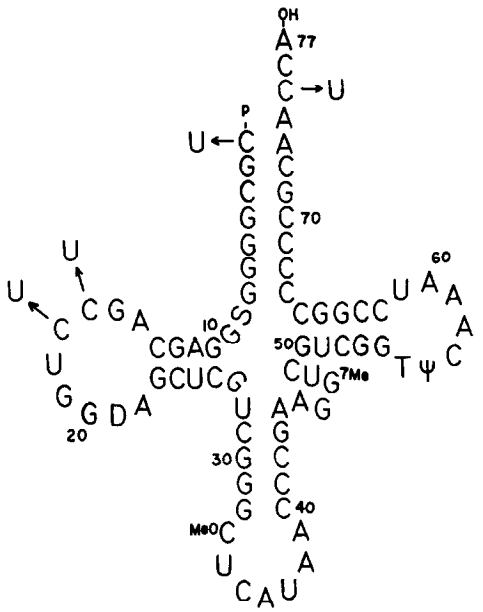

FIGURE 1: Sites of C ——> U conversions in molecules of bisulfite-modified E. coli $tRNA_f^{Met}$ active with respect to methionine and formate acceptance. OMeC, 2'-O-methylcytidine; D, dihydrouridine; S, 4-thiouridine; Ψ, pseudouridine.

We have now examined the ability of bisulfite-modified $tRNA_f^{Met}$ to be recognized by bacterial T factor. The bisulfite reaction was carried out to the extent of 60% loss of methionine acceptor activity. The remaining active molecules were enzymatically aminoacylated and tested for their ability to form a T-GTP-AA-tRNA complex. Figure 2 compares the amount of ternary complex

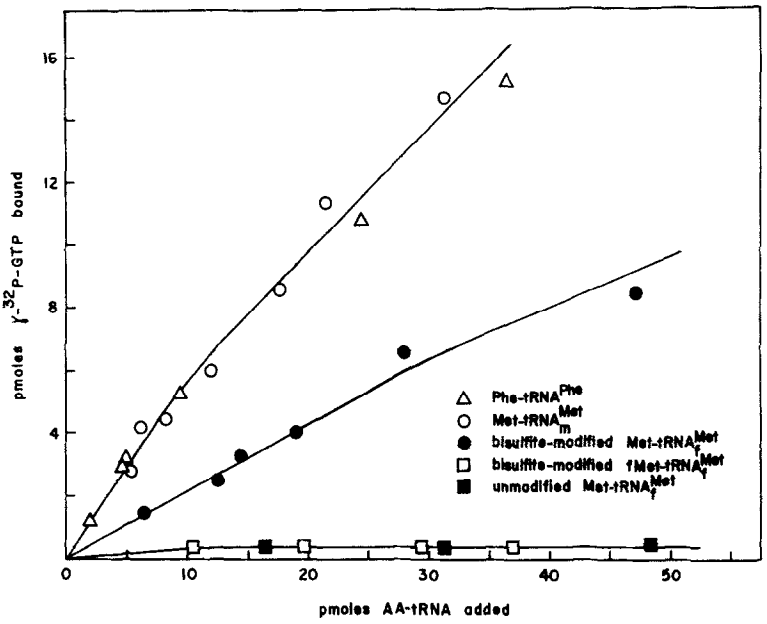

FIGURE 2: Formation of the ternary complex with E. coli T factor, γ-^{32}P-GTP and aminoacyl-tRNAs as measured by Sephadex G-50 gel filtration.

formed, as measured by γ-^{32}P-GTP excluded from a Sephadex G-50 column, for a number of aminoacyl-tRNA species. Phe-tRNA^{Phe} and Met-tRNA$_m^{Met}$ were found to behave identically in the binding reaction. Prior to bisulfite treatment, no ternary complex could be detected with Met-tRNA$_f^{Met}$, however after bisulfite treatment, a substantial fraction of the modified tRNA was bound as T-γ-^{32}P-GTP-Met-tRNA$_f^{Met}$. Formylation of the modified tRNA completely eliminated formation of the ternary complex, in keeping with the known ability of T factor to discriminate against N-acyl aminoacyl-tRNAs.

These results indicate that one or more of the C ⟶ U changes in the structure of bisulfite-modified tRNA$_f^{Met}$ alters the molecule in such a way that it is no longer prevented from binding to T factor. While we have not yet directly determined the modification responsible for this change in biological activity, we consider the C ⟶ U base change at the 5' terminus to be the one most likely to account for our results. E. coli tRNA$_f^{Met}$ is

unique among the tRNAs sequenced to date in having a non-hydrogen-bonded base at the 5' terminus (10). Bisulfite - catalyzed conversion of $C_1 \longrightarrow U_1$ produces a normal U_1 - A_{73} base pair at the end of the acceptor stem of $tRNA_f^{Met}$ and increases the structural resemblance of the initiator tRNA to other tRNAs.

The extent of modification of C_1 in the tRNA used in these experiments is 50-70%. C_{75} is modified to the extent of approximately 40% and C_{16} and C_{17} to less than 30% each. If the $C_1 \longrightarrow U_1$ conversion alone is responsible for the change in T factor recognition of Met-$tRNA_f^{Met}$, this could explain why only a fraction of the modified molecules are able to form the ternary complex.

The initiator tRNA from yeast has been shown have a fully base-paired acceptor stem (18,19). Unlike E. coli $tRNA_f^{Met}$, this tRNA is able to form a ternary complex with GTP and T factor from both yeast and E. coli (20). Richter et al. (21) have shown, however, that while the complex formed with yeast initiator tRNA can readily be detected by Sephadex G-50 gel filtration, it is more unstable than complexes formed with other AA-tRNAs and dissociates during slower gel filtration on G-100. Furthermore, the yeast Met-$tRNA_f^{Met}$-GTP-T complex does not function as an intermediate in the binding of the tRNA to the acceptor site on the ribosomes.

The structural requirements for recognition of AA-tRNAs by T factor have been investigated in a number of laboratories. Ofengand and coworkers have shown that cleavage of the 2',3' -carbon-carbon bond of the 3' terminal adenosine residue of yeast Phe-$tRNA^{Phe}$ prevents formation of the ternary complex (22). The denatured form of yeast Leu-$tRNA_{III}^{Leu}$ has also been shown to have a markedly reduced affinity for wheat germ T factor (23), and a requirement for the 5' half of the tRNA molecule has been demonstrated in the formation of a ternary complex consisting of E. coli Val-$tRNA^{Val}$, E. coli T factor and GTP (24). Modifications in the anticodon loop (24-27), anticodon stem (28), dihydrouridine loop (28) or dihydrouridine stem region (24,29), on

the other hand, have no effect on T factor recognition. Recently, it has also been shown that yeast Phe-tRNA^{Phe} containing CCCA-OH in place of the normal CCA-OH terminus is active in ternary complex formation with E. coli T factor and GTP (30), as is phenyllactyl-tRNA^{Phe} prepared by deamination of Phe-tRNA^{Phe} (31). The present report represents the first example of a structural change which results in an increase in the ability of a tRNA to be recognized by T factor.

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