Lanthanide Fluorescence Studies of Transfer RNA$_f^{Met}$ Conformation†

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ABSTRACT: The possible difference in conformation between aminoacylated and deacylated tRNA is examined using the optical and photochemical properties of the 4-thiouridine residue of *E. coli* tRNA$_f^{Met}$. No differences were seen between fMet-tRNA$_f^{Met}$ and tRNA$_f^{Met}$ observing the native fluorescence of 4-thiouridine, energy transfer from 4-thiouridine to the bound lanthanide ions, Tb^{3+} or Eu^{3+}, or the rates of the photochemical cross-linking reaction of 4-thiouridine. While these results do not necessarily mean that there is no conformational difference between the aminoacylated and deacylated species, they do restrict the possible nature and magnitude of any conformational difference between the two species. In addition, preliminary thermal denaturation studies of tRNA$_f^{Met}$, monitoring 4-thiouridine emission and energy transfer to Tb^{3+}, indicate an unexplained melting phenomenon near 25 °C in the presence of Mg^{2+}.

There are several indications that transfer RNA and aminoacyl-tRNA are functionally different. Valyl-tRNA^{Val} has a lower affinity for valyl-tRNA^{Val} synthetase than does tRNA^{Val} (Lagervist et al., 1966). Charged *E. coli* tRNA^{Val} and yeast tRNA^{Phe} have been shown to bind more oligo(C) than do the uncharged species (Danchin & Grunberg-Manago, 1970). Similarly, progesterone and the tetranucleotides U-C-C-C and C-G-A-A will bind only to Phe-tRNA^{Phe} and not to the deacylated species (Dvorak et al., 1976). Since aminoacylation does not alter the primary structure of tRNA, it would appear that such differences are the result of changes in the secondary or tertiary structures of tRNA.

Physical chemical evidence for such a conformational change has been sought using tritium exchange (Grantt et al., 1969; Englander et al., 1972), NMR[1] (Cohn et al., 1970; Wong et al., 1973), Raman (Thomas et al., 1973), EPR (Caron et al., 1976), and CD (Adler & Fasman, 1970; Wickstrom, 1971; Watanabe & Imahori, 1971). A clear answer to the problem has yet to be found, but a review of the evidence indicates that, if such a conformational change occurs, it is very subtle and apparently localized in a particular region of tRNA.

We have been investigating the 4-thiouridine region of *E. coli* tRNA$_f^{Met}$ because CD (Watanabe & Imahori, 1971) and EPR (Caron et al., 1976) studies have suggested that a conformational change due to aminoacylation occurs in this region. s^4U is present in over 70% of *E. coli* tRNA molecules, usually at sequence position 8 (Venkstern, 1973). In the yeast tRNA^{Phe} structure, position 8 is in a region of the molecule where tertiary interactions occur between the dihydro(U) loop and the GψC loop (Quigley & Rich, 1976). The crystalline *E. coli* tRNA$_f^{Met}$ structure appears compatible with the overall conformation of tRNA^{Phe} (Woo & Rich, 1977). Therefore s^4U-8 in tRNA$_f^{Met}$ should be in a similar structural region as U-8 in tRNA^{Phe}.

The s^4U base absorbs light at longer wavelengths than do the common bases and fluoresces with excitation and emission maxima near 350 and 530 nm, respectively (Favre, 1974; Shalitin & Feitelson, 1973). Energy transfer from s^4U to the bound lanthanide ions, Tb^{3+} or Eu^{3+}, results in enhancement of the rare earth excitation spectra near 350 nm (Kayne & Cohn, 1974; Wolfson & Kearns, 1975). Since the mechanism of energy transfer is not known, the distance dependence may be stronger than the usual inverse sixth power dependence (Förster, 1967; Dexter, 1953; Inokuti & Hirayama, 1965; Darnell et al., 1976).

In the work reported here the local conformations near s^4U in fMet-tRNA$_f^{Met}$ and in tRNA$_f^{Met}$ are compared using: (1) the natural fluorescence of s^4U; (2) energy transfer from s^4U to bound Tb^{3+} or Eu^{3+}; and (3) the photochemistry of s^4U.

Experimental Section

Materials. E. coli MRE-600 (RNase-negative) tRNA$_f^{Met}$ was purchased from Boehringer-Mannheim. L-[*methyl*-^{14}C]Methionine was obtained from New England Nuclear and N^5-formyl tetrahydrofolate (folinic acid) was purchased from General Biochemicals. ATP, dithiothreitol (Cleland's reagent), bovine serum albumin, and Hepes were obtained from Calbiochem. Bovine serum albumin was subsequently carboxymethylated (Yarus & Rashbaum, 1972). An RNase-free mixed aminoacyl synthetase preparation, purified from *E. coli* MRE-600, was generously provided by Dr. Michael Yarus. Terbium and europium oxides samples were given by Molycorp and were converted to their respective chlorides. All other chemicals were reagent grade.

Aminoacylation and Formylation of tRNA$_f^{Met}$. Aminoacylation and formylation of tRNA$_f^{Met}$ were accomplished according to the method of Yarus & Mertes (1973). tRNA$_f^{Met}$ were incubated with mixed aminoacyl synthetase, 1.5 mM [^{14}C]methionine and 1.3 mM folinic acid for 15 min at 37 °C in pH 7.5, 0.1 M Hepes, 2 mM ATP, 0.1 mM dithiothreitol, 0.08 M NH_4Cl, 0.1 mM EDTA, 50 mg/mL carboxymethyl serum albumin buffer. Controls were carried through identical procedures with the exception that either [^{14}C]methionine or aminoacyl synthetase was omitted from the aminoacylation mixture. Following incubation, protein was extracted from both the aminoacylated sample and the control with H_2O-saturated phenol. The aqueous layers were applied to a Sephadex G-25 column and eluted with 0.05 M sodium acetate, 0.025 M sodium borate, 0.1 M NaCl, and 5 mM $Mg(OAc)_2$ pH 5.0 buffer. The extent of formylation of Met-tRNA$_f^{Met}$ was determined by the electrophoretic assay described by Haenni

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[1] Abbreviations used: CD, circular dichroism; EDTA, ethylenediaminetetraacetate; EPR, electron paramagnetic resonance; FDCD, fluorescence detected circular dichroism; Hepes, *N*-2-hydroxyethylpiperazene-*N'*-2-ethanesulfonic acid; NMR, nuclear magnetic resonance; s^4U, 4-thiouridine.

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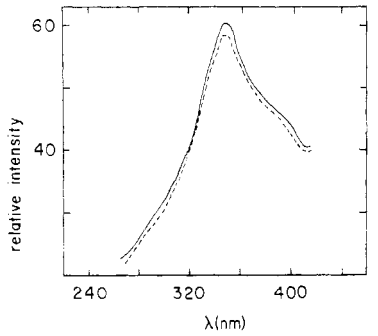

FIGURE 1: Fluorescence excitation spectra of fMet-tRNA$_f^{Met}$ (—) and tRNA$_f^{Met}$ (- - -), monitoring emission at 530 nm. In pH 5.0, 0.05 M NaOAc, 0.025 M $Na_2B_4O_7$, 0.10 M NaCl, 5 mM $Mg(OAc)_2$ buffer at 23 °C.

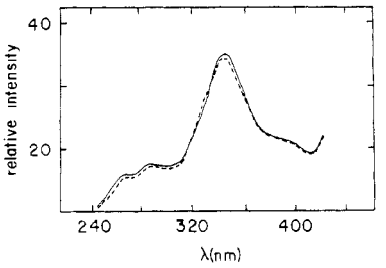

FIGURE 2: Fluorescence excitation spectra of 4 μM Tb^{3+} in the presence of fMet-tRNA$_f^{Met}$ (—) or tRNA$_f^{Met}$ (- - -), monitoring emission at 545 nm. Conditions were the same as in Figure 1.

& Chapeville (1966). The aminoacyl acceptor activity of tRNa$_f^{Met}$ was 1400 pmol/A_{260} and the formyl acceptor activity of Met-tRNA$_f^{Met}$ was 85%.

Fluorescence Measurements. A Perkin-Elmer MPF-2A fluorescence spectrophotometer was used for fluorescence measurements and for photochemical studies. A thermostated cuvette holder was used to maintain constant temperature and all measurements were made with 3-mm pathlength fluorescence cuvettes.

All excitation spectra were pulsed for 5 s every 10 nm to diminish the effects of photochemical processes. In no case were the pulsed spectra significantly different from scanned spectra. Fluorescence was monitored at 530 nm for s^4U, at 545 nm or 585 nm for Tb^{3+} and at 585 nm for Eu^{3+}. The spectra were measured in either the pH 5.0 NaOAc–$Na_2B_4O_7$ buffer described above or in 0.01 M NaOAc, 0.1 M NaCl, 5 mM $Mg(OAc)_2$, pH 5.0 buffer.

The rates of photochemical cross-linking between the s^4U-8 and the C-13 residues in tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ were compared at 5 °C in 0.01 M NaOAc, 0.1 M NaCl, 5 mM Mg^{2+}, pH 5.0 buffer. tRNA$_f^{Met}$ samples were irradiated at 350 nm and native s^4U emission was monitored at 530 nm. Rates of cross-linking were also compared by monitoring the enhancement of Tb^{3+} (4 μM) excitation at 350 nm by tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$.

Fluorescence melting curves were obtained for tRNA$_f^{Met}$ and tRNA$_f^{Met}$–Tb^{3+} complexes in 0.05 M NaOAc, 0.025 M $Na_2B_4O_7$, 5 mM Mg^{2+}, pH 5.0 buffer. Temperature was increased from 5 to 80 °C at a rate of approximately 1.3 °C per min and fluorescence was monitored for 5 s every 2 °C by pulsing the exciting light.

Results

tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ Fluorescence. The native fluorescence excitation spectra at 23 °C for tRNA$_f^{Met}$ and

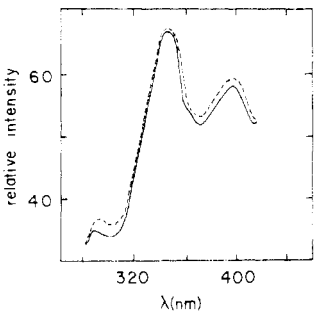

FIGURE 3: Fluorescence excitation spectra of 0.88 mM Eu^{3+} in the presence of fMet-tRNA$_f^{Met}$ (—) or tRNA$_f^{Met}$ (- - -) monitoring emission at 585 nm. Conditions were the same as in Figure 1.

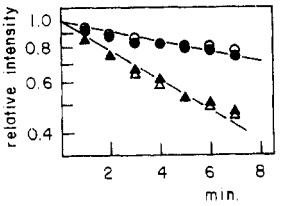

FIGURE 4: Normalized rates of photochemical cross-linking with irradiation at 350 nm. fMet-tRNA$_f^{Met}$ (●) and tRNA$_f^{Met}$ (○) monitoring native s^4U emission at 530 nm (no Tb^{3+} present). fMet-tRNA$_f^{Met}$ (▲) and tRNA$_f^{Met}$ (△) monitoring Tb^{3+} (4 μM) emission at 545 nm. In pH 5.0, 0.01 M NaOAc, 0.10 M NaCl, 5 mM $Mg(OAc)_2$ buffer at 5 °C.

fMet-tRNA$_f^{Met}$ are shown in Figure 1. Concentrations of tRNA samples were approximately 1.0 A_{260}/mL and were identical within 1%. The estimated cumulative error for the fluorescence spectra under the conditions used is between 5 and 10%. The native excitation spectra of the aminoacylated and deacylated tRNA$_f^{Met}$ are identical within experimental error (Figure 1).

Figure 2 shows the Tb^{3+} excitation spectrum in the presence of tRNA$_f^{Met}$ or fMet-tRNA$_f^{Met}$ at 23 °C. tRNA concentrations were adjusted to be equal within 1% at approximately 0.5 A_{260}/mL. A 4:1 lanthanide:tRNA ratio is consistent with the observed conditions for maximum energy transfer (Wolfson & Kearns, 1975; Kayne & Cohn, 1974) and minimum cation-induced hydrolysis of tRNA (Rordorf & Kearns, 1976). Spectra were also obtained at 5 °C and, although greater emission is seen for both species at the lower temperature, the spectra are identical within experimental error at a given temperature.

A comparison of the 23 °C Eu^{3+} excitation spectrum at 350 nm in the presence of tRNA$_f^{Met}$ or fMet-tRNA$_f^{Met}$ (approximately 0.5 A_{260}/mL) again shows no significant difference (Figure 3). The 880:1 Eu^{3+}:tRNA ratio was necessary to achieve an adequate signal. The possibility of Eu^{3+}-catalyzed hydrolysis was eliminated by measuring the spectra within 1 min after the addition of the Eu^{3+} to the tRNA (Rordorf & Kearns, 1976).

All experiments were repeated several times with no consistent difference in excitation spectra and with no difference in spectra greater than the limits of error. Spectra determined in 0.01 M NaOAc, 0.1 M NaCl, 5 mM Mg^{2+}, pH 5.0 buffer yielded similar conclusions.

Photochemical Cross-Linking of s^4U-8 and C-13 in tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$. The rates of photochemical formation of the s^4U-8–C-13 dimer were compared in tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$. Figure 4 shows that the rates of cross-linking are the same in the two species when monitored by either the decay in the native s^4U emission at 530 nm (tRNA concentration 1.0 A_{260}/mL) or by the decay in Tb^{3+}

emission at 545 nm (tRNA concentration 0.5 A_{260}/mL).

tRNA$_f^{Met}$ Melting Studies. Thermal melting curves for tRNA$_f^{Met}$ (approximately 1.0 A_{260}/mL), in the presence of Mg^{2+}, were determined by monitoring native s^4U fluorescence, as well as enhancement of Tb^{3+} fluorescence by s^4U in tRNA$_f^{Met}$. The observed melting profiles at various wavelengths are shown in Figure 5. All samples were excited at 350 nm.

There does not appear to be a sharp change in any of the curves shown in Figure 5 at the expected melting temperature near 70 °C. The Tb^{3+} emission profiles suggest that there may be a melting phenomenon near 25 °C which could also be indicated by the leveling-off of the s^4U native emission at this temperature.

Discussion

The question of whether or not a conformational change occurs in tRNA upon aminoacylation has been left without a concrete answer for some time. Comparison of the native fluorescence excitation of tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ (Figure 1) as well as the energy transfer from the s^4U residue in the two species to bound lanthanide ions (Figures 2 and 3) provides no evidence for the putative conformational change. The similarity in the rates of photochemical cross-linking in tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ also offers no support for the notion that a conformational difference exists between the two species (Figure 4). While it is conceivable that a conformational change could occur which would not cause an observable change in our experiments, our results do restrict the magnitude and the nature of the suggested conformational change. s^4U emission should be sensitive to s^4U environment, and the similarity of the native tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ excitation spectra indicates that little or no change in the polarity or the mobility of the s^4U environment occurs upon aminoacylation. Energy transfer from s^4U to bound lanthanide ions is strongly distance dependent and would be sensitve to a conformational change which results in an altered energy transfer distance. While we cannot quantitate this effect, an intensity change of 10% corresponds to a distance change of 0.1–0.2 Å, if we assume an inverse sixth power distance dependence and a critical transfer distance of 5 Å. It appears, therefore, that the distance between the s^4U residue and the bound lanthanide ions in tRNA$_f^{Met}$ does not change appreciably when tRNA$_f^{Met}$ is aminoacylated. Photochemical dimer formation between s^4U-8 and C-13 should be dependent on the relative orientation of the two residues (Pochon et al., 1971; Favre et al., 1971; Favre & Fourrey, 1974) and the identical rates of cross-linking in tRNA$_f^{Met}$ and fMet-RNA$_f^{Met}$ suggest that the two residues are identically oriented in the two species.

Differences in tRNA$_f^{Met}$ and fMet-tRNA$_f^{Met}$ conformation reported by Watanabe & Imahori (1971) must be carefully considered because of the low magnitude of their CD signal. While spin-labeling studies (Caron et al., 1976) indicate that the conformation near s^4U in *E. coli* tRNA^{Phe} is altered by aminoacylation, the attachment of a spin-label may affect the conformational nature of the macromolecule. Our results are not directly comparable with those of Caron et al. (1976) because of differing tRNA species and differing pH. It is known that tRNA structure is affected by pH (Steinmetz-Kayne et al., 1977; Bina-Stein & Crothers, 1974, 1975). We have used the same tRNA species and pH as Watanabe & Imahori (1971). Their results indicate an altered asymmetry in s^4U environment upon aminoacylation. If the change in asymmetry actually occurs, our results show that it must happen with no appreciable change in the distance between s^4U and the lanthanide ion binding sites or in the relative orientation of s^4U and C-13.

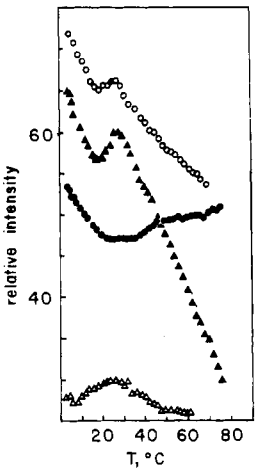

FIGURE 5: Thermal melting profiles of tRNA$_f^{Met}$ (approximately 1.0 A_{260}/mL) in pH 5.0, 0.05 M NaOAc, 0.025 M $Na_2B_4O_7$, 0.10 M NaCl, 5 mM $Mg(OAc)_2$. $TbCl_3$, when present, was at 4 μM concentration. Excitation at 350 nm: s^4U emission at 530 nm, no Tb^{3+} present (●); s^4U emission at 530 nm, Tb^{3+} present (○); emission from Tb^{3+} at 545 nm (▲); emission from Tb^{3+} at 585 nm (△).

It is important to consider the possible structural effect of binding lanthanides to a macromolecule. The different rates of cross-linking in the presence and absence of Tb^{3+} (Figure 4) may indicate that the conformation near s^4U can be affected by Tb^{3+}, but it is also possible that the presence of Tb^{3+} alters the photochemistry without a conformational change. There is evidence that lanthanides can replace divalent cations with little or no effect on structure (Kayne & Cohn, 1972, 1974; Kim et al., 1973; Matthews & Weaver, 1974). With regard to the structures of fMet-tRNA$_f^{Met}$ and tRNA$_f^{Met}$, our results in the presence of Tb^{3+} or Eu^{3+} are consistent with our results in the absence of lanthanides.

tRNA$_f^{Met}$ melting studies were initiated to examine the effect of a known conformational change on native s^4U emission and on energy transfer to Tb^{3+}. However, the melting studies shown in Figure 5 are at present difficult to interpret. The profiles are complicated by possible changes in tRNA–Tb^{3+} binding constants with temperature, and by competing donor electronic processes which are themselves temperature dependent. An understanding of the profiles is also made difficult because the fluorescences from s^4U and from Tb^{3+} are not completely separable under the conditions used, and there may be small contributions from scattered light and background fluorescence. While the melting studies must be considered preliminary, they do exhibit some interesting features, particularly the possibility of a low temperature melting phenomenon.

In the absence of Mg^{2+}, tRNA melting occurs over a wide temperature range indicating that structural features of the molecule are melting independently (Privalov et al., 1975; Crothers et al., 1974; Seno et al., 1969). However, as viewed by UV absorbance (Seno et al., 1969) and calorimetry (Privalov et al., 1975), in the presence of Mg^{2+} the melting occurs over a narrow temperature range near 70 °C. Since the fluorescent properties of the isolated s^4U residue are only slightly temperature dependent (Pavlick, 1977; Shalitin & Feitelson, 1976), the low temperature changes in emission are potentially ascribable to changes in conformation near s^4U in tRNA$_f^{Met}$. Turner et al. (1975) have also observed an apparent low temperature melting for tRNA^{Phe} in the presence of 10 mM Mg^{2+} using CD and FDCD. Their results could indicate a low temperature melting in the vicinity of the Y base in the anticodon arm. While our results suggest the possibility of low temper-

ature melting in the vicinity of s^4U, the two phenomena may be related. Further experimentation will be necessary to resolve this question.

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