CIRCULAR DICHROISM DURING DEACYLATION OF METHIONYL-$tRNA_f^{met}$ AND FORMYLMETHIONYL-$tRNA_f^{met}$ FROM E. COLI

Eric Wickstrom
Department of Chemistry
University of California
Berkeley, California 94720

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SUMMARY

Circular dichroism spectra were taken of [^{14}C]met-$tRNA_f^{met}$ and [^{14}C]fmet-$tRNA_f^{met}$ from E. coli at 37°C in 100 mM NaCacodylate, 10 mM $MgCl_2$, pH 5.5 and 6.5, respectively, conditions in which deacylation proceeded at similar rates. Any difference between the two sets of spectra, which would have converged toward the spectrum of unacylated $tRNA_f$, could have been interpreted as differences occurring between aminoacyl- and peptidyl-tRNA molecules in the A and P sites of ribosomes, as proposed by Woese. No differences could be detected; however, this result does not rule out the Woese model.

INTRODUCTION

Students of protein synthesis have searched for several years for structural differences between aminoacylated and unacylated tRNA* without a consistent pattern of results.[1-6] In addition, the special characteristics of the bacterial initiator, $tRNA_f^{met}$, have led to the suggestion of structural changes in met-$tRNA_f^{met}$ upon formylation.[7,8] The reciprocating ratchet model proposed by Woese[9] suggests that the anticodon loop of met-$tRNA_f^{met}$ is rearranged from a "Hodgson-Fuller" (5'-3' chain extended) structure to a "Fuller-Hodgson" (3'-5'

*Abbreviations used in this paper: tRNA, transfer ribonucleic acid; met, methionine; fmet, formylmethionine; ATP, adenosine triphosphate; CD, circular dichroism; UV, ultraviolet.

chain extended) structure[10] when the methionine N-terminus is blocked by a formyl group.

Circular dichroism is sensitive to composition, structure, and sequence of polynucleotides, and should be able to detect gain or loss of 2-3 base pairs in a tRNA molecule.[11] However, when using optical methods to detect small changes in the state of a large molecule, important errors in concentration, and therefore in magnitude, can arise from trying to compare different solutions. In addition to dilution errors, changes may occur in molar extinction coefficients due to the very structural change one is studying, or due to preparation procedures.

To avoid such problems in this experiment, successive CD spectra were taken of samples of [^{14}C]met-tRNA$_f^{met}$ and [^{14}C]fmet-tRNA$_f^{met}$ in the course of their deacylation. In this manner the CD spectra of the two species could be directly compared with one another and with deacylated tRNA$_f^{met}$ without manipulating the samples. No differences were found between the spectra of met-tRNA$_f^{met}$, fmet-tRNA$_f^{met}$, and tRNA$_f^{met}$. This result implies that any conformational differences among the three species are quite small or nonexistent.

MATERIALS AND METHODS

Aminoacylation and formylation were carried out similar to the method of Hershey and Thach.[12] A 1 ml reaction mixture contained 180 µg tRNA$_f^{met}$ (the gift of Dr. A. D. Kelmers,[13] Oak Ridge National Laboratory), 100 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM dithiothreitol, 2 mM ATP, 0.6 mM folinic acid (Lederle Laboratories), 100 λ S-100, and 0.1 mM [^{14}C]met, 51 Ci/mole (Schwarz). For [^{14}C]met-tRNA$_f^{met}$, the folinic acid was omitted.

Incubation occurred at 37°C for 10 min.; the reaction was stopped by the addition of 1 volume of phenol saturated with 100 mM NaCacodylate, pH 4.5, 10 mM $MgCl_2$, extracted with vigorous shaking, and the aqueous layer removed. tRNA was precipitated from the aqueous layer by adding 0.2 volumes of saturated KOAc, pH 5.3, and 4 volumes of ethanol, and freezing for 2 hr at -10°C. The ethanol precipitate was washed again with ethanol, dissolved in a small amount of 100 mM NaCacodylate, pH 4.5, 10 $MgCl_2$, applied to a 0.6 x 25 cm column of Sephadex G-25 or G-50, and eluted with the same buffer. Resolution of tRNA from ATP and short oligomers was best on G-50. All of the above manipulations were done on ice or at 4°C.

Methionylation was 75-90% complete in the reaction mixture, dropping to nearly 50% after purification, based on TCA-precipitable [^{14}C]met activity and optical density. Formylation of [^{14}C]met-$tRNA_f^{met}$ was 80-90% complete, based on a descending paper chromatography assay using a 90:10:25 n-butanol:glacial acetic acid:H_2O solvent.

[^{14}C]met activity was measured by adding an aliquot of solution to 2 ml cold 10% TCA, followed by filtration on Millipore HAWP 25 filters, with four 1 ml washes of cold 5% TCA. Filters were dried under an infrared lamp and placed in scintillation vials with 10 ml of 4.0% (v/v) Fluor Concentrate II (Research Supplies Laboratory) in toluene, then counted in a Beckman LS-250 liquid scintillation counter with 74% efficiency.

UV absorption measurements were made on a Cary 15 recording spectrophotometer, and CD measurements were made on a Cary 60 recording spectropolarimeter with a Cary 6001 CD attachment. Spectra were digitized and smoothed by a Digital Equipment Corp. PDP8/S computer interfaced to the Cary 60.[14]

For CD spectra, fractions of [^{14}C]met-tRNA$_f^{met}$ or [^{14}C]fmet-tRNA$_f^{met}$ from the above step were diluted to about $A_{260\ nm} = 1$; dead volume fractions to be used as blanks were treated similarly.

1 ml of tRNA solution was adjusted to desired pH; 0.6 ml of this solution was added to a 0.6 ml, 1 cm path cylindrical cell and 0.4 ml was saved for assays. A UV absorption spectrum was taken at 20°C of the tRNA solution in the cell, which was then placed in the sample-holder of the Cary 6001, maintained at 37°C, at the same time the parallel solution was placed in a 37°C water bath. During spectrum-taking, 20λ aliquots of the parallel solution were removed periodically and assayed for [^{14}C]met activity in order to monitor deacylation occurring in the CD cell. 9-12 CD spectra were taken of the tRNA solution in the cell over the course of 12-24 hr, after which another UV absorption spectrum was taken at 20°C. A blank UV absorption spectrum at 20°C and a CD baseline spectrum at 37°C were taken before and after the tRNA runs.

This procedure yielded a series of CD spectra of a tRNA, either [^{14}C]met-tRNA$_f^{met}$ or [^{14}C]fmet-tRNA$_f^{met}$, at identical concentrations over a broad range of aminoacylation levels. Any observed changes between such CD spectra would have been unambiguously due to changes in the molecule, since uncertainty over concentration, composition, and optical differences between two cells were eliminated.

RESULTS AND DISCUSSION

a. Deacylation kinetics. Figures 1 and 2 show semi-logarithmic plots of deacylation kinetics for [^{14}C]met-tRNA$_f^{met}$ and [^{14}C]fmet-tRNA$_f^{met}$. Deacylation which proceeded quickly enough

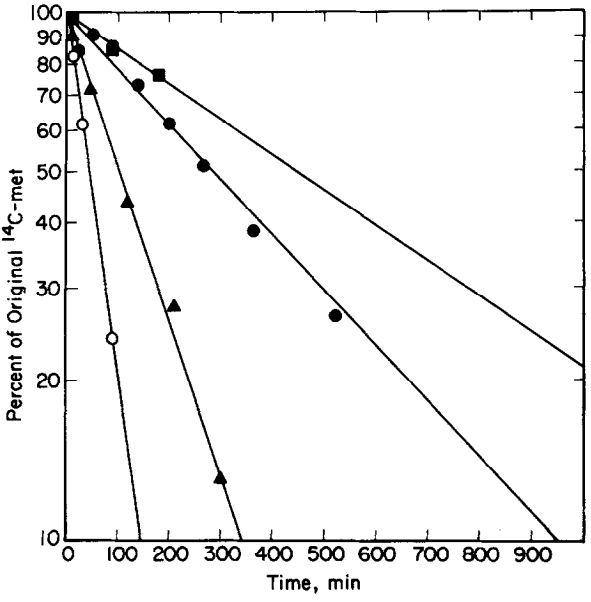

Figure 1. Deacylation kinetics of [^{14}C]met-tRNA$_f^{met}$ in 100 mM NaCacodylate, 10 mM $MgCl_2$ at 37°C. (■), pH 4.5. (●), pH 5.5. (▲), pH 6.0. (O), pH 6.5.

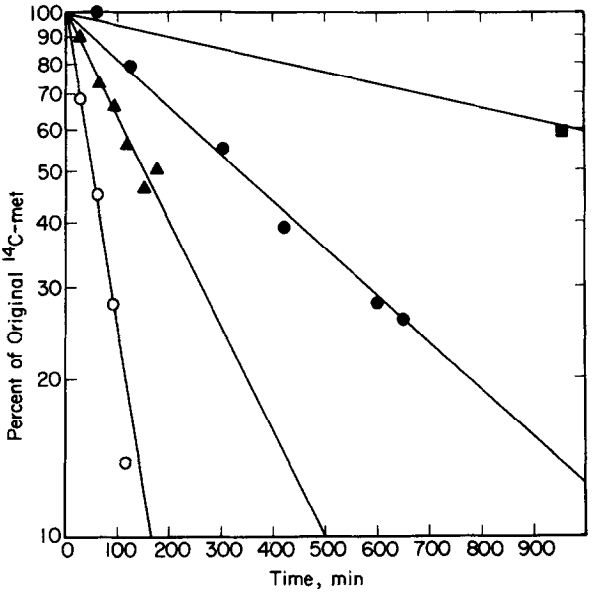

Figure 2. Deacylation kinetics of [^{14}C]fmet-tRNA$_f^{met}$ in 100 mM NaCacodylate, 10 mM $MgCl_2$ at 37°C. (■), pH 5. (●), pH 6.5. (▲), pH 7. (O), pH 9.

to do the spectral series with little evaporation, yet slowly enough that each 10 min spectrum could reasonably be said to represent a constant level of aminoacylation, determined the choice of pH's of the solutions in the two sets of spectra.

At pH 5.5 for [^{14}C]met-tRNA$_f^{met}$, and pH 6.5 for [^{14}C]fmet-tRNA$_f^{met}$, the first 10% of deacylation spread out over about 45 min, while 50% deacylation occurred in about five hr, and 80% in about 12 hr. Solutions used for CD spectra were adjusted to these two pH's for [^{14}C]met-tRNA$_f^{met}$ and [^{14}C]fmet-tRNA$_f^{met}$, respectively.

This work made clear that aminoacylated tRNA remains loaded only a very short time in common assay conditions. The deacylation kinetics of [^{14}C]met-tRNA in mixed tRNA at 37°C in 10 mM Tris HCl, pH 7.0, 60 mM NH_4Cl, 10 mM $MgCl_2$ are virtually the same as in cacodylate buffer, pH 6.5 (Fig. 1). This should be kept firmly in mind when doing binding and incorporation assays with aminoacylated tRNA, where one may be tempted to regard the tRNA as remaining fully loaded.

b. Circular dichroism spectra. Figure 3 shows the circular dichroism spectrum of tRNA$_f^{met}$ in 100 mM NaCacodylate, 10 mM $MgCl_2$, pH 5.5 or 6.5. For both [^{14}C]met-tRNA$_f^{met}$ and [^{14}C]fmet-tRNA$_f^{met}$, 9 successive spectra were averaged every 2.5 nm, or 0.5 nm in the region of the 265 nm peak and plotted. For wavelengths greater than 220 nm, the time-averaged circular dichroism, $\epsilon_L - \epsilon_R$, had an error (2σ) of less than or equal to 0.05 $M^{-1}cm^{-1}$ in each case or less than 1/2% in the region of the 265 nm peak. The two time-averaged CD spectra of [^{14}C]met-tRNA$_f^{met}$ and [^{14}C]fmet-tRNA$_f^{met}$ agreed with each other to the extent that no differences could be shown between fmet-tRNA$_f^{met}$, met-tRNA$_f^{met}$, or -tRNA$_f^{met}$.

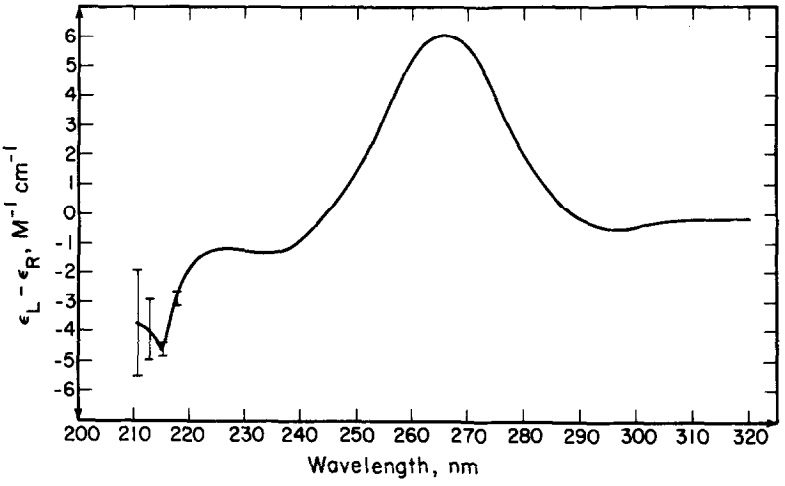

Figure 3. Circular dichroism spectrum of [^{14}C]fmet-tRNA$_f^{met}$ in 100 mM NaCacodylate, 10 mM $MgCl_2$, pH 6.5, 37°C. Average of 9 spectra over 12 hr. Width of line equals random error to two standard deviations, except as indicated by error bars. The same spectrum was found for [^{14}C]met-tRNA$_f^{met}$.

However, this result should not be interpreted as absolute proof that there are no configurational differences among these three species. Rather, one may say that any differences which do exist are limited by instrument error to one or two base pairs, and one or two stacking interactions. For example, the exchange of stacking interactions in the anticodon loop of a tRNA proposed by Woese[9] is not ruled out by this result, though a more drastic rearrangement would be. It should be pointed out for comparison that melting or denaturing of a tRNA substantially alters the shape and magnitude of its circular dichroism spectrum.[15,16]

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