Mapping of the Active Site of *Escherichia coli* Methionyl-tRNA Synthetase: Identification of Amino Acid Residues Labeled by Periodate-Oxidized $tRNA^{fMet}$ Molecules Having Modified Lengths at the 3′-Acceptor End†

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ABSTRACT: Initiator tRNA molecules modified at the 3′-end and lacking either the A_{76} (tRNA-C_{75}), the C_{75}-A_{76} (tRNA-C_{74}), the C_{74}-C_{75}-A_{76} (tRNA-A_{73}), or the A_{73}-C_{74}-C_{75}-A_{76} (tRNA-A_{72}) nucleotides were prepared stepwise by repeated periodate, lysine, and alkaline phosphatase treatments. When incubated with trypsin-modified methionyl-tRNA synthetase (MTS_T), excess amounts of the dialdehyde derivative of each of these shortened tRNAs (tRNA-C_{75}ox, tRNA-C_{74}ox, tRNA-A_{73}ox, and tRNA-A_{72}ox) abolished both the isotopic [^{32}P]PP_i–ATP exchange and the tRNA aminoacylation activities of the enzyme. In the presence of limiting concentrations of the various tRNAox species, the relative extents of inactivation of the enzyme were consistent with the formation of 1:1 complexes of the reacting tRNAs with the monomeric modified synthetase. Specificity of the labeling was further established by demonstrating that tRNA-C_{75}ox binds the enzyme with an equilibrium constant and stoichiometry values in good agreement with those for the binding of nonoxidized tRNA-C_{75}. The peptides of MTS_T labeled with either tRNA-C_{75}ox or tRNA-C_{74}ox were identified. The chymotryptic digestion of the covalent MTS_T·[^{14}C]tRNA-C_{75}ox complex yielded four peptides (A–D). In the case of tRNA-C_{74}ox, only two of the above peptides (C and D) were identified. Peptides A, B, C, and D corresponded to fragments Ser334–Phe340, Lys61–Leu65, Val141–Tyr165, and Glu433–Phe437, respectively, in the MTS_T primary structure. In a previous work [Hountondji, C., Blanquet, S. & Lederer, F. (1985) *Biochemistry 24*, 1175–1180], all these peptides but one (peptide D) had been already found labeled upon MTS_T incubation with [^{14}C]tRNA-A_{76}ox. According to the crystallographic structure of MTS_T, the labeled residues K335, K61, K142, K147, and K149 are within a sphere of about 5.5-Å radius. The present results therefore argue for a marked flexibility of the 3′-end of the enzyme-bound tRNA, enabling it to contact any of the identified reacting residues. Such a cluster of basic amino acids may reflect ionic requirements in the guiding of the negatively charged CCA arm of tRNA toward enzyme-bound methionyl–adenylate.

Recent studies have shown that aminoacyl-tRNA synthetases could be inactivated and affinity labeled with the help of their cognate oxidized tRNA[1] (Fayat et al., 1979; Hountondji et al., 1979, 1980, 1985, 1986a, 1987; Baltzinger et al., 1979; Gerlo & Charlier, 1979; Hill & Schimmel, 1989). In particular, a covalent 1:1 complex between oxidized initiator $tRNA^{Met}$ and trypsin-modified methionyl-tRNA synthetase was obtained (Fayat et al., 1979; Hountondji et al., 1979). Two major labeled chymotryptic peptides were isolated from this covalent complex. They encompassed lysine-335 and lysine-61 of the primary structure of the enzyme (Hountondji et al., 1985). These two lysine residues were found about 11 Å distant from each other, in the crystallographic structure of the enzyme (Brunie et al., 1987). This result was interpreted to reflect a mobility of the CCA arm of enzyme-bound $tRNA^{fMet}$. To investigate the case, we examine, in this work, the reactivity toward *Escherichia coli* trypsin-modified methionyl-tRNA synthetase of a set of oxidized tRNA molecules having reduced lengths at the 3′-acceptor end. Evidence is shown that the activity of the enzyme can be destroyed equally well by incubation with oxidized tRNA molecules lacking either one, two, three, or four nucleotides from the 3′-end. Identification of the peptides of MTS_T labeled by the truncated tRNAox molecules lacking one or two bases suggests that, in addition to the flexibility of the 3′-end of tRNA, there may exist a cluster of basic amino acid residues (lysine and arginine) involved in the binding or the guiding of the CCA arm of tRNA toward the active site of the enzyme. These results are discussed in connection with the known crystallographic structure of MTS_T (Brunie et al., 1987, 1990).

Materials and Methods

Materials

Native methionyl-tRNA synthetase was purified from *E. coli* strain EM20031 carrying the F32 episome (Cassio &

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[1] Abbreviations: The conventional tRNA numbering system of Gauss and Sprinzl (1983) was used. tRNA-A_{76} or $tRNA^{fMet}$, *Escherichia coli* native initiator $tRNA^{Met}$; tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, and tRNA-A_{72}, $tRNA^{fMet}$ species lacking the A_{76}, C_{75}-A_{76}, C_{74}-C_{75}-A_{76}, and A_{73}-C_{74}-C_{75}-A_{76} nucleotides, respectively; [^{14}C]tRNA-C_{75}, tRNA-C_{75} labeled with [^{14}C]CMP on the position of C_{75}; tRNA-X-p, modified tRNA with a 3′-phosphoryl terminus; tRNAox or tRNA dialdehyde, tRNA with the 3′-terminal *cis*-diol group oxidized by periodate to a dialdehyde group; tRNAox-red, tRNAox reduced with sodium borohydride; cytidine-ox-red, the cytidine derivative released by ribonuclease from the 3′-end of tRNA-C_{75}ox-red or of tRNA-C_{74}ox-red; MTS_T, trypsin-modified methionyl-tRNA synthetase. Other aminoacyl-tRNA synthetases are abbreviated as three-letter codes of their specific amino acid followed by RS; and one- and three-letter amino acid codes are those suggested by the IUPAB–IUB Commission on Biochemical Nomenclature.

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Waller, 1971a) or from the overproducing strain PAL1803.5 carrying recombinant plasmid pX1 (Dardel et al., 1984). Homogeneous trypsin-modified methionyl-tRNA synthetase was derived from the purified native enzyme as already described (Cassio & Waller, 1971b). Pure $tRNA^{fMet}$ (1.5 nmol of methionine acceptance/A_{260} unit of tRNA) was obtained according to Blanquet et al. (1973). Methionyl-tRNA synthetase and tRNA concentrations were measured according to their specific absorption coefficients at 280 and 260 nm, respectively (Blanquet et al., 1973; Guéron & Leroy, 1978). Alkaline phosphatase from calf intestine and ribonucleases A, T_1, and U_2 were from Boehringer (Mannheim). Chymotrypsin and phenylmethanesulfonyl fluoride were purchased from Sigma. Partially purified tRNA nucleotidyltransferase was obtained from *E. coli* strain EM20031 according to a procedure similar to that for the purification of methionyl-tRNA synthetase (Cassio & Waller, 1971a). Its specific activity for [^{14}C]AMP incorporation at the 3'-end of tRNA-C_{75} was measured equal to 1.6 nmol·mg^{-1}·s^{-1} at 37 °C in 50 mM Tris-HCl, pH 9, containing 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 150 μM [^{14}C]ATP (10.3 Ci/mol), 37 μM tRNA-C_{75}, and 0.2 mg/mL bovine serum albumin.

Sodium cyanohydridoborate was from Serva, and [^{14}C]ATP (279 mCi/mmol) and [^{14}C]CTP (457 mCi/mmol) were from the Radiochemical Center, Amersham (U.K.). Other chemicals were of the best commercially available grades.

Methods

Preparation of tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, and tRNA-A_{72}: Stepwise Removal of the 3'-Terminal Nucleotides of $tRNA^{fMet}$. Intact $tRNA^{fMet}$ (4 mg/mL) was incubated 30 min at 20 °C in the dark in the presence of 3.3 mM sodium periodate. Oxidation was quenched by the addition of 0.2 volume of 5 M NaCl and of 2.2 volumes of cold ethanol. After centrifugation, the pellet containing the oxidized tRNA was dissolved in 1 mL of 0.5 M L-lysine (pH 8) and then left 2 h at 45 °C. The reaction was stopped by the precipitation of the released tRNA-C_{75}-p with ethanol, as above. After centrifugation, the precipitate was dissolved in 10 mM ammonium bicarbonate (pH 8) containing 15 mM $MgCl_2$ and 25 μg/mL alkaline phosphatase. After 60 min at 37 °C, the reaction mixture was phenol extracted. tRNA-C_{75} was ethanol precipitated and finally dissolved in 1 mM potassium acetate (pH 5) containing 0.1 mM $MgCl_2$. tRNA-C_{75} was assayed for reconstitution by tRNA nucleotidyltransferase in the presence of [^{14}C]ATP as already described (Fayat et al., 1979).

The preparations of tRNA-C_{74} from tRNA-C_{75}, of tRNA-A_{73} from tRNA-C_{74}, and of tRNA-A_{72} from tRNA-A_{73} were carried out stepwise following the above procedure. Each of these tRNA species was tested for its reconstitution by tRNA nucleotidyltransferase in the presence of [^{14}C]ATP or [^{14}C]-CTP as follows: modified tRNAs (0.7 mg/mL) were incubated at 37 °C in 50 mM Tris-HCl (pH 9) containing 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 100 μg/mL bovine serum albumin, and 345 μM unlabeled CTP plus 145 μM [^{14}C]ATP (10.3 Ci/mol), or 190 μM unlabeled ATP plus 350 μM [^{14}C]CTP (7.1 Ci/mol). The reactions were initiated by the addition of catalytic amounts of tRNA nucleotidyltransferase, and the incorporations of [^{14}C]AMP or [^{14}]CMP in tRNA were followed as a function of time by precipitation of aliquots with 5% trichloroacetic acid.

Polyacrylamide Gel Electrophoresis. Electrophoresis of tRNAs on a 10% polyacrylamide [9.5% acrylamide, 0.5% bis(acrylamide)] gel (20 × 40 cm) polymerized in 0.1 M Tris–borate (pH 8.3), 2 mM EDTA, and 8.3 M urea was performed at 20 °C (400 V during 14 h). The gel was soaked for 20 min in an aqueous solution of ethidium bromide (1 μg/mL) and photographed.

Preparation of [^{14}C]tRNA-C_{75}. tRNA-C_{74} was incubated as above in the presence of tRNA nucleotidyltransferase, unlabeled ATP, and [^{14}C]CTP (24.4 Ci/mol). When the [^{14}C]CMP incorporation reached a plateau value corresponding to 85% of the value expected from the tRNA concentration in the mixture, the reaction was quenched by ethanol–NaCl precipitation and the precipitate dissolved in 0.5 mL of 10 mM potassium acetate (pH 5.0) containing 0.1 mM $MgCl_2$. The specific radioactivity of this [^{14}C]tRNA labeled at position C_{75} was found equal to 20.4 Ci/mol. Its aminoacylation capacity was equal to 1200 pmol of methionine/A_{260} unit. In order to probe the homogeneity of the labeling, the following treatments were applied: (i) The labeled aminoacylatable tRNA was submitted to one cycle of periodate, lysine, and alkaline phosphatase treatments. The specific radioactivity of the resulting [^{14}C]tRNA-C_{75} measured by trichloroacetic acid precipitation was 19.4 Ci/mol. This value corresponded to 95% of the ^{14}C label initially present in the aminoacylatable [^{14}C]tRNA. The [^{14}C]tRNA-C_{75} obtained was stored in potassium acetate buffer. (ii) An aliquot of [^{14}C]tRNA-C_{75} (19.4 Ci/mol) was further submitted to one cycle of periodate and lysine treatments. After these treatments, the trichloroacetic acid precipitable radioactivity was reduced to 0.7 Ci/mol. It could be concluded that these treatments, together with the alkaline phosphatase digestion, had well caused the cleavage of more than 95% of the [^{14}C]CMP initially present in the tRNA molecule.

For the preparative tRNAox labeling reaction prior to primary structure determination, [^{14}C]tRNA-C_{75} and [^{14}C]-tRNA-C_{74} were obtained as follows: [^{14}C]tRNA-A_{76} carrying radioactivity on both the C_{75} and C_{74} nucleosides was reconstituted from tRNA-A_{73} (2.6 mg/mL) in the reaction mixture described above containing 300 μM unlabeled ATP, 416 μM [^{14}C]CTP (200 Ci/mol), and catalytic amounts of tRNA nucleotidyltransferase.

The specific radioactivity of the [^{14}C]tRNA-A_{76} thus labeled on positions C_{74} and C_{75} was 360 Ci/mol. This value satisfactorily accounted for the incorporation of 2 mol of [^{14}C]CTP (200 Ci/mol), into $tRNA_{73}$, i.e., tRNA lacking the CCA arm. Chemical removal of the 3'-terminal unlabeled AMP did not affect the specific radioactivity of the resulting [^{14}C]tRNA-C_{75}, as expected. The [^{14}C]tRNA-C_{75} sample was then divided into two equal portions, one of which was kept for further MTS_T·[^{14}C]tRNA-C_{75} covalent complex preparation. The other [^{14}C]tRNA-C_{75} portion (360 Ci/mol) was submitted to one cycle of periodate, lysine, and alkaline phosphatase treatments. The specific radioactivity of the resulting [^{14}C]tRNA-C_{74} (175 Ci/mol) was decreased to 48% that of [^{14}C]tRNA-A_{75} or that of their precursor [^{14}C]tRNA-A_{76}.

For the preparation of the MTS_T·tRNA covalent complexes, isotopic dilution of the truncated [^{14}C]tRNA species was carried out as follows. Three milligrams of freshly prepared unlabeled tRNA-C_{75} was added to 0.2 mg of [^{14}C]tRNA-C_{75} (360 Ci/mol). Similarly, 2.6 mg of tRNA-C_{74} was added to 0.17 mg of [^{14}C]tRNA-C_{74} (175 Ci/mol). This led to a specific radioactivity of 22.5 and 10.7 Ci/mol for [^{14}C]-tRNA-C_{75} and [^{14}C]tRNA-C_{74}, respectively.

Inactivation of Methionyl-tRNA Synthetase by the Oxidized tRNAs. Oxidized tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, and tRNA-A_{72} were prepared following the procedure in Fayat et al. (1979). Kinetics of inactivation of the enzyme by the various oxidized tRNAs were followed at 37 °C in 20 mM imidazole hydrochloride (pH 8.0) containing 25% glycerol,

Table I: Reconstitution of the Various Modified tRNAs by *E. coli* tRNA Nucleotidyltransferase[a]

	tRNA-A_{76}	tRNA-C_{75}	tRNA-C_{74}	tRNA-A_{73}	tRNA-A_{72}
residual aminoacylation capacity (%)	100	0	0	0	0
incorporation (%) of					
[^{14}C]CMP		0	80–90	80–90	80–90
[^{14}C]AMP		80–90	70–80	70–80	60
aminoacylation capacity after repair (%)		90	80	80	40

[a] Incorporation of the ^{14}C-labeled nucleotides was measured as under Methods. The values are expressed in percent of the values expected in the cases where the tRNAs had been completely modified and repaired. The aminoacylation capacities after repair have been measured as follows: the modified tRNAs were incubated at 37 °C in the presence of catalytic amounts of tRNA nucleotidyltransferase (50 mM Tris-HCl, pH 9.0, 10 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 190 μM ATP, 350 μM CTP, and 100 μg/mL bovine serum albumin); at various times, 25-μL aliquots were withdrawn and assayed for tRNA aminoacylation capacity by the addition of 75 μL of buffer, 20 mM imidazole hydrochloride (pH 7.5), containing 2.7 mM ATP, 54 μM [^{14}C]-L-methionine (50 Ci/mol), 200 mM KCl, 7 mM $MgCl_2$, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 200 μg/mL bovine serum albumin, and 200 nM methionyl-tRNA synthetase. After a 10-min incubation at 25 °C, trichloroacetic acid precipitable ^{14}C radioactivity was measured, as in Lawrence et al. (1973).

10 mM $MgCl_2$, and 2 mM $NaBH_3CN$. At various times of the reaction, 10-μL portions were withdrawn and the oxidized tRNA was reduced by the addition of a 2-μL aliquot of 0.5 M $NaBH_4$ in 10 mM NaOH. The resulting 12-μL portions were further diluted at 0 °C in 20 mM imidazole hydrochloride (pH 7.6) containing 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 200 μg/mL bovine serum albumin and then assayed for the methionine-dependent isotopic [^{32}P]PP_i–ATP exchange (Blanquet et al., 1974) and $tRNA^{Met}$ aminoacylation activities (Lawrence et al., 1973).

Stoichiometries of labeling of trypsin-modified methionyl-tRNA synthetase by oxidized [^{14}C]tRNA-C_{75} were measured as in Fayat et al. (1979) and Hountondji et al. (1979).

Preparation of the MTS_T·[^{14}C]tRNA-C_{75} and MTS_T·[^{14}C]tRNA-C_{74} Covalent Complexes. MTS_T (5.7 mg) at a final concentration of 0.57 mg/mL (9 μM) was incubated with 13 μM [^{14}C]tRNA-C_{75}ox or with 11 μM [^{14}C]tRNA-C_{74}ox, in the same conditions as above. At various times, 5-μL aliquots of the incubation mixture were withdrawn and assayed for enzymatic activity. In parallel, 30-μL aliquots were added to a 60-μL mixture of ribonuclease A (5 units/mL), ribonuclease U_2 (5 units/mL) and ribonuclease T_1 (500 units/mL). tRNA digestion was allowed to proceed during 30 min at 37 °C. Incorporation within the enzyme of the ^{14}C radioactivity was followed by precipitation with 5% trichloroacetic acid.

After 150 min, the reaction was stopped by the addition of 25 mM $NaBH_4$. At this time, 65% of the MTS_T activity was lost in the presence of [^{14}C]tRNA-C_{75}ox, while 0.8 mol of ^{14}C was incorporated per mole of enzyme. In the case of [^{14}C]-tRNA-C_{74}ox, 53% of the MTS_T activity was lost after 150 min, in parallel with the incorporation of 0.6 mol of ^{14}C/mol of enzyme. Extrapolation of the curves of residual activity versus ^{14}C incorporation (not shown) indicated covalent attachment of 1.2 mol of [^{14}C]tRNA-C_{75}ox or 1.1 mol of [^{14}C]tRNA-C_{74}ox, upon complete inactivation of 1 mol of MTS_T.

The reaction mixtures reduced with $NaBH_4$ (10 mL) were each dialyzed overnight, against 4 L of 0.1 M NH_4HCO_3 (pH 8.2). Then, each dialysate was treated with α-chymotrypsin for 150 min at 37 °C, at a protease to synthetase ratio of approximately 1/50 (w/w). The chymotryptic digestion was stopped by adding phenylmethanesulfonyl fluoride (0.5 mM final concentration).

Purification of the Labeled Peptides. Peptide purification from the MTS_T·[^{14}C]tRNA-C_{75} and MTS_T·[^{14}C]tRNA-C_{74} covalent complexes was performed by a two-step chromatography on Sephadex G-50 as already described (Hountondji et al., 1985, 1986a, 1987). Radioactive peptides were separated by two-dimensional thin-layer fingerprinting as described in Hountondji et al. (1985). Finally, radioactive peptides recovered from thin-layer plates were loaded on a TSK 2000 SW column (7.5 mm i.d., 30 cm long) with a 7-cm precolumn (Bio-Rad). The eluent was an acetic acid/acetonitrile/water (2/3/5) mixture (Beauvallet et al., 1988).

N-Terminal Sequencing. A Model 470A sequencer (Applied Biosystems) was used for automated Edman degradation of peptides. Samples (30-μL aliquots, 100–500 pmol) were loaded on glass fiber filters that had been previously treated with 2.5 mg of Biobrene (Applied Biosystems) and submitted to three precycles. The O3RPTH program, modified for our on-line PTH identification setup, was used for sequencing. Automated on-line PTH analysis was performed as described in Beauvallet et al. (1988).

Results

Stepwise Modifications of $tRNA^{fMet}$. *E. coli* $tRNA^{fMet}$ species from which all or part of the 3′-acceptor end has been removed were repaired with the help of tRNA nucleotidyltransferase in the presence of various ^{14}C-labeled nucleotides as under Methods, and assayed for their recovery of aminoacylation activity. As shown in Table I, each repaired tRNA recovered 80–90% of its methionine acceptance capacity, except in the case of tRNA-A_{72} which could not be repaired by more than 40%. The latter behavior might reflect abnormal nucleotide incorporation by tRNA-A_{72}, since, as already discussed in Tal et al. (1972), the tRNA nucleotidyltransferase normally synthesizes only the CCA terminus of tRNA.

Homogeneity of the various modified tRNAs was probed by high-resolution gel electrophoresis on polyacrylamide (Figure 1). Each of the modified tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, and tRNA-A_{72} appeared not damaged and homogeneous on the gel. Moreover, the migrations on the gel of the various modified tRNAs with respect to $tRNA^{fMet}$ (77 nucleotides) and *E. coli* $tRNA^{Tyr}$ (85 nucleotides) were in satisfying agreement with their modifications.

Inactivation and Labeling of Methionyl-tRNA Synthetase in the Presence of Oxidized tRNA-C_{75}. The methionine-dependent isotopic [^{32}P]PP_i–ATP exchange and tRNA aminoacylation activities of trypsin-modified methionyl-tRNA synthetase (1 μM) were decreased close to zero within 100 min upon incubation with 5 μM of the dialdehyde derivative of $tRNA^{fMet}$ lacking the 3′-terminal AMP (tRNA-C_{75}). The kinetics of inactivation fitted a single exponential curve, the time constant of which depended on the reacting tRNA concentration (Figure 2). This inactivation was promoted by the shortened oxidized tRNA rather than by contaminating amounts of oxidized tRNA-A_{76} since, in the presence of limiting concentrations of oxidized tRNA-C_{75}, the maximum extent of inactivation related closely to the molar ratio of enzyme to oxidized tRNA (Figure 3): 1 μM oxidized tRNA-C_{75} inactivated at least 48% of 2 μM of the monomeric enzyme. Also, oxidized tRNA-C_{75} inactivated the enzyme according to a K_m value of 4 μM (Figure 2), in close agreement

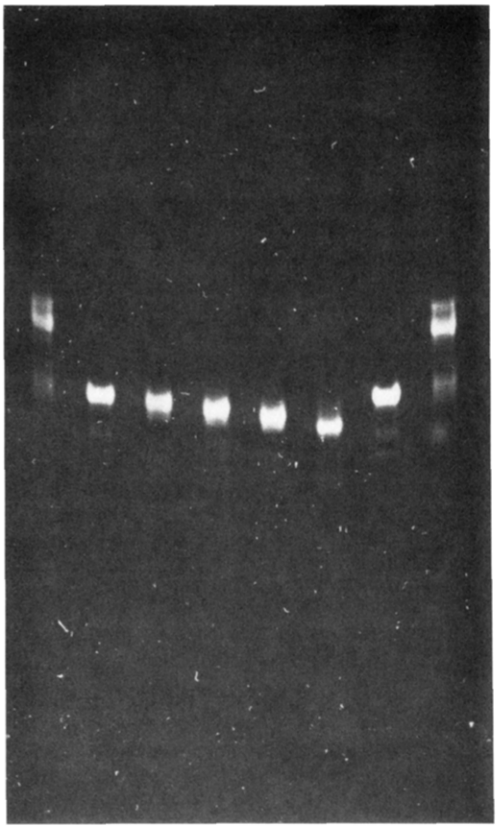

FIGURE 1: Gel electrophoresis on polyacrylamide of the modified $tRNA^{fMet}$ species. From left to right: $tRNA^{Tyr}$ (85 nucleotides), tRNA-A_{76} (77 nucleotides), tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, tRNA-A_{72}, tRNA-A_{76}, and $tRNA^{Tyr}$. The amounts of tRNA loaded onto the gel were about 4 μg for the native or modified $tRNA^{fMet}$ species and about 2 μg for $tRNA^{Tyr}$.

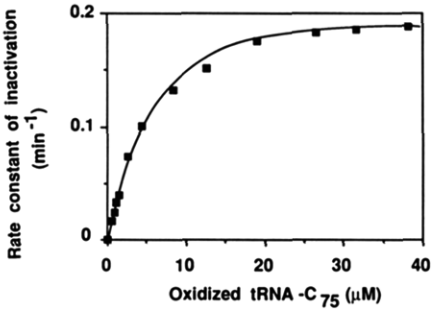

FIGURE 2: Dependence of the rate constant of inactivation on the concentration of oxidized tRNA-C_{75}. Methionyl-tRNA synthetase (100 nM) was incubated in the presence of various concentrations of tRNA-C_{75} dialdehyde. For each oxidized tRNA concentration, the kinetics of inactivation fitted a single exponential curve and a rate constant was measured.

with the dissociation constant of the enzyme·tRNA-C_{75} complex, as measured in Jacques and Blanquet (1977).

Finally, it was shown that 1 mol of trypsin-modified methionyl-tRNA synthetase was fully inactivated upon the incorporation of 1 mol of oxidized [^{14}C]tRNA-C_{75} (Figure 4).

Inactivation of Methionyl-tRNA Synthetase by the Other Modified Oxidized tRNAs. Inactivation of methionyl-tRNA synthetase was observed also in the presence of either oxidized tRNA-C_{74}, tRNA-A_{73}, or tRNA-A_{72}. As in the case of tRNA-C_{75}, we verified that the presence of limiting concentrations of these oxidized tRNAs promoted extents of inactivation relating well to the molar ratio of oxidized tRNA to enzyme in the incubation mixture (Figure 3).

Strategy of Preparation of the Labeled Peptides. The enzyme (89 nmol) inactivated by [^{14}C]tRNA-C_{75}ox (130 nmol)

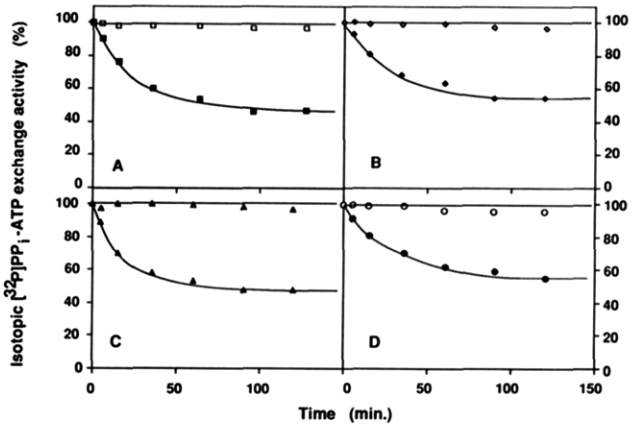

FIGURE 3: Kinetics of inactivation of trypsin-modified methionyl-tRNA synthetase by shortened tRNA dialdehyde species. The enzyme (2.1 μM) was incubated with (A) 1.2 μM tRNA-A_{76}ox, (B) 1.1 μM tRNA-C_{75}ox, (C) 1.2 μM tRNA-C_{74}ox, or (D) 1.2 μM tRNA-A_{73}ox. The kinetics of inactivation of the enzyme with 1.2 μM tRNA-A_{72}ox were superimposable on those with 1.2 μM tRNA-A_{73}ox (D).

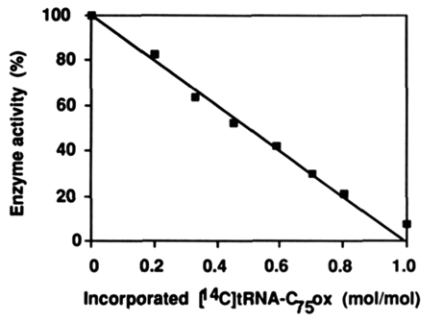

FIGURE 4: Stoichiometry of labeling by [^{14}C]tRNA-C_{75} dialdehyde of trypsin-modified methionyl-tRNA synthetase. The enzyme (4.2 μM) was incubated in the presence of 7.8 μM oxidized [^{14}C]tRNA-C_{75}, as under Methods. ^{14}C incorporation within the enzyme after ribonuclease A digestion was followed by trichloroacetic acid precipitation, as already described (Fayat et al., 1979).

or [^{14}C]tRNA-C_{74}ox (115 nmol), as under Methods, was reduced with sodium borohydride and digested with chymotrypsin. It should be noted that the unreacted species, MTS_T and [^{14}C]tRNA-C_{75}ox-red (or [^{14}C]tRNA-C_{74}ox-red), were not separated from the MTS_T·[^{14}C]tRNA-C_{75}ox (or MTS_T·[^{14}C]tRNA-C_{74}ox) covalent complexe(s), prior to the chymotryptic digestion.

The strategy for peptide purification from the labeled complex consisted of two consecutive separations on Sephadex G-50, as in Hountondji et al. (1985, 1986a, 1987). The unreacted [^{14}C]tRNAox-red and the peptides covalently linked to [^{14}C]tRNA eluted as a single peak in the void volume of the first Sephadex G-50 column (not shown). The radioactive fractions were pooled and further digested with a mixture of three ribonucleases (A, T_1, and U_2) prior to a second chromatography on Sephadex G-50 (Figure 5).

The elution profiles of the peptides linked to [^{14}C]tRNA-C_{75}ox or [^{14}C]tRNA-C_{74}ox (first Sephadex G-50 chromatography) were superimposable. However, the radioactivity in the [^{14}C]tRNA-C_{75}-labeled peptides was more than twice that in the [^{14}C]tRNA-C_{74}-labeled ones. This satisfactorily reflects the ratio of the total radioactivity associated with [^{14}C]tRNA-C_{75}ox (130 nmol, 22.5 Ci/mol) to that associated with [^{14}C]tRNA-C_{74}ox (115 nmol, 10.7 Ci/mol). The same 2/1 ratio was obtained upon comparing the total radioactivity of nuclease-treated [^{14}C]tRNA-C_{75}ox-linked peptides eluting from the second Sephadex G-50 column to that of nuclease-treated [^{14}C]tRNA-C_{74}ox-linked peptides (Figure 5).

Table II: Recovery of Various Radioactive Spots from Thin-Layer Plates[a]

counts recovered from the first Sephadex G-50 column	pool from the second Sephadex G-50 column	spots	counts eluted (10^6)	yield (%)	amino acid content[b]
C_{75}-labeled peptides (5.9×10^6 cpm)	Ia (5×10^6 cpm applied onto the plates)	IaS1	2.7	46.7	−
		IaS2	0.29	5	+
	IIa (0.78×10^6 cpm applied)	IIaS1	0.192	3.2	−
		IIaS2	0.059	1	+
		IIaS3	0.129	2.2	+
C_{74}-labeled peptides (2.6×10^6 cpm)	Ib (0.92×10^6 cpm applied)	IbS1	0.63	24	−
	IIb (1.06×10^6 cpm applied)	IIbS1	0.60	23	−
	IIIb (0.64×10^6 cpm applied)	IIIbS1	0.075	2.8	−
		IIIbS2	0.34	13	+

[a] The various pools from Figure 5 were analyzed by two-dimensional fingerprinting, as in Hountondji et al. (1985). Radioactive spots were eluted with 50% acetic acid. The yield of recovery of each spot is expressed as the ratio of the eluted radioactivity to the total radioactivity recovered from the first Sephadex G-50 column as MTS_T·[^{14}C]tRNA-C_{75} (or MTS_T·[^{14}C]tRNA-C_{74}) covalent complexes. For instance, the eluted counts from spot IIaS1 (0.192×10^6) represent 3.2% of the counts recovered from the first Sephadex G-50 column (5.9×10^6). [b] Amino acid composition on each spot was carried out in order to detect the presence of peptidic material: (+) indicates a peptidic material; (−) indicates a nonpeptidic material.

Specific Radioactivity of [^{14}C]*Cytidine-Labeled Peptides.* As stated under Methods, for the preparation of the MTS_T·[^{14}C]tRNA-C_{75} covalent complex, the kinetics of inactivation was quenched with reducing agent at the time where 13 μM [^{14}C]tRNA-C_{75}ox had inactivated 65% of 9 μM MTS_T. In other words, taking into account the 1:1 tRNA/MTS_T labeling stoichiometry, the sample contained 5.8 μM of MTS_T covalently bound to [^{14}C]tRNA-C_{75}ox (5.8 μM). The excess [^{14}C]tRNA-C_{75}ox (7.2 μM) was reduced to [^{14}C]tRNA-C_{75}ox-red in the presence of $NaBH_4$. Since ^{14}C radioactivity is equally distributed on cytidines-74 and -75 of tRNA-C_{75}, ribonuclease digestion of unreacted [^{14}C]tRNA-C_{75}ox-red (7.2 μM) produces 7.2 μM of [^{14}C]cytidine-ox-red and 7.2 μM of [^{14}C]CMP. In the case of the 5.8 μM peptide-linked [^{14}C]-tRNA-C_{75}ox, ribonuclease action produces 5.8 μM of peptide-linked [^{14}C]cytidine-ox and 5.8 μM of [^{14}C]CMP. Thus, 77% of the total radioactivity is expected to be associated with free nucleotides ([^{14}C]cytidine-ox-red and [^{14}C]CMP, 20.2 μM), and only 23% would correspond to [^{14}C]cytidine-labeled peptides (5.8 μM).

In the tRNA-C_{74} labeling experiments (see Methods), 11 μM [^{14}C]tRNA-C_{74}ox was allowed to inactivate 53% of 9 μM MTS_T. Thus, 4.8 μM MTS_T was covalently labeled with 4.8 μM [^{14}C]tRNA-C_{74}ox, while the unreacted 6.2 μM [^{14}C]-tRNA-C_{74}ox was reduced with sodium borohydride. In that case, 56% of the total radioactivity would be associated with free [^{14}C]cytidine-ox-red, while 44% would represent [^{14}C]-cytidine-ox-labeled peptides.

In conclusion, whatever the tRNA label, ribonuclease action is expected to produce a [^{14}C]cytidine covalently attached to peptides, so that the specific radioactivity of those peptides would be that of [^{14}C]tRNA-C_{74}ox (10.7 Ci/mol, 1 mol of [^{14}C]nucleoside/mol of tRNA) or half that of [^{14}C]tRNA-C_{75}ox (22.5 Ci/mol, 2 mol of [^{14}C]nucleoside/mol of tRNA).

Peptide Purification. The elution profile of the C_{75}-labeled peptides (Figure 5a) was divided into two pools (Ia and IIa) that contained 84% and 13% of the radioactivity initially applied on the column, respectively.

In the case of the C_{74}-labeled peptides, three pools (Ib–IIIb) were recovered that represented 34%, 39%, and 23% of the radioactivity applied on the column, respectively (Figure 5b).

Pools Ia and IIa and pools Ib–IIIb from Figure 5 were each submitted to thin-layer fingerprinting (not shown). Since the two-step size exclusion chromatography ensured that the recovered peptides were ^{14}C-labeled, the peptide maps were only autoradiographed [see also Hountondji et al. (1985, 1986a)]. The detected radioactive spots were further eluted with 50% acetic acid. Table II shows the yield of recovery of each spot,

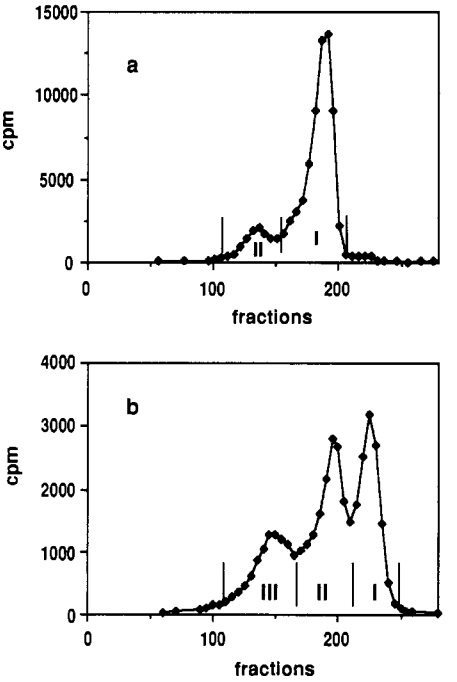

FIGURE 5: Fractionation of the C_{75}-labeled (a) and C_{74}-labeled (b) chymotryptic peptides. The radioactive material recovered from the first G-50 column (5.9×10^6 cpm of [^{14}C]tRNA-C_{75} or 2.6×10^6 cpm of [^{14}C]tRNA-C_{74}) was treated with ribonuclease, lyophilized, dissolved in 50 mM ammonium acetate (pH 8.2), and chromatographed on a column of Sephadex G-50 superfine (56×1.2 cm). The column was equilibrated and run with the same buffer. The flow rate was 6 mL/h, and fractions of 0.3 mL were collected. A 20-μL aliquot from each fraction was counted for ^{14}C radioactivity. Fractions were pooled as indicated.

expressed as the ratio of the eluted radioactivity to the total radioactivity associated with the MTS_T·[^{14}C]tRNA-C_{75} (or MTS_T·[^{14}C]tRNA-C_{74}) covalent complexes. Amino acid compositions of each radioactive spot from the different pools (Ia, IIa, and Ib–IIIb) were carried out in order to probe whether or not they contained any peptidic material (Table II). However, for those spots that were peptidic, amino acid composition as well as N-terminal sequencing indicated that their purity was not sufficient for unequivocal structure determination. Cytidine-labeled peptides were further purified through a last chromatographic step on TSK 2000 SW, as in Beauvallet et al. (1988). Figure 6 shows a typical elution profile obtained with this latter resin, with a clear separation of [^{14}C]cytidine-labeled peptides from nonradioactive nucleotides that had comigrated with the peptides during the

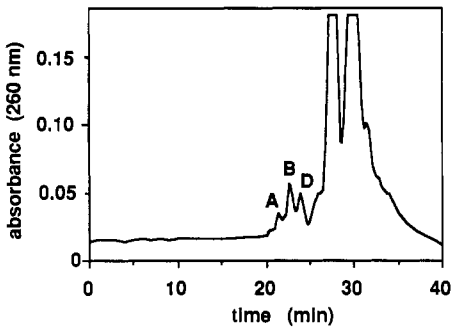

FIGURE 6: Purification on a TSK 2000 SW column of [^{14}C]C_{75}-labeled peptides. The radioactive material from spot IaS2 (Table II) was applied to a TSK 2000 SW column (30 cm × 7.5 mm, with a 7-cm precolumn, Bio-Rad) which was equilibrated and run with acetic acid/acetonitrile/water (2/3/5). The flow rate was 0.4 mL/min. Fractions were collected at 1-min intervals. The absorbance was monitored at 260 nm. Only the peptide peaks A, B, and D were radioactive, as revealed by liquid scintillation counting of aliquots of the collected fractions.

Table III: Percentages of Labeling of Peptides A–D by the Various tRNAox Species[a]

labeled peptides of MTS_T	labeling (%) by tRNA-A_{76}ox 3′-A_{76}	tRNA-C_{75}ox C_{75}	tRNA-C_{74}ox C_{74}
A (K335)	65–75	16	
B (K61)	20–25	32	
C (K142, K147, K149)	2–4	29	55
D (R435)		20	44

[a] The percentages of labeling are expressed as the ratios of the radioactivity associated with each peptide to the total radioactivity eluting as peptides. C represents fragment Val141–Tyr165 as well as its extended form Leu136–Tyr165. For tRNA-A_{76}ox, the data are from Hountondji et al. (1985).

thin-layer fingerprinting. The total recovery of ^{14}C radioactivity from the TSK 2000 SW column was 95–100%.

(1) C_{75}-Labeled Peptides. The peptide map of pool Ia showed two main radioactive spots (IaS1 and IaS2) containing 46.7% and 5% of the total radioactivity recovered from the first Sephadex G-50 chromatography of the MTS_T·[^{14}C]-tRNA-C_{75} covalent complex, respectively (Table II). The peptide map of pool IIa showed three spots IIaS1 IIaS2, and IIaS3, containing 3.2%, 1%, and 2.2%, respectively, of the total radioactivity recovered from the first Sephadex G-50 chromatography of the MTS_T·[^{14}C]tRNA-C_{75} covalent complex.

Spots IaS1 and IIaS1 (Table II) did not contain any peptidic material, as revealed by amino acid composition (not shown). Spot IaS2 contained three ^{14}C-labeled peptides (A, B, and D) that could be separated by chromatography on TSK 2000 SW (Figure 6). Spot IIaS2 contained a unique peptide (C) whereas spot IIaS3 contained, in addition to C, a peptide with a five-residue extension at the N-terminus of C (peptide C*). C_{75}-labeled peptides A, B, C + C*, and D represented 16%, 32%, 29%, and 20% of the total radioactivity from the fingerprint associated with peptidic material, respectively (Table III).

(2) C_{74}-Labeled Peptides. Pools Ib and IIb from MTS_T labeled with [^{14}C]tRNA-C_{74}ox did not contain any peptidic material as revealed by their amino acid composition before and after fingerprinting.

The peptide map of pool IIIb showed two main spots, S1 and S2, one of which, the spot IIIbS2, was shown to contain a peptidic material. The radioactivity associated with spot IIIbS2 represented 13% of the total radioactivity recovered from the first Sephadex G-50 chromatography of the MTS_T·[^{14}C]tRNA-C_{74} covalent complex (Table II). When analyzed on the TSK 2000 SW column, spot IIIbS2 contained two C_{74}-labeled peptides, C + C* and D, that represented 55% and 44% of the total radioactivity eluting as peptides, respectively (Table III).

Table IV: Amino Acid Sequences of Labeled Peptides A–D[a]

peptide	amino acid sequence
A	334 Ser-Lys(O)-Ser-Arg-Gly-Thr-Phe 340
B	61 Lys(O)-Ala-Gln-Gln-Leu 65
C	141 Val-Lys(O)-Gly-Thr-Cys-Pro-Lys(O)-Cys-Lys(O)-Ser-Pro- 156 Asp-Gln-Tyr-Gly-Asp...
C*	136 Leu-Pro-Asp-Arg-Phe-Val-Lys(O)-Gly-Thr-Cys-Pro- 156 Lys(O)-Cys-Lys(O)-Ser-Pro-Asp-Gln-Tyr-Gly-Asp...
D	433 Glu-Ser-Arg(O)-Glu-Phe 437

[a] Solid arrows: residues positively identified as PTH-amino acids. Broken arrows: residues that could not be identified. (O) indicates the cytidine-labeled Lys or Arg (see text).

When chromatographed on the TSK 2000 SW column, the nonpeptidic radioactive spots from Table II showed peaks with retention times equal to or superior to 25 min (not shown). Separation of cytidine-labeled peptides with the TSK 2000 SW chromatography also showed nonradioactive 260-nm-absorbing peaks eluted after 25 min (Figure 6). Control experiments with CMP and ribonuclease-treated bulk tRNA indicated that these peaks corresponded to [^{14}C]CMP, [^{14}C]cytidine-ox-red, and other nucleotides released by ribonuclease digestion of unbound or bound tRNA.

The radioactivity associated with [^{14}C]nucleotides and [^{14}C]C_{75}-labeled peptides represented 50% and 8.2% of the total radioactivity initially used in the [^{14}C]tRNA-C_{75} labeling experiments, respectively (Table II). In the case of C_{74}-labeled peptides, 49.8% and 13% of the total radioactivity initially used in the [^{14}C]tRNA-C_{74} labeling experiments were associated with [^{14}C]nucleotides and peptides, respectively. The discrepancy between these values and those expected (77% and 23%, for MTS_T·[^{14}C]tRNA-C_{75}, and 56% and 44%, for MTS_T·[^{14}C]tRNA-C_{74}) may be explained as follows: first, the overall yield of the thin-layer fingerprinting is only 58–63%; second, the yield of recovery of individual nucleotides or peptides from the cellulose matrix of the plates might be somewhat different.

Peptide Sequencing. The C_{75}-labeled peptides (A–D) and the C_{74}-labeled peptides (C and D) were further analyzed by automated Edman degradation (Table IV). [^{14}C]Cytidine-labeled peptides A, B, C, and D (Table IV), correspond to fragments Ser334–Phe340, Lys61–Leu65, Val141–Tyr165, and Glu433–Phe437, respectively, of the MTS_T primary structure (Dardel et al., 1984; Barker et al., 1982). Peptide C (Val141–Tyr165) was obtained either pure or in a mixture with its extended form (Leu136–Tyr165). In a previous work

(Hountondji et al., 1985), all these peptides but one (peptide D) were already found labeled in MTS_T incubated with [^{14}C]tRNA-A_{76}ox. For clarity, we have named the [^{14}C]cytidine-labeled peptides in the same way as in Hountondji et al. (1985).

When the released phenylthiohydantoins (PTH) were analyzed by RPLC, neither a PTH-Lys peak nor a new PTH peak were observed on the chromatogram at the positions of Lys335 (peptide A) or Lys61 (peptide B). This suggested that the PTH derivative of lysine covalently attached to oxidized cytidine was not eluted from the RPLC column. However, liquid scintillation counting of the collected, noninjected portion of the PTH fraction corresponding to Lys335 or Lys61 revealed that no ^{14}C radioactivity was present at these positions. It was therefore possible that the cleavage by trifluoroacetic acid (TFA) of the PITC-coupled cytidine-labeled lysine did not occur in the course of the Edman degradation. This possibility was ruled out by the fact that positive identification was obtained for all the amino acid residues following these labeled lysines. It is much probable that, after it was cleaved in the presence of TFA, the anilinothiazolinone (ATZ) of the cytidine-labeled lysine could not be extracted and transferred from the filter cartridge to the conversion flask of the gas-phase sequencer. In fact, 35–45% of the radioactivity initially associated with the peptides was still present on the filter at the end of the Edman degradation. As a control, the nonpeptidic spots from the thin-layer plates (Table II) were each submitted to Edman degradation, after repurification on the TSK 2000 SW column. Five degradation cycles were run, and the corresponding PTH fractions were counted for ^{14}C radioactivity (not shown). In any case, none of the five PTH fractions was radioactive, whereas the filter of the sequencer still contained 80–85% of the radioactivity initially submitted to the Edman reaction. Such a situation is not rare since Sejlitz et al. (1990) reported that the ATZ derivative of phosphopyridoxyllysine was not eluted from the sequencer cartridge, due to its strong adsorption to the Polybrene-treated glass fiber filter and/or to its low solubility in the extraction solvent (butyl chloride).

This contrasts with the case of the adenosine-ox-labeled lysine residue, whose PTH derivative eluted from the RPLC column as a single peak separating well from any other PTH (Hountondji et al., 1985, 1986a, 1987).

In the case of peptide C, a small amount of PTH-Lys was observed at the positions of Lys142, Lys147, and Lys149, indicating that these three lysines were fractionally labeled, as previously discussed (Hountondji et al., 1985).

Peptide D (Glu433–Phe437) is devoid of lysine. The Arg435 residue must be therefore the target of the labeling by tRNA-C_{75}ox or tRNA-C_{74}ox. This behavior was rather unexpected in view of our previous affinity labeling studies (Hountondji et al., 1985, 1986a, 1987). However, labeling of arginine residues via Schiff base formation was reported to occur in a few cases (King, 1966; Signor et al., 1971). Similarly to cytidine-labeled lysine, cytidine-labeled arginine was not converted to a PTH derivative, since 40% of the total radioactivity initially applied to the sequencer was recovered with the filter at the end of the Edman degradation of peptide D. However, at the position of Arg435, a small PTH peak was observed which eluted close to PTH-Arg (11.20 min versus 11.43 min) and contained only 3% of the total radioactivity applied to the sequencer as peptide D.

Discussion

Stepwise removal of one, two, three, or four nucleotides from the 3′-end of $tRNA^{fMet}$ is achieved by combining periodate, lysine, and alkaline phosphatase treatments. The resulting shortened tRNA species, when oxidized by sodium periodate, become able to covalently react with MTS_T and to abolish both its ATP–PP_i exchange and tRNA aminoacylation activities. In one case, that of the tRNA-C_{75}ox species, we have verified that all the criteria of an affinity labeling reagent were fulfilled. First, enzyme inactivation parallels the incorporation of 1 mol of [^{14}C]tRNA-C_{75}ox/mol of enzyme. This stoichiometry is in agreement with that of the enzyme·$tRNA^{fMet}$ complex (Blanquet et al., 1973; Dessen et al., 1982; Fayat et al., 1979; Hountondji et al., 1979). Second, enzyme inactivation is a tRNAox concentration dependent process, suggesting that specific binding of tRNA-C_{75}ox to the enzyme takes place prior to the covalent modification. Furthermore, the concentration of free tRNA-C_{75}ox that ensures half of the maximum rate of inactivation of the enzyme in the presence of excess reactant is 4 μM, in good agreement with the previously reported equilibrium constant (5.5 μM) of nonoxidized tRNA-C_{75} (Jacques & Blanquet, 1977).

Covalent modification of MTS_T with the oxidized 3′-nucleotide of the shortened tRNA species (tRNA-C_{75}, tRNA-C_{74}, tRNA-A_{73}, and tRNA-A_{72}) is consistent with the demonstration by ultraviolet photo-cross-linking studies that the 3′-terminal nucleotides of $tRNA^{Met}$ represent one of the contact regions with methionyl-tRNA synthetase (Rosa et al., 1979; Ackerman et al., 1985).

The tRNA-labeled peptides of MTS_T were identified in the cases of tRNA-C_{75}ox and tRNA-C_{74}ox. The chymotryptic digestion of the covalent MTS_T·[^{14}C]tRNA-C_{75}ox complex yielded four peptides, A–D. In the case of the covalent MTS_T·[^{14}C]tRNA-C_{74}ox complex, two labeled peptides, C and D, were isolated.

It should be recalled that upon affinity labeling of MTS_T by tRNA-A_{76}ox, all those peptides but one (peptide D) could be already identified: peptides A (Ser334–Phe340), B (Lys61–Leu65), and C (Val141–Tyr165) represented 65–75%, 20–25%, and 2–4%, respectively, of the total peptidic material (Hountondji et al., 1985). The identification with tRNA-A_{76}ox of several reacting lysine residues of methionyl-tRNA synthetase was interpreted to reflect the flexibility of the CCA arm (15 Å long) of enzyme-bound tRNA (Woo et al., 1980).

In the present work, tRNA-C_{75}-labeled peptides A, B, and C represent 16%, 32%, and 29% of the total peptidic material. An additional peptide D accounts for 20% of the label (Table III). This indicates that removal of the 3′-terminal adenosine from $tRNA^{fMet}$ significantly changes the percentages of labeling of both peptides A and C, while that of peptide B is almost unchanged. The increased percentage of labeling of peptide C, together with the appearance of the labeled peptide D, takes place to the detriment of peptide A, which becomes relatively less reactive. In turn, the significant labeling of K335 and K61 by tRNA-C_{75}ox indicates that a 5-Å-shortened acceptor arm of tRNA is still flexible enough to reach either of these two lysines.

As shown in Table III, peptides A and B were not labeled by tRNA-C_{74}ox. Thus, a 10-Å shortening of the CCA arm totally prevents the 3′-terminal cytidine-74-ox from hitting lysines-61 or -335. Furthermore, the increased percentage of labeling of K142, K147, K149, and R435 suggests that, in the MTS_T·tRNA complex, these amino acid residues lie closer to cytidine-74 of tRNA than to cytidine-75 and, a fortiori, to adenosine-76.

The crystallographic structure of methionyl-tRNA synthetase complexed with ATP has been recently refined at a 2.5-Å resolution (Brunie et al., 1990). The MTS_T polypeptide chain folds into two domains. The amino-terminal 360

residues comprise the N-terminal domain with the $\beta\alpha\beta$ units of a typical "mononucleotide binding fold" (Risler et al., 1981; Blow et al., 1983). The second domain, the C-terminal one, is composed of residues 361–551 and is dominated by α-helices. The active site cleft is located at the junction of the two domains. The carboxyl-terminal end folds back toward the N-terminal domain, so that the segment 520–537 sits over the catalytic crevice (Brunie et al., 1987, 1990).

With the exception of arginine-435, all the identified tRNA-labeled amino acid residues may contribute to the formation of the MTS_T active site. Lysine-61 is located in the middle of a loop (residues 52–69) linking the β_B-strand to the α_B-helix of the nucleotide binding domain. Lysine-335 is located at the end of the fifth β-strand of the nucleotide binding fold, in a loop which is a flank of the putative active site, facing the Lys61 loop which may represent the other flank of the active site (Brunie et al., 1987). Lysines-142, -147, and -149 are located in a large loop (the lysine-142 loop) inserted in the mononucleotide binding fold. The lysine-142 and lysine-61 loops form one wall of the active site cleft (Brunie et al., 1990). The average distance between couples of ϵ-amino groups belonging to either K335, K61, or K142 is approximately 11 Å. Therefore, K335, K61, K142, K147, and K149 are roughly distributed on a sphere of about 5.5-Å radius and form a cluster of positive charges which might favor the binding of the polyanionic amino acid acceptor arm of tRNA. This hypothesis is reinforced by the fact that no other basic amino acid residue (except K332 and R233) is present within this sphere at the interior of the active site crevice (Brunie et al., 1990; S. Brunie, personal communication).

More difficult is to explain the labeling of arginine-435 by either tRNA-C_{75}ox or tRNA-C_{74}ox. Arginine-435 is located in the C-terminal domain in the middle of a loop that connects two α-helices (Brunie et al., 1990). Despite the fact that Arg435 is close to the "mononucleotide binding fold" of the N-terminal domain, this residue lies at 25–30 Å from the other labeled amino acid residues, at the bottom of the catalytic crevice of the enzyme 3-D structure. Leon and Schulman (1987) have shown that lysine residue 439 of the native dimeric methionyl-tRNA synthetase could be cross-linked to a reactive group introduced at position 8 (S_8) of tRNA. Nucleotide S_8 is located at the interior of the L-shaped tRNA conformation, at about 30 Å from the 3′-terminus. Shortening the tRNA molecule of two 3′-nucleotides brings the 3′-terminus of tRNA at a 20-Å distance maximum from nucleotide S_8. Thus, labeling of R435 with the oxidized 3′-terminal cytidine of tRNA-C_{75} or tRNA-C_{74} has to be explained by a distortion of the L-shaped conformation of tRNA capable of bringing the shortened 3′-terminus closer to this amino acid residue. This conclusion is in agreement with the observation by Ferguson and Yang (1986) that the 3′-terminus bends toward the inner loop of the L-shaped $tRNA^{fMet}$ upon binding of methionyl-tRNA synthetase. It should also be noted that recent high-resolution crystallographic studies on the GlnRS/$tRNA^{Gln}$ complex have demonstrated that the acceptor arm of $tRNA^{Gln}$ is drastically bent upon binding its specific enzyme (Rould et al., 1989).

As already discussed in Hountondji et al. (1985, 1986a, 1987) in the case of the labeling of MTS_T by tRNA-A_{76}ox, the identification of several amino acid residues of MTS_T labeled by tRNA-C_{75}ox as well as by tRNA-C_{74}ox, altogether with a 1:1 stoichiometry of labeling, suggests that the covalent modification occurs in a mutually exclusive fashion. Whether or not each of the identified basic amino acid residues is essential to enzyme activity remains an important question which is currently being probed in our laboratory by means of site-directed mutagenesis. Replacement of lysine-61 with glutamine has been recently performed (Brunie et al., 1987; Y. Mêchulam, personal communication). This mutation slightly alters the Michaelis constant of ATP in the $tRNA^{Met}$ aminoacylation reaction. In contrast, substitution of a glutamine for the lysine-335 residue results in the complete loss of both the methionine-dependent isotopic [^{32}P]PP_i–ATP exchange and the tRNA aminoacylation activities (Brunie et al., 1987; Y. Mêchulam, personal communication). Further characterization of the Lys/Gln335 mutant enzyme has indicated that lysine-335 might participate in the pyrophosphate binding site in the enzyme catalytic center, during the production of methionyl–adenylate from ATP (Y. Mêchulam, personal communication). Thus, Lys335 of the CCA binding site of $tRNA^{Met}$ on methionyl-tRNA synthetase appears critical for methionyl–adenylate formation, suggesting that methionine activation and its subsequent transfer to the 3′-adenosine of $tRNA^{Met}$ take place at a same locus in the enzyme structure.

Comparison of the Lys335 region of MTS_T to the amino acid sequence around the $tRNA^{Tyr}$ox-labeled lysines-229, -234, and -237 of *E. coli* tyrosyl-tRNA synthetase has indicated a sequence similarity (Hountondji et al., 1986a). From this, a consensus sequence KMSKS emerged which serves now as a signature sequence of the site of the 3′-end of tRNA among the family of aminoacyl-tRNA synthetases (Hountondji et al., 1986b). Lysines-229, -234, and -237 of *E. coli* tyrosyl-tRNA synthetase were found conserved as Lys225, Lys230, and Lys233 in the homologous enzyme from *Bacillus stearothermophilus* (Hountondji et al., 1986a). The latter two lysines have been shown by mutagenesis to be involved in the stabilization of the transition state during the synthesis of tyrosyl–adenylate (Fersht et al., 1988). Moreover, the recent crystallographic solution of the GlnRS/$tRNA^{Gln}$ complex (Rould et al., 1989) has demonstrated that Lys270, which belongs to the sequence MSK of GlnRS (Hountondji et al., 1986b), is involved in the binding of the phosphate chain of ATP. These results could be expected since the lysine residues designated by the KMSKS consensus box must be close to the active center of aminoacyl-tRNA synthetases, the 3′-end of tRNA having to come in the vicinity of the anhydride bond of the enzyme-bound aminoacyl–adenylate.

The role of lysines-142, -147, and -149 in MTS_T activity is not yet elucidated. However, this cluster of basic amino acids belongs to the segment Val141–Tyr165 which, alone, bears four out of the eight cysteine residues of MTS_T. Interestingly, substitution of alanine for the cysteine-158 residue of *E. coli* methionyl-tRNA synthetase produces a catalytically inactive enzyme (Starzyk et al., 1989). The significance of this cysteine-rich region is further indicated by the observation that the segment Val141–Tyr165 of *E. coli* methionyl-tRNA synthetase shares 60% homology with segment Val333–Leu357 of the homologous cytoplasmic enzyme of *Saccharomyces cerevisiae* (Walter et al., 1983). Further work will be necessary to decipher the functional role of this rather well-conserved region of methionyl-tRNA synthetase.

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CORRECTION

Mechanism of HIV Reverse Transcriptase: Enzyme–Primer Interaction As Revealed through Studies of a dNTP Analogue, 3′-Azido-dTTP, by Padmini S. Kedar, John Abbotts, Terêz Kovâcs, Krystyna Lesiak, Paul Torrence, and Samuel H. Wilson*, Volume 29, Number 15, April 17, 1990, pages 3603–3611.

Page 3604. Under Materials, the specific activity of the HIV-1 reverse transcriptase should be 1000 nmol min^{-1} mg^{-1}.