Edelhoch, H. (1967) *Biochemistry 6*, 1948–1954.
Goodman, W., O'Hern, P. A., Zaugg, R. H., & Gilbert, L. I. (1978) *Mol. Cell. Endocrinol. 11*, 225–242.
Goodman, W. G., Adams, B., & Carlson, R. O. (1982) *Am. Zool. 22*, 977.
Goodman, W. G., Carlson, R. O., & Nelson, K. (1985) *Ann. Entomol. Soc. Am.* (in press).
Kramer, S. J., & Kalish, F. (1984) *J. Insect Physiol. 30*, 311–316.
Kramer, S. J., Mundall, E. C., & Law, J. H. (1980) *Insect Biochem. 10*, 279–288.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
McDonagh, A. F. (1979) *Porphyrins 6*, 293.
McDonagh, A. F., & Palma, L. A. (1980) *Biochem. J. 189*, 193–208.
Morse, D., & Horecker, B. L. (1966) *Anal. Biochem. 14*, 429–433.
Pattniak, N. H., Mundall, E. C., Trambusti, B. G., Law, J. H., & Keždy, F. J. (1979) *Comp. Biochem. Physiol. 63B*, 469–476.
Riddiford, L. M. (1981) *Am. Zool. 21*, 751–762.
Riddiford, L. M. (1982) *Dev. Biol. 92*, 330–342.
Rüdiger, W., Klose, W., Vuillaume, M., & Barbier, M. (1969) *Experientia 24*, 1000.
Sedlak, B. J., & Gilbert, L. I. (1979) *Tissue Cell 11*, 643–653.
Shapiro, J. P., Keim, P. S., & Law, J. H. (1984) *J. Biol. Chem. 259*, 3680–3685.
Weber, K., Pringle, J. R., & Osborn, M. (1972) *Methods Enzymol. 26*, 3–27.
White, C. A., & Kennedy, J. F. (1981) *Techniques in the Life Sciences*, Vol. B3, p 1, Elsevier/North-Holland, Amsterdam.
Wyatt, G. R., & Pan, M. L. (1978) *Annu. Rev. Biochem. 47*, 799–817.
Yphantis, D. A. (1964) *Biochemistry 3*, 297–317.

Methionyl-tRNA Synthetase from *Escherichia coli*: Primary Structure at the Binding Site for the 3′-End of $tRNA_f^{Met}$†

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ABSTRACT: It was previously shown that when the tryptic fragment of methionyl-tRNA synthetase from *Escherichia coli* is incubated with periodate-treated initiator tRNA, it is inactivated due to the formation of a covalent 1:1 complex that could be stabilized by reduction with cyanoborohydride [Hountondji, C., Fayat, G., & Blanquet, S. (1979) *Eur. J. Biochem. 102*, 247–250]. In this work, the residues labeled in the trypsin-modified enzyme have been identified. After chymotryptic digestion of the protein–tRNA complex, two major labeled peptides (A and B) and a minor one (C) were isolated and identified by sequencing. The radioactivity associated with peptides A–C represented 65–75, 20–25, and 2–4%, respectively, of the radioactivity eluted from the peptide maps. Peptides A and B encompassed lysines-335 and -61, respectively. Both these lysines were fully labeled. Peptide C encompassed lysines-142, -147, and -149, each of which was incompletely labeled. The significance of these results is discussed in light of the known crystallographic structure of the enzyme.

Among bacterial aminoacyl-tRNA synthetases, *Escherichia coli* methionyl-tRNA synthetase has been the object of many studies aimed at probing structure–activity relationships [reviewed in Blanquet et al. (1979)]. In particular, a fully active proteolyzed fragment of M_r 64K derived from native methionyl-tRNA synthetase (2 × 76K) was crystallized (Cassio & Waller, 1971a; Waller et al., 1971). The crystallographic structure of the fragment (MTS_T),[1] presently solved at 2.5-Å resolution (Zelwer et al., 1982), indicates an elongated molecule (90 Å × 52 Å × 44 Å) composed of three domains organized in a biglobular structure. The N-terminal globule contains two domains, one of which presents a "mononucleotide binding fold" similar to the cofactor binding site of dehydrogenases (Zelwer et al., 1982; Blow et al., 1983). The primary structure of the enzyme has also been determined (Barker et al., 1982; Dardel et al., 1984), and efforts are being made in order to identify the side chains that belong to the substrate binding sites.

Several affinity labeling methods have been applied to aminoacyl-tRNA synthetases (Schimmel, 1977; Bruton &

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[1] Abbreviations: MTS_T, active crystallized tryptic fragment of methionyl-tRNA synthetase; $tRNA_{ox}$ or tRNA dialdehyde, tRNA oxidized by periodate at its 3′-terminal ribose; ATP_{ox}, ATP oxidized by periodate at the ribose; $ATP_{ox\text{-}red}$, ATP_{ox} reduced with sodium borohydride; N^{α}-acetyl-L-lysyl-ATP_{ox}, α-acetylated lysine derivative in which the ε-NH_2 group has reacted with the dialdehyde group of oxidized ATP; N^{α}-acetyl-L-lysyl-$ATP_{ox\text{-}red}$, N^{α}-acetyl-L-lysyl-ATP_{ox} reduced with sodium borohydride; PMSF, phenylmethanesulfonyl fluoride; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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Hartley, 1970; Akhverdyan et al., 1977; Wetzel & Söll, 1977; Fayat et al., 1978, 1979) among which one method, based on the specific oxidation of the 3′-terminal ribose of tRNA, has emerged as a particularly suitable technique.

Indeed, both the aminoacylation and isotopic ATP–PP_i exchange activities of native and trypsin-modified methionyl-tRNA synthetases from *E. coli* could be destroyed by incubation in the presence of periodate-treated initiator $tRNA^{Met}$ (Fayat et al., 1979). The rate of the covalent modification was shown to parallel the rate at which both ATP–PP_i exchange and aminoacylation activities were lost. The inactivation was shown to proceed through the formation of a reversible Schiff base between the aldehyde groups created at the 3′-end of tRNA and a lysine residue in the active site of the enzyme. The equilibrium for Schiff base formation could be continuously and specifically displaced by reduction with sodium cyanoborohydride (Hountondji et al., 1979). With this technique, a covalent 1:1 complex between tRNA and trypsin-modified methionyl-tRNA synthetase could be obtained.

In this work, the labeled peptides from the covalent MTS_T–[^{14}C]$tRNA_f^{Met}$(ox) complex have been purified and identified by sequencing. The results are discussed in connection with the crystallographic structure of the synthetase.

Materials and Methods

Materials

Homogeneous trypsin-modified methionyl-tRNA synthetase was derived from the purified native enzyme as described earlier (Cassio & Waller, 1971a). Native methionyl-tRNA synthetase was purified from *Escherichia coli* strain EM20031 carrying the F32 episome (Cassio & Waller, 1971b) or from the overproducing strain PAL1803.5 carrying recombinant plasmid pX1 (Dardel et al., 1984). Pure *E. coli* initiator tRNA (1.5 nmol of methionine acceptance/A_{260} unit of tRNA) was obtained according to Blanquet et al. (1973) or purchased from Boehringer-Mannheim (1.2 nmol of methionine acceptance/A_{260} unit of tRNA). Enzyme and tRNA concentrations were determined from their absorbancies at 280 (Blanquet et al., 1973) and 260 nm (Guéron & Leroy, 1978), respectively. Uniformly labeled [^{14}C]ATP (540 mCi/mmol) was purchased from the Commissariat à l'Energie Atomique (Saclay, France). 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 (C. Hountondji, unpublished data). Ribonuclease A (60 Kunitz units/mg), chymotrypsin, and phenylmethanesulfonyl fluoride came from Sigma.

Methods

Preparation of [^{14}C]$tRNA_f^{Met}$. Enzymatic labeling of the initiator tRNA at its 3′-end with [^{14}C]AMP was performed according to Fayat et al. (1979), with the following modification: the [^{14}C]tRNA, after nucleotide incorporation in the presence of tRNA nucleotidyltransferase, was loaded onto a DEAE-Sephadex A-50 column (10 × 1 cm) equilibrated with 20 mM Tris-HCl, pH 7.5. The tRNA was eluted at 0.4 M NaCl with a 200-mL linear gradient of NaCl (0–0.7 M) in the same buffer. The tRNA was then precipitated with ethanol and NaCl and stored in 1 mM potassium acetate buffer (pH 5.5) containing 0.1 mM $MgCl_2$. Its specific radioactivity (15000 counts of ^{14}C min^{-1} $nmol^{-1}$) was determined as follows: the ultraviolet absorbance of tRNA samples was measured at 260 nm in a Zeiss PMQ3 spectrophotometer. After precipitation of known aliquots with 10% trichloroacetic acid, total yeast RNA (400 μg) was added as a carrier and the precipitate filtered on Whatman GF/C filters. The filters were washed 3 times with 20 mL of 5% trichloroacetic acid containing 1 mM ATP and counted in an Intertechnique SL32 counter with the Bray scintillator fluid.

Preparation of MTS_T–$tRNA_f^{Met}$(*ox*) *Covalent Complex.* Periodate oxidation of [^{14}C]$tRNA_f^{Met}$ was performed as in Fayat et al. (1979). Methionyl-tRNA synthetase was allowed to react with [^{14}C]$tRNA_f^{Met}$(ox), at 37 °C, in 20 mM imidazole–HCl buffer (pH 8) containing 10 mM $MgCl_2$, 24% glycerol, and 2 mM sodium cyanoborohydride. Two sets of reaction conditions were used: either excess of $tRNA_{ox}$ over enzyme (3.5 and 1.5 μM, respectively) or slight excess of enzyme over $tRNA_{ox}$ (19 and 18 μM, respectively). At various times, aliquots of the reaction mixture were withdrawn. Half of each aliquot was assayed for the methionine-dependent isotopic ATP–PP_i exchange and $tRNA^{Met}$ aminoacylation activities (Fayat et al., 1979; Hountondji et al., 1979). The remaining half was submitted to ribonuclease digestion and trichloroacetic acid precipitation as above. After completion of the reaction (150–180 min), the MTS_T–$tRNA_{ox}$ complex was separated from the excess of [^{14}C]$tRNA_{ox}$ or of MTS_T by chromatography either on Sephadex G-200 or on DEAE-Sephadex, respectively (not shown). The elution was monitored by radioactivity measurement in the case of the Sephadex G-200 chromatography and by radioactivity and enzymic activity measurements in the case of DEAE-Sephadex. The MTS_T–[^{14}C]$tRNA_{ox}$ complex was lyophilized.

Chymotryptic Cleavage. The MTS_T–[^{14}C]$tRNA_f^{Met}$(ox) complex (8 μM in 0.1M NH_4HCO_3, pH 8.2) was submitted to α-chymotryptic digestion for 150 min at 37 °C, at a protease to synthetase ratio of 1:50 (w/w). Protein cleavage was monitored by withdrawing aliquots and measuring radioactivity in the trichloroacetic acid insoluble material after digestion of the tRNA moiety with nonlimiting amounts of ribonuclease A. The proteolysis was stopped by adding PMSF (80 mM in 2-propanol) to a final concentration of 0.5 mM.

Peptide Mapping. The fingerprinting technique was that of Chen (1976). Aliquots containing 10–20 nmol of peptides were spotted onto cellulose thin-layer plates (Polygram cell 300 UV254, 20 × 20 cm, 0.1 mm; Macherey-Nagel). Electrophoresis was run in the first dimension at 500 V for 2 h at pH 4.4 (2% pyridine–4% acetic acid–16% acetone–78% water, v/v) on a Desaga apparatus. After being dried, the plate was chromatographed in the second dimension for 5 h in pyridine–1-butanol–acetic acid–water (50:75:15:60 v/v). The peptides were detected with 0.005% (w/v) fluorescamine in acetone, after being sprayed with 3% (v/v) pyridine in acetone. Labeled peptides were located by a 15-h autoradiography of the map (Kodak X-Omat-X-Ray film). The peptides were eluted with 50% acetic acid.

Amino Acid Analyses. Samples were hydrolyzed at 110 °C for 24 h in evacuated sealed tubes with 100–200 μL of 5.7 N twice distilled HCl, containing 0.1% phenol. After evaporation, amino acid compositions were determined with an LKB 4400 amino acid analyzer operated at AUFS = 0.1, with a normal ninhydrin system. The recorder of the analyzer was used to calculate proline, and the 570-nm channel was recorded on a Delsi 510 recorder coupled with a Delsi ICAP 10 integrator. The amounts analyzed ranged over 0.3–1 nmol in the case of the peptides and 0.1–0.2 nmol for the intact MTS_T–[^{14}C]-$tRNA_f^{Met}$(ox) complex. All results are expressed in molar ratios, and values for contaminants equal to or less than 0.2 mol/mol are omitted from the tables.

Automatic Edman Degradation. Automatic degradation was carried out in a Beckman 890 C sequenator with 0.1 M

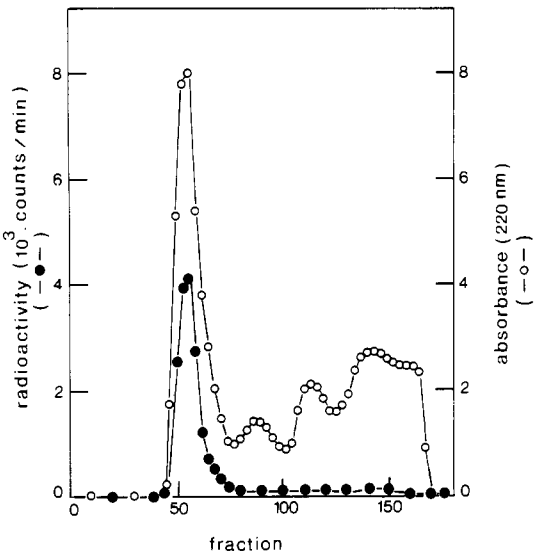


FIGURE 1: Gel filtration of the chymotryptic digest of the MTS_T–[^{14}C]$tRNA_f^{Met}$(ox) complex (5 × 10^6 cpm) on a column of Sephadex G-50 superfine (95 × 2 cm) in 0.1 M ammonium bicarbonate (pH 8.2). Flow rate was 10 mL/h; (○) A_{220nm}; (●) ^{14}C radioactivity (cpm) on 15-μL aliquots of the fractions.

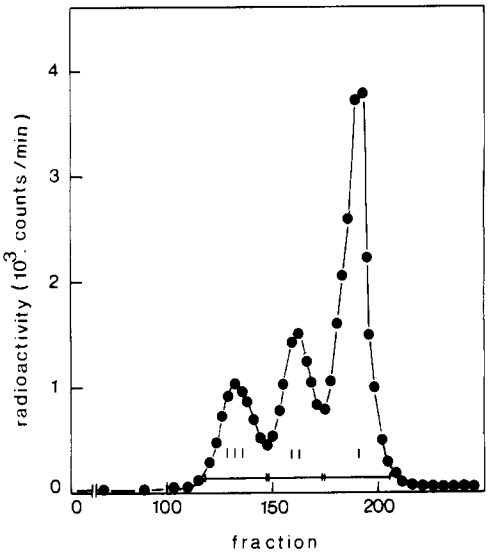


FIGURE 2: Gel filtration on Sephadex G-50 superfine (105 × 1.6 cm) of a 1-h ribonuclease A digest of the radioactive material from Figure 1 [200 μg of RNase A in 0.1 M NH_4HCO_3 (pH 8.2) at 37 °C]. The column was equilibrated with 20% acetic and run at a flow rate of 5 mL/h. Fractions were pooled as indicated.

quadrol in the presence of 2.5 mg of polybrene, and phenylthiohydantoins were identified by high-pressure liquid chromatography as described in Lederer et al. (1983).

RESULTS

Covalent MTS_T–[^{14}C]$tRNA_f^{Met}$(ox) Complex Formation. The stoichiometry of complex formation for the experiment involving excess $tRNA_{ox}$ over enzyme was found equal to 1.04 mol of [^{14}C]$tRNA_f^{Met}$(ox) bound/mol of enzyme, and both aminoacylation and isotopic ATP–PP_i exchange activities were reduced by more than 95% (Fayat et al., 1979; Hountondji et al., 1979). This stoichiometry was based on the known molecular ratio and extinction coefficient of MTS_T (Blanquet et al., 1973). The stoichiometry was further verified by amino acid analysis of the complex. On the basis of the 48 leucine residues present in the MTS_T enzyme (Barker et al., 1982), the amount of protein could be directly estimated. This determination gave 0.96 mol of [^{14}C]$tRNA_f^{Met}$(ox)/mol of enzyme. The overall amino acid composition was in good agreement with that deduced from the DNA sequence.

Strategy for Peptide Isolation. The strategy consisted of separating labeled peptides from the bulk unlabeled ones in only two steps. It was based on the observation that prolonged chymotryptic digestion in 0.1 M NH_4HCO_3 (pH 8.2) did not cause measureable degradation of the tRNA moiety: aliquots of the chymotryptic digest were precipitated with trichloroacetic acid, and the precipitate was filtered. The insoluble radioactivity in the sample indicated that the tRNA had remained intact in the chymotryptic digest.

The chymotryptic digest was first applied on a Sephadex G-50 column (Figure 1). Radioactivity corresponding to [^{14}C]tRNA linked to the peptides was exclusively found in the void volume of the column, as expected. This step separated the tRNA-bound peptides from the bulk of smaller unlabeled peptides. The second step consisted of repeating the Sephadex G-50 column after ribonuclease A treatment of the radioactive material obtained as above. This second chromatography separated the [^{14}C]AMP-labeled peptides from the large unlabeled ones, which might have coeluted in the void volume of the first column. The elution profile, shown in Figure 2, could be divided into three fractions called I–III that contained 48, 32, and 20% of the total radioactivity recovered from the column, respectively. The total recovery from the column was 80%.

Table I: Amino Acid Composition of Labeled Peptides[a]

	fraction I		fraction II		fraction III
amino acid	A	B	A	B	C
Asp					3.3 (3)
Thr	0.8 (1)		1.0 (1)		1.4 (2)
Ser	1.9 (2)	0.4 (0)	1.8 (2)	0.5 (0)	2.3 (1)
Glu		2.6 (2)	0.4 (0)	2.4 (2)	2.8 (2)
Pro					2.2 (2)
Gly	1.6 (1)	0.8 (0)	1.7 (1)	0.8 (0)	3.4 (3)
Ala		1.3 (1)		1.4 (1)	2.1 (1)
Cys					1.8 (4)
Val					1.7 (2)
Met					
Ile					
Leu		1 (1)		1 (1)	0.3 (0)
Tyr					2 (2)
Phe	1 (1)		1 (1)		0.5 (0)
His					
Lys	(1)		(1)		1.7 (3)
Arg	1.0 (1)		1.0 (1)		0.5 (0)
nmol of peptide according to radioactivity	0.78	0.91	0.87	0.95	0.73
amino acid analysis	0.71	0.81	0.83	0.83	0.74
% yield	58	19	21	12	6

[a] Values in parentheses indicate the theoretical compositions for peptides A–C that are derived from the DNA sequence (Barker et al., 1982). Peptide amounts analyzed were calculated from both radioactivity measurements and amino acid analysis. The yield represents the ratio of radioactivity eluted with the peptide to that spotted onto the thin-layer plate.

Purification and Characterization of Labeled Peptides. Fractions I–III from Figure 2 were each submitted to thin-layer fingerprinting. Figure 3 shows the corresponding peptide maps. Several radioactive spots were visible on the maps. The main ones were eluted with 50% acetic acid. Fractions I and II had in common two peptides (A and B). The sum A + B represented 77 and 33% of the spotted radioactivity from fractions I and II, respectively (Table I). Peptide A was also found in fraction III (analysis not shown) together with peptide

Table II: Recovery of Various Peptides[a]

	total enzyme (nmol)	reactant concn		yield (%)				
		protein (μM)	$tRNA_f^{Met}$(ox) (μM)	A	B	C	unidentified (combined)	total
expt 1	340	19	18	17	5	1		23
expt 2	80	1.5	3.5	35.6	12.8	1	4	53.4

[a] The yield was calculated by taking into account (1) the amount of radioactivity found in each fraction from Sephadex and (2) the absolute radioactivity associated with each peptide eluted from the various fingerprints (peptide A in fractions I–III, peptide B in fractions I and II, and peptide C in fraction III). For experiment 1, the thin-layer plates used were Polygram cell 400 instead of cell 300.

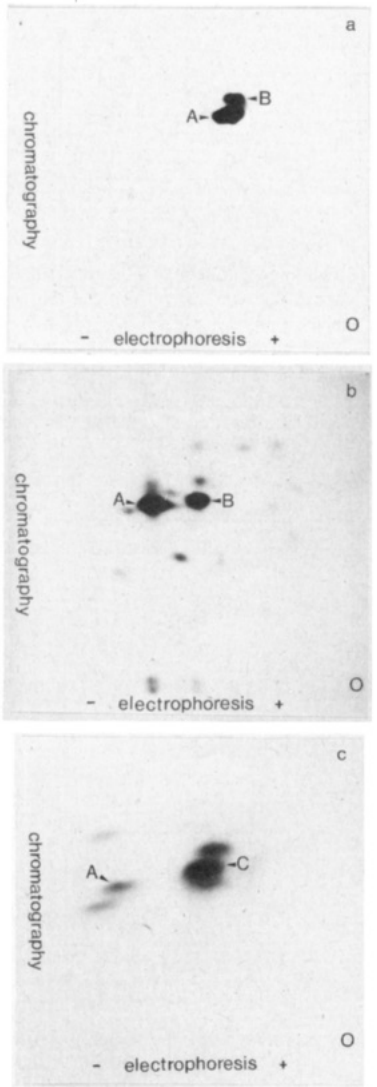


FIGURE 3: Autoradiograms of the two-dimensional peptide maps of fractions I–III. A total of 2×10^5 cpm was spotted onto the thin-layer plate for each peptide. Fluorescamine staining did not indicate the presence of nonradioactive peptides. (O) Origin; (a) fraction I; (b) fraction II; (c) fraction III.

C. None of the weak spots could be identified. The yield of the three peptides A–C summed in the three fractions I–III is given in Table II. The fact that the relative yields of peptides A and B did not significantly change when the enzyme was labeled with either a stoichiometric amount or a 2-fold excess of $tRNA_f^{Met}$(ox) makes it unlikely that either modification could be due to a nonspecific reaction of the tRNA dialdehyde.

The amino acid compositions of peptides A–C, shown in Table I, were in satisfactory agreement with those expected for positions 334–340, 61–65, and 141–165, respectively, of the known MTS_T primary structure (Barker et al., 1982). The lysine peak was absent from peptide A and B analyses, and

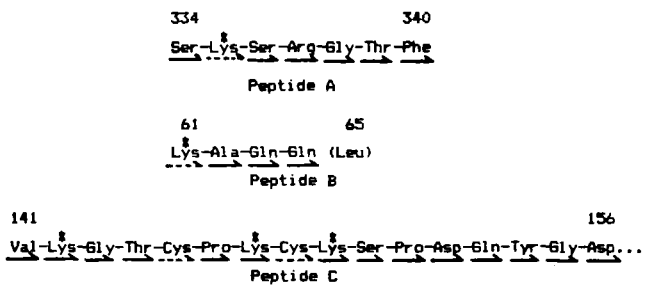
Table III: Automated Edman Degradation of Radioactive Peptides

cycle no.	residue no. in the sequence of MTS_T	amino acid	yield (nmol)	radioactivity (cpm)
		Peptide A (2.3 nmol)		
1	334	Ser		1340
2	335	modified Lys		11120
3	336	Ser		1720
4	337	Arg	0.5	620
5	338	Gly	0.8	
6	339	Thr	0.4	
7	340	Phe	0.3	
		Peptide B (2.5 nmol)		
1	61	modified Lys		14340
2	62	Ala	0.57	320
3	63	Gln	0.56	1660
4	64	Gln	0.30	1080
		Peptide C (2.3 nmol)		
1	141	Val	1.15	260
2	142	modified Lys		5120
		Lys	0.29	
3	143	Gly	0.68	1740
4	144	Thr	0.33	800
5	145			1380
6	146	Pro	0.37	620
7	147	modified Lys		2180
		Lys	0.09	
8	148			1140
9	149	modified Lys		2000
		Lys	0.12	
10	150	Ser		820
11	151	Pro	0.25	560
12	152	Asp	0.18	
13	153	Gln	0.20	
14	154	Tyr	0.15	
15	155	Gly	0.28	
16	156	Asp	0.18	

it was smaller than expected for peptide C. Instead, two small new peaks were observed in the histidine region. One of them only was found in the analysis of a hydrolysate of N^{α}-acetyl-L-lysyl-[^{14}C]$ATP_{ox\text{-}red}$, synthesized as in Easterbrook-Smith et al. (1976). These peaks were radioactive. Furthermore, such an analysis, as well as that of a hydrolysate of [^{14}C]$ATP_{ox\text{-}red}$, showed a significant amount of [^{14}C]glycine; this amino acid must come from the hydrolytic degradation of the adenine ring. This probably explains the systematically high glycine figure in the amino acid compositions of the peptides.

Finally, identification of the labeled peptides A–C was confirmed by automated degradation (Table III and Chart I). When the phenylthiohydantoins were identified by high-pressure liquid chromatography, no PTH-Lys was observed at the positions of lysine-335 (peptide A) or lysine-61 (peptide B). Instead, a new peak appeared close to PTH-Val (19.50 min vs. 19.30 min); radioactivity scanning along the profile showed that this peak contained all the ^{14}C label. In the case of peptide C, both PTH-Lys and the new PTH peak were present at the three lysine positions, and some radioactivity was released at each of those cycles, as indicated in Table

Chart I: Amino Acid Sequence Determined for Peptides A–C[a]



[a] (Solid arrow) Residues positively identified as PTH-amino acids; (broken arrow) residues that could not be identified; (asterisk) residues for which radioactivity was found associated with the PTH fraction. For peptide C, see text.

III. This showed that the three lysines in peptide C were all fractionally labeled.

Discussion

Affinity labeling of MTS_T with $tRNA_f^{Met}$(ox) yielded a covalent 1:1 complex (Fayat et al., 1979; Hountondji et al., 1979), with complete loss of both aminoacylation and isotopic ATP–PP_i exchange activities. Nevertheless, the sequence work described in this paper has led to the isolation of two major labeled peptides, A and B, and a minor one, C. The radioactivity associated with each one represents 65–75, 20–25, and 2–4%, respectively, of the radioactivity eluted from the plates (Table II).

These figures should not however be taken at face value. First of all, each peptide may have a different elution yield from the plate. Second, due to the proximity of the spots on thin layers, their excision from the plate was not equally quantitative for each peptide. Finally, it is clear that the yield of peptide C is underestimated, and this is for two reasons. First, according to the sequence it possesses four cysteines, which were not blocked before proteolysis and purification. They certainly suffered some oxidation, which could easily explain the cloud of spots always observed on the fingerprints of fraction III. Second, peptide C contains two tyrosines. Limit chymotryptic peptides corresponding to peptide C may have been eluted from Sephadex with fraction II. Indeed, fingerprints of fraction II showed a number of weak spots, but they were not identified (Figure 3b).

Another way of estimating the proportion of the peptides comes from the use of amino acid analyses of Sephadex fractions I–III before fingerprinting (not shown). Leucine is present only in peptide B, arginine and phenylalanine are present only in peptide A, and tyrosine is present only in peptide C. Using the amount of each of these amino acids in the various fractions, a proportion of 42% label on peptide A, 39% on peptide B, and 16% on peptide C can be deduced for experiment 1. This calculation assumes that, after the second Sephadex step, all unlabeled peptides had been eliminated and that no other labeled peptides were present than peptides A–C. In conclusion, the amount of labeling of peptide C is certainly not as small as indicated in the first paragraph of this section. Nevertheless, it remains that the bulk of the label attached to the protein was distributed between lysines-61 and -335, with definite preference for the latter.

Peptide B (lysine-61) has been located in the crystallographic structure of MTS_T (Zelwer et al., 1982; Blow et al., 1983). It lies in the middle of a short peptide linking a β-strand (β_B) and an α-helix (α_C) of the "mononucleotide binding fold" of the synthetase (Risler et al., 1981; Blow et al., 1983). It is reasonable to assume that this region represents the catalytic center of the enzyme, since this is where the substrate ATP has been observed to bind (Risler et al., 1981). It can therefore be expected that the 3′-end of an enzyme-bound tRNA points toward this region in order to react with methionyl adenylate.

Peptide A (lysine-335) has not yet been as precisely located in the 3-D structure of the synthetase. However, it is part of the C-terminal domain of the synthetase, which is more than 50 Å away from lysine-61 (S. Brunie, J. L. Risler, and C. Zelwer, personal communication). It is difficult to conceive how the CCA arm of the enzyme-bound tRNA could be mobile enough to allow the 3′-aldehyde groups of $tRNA_{ox}$ to react with such distant residues, unless the enzyme changes conformation upon binding tRNA. It is worthwhile noting that small-angle neutron scattering studies of the MTS_T–$tRNA_f^{Met}$ complex have not indicated gross changes of the protein structure compared with the free enzyme (Dessen et al., 1982). Another possibility can be envisioned where tRNA can adopt several ways of binding to the synthetase. Nevertheless, in this case, the extent of labeling of lysines-61 and -335 may not directly reflect the proportions of the different enzyme–tRNA complexes formed, since the respective lysines may present a different reactivity to Schiff base formation.

On the other hand, it is remarkable that, upon labeling, both activities of the enzyme (ATP–PP_i exchange and $tRNA^{Met}$ aminoacylation) are abolished (Fayat et al., 1979; Hountondji et al., 1979), although distant lysines are hit. This is difficult to explain, in particular in the case of the ATP–PP_i exchange reaction, which involves small substrates. It could reflect that the presence in its binding site of a tRNA molecule covalently attached to the enzyme by any of the identified lysines causes the blocking at a distance of the ATP–PP_i exchange. Such an effect is discussed in Jacques & Blanquet (1977). Alternatively, it may reflect that all the reacted lysines are necessary for catalysis and that modification of one of them abolishes the activation reaction of methionine or its reversal by pyrophosphate.

To investigate the case, the ATP–PP_i exchange activity of the covalent 1:1 enzyme–tRNA complex has been measured after treatment with ribonuclease A. Upon hydrolysis of tRNA, the complex did not recover more than 10% of the initial activity of the synthetase (results not shown). Since this recovery is less than the relative amounts of labeling of either lysine-61 (20–25%) or lysine-335 (65–75%), we are led to conclude that both these lysines must be intact to ensure enzyme activity. It should be added that preliminary experiments carried out with native dimeric methionyl-tRNA synthetase indicated peptide A to be again the most abundant labeled one.

The location of peptide C in the crystallographic structure of the synthetase corresponds to a large peptide linking an α-helix (α_{CD}) to a β-strand (β_D) of the "mononucleotide binding fold" (Risler et al., 1981: Blow et al., 1983). As shown under Results, the three lysines in this peptide can react with oxidized tRNA (lysines-142, -147, and -149). This lysine cluster may present a high reactivity toward the dialdehyde function, and thus, it could be unspecifically labeled by the $tRNA_{ox}$ molecule.

The functional importance of the synthetase region corresponding to lysine-335 is further evidenced by comparison of the primary structure of methionyl-tRNA synthetase from *E. coli* with that from *Saccharomyces cerevisiae* (Walter el al., 1983). The two proteins showed a high degree of homology within the nucleotide binding fold region. This homology includes lysine-61 of the *E. coli* synthetase that is found in the yeast enzyme. Nevertheless, the region of highest homology precisely concerns the lysine-335 region in the C-terminal

domain of the *E. coli* synthetase: the amino acid sequence Ser-Lys-Ser-Arg-Gly is found in both primary sequences.

Only one other example of labeling an aminoacyl-tRNA synthetase with the oxidized form of the cognate tRNA has been published thus far. For yeast phenylalanyl-tRNA synthetase, Renaud et al. (1982) have determined the sequence of the major labeled peptide. It does not resemble the sequences identified in this work. It would be of interest to also compare its location in the complete primary structure, which is not yet available.

Added in Proof

Webster et al. (1984) have just deduced the amino acid sequence of *E. coli* isoleucyl-tRNA synthetase from its DNA sequence. They pointed out the existence of 10 identities in an 11 residue long stretch in isoleucyl-tRNA synthetase (residues 58–68) and in methionyl-tRNA synthetase (residues 16–26). We point out that Lys-335 in methionyl-tRNA synthetase can be matched with Lys-605 in isoleucyl-tRNA synthetase. Residues 330–338 in the former show seven identities with residues 600–608 in the latter.

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References

Akhverdyan, V. Z., Kisselev, L. L., Knorre, D. G., Lavrik, O. I., & Nevinsky, G. A. (1977) *J. Mol. Biol. 113*, 475–501.

Barker, D. J., Ebel, J. P., Jakes, R., & Bruton, C. (1982) *Eur. J. Biochem. 127*, 449–457.

Blanquet, S., Iwatsubo, M., & Waller, J. P. (1973) *Eur. J Biochem. 36*, 213–226.

Blanquet, S., Dessen, P., & Fayat, G. (1979) *Cold Spring Harbor Monogr. Ser. 9B*, 281–294.

Blow, D. M., Bhat, T. N., Metcalfe, A., Risler, J. L., Brunie, S., & Zelwer, C. (1983) *J. Mol. Biol. 171*, 571–576.

Bruton, C. J., & Hartley, B. J. (1970) *J. Mol. Biol. 52*, 165–178.

Cassio, D., & Waller, J. P. (1971a) *Eur. J. Biochem. 20*, 283–300.

Cassio, D., & Waller, J. P. (1971b) *FEBS Lett. 12*, 309–312.

Chen, R. (1976) *Hoppe-Seyler's Z. Physiol. Chem. 357*, 873–886.

Dardel, F., Fayat, G., & Blanquet, S. (1984) *J. Bacteriol. 160*, 1115–1122.

Dessen, P., Fayat, G., Zaccai, G., & Blanquet, S. (1982) *J. Mol. Biol. 154*, 603–613.

Easterbrook-Smith, S. B., Wallace, J. C., & Keech, D. B. (1976) *Eur. J. Biochem. 62*, 125–130.

Fayat, G., Fromant, M., & Blanquet, S. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2088–2092.

Fayat, G., Hountondji, C., & Blanquet, S. (1979) *Eur. J. Biochem. 96*, 87–92.

Guêron, M., & Leroy, J. L. (1978) *Anal. Biochem. 91*, 691–694.

Hountondji, C., Fayat, G., & Blanquet, S. (1979) *Eur. J. Biochem. 102*, 247–250.

Jacques, Y., & Blanquet, S. (1977) *Eur. J. Biochem. 79*, 433–441.

Lederer, F., Ghrir, R., Guiard, B., Cortial, S., & Ito, A. (1983) *Eur. J. Biochem. 132*, 95–102.

Renaud, M., Fasiolo, F., Baltzinger, M., Boulanger, Y., & Rêmy, P. (1982) *Eur. J. Biochem. 123*, 267–274.

Risler, J. L., Zelwer, C., & Brunie, S. (1981) *Nature (London) 292*, 384–386.

Schimmel, P. R. (1977) *Acc. Chem. Res. 10*, 411–418.

Waller, J. P., Risler, J. L., Monteilhet, C., & Zelwer, C. (1971) *FEBS Lett. 16*, 186–188.

Walter, P., Gangloff, J., Bonnet, J., Boulanger, Y., Ebel, J. P., & Fasiolo, F. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 2437–2441.

Webster, et al. (1984) *Science (Washington, D.C.) 226*, 1315–1317.

Wetzel, R., & Soll, D. (1977) *Nucleic Acids Res. 4*, 1681–1694.

Zelwer, C., Risler, J. L., & Brunie, S. (1982) *J. Mol. Biol. 155*, 63–81.