The Structural Basis for the Resistance of Escherichia coli Formylmethionyl Transfer Ribonucleic Acid to Cleavage by Escherichia coli Peptidyl Transfer Ribonucleic Acid Hydrolase*

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SUMMARY

Escherichia coli formylmethionyl-tRNA\textsuperscript{Met} is unique among N-acylaminocyl-tRNAs in its resistance to cleavage by peptidyl-tRNA hydrolase. Chemical modification of tRNA\textsuperscript{Met} with sodium bisulfite converts fMet-tRNA\textsuperscript{Met} into a good substrate for the hydrolase. The products of the enzymatic cleavage are free tRNA\textsuperscript{Met} and formylmethionine. Bisulfite treatment produces cytidine to uridine base changes at several sites in the tRNA structure. One of these modifications results in formation of a new hydrogen-bonded base pair at the end of the acceptor stem of tRNA\textsuperscript{Met}. We have shown that this modification is responsible for the observed change in biological activity.

Enzymatic cleavage appears to be facilitated by the presence of a 5'-terminal phosphate at the end of a fully base-paired acceptor stem, because removal of the 5'-phosphate group from N-acetylphenylalanyl-tRNA\textsuperscript{Phi} or bisulfite-modified fMet-tRNA\textsuperscript{Met} reduced the rate of hydrolysis of these substrates. The unpaired base at the 5' terminus of unmodified fMet-tRNA\textsuperscript{Met} appears to reduce susceptibility of the tRNA to hydrolytic attack both by positioning the 5'-phosphate in an unfavorable orientation and by directly interfering with enzymatic binding. The unusual structure of the acceptor stem of this E. coli tRNA thus plays a critical role in maintaining the viability of the organism by preventing enzymatic cleavage of the fMet group from the bacterial initiator tRNA.

Peptidyl-tRNA hydrolase catalyzes the hydrolysis of N-acylaminoacyl-tRNAs and peptidyl-tRNAs to free tRNA and N-acyl amino acid or peptide (1-7). Unsubstituted aminoacyl-tRNAs are resistant to attack. The enzyme has been found in a wide variety of species, including Escherichia coli, yeast, wheat germ, and mammalian systems (1-10). E. coli peptidyl-tRNA hydrolase has been highly purified (11) and has been shown to be an essential enzyme for normal protein metabolism (12, 13).

Several groups have reported that N-substituted derivatives of the E. coli initiator tRNA are unique in their resistance to cleavage by peptidyl-tRNA hydrolase (2, 3, 5, 14, 15). In this report, we show that the presence of an unpaired base at the 5' terminus of tRNA\textsuperscript{Met} is the structural feature responsible for the resistance of fMet-tRNA\textsuperscript{Met} to hydrolysis by the enzyme.

EXPERIMENTAL PROCEDURE

Materials

Radioactive amino acids and [γ-\textsuperscript{32}P]ATP were obtained from New England Nuclear Corp. N-hydroxyquinuimine and dicyclohexylcarbodiimide were purchased from Eastman Organic Chemicals. Spectro grade ethyl acetate containing under nitrogen was obtained from BDH Chemicals and dimethylsulfoxide from Fisher Chemicals. DEAE-cellulose was purchased from Schleicher and Schuell, benzoylated DEAE-cellulose from Boehringer Mannheim, and carboxymethylcellulose (CM52) from Whatman.

Unlabeled N-acetylphenylalanine and N-formylmethionine were purchased from Sigma Chemicals and unlabeled amino acids from Schwarz BioResearch. N-hydroxyquinuimine acetate acid was prepared as described by Lapidot et al. (16). The crude material was recrystallized four times from water and the resulting product gave a single spot, R\textsubscript{f} 0.74, on paper chromatography in 1-butanol-acetic acid-water. Formyl[\textsuperscript{14}C]methionyladenosine was prepared by incubation of 150 pmol of f[\textsuperscript{14}C]Met-tRNA\textsuperscript{Met} with 5 mg of pancreatic RNase ( Worthington, RNase A) in 0.05 M Tris-HCl, pH 7.5, in a total volume of 0.2 ml at 37° for 30 min.

Polyribonucleotide kinase was the generous gift of Dr. Robert Billoet and Dr. V. O. Malathi, New York University Medical Center. E. coli alkaline phosphatase, electrophoretically purified to remove ribonuclease, was obtained from Worthington Biochemical Corp. Crystalline bovine albumin was purchased from Miles Laboratories.

Crude E. coli K12 tRNA was obtained from General Biochemicals and was used for the purification of E. coli tRNA\textsuperscript{Met} as described previously (17).

Crude E. coli tRNA aminocyl synthetase was prepared from E. coli strain Q13 by the method of Muench and Berg (18). Methionine tRNA synthetase was partially purified as described by Maroker (19).
Methods

Paper Chromatography—Paper chromatography was carried out by the descending technique using Whatman No. 1 filter paper. The solvent system was 1-butanol-acetic acid-water (78:17:17, by volume).

Unlabeled amino acids were detected by ninhydrin. N-blocked amino acids, N-hydroxyxycinnimide, and N-hydroxysucinimide acetic acid ester were detected as described by Lapidot et al. (16). When radioactive compounds were chromatographed, the paper was cut into 25 strips (1.5 cm) and was counted in a liquid scintillation counter using 10 ml of a toluene 2,5-diphenyloxazole (PPO)-1,4-bis[2-(5-phenyloxazoly)]benzene (POPOP) fluor.

Preparation of N-Substituted Aminoacyl-tRNAs—The procedure for bisulfite modification of tRNA\textsuperscript{Met} is reported elsewhere (20, 21). Bisulfite-modified and unmodified f[\textsuperscript{35}S]Met-tRNA\textsubscript{Met} and [\textsuperscript{3}H]Phe-tRNA\textsuperscript{Phs} were prepared as described previously (22). N-Acetyl-[\textsuperscript{3}H]Phe-tRNA\textsuperscript{Phs} was prepared by the method of DeGroot et al. (23) from [\textsuperscript{3}H]Phe-tRNA\textsuperscript{Phs} and the N-hydroxysucinimide ester of acetic acid. The product (44 A\textsubscript{260}) was recovered by ethanol precipitation, was washed twice with 1 ml portions of ethanol, and was dissolved and stored in 5 mM potassium acetate, pH 4.6, at −20°C. An aliquot was treated with 0.5 M NH\textsubscript{4}OH for 1 hour at 37°C and was analyzed by paper chromatography. All of the radioactivity migrated as N-acetyl[\textsuperscript{3}H]phenylalanine and none could be detected in the position of the free amino acid.

Terminal 5′-phosphate groups were removed from tRNA samples by treatment with E. coli alkaline phosphatase at 65°C as described previously (24). The dephosphorylated tRNAs contained no chain breaks as determined by chromatography on Sephadex G-100 at 55°C in 10 mM sodium phosphate, pH 7.5, and 0.1 M NaCl (25, 26) and accepted the same amount of amino acid as the corresponding untreated tRNAs. N-Substituted derivatives of the dephosphorylated aminoacyl-tRNAs were prepared as described above for the untreated tRNAs.

Purification of Peptidyl-tRNA Hydrolase—Peptidyl-tRNA hydrolase was purified by a modification of the method of Kjellén (11). A high speed supernatant (S-150) was prepared from 28 g of E. coli MRE 600 cells as described elsewhere (24) and was added directly to a column (2.4 × 60 cm) of DEAE-cellulose which had been equilibrated with standard buffer containing 10 mM Tris-HCl, pH 7.3, 10 mM MgCl\textsubscript{2}, 10 mM 2-mercaptoethanol, and 0.1 mM EDTA at 4°C. The column was eluted with standard buffer and fractions (7 ml) were collected at a flow rate of 0.5 ml per min. The absorbance at 280 nm was measured and the fractions of the first broad peak were combined (0.1 M). This material is referred to as "partially purified enzyme." The hydrolase was further purified by application of the DEAE-fraction to a CM-cellulose column (1.3 × 30 cm; N\textsubscript{a}H\textsubscript{4}O\textsubscript{4} form) and elution with standard buffer at a flow rate of 0.5 ml per min until the absorbance dropped to 0.1 A\textsubscript{260}. Enzyme with standard buffer containing 0.3 M KCl was started and 1-ml fractions were collected. Peptidyl-tRNA hydrolase activity was assayed by incubating 50 μl of each fraction with 10 μm N-acetyl-[\textsuperscript{3}H]Phe-tRNA\textsuperscript{Phs} and measuring the decrease in trichloroacetic acid-insoluble radioactivity in 20 min at 37°C. The bulk of the hydrolytic activity was found in Fractions 30 to 52 which were pooled and dialyzed twice against 2 liters of standard buffer for 1 hour each. The sample was added to a 5-ml column of CM-cellulose and the column was washed with 15 ml of standard buffer. Elution with standard buffer containing 0.3 M KCl was started and 0.5-ml fractions were collected and assayed as above. The hydrolytic activity was concentrated in Fractions 12 and 13, which were dialyzed against 2 liters of standard buffer for 2 hours, divided into small aliquots, frozen, and stored at −20°C. Protein was assayed by the method of Bucher (27) using crystalline bovine albumin as standard. Peptidyl-tRNA hydrolase (31 μl) having specific activity of 2000 units/ml (isomolar to that reported for the homogeneous enzyme (11) with two different substrates was obtained (see "Results").

Assay for Peptidyl-tRNA Hydrolase Activity—Reaction mixtures (50 μl) contained 0.1 M sodium cacodylate, pH 6.9, 10 mM MgCl\textsubscript{2}, 0.2 mg per ml of bovine albumin, 7 to 10 μm N-substituted [\textsuperscript{3}H]aminoacyl-tRNA, and enzyme as indicated. The reactions were started by the addition of enzyme and simultaneously shifting the temperature from 0°C to 37°C. Aliquots (10 to 40 μl) were removed at various times and were assayed for residual trichloroacetic acid-insoluble radioactivity as described previously (28).

Separation of 5′-32P-Labeled f[\textsuperscript{35}S]Met-tRNA\textsuperscript{Met} and 5′-32P-Labeled Decylated tRNA\textsuperscript{Met} on Benzoylated DEAE-cellulose—Bisulfite-modified 5′-32P-labeled tRNA\textsuperscript{Met} was prepared as described previously (24). The labeled tRNA was aminoclaylated with [\textsuperscript{3}H]methionine and formylated with unlabeled formyltetrahydrofolate (22).

A modification of the procedure of Samuel and Rubinsteinowitz (29) was used to separate fMet-tRNA\textsuperscript{Met} and deacylated tRNA\textsuperscript{Met}. Samples were mixed with 100 A\textsubscript{260} of crude carrier tRNA in 1 ml of starting buffer and were applied to a column (1 × 24 cm) of benzoylated DEAE-cellulose equilibrated at 4°C. Elution was carried out with solutions containing 10 mM MgCl\textsubscript{2} and 10 mM sodium acetate, pH 4.5, using a linear gradient from 0.4 to 2 M NaCl over 600 ml. Fractions (4 ml) were collected at a flow rate of 15 ml per hour. Aliquots (0.5 ml) were diluted with 0.5 ml of water and were counted in 10 ml of Aquasol (New England Nuclear Corp.). Fractions were pooled, deacetylated by dialysis, and concentrated by ethanol precipitation.

6′-Terminal Nucleotide Analyses—\textsuperscript{32}P-Labeled tRNAs were hydrolyzed to nucleotides by incubation with 0.3 n KOH at 37°C for 18 hours. The released \textsuperscript{32}P-nucleoside diphosphates were analyzed as described previously (24, 30).

RESULTS

Bisulfite-modified fMet-tRNA\textsuperscript{Met} is Attacked by Peptidyl-tRNA Hydrolase—Treatment of E. coli formylmethionine tRNA with sodium bisulfite at 25°C results in conversion of 6 cytidine residues in the tRNA to uridine residues (20, 21). We have shown previously (21) that four sites in tRNA\textsuperscript{Met} can be heavily modified without loss of methionine or formate acceptor activity (Fig. 1), whereas modifications at C\textsubscript{15}, C\textsubscript{16}, and C\textsubscript{17} inactive the molecule. Purified tRNA\textsuperscript{Met} was treated with 3 n NaHSO\textsubscript{3} for 18 hours at 25°C, resulting in the loss of approximately 95% of the original methionine acceptor activity, essentially complete modification of C\textsubscript{15} and 50 to 80% modification of C\textsubscript{16}, C\textsubscript{17}, and C\textsubscript{18}. The remaining active molecules were enzymatically aminoacylated and formylated. Fig. 2 compares the rate of hydrolysis of bisulfite modified fMet tRNA\textsuperscript{Met} with the hydrolysis of unmodified fMet-tRNA\textsuperscript{Met} and NAcPhe-tRNA\textsuperscript{Phs} at different
concentrations of purified peptidyl-tRNA hydrolase. The specific activity of our enzyme preparation using NAcPhe-tRNA\(^{\text{Phe}}\) or unmodified fMet-tRNA\(^{\text{Met}}\) as substrate was found to be the same as that reported by Kössel (11) for the homogeneous enzyme. In contrast to the low rate of cleavage of the unmodified initiator tRNA at all enzyme concentrations tested, bisulfite-modified fMet-tRNA\(^{\text{Met}}\) was rapidly hydrolyzed. Hydrolysis of the N-substituted aminoacyl-tRNAs in the absence of enzyme was less than 2% in all cases.

**Products of Cleavage of Bisulfite-modified fMet-tRNA\(^{\text{Met}}\)**

In order to determine whether the cleavage of bisulfite-modified fMet-tRNA\(^{\text{Met}}\) occurred in a manner analogous to that reported for hydrolysis of other N-substituted aminoacyl-tRNAs, the products resulting from treatment of the modified tRNA with peptidyl-tRNA hydrolase were analyzed. The data in Fig. 3 show that one product of the reaction is free tRNA\(^{\text{Met}}\). Bisulfite-modified fMet-tRNA\(^{\text{Met}}\) which had been enzymatically hydrolyzed to the extent of approximately 50% could be recharged with methionine to the same level as a control sample incubated in the absence of hydrolase (Fig. 4). Analysis of the radioactivity attached to the tRNA at the beginning of the experiment showed that it was at least 97% f\(^{\text{14C}}\)Met. After incubation with the hydrolase and recharging, the tRNA was precipitated with trichloroacetic acid. The label bound to the tRNA was released by base hydrolysis and was analyzed by paper chromatography, yielding 47% f\(^{14\text{C}}\)Met and 53% f\(^{\text{35S}}\)Met. The control sample incubated in the absence of hydrolase contained approximately 90% f\(^{14\text{C}}\)Met and 10% f\(^{35\text{S}}\)Met when analyzed in the same way.

The other product obtained on treatment of bisulfite-modified fMet-tRNA\(^{\text{Met}}\) with peptidyl-tRNA hydrolase was free fMet. Fig. 4 shows the analysis of a reaction mixture after approximately 50% enzymatic hydrolysis. Only two radioactive spots were observed on paper chromatography. The unhydrolyzed tRNA was found at the origin and the remaining radioactivity migrated with the same mobility as an authentic fMet marker. A control sample incubated with an equivalent amount of enzyme buffer showed very little nonenzymatic hydrolysis.

These results exclude the possibility of hydrolysis of bisulfite-modified fMet-tRNA\(^{\text{Met}}\) by a nucleolytic cleavage of the modified 3' terminal C-C-A-OH sequence and show that peptidyl-tRNA hydrolase attacks the modified initiator tRNA in the same manner that it attacks other N-substituted aminoacyl-tRNAs. Fully Base-Paired Acceptor Stem is Required for Efficient Cleavage of Bisulfite-modified fMet-tRNA\(^{\text{Met}}\) by Peptidyl-tRNA Hydrolase—One or more of the cytidine to uridine base changes in the structure of bisulfite-modified fMet-tRNA\(^{\text{Met}}\) alters the molecule in such a way that it becomes a good substrate for cleavage by peptidyl-tRNA hydrolase. In order to determine whether the base change at the 5' terminus is responsible for the change in biological activity of the molecule, enzymatic hydrolysis was carried out for various times and the 5'-terminal nucleotides in the remaining (fMet-tRNA\(^{\text{Met}}\)) were analyzed (Table I).

The deacetylated and formylated tRNAs were separated by gradient elution from benzoylated DEAE-cellulose at acid pH (29). Fig. 5 shows the control experiment in which bisulfite-modified \(^{35\text{S}}\)P-labeled tRNA\(^{\text{Met}}\) and f\(^{\text{35S}}\)Met-tRNA\(^{\text{Met}}\) were mixed and co-chromatographed. The deacylated tRNA eluted as a single peak. The \(^{35\text{S}}\)S elution profile showed a small peak at the breakthrough of the column which contained free f\(^{\text{35S}}\)Met. The bulk of the f\(^{\text{35S}}\)Met-tRNA\(^{\text{Met}}\) was separated from tRNA\(^{\text{Met}}\) except for a small shoulder which eluted just after the peak of...
deacylated tRNA. The elution position of this peak is close to that of Met-tRNA$^{Met}$, but no unformylated methionine could be detected in the sample of fMet-tRNA$^{Met}$ used in these experiments, so the small peak may be a different conformational form of bisulfite-modified f$^{[35S]}$Met-tRNA$^{Met}$.

The experiments summarized in Table I were carried out as follows: tRNA$^{Met}$ was treated with sodium bisulfite under conditions leading to partial modification of each of the sites indicated on Fig. 1. The partially modified tRNA was then treated with E. coli alkaline phosphatase under conditions leading to complete removal of 5'-terminal phosphate groups (31). The dephosphorylated tRNA was treated with [γ-$^{32}$P]ATP and polynucleotide kinase, resulting in partial $^{32}$P-labeling of the 5' terminus. The $^{32}$P-labeled tRNA was mixed with excess unlabeled bisulfite-modified tRNA$^{Met}$ and enzymatically aminocylated and formylated. The resulting 5',3' labeled f$^{[35S]}$Met-tRNA$^{Met}$ was hydrolyzed to the extent of approximately 17% or 40% with purified peptidyl-tRNA hydrolase. Reactions were stopped by adjusting the pH to 4.5 and the samples were chromatographed on benzoylated DEAE-cellulose as shown in Fig. 5. Inasmuch as small amounts of $^{32}$P-labeled tRNA were used for these experiments, $^{32}$P radioactivity could not be monitored on the column and fractions containing unhydrolyzed f$^{[35S]}$Met-tRNA$^{Met}$ were pooled, hydrolyzed with 0.5 N KOH, and analyzed for $^{32}$P-labeled nucleoside diphosphates as described under "Methods." The results are compared with those obtained for similar amounts of unreacted fMet-tRNA$^{Met}$ following chromatography on benzoylated DEAE-cellulose. The numbers in parentheses indicate the calculated values for the untreated samples which exactly correspond to the total counts per min used in the hydrolase-treated samples.

**Table I**

| Percent of enzymatic hydrolysis | $^{32}$P in 5'-terminal nucleotides | Ratio of 5'-terminal U/C in bisulfite-modified fMet-tRNA$^{Met}$ |
|--------------------------------|-------------------------------------|--------------------------------------------------------------|
|                                 | pCp                                | pUp                                                         |
| 0                               | 750 (706)                          | 384 (301)                                                   |
| 17                              | 329 (308)                          | 162 (152)                                                   |
| 40                              | 850                                | 206                                                         |
| 253                             |                                     | 23                                                         |

a This value represents a maximum because deacylated tRNA$^{Met}$ is not completely separated from unhydrolyzed fMet-tRNA$^{Met}$ (see Fig. 5). The deacylated fraction in this experiment is approximately 4 times larger than the remaining fMet-tRNA$^{Met}$ fraction and contains bisulfite-inactivated molecules (5% of the total tRNA; $^{32}$P in 5'-terminal U/C = 0.5), active, nonenzymatically deacylated molecules (approximately 7% of the total tRNA; $^{32}$P in 5'-terminal U/C = 0.6), active, bisulfite-modified molecules (49% of the total tRNA; this fraction contains a large peak of 3'-poly(32P)uridylation). A 5% contamination of the remaining fMet-tRNA$^{Met}$ fraction with the deacylated fraction could completely account for the $^{32}$P found as pUp in this experiment. This degree of cross-contamination would not result in a significant error for the fMet-tRNA$^{Met}$ samples containing a higher ratio of 5'-terminal U/C.
pooled using 35S radioactivity. Prior to treatment with hydrolase, the fMet-tRNA^Met fraction contained 5'-terminal uridine and cytidine in a ratio of about 0:1 (Table 1). Enzymatic cleavage was found to result in a rapid decrease in the amount of 5'-terminal uridine in the remaining fMet-tRNA^Met, indicating preferential attack on molecules containing the fully base-paired acceptor stem. After 17% of the total 32P-labeled fMet-tRNA^Met had been hydrolyzed, approximately 43% of the tRNA containing 5'-terminal uridine had been cleaved, whereas only about 3 to 4% of the molecules having a 5'-terminal cytidine had been attacked. After 40% of the total 32P-labeled fMet-tRNA^Met had been hydrolyzed, essentially all of the tRNA containing 5'-terminal uridine had been cleaved, whereas less than 20% of the tRNA having a 5'-terminal cytidine had been attacked.

In order to determine whether the modification at C1 was solely responsible for the observed change in biological activity, we compared the initial rates of cleavage of fMet-tRNA^Met molecules modified with bisulfite for various times. Fig. 6 shows that a linear first order plot is obtained for the rate of enzymatic cleavage as a function of the extent of cytidine to uridine conversion at C1. This result rules out the possibility of a two-hit mechanism in which modifications at both C1 and at another site are required to convert fMet-tRNA^Met into an active substrate for the hydrolase. Similar plots of rate versus mole fraction of C, gave curves which deviated significantly from linearity (not shown). Although we cannot exclude the possibility of a small contribution (either positive or negative) to the rate of cleavage resulting from these other modifications, the major change in the susceptibility of E. coli fMet-tRNA^Met to attack by peptidyl-tRNA hydrolase following bisulfite treatment is accounted for by the cytidine to uridine base change at the 5' terminus.

**Role of 5'-Terminal Phosphate in Substrate Susceptibility to Cleavage by Peptidyl-tRNA Hydrolase**—In order to characterize further the structural requirements for cleavage of N-substituted aminoacyl-tRNA by peptidyl-tRNA hydrolase, we have compared the rates of hydrolysis of substrates containing a 5'-phosphate with those containing a 5'-OH group (Fig. 7).

Removal of the 5'-phosphate from NAcPhe-tRNA^Phe was found to reduce drastically the rate of hydrolysis of this tRNA by the hydrolase (Fig. 7A). Removal of the 5'-phosphate from partially bisulfite-modified fMet-tRNA^Met (70% 5'-uridine, 30% 5'-cytidine; 338 cpm per pmol) had a less dramatic effect but still caused substantial reduction in hydrolytic activity (Fig. 7B). Unmodified fMet-tRNA^Met having a 5'-OH group was cleaved at the same low rate as the tRNA with a 5'-terminal phosphate (Fig. 7C).

In control experiments, the dephosphorylated N-substituted AA-tRNAs were found to be as active as the corresponding untreated tRNA when assayed for initiation of polypeptide chains in an in vitro protein-synthesizing system, indicating that the removal of phosphate groups had not caused nonspecific loss of biological activity.

**DISCUSSION**

The exact role of peptidyl-tRNA hydrolase in vivo is not known; however, it is believed to hydrolyze peptidyl-tRNA molecules carrying incomplete proteins which have been prematurely released from ribosome (32). This processing of free oligopeptidyl-tRNAs regenerates free tRNA and is required for normal protein metabolism (12, 13).

*E. coli* fMet-tRNA^Met is the only N-substituted aminoacyl-tRNA which has been found to be resistant to attack by peptidyl-tRNA hydrolase (2, 3, 5, 14, 15). Inasmuch as formylation is required for initiation of protein synthesis in prokaryotes (33), resistance of tRNA^Met to this enzyme is essential for the survival of bacterial organisms.

Previous studies have shown that it is the tRNA moiety which is responsible for the resistance of N-substituted derivatives of the initiator tRNA to hydrolysis. fMet-, N-acetyl-Met-, and Gly-Gly-Met-derivatives of the noninitiator methionine tRNA, tRNA^Met, are readily cleaved under conditions where little hydrolysis of the corresponding tRNA^Met compounds is observed (11, 14, 15). The data presented here shows that the unpaired
nucleotide at the 5' terminus of the E. coli initiator tRNA is the structural feature responsible for its unique resistance to enzymatic hydrolysis. Bisulfite-modified fMet-tRNA\textsuperscript{Met} is cleaved by purified peptidyl-tRNA hydroxase at the same rate as fMet-tRNA\textsuperscript{Met} (15) and approximately 15 times faster than unmodified fMet-tRNA\textsuperscript{Met}. The bisulfite modification produces a C\textsubscript{1} \rightarrow U\textsubscript{1} base change and results in formation of a normal U\textsubscript{1},A\textsubscript{2} base pair at the end of the acceptor stem. This change in local secondary structure also alters the spatial orientation of the 5'-terminal phosphate group. Previous studies from this laboratory have shown that the presence of the 5'-phosphate at the end of a fully base-paired helix is essential for the formation of stable complexes between the bacterial elongation factor Tu and aminoacyl-tRNAs (24). The data presented in this paper suggest an additional role for the 5'-phosphate of the tRNA moiety in the interaction of N-substituted aminoacyl-tRNAs with peptidyl-tRNA hydroxase. The decrease in susceptibility to hydrolysis of NAcPhe-tRNA\textsuperscript{Phe} and bisulfite-modified fMet-tRNA\textsuperscript{Met} on removal of the 5'-terminal phosphate group is in keeping with data from other laboratories showing that 3'-terminal N-substituted aminoacyl-oligonucleotides are poor substrates for the enzyme (1, 2, 5, 7).

The difference in the effect of dephosphorylation on hydrolysis of NAcPhe-tRNA\textsuperscript{Phe} and bisulfite-modified fMet-tRNA\textsuperscript{Met} may reflect the difference in affinity of the hydroxase for the N-blocking group on each tRNA. The nature of the peptide portion has been found to influence the efficiency of hydrolysis of peptide-tRNAs, those containing two peptide bonds being cleaved more readily than those containing only one peptide bond (2, 23). Formylated derivatives of aminoacyl-tRNAs also have been reported to be hydrolyzed faster than the corresponding N-acetyl compounds (3, 23). Binding to the 5'-phosphate of the tRNA may play a relatively more important role in stabilizing the interaction of the enzyme with poorer substrates such as NAcPhe-tRNA\textsuperscript{Phe}.

Removal of the 5'-phosphate from unmodified fMet-tRNA\textsuperscript{Met} was found to have no effect on the rate of hydrolysis of this tRNA. Thus, the position of the 5'-phosphate cannot be solely responsible for the unique resistance of tRNA\textsuperscript{Met} to hydrolysis. Modified fMet-tRNA\textsuperscript{Met} having a 5'-OH is cleaved at least twice as fast as unmodified fMet-tRNA\textsuperscript{Met} (either 5'-OH or 5'-P), indicating that the unpaired base itself, even without the terminal phosphate, interferes with the interaction between peptidyl-tRNA hydroxase and the initiator tRNA.

Our previous studies on the structural requirements for recognition of aminoacyl-tRNAs by elongation factor Tu showed that a cytidine to uridine base change in the common 3' terminal C-C-A-OH sequence reduced the affinity of AA-tRNA for this protein (24). The bisulfite-modified fMet-tRNA\textsuperscript{Met} used in the present studies contained 70 to 80% uridine at position 75 in the C\textsubscript{2}C\textsubscript{72}A-OH sequence but was cleaved by purified peptidyl-tRNA hydroxase at a rate comparable to that reported for unmodified fMet-tRNA\textsuperscript{Met} (15). Thus, the C\textsubscript{2} \rightarrow U\textsubscript{2} modification appears to have little or no effect on the rate of hydrolysis of fMet-tRNA\textsuperscript{Met} when experiments are carried out in the presence of excess enzyme.

The structural features which appear to be important for efficient hydrolysis of tRNA derivatives by peptidyl-tRNA hydroxase are an amino acid in the L configuration (2), the presence of at least 1 peptide bond (1-4), and a tRNA having a fully base-paired acceptor stem terminated by a 5'-phosphate group.

Acknowledgment—The authors would like to thank John Menninger for a stimulating discussion which initiated this work and for helpful advice on preparation of peptidyl-tRNA hydrolase.

REFERENCES

1. Cuzin, F., Kretschmer, N., Greenberg, R. E., Hurwitz, R., and Chaseville, F. (1967) Proc. Natl. Acad. Sci. 55, 2079-2086
2. Kössel, H., and Raj Bhandary, U. L. (1968) J. Mol. Biol. 36, 539-560
3. Vogel, Z., Zamir, A., and Elson, D. (1968) Proc. Natl. Acad. Sci. 61, 701-707
4. De Groot, N., Panet, A., and Lapidot, Y. (1968) Biochem. Biophys. Res. Commun. 31, 37-42
5. Chaseville, F., Yot, P., and Paulin, D. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 403-408
6. De Groot, N., Groner, Y., and Lapidot, Y. (1969) Biochim. Biophys. Acta 185, 296-296
7. Menninger, J. R., Mulholland, M. C., and Stirewalt, W. S. (1970) Biochim. Biophys. Acta 217, 496-511
8. Jost, J. P., and Bock, R. M. (1969) J. Biol. Chem. 244, 5866-5870
9. Paulin, D., Yot, P., and Chaseville, F. (1968) Fed. Eur. Biochem. Soc. Lett. 1, 163-165
10. Beun, C., Paulin, D., Yot, P., and Chaseville, F. (1971) Biochimie 53, 225-231
11. Kössel, H. (1970) Biochim. Biophys. Acta 204, 191-202
12. Atherly, A. G., and Menninger, J. R. (1972) Nature New Biol. 240, 245-246
13. Menninger, J. R., Walker, C., Tan, P. F., and Atherly, A. G. (1972) Mol. Gen. Genet. 121, 307-324
14. Vogel, Z., Zamir, A., and Elson, D. (1968) Biochem. Biophys. Res. Commun. 33, 94-98
15. Lapidot, Y., Inbar, D., DeGroot, N., and Kössel, H. (1969) Fed. Eur. Biochem. Soc. Lett. 3, 253-256
16. Lapidot, Y., De Groot, N., and Fat-Shapsher, I. (1967) Biochim. Biophys. Acta 145, 292-299
17. Schulman, L. H. (1971) J. Mol. Biol. 58, 117-131
18. Munch, K. H., and Berg, P. (1969) in Proceedings in Nucleic Acid Research (Cantoni, G. L., and Davies, D. R., eds) pp. 375-383, Harper and Row, New York
19. Marcker, K. (1966) J. Mol. Biol. 14, 63-70
20. Goddard, J. P., and Schulman, L. H. (1972) J. Biol. Chem. 247, 3864-3867
21. Schulman, L. H., and Goddard, J. P. (1973) J. Biol. Chem. 248, 1341-1345
22. Schulman, L. H., and Hecht, M. O. (1973) Biochim. Biophys. Res. Commun. 51, 275-282
23. DeGroot, N., Groner, Y., and Lapidot, Y. (1969) Biochim. Biophys. Acta 190, 296-298
24. Schulman, L. H., Pelka, H., and Sundari, R. M. (1974) J. Biol. Chem. 249, 7102-7110
25. Imura, N., Schwam, H., and Chambers, R. W. (1969) Proc. Natl. Acad. Sci. 62, 1203-1209
26. Schmidt, J., and Reid, B. R. (1970) Biochem. Biophys. Res. Commun. 41, 1261-1265, (1970) Biochem. Biophys. Acta 213, 539-541
27. Büchler, T. (1947) Biochim. Biophys. Acta 1, 292-314
28. Reeves, R. H., Imura, N., Schwam, H., Weiss, G. B., Schulman, L. H., and Chambers, R. W. (1968) Proc. Natl. Acad. Sci. 56, 1450-1457
29. Samuel, C. E., and Rabinowitz, J. C. (1972) Anal. Biochem. 47, 244-252
30. Sjölander, R., Malkaitis, V. C., Schulman, L. H., Hurwitz, J., and Duesberg, P. H. (1973) Biochim. Biophys. Res. Commun. 50, 467-472
31. Haranessis, D. R., and Hilmo, R. J. (1962) Biochem. Biophys. Res. Commun. 9, 393-397
32. Lapidot, Y., and DeGroot, N. (1972) in Progress in Nucleic Acid Research and Molecular Biology (Davidson, J. N., and Cohn, W. E., eds) Vol. 12, pp. 189-228, Academic Press, New York
33. Lucas-Lenard, J., and Lipmann, F. (1971) Annu. Rev. Biochem. 40, 409-448
The structural basis for the resistance of Escherichia coli formylmethionyl transfer ribonucleic acid to cleavage by Escherichia coli peptidyl transfer ribonucleic acid hydrolase.

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*J. Biol. Chem.* 1975, 250:542-547.

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