Structural Requirements for Aminoacylation of *Escherichia coli* Formylmethionine Transfer RNA†

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ABSTRACT: Previous studies from this laboratory have described the effects of chemical modification of specific cytidine, uridine, and guanosine residues in *Escherichia coli* formylmethionine tRNA on methionine acceptor activity. In the present work, we have determined the effects of chloroacetaldehyde modification of five adenosine residues on the ability of the tRNA to be aminoacylated. Modification of the A residue in the middle of the anticodon (A_{36}) by this reagent inactivates the molecule, while modification of an A residue in the TψC loop (A_{58}) or of A residues at the acceptor end (A_{73}, A_{74}, and A_{77}) does not. Alteration of the 3′-CCA terminus reduces the rate of aminoacylation. Consideration of all the available modification data indicates that essential structural features for recognition of $tRNA^{fMet}$ by *E. coli* methionyl-tRNA synthetase are present in the anticodon, the acceptor stem, and possibly the variable loop. Modifications at any one of the essential sites are accompanied by loss of ability to effectively interact with the enzyme in addition to loss of methionine acceptor activity. Inactivating modifications which occur in the 3′-terminal CCA sequence do not prevent interaction of $tRNA^{fMet}$ with methionyl-tRNA synthetase, but appear to interfere with the catalytic step. Other structural features play an important but nonessential role in aminoacylation, possibly by orienting the tRNA in a manner which facilitates access of the enzyme to essential ligands. A large number of modifications have no effect on the reaction, indicating that many nucleotides in the tRNA play no role in aminoacylation.

Previous work from this laboratory has been concerned with determination of the structural requirements for recognition of *Escherichia coli* $tRNA^{fMet}$[1] by *E. coli* methionyl-tRNA synthetase (Schulman and Pelka, 1977; Stern and Schulman, 1977; Schulman and Goddard, 1973; Schulman, 1970, 1971, 1972). Earlier studies described the effects of chemical modification of specific cytidine, uridine, and guanosine residues in $tRNA^{fMet}$ on methionine acceptor activity. In this paper, we report the effect of modification of five adenosine residues on the ability of the tRNA to be enzymatically aminoacylated, and discuss the presently available modification data in terms of the overall structural requirements for this reaction.

Experimental Procedure

Chloroacetaldehyde was purchased from Pfaltz and Bauer, diluted to a concentration of 2 M with water, and distilled under reduced pressure. Purified *E. coli* $tRNA^{fMet}$ and *E. coli* methionyl-tRNA synthetase were prepared as described previously (Schulman and Pelka, 1977; Schulman, 1971). Snake venom phosphodiesterase was purified from crude venom of *Crotalus adamanteus* by the procedure of Dolapchiev et al. (1974) and tRNA nucleotidyltransferase was purified from baker's yeast using the method of Rether et al. (1974).

Reaction mixtures for chloroacetaldehyde modification of $tRNA^{fMet}$ contained 20 A_{260} units/mL of tRNA and 1.0 M chloroacetaldehyde in 20 mM potassium acetate, 10 mM magnesium acetate, pH 6.0. After incubation at 25 °C for various times, aliquots were removed, and 0.1 vol of 1 M KCl, 0.5 M Tris-HCl (pH 7.5) and 2 vol of ethanol were added. The tRNA was collected by centrifugation and reprecipitated twice from 0.1 M KCl, 10 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.5) with 2 vol of ethanol. Assays for methionine acceptor activity were carried out as previously described (Schulman, 1970). The initial rate of aminoacylation of $tRNA^{fMet}$ was measured under conditions where methionine acceptance increased linearly with time and with methionyl-tRNA synthetase concentration, as described earlier (Schulman and Pelka, 1977).

Large scale aminoacylation and separation of active and inactive chloroacetaldehyde-modified $tRNA^{fMet}$ were carried out as described before (Schulman and Pelka, 1977). The procedures used for ribonuclease digestion of modified tRNA, chromatography of oligonucleotides on RPC-5 columns, and nucleoside analysis were as previously reported (Schulman and Pelka, 1976).

The modified 3′ terminus of chloroacetaldehyde-treated $tRNA^{fMet}$ was removed by limited digestion with purified snake venom phosphodiesterase and a normal 3′-terminal CCA sequence was resynthesized as described earlier (Schulman et al., 1974) using tRNA nucleotidyltransferase purified from yeast.

Results

Modification of $tRNA^{fMet}$ with Chloroacetaldehyde. Chloroacetaldehyde reacts with adenosine and cytidine residues in nucleic acids to yield 1,N^6-ethenoadenosine (ϵA) and 3,N^4-ethenocytidine (ϵC) derivatives (Kochetkov et al., 1971; Barrio et al., 1972; Secrist et al., 1972). Treatment of purified *E. coli* $tRNA^{fMet}$ with 1 M chloroacetaldehyde in 10 mM magnesium acetate, 20 mM potassium acetate (pH 6.0) was found to result in loss of methionine acceptor activity. Inactivation occurred with pseudo-first-order kinetics, indicating that modification at a single site is sufficient to eliminate activity. The half-life of the reaction was 93 min at 25 °C.

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[1] Abbreviations used are: $tRNA^{fMet}$, the *E. coli* initiator methionine tRNA (a mixture of $tRNA^{fMet}_1$ and $tRNA^{fMet}_3$, which differ from each other by an m^7G to A base change at position 47 from the 5′ terminus); $tRNA^{Met}_m$, the *E. coli* noninitiator methionine tRNA; MetRS, *E. coli* methionyl-tRNA synthetase; ϵA, 1,N^6-ethenoadenosine; ϵC, 3,N^4-ethenocytidine; DEAE, diethylaminoethyl; ac^4C, N^4-acetylcytidine; NMR, nuclear magnetic resonance; UV, ultraviolet; dansyl, 8-dimethylamino-1-naphthalenesulfonate; Tris, tris(hydroxymethyl)aminomethane.

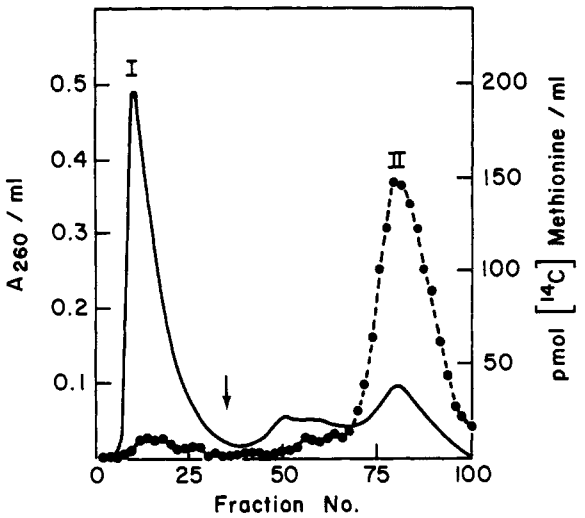

FIGURE 1: Separation of active and inactive molecules of tRNA^{fMet} after chloroacetaldehyde treatment; 84 A_{260} units of chloroacetaldehyde-modified tRNA^{fMet} was aminoacylated with methionyl-tRNA synthetase and then reacted with phenoxyacetic acid *N*-hydroxysuccinimide ester as described before (Schulman and Pelka, 1977). The derivatized sample in 5 mL of a solution containing 0.3 M NaCl, 10 mM $MgCl_2$, and 10 mM sodium acetate (pH 4.5) was added to a column (2.2 × 17 cm) of benzoylated DEAE-cellulose. The column was washed with 100 mL of the same buffer. The inactive fraction was then eluted with a solution containing 0.8 M NaCl, 10 mM $MgCl_2$, and 10 mM sodium acetate (pH 4.5) and 9-mL fractions were collected at a flow rate of 1 mL/min. At the position marked by an arrow, elution was started with solutions containing 1 M NaCl, 10 mM $MgCl_2$, and 10 mM sodium acetate (pH 4.5) using a linear gradient from 0 to 40% ethanol over 1 L: (—) A_{260} units per mL; (●) pmol of [^{14}C]methionine per mL.

Separation of Active and Inactive Molecules of Chloroacetaldehyde-Modified tRNA^{fMet}. In order to determine which modifications are responsible for the observed inactivation, 100 A_{260} units of purified tRNA^{fMet} were modified with chloroacetaldehyde to an extent which resulted in 64% loss of activity. The remaining active molecules were enzymatically aminoacylated with methionine. The resulting Met-tRNA^{fMet} was converted to *N*-phenoxyacetyl-Met-tRNA^{fMet} as described by Gillam et al. (1968) and separated from unacylated tRNA^{fMet} by chromatography on benzoylated DEAE-cellulose (Figure 1). Peak I contained the chloroacetaldehyde-inactivated tRNA plus a small amount of acylated tRNA which had failed to be derivatized with the phenoxyacetic acid ester and eluted with the inactive fraction. After isolation, the methionine acceptor activity of tRNA^{fMet} from peak I fractions 8–28 was 19% of that of the starting material.

Peak II contained *N*-phenoxyacetyl-Met-tRNA^{fMet}. A portion of the inactive tRNA eluted just ahead of the active molecules and contaminated the front portion of peak II. Fractions 78–100, having the highest methionine/A_{260} ratio, were therefore pooled. After isolation and removal of the *N*-phenoxyacetyl group, the methionine acceptor activity of modified tRNA^{fMet} from peak II was 94% of that of the starting material.

Sites of Modified Residues in Active and Inactive Chloroacetaldehyde-Modified tRNA^{fMet}. The locations of modified A and C residues following chloroacetaldehyde treatment of tRNA^{fMet} have been reported (Schulman and Pelka, 1976). The active and inactive fractions of modified tRNA^{fMet} were each analyzed in a similar manner for the sites of ϵA and ϵC residues following digestion with RNase T_1 and fractionation of the oligonucleotides by chromatography on RPC-5. Figure 2 compares the oligonucleotide profiles obtained from digestion

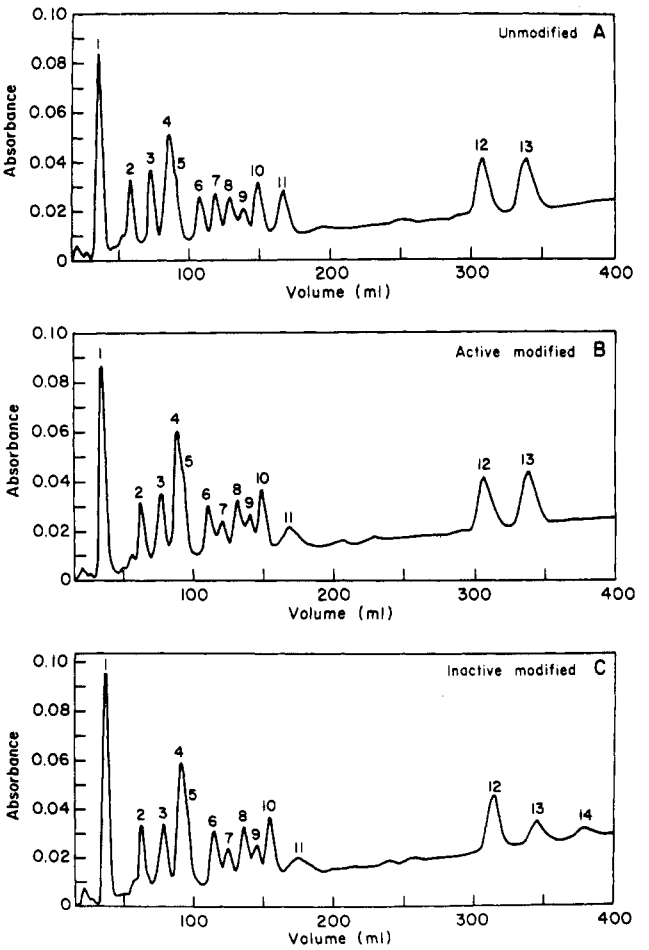

FIGURE 2: Fractionation of oligonucleotides obtained from RNase T_1 digestion of tRNA^{fMet} on RPC-5 at pH 7.5: (A) unmodified tRNA^{fMet}; (B) active chloroacetaldehyde-modified tRNA^{fMet} (fractions 78–100, peak II, Figure 1); (C) inactive chloroacetaldehyde-modified tRNA^{fMet} (fractions 8–28; peak I, Figure 1). Chromatography was carried out as described previously (Schulman and Pelka, 1976).

of unmodified tRNA^{fMet}, active modified tRNA^{fMet}, and inactive modified tRNA^{fMet}. A decrease in absorbance of peak 7 indicated partial reaction of the C residues in the oligonucleotide C_{16}-C_{17}-U-Gp in both the active and inactive modified tRNA. The modified oligonucleotide was found to elute near the trailing edge of peak 6; thus the loss of absorbance from peak 7 can be taken as a measure of the total reaction at C_{16} + C_{17}.

Peak 11, containing the 3′-terminal C-A-A-C-C-A sequence, was also extensively modified in both active and inactive molecules. The broadness of the peak containing the modified oligonucleotide suggested the presence of multiple modifications, as had been observed previously following more extensive treatment of tRNA^{fMet} with chloroacetaldehyde (Schulman and Pelka, 1976). The broad peak obtained from RNase T_1 digestion of 20 A_{260} units of modified tRNA was pooled and further digested with pancreatic RNase and alkaline phosphatase. Modified A_{73}-A_{74}-C isolated by this procedure was analyzed for the location of ϵA residues by digestion of the oligonucleotide with purified snake venom phosphodiesterase in the presence and absence of alkaline phosphatase (Schulman and Pelka, 1976). In the absence of phosphatase, the only residue released as a nucleoside is A_{73}, while both A_{73} and A_{74} yield nucleosides in the presence of phosphatase. The extent of modification of A_{77} was taken as the difference between the total ϵA in C-A_{73}-A_{74}-C-C-A_{77} and that present in A_{73}-A_{74}-C.

TABLE I: Summary of the Modifications Present in Active and Inactive Chloroacetaldehyde-Treated $tRNA^{fMet}$.

Residue	mol of modified residue/mol of $tRNA^{fMet}$	
	Active	Inactive[a]
C_1	+[c]	+
$C_{16} + C_{17}$	0.30[b]	0.30[b]
C_{35}	0	0.35[b]
A_{36}	0	0.14
A_{58}	0.18	0.23
A_{73}	0.07	0.11
A_{74}	0.08	0.13
C_{75}	+	+
C_{76}	n.d.[d]	n.d.
A_{77}	0.15	0.25

[a] The "inactive" fraction (Figure 1, peak I, fractions 8–28) contains 19% active modified molecules. [b] Based on the amount of oligonucleotide shifted away from the normal elution position in the RNase T_1 oligonucleotide profile. [c] A plus sign indicates that the residue is modified. Quantitative data are not available (see text). [d] n.d. indicates not determined.

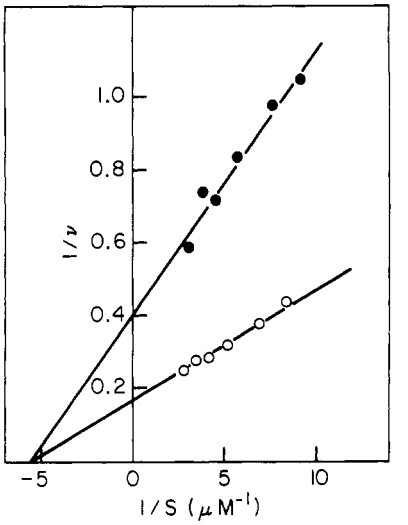

FIGURE 3: Kinetics of aminoacylation of chloroacetaldehyde-modified $tRNA^{fMet}$. Assay conditions are as described previously (Schulman and Pelka, 1977): (○) unmodified $tRNA^{fMet}$; (●) active chloroacetaldehyde-modified $tRNA^{fMet}$.

Previous studies have shown that there is no modification of C_{72} following chloroacetaldehyde treatment of $tRNA^{fMet}$, but that both C_{75} and C_{76} react with this reagent. The quantitative amount of ϵC at each position could not be determined, however, since it was found that the repeated chromatography of the oligonucleotide required for structural analysis resulted in progressive loss of the modified C derivative. Several unknown products were formed, one of which cochromatographed with unmodified cytidine on nucleoside analysis. Both active and inactive chloroacetaldehyde-modified $tRNA^{fMet}$ were found to show a loss of C_{75} by direct analysis of modified A-A-C_{75}; however, the relative amount of modification in the two tRNA fractions could not be determined and no definitive data on the modification of C_{76} could be obtained. Chloroacetaldehyde-modified adenosine residues were found to be completely stable to the experimental manipulations required for structural analysis.

The large oligonucleotide, C^m-U-C-A-U-A-A-C-C-C-Gp (peak 13, Figure 2), obtained from RNase T_1 digestion of the active fraction of modified $tRNA^{fMet}$ was found to contain no modifications and to have the same nucleoside content as the oligonucleotide isolated from unmodified $tRNA^{fMet}$. Previous studies (Schulman and Pelka, 1976) have shown that chloroacetaldehyde treatment of $tRNA^{fMet}$ results in modification of C_{35} and A_{36} in the anticodon of the tRNA, and both of these modifications were present in the inactive molecules. Modification of C_{35} resulted in elution of the oligonucleotide at higher salt concentration, giving rise to a new peak in the oligonucleotide profile (peak 14, Figure 2C). Modified A_{36} was approximately equally distributed between peaks 13 and 14; thus the formation of ϵA_{36} did not alter the elution position of the oligonucleotide. The loss of peak 13 was therefore taken as a measure of the amount of ϵC_{35}, while the amount of ϵA_{36} was determined by direct nucleoside analysis of the modified oligonucleotides.

Analysis of the other large oligonucleotide obtained from RNase T_1 digestion of $tRNA^{fMet}$, T-ψ-C-A-A-A-U-C-C-Gp (peak 12, Figure 2), revealed that ϵA was present in this sequence in both the active and inactive modified tRNA. Further structural analysis, carried out as previously described (Schulman and Pelka, 1976), showed that the site of the modified residue was A_{58} in both cases.

Examination of the oligonucleotide profiles obtained following pancreatic RNase digestion of chloroacetaldehyde-modified $tRNA^{fMet}$ revealed the presence of one additional modification at C_1. Loss of absorbance of pCp, containing the 5′-terminal cytidine residue, was observed in the pancreatic RNase profile of both the active and inactive modified tRNA. Structural alterations at this site have previously been shown to have no effect on aminoacylation of $tRNA^{fMet}$ (Schulman and Goddard, 1973).

A summary of the chloroacetaldehyde modifications present in active and inactive $tRNA^{fMet}$ is given in Table I. Since the inactive fraction of chloroacetaldehyde modified $tRNA^{fMet}$ (peak I, Figure 1) is contaminated with 19% active modified molecules, the anticodon modifications account for 60% of the observed loss of methionine acceptor activity. We have previously observed that modification of one or both of the C residues in the 3′-terminal CCA sequence of $tRNA^{fMet}$ by ultraviolet (UV) light or by treatment with sodium bisulfite inactivates this tRNA with respect to methionine acceptance (Schulman and Goddard, 1973; Schulman, 1970). It therefore seems likely that the additional loss of activity following chloroacetaldehyde treatment of $tRNA^{fMet}$ is also due to modification of C residues in the CCA sequence, although this has not been directly determined.

Kinetics of Aminoacylation of Chloroacetaldehyde-Modified $tRNA^{fMet}$. Modifications which are completely absent from active modified $tRNA^{fMet}$ prevent aminoacylation. In addition, a number of other modifications are found to be unequally distributed between the active and inactive molecules. This is particularly apparent in the case of the A residues in the 3′-terminal region of the molecule, and suggests that these structural modifications may alter the rate of aminoacylation. Examination of the isolated active fraction (peak II, Figure 1) showed that 94% of the molecules could be aminoacylated with methionine under ordinary assay conditions; however, measurement of the kinetics of aminoacylation revealed a 2.4-fold reduction in the rate of amino acid acceptance compared to unmodified $tRNA^{fMet}$ (Figure 3). In order to investigate the effects of chloroacetaldehyde modifications at the 3′ end of $tRNA^{fMet}$ on aminoacylation kinetics, the modified CCA sequence was removed by exonucleolytic digestion with snake venom phosphodiesterase and a normal CCA terminus was synthesized using tRNA nucleotidyltransferase.

The rate of aminoacylation of active modified tRNA^{fMet} following CCA repair was then compared with that exhibited by a sample of unmodified tRNA^{fMet} which had been treated in the same manner. The kinetic data obtained with the modified and unmodified tRNAs were indistinguishable, suggesting that alterations in the CCA sequence are largely responsible for the reduced rate of aminoacylation of active chloroacetaldehyde-modified tRNA[2].

McCutchan et al. (1976) have reported that incorporation of ϵA into the 3′ terminus of *E. coli* tRNA^{fMet} in place of the normal adenosine residue leads to loss of about half the methionine acceptor activity. Our data show that tRNA^{fMet} containing 0.15 mol of ϵA_{77}/mol of tRNA is not inactive, but has a substantially reduced rate of aminoacylation. Introduction of 1 mol of ϵA_{77}/mol of tRNA would be expected to have a much larger effect on the rate of the reaction. This could account for the apparent loss of methionine acceptor activity seen by McCutchan et al. since substantial reduction in the rate of aminoacylation of tRNAs often produces incomplete charging with amino acid due to an equilibrium between aminoacylation and a competing synthetase-catalyzed deacylation of aminoacyl-tRNA (Bonnet and Ebel, 1972). A decrease in the extent of charging of tRNA^{fMet} has previously been observed to result from an anticodon base modification which alters the kinetics of aminoacylation (Schulman and Pelka, 1977). The 6% lower extent of methionine acceptance exhibited by active chloroacetaldehyde-modified tRNA^{fMet} may therefore be due to the presence of ϵA_{77} in 15% of the molecules.

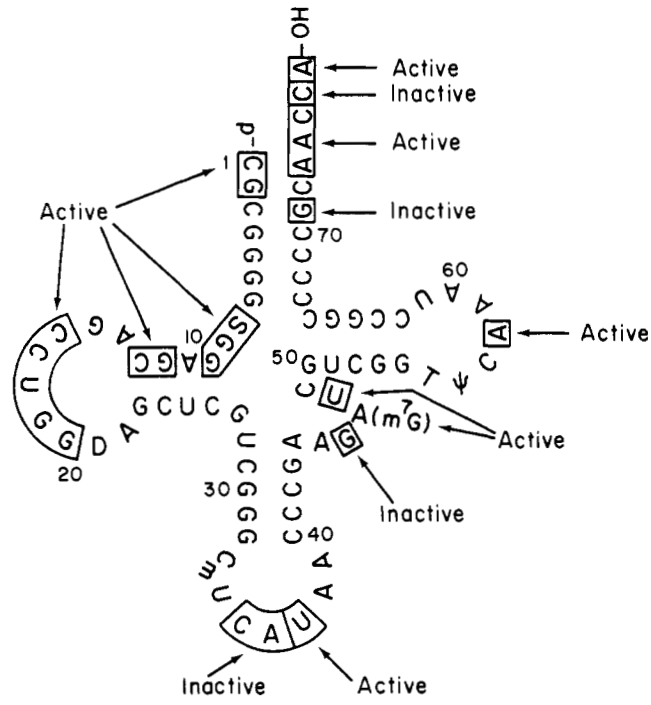

FIGURE 4: Sites of modified nucleotides in tRNA^{fMet} which have been studied in this laboratory. "Active" indicates residues which have been modified without loss of methionine acceptor activity. "Inactive" indicates sites where modification has resulted in complete loss of methionine acceptance.

Discussion

Structural alterations of 34 of the 77 nucleotides in tRNA^{fMet} have now been examined for their effect on methionine acceptor activity. The sites of chemical modifications which have been studied in this laboratory are indicated in Figure 4. Complete data on the effects of structural changes in tRNA^{fMet} on aminoacylation are summarized in Table II.

We have carried out our chemical modification studies under experimental conditions where the biologically active conformation of the tRNA can be maintained, i.e., in aqueous solution, near neutral pH, in the presence of Mg^{2+} and at moderate temperatures. In addition, we have used reagents which yield products that do not greatly increase the size of the nucleotide base. Under these conditions, a large number of chemical modifications have been found to have little or no effect on methionine acceptance by tRNA^{fMet}. These modifications provide the most clear-cut structure–function data since they require no interpretation. If alteration of a functional group is without effect, that functional group is not required either for recognition of the tRNA or for enzyme catalysis.

Meaningful analysis of inactivating modifications requires additional information about the effect of the structural alteration on the local conformation of the tRNA in the vicinity of the modified base, as well as its effect on the binding affinity of tRNA and enzyme. In cases where denaturing conditions have been used during the modification procedure, the effect of the structural change on the ability of the tRNA to refold into its native conformation must also be considered.

Inactivating modifications which do not produce indirect conformational effects may cause loss of biological activity by blocking or removing an essential enzyme binding site or by introducing a functional group which has a negative effect on the tRNA–protein interaction due to electrostatic or steric repulsion.

Only in the case of the anticodon base C_{35} has sufficiently complete information been obtained to justify identification of a specific functional group in tRNA^{fMet} as a probable ligand of MetRS. The remaining data indicate several other specific regions which are likely to contain binding sites for the enzyme and exclude a number of others.

The Anticodon Region. Conversion of C_{35} to U_{35} results in complete loss of methionine acceptor activity (Table II). The tRNA containing this modification is not an inhibitor of aminoacylation when present in 30-fold excess over active tRNA^{fMet}, indicating that the modified tRNA is unable to bind effectively to the synthetase, in addition to being catalytically inactive. The modified tRNA has a conformation which retains all of the secondary and tertiary N–H hydrogen bonds of unmodified tRNA^{fMet} as measured by high-resolution NMR spectroscopy (R. Römer, L. H. Schulman, and R. G. Shulman, to be published); thus the loss of ability to interact with MetRS is not due to loss of ordered structure. This indicates that C_{35} is an essential ligand of MetRS or that U_{35} has a strong negative effect on interaction with the enzyme. The structural differences between cytidine and uridine are confined to the N_3 and C_4 positions of the pyrimidine ring. Not surprisingly, formation of ϵC_{35} by modification with chloroacetaldehyde also inactivates tRNA^{fMet} since it alters both of these positions in the tRNA. The noninitiator methionine tRNA from *E. coli*, tRNA$^{Met}_m$, contains the minor base N^4-acetylcytidine (ac^4C) in the wobble position of the anticodon in place of the parent cytosine base found in tRNA^{fMet}. In order to investigate the effect of this structural change on recognition of the tRNA by MetRS, we prepared tRNA$^{Met}_m$ containing C_{35} in place of ac^4C_{35} and compared the kinetics of aminoacylation of the two species (Stern and Schulman,

[2] It is not clear whether or not tRNA^{fMet} containing ϵA_{73} or ϵA_{74} serves as a substrate for CCA repair by tRNA nucleotidyltransferase; thus these modifications could also contribute to the decreased rate of aminoacylation.

TABLE II: Effect of Modifications in the Structure of $tRNA^{fMet}$ on Methionine Acceptor Activity.

Site in $tRNA^{fMet}$	Modification	mol/mol of $tRNA^{fMet}$	Effect on methionine acceptor act.		Ref.[x]
			Extent[a] (%)	Kinetic parameters	
5′-P	5′-OH	1	Normal[b]	n.d.[c]	1, 2
	5′-Dansyl-p-p	1	30	n.d.	3
	5′-Ant-p-p[d]	1	30	n.d.	3
C_1	U_1	0.7	Normal	Normal	4
	ϵC_1	Min. 0.2	Normal	n.d.	5
G_2	Photooxidation	0.5	70–100[e]	Threefold higher K_m; normal V_{max}[e]	6
pC_1-G_2	Nucleolytic excision[f]	1	30–50	Tenfold higher K_m; ~20% lower V_{max}	7, 8
S_8	U_8	0.75–1	84	n.d.	9
	S_8 C_{13} cross-link	1	Normal	50% of normal rate	10
		1	Normal	Normal	11
	Cyanoethyl-S_8[g]	*h*	63	n.d.	12
	Dye-labeled S_8[i]	0.5	Normal	n.d.	13
	Spin-labeled S_8[j]	n.d.	Normal	n.d.	14
		1	$tRNA^{fMet}_1$: normal	n.d.	15, 16
		1	$tRNA^{fMet}_3$: 39	Initial rate 43% of normal	16
G_9	Photooxidation	0.3–0.5	70–100[e]	*e*	6
G_{10}	Photooxidation	0.5	70–100[e]	*e*	6
G_{12}	Photooxidation	0.5	70–100[e]	*e*	6
C_{13}	S_8 C_{13} cross-link	1	Normal	See S_8 above	10, 11
	UV photoproduct	0.4–0.6	Normal	Normal	17
C_{16}	U_{16}	0.3–0.5	Normal	Normal	4
	UV photoproduct[k]	0.3	Normal	Normal	*k*
C_{17}	U_{17}	0.3–0.5	Normal	Normal	4
C_{16}, C_{17}	One or both ϵC	0.3	Normal	n.d.	5
U_{18}	U_{18}-HSO_3^- adduct	1	70–100	Normal	18
	U_{18}-photohydrate	Min. 0.3	Normal	Normal	*k*
G_{19}	Photooxidation	0.3	70–100	*l*	19
G_{20}	Photooxidation	0.3	70–100	*l*	19
	AAF-G_{20}[m,n]	1[n]	40	Threefold higher K_m; normal V_{max}	20
	Single-strand break	1	Normal	Normal	21
C_{17}-G_{20}	Nucleolytic excision	1	Normal	n.d.	2
G_{15}-G_{20}	Nucleolytic excision[o]	1[o]	55–60	n.d.	2, 8
A_{14}-G_{20}	Nucleolytic excision	1	41–50	n.d.	2, 8
C_{13}-G_{20}	Nucleolytic excision	1	11	n.d.	2
G_{12}-G_{20}	Nucleolytic excision	1	4	n.d.	2
D_{21}	$NaBH_4$ reduction[g,p]	*h*	80	n.d.	22
	Removal of D base; attachment of PFSH[q,t]	1	21	n.d.	23
D_{21}-A_{22}-G_{23}	Nucleolytic excision	1	~20	Twofold higher K_m; ~20% lower V_{max}	8
G_{27}	m^2G_{27}	1	Normal	Little or no effect	24, 25
C_{35}	U_{35}	1	Inactive	30-fold excess causes no inhibition	4, 26
	ϵC_{35}	0.4[r]	Inactive		5
A_{36}	ϵA_{36}	0.2[r]	Inactive		5
U_{37}	U_{37}-photohydrate	Min. 0.5	Normal	Normal	17
	U_{37}-HSO_3^- adduct	1	70–80	One specific stereoisomer: four- to sixfold higher K_m; four- to sixfold lower V_{max}	18
G_{46}	Photooxidation	0.6[r]	Inactive	Ninefold excess causes no inhibition	6
m^7G_{47}	A_{47}	1	Normal	Higher K_m; higher V_{max}[s]	16, 18
U_{48}	U_{48}-photohydrate	Min. 0.5	Normal	Normal	17
	U_{48}-HSO_3^- adduct	1	70–100	Little or no effect	18
C_{49}	m^5C_{49}	0.6	Normal	n.d.	25
ψ_{56}	N_1-Cyanoethyl-ψ_{56}[g]	*h*	Inactive		12, 27
	N_1-BMB-ψ_{56}[i,t]	1	30	n.d.	28
A_{58}	ϵA_{58}	0.2	Normal	n.d.	5
A_{59}	m^1A_{59}	0.6	Normal	n.d.	25
		1	77	Little or no effect	24
G_{71}	Photooxidation	0.5[r]	Inactive	Threefold excess causes no inhibition	19
A_{73}	ϵA_{73}	0.07	Normal	n.d.	5
A_{74}	ϵA_{74}	0.08	Normal	n.d.	5
C_{75}	U_{75}	0.4	Normal	0–30% higher K_m; normal V_{max}	4, 26
	ϵC_{75}	n.d.	Normal	n.d.[u]	5
C_{76}	U_{76}	0.6[r]	Inactive	Inhibitor of aminoacylation[v]	4, 26
C_{75}-C_{76}	UV photoproduct[w]	0.8[r]	Inactive	Inhibitor of aminoacylation[v]	17, 29
A_{77}	ϵA_{77}	0.15	Normal	Normal K_m; ~twofold lower V_{max}[u]	5
		1	~50	n.d.	30

TABLE II (continued)

[a] Level at which aminoacylation plateaus in the presence of excess enzyme. [b] Changes of 10% or less have been ignored. [c] Not determined. [d] 5′-Anthraniloyl-p-p. [e] tRNA^{fMet} modified to the extent of 50% at each of the sites G_2, G_9, G_{10}, and G_{12} can be 100% aminoacylated under standard assay conditions; however, the extent of aminoacylation drops to 70–75% when the modified tRNA is stripped of methionine and reassayed. This modified tRNA has a threefold higher K_m than normal; V_{max} is unchanged. [f] The molecule also contains a single-strand break at G_{20}. [g] The site of reaction is based on the known specificity of the reagent. [h] Quantitative reaction is expected based on model compounds. [i] Modified with 4-bromomethyl-7-methoxy-2-oxo-2*H*-benzopyran. [j] N-(2,2,5,5-tetramethyl-3-pyrrolidinyl-1-oxy)-S_8. [k] Reaction at C_{17} may also occur but has not been directly demonstrated. An early report (Schulman, 1970) of UV modification of the CUCG sequence in tRNA^{fMet} was incorrect. [l] Modification of G_{19} and G_{20} causes no further change in the kinetic parameters for aminoacylation of photooxidized tRNA^{fMet} (see footnote *e*). [m] Modification of the 8 position of G_{20} with 2-acetylaminofluorene. [n] The molecule also contains 0.3 mol of AAF at other site(s). [o] Mixture of tRNA^{fMet} missing C_{16} through G_{20} and G_{15} through G_{20}. [p] S_8 is presumably also reduced. [q] Proflavinylsuccinylhydrazide. [r] Single hit kinetics indicate that modification at this site alone can inactivate tRNA^{fMet}. [s] 1.3- to 4-fold higher K_m and 0- to 2-fold higher V_{max} depending on assay conditions; also, L. H. Schulman and H. Pelka, unpublished observations. [t] Also $S_8 \rightarrow U_8$. [u] See text. [v] Significant inhibition of aminoacylation of active tRNA^{fMet} is observed when the inhibitor is present in equal concentration. [w] One or both are modified. [x] References to the table: (1) Schulman et al., 1974; (2) Seno et al., 1970; (3) Yang and Söll, 1973a; (4) Schulman and Goddard, 1973; (5) this work; (6) Schulman, 1971; (7) Seno et al., 1971; (8) Seno and Sano, 1971; (9) Walker and RajBhandary, 1972; (10) Berthelot et al., 1972; (11) Blanquet et al., 1973; (12) Siddiqui and Ofengand, 1970a; (13) Yang and Söll, 1973b; (14) Hara et al., 1970; (15) Daniel and Cohn, 1975; (16) Daniel and Cohn, 1976; (17) Schulman, 1970; (18) Schulman and Pelka, 1977; (19) Schulman, 1972; (20) Fujimura et al., 1972; (21) Seno et al., 1969; (22) Shugart and Stulberg, 1969; (23) Yang and Söll, 1974a; (24) Spremulli et al., 1974; (25) Shershneva et al., 1974; (26) Stern and Schulman, 1977; (27) Siddiqui and Ofengand, 1970b; (28) Yang and Söll, 1974b; (29) Schulman, unpublished results; (30) McCutchan et al., 1976.

1977). Identical K_m and V_{max} values were obtained for acetylated and unacetylated $tRNA^{Met}_m$ and both had a twofold greater affinity for MetRS than tRNA^{fMet}. Thus, the presence or absence of a free exocyclic amino group at C_{35} is of no importance for the interaction of tRNA^{Met} with the enzyme. Furthermore, it is evident that MetRS is not sensitive to small structural changes at this site, including the presence of a carboxyl group. The overall results therefore support a positive role for C_{35} in interaction with MetRS and point to the N_3 position of the cytidine base as a probable binding site for the enzyme.

Loss of methionine acceptor activity by formation of ϵA_{36} in the middle position of the anticodon of tRNA^{fMet} may be due to a requirement for interaction of the enzyme with N_1 or the exocyclic amino group of this purine base; however, a negative effect of this modification on interaction of MetRS with the adjacent base (C_{35}) cannot be ruled out at the present time.

Modifications at the 3′ end of the anticodon have not been found to inactivate tRNA^{fMet}. Addition of water to the 5,6-double bond of the pyrimidine base U_{37} by photohydration has no effect on the rate or yield of methionine acceptance. Saturation of the pyrimidine ring changes the electron distribution, as indicated by a shift in the pK of the N–H proton, and converts the planar base to a nonplanar puckered ring structure. It is also likely that this modification results in conversion of U_{37} from an anti to a syn nucleoside conformation in which the C_6 group is rotated away from the sugar, as has been reported for other C_6-substituted pyrimidines (Schweizer et al., 1971). Since these drastic changes in conformation and hydrogen bonding properties have no effect on aminoacylation, it can be concluded that MetRS does not interact with this anticodon base. In binding to the adjacent region of the anticodon, the enzyme must closely approach U_{37}, however, and recently it has been found that addition of bisulfite to the 5,6-double bond of this base alters the kinetic parameters for aminoacylation of tRNA^{fMet} (Table II). The bisulfite adduct has a structure analogous to the water adduct, but with an $-SO_3^-$ group at the C_6 position of the pyrimidine ring rather than an –OH group. Both types of adducts exist in two diastereomeric forms which differ only in the orientation of the C_6 substituent. Interestingly, it was observed that the effect on aminoacylation is due to one particular stereoisomer of the U_{37}-bisulfite adduct, suggesting steric or electrostatic interference with the approach of MetRS from one particular side of the anticodon loop.

A summary of the structural changes in the anticodon bases of the *E. coli* methionine tRNAs and their effect on methionine acceptor activity is given in Figure 5.

The Variable Loop. The variable loop joining the anticodon stem and the TψC stem in tRNA^{fMet} contains five nucleotides. The effects on aminoacylation of structural alterations in four of these bases have been investigated (Table II).

The sequence of nucleotides in the variable loop of yeast tRNA^{Phe} is identical with that found in *E. coli* $tRNA^{fMet}_1$ and the two tRNAs show the same pattern of reactivity in this region with single-strand specific chemical reagents (Rich and RajBhandary, 1976; Schulman and Pelka, 1976). The crystal structure of yeast tRNA^{Phe} shows that the variable loop is a structurally complex region which is connected to several other parts of the molecule through base–base and base–backbone interactions; thus most modifications in this region can be expected to be accompanied by changes in tertiary structure.

Photooxidation of G_{46} in the variable loop of tRNA^{fMet} destroys methionine acceptor activity and prevents effective binding of the tRNA to MetRS. In yeast tRNA^{Phe}, this G residue is stacked on the adjacent A residue and is hydrogen bonded through its exocyclic NH_2 group to the O_6 position of G_{10} in the dihydrouridine stem (Quigley and Rich, 1976). Photooxidation of G_{46} in tRNA^{fMet} would eliminate both of these interactions since it destroys the six-membered ring of the purine structure. Base–base interactions between G_{46} and G_{10} cannot be critical for aminoacylation of tRNA^{fMet}, however, since photooxidation of G_{10} would also eliminate such interactions, but is found not to inactivate the tRNA. The structural complexity of the variable loop region leaves several possible interpretations for the loss of activity due to modification of G_{46}: this base may be an essential ligand of MetRS, the photooxidized product may have a strong negative effect on interaction of the tRNA with MetRS, or the structural change may be accompanied by a loss of some nearby essential conformational feature.

Evidence that MetRS is sensitive to conformational changes in the variable loop region comes from a comparison of the two tRNA^{fMet} isomers, which differ by a single base substitution at position 47 and have different kinetic parameters for aminoacylation. Yeast tRNA^{Phe}, like $tRNA_1^{fMet}$, contains an m^7G residue in the variable loop. This base forms two hydrogen bonds with the G residue of the C_{13}-G base pair in the dihydrouridine stem and also stabilizes the association of these two structural regions through electrostatic interaction of the positively charged methylated base and negative phosphate

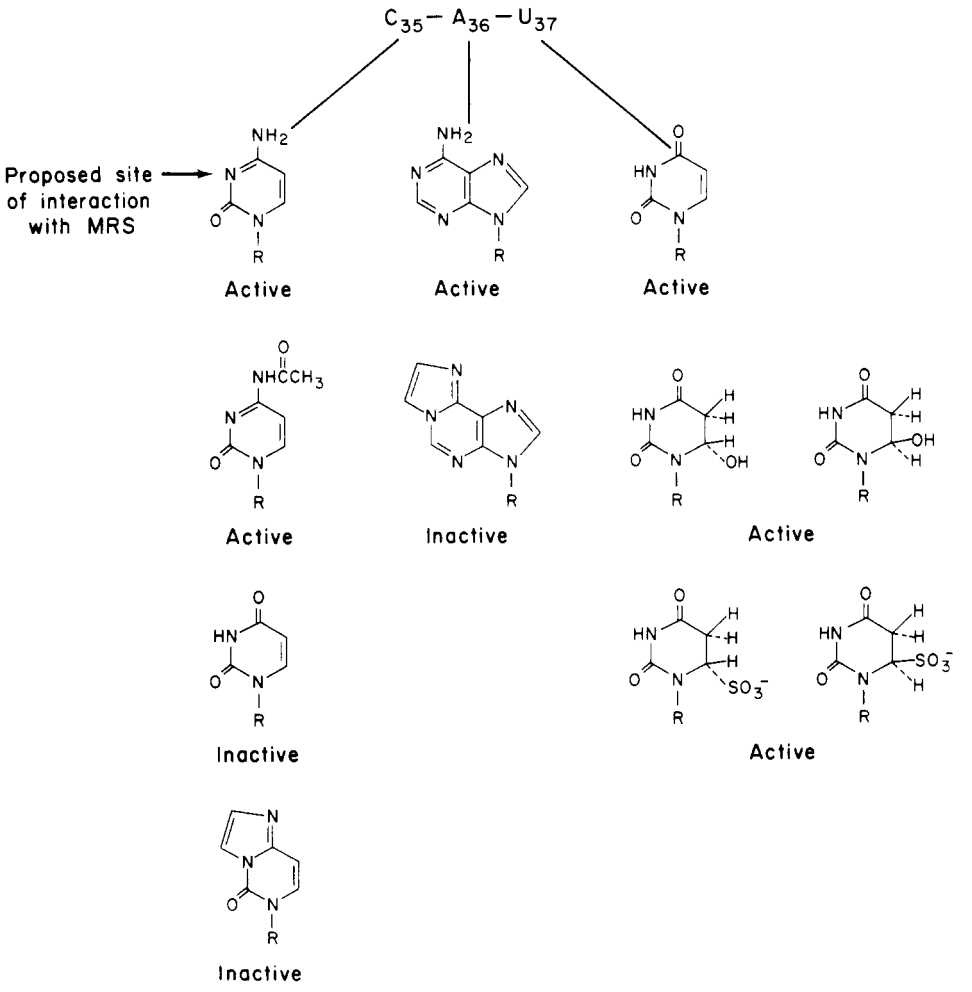

FIGURE 5: Effects of structural changes in the anticodon bases of *E. coli* methionine tRNAs on methionine acceptor activity.

groups in the stem (Rich and RajBhandary, 1976). Daniel and Cohn (1976) have demonstrated the expected tertiary structure hydrogen bond between m^7G_{47} and C_{13}-G_{23} in $tRNA^{fMet}_1$ by high-resolution NMR spectroscopy and shown that this interaction is absent from $tRNA^{fMet}_3$, containing A_{47} in the variable loop. Evidence that the two $tRNA^{fMet}$ isomers differ in tertiary structure in this central core region also comes from comparisons of the rate of UV-induced cross-linking of S_8 and C_{13} (Delaney et al., 1974) and from temperature-jump studies of the conformation of $tRNA^{fMet}_1$ and $tRNA^{fMet}_3$ (Crothers et al., 1974). The differences in kinetic parameters for aminoacylation of the two isomers vary with the assay conditions, showing a maximal difference of about fourfold in K_m and twofold in V_{max}. The N_3 position of the purine base is a potential hydrogen bonding site in both A_{47} and m^7G_{47}; however, the relatively small effect of this base change on aminoacylation suggests that the observed changes are probably due to local ordered structure differences which alter the interaction of the enzyme with some other ligand. Modifications at the adjacent base, U_{48}, are not sensed by MetRS, suggesting that this base is pointed away from any binding site. Methylation of C_{49} at the 5 position of the pyrimidine ring also causes no loss of methionine acceptor activity; however, the kinetic parameters for aminoacylation of the tRNA modified at this site have not been determined.

The Dihydrouridine Loop and Stem Region. Chemical modifications of six of the nine nucleotides in the dihydrouridine loop have been studied with respect to their effect on aminoacylation of $tRNA^{fMet}$ (Table II). In many cases, several different modification procedures have been used and none has been found to inactivate $tRNA^{fMet}$. In cases where no substantial increase in the size of the nucleotide base results from the modification, the tRNA shows normal aminoacylation kinetics and can be fully charged with methionine. Attachment of bulky substituents to G_{20} or D_{21} reduces the amount of amino acid which can be attached to $tRNA^{fMet}$ in the presence of excess MetRS, and in the case of G_{20}, this effect has been shown to be accompanied by an increased K_m for aminoacylation. Structural changes which alter the rate of aminoacylation compared to the competing synthetase-catalyzed deacylation of aminoacyl-tRNA can lead to a suboptimal steady-state level of charged tRNA (Bonnet and Ebel, 1972). In all cases which have been tested, changes in kinetic parameters have been found to accompany substantial reductions in the yield of methionine accepted by modified $tRNA^{fMet}$ (Table II). The lower level of aminoacylation caused by attachment of a bulky aromatic group to D_{21} may therefore also reflect a change in the rate of the forward reaction. The possibility that a portion of the altered tRNA is trapped in an inactive conformation cannot be excluded, however, since denaturing conditions were used during the modification procedure.

In addition to the chemical modifications which have been studied, the effect on aminoacylation of complete removal of nucleotides from the dihydrouridine loop by nucleolytic excision has been investigated (Table II). The combined data clearly indicate that none of the bases in the dihydrouridine loop is required for recognition by MetRS. Tertiary structure

interactions between the constant G residues at positions 19 and 20 and nucleotides in the TψC loop are also nonessential. In addition, the phosphate groups of residues 17 through 20 appear not to be involved in binding to the enzyme. Reduction in the level of aminoacylation of tRNA^{fMet} from which residues 14 and 15 have been excised could be due to failure of some of the tRNA fragments to reassociate properly, since both of these residues are believed to participate in tertiary structure interactions. Removal of residues D_{21}, A_{22}, and G_{23} has an even larger effect on aminoacylation, reducing the level to about 20% of normal. This could also reflect failure of some tRNA fragments to reconstitute properly; however, similar results are obtained by attaching bulky substituents to G_{20} and D_{21}, suggesting that the enzyme may closely approach this region of the molecule. Structural alterations here affect K_m with little change in V_{max}. Since specific nucleotide bases in the loop are not required for enzyme recognition, the data suggest the possibility of one or more phosphate–protein binding sites in this part of the sequence.

Three of the bases in the double-stranded stem adjacent to the dihydrouridine loop have been chemically modified without inactivation of tRNA^{fMet} (Table II). The modifications at C_{13} may not significantly change the ordered structure in this region; however, photooxidation of G_{10} and G_{12} leads to destruction of the purine bases and disruption of two base pairs in this stem. None of these modifications inactivate tRNA^{fMet}. Nucleolytic excision of C_{13} and G_{12} (in addition to residues 14 through 20) has a drastic effect on biological activity. Since these bases are not required for recognition by MetRS, this loss of activity is probably due to cumulative loss of ordered structure and/or protein–backbone interactions. Molecules of tRNA^{fMet} modified to the extent of 70% at $G_{10} + G_{12}$ are aminoacylated with normal V_{max} and a threefold higher K_m. These molecules are also modified at G_2, a site where structural alteration is known to affect K_m; therefore the modifications in the dihydrouridine stem cannot have a very large effect on interaction of tRNA^{fMet} with MetRS. In addition, photooxidation of G_9 and several types of modifications at S_8 fail to significantly affect aminoacylation, although both of these residues are believed to be involved in tertiary structure interactions which stabilize the conformation of the central core region of the molecule. It is possible that pyrimidine residues 24 through 26 contain a site which interacts with MetRS; however, the presently available data suggest that this stem region, like the adjoining loop, does not contain specific information for recognition of tRNA^{fMet}, but may contain nonspecific binding sites.

One modification near the dihydrouridine stem has been found to affect aminoacylation. Attachment of a bulky substituent to S_8 specifically alters the rate and extent of methionine acceptance by tRNA$^{fMet}_3$, but not tRNA$^{fMet}_1$. Daniel and Cohn (1976) have suggested that this loss of activity results from loss of the S_8·A_{14} base pair in tRNA$^{fMet}_3$, which already has a weaker tertiary structure than tRNA$^{fMet}_1$ due to the absence of stabilizing interactions involving m^7G_{47}.

The TψC Loop. The resistance of the TψC loop to chemical modification under nondenaturing conditions due to its participation in tertiary structure interactions has limited our ability to study this structural region. The only site where we have obtained a modification is A_{58} in the center of the loop. Blocking the N_1 and exocyclic NH_2 of this purine base does not inactivate tRNA^{fMet}. Enzymatic methylation of the N_1 position of the adjacent A_{59} residue also has little or no effect on interaction of tRNA^{fMet} with MetRS (Table II). Other investigators have used denaturing conditions in order to modify ψ_{56}, and conflicting results have been obtained with respect to the effect of these modifications on methionine acceptor activity (Table II). Attachment of a bulky aromatic dye to the N_1 position of ψ_{56} does not inactivate tRNA^{fMet}; thus it seems likely that the loss of activity observed following cyanoethylation of N_1 is due to a failure of the denatured tRNA to properly refold into a biologically active conformation. The dye-labeled tRNA exhibits lower than normal methionine acceptance, indicating that part of this modified tRNA may also be trapped in an inactive conformation or that the modification significantly reduces the rate of aminoacylation by interfering with tRNA–protein binding.

The Acceptor Stem Region. The effects on aminoacylation of chemical modifications of five nucleotides in the acceptor stem region of tRNA^{fMet} have been studied (Table II). This tRNA contains an unpaired base at the 5′ terminus. Conversion of $C_1 \rightarrow U_1$ leads to formation of a new U_1·A_{73} hydrogen bonded base pair and has no effect on the rate or extent of methionine acceptance. Photooxidation of the adjacent G residue, which results in disruption of the G_2·C_{72} base pair, can also occur without inactivation of tRNA^{fMet}; thus neither specific nucleotide bases at the 5′ end of the molecule nor the presence or absence of base pairs in the first two positions of the stem is essential for recognition of the tRNA. Nucleolytic excision of the pC_1pG_2p sequence causes a ten-fold increase in the K_m for aminoacylation. Since removal of the 5′ phosphate or chemical modification of C_1 has little or no effect on the reaction, this change in binding affinity can be attributed to removal of pG_2p from the structure. The purine base itself is not required for recognition of tRNA^{fMet}, but its removal or modification may alter interaction of MetRS with an essential nearby site. It is also possible that the pG_2p sequence contains a phosphate group involved in protein–tRNA binding.

Photooxidation of G_{71} on the 3′ side of the acceptor stem has a much more drastic effect on aminoacylation, completely inactivating tRNA^{fMet}. This modification destroys the purine base and breaks the C_3·G_{71} base pair. Modification of two other bases on the 3′ side of the acceptor stem region, A_{73} and A_{74}, does not inactivate tRNA^{fMet}. Although the effect of these modifications on the rate of aminoacylation is not known, the functional groups at positions 1 and 6 of these purine bases cannot be essential for enzyme binding or catalysis. The overall results suggest a specific role for G_{71} in interaction with MetRS; however, strong interference by the photooxidized residue with enzyme binding to an adjacent essential base such as C_{72} or C_3 cannot be excluded.

The CCA Terminus. Since aminoacylation results in attachment of the amino acid to the 3′ terminus, this part of the molecule must come in close contact with the aminoacyl-tRNA synthetase. It is therefore not surprising to find that chemical modifications in the 3′-terminal region of tRNA^{fMet} affect methionine acceptance (Table II). Modification of the 3′-adenosine, A_{77}, by chloroacetaldehyde blocks both N_1 and the exocyclic NH_2 of the purine base. This modification does not inactivate tRNA^{fMet} but significantly lowers V_{max} for aminoacylation. Since these functional groups are not required for catalysis, the decreased rate may be due to steric interference of the etheno bridge in ϵA_{77} with the correct positioning of the acceptor end in the catalytic site.

A cytidine to uridine base change at C_{75} has only a small effect on methionine acceptance by tRNA^{fMet}. In contrast, modification of the adjacent cytidine residue inactivates the tRNA, indicating an important role for this base in aminoacylation. Inactivating modifications at C_{76} are qualitatively different from those found at other sites in the tRNA, however, since the modified molecules are still able to bind to MetRS

TABLE III: Categories of Structural Alterations in $tRNA^{fMet}$.

Site of modification and effect on methionine acceptor act.			
Inactive	Active (no kinetic data avail.)	Altered kinetics parameters	No effect[a]
C_{35}	G_9	G_2 (K_m)	C_1
A_{36}	G_{10}	G_{20}[b] (K_m)	S_8[c]
G_{46}	G_{12}	D_{21}-A_{22}-G_{23} (K_m)	C_{13}
ψ_{56}[b]	A_{14}	U_{37}[b] (K_m and V_{max})	C_{16}
G_{71}		A_{47} (K_m and V_{max})	C_{17}
C_{76}	G_{15}	A_{77} (V_{max})	U_{18}
	G_{19}		G_{27}
	G_{20}[b]		U_{37}[b]
	D_{21}		U_{48}
	A_{22}		A_{59}
	G_{23}		C_{75}
	C_{49}		
	ψ_{56}[b]		
	A_{58}		
	A_{73}		
	A_{74}		

[a] Less than twofold change in kinetic parameters when present in 1 mol/mol of $tRNA^{fMet}$. [b] Variable depending on the type of modification. [c] Change in aminoacylation rate observed with $tRNA^{fMet}_3$ but not $tRNA^{fMet}_1$

in a manner which inhibits aminoacylation of active tRNA. This indicates that structural changes at this site do not prevent recognition of $tRNA^{fMet}$ by MetRS but rather interfere with the catalytic step in the reaction.

General Remarks. The structural alterations in $tRNA^{fMet}$ can be grouped into categories according to their effect on methionine acceptor activity (Table III). A significant number of modifications have no affect on aminoacylation. A number of other structural changes are not inactivating, but alter the rate of the reaction. Lawrence et al. (1973) have pointed out that the Michaelis constants for aminoacylation of $tRNA^{Met}$ by MetRS cannot be simply related to single kinetic dissociation and association constants. In addition, the effect of structural changes on the Michaelis constants has been found to vary significantly with the experimental conditions of the aminoacylation assay. For example, $tRNA^{fMet}_1$ and $tRNA^{fMet}_3$ differ in K_m but have the same V_{max} when assayed in the presence of 0.15 M NH_4Cl, while the K_m values are similar and there is a small difference in V_{max} when NH_4Cl is omitted from the assay (Daniel and Cohn, 1976; L. H. Schulman and H. Pelka, unpublished observations). In the absence of a more detailed knowledge of the mechanism of the reaction, it is not possible to interpret the observed changes in K_m and V_{max} in terms of individual steps in the overall aminoacylation process. Structural changes which alter the rate are obviously of more importance than those which have no effect on the reaction, however, and less important than those which eliminate activity.

The inactivating modifications appear to fall into two classes: those which affect recognition and those which affect catalysis. The available data indicate that essential structural features for recognition of $tRNA^{fMet}$ by MetRS are present in the anticodon, the acceptor stem, and possibly the variable loop. The critical interactions which occur between the enzyme and the tRNA appear to involve binding to one particular side of each of these regions. This is suggested by the much greater sensitivity of the enzyme to a structural change on the 3′ side of the acceptor stem than to changes on the 5′ side, the much greater sensitivity to a structural change in the variable loop than to changes in the closely associated dihydrouridine stem and loop regions, and the much greater sensitivity to one particular stereoisomer of the U_{37}–bisulfite adduct in the anticodon than to the other. Modifications at any one of the essential sites are accompanied by loss of ability to effectively interact with MetRS in addition to loss of methionine acceptor activity, indicating that all such sites must be simultaneously available in order for the tRNA to be recognized by the enzyme.

It has been most convincingly demonstrated in the case of the anticodon base C_{35} that structural alteration of a single nucleotide base can drastically affect recognition by MetRS. Similar observations have been made in the case of *E. coli* $tRNA^{Tyr}$ (Shimura et al., 1972; Hooper et al., 1972; Smith and Celis, 1973; Celis et al., 1973; Ghysen and Celis, 1974), yeast $tRNA^{Val}_1$ (Chambers et al., 1973), *E. coli* $tRNA^{Trp}$ (Yaniv et al., 1974), and *E. coli* $tRNA^{Arg}$ (Chakraburtty, 1975). The small differential increment to the binding specificity provided by interaction with a single base seems insufficient to explain the observed loss of aminoacylation activity and loss of inhibitory capacity, and a direct effect of a structural change far removed from the 3′ terminus on the catalytic step also seems unlikely. Such results are more easily understood in terms of a model in which simultaneous interaction of MetRS with C_{35} in the anticodon and a number of other specific recognition sites in $tRNA^{fMet}$ induces a structural rearrangement of the complex leading to movement of the terminal adenosine into the catalytic site. Data supporting a multistep model of tRNA-aminoacyl-tRNA synthetase recognition have recently been reported by a number of laboratories (von der Haar and Gaertner, 1975; Bartmann et al., 1975; Rigler et al., 1976; Riesner et al., 1976; Krauss et al., 1976; Remy and Ebel, 1976; Yarus et al., 1977).

In addition to the essential recognition sites, which determine whether or not the 3′ terminus is correctly positioned in the active site, there are other structural elements which appear to influence the catalytic step. For example, inactive $tRNA^{fMet}$ modified at C_{76} is able to significantly inhibit aminoacylation of active tRNA when present in a 1:1 molar ratio, suggesting that the recognition step can still occur with this altered tRNA, and that the structural modification near the 3′ end of $tRNA^{fMet}$ interferes with catalysis. Certain modifications of the 3′-terminal A residue may also be found to fall into this class.

Other structural features play an important but nonessential role in aminoacylation. These may include phosphate–protein interactions which position the tRNA in a manner that facilitates binding of the enzyme to the critical recognition sites, or elements of tRNA secondary or tertiary structure which similarly ensure proper access of MetRS to essential ligands.

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