An RNA Structural Determinant for tRNA Recognition†

Christian S. Hamann and Ya-Ming Hou*

Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, 233 South 10th Street, Philadelphia, Pennsylvania 19107

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ABSTRACT: *Escherichia coli* $tRNA^{Cys}$ contains an unusual G15·G48 tertiary base pair that is important for recognition and aminoacylation by cysteine tRNA synthetase. This G15·G48 tertiary base pair has a distinctive chemical modification signature that suggests an N2·N3 base pairing. The N2·N3 pairing of a G·G base pair has not been described in any existing RNA structures. Identification of the structural determinant of G15·G48 is of fundamental importance for understanding the formation of an RNA tertiary base pair, as well as the role of RNA tertiary structure in tRNA recognition. We show here that the structural determinant for G15·G48 is an A13·A22 mismatch in the dihydrouridine stem. Introduction of A13·A22 to an unrelated tRNA confers the distinctive chemical modification signature of G15·G48 while substitution of A13·A22 eliminates this signature. The relationship between G15·G48 and A13·A22 enables the unrelated tRNA to be efficiently recognized by cysteine tRNA synthetase. Modeling studies show that A13·A22 has the potential to form a base triple with A46, which is directly connected to G48 in the G15·G48 base pair. The proposed A13·A22·A46 base triple provides a framework for understanding how two RNA structural elements may be related to each other in playing an important role in tRNA aminoacylation.

RNAs can fold into distinct tertiary structures that arise from noncanonical tertiary hydrogen base pairs. The crystal structures of tRNAs (Kim *et al.*, 1974; Robertus *et al.*, 1974; Schevitz *et al.*, 1979; Woo *et al.*, 1980; Moras *et al.*, 1980), the hammerhead ribozymes (Pley *et al.*, 1994; Scott *et al.*, 1995), and a domain of the group I self-cleaving intron of *Tetrahymena thermophila* (Cate *et al.*, 1996a,b) provide a paradigm of these tertiary hydrogen base pairs. Although many of these tertiary base pairs are well defined in the crystal structures, we do not yet know the structural context which permits their formation. A second question is whether we have the ability to specifically alter their hydrogen pairings so as to manipulate RNA structure and function. We attempted to address these two questions in this study, using an RNA tertiary base pair in *Escherichia coli* $tRNA^{Cys}$ as an example. The ability to address these questions will become increasingly important as more structural information about small and large RNAs is obtained and more noncanonical RNA tertiary base pairs that are critical to RNA structure, function, and catalysis are identified.

We focused on the G15·G48 tertiary base pair of *E. coli* $tRNA^{Cys}$. This base pair connects the dihydrouridine (D) loop with the variable loop to stabilize the L-shaped tRNA tertiary structure. Although the structural details of G15·G48 are unknown, chemical modifications of this base pair revealed a distinctive signature pattern that allowed a straightforward modeling of this base pair within the crystal structures of tRNAs. More importantly, this base pair is a critical element for recognition of *E. coli* $tRNA^{Cys}$ by cysteine tRNA synthetase. Substitution of G15·G48 with G15·C48 or C15·G48 reduced the catalytic efficiency (k_{cat}/K_m) of aminoacylation with cysteine by almost 2 orders of magnitude (Hou *et al.*, 1993). In contrast, most tRNAs have a G15·C48 or an A15·U48 tertiary base pair (Steinberg *et al.*, 1993). Although the structural details of G15·C48 and A15·U48 are known (Kim *et al.*, 1974; Robertus *et al.*, 1974; Moras *et al.*, 1980), these two base pairs are usually not important for aminoacylation in most tRNAs; substitutions of G15·C48 or A15·U48 usually have no major effect on aminoacylation (Sampson *et al.*, 1990; Giegé *et al.*, 1993; Hou *et al.*, 1995). These comparisons emphasize the significance of G15·G48 in aminoacylation with cysteine and suggest that this base pair is in a functional context that allows its structural determinant to be elucidated and tested.

Structural modeling and chemical modifications suggest an N2·N3 pairing for G15·G48 of *E. coli* $tRNA^{Cys}$ (Figure 1). This pairing is distinct from an N1·O6 pairing that would mimic the structure of G15·C48 or A15·U48 in most tRNAs (Hou *et al.*, 1993; Hou, 1994). The chemical modification signature of this base pair is (1) that G15 and G48 both react with kethoxal and (2) that G15 reacts with dimethyl sulfate (DMS). Kethoxal attacks N1 and one of the N2 hydrogens of guanines (Ehresmann *et al.*, 1987). The reactivity with kethoxal indicates an accessible N1 and eliminates the possibility of the N1·O6 pairing. The N2·N3 pairing, which uses one of the N2 hydrogens as the hydrogen donor and the ring N3 as the acceptor, leaves N1 and the other N2 hydrogen free and thus provides a rationale for the accessibility of G15·G48 to kethoxal. The geometry of the N2·N3 pairing in *E. coli* $tRNA^{Cys}$ suggests that the N7 of G15 is protruded from the purine ring of A14 (Figure 1). This protrusion explains the accessibility of G15 to DMS, which attacks N7 of unstacked G's (Ehresmann *et al.*, 1987).

We previously showed that the N2·N3 pairing of G15·G48 in *E. coli* $tRNA^{Cys}$ is dependent on other nucleotides in the

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* To whom correspondence should be addressed. Telephone: 215-503-4480. Fax: 215-923-9162. E-mail: houl@jeflin.tju.edu.

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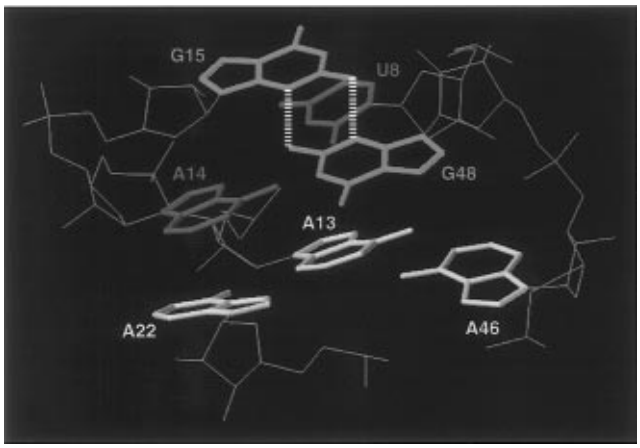

FIGURE 1: Three-dimensional modeling of the spatial relationship between G15·G48, U8·A14, and A13·A22·A46 in *E. coli* $tRNA^{Cys}$. G15·G48 is shown as the N2·N3 pairing, while A13·A22·A46 is shown as a potential base triple. The tRNA model was first generated by building the sequence of *E. coli* $tRNA^{Cys}$ into the coordiates of yeast $tRNA^{Phe}$ using the FRODO program (Bush *et al*., 1987) and incorporating the results of chemical modifications. The A13·A22·A46 base triple was modeled according to the A13·A22·A45 base triple in the ternary complex of $tRNA^{Gln}$ bound to *E. coli* glutamine tRNA synthetase and ATP (Rould *et al*., 1989) using the program *O* (Jones *et al*., 1991). The rebuilt tRNA structure was exhaustively minimized in X-PLOR (Brunger, 1992) using the Berman parameter set (Parkinson *et al*., 1996) with the atoms belonging to the bases (but not the sugar phosphate backbone) of nucleotides 8, 13−15, 22, 46, and 48 kept fixed during minimization. Hydrogen bonds for G15·G48 are indicated with dashed lines, and some atoms in the sugar phosphate backbone are omitted for clarity. This figure was prepared using Sybyl (Tripos, St. Louis, MO) on a Silicon Graphics workstation.

D stem−loop. Alteration of A13·A22 to U13·A22 and of U21 to A21 eliminated the accessibility of G15 to kethoxal and reduced its accessibility to DMS (Hou, 1994). The loss of the chemical modification signature of G15·G48 was accompanied by a decrease of almost 2 orders of magnitude in k_{cat}/K_m. This decrease in k_{cat}/K_m is the same as that of the G15·C48 or C15·G48 variant of $tRNA^{Cys}$. These results imply a structural and functional correlation between G15·G48 and A13·A22/U21.

To determine if A13·A22/U21 were sufficient determinants for the N2·N3 pairing of G15·G48, we report here the study of introducing G15, G48, and variations of A13·A22/U21 to an unrelated tRNA to evaluate their structure/function relationship. We prepared all tRNAs as T7 transcripts. Although these transcripts lacked modified bases, we have shown that this lack of modification did not interfere with our ability to perform structure/function analysis (Hou *et al.*, 1993; Hou, 1994). All tRNA variants were structurally characterized by chemical probes and functionally analyzed by kinetics of aminoacylation. Together, these two methods allowed us to establish A13·A22, but not U21, as the determinant for the N2·N3 pairing of G15·G48. The ability of A13·A22 to determine the structure of G15·G48 provides an example of an RNA tertiary base pair that can be manipulated through another RNA element.

MATERIALS AND METHODS

Preparation of tRNA Transcripts and Aminoacylation with Cysteine. All tRNA genes were constructed and site-specifically mutagenized in plasmid pTFMa (Hou *et al.*, 1993; Hou, 1994). Mutagenesis followed the procedures of Kunkel *et al.* (1987). Restriction of pTFMa-derived plasmids with *Bst*NI generated the template for T7 transcription. Conditions for T7 transcription, purification of T7 transcripts, and aminoacylation with cysteine were described previously (Hou *et al.*, 1993; Hou, 1994). We used purified T7 RNA polymerase for T7 transcription (Grodberg & Dunn, 1988) and *E. coli* cysteine tRNA synthetase (Hou *et al.*, 1991) for aminoacylation assays.

Chemical Modifications with Kethoxal (N2 and N3 of G), CMCT (N3 of U), and DMS (N1 of A). Each tRNA (120 pmol) was briefly denatured (80 °C, 3 min) in 50 μL of 50 mM sodium cacodylate (pH 7.5) and annealed to native (10 mM $MgCl_2$) or semidenaturing (1 mM EDTA) conditions before being treated with kethoxal (1.8 mg/mL), CMCT (3.8 mg/mL), or DMS (0.4%). Modification reactions followed the established conditions (Moazed *et al.*, 1986) and were incubated at room temperature for 5−20 min before being stopped by ethanol precipitation. AMV-reverse transcriptase (Life Sciences, 2 units) and an appropriate oligonucleotide primer (5′-labeled with ^{32}P, approximately 2×10^5 cpm, 2.4 pmol) were added to initiate primer extension [27 mM potassium borate (pH 7.0) and each dNTP at 0.33 mM] at 42 °C for 30 min. Primer extension was stopped by 10 mM EDTA, and the extension products were purified by a C18 cartridge (Waters, Sep-Pak Vac 1cc) before being analyzed by electrophoresis on a 10% polyacrylamide/7 M urea gel. Under the established conditions, nucleotides in the helical stems were protected from chemical modifications, whereas those in the anticodon loop were accessible.

Chemical Modifications with DMS (N7 of G). Each tRNA (3′-end-labeled, approximately 2×10^5 cpm), mixed with 160 pmol of carrier tRNAs, was briefly denatured and annealed to either native or semidenaturing conditions as described above. Modification with DMS (at 0.4%) followed the established conditions (Peattie & Glibert, 1980), was carried out at room temperature for 10 min, and was stopped by ethanol precipitation. The DMS-modified tRNA was treated with sodium borohydride, followed by aniline scission (at 10%) at 60 °C for 10 min to generate tRNA fragments that terminate at the site of modification. These tRNA fragments were separated by electrophoresis on an 8% polyacrylamide/7 M urea gel, which was analyzed after autoradiography.

RESULTS AND DISCUSSION

Aminoacylation of tRNA Mutants. For ease of kinetic analysis, the rationale was to start with an unrelated tRNA and to add back all the required elements for aminoacylation with cysteine. Besides G15·G48, the U73 nucleotide and the GCA anticodon of $tRNA^{Cys}$ are important for aminoacylation (Pallanck *et al*., 1992; Hou *et al.*, 1993; Komatsoulis & Abelson, 1993). We chose the sequence framework of *E. coli* $tRNA^{Gly/UCC}$ (Figure 2), which already has a U73. The wild type transcript (Gly01) was not a substrate for the cysteine enzyme; its k_{cat}/K_m of aminoacylation with cysteine was at least 10^4-fold below that of the $tRNA^{Cys}$ transcript (Cys01, Table 1). Introduction of the GCA anticodon for $tRNA^{Cys}$ to Gly01 significantly improved its cysteine acceptance (Gly06; relative $k_{cat}/K_m = 0.30$). Gly06 contains A15·U48. Additional substitution of A15·U48 with G15·G48 created Gly11, which had a k_{cat}/K_m of aminoacylation with cysteine identical to that of Gly06 (Table 1). We created

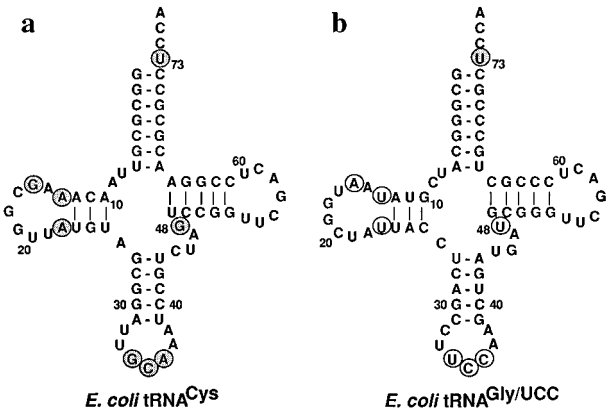

FIGURE 2: Sequence and the cloverleaf structure of *E. coli* $tRNA^{Cys}$ (a) and of *E. coli* $tRNA^{Gly/UCC}$ (b). U73, G15·G48, A13·A22, and the GCA anticodon in *E. coli* $tRNA^{Cys}$ that are important for aminoacylation are indicated by shaded circles. Among these, U73 is common to the two tRNAs, whereas others that differ are indicated by open circles in $tRNA^{Gly/UCC}$. The sequence framework of $tRNA^{Gly/UCC}$ contains two nucleotides (rather than one) between positions 19 and 21 and thus provides a D stem−loop structure distinct from that of $tRNA^{Cys}$.

four variations at 13·22 in Gly06 to include U·U, A·U, U·A, and A·A. With each variation at 13·22, we generated an A21 and a U21 mutant. These eight variants were kinetically screened for the mutant that had the highest enzymatic activity of aminoacylation with cysteine.

We showed that A13·A22 was the only requirement for efficient aminoacylation with cysteine in $tRNA^{Gly}$ (Table 1). The Gly21 and Gly24 mutants that contained A13·A22 but differed in U21 or A21 had a similar k_{cat}/K_m for aminoacylation with cysteine, which is at least as good as that of $tRNA^{Cys}$. The remaining six mutants suffered varying defects in k_{cat}/K_m. The weakest mutant was Gly20, which contained U13·A22/A21 and had a k_{cat}/K_m of aminoacylation with cysteine almost 2 orders of magnitude below that of $tRNA^{Cys}$. The kinetics of aminoacylation were such that the Michaelis−Menten constant K_m reflected the binding of the tRNA to the synthetase. Examination of kinetic parameters showed that nucleotides at position 13·22 largely influenced K_m and thus the ability of the tRNA to bind cysteine tRNA synthetase. The fully active Gly21 and Gly24 each had a K_m (2.8 and 3.7 μM, respectively) similar to that of $tRNA^{Cys}$ ($K_m = 2.2$ μM). In contrast, the less active mutants all had K_m values at least 10-fold higher than the K_m of $tRNA^{Cys}$. All variants showed a small increase in k_{cat} from that of $tRNA^{Cys}$. Thus, the primary defect for the less active mutants was in K_m. The major contribution of A13·A22 was presumably to confer a binding strength of Gly21 and Gly24 for the cysteine enzyme as that of $tRNA^{Cys}$.

Chemical Modifications of tRNA Mutants at G15·G48. We next performed chemical modification studies with these eight variants to determine if the kinetic effect at 13·22 correlated with the structural change at G15·G48. The sequence framework of $tRNA^{Gly}$ provided a valid model for these studies. Not only did the fully active Gly21 and Gly24 mutants retain the kinetic behaviors of $tRNA^{Cys}$, but the least active Gly20 mutant retained behaviors identical to those of a mutant of $tRNA^{Cys}$ which contained U13·A22 and A21 (Hou, 1994). The kinetic parallel suggested a structural parallel. We therefore tested the chemical accessibility of G15·G48 in these eight variants to kethoxal and to DMS. We showed that the fully active mutants recapitulated the chemical modification signature of $tRNA^{Cys}$ by reacting with both kethoxal and DMS, whereas the less active mutants failed to react with either kethoxal or DMS.

The results of chemical modifications are summarized in Table 2. There is a clear distinction between the active and the less active mutants. First, the fully active mutants reacted with kethoxal at both G15 and G48, whereas none of the less active mutants reacted with kethoxal. The complete lack of kethoxal modification in the less active mutants suggested a base pairing of G15·G48 that involved N1. This base pairing, for example, could consist of N1·O6. In the fully active mutants, the kethoxal modification is the same as that of $tRNA^{Cys}$ and is consistent with the N2·N3 pairing of G15·G48. Second, the fully active mutants readily reacted with DMS for methylation at the N7 position. This signal is strong and is reminiscent of that of $tRNA^{Cys}$ (Figure 3). The signal for DMS suggested that G15 in the fully active mutants is not protected by stacking interactions, which is consistent with the modeling studies of the N2·N3 pairing of G15·G48. The less active mutants displayed two patterns of DMS activity at G15. Gly11 and Gly25, which shared U13·U22 but differed at position 21, weakly reacted with DMS at G15 (shown for Gly11 in Figure 3). Gly19 and Gly22, which shared A13·U22 but differed at position 21, also failed to react with DMS at G15 (not shown). For these four variants, the lack of DMS activity at G15 correlated with the lack of kethoxal activity at G15. However, Gly20 and Gly23, which shared U13·A22 but differed at position 21, both positively reacted with DMS at G15 (Figure 3). Although their DMS activity indicated an alteration of G15·G48 from that of the other four mutants, this alteration did not simultaneously lead to a kethoxal activity.

Thus, A13·A22 was the only determinant that conferred the chemical modification signature of kethoxal and DMS of G15·G48 in $tRNA^{Cys}$ to that in $tRNA^{Gly}$. This was demonstrated in Gly21 and Gly24. In these two $tRNA^{Gly}$ mutants, as in *E. coli* $tRNA^{Cys}$, the correlation of A13·A22 with the chemical modification signature of G15·G48 was accompanied by full enzymatic aminoacylation with cysteine. Gly21 and Gly24 differed in U21 and A21. This suggests that, at least in the context of $tRNA^{Gly}$, the nucleotide identity at 21 is not critical for aminoacylation with cystiene. We note that, although Gly21 and Gly24 were both fully active with aminoacylation with cysteine, they were structurally distinct outside of G15·G48 and A13·A22. For example, Gly24 reacted strongly with DMS at G10 whereas Gly21 was inactive (Figure 3). Including *E. coli* $tRNA^{Cys}$, the ability of A13·A22 to manipulate G15·G48 is therefore demonstrated in three tRNA structural frameworks.

Chemical Modification of tRNAs at A13·A22. We characterized the base pairing nature of A13·A22 and showed that it was a mismatch with no apparent hydrogen bonds that involved N1 of either A. Additionally, we showed that the 13·22 nucleotides in the less active mutants all had characteristics of a base pair. We probed the eight variants of $tRNA^{Gly}$ for the accessibility of N1 of A with DMS and the accessibility of N3 of U with CMCT [1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluene sulfonate]. The chemical modifications were such that N1 of A and N3 of U not protected by Watson−Crick base pairing, tertiary interactions, or steric effects would be modified (Ehresmann *et al.*, 1987). Both A13 and A22 in Gly21 and Gly24 were accessible to DMS (Table 2), indicating that their N1

Table 1: Kinetic Parameters for Variants of G15·G48 tRNA$^{Gly/GCA}$ [a]

		nucleotide position						
	name	13	22	21	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (M^{-1} s^{-1})	relative to Cys01
G15·G48	Cys01	A	A	U	0.470 ± 0.090	2.24 ±1.20	210 000	1.00
A15·U48	Gly01	U	U	A				3.0×10^{-5}
A15·U48	Gly06	U	U	A	0.940 ± 0.480	14.8 ± 8.1	63 500	0.30
G15·G48	Gly11	U	U	A	2.10 ± 0.14	34.7 ± 4.3	60 500	0.29
	Gly25	U	U	U	1.23 ± 0.17	43.4 ±6.8	28 300	0.13
	Gly19	A	U	A	0.733 ± 0.043	41.0 ± 13.7	17 900	0.085
	Gly22	A	U	U	1.07 ± 0.09	36.6 ± 8.7	29 200	0.14
	Gly20	U	A	A	0.451 ± 0.069	70.4 ± 14.4	6 410	0.031
	Gly23	U	A	U	2.33 ± 0.12	32.0 ± 4.8	72 800	0.35
	Gly21	A	A	A	1.82 ± 0.16	2.76 ± 0.18	659 000	3.1
	Gly24	A	A	U	1.90 ± 0.23	3.68 ± 0.85	516 000	2.5

[a] Wild type *E. coli* tRNA^{Cys} (Cys01), wild type *E. coli* tRNA$^{Gly/UCC}$ (Gly01), the GCA anticodon variant tRNA$^{Gly/GCA}$ (Gly06), and the eight variants of Gly06 were prepared as T7 transcripts, and their kinetic parameters K_m and k_{cat} were determined according to the Michaelis−Menten equation. Each parameter was the average of at least three determinations. Aminoacylation was assayed at 37 °C and was initiated by adding purified *E. coli* cysteine tRNA synthetase (4.2 nM) to a series of tRNA concentrations that ranged from 1 to 12 μM for Cys01, Gly21, and Gly24 or by adding the enzyme (12.6 nM) to concentrations that ranged from 10 to 120 μM for the less active mutants.

Table 2: Relative Chemical Accessibilities to Kethoxal (N1 and N2 of G), CMCT (N3 of U), and DMS (N1 of A) for *E. coli* tRNA^{Cys} and Variants of tRNA^{Gly} [a]

tRNA	nucleotides and chemical accessiblities				
Cys01	G15 +	G48 (+)	A13 −	A22 +	U21
Gly11	G15 −	G48 −	U13 −	U22 −	A21 −
Gly25	G15 −	G48 −	U13 −	U22 −	U21 −
Gly19	G15 −	G48 −	A13 −	U22 nd[b]	A21 −
Gly22	G15 −	G48 −	A13 −	U22 nd	U21 nd
Gly20	G15 −	G48 −	U13 nd	A22 −	A21 −
Gly23	G15 −	G48 −	U13 nd	A22 −	U21 nd
Gly21	G15 +	G48 +	A13 +	A22 +	A21 +
Gly24	G15 +	G48 +	A13 +	A22 +	U21 nd

[a] *E. coli* tRNA^{Cys} (Cys01) and the eight variants of Gly06 were prepared as T7 transcripts, and their nucleotides at positions 15·48, 13·22, and 21 are indicated. Each nucleotide position was tested by the appropriate chemical probe at least two times. A positive signal means a consistent chemical accessibility, while a negative signal means inaccessibility. All these chemical modifications were mapped by primer extension (Moazed *et al.*, 1986). It should be noted that DMS also modified N7 of G (Peattie & Gilbert, 1980). This modification, however, does not interfere with primer extension but can be detected after aniline scission as described in the legend to Figure 3. [b] Not determined.

positions were free and that A13·A22 was a mismatch. In contrast, A13 in the A13·U22 variants (Gly19 and Gly22) and A22 in the U13·A22 variants (Gly20 and Gly23) were inaccessible to DMS, indicating that A13·U22 and U13·A22 were Watson−Crick base pairs. Even U13 and U22 in the U13·U22 variants (Gly11 and Gly25) were inaccessible to CMCT, indicating that their N3 was involved in hydrogen bonds. One possibility is that U13·U22 formed a homopyrimidine base pair, as previously described in other RNAs (Baeyens *et al.*, 1995; Wahl *et al.*, 1996; Fourmy *et al.*, 1996). This U·U base pair could use N3 as the hydrogen donor and O2 or O4 as the acceptor.

We also investigated the mismatch nature of A13·A22 in *E. coli* tRNA^{Cys}. Table 2 showed that N1 of A22 was accessible to DMS, indicating A22 was not base paired. However, N1 of A13 was inaccessible. Structural modeling suggested that the inaccessible N1 of A13 might be due to an additional hydrogen interaction with N6 of A46. This was supported by other chemical accessibilities of A46 (not shown). In our model of tRNA^{Cys}, A46 is in the same plane with A13·A22 (Figure 1) and is at a position that can afford an A13·A22·A46 base triple. The possibility of an A13·A22·A46 base triple was suggested from the crystal structure of *E. coli* tRNA^{Gln} (Rould *et al.*, 1989). In the latter, A13·A22 is in a coplanar relationship with A45, where A13 makes two symmetric hydrogen bonds with A45 through N1 and N6, while using its N3 to maintain one hydrogen bond with N6 of A22. The mismatch of A13·A22 is then stabilized by a base−backbone interaction between N6 of A22 and 2′-OH of A13. A similar base triple can be proposed for A13·A22·A46 in *E. coli* tRNA^{Cys} (Figure 4a).

A Model for the Relationship between A13·A22 and G15·G48. *E. coli* tRNA^{Cys} lacks the nucleotide at position 47, and therefore, its A46 is directly linked to G48. The possibility of an A13·A22·A46 base triple in tRNA^{Cys} thus provided a model for understanding how A13·A22 could act as a structural determinant of G15·G48 through A46. This model proposes that A46 forms a base triple with nucleotides at 13·22 and that, depending on the geometry of the base triple, the backbone of A46 moves relative to the center of the base triple. The movement of A46 in turn influences the backbone of G48 and as such changes the base pairing of G15·G48. In the active structure, the model proposes that A46 pairs with A13 to stabilize an A13·A22·A46 base triple (Figure 4a) that is similar to the A13·A22·A45 base triple in *E. coli* tRNA^{Gln}. In the inactive structure, when A13·A22 is mutated to U13·A22, the model proposes that A46 switches its position to pair with A22 to stabilize a U13·A22·A46 base triple (Figure 4b). The proposed U13·A22·A46 base triple is reminiscent of the C13·G22·m^{7}G46 base triple of yeast tRNA^{Phe} (Kim *et al.*, 1974; Robertus *et al.*, 1974). While a base triple is maintained in both structures, the geometry of the triple is altered depending on the position of A46 and the nucleotides at 13·22. We suggest that the geometry of A13·A22·A46 favors the N2·N3 pairing of G15·G48 whereas that of U13·A22·A46 may instead favor the N1·O6 pairing.

The DMS modification of A13 and A22 in Gly21 and Gly24 does not completely support an A13·A22·A46 base

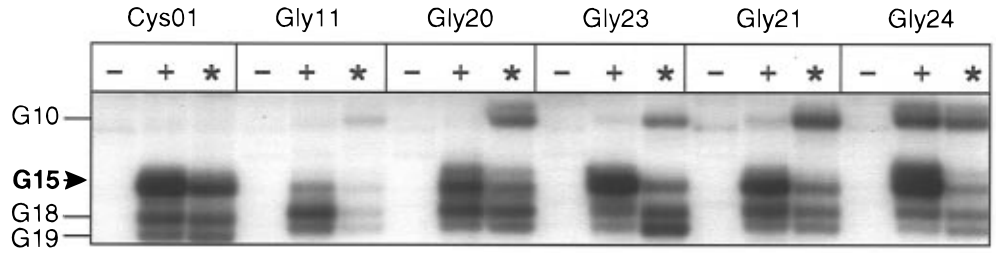

FIGURE 3: DMS accessibility at G15 of the wild type tRNA^{Cys} (Cys01) and five variants of Gly06. Each tRNA was tested by DMS modification at least twice, and results are shown for modification under the native (+) or the semidenaturing (*) conditions or under a control where no DMS was added (−). The bands that correspond to cleavage at G10, G15, G18, and G19 are indicated.

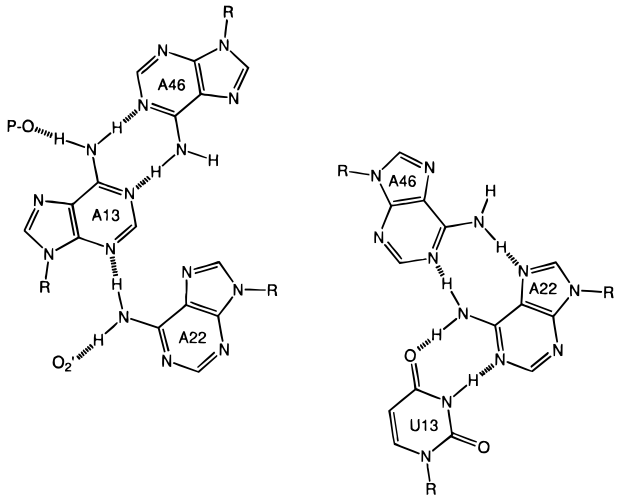

FIGURE 4: A13·A22·A46 base triple (a) proposed in *E. coli* tRNA^{Cys} and in the fully active mutants of tRNA^{Gly} and the U13·A22·A46 base triple (b) proposed in the U13·A22/A21 mutantof tRNA^{Cys} and in the least active mutants of tRNA^{Gly}.

triple as proposed for tRNA^{Cys}. However, the accessibility of A13 in these two tRNAs does not exclude the possibility of a similar active structure as proposed in our model. It is possible that the orientation of A13 may be sufficiently different in these two tRNA mutants than that in tRNA^{Cys} such that A13 can maintain one hydrogen bond with A46 through N6 while its N1 is accessible to solvent. This alternative structure may then be stabilized by water or metal ion-mediated hydrogen bonds with the N1 of A13. Further structural analysis of these tRNA mutants will be necessary to distinguish these possibilities.

Our model of the relationship between A13·A22·A46 and G15·G48 provides a framework for future studies of *E. coli* tRNA^{Cys}. This model raises questions of whether other mismatches at 13·22 can mimic the function of A13·A22 and whether other nucleotides at 13·22·46 can maintain the N2·N3 pairing of G15·G48. The answers to these questions will help to refine the model and will further highlight the importance of tRNA structural elements for aminoacylation by cysteine tRNA synthetase. Among the 20 aminoacyl tRNA synthetases in *E. coli*, the cysteine enzyme is the smallest monomer of 461 amino acids (Hou *et al.*, 1991; Eriani *et al.*, 1991; Avalos *et al.*, 1991). However, this enzyme must contact U73 and the GCA anticodon for aminoacylation, which are located at the two ends of the L-shaped tRNA structure that are separated by about 75 Å. The small size of the enzyme may limit its ability to reach the two ends of the L. One solution to this problem may be to utilize the relationship between A13·A22 and G15·G48 to modulate the overall structure of the tRNA so as to facilitate the interaction with the cysteine enzyme. Our kinetic analysis supports this hypothesis. Substitutions of A13·A22 result in an increase in K_m, suggesting that the relationship between A13·A22 and G15·G48 works to facilitate the binding of the tRNA to the synthetase, possibly to optimize the presentation of U73 and the GCA anticodon to the enzyme. Most other synthetases do not use tRNA structural elements as determinants for aminoacylation (Sampson *et al.*, 1990; Giegé *et al.*, 1993; Hou *et al.*, 1995). This distinguishes the cysteine enzyme from all other aminoacyl tRNA synthetases and establishes the ability of the cysteine enzyme to develop a different strategy of tRNA recognition that depends on a structural determinant to increase overall tRNA selection before committing to catalysis.

The N2·N3 pairing of a G·G tertiary base pair proposed for *E. coli* tRNA^{Cys} has been described in the structure of a DNA dodecamer (Wing *et al.*, 1980). Phylogenetic and molecular modeling studies indicate that G·G base pairs are commonly present in naturally occurring RNAs, as well as in RNAs isolated from *in vitro* selection of random sequences (Gutell *et al.*, 1985). Of those with functional significance and structural information, G·G pairs are characterized as N1·O6, Hoogsteen, or reverse Hoogsteen hydrogen bonds (Jiang *et al.*, 1996; Battiste *et al.*, 1994). Although the N2·N3 pairing of G15·G48 in tRNA^{Cys} has not been documented previously, its dependence on A13·A22 suggests the ability to manipulate a G·G base pair through another RNA structural element. The relationship between G15·G48 and A13·A22 in *E. coli* tRNA^{Cys} illustrates a means of manipulation of an RNA tertiary base pair to alter the function of the RNA. This raises the possibility that known G·G base pars of the N1·O6, Hoogsteen, or reverse Hoogsteen base pairing nature can also be manipulated, provided that their structural determinants are known. As more RNA tertiary hydrogen base pairs are identified, biochemical studies of these base pairs that elucidate the structural determinants of their formation will provide a most critical insight into higher-order RNA structures.

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