Molecular Recognition of tRNA by tRNA Pseudouridine 55 Synthase†

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Received July 1, 1997; Revised Manuscript Received October 24, 1997⊗

ABSTRACT: *Escherichia coli* tRNA pseudouridine 55 synthase catalyzes pseudouridine formation at U55 in tRNA. A 17 base oligoribonucleotide analog of the T-arm was equivalent to intact native tRNA as a substrate for pseudouridine 55 synthase, viz., the features for substrate recognition by this enzyme are completely contained within the T-arm. The structures and activities of mutant tRNAs and T-arms were used to analyze substrate recognition by pseudouridine 55 synthase. The 17-mer T-arm was an excellent substrate for the synthase, while disruption of the stem structure of the 17-mer T-arm eliminated activity. Kinetic data on tRNA mutants lacking single T-stem base pairs indicated that only the 53:61 base pair, which maintains the 7 base loop size, was essential for activity. The identities of individual bases in the stem were unimportant provided base pairing was intact. A major function of the T-stem appears to be the maintainence of a stable stem-loop structure and proper presentation of the T-loop to pseudouridine 55 synthase. The 7 base T-loop could be expanded or contracted by 1 base and still retain activity, albeit with a 30-fold reduction in k_{cat}. Kinetic analysis of T-loop mutants revealed the requirement for U54, U55, and A58, and a preference for C over U at position 56. Base substitutions at loop nonconserved position 59 or semiconserved positions 57 or 60 were well tolerated. Comparison of pseudouridine 55 synthase and tRNA (m^5U54)-methyltransferase revealed that both enzymes required the stem-loop structure. However, pseudouridine 55 synthase was not stringent for a 7 base loop and recognized a consensus base sequence within the T-loop, while tRNA (m^5U54)-methyltransferase recognized the secondary structure of the 7 member T-loop with only a specific requirement for U54, the T-loop substrate site. We conclude that recognition of tRNA by pseudouridine 55 synthase resides in the conformation of the T-arm plus four specific bases of the loop.

There are two post-transcriptional modifications which occur in the T-arm of all *Escherichia coli* and most other tRNAs: U54 to m^5U, and U55 to Ψ (*1*).[1] The enzymes that catalyze these modifications provide interesting tools for the study of protein-RNA recognition, because both recognize features which are common to all tRNA substrates. This contrasts with enzymes such as the amino acyl tRNA synthetases which only recognize cognate tRNAs among all tRNA species (*1, 2*), and tRNA-guanine transglycosylase, which recognizes several tRNAs (*3*−*5*).

The T-arm is one of the most highly conserved regions of tRNA, and in *E. coli* tRNA possesses the following features (Figure 1A). First, all *E. coli* tRNAs have a T-stem which has five base pairs. Second, the bases in the three base pairs of the stem which are distal from the loop are not conserved. Third, the Pu52:Py62 and G53:C61 bases in the stem positions adjacent to the loop are conserved in all *E. coli* tRNAs. Finally, the 7 base T-loop is conserved in size and contains four completely conserved bases, two semiconserved bases, and one nonconserved base (*5*). Since T-arm modifying enzymes recognize features common to all tRNA substrates, the recognition of multiple tRNAs by these enzymes presumably involves one or more of the conserved features of the T-arm.

We have recently reported studies on substrate recognition and catalysis by *E. coli* tRNA (m^5U54)-methyltransferase (RUMT),[1] which methylates U54 of the T-arm of tRNA (*6*). The most remarkable findings were that the features that governed substrate specificity were completely contained within the T-arm and that specificity was governed primarily by elements of the secondary and tertiary structure of the T-arm, rather than by its primary sequence (*7*).

Pseudouridine synthases (ΨSs) catalyze the post-transcriptional conversion of U to Ψ in a variety of prokaryote and eukaryote RNAs (*8*−*11*). There are two characterized ΨSs which catalyze the reaction at different sites of *E. coli* tRNA. tRNA ΨSI, the TruA gene product, catalyzes conversion of uridine to Ψ at positions 38, 39, and/or 40 in the anticodon loop, and Ψ55S, the *truB* gene product, catalyzes Ψ55 formation in most tRNAs (*12*).

† This work was supported by USPHS Grant GM-51232 (D.V.S.).

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⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1997.

[1] Abbreviations: Ψ55S, *E. coli* tRNA pseudouridine 55 synthase; RUMT, *E. coli* tRNA (m^5U54)-methyltransferase; $tRNA^{Phe}$, unmodified yeast $tRNA^{Phe}$ prepared by *in vitro* transcription; T-arm, the 17-mer oligoribonucleotide corresponding to nucleotides 49−65 of *E. coli* $tRNA_1^{Val}$ (GGCGGUUCGAUCCCGUC); 11-mer T-arm, the 11-mer oligoribonucleotide corresponding to nucleotides 52−62 of *E. coli* $tRNA_1^{Val}$ (GGUUCGAUCCC); tRNA mutants are designated by the wild-type base, followed by the position (tRNA numbering), followed by the mutation; Ψ, pseudouridine; ΨMP, pseudouridine monophosphate; Py, pyrimidine; Pu, purine.

S0006-2960(97)01590-0 CCC: $15.00
Published on Web 01/06/1998

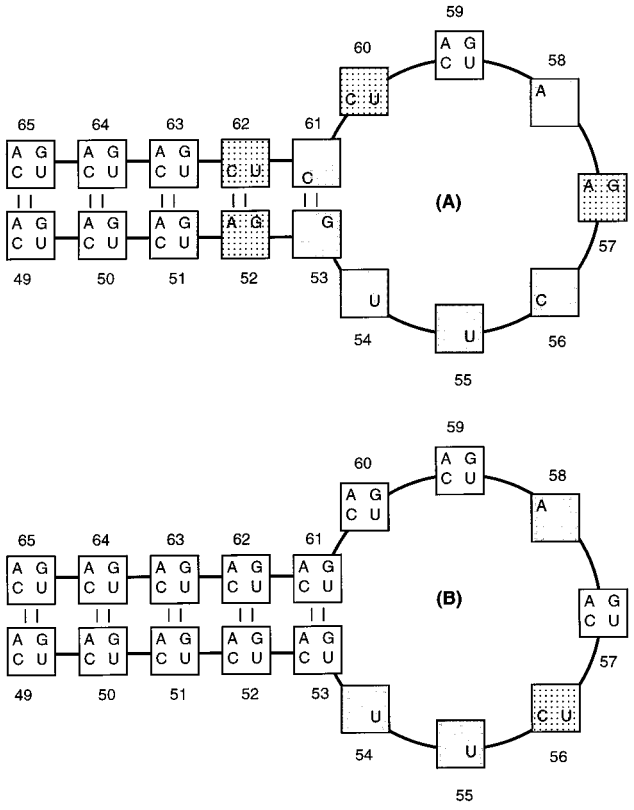

FIGURE 1: (A) Consensus sequence of the T-arm of tRNA derived from the structures of all *E. coli* tRNAs with Ψ55 (*5*). Dark shading indicates complete conservation of the base, light shading indicates conservation of Py or Pu, and no shading indicates nonconserved positions. (B) Consensus sequence derived from the data for tRNA mutants in Table 1.

In the present work, we describe studies which seek to understand the features involved in the molecular recognition of tRNA by Ψ55S and to compare and contrast such features with those involved in the interaction of tRNA with RUMT. Specifically, we have determined the minimal structure requirements for tRNA substrates and investigated the effects of single base or base pair mutations in the T-arm on substrate activity for Ψ55S.

MATERIALS AND METHODS

The plasmids p67YF0−82 used for preparation of mutant yeast $tRNA^{Phe}$ substrates were a gift from O. C. Uhlenbeck (Department of Chemistry and Biochemistry, University of Colorado). The plasmids p67YF101−137 containing variant tRNA mutant genes were constructed by cassette mutagenesis of yeast $tRNA^{Phe}$ gene in plasmid p67YF0 (*13*). The appropriate oligonucleotide duplex cassettes were inserted into the *Bgl*II and *Eco*RI sites of p67YF0 to give the plasmids containing tRNA mutant genes. T4 RNA ligase was from New England Biolabs. T7 RNA polymerase was isolated from *E. coli* BL21 harboring the plasmid pAR1219 (J. J. Dunn, Brookhaven National Laboratory, Upton, New York) and purified as described (*14*), except that S-Sepharose (Pharmacia) was used instead of Trisacyl SP.

Oligonucleotides were prepared at the UCSF Biomolecular Resource Center and purified as described (*15*). [5-^{3}H]UTP (20 Ci/mmol) was from Moravec Biochemicals Inc. The plasmid from pET-15b vector with the insertion of the *E. coli* Ψ55S gene was a gift from J. Ofengand (Roche Institute of Molecular Biology, Roche Research Center). Ψ55S was purified as described (*12*) and was homogenous (>95%) as assessed by SDS−PAGE. All molecular biology procedures not described were according to Sambrook *et al.* (*16*).

T-Arm and tRNA Synthesis. T7 RNA polymerase-catalyzed *in vitro* synthesis of the [5-^{3}H]Ura-T-arm or [5′-^{32}P]CMP-T-arm was performed using appropriate template and primer, nucleotide triphosphates {4 mM ATP, 4 mM CTP, 4 mM GTP and 0.1 mM [5-^{3}H]UTP (0.5 Ci/mmol) or 4 mM ATP, 4 mM GTP, 4 mM UTP and 0.1 mM [5′-α-^{32}P]CTP (0.5 Ci/mmol)} and T7 RNA polymerase. The [5′-^{32}P]UMP-11-mer T-arm was prepared using appropriate template and primer, nucleotide triphosphates {4 mM ATP, 4 mM CTP, 4 mM GTP and 0.1 mM [5′-α-^{32}P]UTP (0.5 Ci/mmol)} and T7 RNA polymerase (*17*). The labeled T-arms were purified on 7 M urea-20% PAGE. The concentration of the 17-mer T-arm or 11-mer T-arm was determined from the sum of extinction coefficients of the component nucleotides with correction for a hyperchromic effect of 1.21 or 1.14 (unpublished data).

T7 RNA polymerase-catalyzed *in vitro* synthesis of [5-^{3}H]-Ura-tRNA mutants and [5-^{32}P]CMP-tRNA mutants was performed with appropriate *BstNI* linearized plasmids, nucleotide triphosphates {2 mM ATP, 2 mM CTP, 2 mM GTP, 0.1 mM [5-^{3}H]UTP (0.5 Ci/mmol) or 2 mM ATP, 2 mM UTP, 2 mM GTP, 0.1 mM [5′-α-^{32}P]CTP (0.5 Ci/mmol)} and T7 RNA polymerase (*13*). The *in vitro* transcribed [5-^{3}H]Ura-tRNAs or [5′-^{32}P]CMP-tRNAs were purified on 7 M urea-15% PAGE. The concentration of the tRNAs was calculated assuming 1600 pmol/1 A_{260} (*18*).

Tritium Release Assay. Typically, reaction mixtures (40 μL) containing varying amounts of [5-^{3}H]Ura-T-arm or [5-^{3}H]Ura-tRNA (0.1−6 μM) plus 0.02−0.2 μM Ψ55S in assay buffer (20 mM Tris-HCl, pH 8.0, 100 mM NH_4Cl, and 2 mM EDTA) (*8*) were incubated at 37 °C. Aliquots (18 μL) were quenched at 0.5, 1, or 2 min by adding to 1 mL of 5% Norit A in 0.1 N HCl. The quenched sample was centrifuged briefly, and 0.8 mL of the supernatant was removed and mixed with 0.5 mL of 5% Norit A in 0.1 N HCl. This mixture was centrifuged for 2 min, the resulting supernatant was filtered through a glass wool plug in a 1 mL disposable plastic pipette tip, and 1 mL of the filtrate was counted in 6 mL Bio-Safe II (Research Products International Corp.) (*19*). Kinetic parameters were assessed by nonlinear least-squares fit of the data to the Michaelis−Menten equation.

Nucleotide Analysis. The pseudouridine formation reactions (20 μL) contained 1 μM [5′-^{32}P]CMP-T-arm (1×10^5 cpm), [5′-^{32}P]CMP-tRNA (3 x10^5 cpm) or [5′-^{32}P]UMP-11-mer T-arm (6×10^4 cpm), and 0.5 μM Ψ55S in assay buffer and were incubated at 37 °C (for T-arm or tRNAs) or 20 °C (for 11-mer T-arm) for 30 min. The samples were then diluted with 80 μL of water, extracted with an equal volume of phenol, and precipitated with 3 vol of ethanol (5−10 μg of carrier DNA and 10 μL of 3 M sodium acetate, pH 6, added). After the precipitate was washed with 80% ethanol and dried, the pellet was dissolved with RNase T2 digestion buffer and digested to completion with RNase T2 (for [5′-^{32}P]CMP-T-arm and [5′-^{32}P]CMP-tRNA) or with nuclease P1 digestion buffer and digested to completion with nuclease P1 for [5′-^{32}P]UMP-11-mer T-arm. The [3′-^{32}P]NMPs or [5′-^{32}P]NMPs produced were subjected to 2d-TLC on

Table 1: Activity of Mutants of Yeast $tRNA^{Phe}$ for Pseudouridine 55 Synthase[a]

mutant	plasmid	mutation	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (10^4 M^{-1} s^{-1})
A0	P67YF0	wild-type	0.78	0.24	31
A1	P67YF137	U52A/A62U	0.91	0.18	20
A2	P67YF122	U52C/A62G	0.91	0.011	1.2
A3	P67YF132	U52G/A62C	0.73	0.20	27
A4	P67YF121	U52C/A62C	1.4	0.011	0.79
A5	P67YF125	G53A/C61U	1.1	0.014	1.3
A6	P67YF127	G53C/C61G	0.96	0.006	0.63
A7	P67YF128	G53U/C61A	0.76	0.018	2.4
A8	P67YF138	C61A	*c*	*c*	*c*
A9	P67YF130	ΔU59[b]	2.2	0.008	0.45
A10	P67YF131	U60.1[d]	2.6	0.008	0.31
A11	P67YF40	U54A	*c*	*c*	*c*
A12	P67YF30	U54C	*c*	*c*	*c*
A13	P67YF101	U54G	*c*	*c*	*c*
A14	P67YF46	U55A	*c*	*c*	*c*
A15	P67YF13	U55C	*c*	*c*	*c*
A16	P67YF102	U55G	*c*	*c*	*c*
A17	P67YF103	C56A	*c*	*c*	*c*
A18	P67YF1	C56G	*c*	*c*	*c*
A19	P67YF104	C56U	0.97	0.006	0.62
A20	P67YF133	G57A	0.64	0.23	36
A21	P67YF105	G57C	0.81	0.11	14
A22	P67YF82	G57U	1.1	0.11	10
A23	P67YF106	A58C	*c*	*c*	*c*
A24	P67YF31	A58G	*c*	*c*	*c*
A25	P67YF107	A58U	*c*	*c*	*c*
A26	P67YF135	U59A	0.95	0.10	11
A27	P67YF34	U59C	0.91	0.11	12
A28	P67YF136	U59G	1.0	0.10	10
A29	P67YF108	C60A	0.79	0.21	27
A30	P67YF109	C60G	0.80	0.13	16
A31	P67YF35	C60U	0.75	0.15	20
B0		17 mer T-arm, wild-type	0.80	0.24	30
B1		18 mer T-arm mutant G48/A49−53/U61−65[e]	1.1	0.015	1.4
B2		18 mer T-arm mutant G48/A49−53/C61−65[e]	*c*	*c*	*c*

[a] Reactions were performed at pH 8.0 and 37 °C. [b] U59 was deleted. [c] Undetectable under the conditions used. The assay limit for k_{cat} was estimated as 0.002 s^{-1}. [d] U60.1 has a U inserted between C60 and C61. [e] G was added to the 5′ end of the T-arm to increase the yield of transcripts (*17*).

cellulose plates, using the following mobile phases: (first direction) isobutyric acid-concentrated NH_4OH-H_2O (66/1/33, v/v/v), (second direction) *tert*-butanol-concentrated HCl-H_2O (70/15/14, v/v/v) (*20*). The nucleotides were detected by autoradiography.

RESULTS

Ψ55S Specifically Modifies U55 of the 17-mer T-Arm and tRNA. When the 17-mer T-arm or tRNA were labeled with [5-^{3}H]UTP, treatment with Ψ55S resulted in the loss of 1.0 mol of tritium per mole of substrate; [5-^{3}H]Ura-tRNA mutants containing A, C or G at position 55 (A14-A16) (Table 1) showed no loss of tritium upon treatment with Ψ55S. Further, when the [^{32}P]T-arm was modified with Ψ55S and digested to with RNase T2, 2d-TLC analysis yielded the following ratios of [3′-^{32}P]NMP products: observed [3′-^{32}P]CMP/[3′-^{32}P]GMP/[3′-^{32}P]UMP/[3′-^{32}P]ΨMP, 1.9/0.9/2.4/1.0; calculated, 2/1/2/1.

11-mer T-Arm. The 11-mer T-arm, which does not contain the three base pairs of T-arm stem furthest from the loop, is a substrate for Ψ55S: when the [5′-^{32}P]UMP-11-mer-T-arm was treated with Ψ55S and digested with nuclease P1, [5′-^{32}P]ΨMP was observed by thin layer chromatogaphy (data not shown). Since we were unable to synthesize the [^{3}H]-UMP-labeled 11-mer in high enough specific activity and yield, we were unable to perform kinetic analysis on this compound under conditions of saturating substrate concentration, in order to assess the activity of the 11-mer.

Kinetic Measurements. Steady state kinetic parameters for Ψ55 formation were determined by varying the concentration of the 17-mer [5-^{3}H]Ura-T-arms or [5-^{3}H]Ura-tRNAs in the presence of limiting concentrations of Ψ55S. The kinetic parameters are given in Table 1.

Substrate Specificity of the T-Arm. Steady state kinetic analysis of tritium release from [5-^{3}H]U-labeled substrates indicated that the 17-mer T-arm from $tRNA_1^{Val}$ was as good a substrate for Ψ55S as the entire 76 base $tRNA^{Phe}$ (Table 1). Thus, the determinants for the binding and substrate specificity of Ψ55S for tRNA appear to be contained entirely within the T-arm.

T-Stem Mutations. The elimination of all of the base pairs in the stem structure (B2) totally eliminated Ψ55S activity, confirming the need for the stem-loop structure for Ψ55S activity.

The bases of the three base pairs of the T-arm stem furthest from the loop are not conserved in *E. coli* tRNAs nor are they necessary (Figure 1A) and cannot be primary determinants in specificity. All *E. coli* tRNAs contain Pu52:Py62 and G53:C61 stem base pairs adjacent to the loop (Figure 1A), and *a priori*, these could be important for Ψ55S activity. $tRNA^{Phe}$ mutants with all possible base pairs at 52:62 were prepared and examined as substrates for Ψ55S (Table 1, A1−A3). Two of the mutants, A52:U62 and G52:C62 tRNA (A1, A3), showed K_m and k_{cat} values similar to native $tRNA^{Phe}$ which contains the U52:A62 base pair, while the C52:G62 mutation (A2) had an unchanged K_m, but showed a 22-fold reduction in k_{cat}. The mutant containing C at both positions 52 and 62 of tRNA (A4), which cannot form a 52:62 base pair but has all other stem base pairs intact, was a substrate for Ψ55S with an unaffected K_m and a 20-fold reduced k_{cat}. Thus, Ψ55S has a preference for U52:A62 or Pu52:Py62 base pairs, but a 52:62 base pair was not required for substrate activity when the other stem base pairs were intact.

The substitution of the conserved G53:C61 base pair of tRNA by other base pairs (A5−A7) resulted in little change in the K_m, but in a 13−40-fold reduction in k_{cat}. However, disruption of this base pair (C61A-tRNA, A8) totally eliminated substrate activity. It was concluded that any base pair can suffice at 53:61, but that the conserved G53:C61 is preferred for optimal catalysis.

T-Loop Size Mutations. All native tRNAs possess a 7 base T-loop. The disruption of the 53:61 base pair in the C61A tRNA mutant described above (*A8*) resulted in a 9 base loop structure, which was not a substrate for Ψ55S. However, insertion of a U between positions 60 and 61 of tRNA (A10) to give an 8 base loop or deletion of the nonconserved nucleotide at position 59 (A9) to give a 6 base loop provided substrates with a 3-fold increase in K_m and a 30-fold decrease in k_{cat}. Thus, it appears that the 7 base loop size is optimal for activity, but the addition or subtraction of 1 base decreases but does not eliminate Ψ55S activity.

T-Loop Base Mutations. We prepared and examined the kinetic properties of individual T-arm mutants in which each

base of the loop was mutated to every other base (A11−A31).

U54 is conserved in all *E. coli* tRNAs (Figure 1A), and its substitution by any other base resulted in complete loss of activity (A11−A13). These observations indicate that U54 is essential for Ψ55S activity.

C56 is conserved in *E. coli* tRNAs and forms hydrogen bonds with G19 of the D-loop (*21*, *22*). The C56A and C56G tRNA mutants (A17−A18) were inactive, but the C56U mutant (A19) showed a normal K_m and 40-fold decrease in k_{cat}. Thus, Ψ55S prefers C at position 56, but accepts the pyrimidine U at that position with lowered catalytic efficiency.

There is a Pu57 in all tRNAs, and as expected, the G57 (A0) and A57 (A20) tRNAs were good substrates for Ψ55S. The Pu57Py mutants G57C and G57U (A21, A22) were also good substrates for Ψ55S.

All tRNAs contain a conserved A58 which forms a reverse-Hoogstein base pair with U54 that stacks over the G53:C61 base pair to stabilize the conformation of the T-arm (*23*). Thus, A58 plays a role in maintaining the secondary and tertiary structure of tRNA. In accord with this hypothesis, any mutation at position 58 of tRNA resulted in complete loss of Ψ55S activity (A23−A25).

Nucleotide 59 is the only completely nonconserved base in the T-loop and, as expected, tRNA mutants with any base at this position (A26−A28) served as substrates for Ψ55S.

The conserved Py60 in tRNA forms a H-bond with C61 (*23*). Since tRNAs containing any base at position 60 served as excellent substrates for Ψ55S (A29−A31), the conserved Py is not essential for Ψ55S activity.

DISCUSSION

We have been interested in determining the requirements for substrate recognition for enzymes involved in the modification of the T-arm of tRNA. Recently, we described studies on the recognition of mutants of intact tRNA and the 17-mer T-arm by RUMT, the enzyme which methylates U54 of tRNA (*7*). It was found that the 17-mer T-arm in itself was sufficient for binding and catalysis, albeit showing a lower k_{cat} than the intact tRNA substrate. Further, contrary to our expectations, we found that recognition of tRNA by RUMT did not involve the primary sequence of the tRNA. Except for U54, recognition relied on elements of the secondary and tertiary structure. In the present work, we describe similar studies on the binding and substrate properties of tRNA mutants by Ψ55S. The objectives of this study were to identify aspects of the molecular recognition of tRNAs and analogs by Ψ55S and compare them to those previously elucidated for RUMT.

Initially, we sought to determine a minimal structure within tRNA which would serve as a substrate for Ψ55S. We found that the 17 base oligoribonucleotide corresponding to the T-arm of tRNA had kinetic properties (K_m and k_{cat}) for Ψ55S that were almost identical to those of intact tRNA (Table 1). Since the T-arm is associated with other components of the tRNA molecule (e.g. H-bonding to the D-loop) (*21*, *22*), we expected that some perturbation of the kinetics would result from the energy necessary to disrupt intramolecular interactions and fully expose the T-arm to the enzyme. The similarity of catalytic properties of the T-arm and intact $tRNA^{Phe}$ suggests either that disruption of tRNA is not necessary for Ψ55S conversion of U55 to Ψ or that the energetics of the disruption of the tertiary structure in tRNA are negated by an even more favorable binding of the free T-arm. Regardless, the features of tRNA necessary for recognition and catalysis by Ψ55S are clearly contained within the T-arm.

Next, we systematically modified the structure of the T-arm of $tRNA^{Phe}$ by mutagenesis in an attempt to define the elements essential for Ψ55S activity. First, we modified the 7 base loop size of the T-arm, and found that, although the 7 base loop was optimal, the enzyme would accept tRNAs with 6 and 8 base loops (Table 1, A9, A10). Our study then focused on analysis of the requirements for specific bases in the T-arm for catalytic efficiency.

As anticipated, the disruption of all base pairs in the T-stem of the 17-mer (B2) totally eliminated Ψ55S activity, confirming the requirement for the stem-loop structure. At the outset, we anticipated that all individual bases in the 3 base pairs of the stem furthest from the loop were equivalent in terms of substrate activity, since these bases are not conserved in *E. coli* tRNAs which all contain Ψ55 (Figure 1A). We subsequently demonstrated that the 3 base pairs of the stem furthest from the loop could be deleted from the 17-mer T-arm and the resulting 11-mer retained Ψ55S activity, i.e., when the U52:A62 and G53:C61 base pairs were present, the other three base pairs were not required for Ψ55S activity. When the other stem base pairs were intact, the 52:62 and 53:61 base pairs proximal to the loop could be substituted by any other base pair (A1−A3, A5−A7) without total loss of activity, although some substitutions resulted in an up to 50-fold decrease in activity (k_{cat}/K_m). The disruption of the 52:62 base pair (A4) resulted in a 40-fold decrease in activity, while disruption of the 53:61 base pair (A8) totally eliminated Ψ55S activity. We conclude that, in general, the base pairs of the T-stem, rather than the specific bases, are important for maintaining the stem-loop structure necessary for Ψ55S recognition and catalysis. This is particularly true for the 53:61 base pair which maintains the 7 base size of the loop. As with the RUMT−tRNA interaction (*7*), it appears that the secondary structure of the T-stem is the key feature in maintaining substrate activity, and that a main function of the T-stem is to maintain an appropriate loop structure for presentation to Ψ55S and RUMT.

The results from mutation of each base of the T-loop of $tRNA^{Phe}$ and assessment of the substrate properties of the analogs were as follows: (i) conserved U54, which is immediately 5′ to the site of reaction and is the site of RUMT methylation to form m^5U (*1*), was essential for the activity of Ψ55S (Table 1, A11−A13); (ii) conserved C56, which is immediately 3′ to the site of reaction, could not be substituted by Pu, but accepted U with a 50-fold decrease in k_{cat}/K_m (A17−A19); (iii) Pu57 could be substituted by any base (A20−A22); (iv) conserved A58 could not be substituted without total loss of activity (A23−A25); (v) as in natural tRNA substrates, any base was acceptable at position 59 (A26−A28); and (vi) Py 60 could be substituted by any base (A29−A31). Thus, as depicted in Figure 1, the consensus T-arm sequence for Ψ55S activity is more relaxed at positions Pu52:Py62, G53:C61, C56, Pu57, and Py60 than is the natural consensus sequence of tRNA. The consensus

sequence for Ψ55S shows two completely conserved bases (U54 and A58) and one semiconserved base (Py56), in addition to the conserved substrate site U55 (Figure 1A).

The results of this work, together with analogous studies of tRNA mutants with RUMT (*7*) allow us to directly compare the features that govern substrate recognition and catalysis by Ψ55S to another T-arm modifying enzyme. The enzymes are similar in that (i) the structural features of tRNA which govern recognition and catalysis are contained within the 17 base sequence of the T-arm, and (ii) the function of the T-stem appears to mainly involve the appropriate presentation of the loop. The enzymes are different in their molecular recognition of the loop size and bases: (i) the recognition of tRNA by RUMT is stringent for a 7 base loop, whereas Ψ55S can accept loops one base smaller or larger, albeit with deletory effects on catalysis; and (ii) the recognition of the T-arm by RUMT is governed by elements of the secondary and tertiary structure of the T-arm rather than primary base sequence. In contrast, in addition to certain requirements for secondary structure, i.e. a stem-loop structure, Ψ55S are also stringent for specific bases in the loop, with Ψ55S showing a requirement for at least 2 conserved bases and 1 semiconserved base in addition to the conserved substrate site U55.

It is of interest to compare the results on RNA recognition by RUMT and Ψ55S to that of tRNA-guanine transglycosylase which modifies the G34 residue in the anti-codon loop of several *E. coli* tRNAs. As with the T-arm modifying enzymes, this enzyme recognizes and modifies small minihelices analogs of the anticodon stem-loop containing a stem, 7 base loop structure; hydrogen-bonded bases of the stem are required, but specific bases of the stem are not, and 3 bases of the loop (U^{33}-U^{34}-G^{35}) which are conserved in tRNA substrates are essential for activity (*4*, *5*). The general aspects of RNA recognition are thus quite similar to that of the T-arm modifying enzymes, in particular Ψ55S.

The fact that the T-arm of tRNA is more conserved than required for either RUMT or Ψ55S recognition raises the question of why elements of the T-arm of tRNA were conserved through evolution. The proposal that the conserved structural elements of the T-arm represents a composite of the recognition requirements of several systems (*7*) is supported by the present work. Thus, the combined requirements of Ψ55S and RUMT account for the conserved 7 base T-loop, U54, U55, Pyr 56, and A58. The remaining conserved elements in the T-arm, C56, Pu57, and Py60, may be required by other functions of tRNA.

ACKNOWLEDGMENT

We wish to thank Drs. O. C. Uhlenbeck and J. Ofengand for the plasmids used in this study.

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BI971590P