NMR Study of Isoleucine Transfer RNA from *Thermus thermophilus*†

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ABSTRACT: An NMR and nuclear Overhauser effect (NOE) analysis of *Thermus thermophilus* $tRNA^{Ile}_{1a}$ is presented. This species contains modifications including s^2T54 and s^4U8 [Horie, N., Hara-Yokoyama, M., Yokoyama, S., Watanabe, K., Kuchino, Y., Nishimura, S., & Miyazawa, T. (1985) *Biochemistry 24*, 5711–5715]. All the expected secondary and reverse Hoogsteen AU pairs were identified, with one possible exception. The general geometry of the TΨC loop is the same as the *Escherichia coli* species, and there is NOE evidence for an A9-UA12 triple. Preliminary measurements of solvent exchange rates of internally hydrogen-bonded bases suggest that this tRNA is more stable than previously studied *E. coli* and yeast tRNAs.

It is generally desirable to perform NMR studies of transfer RNAs (Reid, 1981), and their interactions with proteins, at the highest possible temperatures and often for long times. For this reason, it is of interest to study the proteins and nucleic acids of thermophilic bacteria. Such studies may also provide insight into the reasons for the thermal stability of these macromolecules. As a first step in this direction, we have performed an analysis of the NMR spectrum of a species of $tRNA^{Ile}$ from *Thermus thermophilus*, an extreme thermophile, by means of the nuclear Overhauser effect (NOE).[1] We have also performed preliminary NMR studies of the thermal stability of this molecule.

While this work was in progress, the primary structures of the isoleucine acceptors in *T. thermophilus* were determined by Horie et al. (1985), who kindly sent these structures to us and also analyzed our sample at the end of our research. The structures are remarkably similar to the abundant *Escherichia coli* species $tRNA^{Ile}_1$, differing only in substitution of GC pairs in place of AU1, GU5, and GΨ49, substitution of UA for GC7, substitution of G for C at position 16, substitution of unmodified U at position 47, and, in the case of the species we isolated, modification of positions 8, 18, 54, and 58 to s^4U, Gm, s^2T, and m^1A, respectively.

Materials and Methods

Purification of *T. thermophilus* $tRNA^{Ile}$ was similar to that described by Watanabe et al. (1980). Strain HB8 (ATCC 27634) was grown in a 100-L fermentor at 72 °C for 7 h after inoculation with 1.5 L of a culture grown for 15 h. The fermentor medium contained 0.8% polypeptone (BRL), 0.4% yeast extract (Difco, dehydrated), 0.2% NaCl, and 20 mg/L Antifoam A (Dow-Corning) and was maintained at pH 7 (as measured at room temperature). The harvest at the end of log phase yielded 4 g of wet cells/L.

Transfer RNA was phenol extracted and precipitated in ethanol buffered with potassium acetate at pH ~5. It was dissolved in 0.1 M Tris-HCl and 0.1 M NaCl at pH 7.5 and applied to a DEAE-cellulose column. It was eluted by a step to 1 M NaCl, ethanol precipitated, dialyzed against water, lyophilized, and stored at −20 °C. Subsequently, it was chromatographed on DEAE-Sephadex A-50 in 20 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, and an NaCl gradient from 0.375 to 0.525 M, followed by a DEAE-Sephadex G-50 column in 20 mM sodium acetate (pH 4), 10 mM $MgCl_2$, and a gradient from 0.4 to 0.7 M NaCl. The final chromatography was on a BD-cellulose column in 20 mM sodium acetate (pH 6) and a gradient from 0.4 to 1.1 M NaCl, followed by dialysis against water and lyophilization. Assays were performed using *T. thermophilus* aminoacyl-tRNA synthetase prepared by a procedure similar to that described for the *E. coli* enzyme (RajBhandary & Ghosh, 1969). Cells were ground in alumina, which was washed and centrifuged, and the supernatant was applied to DEAE-cellulose (Whatman DE52) in 20 mM potassium phosphate (pH 7.5), 20 mM mercaptoethanol, 1 mM $MgCl_2$, and 10% glycerol. Crude synthetase was eluted by a step to 250 mM potassium phosphate (pH 6.5), concentrated, and stored in 50% glycerol. Further details of the preparation can be found elsewhere (Choi, 1984). The tRNA accepted 1.6 nmol of isoleucine per A_{258} unit.

NMR samples were prepared by dissolving approximately 80 OD_{258} units of tRNA (~4 mg) in 160 μL of 20 mM EDTA, 10 mM sodium phosphate, 100 mM NaCl, and 1 mM $Na_2S_2O_3$ at pH 7. This was dialyzed in a microcell against 100 mL of the same buffer for 24 h with two changes of buffer to attempt to remove Mg^{2+}. It was then dialyzed twice against the same buffer but containing only 1 mM EDTA followed by a final dialysis against the same 1 mM EDTA buffer with 5% D_2O for lock. Unfortunately, we did not analyze the sample for Mg^{2+} but have done so with samples of yeast $tRNA^{Phe}$ and $tRNA^{Asp}$, and *E. coli* $tRNA^{Val}$, dialyzed in the same way, and found them to be nearly magnesium free (i.e., less than 0.4 mol of Mg^{2+}/mol of tRNA). $MgCl_2$ was added as a concentrated solution directly to the NMR tube.

Real-time solvent exchange was studied as described in greater detail by Figueroa et al. (1982). A pellet of tRNA was produced by alternate washing, spinning, and lyophilization of the sample in a microcentrifuge tube, and this pellet was then dampened with a small amount of H_2O buffer. The sample was then dissolved rapidly in the final D_2O buffer while

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[1] Abbreviations: NOE, nuclear Overhauser effect; Ψ, pseudouridine; s^2T, 2-thiothymidine; s^4U, 4-thiouridine; m^1A, 1-methyladenosine; Gm, 2′-*O*-methylguanosine; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; 2D, two dimensional; DSS, 4,4-dimethyl-4-sila-1-pentanesulfonate.

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on ice, just before being put in the NMR tube and spectrometer. The pH (=7) of the D_2O buffer is the uncorrected pH meter reading.

NMR methods were generally as described previously in this laboratory. Spectra were obtained on our homemade LDB-500 500-MHz spectrometer constructed by S. Kunz and A. Redfield, based on an Oxford magnet, probes from Cryomagnet Systems, and a computer programmed by A. Redfield.

After completion of all the NMR spectroscopy, which was performed on a single 80 OD_{256} unit sample, the sample was kindly analyzed by N. Horie and Dr. S. Yokoyama of the University of Tokyo, Japan, and Dr. Y. Kuchino of the National Cancer Center Research Institute, Japan. They further purified the sample by means of large-scale polyacrylamide gel electrophoresis and analyzed the nucleotide sequence by the postlabeling method (Horie et al., 1985). The sample was found to have the same sequence as *T. thermophilus* $tRNA^{Ile}_{1a}$ bearing s^4U8 as well as the modified base s^2T at position 54.

Results and Assignments

NMR Spectra. Figure 1 shows the downfield region of the spectrum of *T. thermophilus* $tRNA^{Ile}_{1a}$ for nominally zero, moderate, and high Mg^{2+} concentrations. As with other tRNAs, added Mg^{2+} produces definite but minor changes in the spectrum: peak I moves upfield, peaks O and V move downfield to merge with peaks N and U, respectively, and peak C splits into three peaks. Unfortunately, these shifts are not readily interpretable, and the purpose of such a Mg^{2+} concentration study is to find suitable concentrations for an NOE study. The sequence of this tRNA, as determined by Horie et al. (1982), is also shown in Figure 1.

The upfield NMR spectrum (not shown) contained the expected distinctive methyl resonance of s^2T54 at 0.96 ppm, in the same place where the T54 methyl resonance has been found in most other tRNAs. As expected, no other resonances (except for those from EDTA) were found upfield of 2.2 ppm. The methyl resonances of m^7G46, m^1A48, and Gm18 are all expected considerably further downfield, and we did not attempt to find or assign them. The resonance X in Figure 1 is almost certainly from the C8 proton of m^7G46, from its position and its slow exchange with solvent (see below), as found in other tRNAs.

The spectra in Figure 1 contain an unusual number of peaks in the range from 9 to 12 ppm. It is generally believed that this region contains ring NH protons not internally bonded in Watson–Crick or similar base pairs. Of these, only four are assigned (peaks M, P, W, and T), and three of these are internally bonded. There remain about eight other discernible peaks between M and X, which is nearly the same as the number of U's and G's that are not likely to be internally paired judging from the sequence and the yeast $tRNA^{Phe}$ crystal structure.

NOE Spectral Analysis. NOE data (Table I) were obtained in zero added Mg^{2+}, except for a few NOE observations with approximately 4 mol of Mg^{2+} added per mole of tRNA in solution. The addition of Mg^{2+} was found to split peak C into three peaks, C_1, C_2, and C_3, with useful NOE's. As usual, an AU pair is signaled by a strong imino to aromatic NOE, where the aromatic resonance is the adenine C2 proton of the AU pair.

We will not describe the NOE analysis in detail because for the most part it was a straightforward repeat of methodology described in detail in previous papers on tRNA NMR (Heerschap et al., 1982, 1983a,b; Hare & Reid, 1982a,b; Roy et al., 1982ab; Roy & Redfield, 1983; Tropp & Redfield, 1981). The data in Table I are grouped to make clear the three

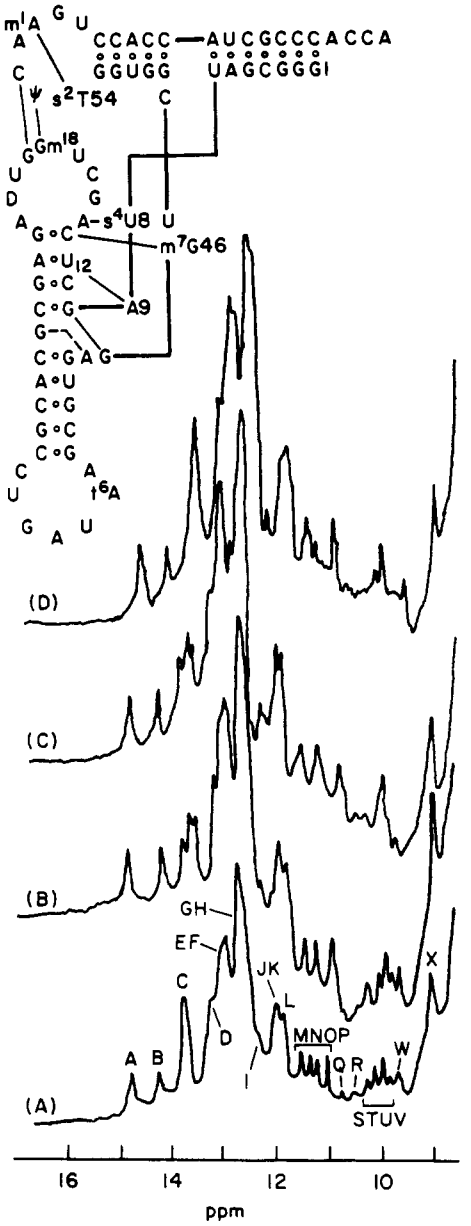

FIGURE 1: (A) Proton NMR spectrum of the downfield region of ~1 mM *T. thermophilus* $tRNA^{Ile}$ at 20 °C in 0.1 M NaCl, 0.01 M sodium phosphate buffer, pH 7, and zero magnesium. (B and C) Same as (A) with 4 and 15 equiv of Mg^{2+} added per tRNA. (D) Same as (A), zero Mg^{2+} at 37 °C. (Top) Sequence of the tRNA studied as determined by Horie et al. (1985). Likely secondary structure interactions, based on the yeast $tRNA^{Phe}$ crystal structure, are indicated by light lines. This structure is in the L-shaped representation proposed by Kim (1978), which approximates the yeast $tRNA^{Phe}$ crystal structure.

major NOE networks that we found in this tRNA. First, there is the unique acceptor stem sequence of the imino proton resonances of GC5-AU6-AU7. Second, there is a sequence starting from AU51 and including several protons in or near the TΨC loop. These were readily identified by their similarity to previously elucidated NOE's in other tRNAs involving the methyl protons of T54. Finally, there is a sequence starting with the tertiary imino proton resonance of s^4U8 and proceeding through the imino resonances of GC13, AU12, GC11, and GC10, which can be identified because there is no secondary AU-GC-AU-GC-GC sequence in this tRNA and because the first resonance in the sequence and its aromatic NOE partner have the typical relatively downfield shifts of the s^4U8-A14 tertiary base pair found in most *E. coli* tRNAs.

Table I: Summary of NOE Data[a]

label of irradiated peak	peak assignment[b]	ppm	transfer %[c]	NOE (ppm)	assignment[b]
B	AU6	14.35	5	12.03	GC5
			5	12.67	AU7
			30	7.64	A6 C2H
GH	AU7	12.67	30	7.15	A63 C2H
JK + L	GC5	12.03	5	14.35	AU6
	GC52		5	13.28	AU51
			5	13.77	
			5	13.0	
			8	12.67	
			5	10.91	
D	AU51	13.32[d]	5	12.03	GC52
			20	7.49	A63 C2H
Z	s^2T54 methyl	0.93	10	12.83	GC53, s^2T54 N3H
			5	12.03[e]	GC52
			8	11.56[e]	Ψ55 N3H
			15	11.08	Ψ55 N1H
			5	9.74[e]	G18
			30	7.53	s^2T54 C6H
W	Gm18 N1H	9.74	5	12.83	s^2T54 N3H
			5	11.56	Ψ55 N3H
P	Ψ55 N1H	11.08	<5	12.83	s^2T54 N3H
			5	11.56	Ψ55 N3H
			20	7.37	Ψ55 C6H
M	Ψ55 N3H	11.56	5	12.83	s^2T54 N3H
			5	11.08	Ψ55 N1H
			5	9.74	G18 N1H
EF	s^2T54 N3H	12.83	~30	8.34	m^1A58 C8H
C_3	GC11	13.76[d]	10	12.78	GC10
			~5	13.90	AU12
C_1	AU12	13.90[d]	5	13.0	GC13
			20	7.42	A23 C2H
			~5	13.76	GC11
A	s^4U8-A14	14.84	5	13.0	GC13
			15	8.6	A14 C8H
T	A9·A23·U12[f]	10.17	10	8.6	A9 or A23 C8H
C_2	AU28 or G26-A44?	13.84[d]	10	6.18	
			<5	13.32	
			<5	12.03	

[a] Done with zero added Mg^{2+} at 20 °C, and with 300-ms preirradiation time except as noted. Other conditions are given in the text. [b] Assignments to imino NH···N resonances in Watson–Crick base pairs are denoted by stating the pair only. [c] Estimate. [d] Observed with 5 equiv of Mg^{2+} added/tRNA. In zero added Mg^{2+}, peak D is at 13.28 ppm, and peaks C_1, C_2, and C_3 coalesce into peak C at 13.76 ppm. [e] Preirradiation time 0.75 s to promote multistep (spin diffusion) NOE's. [f] See text for detailed location.

In addition to these NOE networks, there is a single unusual NOE from 10.17 ppm which is tentatively assigned to protons in a presumed triplet structure consisting of A9, A23, and U12. This triplet structure was found in the X-ray structure of yeast $tRNA^{Phe}$ and is a Watson–Crick U12-A23 pair with the amino proton of A9 hydrogen bonded to N7 of A23 and a similar bond between the amino proton of A23 and N7 of A9. The NOE-connected pair of resonances are tentatively assigned to one of these amino protons and the nearest adenine C8 proton in the triplet. We have previously assigned a resonance in yeast $tRNA^{Phe}$, at 9.76 ppm, to the amino proton of A12 that is hydrogen bonded to N7 of A9 (Choi & Redfield, 1985). Unfortunately, in the present case we could find no NOE connection between these resonances and the AU12 uracil N3H resonance, as we did in yeast $tRNA^{Phe}$. This NOE assignment is probable but does not unequivocably establish the existence of the 9-13-12 triplet in the structure. On the other hand, the 10.17 ppm resonance exchanges with solvent moderately slowly (below) and must come from some internally bonded proton close to a ring carbon proton, and most likely not in a ring to ring NH···N hydrogen bond, as judged from its chemical shift.

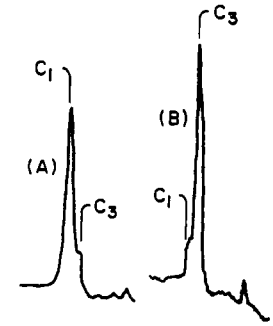

FIGURE 2: (A) NOE from peak C_1 to peak C_3, with four Mg^{2+} ions added per tRNA. (B) NOE from peak C_3 to C_1 under the same conditions.

The remaining AU pair (AU28) was assigned by default to be in peak C_2 (or peak C in zero Mg^{2+}), in view of the sharp aromatic NOE to 7.18 ppm from this peak. Another possible assignment for this resonance is the N1 proton of G26 base paired with A44, similar to the 26-44 base pairs in yeast $tRNA^{Phe}$ and $tRNA^{Asp}$ (Kim, 1978; Moras et al., 1980). We do not have a plausible explanation for the interesting NOE at 6.18 ppm from this peak.

Of the NOE connectivities in Table I, the most difficult to establish experimentally was the mutual connection between peaks C_1 and C_3. Data are shown in Figure 2. The connections are based on the small shoulders next to the direct irradiation peak. The lack of these shoulders when peak C_2 was irradiated shows that the shoulders are real NOE's and not spillover of saturation.

The assignment of GC53 also needs to be described in some detail. The assignments of s^2T54 N3H, AU51, and GC52 are solidly based on mutual NOE's and NOE's from Gm18 N1H and the s^2T54 methyl group. There should be clear NOE's between GC53 and the s^2T54 methyl resonance and the GC52 imino resonance. There is no strong common NOE in the expected region from the peaks including the latter resonances. The possibility that the GC52 and -53 resonances coincide tends to be ruled out by the lack of an NOE from peak JK, which contains the GC52 resonance, to the s^2T54 N3 proton resonance. The only remaining possibility is to assign GC53 to the composite peak which includes the s^2T54 N3 proton.

Solvent Exchange Rates. Solvent exhange rates were surveyed by the saturation–recovery method, between 24 and 37 °C in zero Mg^{2+}. All of the peaks assigned as internally bonded in Table I, as well as peaks assigned to Ψ55 and Gm18, showed recovery rates that were not faster, from 24 to 37 °C, than the roughly 5 s^{-1} expected from magnetic relaxation (Johnston & Redfield, 1981b), with the exception of peaks A and B (s^4U8-A14 and AU6) which showed no change up to 34 °C and then showed a measurable 5–10 s^{-1} increase in exchange rate between 34 and 37 °C. Of the unidentified resonances which we believe to arise from noninternally bonded ring N protons, resonances O, R, U, and especially V showed more rapid and highly temperature-dependent exchange and were undetectable above 34 °C. Resonances N and S showed weak temperature dependence in their exchange kinetics, ranging from about 10 s^{-1} at 24 °C to about 25 s^{-1} at 37 °C. Figure 1D shows the spectrum of the tRNA at 37 °C, illustrating the relatively few changes which occur.

Figure 3 shows a series of spectra taken at intervals after protonated tRNA was dissolved in a deuterated high Mg^{2+} concentration solvent, in order to observe real-time exchange of the imino protons. The protons resonating at peaks C_3, EF, and GH disappear in the order of 24–36 h whereas all the other imino protons in the structure are gone within 10 min. Peak

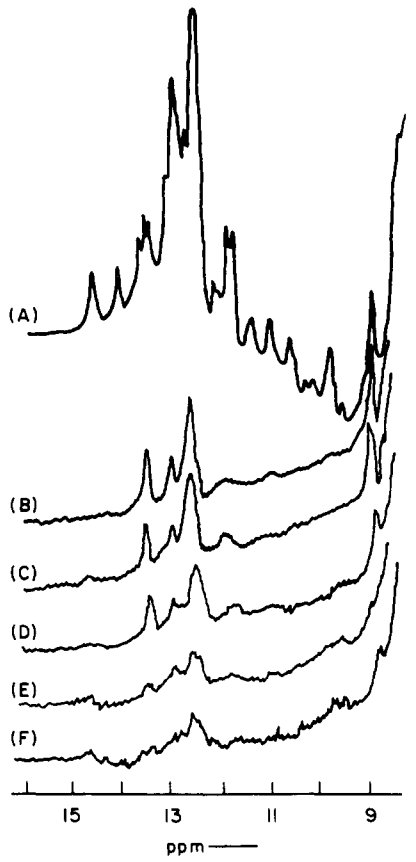

FIGURE 3: Spectra taken as a function of time after a wet protonated pellet of tRNA was dissolved in D_2O. (A) Control spectrum on the sample in H_2O buffer obtained before lyophilization. (B–F) Spectra 10, 64, 168, 1590, and 2160 min after dissolving in D_2O. The final spectrum shows some intensity due to the 10–20% H_2O content from the starting pellet.

C_3 is the resonance position assigned to the GC11 proton. Peaks EF and GH are composite peaks containing the imino proton resonances of GC13 and GC10, respectively, and it is most likely that these two bases contribute to the intensity of the slowly exchanging peaks seen in Figure 3 because these base pairs are located in the D stem which is generally the most stable part of the molecule by far (Figueroa et al., 1983; Leroy et al., 1985). Peak GH contains the intensity of at least two proton's resonances, and there could be another slowly exchanging resonance in this peak.

Resonance X disappears in about 2 h and is almost certainly the C8 proton of m^7G46, since a similar exchangeable resonance is seen in other tRNAs having m^7G at this position (Reid, 1981) and in one case its assignment has been strengthened by NOE from a methyl presumed to be the C8 proton of m^7G46 (Johnston & Redfield, 1981a). Monomer m^7G has a rapidly exchangeable C8 proton resonance at this chemical shift (Hurd & Reid, 1979).

Discussion

Spectral Analysis. We have found imino resonances of at least six out of seven Watson–Crick and reverse Hoogsteen AU base pairs in *T. thermophilus* $tRNA^{Ile}_{1a}$ and identified these six together with their NOE-coupled C2 or C8 proton resonances. We have also identified the upfield NH resonances of Ψ55 and Gm18, the s^2T54 methyl resonance, and, tentatively, an amino proton hydrogen bonded to a ring nitrogen, possibly in the A9-AU12 triple.

Studies on other tRNAs have appeared (Hare & Reid, 1982a,b; Roy & Redfield, 1983; Heerschap et al., 1982, 1983a,b; Hare et al., 1985) in which nearly all the downfield resonances are assigned and which may convey the impression that nearly complete assignments should generally be feasible. Although we identified most of the AU-type resonances, we could only locate 5 out of the 15 GC pairs. However, the nearly completely analyzed tRNAs are unusually favorable in having well-dispersed spectra and/or spatially closely spaced markers (GU pairs, methyl groups, or reverse Hoogsteen pairs that can be identified with deuteron labels) (Sanchez et al., 1980; Roy et al., 1982). *T. thermophilus* $tRNA^{Ile}$ contains only a limited number of such features and is considerably more difficult to study than yeast $tRNA^{Asp}$ in which the first massive interbase NOE study was performed (Roy & Redfield, 1981; Roy et al., 1982) and in which only 1 of 11 CG resonances was identified. These latter two tRNAs are more typical in their resistance to complete NMR identification, in our opinion, but in all cases despite the partial state of analysis, there is sufficient spectral identification to use for further study. Reports of two-dimensional (2D) NOE studies of tRNA have appeared (Hilbers et al., 1983; Hare et al., 1985), but 2D NOE in tRNA has not yet presented information not previously known from traditional one-dimensional NOE although 2D NOE may save some time. Isotope labeling has proven crucial in developing and testing the methodology of NMR analysis in tRNAs (Sanchez et al., 1980; Griffey et al., 1983) and may be useful here, especially in the case of ^{15}N labeling, for elucidation of the identities of ring NH and amino resonances between 9 and 11.5 ppm. However, the present analysis goes nearly as far as can be expected despite the limited sample quantity available to us.

Two of the chemical shifts of lines we have identified deserve further mention. At 12.83 ppm, the position of the s^2T54-m^1A58 imino proton resonance is unexpectedly far upfield for a reverse Hoogsteen AU pair; in several other tRNAs, the T54-A58 resonance is found around 13.7 ppm (Hare & Reid, 1982a; Roy et al., 1982b). However, the T54-m^1A58 resonance in yeast $tRNA^{Phe}$ is also found at roughly the same position, 12.4 ppm (Roy et al., 1984; Heerschap et al., 1983), and these observations suggest that methylation of A58 is responsible for the upfield shift of T54 N3H. The corresponding C8 protons of A58 in *E. coli* $tRNA^{Val}$ and m^1A58 in yeast $tRNA^{Phe}$ both resonate at 8.4 ppm (B.-S. Choi, unpublished results), suggesting that methylation of A58 does not change the aromaticity of its six-membered ring and that the effect on the s^2T54 N3 proton may be a steric interaction.

Another unusual upfield shift is that of AU7 (12.67 ppm instead of the usual secondary AU imino resonance position of 13–14.5 ppm). A nearly identical shift is found in *E. coli* $tRNA^{Val}$ for this resonance, of 12.6 ppm (Hare & Reid, 1985; Choi, 1984). Both tRNAs have the same three base pairs at the bottom of the acceptor stem and otherwise generally similar surroundings for AU7. We do not know the reason for this anomalous shift.

Table II summarizes the assignments of N protons made in this study and compares them to assignments of corresponding bases in the similar *E. coli* species, where the bases correspond (Hare & Reid, 1982b; Hare, 1983). All chemical shifts are the same within 0.2 ppm except those of AU6, GC11, and Ψ55 N1H. The difference in the case of AU6 is easily explained by the differences at positions 5 and 7 (GU and GC, respectively, in the *E. coli* tRNA). The relative shift of Ψ55 N1 could be an indirect result of modification of T54 to s^2T54 in *T. thermophilus*. The assignment of GC11 in the *E. coli* species is tentative (D. Hare, private communication). In yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Val}$, GC11 resonates in nearly the

Table II: Nitrogen Proton Resonance Shifts[a] in $tRNA^{Ile}$

location[b]	*T. thermophilus*[c]	*E. coli*[d]
GC5	12.03	
AU6	14.35	14.0
AU7	12.67	
S^4U8–A14	14.84	
GC10	12.78	12.55[e]
GC11	13.76	12.65(??)[e]
AU12	13.9	13.8[e]
A9 or A23 NH_2	10.17	
AU28	13.87(?)	13.75[e]
AU51	13.32	13.4
GC52	12.03	12.18
GC53	12.83	12.9
s^2T54 N3	12.83	
Ψ55 N1	11.08	10.6
Ψ55 N3	11.56	11.35
Gm18 N1	9.74	

[a] In ppm downfield from DSS. [b] Unless otherwise stated, the proton is at G N1 or U C2. [c] This research, $tRNA_{1a}^{Ile}$. [d] From Hare and Reid (1982b), $tRNA_{I}^{Ile}$. [e] Hare (1983).

Table III: Carbon Proton Resonance Shifts[a] in $tRNA^{Ile}$

location[b]	*T. thermophilus*[c]	*E. coli*[d]
AU6	7.64	6.9
AU7	7.15	
AU28	7.18(?)	7.2
AU12	7.42	
AU51	7.49	7.45
T54 C6	7.53	7.5
Ψ55 C6	7.37	7.3
A58 C8	8.34	8.3
A14 C8	8.6	

[a] In ppm downfield from DSS. [b] Unless otherwise stated, the proton is at A C2. [c] This research. [d] From Hare (1983) and Hare and Reid (1984).

same place (~13.6 ppm) as in *T. thermophilus* $tRNA^{Ile}$ (Roy & Redfield, 1983; Hare & Reid, 1982a). All four tRNAs have the same D-stem sequence.

Table III presents a similar summary and comparison for the carbon protons. The agreement between the two species is even better for this group of protons, except for AU6 which can again be explained by neighboring sequence differences. These results strongly suggest that there are no major conformation differences between these molecules.

Thermal Stability. Our studies of thermal stability were limited and preliminary. Furthermore, Leroy et al. (1984) have recently shown the incorrectness of the previously widely held belief that the measured proton solvent exchange rate is equal to the opening rate for internally bonded protons in tRNA. They have found and studied buffer catalysis, especially by imidazole, for solvent exchange of yeast $tRNA^{Phe}$ imino protons, and have shown convincingly that the opening time is in the several millisecond range for the acceptor stem and several minutes for the D-stem base triples in zero Mg^{2+} at room temperature. Proton exchange from an open state is rate limiting for overall exchange. In the absence of such buffer catalysis, and study thereof, proton exchange rates are obscure in interpretation, and in our following discussion, we hope and assume that they are nevertheless useful at least for rough comparisons.

As was already noted, there are an unusual number of resolved lines in the spectral region between 9 and 12 ppm, but this could be the result of fortuitous lack of resonance overlap compared to other tRNAs, rather than exceptionally slow exchange. We also have the impression that these resonances remain unbroadened to a higher temperature than do resonances in this spectral region in other tRNAs, but again this is hard to quantitate given the limited data. However, a direct comparison can be made in the case of the Ψ55 N1 and N3 protons, and the Gm18 N1 proton, where we found no detectable ($\sim$<5 s^{-1}) solvent exchange up to 37 °C by saturation–recovery. In contrast, in other tRNAs prepared similarly (Tropp & Redfield, 1983), there is definitely increasing exchange for these protons at 37 °C, ranging from a rate of about 60 s^{-1} in yeast $tRNA^{Phe}$ for both Ψ55 N protons down to about 15 s^{-1} in *E. coli* $tRNA^{Val}$. Yeast $tRNA^{Phe}$ might be considered the closest analogue for the Ψ55 N1 and N3 protons since both it and *T. thermophilus* $tRNA^{Ile}$ are methylated at A58. In the case of the Gm18 N1 proton, we can make a comparison with the G18 N1 proton of yeast $tRNA^{Phe}$ which has been identified (B.-S. Choi, unpublished results; Heerschap et al., 1983b). It is noticeably exchange broadened at pH 7 though much less so at pH 6, at 20 °C. It disappears by 37 °C at pH 7 (B.-S. Choi, unpublished results).

The internally paired protons also exchange more slowly than in other tRNAs. For example, the imino proton of AU6 exchanges at a rate of about 10 s^{-1} in this tRNA at 37 °C (Tropp & Redfield, 1983), whereas in *E. coli* $tRNA^{Val}$, where AU6 has nearly identical surroundings, the same proton exchanges at a rate of around 100 s^{-1}. However, this corresponds to a difference in temperature, for the same rate, of only about 5 °C between the two species. A similar difference is seen for the s^4U8-A14 proton. Other internally bonded protons cannot be compared so easily because the present studies were not carried out at high enough temperature.

Turning to the extremely slow exchangers, again *T. thermophilus* shows the expected signs of high thermal stability. There are three imino protons in it that exchange in a day or longer, whereas no other tRNA is known to possess more than one such extreme slow exchanger. Other such cases are the protons of CG11 in yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Val}$, and of U8-A14 in yeast $tRNA^{Asp}$. In *T. thermophilus*, these three protons are likely to belong to the D stem which is always found to be the most stable part of other tRNAs. Most likely, therefore, they are GC10, -11, and -13.

The behavior of the presumed three D-stem slowly exchanging protons is in marked contrast to that in *E. coli* $tRNA^{Val}$ (N. Figueroa, unpublished results; LeRoy et al., 1985) or yeast $tRNA^{Phe}$ (Johnston et al., 1979; LeRoy et al., 1984), both of which have identical D stems and the likelihood of similar base triples in the D stem. Both contain one extremely (>24 h) slow exchanger, identified to be the GC11 proton, but the next longest exchange times found are in the range of 4 h in yeast $tRNA^{Phe}$ and less than 0.5 h in *E. coli* $tRNA^{Val}$.

In summary, we have preliminary evidence for high thermal stability of *T. thermophilus* $tRNA^{Ile}$, compared to other tRNAs, which seems most dramatic in the region of the D stem and the T loop (as judged from the behavior of Ψ55 and Gm18). These studies should be repeated and extended by studying the effect of added exchange catalysts in order to obtain actual opening rates and estimates of equilibria (Leroy et al., 1984). Comparisons using these methods, between the various tRNAs mentioned in this paper, should be feasible and interesting. One comparison, of optically detected melting between *T. thermophilus* $tRNA^{Ile}$ species 1a and 1b differing only in modification of s^2T54, has already been reported (Horie et al., 1985).

Conclusion

We have presented the first NMR–NOE analysis of a tRNA from thermophilic bacteria. We have shown that,

judged by a comparison of proton exchange rate estimates with yeast and *E. coli* tRNAs, this tRNA is relatively thermally stable. A comparison of chemical shifts with a similar *E. coli* tRNA shows very minor differences in secondary structure and the structure of the TΨC loop. Thus, the greater stability of the *T. thermoplilus* tRNA is not reflected in a quantifiable change in structure detectable from chemical shifts of resonances we have identified.

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