Assignment of Imino Proton Spectra of Yeast Phenylalanine Transfer Ribonucleic Acid†

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ABSTRACT: Yeast $tRNA^{Phe}$ has been studied by using proton NMR and nuclear Overhauser effect (NOE) with deuterium substitution. Direct NOE evidence is presented for assignment of imino resonances of 23 of 27 base pairs in this tRNA. Other indirect evidence is presented for tentative assignment of four other base pairs. Almost total assignment also has been made of the important noninternally bonded imino protons and tertiary interactions (however, G18-Ψ55 remains unassigned). The most surprising result has been identification of GC11 at −13.68 ppm; this is the first time a GC base pair has been identified so far downfield. This peak (GC11) is also identified as the resonance of the unique imino proton that exchanges in a time of more than 1 day, as previously described. These identifications of imino proton resonances made it possible to reinterpret the proton solvent exchange rate data previously published on this tRNA and understand them better. The assignments of resonances should pave the way for more detailed solution study of this tRNA and its interaction with biologically relevant molecules.

NMR is used in biopolymers in general, and tRNAs[1] in particular, to assay mobility and, potentially, interactions and conformation changes (Jardetzky & Roberts, 1981; Reid, 1981). A necessary preliminary to such studies is the correct assignment of resonances to nuclei in the structure, and such assignment studies often lead to a test of the truth of the structure as seen by X-ray crystallography.

In the tRNAs, assignment studies of the downfield imino region of the proton NMR spectrum have had a checkered history. Early attempts based on predicted ring-current shifts did not survive a rigorous test based on nuclear Overhauser effect combined with semispecific deuteration (Sânchez et al., 1980). However, chemical modification was successful in a few cases. The assignment problems advanced steadily with the interpretation of NOE of unusual base pairs (such as GU pairs or pairs containing methylated bases) and of purine C8 deuterated tRNAs (Johnston & Redfield, 1981a; Tropp & Redfield, 1981). Most recently we have made large-scale use of NOEs between adjacent base pairs to assign a large region of tRNA (Roy & Redfield, 1981). These studies were carried out largely at 270 MHz, but in an optimum 500 MHz instrument it is sometimes possible to assign nearly all the imino resonances of tRNA in a relatively short time without the tedious task of preparing deuterated tRNAs (Hare & Reid, 1982). The same techniques are becoming extensively used for experiments on DNA fragments (Patel et al., 1982).

Here we describe the conclusions drawn from such interbase-pair NOE's observed in yeast $tRNA^{Phe}$ at 270 and 500 MHz. This is the fourth article based on NOEs in this species from this laboratory and in it we correct a few of our previous assignments that were clearly labeled as speculative in our earlier papers. We have now firmly assigned all but about three of the secondary imino protons; all the expected internal tertiary imino protons except G19-C56 and G18-Ψ55; and two noninternally hydrogen-bonded imino protons of Ψ55 and Ψ39. We have also assigned numerous carbon proton resonances in addition to the methyl resonances assigned by others. We will not describe all this earlier work in detail except as needed to justify arguments used herein. Our most recent previous paper in the series described NOE's of purine C2 deuterated tRNA and showed that the glycosidic bond of Ψ39 is in the syn conformation (Roy et al., 1982a). A study similar to the present one, on yeast $tRNA^{Asp}$, has also been published (Roy et al., 1982b). After the present paper was submitted for publication we learned of a similar assignment study of the same tRNA based on NOE at 500 MHz (Heerschap et al., 1982; C. W. Hilbers, personal communication). Although this set of assignments and ours were completely independent, at this writing there are only two disagreements, namely, the assignments of the GC1 and the T54-m^1A58 imino protons. We were also recently able to produce yeast $tRNA^{Phe}$ labeled at all purine N1 positions with ^{15}N and perform decoupling of the ^{15}N splitting of proton peaks. This experiment confirms the positions of several GC resonances (below). A similar experiment was previously performed by Griffey et al. (1982) on *Escherichia coli* tRNA fMet.

Materials and Methods

Yeast $tRNA^{Phe}$ was purchased from Boehringer Mannheim and used without further purification. Samples were not assayed, but we have previously repeatedly assayed such samples and found activities in the range of 1.5 nmol of acceptance/A_{258}. NMR samples were prepared by dissolving 5 mg of tRNA in 190 μL of buffer containing 20 mM EDTA, 10 mM phosphate (Na^+), and 0.1 M NaCl at pH 7.0. This was dialyzed twice in a microcell against 100 mL of the same buffer and twice against the same buffer but containing 1 mM EDTA. Final dialysis was against 100 mL of the same buffer containing, when desired, 1 mM EDTA and 5% D_2O for lock. $MgCl_2$ was added as required to samples that were dialyzed against zero EDTA. In what follows, "high magnesium" refers to samples to which 15 mM $MgCl_2$ has been added. All spectra were recorded on a homemade 270-MHz instrument

† From the Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254. *Received September 1, 1982.* This research was partially supported by U.S. Public Health Service Grant GM 20168. This is Publication No. 1433 of the Brandeis University Biochemistry Department.

[1] Abbreviations: tRNA, transfer ribonucleic acid; NOE, nuclear Overhauser effect; EDTA, ethylenediaminetetraacetic acid; T, ribothymidine; m^5C, 5-methylcytidine; Ψ, pseudouridine; m^1A, 1-methyladenosine; m^2G, 2-methylguanosine; m_2^2G, 2,2-dimethylguanosine; ppm, parts per million; m^7G, 7-methylguanosine.

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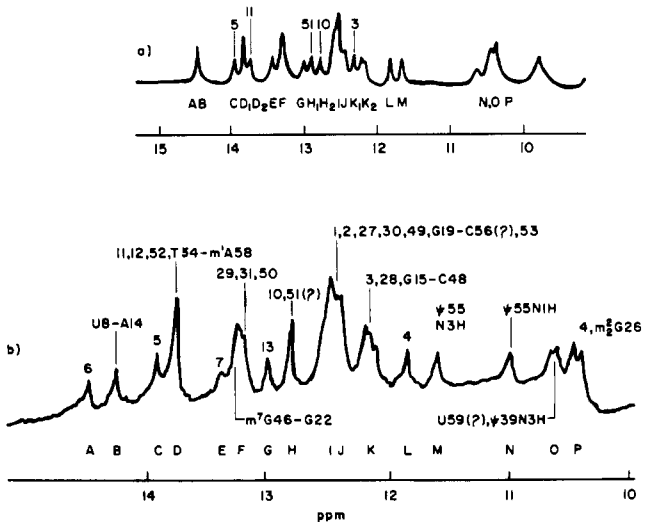

FIGURE 1: (a) NMR spectrum of yeast tRNA^{Phe} in buffer containing 15 mM added $MgCl_2$. (b) NMR spectrum in the absence of magnesium. The NMR spectra shown here are taken at 500 MHz. The peaks are lettered as referred to in the text. The set of assignments discussed is also given. Spectrum a was kindly provided by Dr. B. R. Reid.

as described previously (Johnston & Redfield, 1981a) or on the 500-MHz instrument at the Francis Bitter National Magnet Laboratory at the Massachusetts Institute of Technology.

Results and Assignments

Table I summarizes the most important new imino–imino and methyl–imino NOEs. Several other less important or less well-defined NOEs are mentioned in the text. We have presented those NOEs which establish connections between imino protons or are otherwise important for assignments, e.g., methyl to imino NOEs. NOEs to the aromatic region were described by Johnston & Redfield (1981a). Below we discuss the assignments in sections of the molecule. Figure 1 summarizes these assignments.

Almost all the weak NOEs reported here are interpreted as direct one-step NOEs over distances of 3.5–4 Å, but a few are attributed to second-order or domino-effect NOEs which we call spin diffusion. We and others have discussed ways to experimentally distinguish between spin diffusion and direct NOEs. However, in the present paper the distinction was made on the basis of the tRNA X-ray structure and by elimination of alternatives. Spin diffusion is relatively unimportant and unusual in NOE of the imino spectrum of tRNA because of the relatively few protons surrounding each imino proton.

Acceptor Stem. GU4 was the first imino base pair to be positively assigned in this tRNA (Johnston & Redfield, 1978), to peaks L and P (Figure 1). Johnston & Redfield (1981a) also tentatively observed an NOE from peak L to peak C and assigned AU5 to peak C. We have confirmed this NOE and observed that when peak C is irradiated NOEs to peaks L and P can be observed. Peak C is certain to be an AU pair, not only because of its position but also because there is a sharp NOE to the aromatic region from peak C which vanishes on C2 deuteration. Thus, we confirm the assignment of AU5 to peak C. From its intensity peak C was calculated to have one proton. When peak C is irradiated, in addition to the NOEs mentioned there is an NOE to peak A. Again peak A is an AU pair, by the same criterion used for peak C, and is assigned to the next pair AU6. When peak A is irradiated there is the expected back NOE to peak C and an NOE to peak E. This NOE can best be seen in the presence of one Mg^{2+} per tRNA

Table I: Summary of NOE Data[a]

assignment	peak irradiated	ppm	fractional transfer	NOE ppm	NOE assignment
AU6	A	14.35	0.05	13.80	AU5
			<0.05	13.25[b]	AU7
U8-A14	B	14.20	<0.05	13.20[b]	m^{7}G46
			0.05	12.90	GC13
			<0.05	12.20[b]	G15-C48
AU5	C	13.80	0.05	14.35	AU6
			0.05	11.80	GU4
			0.05	10.45	GU4
AU12	D	13.70	0.05	12.90	GC13
GC11	D	13.70	0.08	12.75[c]	GC10
			0.05	12.45	
UA7	E	13.25	0.05	14.35	AU6
AU29	F	13.18	0.05	12.20	GC28
			0.05	12.45	
GC13	G	12.90	0.05	14.20	U8-A14
			0.05	13.70[d]	AU12
GC10	H	12.75	0.05	13.70[e]	GC11
GC28	K	12.20	0.05	13.20	AU29
GU4	L	11.80	0.05	13.80	AU5
			0.05	12.20[f]	GC3
m^{2}G10	V	2.75	0.20	12.75[g]	GC10
			0.05	13.70[h]	GC11
m$_2^2$G26	W	2.44	0.25	10.40	m$_2^2$G26-A44
			0.08	12.45	GC27
			0.05	12.20[i]	GC28
T	Z	0.95	0.08	10.90	Ψ55 N1H
			0.05	11.55[j]	Ψ55 N3H
			0.05	13.70	T54-A58

[a] Conditions: NOE studies were done at zero magnesium chloride concentration and 20 °C, except as noted. All buffers contained 1 mM tRNA, either 1 mM EDTA or $MgCl_2$, 10 mM phosphate, pH 7.0, and 0.1 M NaCl. Preirradiation time was 300 ms. NOEs between imino protons of two base pairs are generally small and difficult to measure accurately. They are estimated to be approximately 0.05 of a single proton and therefore are given as 0.05. NOEs distinctly smaller are given as <0.05. [b] These NOEs are best seen in the presence of at least 1 mM Mg^{2+}. [c] In high magnesium, when peak D_2 is irradiated, NOE can be seen at peak H_2. [d] In high magnesium this NOE is observed at peak D_1. [e] In high magnesium irradiation of peak H_2 produces NOE at peak D_2. [f] In high magnesium the NOE is at peak K_1. [g] In high magnesium the NOE is at peak H_2. [h] In high magnesium the NOE is at peak D_2. [i] In high magnesium the NOE is at peak K_2. [j] In high magnesium the NOE is at peak D_1.

and at $T < 20$ °C. We believe that this Mg^{2+} dependence may be explained by relatively rapid chemical exchange of the proton resonating at E which is suppressed by magnesium and lower temperature. The assignment of AU7 to peak E is thus straightforward. We have also observed the reverse NOE from peak E to peak A. There is also an aromatic NOE to 7.18 ppm from peak E which must arise from the C2 proton of adenosine-66. Increased chemical exchange of AU7 may be due to an end fraying effect, and a similar increased exchange was observed for AU7 in yeast tRNA^{Asp} (Roy et al., 1982b). We have also irradiated peak L at 500 MHZ in high magnesium and find, in addition to the previously mentioned NOE to peak C, another NOE (Table I) to peak K_1 which can be assigned unambiguously to GC3. Johnston & Redfield (1981a) reported a heat-labile resonance in peak IJ and assigned it to GC1, and we have no new evidence concerning this assignment. In yeast tRNA^{Asp} similar behavior of AU1 was noted (Roy et al., 1982b). On the other hand, Heerschap et al. (1982) assigned GC1 to peak H_1. We have not obtained a direct NOE to GC2. However, there is a small NOE to peak IJ in addition to NOEs to peak C and K_1 when peak L is irradiated. Since peak L belongs to the GU4 imino proton and is a single proton peak, we interpret this as spin diffusion

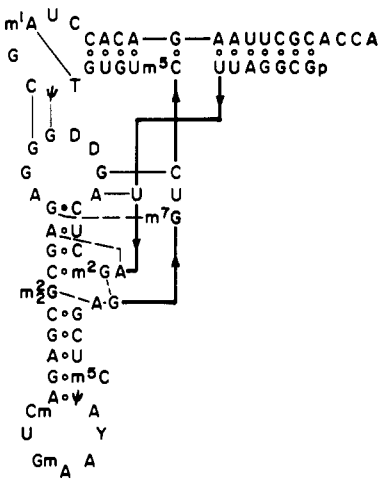

FIGURE 2: Nucleotide sequence of yeast tRNA^{Phe}. The sequence is shown in the form introduced by Kim to emulate the tertiary structure.

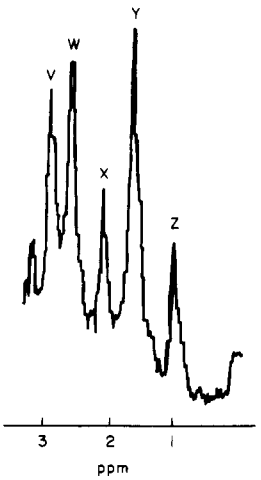

FIGURE 3: Methyl region spectrum of yeast tRNA^{Phe}. Solution conditions are 10 mM phosphate, pH 7.0, 0.1 M NaCl, and 1 mM EDTA.

to GC2 via GC3. Thus, we tentatively assign GC2 to peak IJ.

Observations on [1-^{15}N]purine-labeled tRNA, which we made after submitting this article for publication, show that peak L is unlabeled, so it is U69, and G4 is at peak P.

Dihydrouridine Stem and Nearby Tertiary Interactions. The m^{2}GC10 imino proton resonance has been reliably assigned to peak H in zero magnesium and peak H_2 at high magnesium (Johnston & Redfield, 1981a). We have also assigned U8-A14 (Figure 2) to peak B, GC13 to peak G, and AU12 to peak D in zero magnesium (Roy et al., 1982a). The same NOEs repeated at high magnesium place AU12 in peak D_1. This contradicts a previous assignment of AU12 to the slow exchanging peak D_2 (Johnston et al., 1979). When peak B is saturated by using longer irradiation times (≥300 ms), we observed two small NOEs to peaks F and K, in addition to the NOE to peak G already assigned to GC13. These small NOEs are best seen in the presence of 1 mM magnesium. These two NOEs probably are m^{7}G46 and G15-C48 imino protons, the two other imino protons not assigned around U8-A14. At present we do not have direct NOE evidence to differentiate between them, but we note the chemical modification experiment done by Hurd & Reid (1979) which places the m^{7}G46 imino proton resonance at peak EF. Our recent observations on [1-^{15}N]purine-labeled tRNA also show that there is a G imino proton resonance at peak EF, reinforcing this assignment. One other piece of indirect evidence reinforces the hypothesis that G15-C48 is at peak K. When peak K is irradiated there is a broad NOE to peak O. As seen in Figure 1 peak K is composed of three imino protons. The assignments to this peak are GC3, either G15-C48 or m^{7}G46 imino proton, and GC28 (as will be shown later). Now, neither GC3, GC28, nor m^{7}G46 has a noninternally hydrogen-bonded imino proton close by (assuming peak O is the resonance of such protons), whereas G15-C48 is close to the imino proton of U59, which is not internally paired. Therefore, assuming peak O is the resonance of a noninternally bonded imino proton, we assign it to U59. A similar NOE from A15-U48 to U59 has been observed in yeast tRNA^{Asp} (Roy et al., 1982a).

We assign GC11 to peak D_2 in high magnesium. The evidence is straightforward: in high magnesium both peak D_2 and peak H_2 are single proton peaks. There is an NOE from peak H_2 to D_2 and vice versa. Peak H_2 has been assigned to m^{2}GC10 and hence peak D_2 must represent an adjacent base pair. One adjacent base pair, m_2^2G26-A44, has already been assigned elsewhere to peak P. Thus, peak D_2 must be the other adjacent base pair, GC11. This is also reinforced by the observation that when the m^{2}GC10 methyl is saturated, in addition to the NOE to peak H_2 a weaker NOE is observed to peak D_2. Our recent ^{15}N labeling experiments confirm that a GC pair resonates at this point. Peak D_2 is unusually downfield for a GC proton and represents an exception to the rule that GC protons resonate upfield of 13.5 ppm. It is interesting that the AU11 imino proton in yeast tRNA^{Asp} (which is the base pair in the corresponding position in that tRNA) resonates at 14.65 ppm, further downfield than the rest of the AU resonances. Thus, the identity of the proton that exchanges in 1 day or more is GC11, not AU12 as initially assigned (Johnston et al., 1979).

Anticodon Stem. The N1 proton of Ψ39 has already been assigned to peak F and Ψ39 found to be bonded to the adenosine via ΨN1H. m^{5}CG40 has also been assigned to peak IJ (Roy et al., 1982a). We will not attempt to summarize the previously published reasoning which led to these assignments except to say that they were based largely on elucidation of the NOE patterns by observations on purine C2 deuterated tRNA. We have assigned GC27 to peak IJ at 12.45 ppm on the basis of an NOE from m_2^2G26 methyl. When the m_2^2G26 methyl (peak W, Figure 3) was irradiated, a large NOE was observed at peak P. This was assigned to the imino proton of m_2^2G26. There were two smaller NOEs to peak H and peak IJ at 12.45 ppm. These two NOEs were interpreted as NOEs to two adjacent base pairs, m^{2}GC10 and GC27 (Johnston & Redfield, 1981a). m^{2}GC10 has already been assigned to peak H and hence GC27 is assigned to peak IJ at 12.45 ppm. On longer irradiation times (≥300 ms) another NOE becomes evident at peak K at zero magnesium and peak K_2 at high magnesium. This is presumably spin diffusion to the next-nearest-neighbor base pair. Similar effects have been observed in *E. coli* tRNA^{Val} (Hare & Reid, 1982). Since GC11 on one side has already been assigned elsewhere, this NOE is assigned to GC28. One might conceivably argue that this NOE could be to the imino proton of G45, which hydrogen bonds to m^{2}GC10 in the crystal structure. We discount this possibility because there is another NOE from peak K to peak F at 13.2 ppm. In Figure 1 it can be clearly seen that there are three imino protons in peak K. Our area estimates also yield the same value. We have already assigned GC3 and G15-C48 there. Neither GC3 nor G15-C48 has one base pair adjacent which has been previously assigned to peak F (e.g., GU4 and GC2 adjacent to GC3 or U8-A14 adjacent to G15-C48 has been assigned elsewhere). So this NOE must arise from the

third imino proton of the three protons which resonate in peak K. If this were G45, then an adjacent base pair has to be assigned to peak F. Again, adjacent base pairs GC11, m_2^2G26-A44, m^2GC10, AU12, and GC27 all have been assigned elsewhere, so this must be the GC28 to AU29 NOE. Thus, we assign AU29 to peak F.

TΨC Stem and Nearby Imino Protons. m^5CG49 and GC53 have been assigned to peak IJ at zero magnesium by virtue of NOE from m^5C and T methyls, respectively (Roy et al., 1982a). NOEs from the T methyl resonance have been analyzed in detail (Tropp & Redfield, 1981), and the Ψ55 N1H has been assigned to peak N. On longer irradiation times (≥300 ms) another NOE to peak M can be clearly seen. The only noninternally hydrogen-bonded imino proton within a reasonable distance is Ψ55 N3H. Thus, we assign the Ψ55 N3H to peak M. Ψ55 N3H has been assigned previously in other tRNAs in similar positions [e.g., see Roy et al. (1982b)].

We have tentatively assigned T54-m^1A58 to peak D in zero magnesium and to peak D_1 in high magnesium on the basis of an NOE from the T54 methyl resonance. When the methyl resonance is saturated (≥300 ms), an NOE can be observed at peak D in zero magnesium and peak D_1 in high magnesium. All other imino protons within a reasonable distance (Ψ55 N1H, N3H; GC53) have been assigned elsewhere. Peak D is also at a reasonable position for an AU pair, and T54-A58 base pairs in two tRNAs have been assigned at about −13.8 ppm [yeast tRNA^{Asp}, see Roy & Redfield (1981); *E. coli* tRNA^{Val} observed by B. R. Reid (personal communication)]. However, T54-$m^1$58 has been assigned to a different line in yeast tRNA^{Phe} by Hilbers and co-workers (unpublished experiments).

There is another sharp NOE (Johnston & Redfield, 1981a) from peak D to a C2 proton in the aromatic region which remains unassigned. There are actually two NOEs and one of them belongs to AU12. This places either AU52 or AU50 at peak D. From the intensity of peak D it seems unlikely that a third imino proton can be accommodated in peak D and hence the other base pair (AU50 or AU52) must resonate at peak F (there are no other peaks in the AU region that can accommodate this extra intensity).

In high magnesium the methyl region peak Y (m^5C40 and m^5C49) is split into two peaks. Recently, we have observed NOE from both of these peaks to peak F at 500 MHz. We tentatively interpret this as NOE from m^5C40 to AΨ31 or AU29 or both and m^5C49 to AU50 (another adjacent base pair AU7 has been assigned to peak E). This would assign AU52 to peak D.

Peak H contains two imino protons, one of which is m^2GC10. The other proton must be one of the two unassigned pairs GC51 and G19–C56. Peak H_2 is heat stable and does not melt until all tertiary and acceptor stem resonances have melted (Johnston & Redfield, 1981b). If G19-C56 shows similar melting behavior to other tertiary resonances, GC51 must be assigned to peak H. Since no other peak in the GC region can accommodate the intensity of an extra base pair, we tentatively place G19-C56 at peak IJ.

Discussion

As mentioned in the introduction, Johnston & Redfield (1981b) have studied the thermal behavior of this tRNA in detail. At that time only a few reliable assignments were available and the data were interpreted on the basis of the assumption that these pairs in the same structural position (e.g., acceptor stem) melt in an organized cooperative fashion. Now that the assignments are available we can reexamine this hypothesis and reinterpret the data in detail. We should caution though that with so many overlapping lines, this reinterpretation of the data is not unambiguous. But with proper caution we can provide an interpretation that is essentially sound. For the actual data we refer to Johnston & Redfield (1981b).

In zero magnesium, between 23 and 29 °C there is melting of only one resonance at peak IJ. As discussed previously this may be GC1 and its thermolability may be due to end fraying effects. However, this assignment is not solid, and Heerschap et al. (1982) dispute it. Between 29 and 33 °C there are several resonances that show increased exchange. Most of these resonances belong to acceptor stem or tertiary protons.

Increased exchange could be observed for AU7 (peak E), AU6 (peak A), and AU5 (peak C), in that order. Additional melting of peak IJ could be interpreted as lability of GC2. Loss of intensity of peak K could be attributed to either GC3 or G15-C48 or both. It is interesting to note that at this temperature (33 °C) peak L, assigned to one of the GU4 imino protons, has not yet shown any intensity loss. This would seem to imply that the acceptor stem is showing increased lability toward both ends. On the other hand, if Heerschap et al. (1982) are correct in assigning GC1 in peak H_1 (which is quite thermostable), only one end, i.e., AU7, is showing increased lability. Indeed, from NOE experiments it appears that at 20 °C and zero magnesium, AU7 is more rapidly exchanging than AU6, AU5, or GU4. Thus, the early assumption [e.g., Johnston & Redfield (1981b)] that individual stems would melt concertedly is incorrect, as already indicated by other NMR studies (N. Figueroa et al., unpublished experiments).

Among other protons showing increased exchange are peaks M, N, D, and F. Increased exchange at peak F may reflect lability of the m^7G46 imino proton or AΨ31 or both. Peaks M and N represent Ψ55 N3H and N1H, respectively. Peak D is composed of four resonances, GC11, AU12, T54-m^1A58, and AU52. It is unlikely that GC11 or AU12 would be the labile proton because other D stem resonances GC13 (peak G) and GC10 (peak H) have not melted. None of the TΨC stem markers are single proton peaks so we cannot make a similar argument about AU52 which is in this stem. However, our general experience is that the TΨC stem is rather stable (Roy et al., 1982b). So most likely the labile resonance is T54-m^1A58 though AU52 cannot be completely ruled out. If this assignment is correct, there is increased exchange around the T54-m^1A58 tertiary interaction. At a slightly elevated temperature (36 °C) peak B (U8-A14) begins to melt.

At 43 °C GU4 is completely melted. In addition to the heat-labile peaks already mentioned, peak G (GC13) loses intensity. At 47 °C peak H (m^2GC10) also shows an intensity loss. The loss of resonances at peak D may be interpreted as melting of GC11 and AU12.

We now have unambiguously assigned GC11 to the slowly exchanging peak D_2 in high magnesium. The other slowly exchanging peak (~1 h) has also been assigned to GC13. The whole exchange experiment thus now can be interpreted as slow exchange of D stem protons: GC11 (peak D_2), GC13 (peak G), m^2GC10 (peak H), and extra intensity at the slow exchanger [reported as 1.5 protons by Johnston et al. (1979)] possibly from AU12.

Thus, we conclude that in zero magnesium acceptor stem and tertiary resonances melt almost concurrently followed by the dihydrouridine stem, whereas in high magnesium the dihydrouridine stem becomes the most stable structure.

In conclusion, we summarize the history and status of the assignment problem in this tRNA, restricting our statements to work from this laboratory. No assignment previously la-

beled by us as certain has had to be revised. Previous incorrect tentative assignments that are now revised and solid are AU7, AU12, GC11, and GC3. All other internal imino assignments are solid, in our opinion, except the following: two GC pairs GC1 and GC51 could be interchanged since they are based on melting data; T54-m^{1}A58 is in dispute; G19-C56 has not been located at all. Among noninternally bonded imino proton resonances only three out of eight are assigned, namely, Ψ55N3, Ψ39N3, and U59. Some of the other five may exchange too rapidly to be seen by NMR at neutral pH.

Thus, on the basis of our work on this tRNA and on yeast tRNA^{Asp} published elsewhere, and on the work cited from other laboratories at 500 MHz, we conclude that reliable assignments in these molecules can now be made relatively rapidly and in adequate numbers for use in studies of conformation change and mobility and interactions with proteins.

Acknowledgments

We thank Mary Z. Papastavros for excellent technical assistance and Dave Ruben and Ron Haberkorn for helping us to use the NMR facility at Francis H. Bitter National Magnet Laboratory at Massachusetts Institute of Technology, supported by Grant RR00995 from the Division of Research Resources of the National Institutes of Health and by the National Science Foundation under Contract C-670. We also thank C. Hilbers and R. Griffey and their co-workers for communicating their results prior to publication and for permission to quote them.

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Cyclobutane-Type Pyrimidine Photodimer Formation and Excision in Human Skin Fibroblasts after Irradiation with 313-nm Ultraviolet Light†

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ABSTRACT: The formation and excision of 313-nm light-induced cyclobutane-type pyrimidine photodimers were determined in confluent cultures of human fibroblasts. A new method was developed for the resolution and determination of cytosine–thymine (CT) and thymine–thymine dimers (TT) by using sodium borohydride reduction and high-pressure liquid chromatography. This assay can detect as little as 1.8 TT or 5.6 CT per 10^8 daltons, levels induced in monolayers of human skin fibroblasts by doses of 1 and 2 kJ m^{-2} of 313-nm light, respectively. CT formation was 20% more efficient than TT formation in the physiological dose range of 2.25–15 kJ m^{-2} at 37 °C. Normal fibroblasts removed 61% TT within the first 8 h of incubation following a dose of 5.5 kJ m^{-2}. CT was removed approximately twice as efficiently as TT during the same time period following exposure to 10 kJ m^{-2}. The lack of removal of CT as well as TT observed in xeroderma pigmentosum fibroblasts indicates that the repair deficiency in these cells affects the repair of both classes of dimers.

Ultraviolet light in the range from 290 to 320 nm (UV-B)[1] is mostly responsible for sunlight-induced skin cancer (Blum, 1959; Urbach, 1975). DNA remains the most important target for the biological effects of UV light in this region of the spectrum, although its absorption decreases preciptously toward longer wavelengths (Setlow, 1974; Sutherland & Griffin, 1981). The major mechanism of the formation of DNA damage by UV-C in the region of the absorption maximum of DNA involves the electronic excitation of the heterocyclic

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[1] Abbreviations: CT, cyclobutane-type photodimer between cytosine and thymine; CC, cyclobutane-type cytosine–cytosine photodimer; DMEM, Dulbecco's modified Eagle's medium; HPLC, high-pressure liquid chromatography; $NaBH_4$, sodium borohydride; NF, normal human skin fibroblasts; PyT, cyclobutane-type pyrimidine dimer containing thymine; TT, cyclobutane-type thymine–thymine photodimer with cis-syn stereochemistry; UT, cyclobutane-type photodimer between uracil and thymine; UV, ultraviolet light; UV-B, UV light from 290 to 320 nm; UV-C, UV light of wavelength shorter than 290 nm; XPA, xeroderma pigmentosum skin fibroblasts of complementation group A; Me_2SO, dimethyl sulfoxide.

0006-2960/83/0422-1390$01.50/0