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Nuclear Overhauser effect study of yeast tRNA^{Val} 1: evidence for uridine-pseudouridine base pairing

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ABSTRACT

The proton NMR spectrum of yeast tRNA^{Val} 1 has been studied using nuclear Overhauser effect (NOE), including comparison of NOE patterns between purine C8 deuterated and nondeuterated samples. Studies of the downfield region enable us to reliably assign many resonances in the acceptor and D stems. Prominent among these reliable assignments is that of the unusual base pair U¥, which is made here for the first time. Other identifications include GU2, U8-A14, the three AU base pairs of the acceptor stem, and N1 and N3 protons of ¥55.

INTRODUCTION

NMR studies utilizing the nuclear Overhauser effect (NOE) have been directed in recent years to a growing variety of biological molecules, aiding in the elucidation of both structural and binding properties. Nucleic acids which have been investigated by this method include several tRNA species among which are the phenylalanine[1] and aspartate[2] accepting species from yeast, and the valine 1 accepting species from E. coli.[3] The NOE has been used to study DNA conformation and dynamics as well.[4]

Briefly, the NOE experiment involves saturation of specific lines in the proton NMR spectrum. Partial transfer of saturation to resonances of nearby protons (generally < 4Å away) can be detected by difference spectroscopy. A discussion of NOE theory and its practical applications may be found elsewhere.[1,5]

The major result of NOE studies on tRNA has been the successful assignment of lines in the downfield region of the proton NMR spectrum to counterparts in the tRNA structure. These assignments have included both previously unassigned, and also erroneously assigned lines. Use of the NOE approach has allowed researchers in this laboratory to demonstrate conclusively the existence of the wobble GU base-pair,[6] to identify and distinguish between standard Watson-Crick and reverse Hoogstein AU base-pairs,[7] and to use interbase interactions as criteria for assignment.[2,3] We report here the application

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of the NOE studies to yet another tRNA species, valine isoacceptor 1 tRNA from yeast, whose secondary structure is shown in Fig. 1.

The approach taken here is to compile a list of NOE's resulting from saturation of NMR lines in the relatively uncluttered imino and methyl regions of the spectrum and correlate these with structural information, to yield a set of assignments. Two assumptions are made in this process: 1. The solution structure of yeast $tRNA^{Val}$ 1 is assumed to be reasonably similar to the X-ray structure[8] of yeast $tRNA^{Phe}$. Similarity between the two species has been inferred, for example, based on the susceptibility of their phosphodiester bonds to the action of ethylnitroseurea,[9] as well as the usual base and secondary structure similarities.

2. tRNA imino protons are assumed to resonate in spectral regions determined by the base pair they belong to:[6,10] AU protons resonate at 13.1 to 14.5 ppm, GC protons at 11.8 to 13.3 ppm and GU protons at 10 to 12.5 ppm.

Once the assignments have been made, additional information pertaining to properties of the tRNA can be gathered by studying the spectrum under altered conditions of temperature, magnesium concentration, etc.

MATERIALS AND METHODS

$tRNA^{Val}$ 1 was prepared from crude yeast tRNA (Boehringer), using the method described by Kryukov et al.[11] with some variations. The sequence of chromatographic steps was as follows:

1. BD cellulose column at pH 5.[12]

2. Sephadex A-50 column. 2,940 A_{258} units in 50% starting buffer at a concentration of 200 units/ml. were loaded on a 1.43 × 81 cm column. The elution rate was 5 ml/hr/cm^2 and fractions were collected 30 minutes. Starting buffer: 500 ml 0.325M NaCl, 0.008M $MgCl_2$, 0.02M Tris (pH 7.5). Ending buffer: 500 ml 0.5M NaCl, 0.016M $MgCl_2$, 0.02M Tris (pH 7.5). The valine fraction eluted at about 0.45M NaCl.

3. BD cellulose column at pH 3.5 which resolves the isoacceptors. The elution profile of the valine accepting peaks off this column was somewhat different than that reported in the original study,[11] with Val 1 eluting at 0.85M - 0.95M NaCl. Specific activity of the Val 1 was 1.2 nmole/O.D. unit. The purine C8 deuterated sample was prepared similarly from a side fraction of the BDC step of the preparation described by Sánchez et al.[7] with a Sepharose 4B column replacing the Sephadex A50 step. Specifications for this column were as follows: 3,600 A_{258} units in starting buffer at a concentration of 400 buffer: 500 ml 0.5M NaCl, 0.016M $MgCl_2$, 0.02M Tris (pH 7.5). The valine

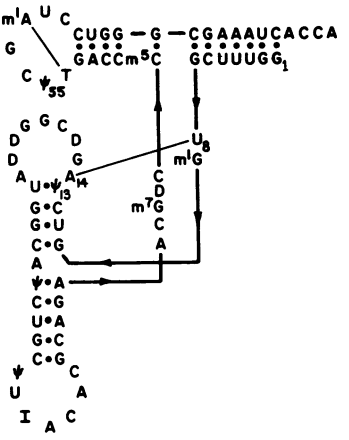

Figure 1. Nucleotide sequence of yeast $tRNA^{Val}$ 1. The sequence is shown in the form introduced by Kim, to emulate the tertiary structure. The two expected reverse-Hoogsteen pairs are shown as light solid lines. Other likely tertiary interactions are omitted.

fraction eluted at about 0.45M NaCl. 3. BD cellulose column at pH 3.5 which resolves the isoacceptors. The elution profile of the valine accepting peaks off this column was somewhat different than that reported in the original study,[11] with Val 1 eluting at 0.85M - 0.95M NaCl. Specific activity of the Val 1 was 1.2 nmole/O.D. unit. The purine C8 deuterated sample was prepared similarly from a side fraction of the BDC step of the preparation described by Sánchez et al.[7] with a Sepharose 4B column replacing the Sephadex A50 step. Specifications for this column were as follows: 3,600 A_{258} units in starting buffer at a concentration of 400 units/ml were loaded on a 0.9 × 88 cm column. Elution rate was 5 - 6 ml/hr/cm^2 and fractions were collected every 60 minutes. Starting buffer: 30 ml 1.3M $(NH_4)_2 SO_4$, 0.01M $MgCl_2$, 0.01M sodium acetate (pH 4.5). Ending buffer: 300 ml 0.4M $(NH_4)_2 SO_4$, 0.01M $MgCl_2$, 0.01M sodium acetate (pH 4.5). The valine fraction eluted at about 1.15M $(NH_4)_2 SO_4$.

NMR samples were prepared by dialyzing the tRNA in a microcell against several changes of buffer containing 10mM NaCl, 10 mM sodium phosphate, and 20mM EDTA at 7.0, followed by successive dialysis against otherwise identical buffers containing 1mM, and then no, EDTA. The dialysis against the zero EDTA buffer was performed with the dialysis cell placed in series with a small Chelex 100 column. The final buffer contained 5% D_2O for heteronuclear lock.

NMR spectra were recorded on a 270 MHz instrument at 20°C. Some spectra and NOEs were obtained on the 500 MHz spectrometer at the Francis Bitter National Magnet Laboratory of the Massachusetts Institute of Technology. NMR methods have been described elsewhere.[1,5,6]

RESULTS

The lowfield NMR spectrum of yeast $tRNA^{Val}$ 1 is shown in Fig. 2. The

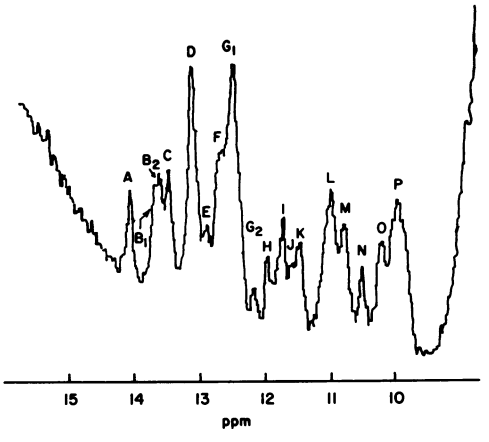

Figure 2. 270 MHz downfield spectra of yeast $tRNA^{Val}$ 1. The sample contains 5% D_2O, 10 mM phosphate (pH 7.0) and no magnesium. Spectra was recorded at 20°C. Labeling of peaks is arbitrary and corresponds to the text.

majority of studies were carried out at 20°C and at zero Mg^{+2}.

Integration of peaks A, B, and C, which are in a region of the spectrum which contains AU protons exclusively, gives a ratio of one to five or six between the area of peak A and the combined area of peaks B and C. Since the molecule contains only eight AU or AΨ base pairs, each contributing one imino proton, peak A is most likely a single proton peak. One or two additional AU imino proton resonances may be in the borderline peak D. AU base pairs contain an imino proton (N3H of uracil) close to a carbon ring proton of adenine (C2 or C8) so that an NOE from the imino peak to the aromatic region of the spectrum can usually be observed. The aromatic resonance arises from the adenine C2 proton in conventional Watson-Crick base pairs and to the C8 proton in reverse Hoogstein base pairs. In order to distinguish between the two types of base pairing, a purine C8 deuterated tRNA was prepared, as described in Materials and Methods. NOEs to the C8 position will disappear in this deuterated sample.

Six of the expected eight imino to aromatic NOE's show the characteristic narrow NOE linewidth of carbon protons: peak A shows a 20% NOE at 7.79 ppm, peak B_1 a 30% NOE at 7.12 ppm, peak B_2 a 30% NOE at 7.00 ppm, peak C shows NOEs at 6.84 ppm (10%) and 7.67 ppm (5%), and peak D shows a 20% NOE at 6.94 ppm. Of these six NOEs, only that from peak C to 7.67 ppm disappears in the C8 deuterated sample. Since the AU imino peaks are close in frequency to each other, irradiation of the region must proceed in small steps to ensure that the effects observed are indeed due to the NOE and not a result of saturation spillover.

GU base pairs are the only type of secondary base pairs that place two imino protons (N3H of uracil and N1H of guanine) close enough to observe an

NOE between them,[6] and the protons involved resonate typically between 10 to 12.5 ppm. Two pairs of peaks connected by NOEs are observed in this region, the pairs IK and LN (Fig. 3). The size of the LN NOE varies considerably with temperature, as discussed below. At 15°C this NOE reaches 20%.

To ensure that the effect observed was indeed a genuine NOE and not due to saturation spillover, an experiment was performed in which irradiation of one of the assumed GU peaks was compared to irradiation at a frequency equidistant to the proposed NOE, but opposite in frequency direction (Fig. 3). If the NOE is genuine, the effect observed due to the irradiation of the GU peak will exceed that of the other irradiation by the NOE's true magnitude. With respect to the IK pair, the question could not be resolved on the 270 MHz spectrometer, but was successfully answered affirmatively on the 500 MHz instrument.

The NOEs described so far are typical of intra-base pair effects. Smaller effects which typify NOEs between protons on different, adjacent base pairs[2,3] have also been observed in this tRNA. They include NOEs from peak A to peak B_1 (8%), and to I and K (<5%), and between peak C and peaks L and N (5%).

The methyl region of the NMR spectrum (0 - 4 ppm), like the imino region, is amenable to the NOE approach as it is relatively uncluttered. In yeast $tRNA^{Val}$ we have concentrated so far on the T54 methyl protons, whose resonance at 0.96 ppm is well established.[10] NOEs from this peak to the downfield region include those at 11.61 ppm (<5%), 10.94 ppm (5%), 8.76 ppm (10%), and at 7.44 ppm (50%).

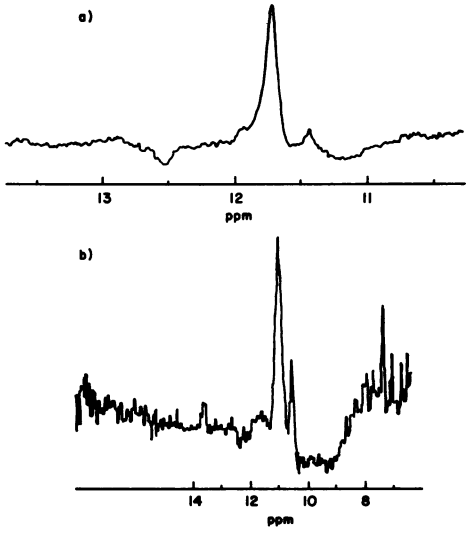

Figure 3. NOEs between GU or UΨ peaks. (a) Irradiation at peak I, using the 500 MHz spectrometer. As described in Results, saturation spillover has been eliminated by subtraction of the spectra obtained from irradiation at a frequency equidistant to peak K but opposed in field direction; (b) Irradiation at peak L. Besides the NOE to peak N an inter base-pair NOE is observed at 13.5 ppm (peak C).

TABLE 1: Summary of NOE intensities and assignments.

Irradiated Resonances	A	B_1	B_2	C	D	I	K	L	N	T methyl
ppm	14.15	13.76	13.70	13.55	13.15	11.73	11.44	10.97	10.47	0.96
Assignments	U3 (U3A70)	U4 (U4A69)	(AU)	U8 (U8A14)	(AU)	-----GU2-----		----U2-Ψ13----		T54
NOEs/Assignments (ppm)	NOE Intensities (%)									
14.16 U3 N3 (UA3)		8				<5	<5			
13.76 U4 N3 (UA4)	8									
13.55 U8 N3 (U8-A14)								5	<5	
11.73 GU2 imino	<5						*			
11.61 Ψ55 N3										<5
11.44 GU2 imino	<5					*				
10.97 Ψ13-U22 imino				5					20	5
10.47 Ψ13-U22 imino				<5				20		
(8.76)										(10)
7.79 A70 C2 (UA3)	20									
7.67 A14 C8 (U8-A14)				5						
7.44 T54 C6										50
7.12 A69 C2 (UA4)		30								
7.00			30							
6.94					20					
6.84				10						

*NOE intensity not determined (see Results).

DISCUSSION

A summary of the assignments made in this paper is shown in Table 1.

Yeast $tRNA^{Val}$ 1 has two or three expected GU type base pairs (GU2, UG11, and possibly UΨ13), and eight AU type base pairs (UA3, UA4, UA5, U8-A14, AΨ27, UA29, AU52, AU41, AU62, AU62 and T54-m^1A58), two of which (U8-A14 and T54-m^1A58) are tertiary reverse Hoogstein basepairs. It should be noted that the base pair AΨ27 can form in either of two ways,[13] in a manner that would place either the N1 or the N3 proton of Ψ27 within close proximity of the C2 proton of A43. In the case that N1H of Ψ27 is internally hydrogen bonded, a total of nine imino to aromatic NOEs should be found.

Two of the GU and six of the AU base pairs have been located in the NMR spectrum. Specific identification of some of these base pairs is possible when the NMR data is correlated with the assumed tertiary structure of the tRNA.

Study of the structure reveals two instances of spatially close AU and GU type base pairs: U8-A14 is situated close to UΨ13 and UA3 is close to GU2.

As reported in Results, two sets of AU to GU inter-base pair NOEs are observed: peak A is connected to the GU pair IK and peak C is connected to the pair LN.

We can now proceed to assign these peaks as follows: U8-A14 is a reverse-Hoogstein base pair while UA3 is a standard Watson-Crick base pair. Thus the imino to aromatic NOE for U8-A14 is expected to disappear in the C8 deuterated sample, but this should not be the case for the UA3 NOE. Indeed, peak C shows an NOE (to 7.67 ppm) which disappears in the C8 deuterated sample, while the NOE from the single proton peak A does not. Peak C is thus identified as the N3H resonance of U8, the NOE at 7.67 as C8H of A14, and the LN pair as resonances of the imino protons of the unusual base pair U¥13. The assignment of the 7.67 ppm peak is not irrefutable since peak C is a multiproton peak and could conceivably arise from both reverse-Hoogstein imino protons. Thus, the NOE at 7.67 ppm may be the T54-m^1A58 C8 proton, and the NOE to the C8 proton of A14 may be unobserved as of yet. In any case, the assignment of peak C as N3H of U8 and other imino assignments hold.

As a consequence of the assignment scheme outlined above, the NOE between peaks A (UA3) and B_1 identifies B_1 as UA4. Thus, three of the eight AU type NOEs have been identified. Of the remaining five base pairs, three have been detected by imino to aromatic NOEs (peaks B_2, C and D). Since none of these NOEs disappears upon C8 deuteration, we have as yet failed to find the NOE of one of the tertiary base pairs, probably T54-m^1A58.

The scheme given above provides conclusive evidence for the existence of the unusual base pair U¥13 at the loop end of the D stem. This region of yeast tRNA is highly variable, with positions 13 and 22 populated by a variety of both purine and pyrimidine bases, leading to both conventional Watson-Crick and unconventional wobble base-pairing possibilities. This has led authors to draw the secondary structure of tRNA (including that of yeast $tRNA^{Val}$)[13] without a base-pair between positions 13 and 22, although such a base pair has been observed in the crystal structure of yeast $tRNA^{Phe}$ (where it is a conventional GC pair)[14] and despite the assertion that the three-dimensional structure of tRNA, based on the yeast $tRNA^{Phe}$ example, allows for a wobble base pair in the 13-22 position without distortion of the tertiary structure.[15] The evidence presented here has thus served to clarify a structural problem. NOE methods[2] have similarly shown the existence of a G22-¥13 base pair in yeast $tRNA^{Asp}$, proving NOE to be a consistent tool in the structural determination of tRNA regions that are amenable to this approach. Nonstandard pairing in RNA pentamer duplexes has also been studied by NMR.[16]

The significance of this first observation of a pyrimidine-pyrimidine-

pair in tRNA extends beyond the study of tRNA structure. The observation lends support to the model for wobble interactions proposed by Grosjean et al.[17] and to the proposed scheme for mitochondrial codon-anticodon base-pairing,[18] both of which include the possibility of U-U base pairs.

The NOE between peaks L and N, which we have shown to come from the UΨ13 base-paired imino protons, exhibits particularly early melting behavior; the size of the NOE is reduced sharply between 15°C and 20°C and the peaks themselves are greatly reduced in size at 28°C. This behavior may be a result of weak bonding between the bases due to structural constraints which do not allow for optional hydrogen-bonding and/or due to the unusual nature of the base pair itself. The appreciably higher melting temperatures of[19,20] the more conventional base pairs GΨ13 in yeast $tRNA^{Asp}$ and GC13 in yeast $tRNA^{Phe}$ lend impetus to these arguments. Additional instability may be caused by the lack of magnesium in the samples. The base pair in the same position in yeast $tRNA^{Phe}$, GC13, is stabilized by addition of magnesium.[21,22]

Finally, the NOEs from the thymidine-54 methyl protons are identified by comparison with similar NOEs observed in other tRNA species.[5] The NOE at 10.94 ppm (peak L) is identified as the N_1H imino proton of Ψ55 and the NOE to 7.44 ppm is identified as the C6 proton of T54. The NOE to 11.61 (Peak J) is identified as the N3H imino proton of Ψ55. This last assignment is bolstered by observation of early melting (as low as 28°C) of peak J. Similar NOEs and melting behavior have been observed[21,22] in yeast $tRNA^{Asp}$ and yeast $tRNA^{Phe}$.

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