Nuclear magnetic resonance observation of the triple interaction between A9 and AU12 in yeast tRNA^{Phe}

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ABSTRACT

The nuclear Overhauser effect (NOE) was used to identify one of the amino proton resonances of base A23 in interaction with A9. These bases form a triple with U12 in the D stem of yeast tRNA^{Phe}. The identification was verified by finding an NOE from this amino proton to the C8 proton of A9, as determined by comparisons of NOE's in a native and a C8-deuterated sample.

INTRODUCTION

One of the reasons for studying nucleic acids by NMR is to be able to detect unusual or unexpected local tertiary interactions. To do so requires first characterizing fingerprints for such features in nucleic acids of more or less known structure. Examples of such structural features previously detected in yeast tRNA^{Phe} and several other tRNA's include GU, Gγ, Uγ, and Aγ pairs^{1,2,3} that take the place of secondary base pairs; reverse Hoogsteen tertiary pairs^{2,4}; and the general area of the TγC loop⁵. As a byproduct of a brief study of the pH dependence of tRNA NMR spectra we were able to find evidence for another one of these interactions, namely the A9-UAl2 triple in yeast tRNA^{Phe}. Such a triple could easily occur as a tertiary feature in other nucleic acids and might be detectable by NMR. The NMR fingerprint of this triple is unusual in that it involves an amino proton rather than one of the imino protons that are most often studied by NMR in nucleic acids.

RESULTS AND DISCUSSION

Figure 1a shows the 500 MHz NMR spectrum of yeast tRNA^{Phe} at pH 7. The only obvious NMR spectral feature which changes on lowering the pH from 7 is a bump denoted QR, at 9.75 ppm, that shows two distinct side peaks which sharpen at pH 6 as well as a broader underlying peak between them. A nuclear Overhauser experiment irradiating this peak on a 270 MHz instrument gave the reproducible NOE's shown in Table 1. These NOE's were not fully convincing at 270 MHz so we repeated the observations at 500 MHz, on a pH 7 sample with zero

Figure 1. A. Downfield NMR spectrum of yeast tRNA^{Phe} in 10 mM phosphate (pH 7), 0.1 M NaCl, 10°C, rigorously dialyzed to remove magnesium. Peak labelling is the same as in references 1 and 7. B. NOE observed when the center of peak QR is irradiated. C. The same when the upfield shoulder of peak QR is irradiated. Inset: The A9-U12-A23 triple.

Mg⁺⁺, 0.1 M NaCl, at 10°C, as shown in Figures 1b and 1c. Methods were as described elsewhere.^{5,6}

The NMR region between 9 and 11.5 ppm contains resonances that have been generally believed to belong to imino protons of unpaired bases or GU pairs, and a number of such resonances have been identified. We were able to identify two sources for the NOE's in Table I. One of them, the N1 proton of

Table I. NOE's observed when peak QR is irradiated.

Peak	ppm	Percent NOE	Assignment
EF	13.2	~2	m ⁷ G46-G22 (G22N1H)
IJ	12.4	5	T54-m ¹ A58 (T54N3H)
M	11.5	5	ψ55N3H
N	10.9	2	ψ55N1H
D	13.7	5	AU12 (U12N3H)
	8.4 ^a	~8	A9C8H
	7.24 ^b	~5	ψ55 and/or T54 C6H
	7.72 ^b	~5	A21 C2H?

a. Disappears in purine C8 deuterated sample.

b. Obscure except in purine C8 deuterated sample.

G18, was independently identified by Heerschap et al⁸, and accounts for the first four NOE's listed in Table I. Our reasons for identification of the G18N1 proton as a component of peak QR are essentially the same as those of Heerschap et al and we will not repeat them. The NOE's in Figures. 1b and 1c, and another one not shown from the downfield shoulder of peak QR, indicate that the G18 N1 proton resonates in the central hump of peak QR.

The other NOE-active component of peak QR, localized at its upfield shoulder (Figure 1c), could be identified by considering the NOE to peak D, which has been shown to contain the secondary base pairs GC11, AU12, and AU52. According to the x-ray structure there are no imino protons from unpaired bases spatially near any of these three base pairs. In any case all imino protons from base pairs would not resonate so far upfield as peak QR, with the conceivable exception of the imino proton of G45 which is involved in the tertiary interaction with m²G10. This proton is too far from any of the three base pairs resonating in peak D (pairs 11,12, and 52) to give a first or higher order NOE.

Therefore we must look further for an explanation of the NOE from peak QR to peak D. An obvious candidate presents itself in the x-ray structure when we examine the triple A9-UA12 (Figure 1). The amino proton of A23 that is hydrogen bonded to N7 of A9 could be shifted from the normal amino position of about 8 ppm, to 9.75 ppm, by the A9 ring current shift. The NOE to the AU12 imino proton could be a first order NOE, or more likely a second-order NOE via the other amino proton between the two.

As a test of this hypothesis we first sought a sharp aromatic NOE from peak QR, to the C8 proton of A9. This was found (Figure 1), at 8.4 ppm, and

Figure 2. NOE spectra of a purine C8-deuterated tRNA sample; otherwise similar to the runs of Figure 1. A. Central part of peak QR irradiated. B. Upfield shoulder of peak QR irradiated.

we repeated the experiment on a sample, previously used to test identifications of reverse Hoogsteen resonances, that had been biosynthetically deuterated at all purine C8 positions and dialized in the same way as the unlabeled sample of Figure 1. The spectrum of this sample was normal despite its age, but the NOE from peak QR to 8.4 ppm was missing (Figure 2), confirming that it was from a purine C8 proton, and confirming the identification of the A9-UA12 triple.

Also clearly visible in Figure 2 are two sharp NOE's at 7.72 and 7.24 ppm, which appear to arise from the central part of peak QR. The one at 7.24 ppm is likely to be from the G15 N1 proton to the T54 and/or Ψ 55 C6 protons, via spin diffusion⁵. The one at 7.72 ppm has no obvious identification. One speculation is that it arises from NOE from the G15 amino proton hydrogen bonded to C48 N3, to the C2 proton of A21, an invariant base stacked above the tertiary G15-C48. This is consistent with a small NOE in Figure 2, to peak K which contains the G15 imino resonance of a proton hydrogen bonded to the C48 carbonyl. However, the latter NOE seems too small, and this speculation calls for further study.

As a byproduct of this experiment we also located the NOE from the

reverse Hoogsteen T54-m¹A58 imino proton at 12.4 ppm to the m¹A58 C8 proton, also at 8.4 ppm (not shown). We verified that this NOE was also missing in the purine C8 deuterated sample, providing further proof of the T54-m¹A58 identification. The identification of this resonance was essentially the only important difference between two independent nearly full assignments of the yeast tRNA^{Phe} imino spectrum,^{7,8} and was correctly made by Heerschap *et al*⁸ and later strongly supported by use of ¹⁵N labeling.⁹ The NOE from peak QR to 8.4 ppm is far too strong to be a multiple order NOE to the m¹A58 C8 proton which resonates at the same place.

The exceptional lability of the peak QR in the spectrum at pH 7.0 has been pointed out above. A comparison of solvent exchange rate of this peak at pH 7.0 and 6.0 as a function of temperature and magnesium shows significant differences in the melting behavior (data not shown). The interpretation of such differences produced by one pH unit is not simple, but the stabilization at pH 6.0 could be the result of protonation of the A9N1 position and resulting in a decrease in general base catalysis of the G18N1 proton. Long ago Steinmetz-Kayne *et al.*¹⁰ proposed that a proton on ring N1 of A9 could interact with nearby phosphates electrostatically and stabilize residue A23 and A9 in a salt bridge at acidic pH. Our observations are consistent with these ideas.

The type of amino-to-ring (N7) hydrogen bond exemplified by the A9 to A23 interaction is also likely to occur when nucleic acids bind to proteins. Therefore the present identification suggests that NOE's similar to the amino-imino NOE reported here might be worth looking for in protein-nucleic acid complexes.

In conclusion, the utility of isotopic substitution in combination with NOE has again been demonstrated. We have verified a potentially widely occurring feature, namely the hydrogen bonding mode in the A9·A23·U12 triple as predicted by x-ray crystallography.

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