Assignment of resonances in the *Escherichia coli* 5 S RNA fragment proton NMR spectrum using uniform nitrogen-15 enrichment

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The downfield proton NMR spectrum of aqueous uniformly nitrogen-15 enriched 5 S RNA fragment is presented. Selective nitrogen-15 decoupling difference proton spectroscopy revealed nitrogen-15 chemical shifts of fragment imino nitrogens. Nitrogen chemical shifts of nucleic acid guanine and uracil imino nitrogens have separate small ranges. Nitrogen-15 and proton chemical shift correlation by the heteronuclear decoupling permitted the identification of the base type of some previously unassigned imino proton resonances in the 5 S RNA fragment spectrum. Corresponding resonances in the natural isotopic abundance 5 S RNA fragment spectrum are assigned to base types by comparison with the enriched sample spectrum.

5 S RNA fragment *Proton NMR* *Uniform ^{15}N enrichment* *Heteronuclear decoupling* *Chemical shift correlation* *Assignment strategy*

1. INTRODUCTION

The potential of NMR spectroscopy to reveal detailed information about macromolecular structure and dynamics is only realised after the macromolecule resonances are assigned. Recently nucleic acid imino proton resonances have been assigned using the heteronuclear proton-nitrogen-15 coupling in the downfield proton spectrum of aqueous tRNA labelled with nitrogen-15 at position N(3)H in uracil and related bases [1,2]. Selective heteronuclear J-decoupling difference spectroscopy revealed the imino proton resonances with nitrogen-proton coupling [3]. Subsequently nitrogen and proton chemical shift correlation maps were obtained for the labelled tRNA [4] using multiple quantum NMR spectroscopy [5,6].

These chemical shift correlations revealed that the uracil and uracil-related position N(3) nitrogen-15 chemical shifts were similar in the tRNA studied. The two uracil N(3) nitrogens involved in AU base-pairs possessed almost identical nitrogen-15 chemical shifts. In nitrogen-15 NMR spectra of a mixture of uniformly nitrogen-15 enriched tRNAs the nitrogen-15 resonances of the tRNAs were grouped into several discrete peaks with U and G imino nitrogens each at two different chemical shifts [7]. It appears that the nitrogen-15 chemical shifts of nitrogen atoms in nucleic acids are dependent upon the nucleic acid base type, the position in the base and less dependent upon the base-pair in which they participate but almost independent of the neighbouring base or base-pairs. If this were true, regiospecific nitrogen-15 labelling would be unnecessary: nitrogen-15 resonances of a uniformly nitrogen-15 enriched nucleic acid could be assigned as to base type and position within the base on the basis of nitrogen-15 chemical shifts and therefore a proton which is coupled to nitrogen could be assigned by heteronuclear chemical shift correlation. There have been other NMR studies of uniformly nitrogen-15 enriched nucleic acids in solution [8–10].

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Here the nitrogen-15 and proton chemical shift correlations are examined for uniformly nitrogen-15 enriched rrnB 5 S RNA fragment.

2. MATERIALS AND METHODS

2.1. *5 S RNA fragment*

This was prepared as in [11] from rrnB 5 S RNA overproduced in the presence of chloramphenicol and has been well characterised [12,13]. The nitrogen-15 enriched rrnB 5 S RNA fragment was provided by Professor P. Moore.

2.2. *NMR spectroscopy*

A Bruker WM-500 spectrometer with an additional Programmed Test Sources 500 frequency synthesiser was used (with a 10 MHz frequency input from the spectrometer and under software control for pulse triggering) to generate the proton frequency. The WM-500 frequency synthesiser generated the nitrogen-15 frequency and was amplified by an ENI Model 310L radiofrequency amplifier. A 5 mm diameter proton observe, phosphorus-31 decouple probe was retuned to nitrogen-15 decouple.

A 45°–delay–45° observe pulse train was used to observe the proton spectra in 95% H_2O/5% D_2O solvent [11]. The nitrogen-15 decoupling field strength was 91 Hz and was continuously applied at this low power (<0.1 W). Nitrogen-15 chemical shifts are relative to the middle (at 50.67955 MHz) of the 4 intense nitrogen-15 resonances of a standard ammonium chloride sample (Wilmad WGN-01). The proton chemical shift of added dioxan was taken as 3.741 ppm giving a frequency of 500.13672 MHz at 0 ppm.

3. RESULTS

3.1. *The proton NMR spectrum of uniformly nitrogen-15 enriched 5 S RNA fragment*

Fig.1 shows the downfield spectrum of nitrogen-15 enriched 5 S RNA fragment (a) which has slightly asymmetrical doublets [9,14] with a splitting of approximately 87 Hz at the positions of resonances in the natural isotope abundance fragment spectrum (b) (shown resolution enhanced). The absence of resonances at the middle of each doublet in (a) indicates that the nitrogen-15 enrichment is effectively total. The resolution enhanced nitrogen-15 enriched fragment spectrum (c) shows the doublet relationships. The resonances are in the same order as the lettering in (b). The simulation of the resolution enhanced nitrogen-15 enriched spectrum (d) was computed by adding two normal fragment spectra (similar to b), each of which had been resolution enhanced using different parameters.

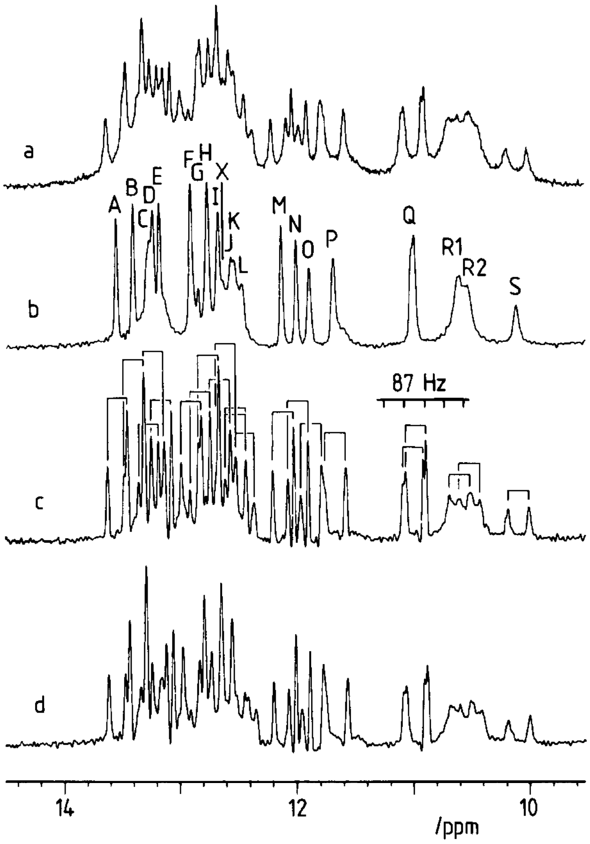

Fig.1. Downfield regions of 1.2 mM rrnB 5 S RNA fragment proton spectra recorded with a 2 s cycle time at 303 K in 100 mM KCl, 5 mM cacodylate, 4 mM $MgCl_2$, pH 7.1: (a) nitrogen-15 enriched; (b) resolution enhanced normal; (c) resolution enhanced nitrogen-15 enriched; (d) simulation using two resolution enhanced normal fragment spectra.

3.2. *The nitrogen-15 and proton chemical shift correlation for nitrogen-15 enriched 5 S RNA fragment*

Double resonance difference spectra were recorded with selective irradiation at nitrogen-15 frequencies at 100 Hz frequency intervals over the

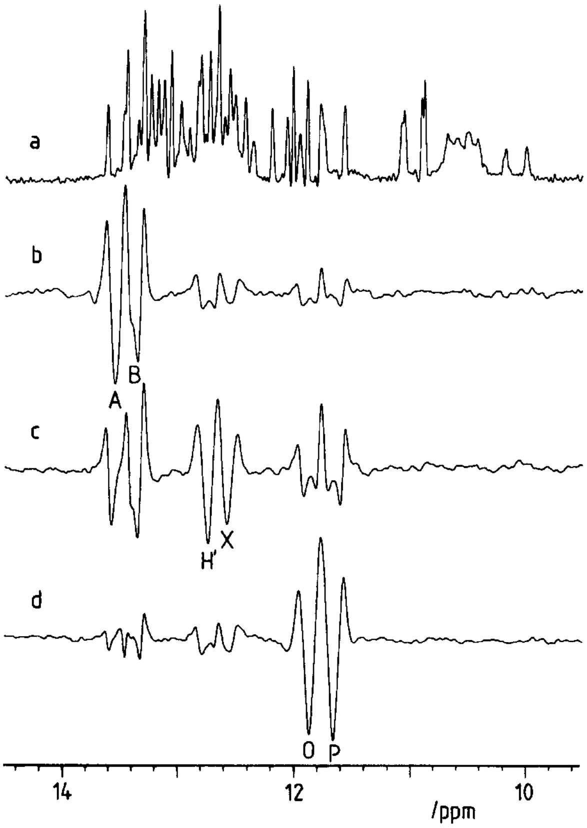

Fig.2. The uracil N(3)H nitrogen-15 decoupling difference spectra of the nitrogen-15 enriched fragment (b,c,d) with the spectrum of fig.1c (a) for comparison.

200 ppm range in which nucleic acid nitrogen-15 resonances occur [7]. Figs.2 and 3 show selected difference spectra in which the decoupling is efficient for labelled resonances. This occurs if the nitrogen-15 resonance of the nitrogen to which the proton is covalently bonded is irradiated and in this way the nitrogen and proton chemical shifts are correlated. The correlations are presented in table 1. Other correlations were made including amino systems and carbon-bound proton to nitrogen systems.

4. DISCUSSION

In table 1 previous imino proton resonance assignments [12,13] are given to assist examination of the data. Fig.4 shows the secondary structure of the fragment and indicates reliable imino proton resonance assignments to primary structure positions. The uracil base imino nitrogens have an approximately 4 ppm chemical shift range: the strongly hydrogen bond stabilised AU resonances occur at the higher frequency with the wobble GU

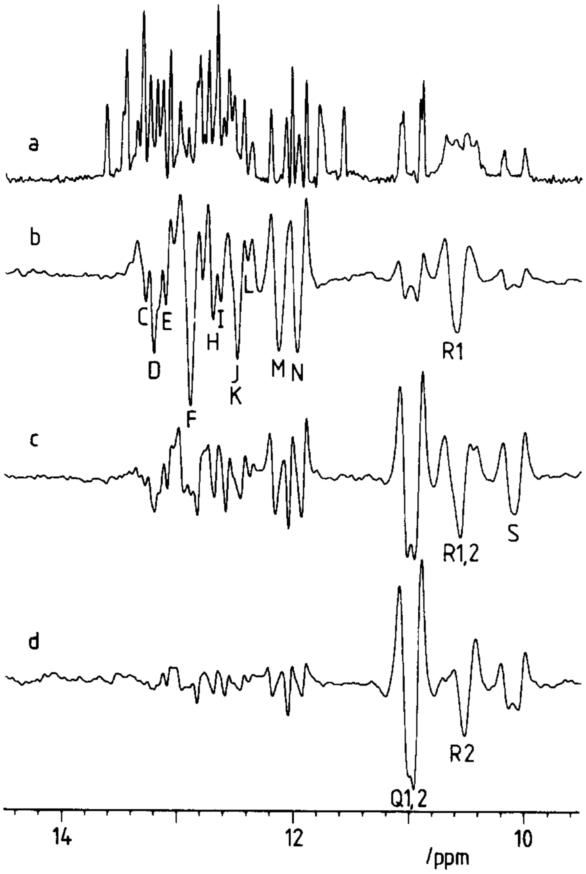

Fig.3. The guanine N(1)H nitrogen-15 decoupling difference spectra of the nitrogen-15 enriched fragment (b,c,d) with the spectrum of fig.1c (a) for comparison.

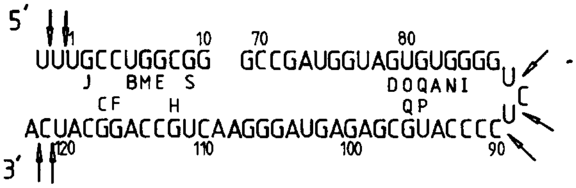

Fig.4. The structure of fragment obtained by limited RNase A digestion of rrnB 5 S RNA overproduced in the presence of chloramphenicol. Sites frequently subject to further nicking in the RNase digestion are indicated by arrows [13]. Assigned imino proton resonances are indicated by the resonance lettering between RNA strands.

Table 1

Nitrogen-15 and proton chemical shift correlations for imino protons of rrnB 5 S RNA fragment

Resonance	Base[a]	Base-pair[a]	Base type	Nitrogen-15	
				Frequency (MHz)	Chemical shift (ppm)
A	U82	AU	U	50.6866(6)	140.2 ± 1
B	U5	AU	U	50.6866(6)	140.2 ± 1
H′			U	50.6865(6)	138.3 ± 1
X			U	50.6865(6)	138.3 ± 1
O	U80	GU	U	50.6864(6)	136.3 ± 1
P	U95	GU	U	50.6864(6)	136.3 ± 1
C	G117	GC	G	50.6858(6)	124.4 ± 1
D	G79	GC	G	50.6858(6)	124.4 ± 1
E	G7	GC	G	50.6858(6)	124.4 ± 1
F	G116	GC	G	50.6858(6)	124.4 ± 1
H	G112	GC	G	50.6858(6)	124.4 ± 1
I	G84	GC	G	50.6858(6)	124.4 ± 1
J	G2	GC	G	50.6858(6)	124.4 ± 1
K			G	50.6858(6)	124.4 ± 1
L			G	50.6858(6)	124.4 ± 1
M	G6	GC	G	50.6858(6)	124.4 ± 1
N	G83	GC	G	50.6858(6)	124.4 ± 1
R1			G	50.6858(6)	124.4 ± 1
S	G9/U111		G	50.6857(6)	122.5 ± 1
Q1	G81/G96	GU	G	50.6856(6)	120.5 ± 1
Q2	G81/G96	GU	G	50.6856(6)	120.5 ± 1
R2			G	50.6856(6)	120.5 ± 1

[a] From [12,13]

resonances at the lower frequency. The guanine base imino nitrogens show the same effects with the middle of this guanine chemical shift range 16 ppm from the middle of the uracil range. The nitrogen chemical shifts are determined primarily by the nature of, and position in, the base ring (the influence of the hydrogen bond is less important) and not, at this level of resolution, by neighbouring ring current or anisotropy effects.

Nitrogen-15 coupling is seen on all the nitrogen-15 bound proton resonances detected, including those broadened by intermediate exchange with the aqueous solvent, and therefore, unlike NOE connectivity methods, this tactic of nucleic acid imino proton resonance assignment by nitrogen and proton chemical shift correlation will be able to identify the base type of imino protons in non-double-helix base pairs such as tertiary base-pairs or isolated slowly exchanging imino protons. NOE connectivity data from double-helices will complement this general method to identify the base type of imino proton resonances by the further assignment of some imino proton resonances to the primary structure positions.

Heteronuclear chemical shift correlations may be examined by two-dimensional NMR techniques. The two possibilities of (a) further effective enhancement of the NMR sensitivity of detection of the lower NMR sensitivity nucleus (^{15}N) and (b) improved resolution of resonances in ^{15}N and/or ^{1}H dimensions strongly suggest that the technically demanding two-dimensional NMR methods described by Bax et al. [6] and related methods offer advantages over the correlation method described above.

Note that the proton resonances of slowly exchanging isolated base imino protons, and the GU imino protons which engage in nitrogen-proton to oxygen hydrogen bonds, are asymmetric doublets in the nitrogen-15 enriched sample spectrum with the upfield component the sharper (which supports Redfield's explanation for the asymmetry [9,14]).

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