Assignment of resonances of exchangeable protons in the NMR spectrum of the complex formed by *Escherichia coli* ribosomal protein L25 and uniformly nitrogen-15 enriched 5 S RNA fragment

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The downfield proton NMR spectrum of the aqueous nucleoprotein complex formed by *Escherichia coli* ribosomal protein L25 and uniformly nitrogen-15 enriched 5 S RNA fragment is presented. Many proton resonances show the effects of scalar coupling to nitrogen-15 and these resonances are assigned to nucleic acid imino protons. Selective nitrogen-15 decoupling difference proton spectroscopy revealed nitrogen-15 and proton chemical shift correlations from which the base types of nucleic acid imino proton resonances could be assigned because the nitrogen-15 chemical shifts of nucleic acid guanine and uracil imino nitrogens have separate small ranges for both nucleoproteins and isolated nucleic acids.

5 S RNA fragment-L25 complex *Uniform nitrogen-15 enrichment of RNA* *Proton NMR* *Heteronuclear decoupling* *Chemical shift correlation* *Assignment to base type*

1. INTRODUCTION

Proton NMR spectra of macromolecules contain information describing molecular structure and dynamics but access to this information requires the assignment of resonances to positions in the molecular structure. For nucleoprotein NMR spectra a first step towards spectral assignment is to sort resonances as to macromolecular type. The most tractable region of aqueous nucleoprotein proton NMR spectra is downfield of chemical shift $\delta = 10$ ppm and is dominated by nucleic acid imino proton (hydrogen-bonded or merely slowly exchanging) resonances. The NOE connectivity method for assignment of resonances (e.g., see [1]) appeared inadequate to unambiguously make the distinction between protein protons and nucleic acid protons for all downfield resonances in the spectrum of the modestly sized complex formed by *Escherichia coli* 5 S RNA fragment with its cognisant ribosomal protein L25 [2].

Here uniform nitrogen-15 enrichment of the nucleic acid moiety of the L25-5 S fragment complex is employed to reveal protons scalar coupled to nucleic acid nitrogens, as opposed to protein protons. The effects of this nitrogen-15 coupling on the lineshape of a nucleic acid imino proton resonance, which is a singlet in natural isotopic abundance spectra (with less than 0.4 atom % nitrogen-15 isotope) and is a doublet with approximately 87 Hz coupling in nitrogen-15 enriched spectra, may be detected in the complex proton NMR spectrum.

The base types of nucleic acid imino proton resonances may be distinguished by nitrogen-15 and proton chemical shift correlation because the imino nitrogen-15 chemical shifts of guanine and uracil bases have separate small ranges [3]. This assignment tactic is applied here to the nucleoprotein spectrum.

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2. MATERIALS AND METHODS

These were as described in [3]. The uniformly nitrogen-15 enriched 5 S RNA fragment is identical to that used to obtain a nitrogen-15 and proton chemical shift correlation for isolated 5 S fragment [3]. This was provided by Professor P. Moore.

The ribosomal protein L25 had natural isotopic abundance and was prepared as in [4,5].

Complex was prepared as in [2] with the solution sample decanted from over a fluffy white precipitate.

3. RESULTS

3.1. *The proton NMR spectrum of 5 S RNA fragment–L25 complex*

Fig.1(a) shows the downfield spectrum of the complex of protein L25 and uniformly nitrogen-15 enriched rrnB 5 S RNA fragment. As in several other preparations of the complex, there remains some free 5 S fragments notwithstanding that a calculated stoichiometric excess of 5% protein L25 was added to the isolated fragment. Making allowance for the increased linewidths of protein-bound 5 S fragment resonances, there is an estimated bound-to-free RNA ratio of 1:1. At the concentrations employed (approximately 0.1 mM at the mixing stage and 1 mM in the final sample) and with an association constant of $1.5 \times 10^7\ M^{-1}$ for the comparable binding of protein L25 to intact ribosomal 5 S RNA [6], the binding should be effectively quantitative. Examination of the upfield shifted methyl region of the sample spectrum reveals no methyl resonances at the chemical shift positions previously shown [4,7] to be characteristic of free protein L25 (not shown). Some irreversible local aggregation of protein L25 is believed to occur upon addition of the protein to the RNA when complex is prepared. Note that binding difference spectra (complex minus isolated RNA) show that protein L25 binding perturbs the rrnB 5 S fragment spectrum in the same way as it does either of ribosomal 5 S fragment or intact ribosomal 5 S RNA (not shown). The resolution-enhanced spectrum of natural isotopic abundance rrnB 5 S fragment-L25 complex is shown in fig. 1(b). There is an estimated bound-to-free RNA ratio of 2:1 for this sample. The data from (a) are

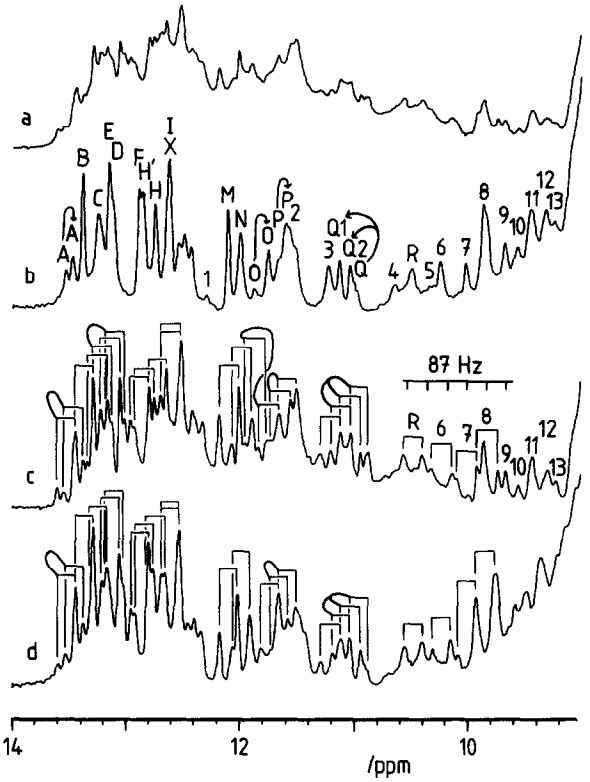

Fig.1. Downfield regions of proton spectra, recorded at 303 K, of fragment-L25 nucleoprotein samples made to 1.2 mM of rrnB 5 S RNA fragment in 100 mM KCl, 5 mM cacodylate, 4 mM $MgCl_2$, 0.1% [$^2H_6$]ethanol, pH 7.1: (a) uniformly nitrogen-15 enriched RNA, (b) resolution-enhanced natural isotopic abundance RNA, (c) resolution-enhanced nitrogen-15 enriched RNA and (d) simulation from two resolution-enhanced normal RNA spectra.

shown in resolution-enhanced form in fig.1(c). The simulation in fig.1(d) was computed by shifting and adding two weighted spectra identical to those in (b).

Comparison of the simulated spectrum (d), which was straightforwardly derived from spectrum (b) (spectrum (b) is essentially identical to ribosomal 5 S fragment-L25 complex spectra analysed previously [2]), with spectrum (c) allows some assignments of complex resonances which have not been shown previously to be derived from RNA fragment protons [2]. Resonances 3, 6, 7 and some of the intensity of resonance 8 may be seen to be doublets in spectrum (c) and spectrum (d): they must be resonances from the nucleic acid

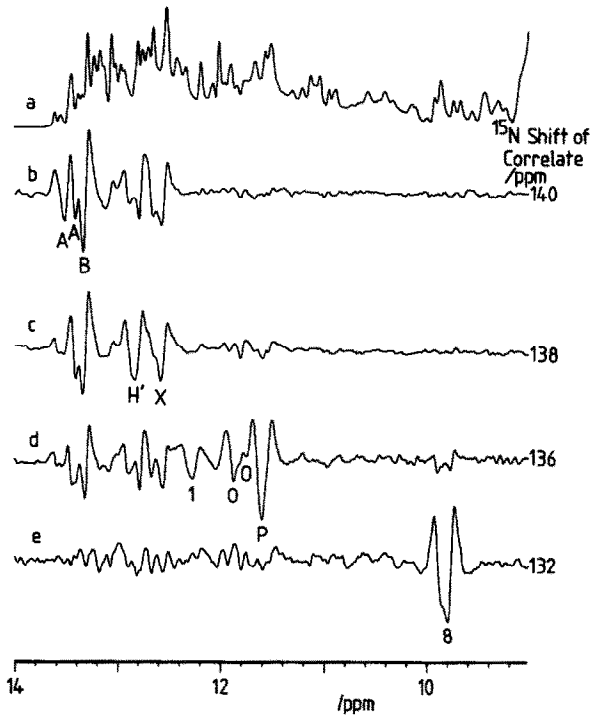

Fig.2. The uracil N(3)H nitrogen-15 decoupling difference spectra of the nitrogen-15 enriched fragment complex (b–e) with fig.1c (a) for comparison.

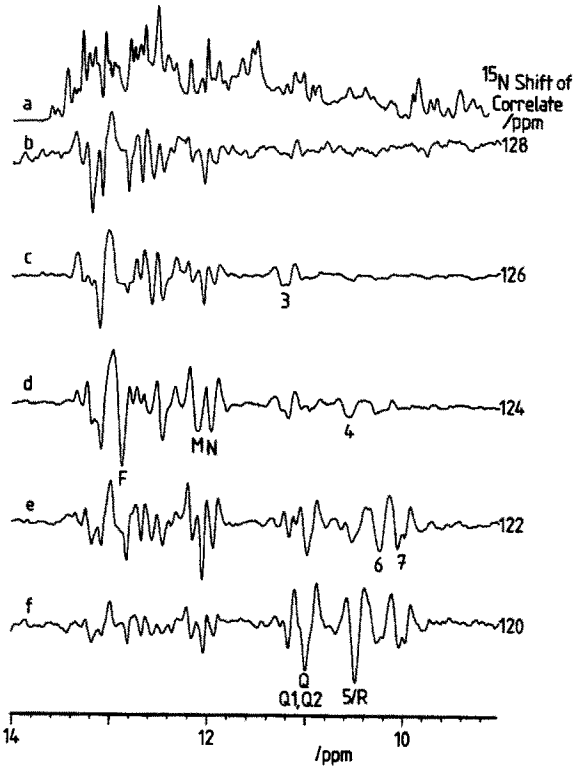

Fig.3. The guanine N(1)H nitrogen-15 decoupling difference spectra of the nitrogen-15 enriched fragment complex (b–f) with fig.1c (a) for comparison.

Table 1

Nitrogen-15 and proton chemical shift correlations for imino groups of nitrogen-15 enriched 5 S fragment nucleoprotein complex

Resonance	Base[a]	Base-pair[a]	Base type	Nitrogen-15[b] chemical shift (ppm)
A	U82	AU	U	140.2 ± 1
B	U5	AU	U	140.2 ± 1
H'			U	138.3 ± 1
X			U	138.3 ± 1
P	U95	GU	U	136.3 ± 1
O	U80	GU	U	136.3 ± 1
1			U	136.3 ± 1
8			U	132.3 ± 1
3			G	126.4 ± 1
4			G	124.4 ± 1
6			G	122.5 ± 1
7			G	122.5 ± 1
Q1	G81/G96	GU	G	120.5 ± 1
Q2	G81/G96	GU	G	120.5 ± 1
R			G	120.5 ± 1
5/R(free)/R			G	122.5 ± 1

[a] This information is from previous publications, see [3]

[b] This is relative to a standard ammonium chloride sample resonance, see [3]

moiety of the complex. Most of the intensity of resonance 8 and resonances 9–13 remain unaffected by the nitrogen-15 enrichment of the RNA: they are protein resonances.

3.2. *Selective nitrogen-15 decoupling difference proton spectra of complex*

Figs 2 and 3 show difference spectra in which decoupling is efficient. This occurs if the decoupling frequency coincides with the nitrogen-15 resonance of the nitrogen to which the proton is covalently bonded and in this way the nitrogen and proton chemical shifts may be correlated.

Correlations are presented in table 1. Residual free fragment resonances were identified by comparison with isolated fragment data [3]. Resonance H′ has a nitrogen chemical shift of 140 ppm for both free fragment and complex correlates but in the complex spectrum the proton resonance is downfield shifted by 0.1 ppm from the free fragment position. Resonance 0 in the bound form is not clearly seen in the difference spectrum recording decoupling at 136 ppm because of fortuitous difference overlap. Some of the resonance 8 intensity (there are several resonances superimposed which have been labelled 8) correlates with a nitrogen having a chemical shift of 132 ppm.

If resonance 2 is an imino proton, it is a uracil with a nitrogen correlate at 136.3 ± 1 ppm with the decoupling effect obscured in fig.2(d) by bound P resonance overlap.

4. DISCUSSION

The nitrogen-15 and proton chemical shift correlations for the fragment-L25 complex may be rationalised similarly to free fragment results [3]: The chemical shifts of nucleoprotein guanine and uracil imino nitrogens-15 occur in separate small ranges. The RNA imino nitrogen correlated to resonance 8 is noteworthy: the 132 ppm nitrogen chemical shift is outside the range for uracil N(3) nitrogens of isolated fragment [3]. The apparent correlation between imino nitrogen-15 chemical shifts and hydrogen-bond strength [3] indicates that the 132 ppm resonance is a uracil N(3) which is not participating in an inter-base hydrogen-bond but is bound to a proton less accessible to the aqueous solvent in the complex than in the free fragment. The proton chemical shift of resonance 8 is 9.8 ppm, too far upfield for a hydrogen-bonded imino proton. The possibility that the 132 ppm nitrogen is an imino nitrogen of a tautomeric base structure, not a uracil N(3), induced by protein binding cannot be excluded. The resonances labelled 8 include the most upfield RNA imino proton resonance, and the most downfield resonance which is definitely not that of a RNA imino proton (no nitrogen-15 coupling): there is no demonstrated overlap between the proton chemical shift ranges of RNA imino protons and other protons in this nucleoprotein complex.

Resonance 3 correlates with a nitrogen having a chemical shift of 126 ppm, which slightly extends the shift range of nitrogens attributed to guanine imino groups and suggests this is a strongly hydrogen-bonded guanine, but the proton chemical shift is not consistent with the proton bridging a strongly hydrogen-bonded GC base-pair.

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REFERENCES

[1] Hare, D.R. and Reid, B.R. (1982) Biochemistry 21, 1835–1842.
[2] Kime, M.J. and Moore, P.B. (1983) Biochemistry 22, 2622–2629.
[3] Kime, M.J. (1984) FEBS Lett. 173, 342–346.
[4] Kime, M.J., Ratcliffe, R.G., Moore, P.B. and Williams, R.J.P. (1981) Eur. J. Biochem. 116, 269–276.
[5] Kime, M.J. and Moore, P.B. (1982) Nucleic Acids Res. 10, 4973–4983.
[6] Spierer, P., Bogdanov, A.A. and Zimmermann, R.A. (1978) Biochemistry 17, 5394–5398.
[7] Kime, M.J. and Moore, P.B. (1984) Biochemistry 23, 1688–1695.