^{1}H NMR STUDY OF ^{15}N-LABELED tRNA

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1. Introduction

The structure and conformation of various tRNAs has been studied using the imino proton resonances of the base pairs as NMR probes [1–4]. Here, we report corresponding ^{1}H NMR studies of ^{15}N-labeled tRNAs of *Escherichia coli*. The interaction of the bridge nitrogens with the hydrogen bond protons should lead to a characteristic multiplet structure of the $^{15}N-^{1}H-{}^{15}N$ proton resonances. Since the ^{14}N-nucleus in the tRNA of natural isotope abundance produces a considerable line broadening of the imino proton resonances because of quadrupolar relaxation, reduced linewidths and a better resolution are expected to occur in the absorption region of the t[^{15}N]RNA imino proton resonances. Furthermore, deeper insight into the structure of the hydrogen bonds forming the basepairs, and into their dynamics may be gained. No $^{1}J_{^{1}H-^{15}N}$-couplings have been reported for a basepair system in water. The question arises as to whether the hydrogen bond proton is fluctuating from one of the bridge nitrogens to the other. In the tRNA we should be able to study this dynamic process since the hydrogen bonds are fairly stable with respect to time.

2. Materials and methods

2.1. *Isolation of t[^{15}N]RNA*

Escherichia coli MRE 600 was grown on a minimal medium containing $(^{15}NH_4)_2SO_4$ (95% ^{15}N-isotope content) as the only nitrogen source [11]. tRNA was extracted from the bacteria according to [10]. The tRNAs were separated on BD-cellulose [7]. Purification to homogeneity was obtained by Sepharose 4B- and RPC-5-chromatography [8,9]. The purified species accepted >1700 pmol/A_{260}-unit when assayed.

2.2. *NMR samples*

t[^{15}N]RNA_1^{Val} (6 mg), t[^{15}N]RNA_1^{fMet} (6 mg) and t[^{15}N]RNA_m^{Met} (1.5 mg) were extensively dialyzed against quartz-distilled water and the appropriate buffers. Samples were 220 μl (for t[^{15}N]RNA_1^{Val} and t[^{15}N]-RNA_1^{fMet}) and 180 μl (for t[^{15}N]RNA_m^{Met}). The t[^{15}N]RNA_1^{Val}-solution contained 100 mM NaCl, 1 mM $MgCl_2$, 10 mM sodium cacodylate (pH 7.0) and 5% $^{2}H_2O$; the t[^{15}N]RNA_1^{fMet} and t[^{15}N]RNA_m^{Met} solutions contained 100 mM NaCl, 15 mM $MgCl_2$, 10 mM sodium cacodylate (pH 7.0) and 5% $^{2}H_2O$. The samples were transferred to 5 mM NMR sample tubes (Wilmad). Sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) was used as reference.

2.3. *^{1}H NMR spectra*

These were obtained with Bruker WM 400- and WM 500-NMR spectrometers using the 2–1–4 Redfield pulse technique [12] to suppress the water signal. Acquisition times were generally 0.5 s with 0.1 s delay which led to usable spectra within 6–15 min.

3. Results

The absorption regions of the basepair imino proton resonances of t[^{15}N]RNA_1^{Val}, t[^{15}N]RNA_1^{fMet} and t[^{15}N]RNA_m^{Met} are shown in fig.1. Due to the coupling of the hydrogen bond protons to the corresponding ^{15}N-nuclei the signals appear as doublets. In addition the doublet signals are asymmetric with respect to signal intensity and linewidth. This asymmetry is more pronounced at higher field strength and lower temperatures (fig.1,2). The ^{1}H NMR spectra were obtained at between 20–65°C (fig.2). For t[^{15}N]RNA_1^{fMet} 23 resolved doublet signals can be detected. In spite of the high magnetic field and the

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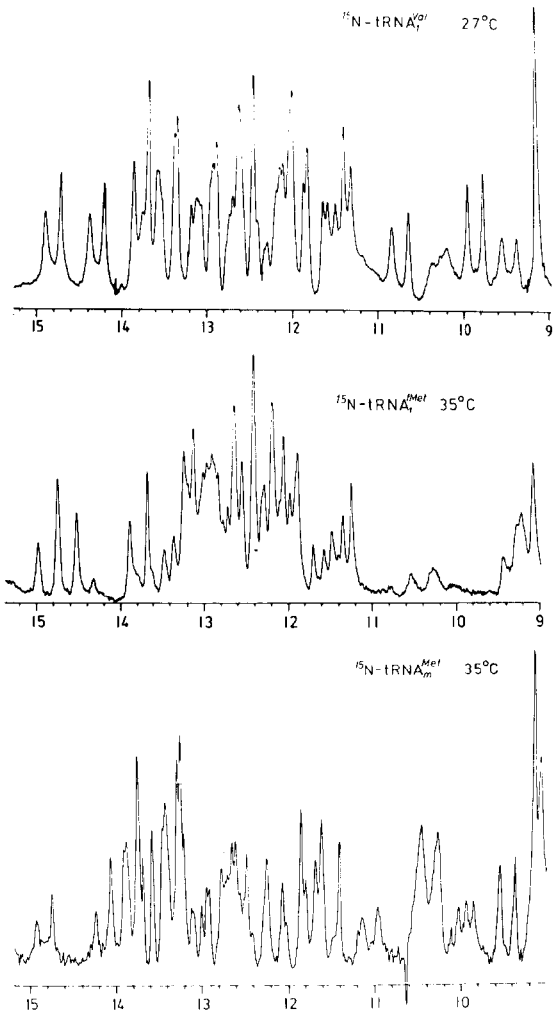
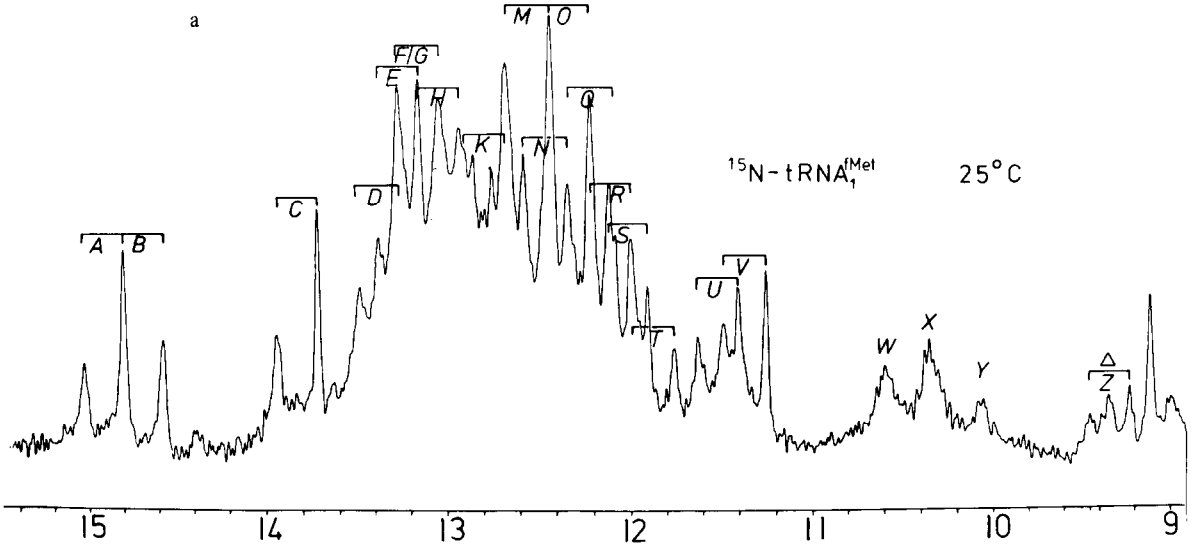


Fig.1. Above: 500 MHz ^{1}H NMR spectrum of low field N–H resonances of t[^{15}N]RNA$_1^{Val}$ at 27°C; 2100 scans; exponential multiplication by +2 Hz. Middle: 400 MHz ^{1}H NMR spectrum of low field N–H resonances of t[^{15}N]RNA$_1^{fMet}$ at 35°C; 1150 scans; exponential multiplication by +2 Hz. Below: 500 MHz ^{1}H NMR spectrum of low field N–H resonances of t[^{15}N]RNA$_m^{Met}$ at 35°C; 4700 scans; exponential multiplication by +2 Hz. Abscissae: units in ppm.

remarkably narrow lines, not all of the expected 25 ± 1 resonances between 15–11 ppm (from TSP) are resolved. For the tentative identification of the resonances we made use of the coupling constants (89 ± 4 Hz), the individual shift of some resonances with temperature as well as of the simultaneous loss of intensity of both doublet signals during the temperature-induced helix–coil transition [4]. The variation of the chemical shift of t[^{15}N]RNA$_1^{fMet}$ imino resonances with temperature is shown in fig.3. Most of the resonances exhibit a slight upfield shift of 0.05 ppm with increasing temperature. Six doublets (A,B,C,Q,T,U in fig.3) and the resonance Δ, shift up to 0.3 ppm in the same temperature range. The intensity of doublet *F*, already low at 45°C has almost disappeared at 55°C. The doublets Q and U also start to 'melt' at ~45°C, M and T at 55°C and R, S and V at 65°C, respectively.

The resonances W, X and Y are already broad at low temperatures, coalesce at 45°C and disappear at higher temperature. Most other doublets are more or less stable with respect to temperature up to 65°C.



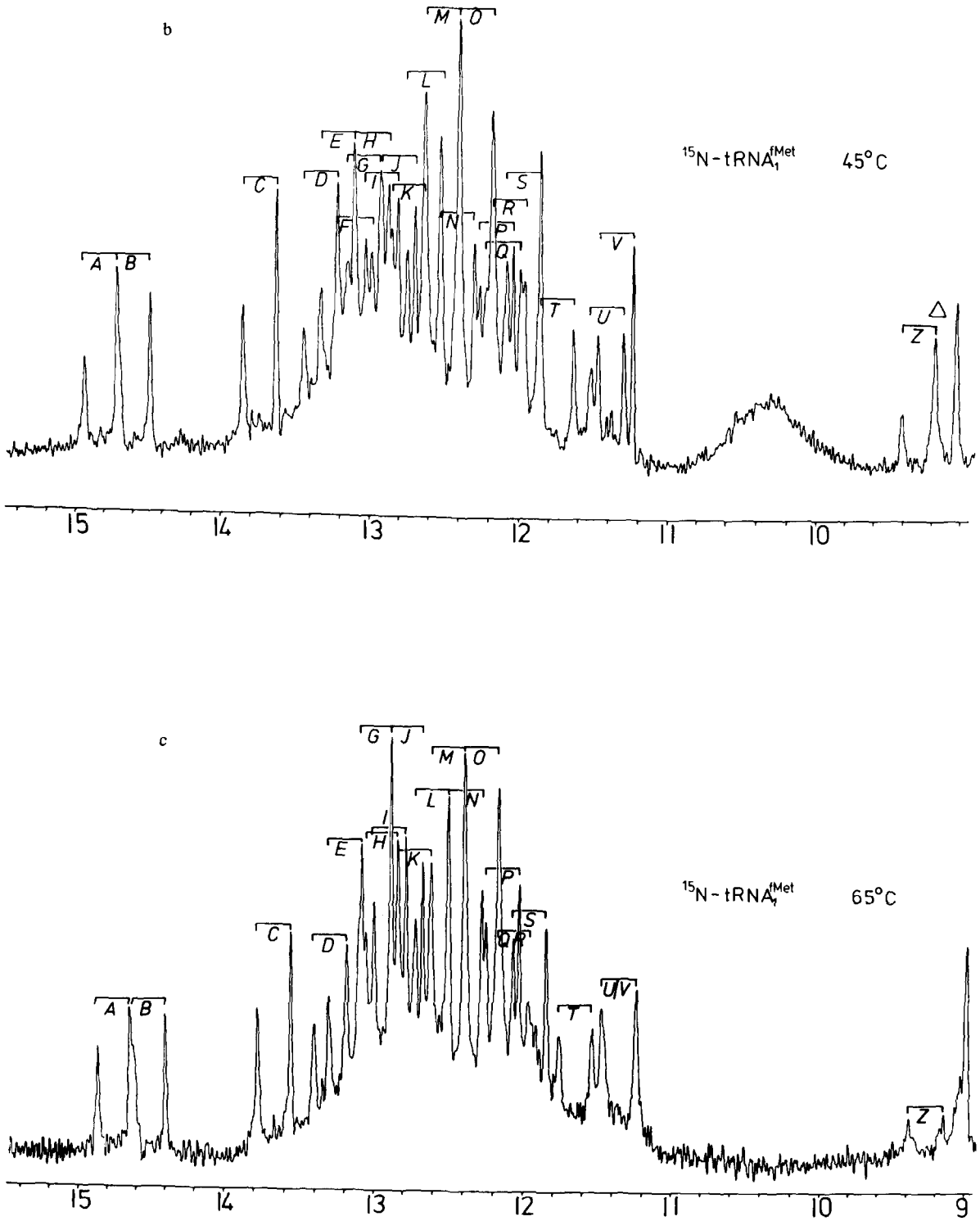


Fig.2. 400 MHz ^{1}H NMR spectra of N–H resonances of t[^{15}N]RNA$_1^{fMet}$ at various temperatures. The spectra are the results of 1600–2000 scans, resolution-enhanced by Gaussian multiplication (*LB* = −10 Hz, *GB* = 0.17): (a) 25°C; (b) 45°C; (c) 65°C. Abscissae: *units in ppm*.

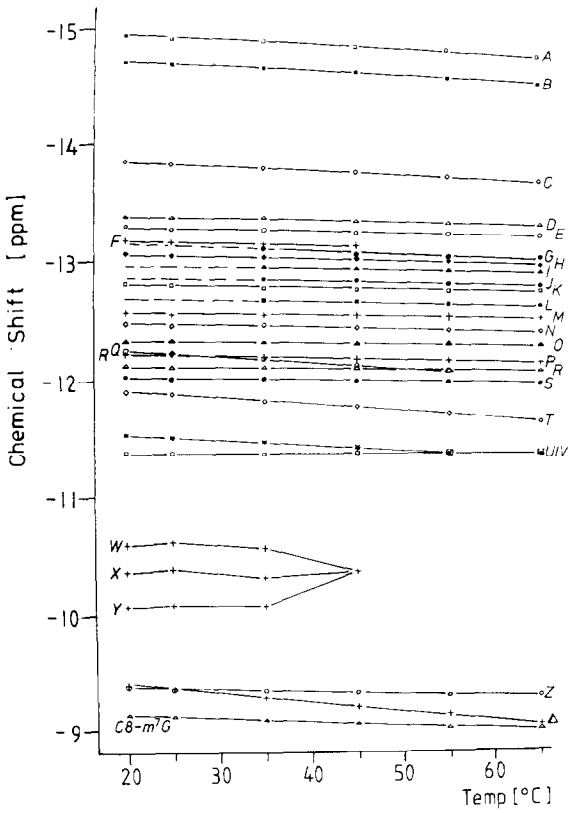


Fig.3. Temperature dependence of the chemical shifts of imino proton resonances of t[^{15}N]RNA$_i^{fMet}$ at 400 MHz between 20–65°C. For each doublet the average chemical shift value is given.

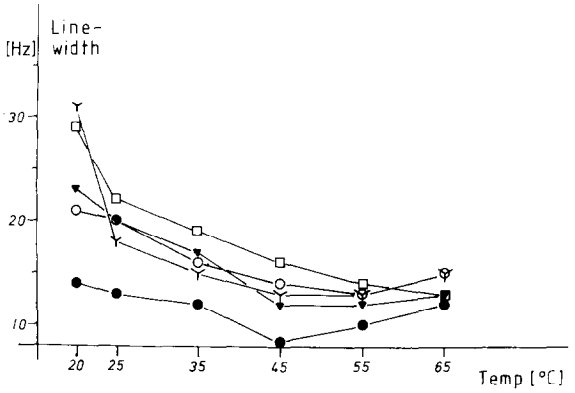


Fig.4. Linewidths of some selected imino proton resonances of t[^{15}N]RNA$_i^{fMet}$ plotted against temperature: (□) $A_{downfield}$; (▼) $B_{upfield}$; (○) $C_{downfield}$; (●) $C_{upfield}$; (γ) C8-m^{7}G.

Table 1
Coupling constants ($^1\overline{J_{NH}}$) of the imino protons of t[^{15}N]RNA$_i^{fMet}$

Resonance	$^1\overline{J_{^{15}N-^1H}}$ (Hz)	Resonance	$^1\overline{J_{^{15}N-^1H}}$ (Hz)
A	90.0 ± 0.8	M	91.0 ± 2.6
B	89.4 ± 1.6	N	90.0 ± 1.8
C	88.3 ± 0.4	O	88.0 ± 1.8
D	87.7 ± 0.8	P	87.7 ± 0.8
E	89.4 ± 1.4	Q	87.5 ± 2.4
F	93.4 ± 0.6	R	84.8 ± 0.6
G	87.7 ± 1.4	S	87.5 ± 1.4
H	89.9 ± 3.6	T	91.0 ± 1.8
I	86.6 ± 0.2	U	90.0 ± 3.6
J	90.1 ± 4.2	V	93.2 ± 0.4
K	91.2 ± 3.4	Z	90.1 ± 2.2
L	88.4 ± 0.6		

The values of the coupling constants $^1J_{^1H-^{15}N}$ were determined with fairly good accuracy depending on linewidths (table 1). The doublet signals are asymmetric: a smaller but broader signal appears at lower field, a taller but more slender signal appears upfield within the distance of the coupling constants. Despite of the relatively well-resolved spectra, an exact determination of the linewidth was possible for only a few resonances with no or insignificant overlap. In fig.4 the linewidth of resonances A(downfield), B(upfield), C(downfield), C(upfield) and of the C8-H of m^{7}G (2) is plotted against temperature. Generally the linewidths decrease with increasing temperature up to 55°C. Above 55°C the linewidths increase again.

4. Discussion

The introduction of the ^{15}N-isotope into tRNA leads to a unique multiplet structure of the N–H resonances. The coupling of the bridge nitrogens with the hydrogen bond protons results in a doublet signal of each of the detectable low field resonances regardless of whether the hydrogen bond is involved in secondary or tertiary base pairing. The coupling constants vary over 85–93 Hz [6]. In addition, the line shapes are asymmetric, the asymmetry depending on field strength and temperature. The varying $^1J_{^1H-^{15}N}$ coupling constants and the asymmetric lineshapes can be explained with a tautomerism of the base pairing systems. In this tautomerism, the proton in the hydro-

gen bond fluctuates between the two nitrogen sites ($^{15}N-^{1}H\cdots{}^{15}N \rightleftharpoons {}^{15}N\cdots{}^{1}H-{}^{15}N$). It is assumed that in the N–H- -O hydrogen bonds of the GC or AU basepairs corresponding fluctuations take place leading to the well-known keto–enol tautomerism of the bases. Assuming this two-site exchange model for the $^{15}N-^{1}H\cdots{}^{15}N$ proton resonances it is possible to simulate the asymmetric shape of the doublet signals. The rate constant as one of the fit parameters varies within 100–300 s^{-1} the mole fraction of the enol tautomer, being another fit parameter, varies within 0.05–0.15. Both fit parameters strongly depend on temperature and the nature of the basepair. Further details of the results of the calculation will be described elsewhere. It seems that after decades of nucleic acid research, a quantitative description of the tautomerism in basepairing systems is now possible. Needless to say, the presence of presumably as large an amount of the unusual enol tautomers in DNA will be of considerable importance in molecular biology.

The ^{14}N-isotope usually produces considerable line broadening of the N–H resonances because of its quadrupolar momentum [5]. Therefore an advantage of the substitution of ^{14}N by ^{15}N should be a reduction in linewidth and hence a better resolution of the corresponding ^{1}H NMR spectra. We found a reduction in linewidth by a factor of 2–3.

However, selective decoupling of the corresponding ^{15}N-resonances may lead to a simplification of the spectra and to an identification of the imino resonances (in preparation). The increase in linewidth at higher temperatures (fig.4) is generally believed to be caused by an increasing rate of the helix–coil transition [13]. In the dynamic range observable by NMR, the doublet structures do not coalesce into single resonances, but disappear as doublets when the basepairs reach their melting temperature.

Acknowledgement

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