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^{15}N NMR study of a mixture of uniformly labeled tRNAs

(**^{15}N chemical shift/nuclear Overhauser effects/nucleotide-base nitrogen resonances/denaturation of tRNA**)

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ABSTRACT **^{15}N NMR spectra were taken of ^{15}N-enriched tRNA extracted from bakers yeast; ammonium sulfate was used as a nitrogen source. The increase in the degree of denaturation of tRNA, which occurs with increase in temperature from 30°C to 70°C, resulted in no large changes in ^{15}N chemical shifts at acidic and neutral pH but quite pronounced changes in proton–^{15}N nuclear Overhauser effects.**

^{15}N NMR spectroscopy is becoming a very important probe of biochemical molecules, even though ^{15}N NMR studies at the natural-abundance level of large molecules have rather severe limitations because the signal intensities are weak (1). A further complication with large molecules is that they usually have rather long rotational correlation times (τ_c). As τ_c becomes longer, dipolar relaxation processes tend to speed up, which has a favorable effect on signal accumulation in the Fourier-transform mode (at least until line broadening is serious) because T_1 becomes smaller. However, this advantage may be offset at large τ_c values by a decrease in that part of the signal intensity which is associated with a favorable nuclear Overhauser effect (NOE) and arises when the protons in the sample are irradiated while observing the nitrogen signals. Diminishment of the proton NOE results in an actual nulling of ^{15}N resonances at particular τ_c values (1).

tRNAs are relatively small, soluble nucleic acids ($M_r \approx$ 30,000) for which ^{15}N NMR spectra have been obtained at the natural-abundance level (1). However, large amounts of material and long observation times were required to obtain a spectrum in which the signal-to-noise ratios were, in fact, not very favorable. The solution to this problem is enrichment in ^{15}N, and spectra of 95% ^{15}N-enriched DNA have been reported for which assignments could be made that would be difficult or impossible at the natural-abundance level (2). In this paper, we report a similar study of a ^{15}N-enriched mixture of tRNAs obtained from yeast grown in a carbon-base medium to which was added ^{15}N-enriched ammonium sulfate as the nitrogen source (3).

The substantially greater range of NMR chemical shifts for nitrogen compared to hydrogen and carbon, make ^{15}N NMR an especially attractive way to study the characteristics of tRNA in solution. The sensitivity of the resonances of many of the nucleotide-base nitrogens to hydrogen bonding (unpublished data) provides hope that ^{15}N NMR could be useful for monitoring the conformations and dynamics of individual base pairs in tRNA molecules.

EXPERIMENTAL SECTION

Preparation of 99% ^{15}N-enriched tRNA was as described (3). The medium on which the yeast was grown consisted of water (2.5 liters), glucose (40 g), a commercially available (Difco) carbon base (14.5 g), and 99% ^{15}N-enriched ammonium sulfate (2 g). Five 2-liter flasks, each containing 500 ml of autoclaved medium, were inoculated with 1 ml of yeast cells growing in a broth. The flasks were placed in a shaker-incubator and maintained at 30°C for 24 hr. The yeast was then separated by centrifugation at 10,000 × *g* for 10 min.

The tRNA was extracted from the yeast by a modification of a published procedure (4). The cells were first suspended in 3 times their volume of 0.1 M sodium acetate solution containing 1% sodium dodecyl sulfate (pH 5). One-half volume of water-saturated phenol was then added, and the mixture was shaken vigorously at room temperature for 1–2 hr. The phases were separated by centrifugation for 10 min at 10,000 × *g*. The cells and aqueous phase were removed and reextracted with an equal volume of water-saturated phenol. Two volumes of absolute ethanol were added to the phenolic extract, and the tRNA mixture was allowed to precipitate overnight at −20°C. The precipitated tRNA was separated by centrifugation at 10,000 × *g* for 70 min. The resulting pellet was dissolved in 17 ml of water and centrifuged at 10,000 × *g* for 1 hr. The supernatant, on lyophilization, afforded 50 mg of 99% ^{15}N-labeled tRNA. Only adventitious magnesium was present in the preparation.

The ^{15}N spectra of the ^{15}N-enriched tRNA mixture (0.33 mM) were taken in 0.15 M sodium chloride at pH 4 and 7. The pH was adjusted with 6 M sodium hydroxide and measured with a Radiometer pH meter. Proton-coupled and -decoupled ^{15}N NMR spectra were obtained at 18.25 MHz with a Bruker WH-180 spectrometer (1) and at 50.65 MHz with a Bruker WM-500 spectrometer. A capillary made up to contain 1 M $H^{15}NO_3$ in ^2H_2O provided the reference standard, and ^2H_2O was used as an internal field-frequency lock. The reported chemical shifts are in ppm, upfield from the resonance of external $^2H^{15}NO_3$. The normal operating conditions used a pulse angle of 90° and pulse delays of 0.7–5 sec. From 5,000 to 17,000 transients were required. The sample temperatures ranged from 30°C to 70°C. The tRNA was renatured by heating the sample to 40°C for 2 hr and then cooling to room temperature over the course of 5 hr.

RESULTS AND DISCUSSION

The ^{15}N chemical shifts determined for nitrogens in the mixture of yeast tRNAs in 0.15 M sodium chloride with the Bruker WH-180 spectrometer are given in Table 1. These shifts correspond to the resonances in the spectra shown in Fig. 1. The chemical shifts obtained on the Bruker WM-500 spectrometer are given in Table 2, and these correspond to the resonances shown in Fig. 2. Because the WM-500 spectrometer has greater sensitivity than the WH-180 has, more peaks were observed, and the numbering system for the resonances in Table 1 and Fig. 1 has been adjusted to correspond to that used for Table 2 and Fig. 2. That there are striking differences in the appearance and relative intensities of the peaks of the spectra taken at the substantially different magnetic fields of the two spectrometers is hardly due to the disparity of sensitivity alone.

Abbreviation: NOE, nuclear Overhauser effect.

Table 1. ^{15}N chemical shifts from the broad-band proton-decoupled spectra*

Peak no.	pH 4.0 30°C	pH 4.0 45°C	pH 4.0 61°C	pH 4.0 75°C	pH 7 30°C	Assignments†
2	342				343.2	‡
4	335.3				335.1	‡
	334.9					‡
5	330.2				330.2	‡
6	303–302	302.3	302	299.8	303.0	G NH^2
		301.8		299.7	302.6	
7	298–296.8	297.4–296.4	296.8	292.1	297.5	A NH_2
				291.7		
7a			294.4			
8	282.7–278.9	281.3	280.8	272.8	282.5	C NH_2
		280.5			282.3	
8a			275.7			
10	228.5	228.2	227.6	224.6	228.4	G1, U1
11	224.1	223.9	222.6		224.2	C1
		223.7			224.0	
12	217.2	217.1	216.7	214.2	217.2	U3
		216.9				
14	206.9	206.5	205.7		207.2	A9, G9
		206.3			206.8	
15	173.5				172.8	C3
					173.3	
18	149	148.4				A1
19	144.6–140.5	143.2	140.1			A7
20	139.6–138.2	138.4			139.8	G7
					139.0	

* Taken with a WH-180 spectrometer.
† In one-letter nucleotide code.
‡ α-Amino acid nitrogen(s).

Considerable effects of chemical-shift anisotropy of the T_1 relaxations are expected and are worthy of further investigation.

The observed resonances were assigned by reference to the corresponding resonances of the 5′-monophosphates of guanosine, cytidine, adenosine, and uridine (5, 6).

Two peaks at 156.8 ppm and 109 ppm, corresponding to N3 of adenosine and N3 of guanosine, respectively, of largely helical tRNA appear in the proton-decoupled spectrum c in Fig.

Table 2. ^{15}N chemical shifts of tRNA spectra*

Peak no.	30°C Decoupled pH 4	30°C ^1H-Coupled renatured	65°C ^1H-Coupled	65°C Decoupled	Assignments†
1		354.5	354.5		‡
2		342.8	342.8		‡
		343.1			‡
3		337.7	337.7		‡
4		335.2	335.3		‡
5		330.1	330.1		‡
6	301.8–302.0	301.7–303.3	303.3	302.4	G NH_2
7	295.7–297.1	297.0–297.5	299.1	297.3	A NH_2
8	281.4	281.8	282.5	282.3	C NH_2
9		242.6		238.3	
10	227.6	228.4	227.9	227.2	U1, G1
11	223.5	223.0		223.2	C1
12	216.3	217.0	216.8	216.4	U3
13	208.3	209.6	208.3		G3
14	207.6	207.0	207.5		G9, A9
15	173.5	174.9	172.5	171.1	C3
16		165.7			
17	156.8	158.3	156.2		A3
18	148.8	150.0	147.6	146.5	A1
19	141.7–143.6	144.8–144.5	142.6	141.7	A7
20	137.0–139.4	140.3–139.8	138.5	137.6	G7

* Taken with a WM-500 spectrometer.
† In one-letter nucleotide code.
‡ α-Amino acid nitrogen(s).

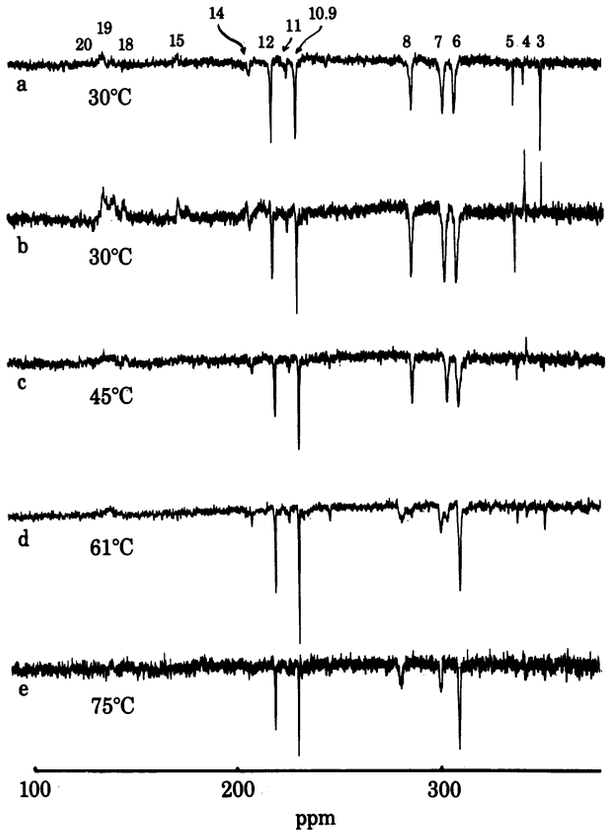

FIG. 1. ^{15}N NMR broad-band proton-decoupled spectra taken with a Bruker WH-180 spectrometer. Spectrum a was obtained at pH 7, whereas spectra b, c, d, and e were at pH 4.

2, taken with the WM-500 spectrometer. These peaks are not present in spectrum b in Fig. 1 taken with the WH-180 spectrometer—a result which might be attributed to greater sensitivity at the higher magnetic field or to a more favorable relaxation time.

Interestingly, changing the pH from 4 to 7 of the largely helical mixture of tRNAs* does not significantly alter the nitrogen chemical shifts (Table 1; Fig. 1, spectra a and b).

The degree of denaturation of the tRNA sample was increased by heating it slowly at pH 4, and spectra c, d, and e of Fig. 1 were obtained at temperatures of 45°C, 61°C, and 75°C, respectively. At higher temperatures, the NH_2 signals for adenosine and cytidine decreased in intensity, and new peaks emerged ≈5 and ≈9.2 ppm downfield, respectively. These changes with temperature can be interpreted as the consequence of disrupting the hydrogen bonds between the base pairs as the tRNA becomes denatured, thus allowing partial protonation of adenosine at N1 and cytidine at N3. Such protonations have been shown to cause downfield shifts of the NH_2 resonances of adenosine and cytosine (5, 6; unpublished data). At pH 4, the peaks for N1 of adenosine and N3 of cytidine could not be observed because of unfavorable NOE effects, which are discussed in more detail below. For this reason, the protonation effects on the ring-nitrogen resonances could not be determined.

At pH 7, the resonances of N1 of adenosine and N3 of cytidine move 2.4 ppm downfield when the tRNA is denatured by heating (Table 2; Fig. 2, spectra a and b). The changes are in the direction expected for decreases in hydrogen bonding to azine-type nitrogens. However, because the resonances of N3 and N7 of adenosine and N7 of guanosine, which correspond to nitrogens not involved in interbase hydrogen bonding, also move downfield a few ppm on heating, other factors such as temperature effects on the ^{15}N shifts may be responsible.

The coiling/uncoiling effects on the NOE are strikingly illustrated in Fig. 2. In decoupled spectrum c, the upfield peaks, which have large negative NOE enhancements of their signal intensities, correspond to the NH_2 and ring nitrogens 1 of guanosine and 3 of uridine, which bear protons. The downfield resonances, which correspond to non-proton-bearing ring nitrogens, have NOE values between 0 and +1 and give positive signals of various intensities. When the sample is warmed to 65°C (Fig. 2, spectrum d), the downfield resonances of the ring nitrogens become relatively weaker. This is the result of an algebraic decrease in NOE, as expected for a decrease in correlation time (τ_c) (1). Some of the decrease in τ_c is expected for an increase in temperature alone, but the dramatic change in the spectra is almost surely due more to uncoiling of the chains, which is known to be extensive at 65°C. Uncoiling results in greater chain mobility and shorter effective τ_c values for the nitrogen nuclei. The relative importance of denaturation relative to temperature on the NOE is further demonstrated by the spectrum (Fig. 2, spectrum e) of denatured tRNA taken at 30°C which, when compared with the spectrum of the largely helical

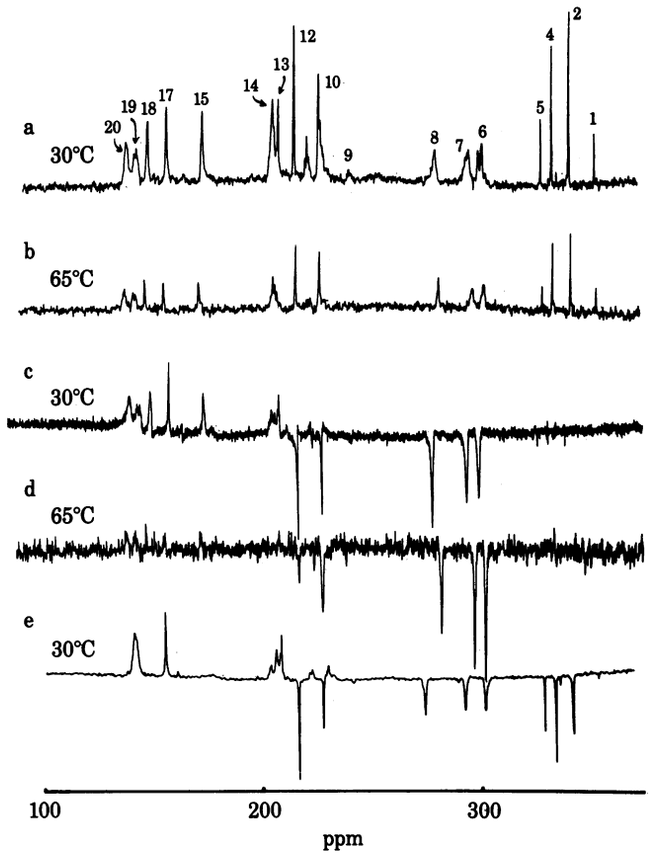

FIG. 2. ^{15}N NMR spectra taken with a Bruker WM-500 spectrometer: a and b, broad-band proton-coupled; c, d, and e, proton-decoupled. Spectra a, b, and d were observed at pH 7, whereas spectra c and e were at pH 4. Spectrum e was of a sample heated to 65°C and then cooled to 30°C.

* The degree of helicity of the yeast tRNAs in the mixture used here is not easily assessed. The changes in conformation measured by hypochromicity are reported to occur at different temperatures for different yeast tRNAs (7), but there seems little doubt that the degree of helicity at 30°C must be relatively large.

tRNA (Fig. 2, spectrum c), shows a nulling of azine-type resonances at positions 3 of cytidine, 1 of adenosine, and 7 of guanosine.

It is interesting to note that there are no large changes in nitrogen chemical shifts on going from the double helical structure to the "melted" form (Table 2). This indicates either that stacking and unstacking effects are not large on the ^{15}N shifts, or that replacement of interbase hydrogen bonds by hydrogen bonds to water does not result in much change in shift.

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