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Multiple quantum two-dimensional ^{1}H—^{15}N nuclear magnetic resonance spectroscopy: Chemical shift correlation maps for exchangeable imino protons of *Escherichia coli* $tRNA_f^{Met}$ in water

(nucleic acids/biopolymers)

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ABSTRACT **A procedure based on multiple quantum two-dimensional nuclear magnetic resonance spectroscopy is described for generation of ^{1}H—^{15}N chemical shift correlation maps. The method is used to obtain ^{15}N chemical shifts for the exchangeable imino protons in ^{1}H—^{15}N units of site-specifically labeled *Escherichia coli* $tRNA_f^{Met}$ in water. The high sensitivity and excellent chemical shift dispersion of the multiple quantum two-dimensional technique make it ideally suited for studying protonated nitrogens by NMR.**

Nuclear magnetic resonance (NMR) spectroscopy is a powerful, nondestructive technique to study structure or catalysis in biopolymers such as nucleic acids and proteins in solution (1, 2). Of the various magnetically active nuclei available, ^{1}H is most frequently observed because of its widespread distribution and high sensitivity. Protons, however, have a rather narrow range of chemical shifts, with attendant problems of overlapping signals and peak assignments (3). As a result there is considerable interest in other nuclei such as ^{13}C, ^{15}N, and ^{31}P with greater chemical shift dispersion.

Nitrogen is especially attractive because of its involvement in numerous biochemical processes and its large range of chemical shifts, approximately 900 ppm (4). Applications of ^{15}N NMR to biological problems, however, have not flourished to the extent of the other magnetically active nuclei because of problems with sensitivity. ^{15}N has a low natural abundance, 0.37%, and even when incorporated at >99% levels, is still only about 10^{-3} as sensitive as ^{1}H (5). Additional losses in sensitivity for biopolymers are observed because of line broadening and unfavorable nuclear Overhauser effects (6).

Chemical shifts for ^{15}N, and other heteroatomic nuclei as well, can be extracted from the NMR signals of a directly attached proton by two-dimensional (2D) chemical shift correlation spectroscopy (7). In the 2D experiment, observation of the nucleus with the highest gyromagnetic ratio (γ) provides maximal sensitivity (8). In the case of ^{15}N, "indirect" detection of chemical shifts through the high γ ^{1}H signals theoretically provides a 10^{3}-fold improvement in signal-to-noise ratio relative to a method based on direct detection of the less sensitive nucleus (9).

We now report a method for observing ^{1}H signals with ^{15}N chemical shift correlation in directly bonded ^{1}H—^{15}N units by multiple quantum 2D NMR. In the specific application described, the 2D procedure is used to generate a ^{1}H—^{15}N chemical shift correlation map for the slowly exchanging imino protons in a 0.8 mM aqueous solution of *Escherichia coli* $tRNA_f^{Met}$ that is labeled with ^{15}N at N3 of all the uridine-related bases. The technique not only provides ^{15}N chemical shifts at ^{1}H sensitivity but eliminates all ^{1}H signals for protons not spin coupled to ^{15}N. Site-specific labeling thus provides a powerful tool for detection and assignment of the ^{1}H NMR signals from the biopolymer. The 2D multiple quantum technique should be applicable to other heteronuclear spin coupled systems as well, including the biologically important ^{1}H—^{13}C unit.

MATERIALS AND METHODS

Synthesis and purification of ^{15}N-labeled tRNA with 65% incorporation at N3 of uridine and all bases derived from uridine biosynthetically was described previously (10). In the present study, 5 mg of *E. coli* $tRNA_f^{Met}$ (M_r 25,000) was dialyzed against 0.1 mM sodium thiosulfate, lyophilized, and dissolved in 250 μl of 10 mM sodium cacodylate buffer, pH 7.0, containing 8% (vol/vol) deuterium oxide, 50 mM sodium chloride, 10 mM magnesium chloride, and 1 mM EDTA. The sample was placed in a Wilmad 508 CP microcell, and spectra were recorded on a Nicolet 360-MHz spectrometer equipped with a probe triply tuned for ^{1}H, ^{2}H, and ^{15}N and an NTC-1180 data processor. The 360-MHz ^{1}H decoupler frequency was mixed down to 36.49 MHz, filtered, amplified, and used for ^{15}N pulses and decoupling. ^{15}N decoupling utilized a WALTZ-16 sequence [ref. 11; the ^{15}N phase variations were controlled by an AdNic Products (Ft. Collins, CO) Black Box].

RESULTS AND DISCUSSION

The pulse sequence (see Fig. 1) begins with a 90° Redfield "2-1-4" ^{1}H pulse (12). The "2-1-4" was used to minimize the size of the water signal in our specific application, but any semiselective pulse could be substituted for the initial interval. Alternatively, a hard 90° ^{1}H pulse would suffice for spin systems in which proton exchange did not occur (i.e., ^{1}H—^{13}C) and deuterated solvent was used. After a delay time $\Delta = 1/2J$ ($J_{^{1}H-^{15}N} \approx 90$ Hz), a 90° nitrogen pulse changes the ^{1}H magnetization into zero quantum (ZQ) and double quantum (DQ) coherences in the ^{1}H—^{15}N spin system. During the evolution period t_1, the ZQ coherence (M_{ZQ}) is frequency labeled as $\nu_{^{1}H} + \nu_{^{15}N}$, and the DQ coherence ($M_{DQ}$) as $\nu_{^{1}H} - \nu_{^{15}N}$, in which $\nu_{^{1}H}$ and $\nu_{^{15}N}$ are the respective proton and nitrogen chemical

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Abbreviations: 2D, two-dimensional; DQ, double quantum; ZQ, zero quantum.

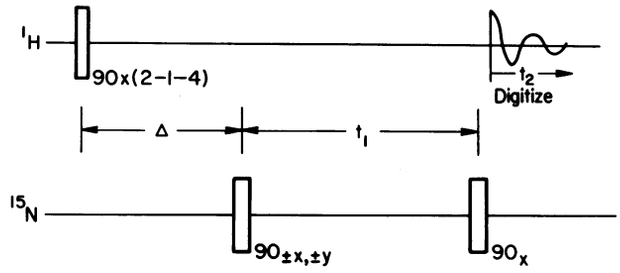

FIG. 1. Pulse sequence used for multiple quantum 2D ^{1}H—^{15}N NMR. ^{1}H—^{15}N decoupling can be accomplished by introducing a second delay Δ following the second 90° nitrogen pulse and irradiating ^{15}N during t_2.

shift frequencies expressed as differences between the resonance frequency for the nucleus and its transmitter frequency. The second 90° nitrogen pulse converts M_{ZQ} and M_{DQ} into phase-modulated ^{1}H signals (13). These ^{1}H signals are recorded during the acquisition period t_2 and stored in a 2D data set $s(t_1, t_2)$. The ^{1}H signals, modulated by the frequencies of the ZQ ($\nu_{^1H} + \nu_{^{15}N}$) and DQ ($\nu_{^1H} - \nu_{^{15}N}$) transitions, may be distinguished by acquiring two data sets that have a 90° phase difference for the first 90° nitrogen pulse. The signals for protons not spin coupled to ^{15}N may also be cancelled by using four phase variations ($\pm x$, $\pm y$) for the ^{15}N pulse and subtracting alternate scans for the two sets $\pm x$ and $\pm y$ (14).

ZQ and DQ contour maps for ^{15}N-labeled *E. coli* $tRNA_f^{Met}$ obtained by using the pulse sequence given in Fig. 1 are shown in Fig. 2 *A* and *B*, respectively. The F_2 dimension of the Fourier-transformed data contains the ^{15}N-coupled ^{1}H spectrum expressed in units of $\nu_{^1H}$. The F_1 dimension has ZQ resonances at $\nu_{^1H} + \nu_{^{15}N}$ (Fig. 2*A*) and DQ resonances at $\nu_{^1H} - \nu_{^{15}N}$ (Fig. 2*B*). The ^{15}N frequency, $\nu_{^{15}N}$, in any ^{1}H—^{15}N unit is obtained

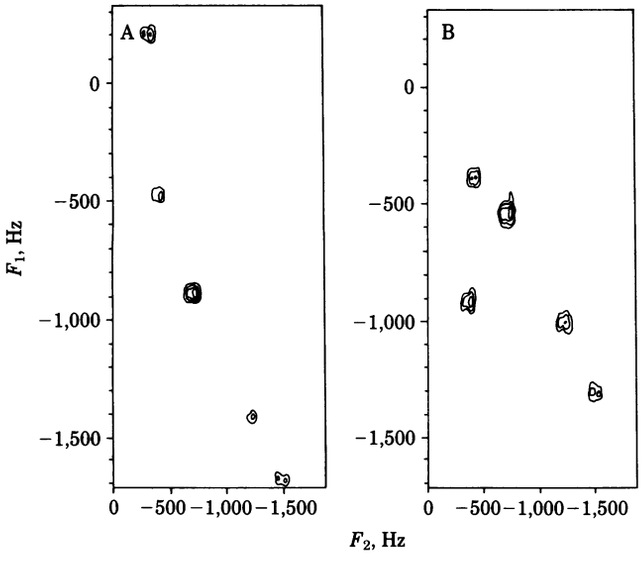

FIG. 2. Absolute value contour plots (25% contours) of the ZQ (*A*) and DQ (*B*) frequencies in F_1 versus ^{1}H frequency in F_2 for ^{15}N-labeled *E. coli* $tRNA_f^{Met}$ at 15°C. A 300-μs "2-1-4" proton pulse was used; Δ = 4.5 ms; and t_1 was varied from 0 to 15 ms in 1-ms intervals. A 150-ms delay was used between scans. The ^{1}H transmitter was located at 360.064955 MHz (15.80 ppm), and the ^{15}N transmitter, at 36.490500 MHz (164.4 ppm). The ^{1}H spectral width was ±5,000 Hz, and four sets of 16 × 800 scans were acquired in 512 real and 512 imaginary data points. The t_1 data were zero-filled to 128 real and imaginary points, and 15-Hz line broadening was applied in both dimensions before transformation. The acquisition time was 1.8 hr.

Table 1. ^{1}H and ^{15}N chemical shifts for *E. coli* $tRNA_f^{Met}$

Resonance	^{1}H δ, ppm*	^{15}N (2D) δ, ppm†	^{15}N δ, ppm‡
s^4U8A14	14.90	178.1	181.6
A11U24	14.60	162.0	162.9
rT54A58	13.75	158.6	159.4
U27A43	12.70	159.7	160.5
G64U50	12.18	158.2	158.3
Ψ55P58	11.39	158.7	158.9

All shifts are corrected external C^2HCl_3 versus internal ^2H_2O.
* Relative to 2,2-dimethyl-2-silapentane-5-sulfonate at 25°C.
† Relative to NH_3 at 25°C.
‡ From Griffey *et al.* (16).

from either map by subtracting the known value of $\nu_{^1H}$ from the F_1 shift. ^{15}N chemical shifts in δ (ppm) are obtained from $\nu_{^{15}N}$ by calibration with a deuterated chloroform solution 0.1 M in both 2′,3′,5′-tri-*O*-benzoyl[3-^{15}N]uridine and 2′,3′-*O*-isopropylidene-5′-*O*-acetyladenosine (15). The ^{15}N resonance frequency of 36.490370 Hz (C^2HCl_3 lock) measured for N3 in the standard by the 2D experiment corresponds to a directly observed δ value of 162.8 ppm relative to ammonia at 25°C with an external reference of 2.9 M $^{15}NH_4Cl$ in 1 M HCl. A frequency shift of 70 Hz is observed for the ^{1}H signal in water for an external C^2HCl_3 lock versus internal ^2H_2O. This difference corresponds to approximately 2 ppm in $\delta_{^{15}N}$, and a correction is applied to all of the values listed in Table 1.

A more appealing presentation of ^{1}H—^{15}N chemical shift data is shown in Fig. 3, in which ^{15}N δ is plotted versus ^{1}H δ for the DQ coherences. Using the procedure of Muller (17), we applied a constant phase shift to each ^{1}H data set transformed

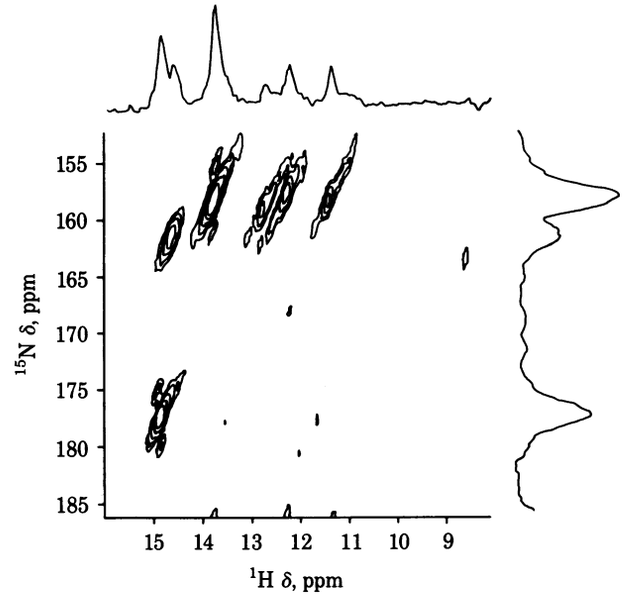

FIG. 3. Absolute value contour plots (20% contours) of ^{15}N δ versus ^{1}H δ obtained from DQ frequencies for ^{15}N-labeled *E. coli* $tRNA_f^{Met}$. A 350-μs "2-1-4" pulse was used; Δ = 4.0 ms; and t_1 was varied from 0 to 12.8 ms in 0.8-ms increments. A 150-ms delay was used between scans. The ^{1}H transmitter was located at 360.064415 MHz (14.30 ppm), and the ^{15}N transmitter, at 36.490700 MHz (169.8 ppm). The ^{1}H spectral width was ±5,000 Hz, and four sets of 16 × 3,200 scans were acquired in 512 real and 512 imaginary data points. The t_1 data were zero-filled to 128 real and imaginary points, and 15-Hz line broadening was applied in both dimensions before transformation. The acquisition time was 6 hr. ^{1}H and ^{15}N projections are shown opposite the ^{1}H and ^{15}N axes, respectively.

in the t_2 dimension, $s(t_1, F_2)$, to compensate for the increasing length of t_1 before the onset of ^1H acquisition, yielding a new set $s(t'_1, F_2)$. Transformation in the t'_1 dimension then yields $s(F'_1, F_2)$, in which $F'_1 = F_1 - F_2$, with resonances in the 2D map at $\pm\nu_{^{15}N}$, $\nu_{^1H}$ rather than $\nu_{^1H} \pm \nu_{^{15}N}$, $\nu_{^1H}$. A similar map can be generated from the ZQ data set as well.

An additional simplification, also shown in Fig. 3, is removal of the ^1H—^{15}N coupling interaction in the F_1 dimension. This is accomplished by including an additional delay $\Delta = 1/2J$, after the second 90° nitrogen pulse and irradiation of the ^{15}N region during t_2. The extra delay is necessary to allow the two parts of the ^{15}N-coupled ^1H doublets, which are 180° out of phase after the second nitrogen pulse, to rephase before decoupling. Without the second delay, the two anti-phase components are summed, and the ^1H signal disappears. In theory, ^{15}N decoupling enhances sensitivity by a factor of 2, although in practice the increase is slightly less.

Multiple quantum 2D NMR spectroscopy offers several advantages for studying protonated heteroatoms in biopolymers. Undoubtedly, the most exciting aspect of the method is its sensitivity for determining ^{15}N chemical shifts. The 2D map shown in Fig. 3 required only 6 hr to acquire with a sample containing 0.5 mM ^{15}N and is representative of the least sample used to date for a ^{15}N spectrum (for a comparison with direct observation of ^{15}N-enriched tRNA, see ref. 18). Even the weak signal for the uridine-27–adenine-43 pair at 12.70, 159.7 ppm, not apparent in the spin-coupled spectra shown in Fig. 2, is clearly visible. We estimate that ^{15}N shifts for ^1H—^{15}N units can be obtained *at natural abundance* in only 2–4 hr with a 100 mM sample of biopolymer. The factors that contribute to the high sensitivity include detection of ^{15}N through the higher γ ^1H nucleus, elimination of ^1H—^{15}N coupling in the ^1H signals, and the short duration of the pulse sequence, which avoids excessive loss from rapid relaxation of the imino protons. Additional advantages of the method include the chemical shift dispersion inherent in 2D maps, the suppression of ^1H signals for protons not attached to ^{15}N, and the increased reliability of assignments for molecules with site-specific substitution of ^{15}N.

CONCLUSIONS

Multiple quantum 2D NMR spectroscopy is a powerful tool for obtaining ^1H—^{15}N chemical shift correlation maps of biopolymers in aqueous solution. The pulse sequence is relatively simple, and the 2D experiment can be performed on most modern high-field NMR spectrometers with minimal modifications. Although the specific application presented uses ^{15}N-labeled tRNA, the high nitrogen sensitivity of the multiple quantum method makes it ideally suited for chemical shift studies of protonated nitrogens at natural abundance in both small-molecule and macromolecular systems. Other logical extensions of the technique include ^1H—^{13}C chemical shift correlation for protonated carbons and metabolic studies with ^{15}N or ^{13}C *in vivo*.

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