JOURNAL OF MAGNETIC RESONANCE **133,** 204–206 (1998)
ARTICLE NO. MN981442

# A Simple One-Dimensional Solid-State NMR Method to Characterize the Nuclear Spin Interaction Tensors Associated with the Peptide Bond

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Received November 6, 1997; revised March 6, 1998

**We propose a simple one-dimensional RF pulse sequence for the study of chemical shift and heteronuclear dipolar coupling tensors of oriented as well as unoriented solids. An off-resonance RF decoupling of protons during the signal acquisition of less sensitive nuclei is used to suppress homonuclear $^1$H–$^1$H dipolar interactions. This method is experimentally demonstrated on peptide samples selectively labeled with $^{15}$N isotope.** © 1998 Academic Press

*Key Words:* **solid-state NMR; tensors; Lee–Goldburg; SLF; peptide bond.**

Multidimensional NMR experiments have become important to study the structure and dynamics of molecules in the solid state (*1–5*). One of the commonly used solid-state NMR methods is the separated-local-field (SLF) experiment (*1*), yielding two-dimensional correlation of the chemical shift and dipolar interactions. The magnitudes and orientations of spin interaction tensors measured from an SLF experiment provide very powerful information for characterizing rapid, large-amplitude motions in rigid solids and also to interpret the relaxation rates measured through solution NMR spectroscopy (*6*). Several versions of high resolution two-dimensional SLF experiments (*2–5*) have been developed based on the use of multiple RF pulse sequences to suppress $^1$H–$^1$H dipolar couplings. These high-resolution two-dimensional experiments are also routinely used to measure $^{15}$N chemical shifts as well as $^1$H–$^{15}$N dipolar couplings of peptides oriented in lipid bilayers in order to study the backbone conformation and dynamics of the peptide (*7, 8*). In this paper, we present a one-dimensional experiment to study the chemical shift and heteronuclear dipolar coupling tensors from oriented as well as unoriented solid samples. This method is experimentally demonstrated on a model peptide sample selectively labeled with $^{15}$N isotope. It may be mentioned here that similar one-dimensional experiments have been used to study the chemical shift and heteronuclear dipolar coupling tensors pertaining to directly bonded heteronuclei not involving protons (*9–18*).

In the one-dimensional experiment, transverse magnetization of protons prepared with a 90° pulse is cross-polarized to $^{15}$N nuclei using the standard Hartmann–Hahn procedure. After cross-polarization, the $^{15}$N magnetization is sampled under an off-resonance irradiation of protons. It is well known that an off-resonance irradiation of protons changes both homonuclear and heteronuclear dipolar interactions (*19–22*). On the other hand, an on-resonance decoupling of protons results in a complete suppression of heteronuclear dipolar interaction present in the system. The effect of an off-resonance irradiation on dipolar couplings can be varied by changing either the offset frequency or the RF field strength of the irradiation. At the same time, by proper selection of an off-resonance RF field with an effective field pointing at the magic angle, $^1$H–$^1$H dipolar coupling can be suppressed to a great extent and $^1$H–$^{15}$N dipolar couplings can be scaled by a factor of 0.58.

The time evolution of the observable part of the final density matrix under the one-dimensional pulse sequence can be written as

$$S_x \rightarrow \cos(0.58\pi\omega_{IS}t\{S_x\cos(\delta_S t) + S_y\sin(\delta_S t)\} + 2\sin(0.58\pi\omega_{IS}t)\{S_yI_z\cos(\delta_S t) + S_xI_z\sin(\delta_S t)\}, \quad [1]$$

where $I$ and $S$ represent the $^1$H and $^{15}$N nuclei, respectively, $\delta_S$ is the chemical shift of $S$ spins, and $\omega_{IS}$ is the $I$–$S$ dipolar coupling. From Eq. [1], it is clear that two absorbance mode signals result, at frequencies $(\delta_S + 0.58\pi\omega_{IS})$ and $(\delta_S - 0.58\pi\omega_{IS})$ radians per second. In the case of a single crystal or an oriented sample, $\delta_S$ depends on the orientation of the sample with respect to the external magnetic field. On the other hand, for powders, $\delta_S$ represents the anisotropic chemical shift, and the expression for $\delta_S$ is

$$\delta_S = \sigma_{11N}\cos^2\phi\,\sin^2\theta + \sigma_{22N}\sin^2\phi\,\sin^2\theta + \sigma_{33N}\cos^2\theta, \quad [2]$$

where $\sigma_{iiN}$ ($ii$ = 11, 22, 33) are the principal elements of the $^{15}$N chemical shift tensor, $\theta$ is the angle between the $\sigma_{33N}$ axis and the applied external magnetic field direction, and $\phi$ is the angle between the projection of the vector representing the external magnetic field direction on the $\sigma_{11N}$–$\sigma_{22N}$ plane and

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1090-7807/98 $25.00

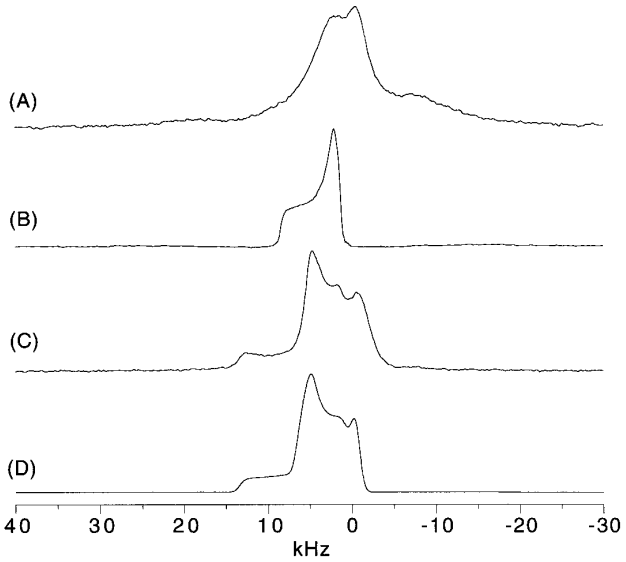


**FIG. 1.** $^{15}$N signal of a 50-mg polycrystalline sample of *N*-acetylvaline labeled with $^{15}$N isotope obtained with (a) no $^{1}$H decoupling, (b) on-resonance $^{1}$H decoupling, and (c) irradiation of protons at the magic angle. (d) is the simulated spectrum best fitting with the dipolar-chemical shift spectrum in (c). Spectra (a), (b), and (c) are the result of coadding 20,000, 256, and 2500 transients, respectively, from cross-polarization with 1-ms mix time. A relaxation delay of 3 s was used in all the experiments. Interestingly, a one-dimensional dipolar-chemical shift spectrum obtained with even 40 scans is sufficient to characterize the reported spin interaction tensors.

the $\sigma_{11N}$ axis. The dipolar coupling frequency, $\omega_{IS}$, under the off-resonance decoupling condition is expressed as

$$\omega_{IS} = \frac{0.58}{2} D_{IS}[1 - 3\{\sin\theta \sin\beta \cos(\phi - \alpha) + \cos\theta \cos\beta)\}^2], \quad [3]$$

where $D_{IS}$ is given as $\mu_o \hbar \gamma_I \gamma_S / 4\pi r_{IS}^3$, $\beta$ is the angle between the $\sigma_{33N}$ and the N–H bond vectors, and $\alpha$ is the angle between the $\sigma_{11N}$ axis and the projection of the N–H bond on the $\sigma_{11N}$–$\sigma_{22N}$ plane (*2*). $\theta$ and $\phi$ are the same as defined in Eq. [2]. Therefore, $^{15}$N signal acquired under off-resonance decoupling of protons will be useful to characterize the $^{15}$N chemical shift and $^{1}$H–$^{15}$N dipolar coupling tensors in the molecular frame.

All of the experiments were performed on a Chemagnetics Infinity 400-MHz spectrometer with a 9.4-T wide-bore JMT 400/89 magnet. A 5-mm Chemagnetics probe double-tuned to $^{15}$N and $^{1}$H nuclei resonating at frequencies 40.59 and 400.56 MHz, respectively, were used. An RF field strength of 55.6 kHz was used in both the RF channels. During $^{15}$N signal acquisition an offset of 38.5 kHz was used to set the Lee–Goldburg condition. Experiments were performed on a powder sample of a model peptide, *N*-acetyl-[$^{15}$N] DL-valine (NAV), labeled with $^{15}$N isotope at the amide site. Experimental spectra are given in Fig. 1. $^{15}$N chemical shift spectra obtained without any $^{1}$H decoupling, with on-resonance $^{1}$H decoupling, and with an off-resonance decoupling (*19*) are given in Figs. 1A, B, and C, respectively. The experimental spectrum in Fig. 1c was simulated to determine the $^{15}$N chemical shift and $^{1}$H–$^{15}$N dipolar coupling tensors in the molecular frame. The best-fitting simulated spectrum is given in Fig. 1d. The simulated spectrum was obtained with the following parameters: $\sigma_{11N} = 56.9 \pm 0.5$, $\sigma_{22N} = 78.3 \pm 0.5$, $\sigma_{33N} = 235.3 \pm 1$ ppm, $D_{IS} = 9.7 \pm 0.1$ kHz, $\alpha = 0°$, and $\beta = 19° \pm 2°$. Principal elements of the chemical shift tensor were directly measured from Fig. 1B and the spectrum was referenced to $NH_3$ (liquid, 25°C) by setting the observed $^{15}$N signal of solid $(^{15}NH_4)_2SO_4$ to 26.8 ppm. $\sigma_{iiN}$ values were confirmed from the isotropic chemical shift frequencies of the $^{15}$N MAS spectrum of the NAV powder sample obtained with a 6-kHz spinning frequency. Since there are two magnetically inequivalent molecules in a unit cell of NAV, two isotropic chemical shift peaks were observed at 123.5 and 119 ppm. Error in the tensor values presented in this work due to two different $^{15}$N resonances is negligible as the signal intensity of the high-field resonance peak is about 8% of the low-field resonance peak.

The best-fitting simulated spectrum shown in Fig. 1D was obtained by comparing the intensity ratios of the shoulders as well as their frequency separations with the experimental spectrum given in Fig. 1C. The intensity ratio of the shoulders of the powder pattern is highly sensitive to the change in $\beta$ value. The spectrum is somewhat less sensitive to the $\alpha$ angle when the $\beta$ is a small value; however, it is possible to estimate $\alpha$ to be in the range of 0–10°. The frequency separation of the shoulders is sensitive to the $^{1}$H–$^{15}$N dipolar coupling and the widths of the low-frequency shoulders are sensitive to $\alpha$ angle. As a result, a change of 1° for $\beta$, 5° for $\alpha$, and 100 Hz for the $^{1}$H–$^{15}$N dipolar coupling is transparent from the one-dimensional dipolar-chemical shift spectrum.

In the simulations, we assumed that the $^{1}$H–$^{15}$N dipolar coupling is collinear with the N–H bond. Therefore, the least shielded component of the $^{15}$N chemical shift tensor, $\sigma_{33N}$, is 19° away from the direction of the N–H bond. This value agrees well with other reported studies in the literature (*11–13*, *23–26*).

We have also analyzed the performance of this method on several peptide samples, both single-crystalline and polycrystalline, selectively labeled with $^{15}$N isotope. In the case of a single-crystal sample, DANTE-90° was used before the signal acquisition to select an $^{15}$N resonance in order to measure the dipolar coupling directly from the resultant Pake doublet shifted by the $^{15}$N chemical shift. The measured $^{15}$N chemical shift and $^{1}$H–$^{15}$N dipolar coupling could be used to determine the orientation of the peptide plane relative to the external magnetic field of the spectrometer (*7*, *8*). Linewidth measured from the dipolar-chemical shift spectrum of a single crystal was on the order of a kilohertz. This is not as narrow as the dipolar spectral lines obtained through the PISEMA experiment (*4*). Nevertheless, these line widths are comparable with most of the high-resolution two-dimensional SLF methods (*4*).

There are several advantages in using this method to char-

acterize nuclear spin interaction tensors. Since this is a one-dimensional experiment, resolution of the dipolar spectral lines depends on the acquisition time and the number of points sampled during the acquisition of the $^{15}N$ signal. On the other hand, in a two-dimensional SLF experiment, it depends on the number of points sampled in the indirect frequency, or heteronuclear dipolar, dimension. Therefore, determination of tensors using this method is faster and easier to implement compared to the traditional 2D SLF experiments. The one-dimensional dipolar-chemical shift spectrum obtained using this method is simple to interpret and to measure for tensor values. This method would be highly useful in studying biological solids labeled with specific isotopes, for example, $^{15}N$ or $^{13}C$, as there exists a common difficulty in obtaining sizable amount of such samples for NMR experiments (*27*). For example, $^{15}N$ chemical shift and $^{1}H$–$^{15}N$ dipolar coupling pertaining to an amide site of a membrane-associated peptide or protein labeled with $^{15}N$ at a specific site, oriented in phospholipid bilayers either mechanically or magnetically, could be measured in order to determine its backbone conformation.

## ACKNOWLEDGMENTS

This research was supported by the research funds from the Department of Chemistry and Biophysics Research Division at the University of Michigan.

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