Molecular Dynamics and Magic Angle Spinning NMR

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Received February 14, 1994®

Abstract: The observation of "disappearing lines" or the inability to observe expected resonances in magic angle spinning (MAS) NMR spectra suggest an interference phenomenon between molecular dynamics and the characteristic frequencies of pulse trains and radiofrequency (RF) fields employed in the NMR experiments. In this paper, we present a systematic study of 3-fold hopping of the $-NH_3^+$ group in L-alanine, and the motion's interference with two widely-used techniques for homo- and heteronuclear decoupling. It is demonstrated experimentally and theoretically that rates of 3-fold hopping in the vicinity of the cycle times of homonuclear pulse trains or the reciprocal of the heteronuclear decoupling field strengths lead to dramatic broadening of NMR lines in MAS spectra.

Introduction

Inter- and intramolecular dynamic processes can profoundly affect spectral features (lineshapes and relaxation times) when the rate of a process becomes equal to a characteristic frequency of the experiment. Several well-known examples of these phenomena occur in solution NMR when the correlation times governed by chemical exchange processes reach a minimum.[1] The increased use of multiple pulse and multiple resonance experiments presents additional opportunities for these effects to occur. In particular, when the correlation times of the motional processes become comparable to the cycle time of a RF perturbation, spectral broadening is observed. For example, motion at a rate equal to the inverse of the size of a heteronuclear decoupling field or on the timescale of a homonuclear multiple pulse train will have deleterious consequences on the intended effects of the applied RF field/pulse train. This interaction can severely reduce resolution, thus rendering certain resonances undetectable in chemical shift spectra. This phenomenon was used to explain the loss of resolution observed in ^{13}C CPMAS spectra of proteins and peptides in membranes.[2,3] Specifically, diffusional motion of the peptide or protein in the membrane interfering with the decoupling could explain the failure to observe several of the expected resonances. More recently, line intensities in chemical shift spectra of the tyrosine side chain carbons in a cyclic hexapeptide, Gly-Tyr-Gly-Pro-Leu-Pro,[4] and the coordinated methyl groups in $\{W(\eta^5\text{-}C_5Me_5)Me_4\}^+$ [5] have displayed strong temperature dependencies. Thus, it appears that the interference of molecular motion with RF fields is present in a wide variety of systems.

In this paper, we study this effect in detail using a combination of solid-state NMR experiments. Namely, the temperature dependence of the kinetics of the ammonium group in L-alanine is determined via deuterium NMR lineshape experiments and simulations. Using these data, we theoretically calculate and experimentally confirm the interference between coherent spin motion, induced by a decoupling field, and incoherent hopping of the protons of the ammonium group of L-alanine using two separate techniques: continuous wave (CW) proton decoupling when observing ^{15}N and multiple pulse homonuclear decoupling when observing ^1H. As expected, the spectra exhibit dramatic lineshape and linewidth effects when the hopping rate is comparable to the reciprocal of the decoupling field or the cycle time of the multiple pulse train. This behavior is further confirmed by computer simulations which model the dependence of the lineshape and linewidth on hopping rates.

Experimental Section

Sample Preparation. L-Alanine was purchased from Aldrich Chemical Co. (Milwaukee, WI). Deuterated samples were prepared by proton/deuteron exchange in D_2O purchased from CIL (Woburn, MA). The saturated solution was placed in a nitrogen atmosphere and ND_3-labeled L-alanine was recovered by slow evaporation at room temperature. Crystals were redissolved in D_2O and recrystallized a second time, dried, and then pulverized. The powder was dried over P_2O_5 to remove occluded D_2O molecules; nevertheless, the deuterium NMR spectra do show a residual D_2O peak. ^{15}N-labeled L-alanine, also purchased from CIL, was used without further purification.

Deuterium NMR Measurements. Deuterium quadrupole NMR spectra of ND_3-labeled L-alanine were recorded at 61.05 MHz on a home-built spectrometer. Pulse lengths and phases were optimized prior to recording spectra, and a quadrupole echo pulse sequence (Figure 1a), using a pulse spacing of 40 μs, was used to record lineshapes. Quadrature detection with a dwell time of 2 μs was used and 90° pulse lengths were typically $\leq$2.2 μs. Pulses were phase cycled through a set of eight quadrupole echoes during signal averaging to eliminate DC offset and quadrature phase error artifacts. Recycle delays were greater than five times T_1, ranging from 0.5 s at higher temperatures to 400 s at lower temperatures. T_1 was measured using the inversion recovery method with a quadrupole echo sequence for observation, i.e., $180°-\text{delay(ms)}-90°_x-\tau-90°_y-\tau-\text{echo}$.

Proton NMR Measurements. Proton multiple-pulse NMR spectra[6-8] of natural abundance L-alanine were obtained at 397.6 MHz on the same spectrometer. A standard double resonance probe was used with proton 90° pulse lengths $\leq$4 μs. Multiple pulse parameters were optimized using malonic acid and cycle times for the MREV-8 sequence were typically 70 to 75 μs (Figure 1b). Spectra of L-alanine were obtained at spinning speeds of 1.5-1.7 kHz, with recycle delays ranging

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® Abstract published in *Advance ACS Abstracts*, November 15, 1994.

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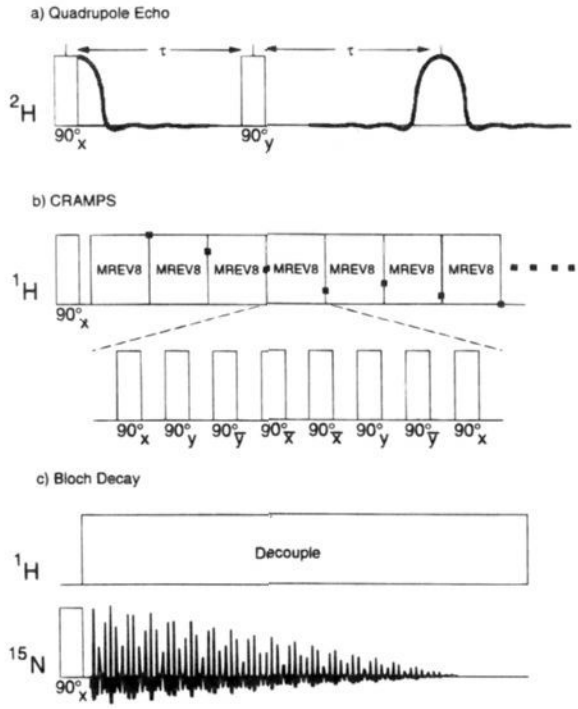

Figure 1. Schematics of NMR pulse sequences used to obtain spectra: (a) quadrupole echo sequence for deuterium spectra, (b) combined rotation and multiple pulse spectroscopy (CRAMPS) for proton spectra, and (c) Bloch decay sequence for nitrogen spectra.

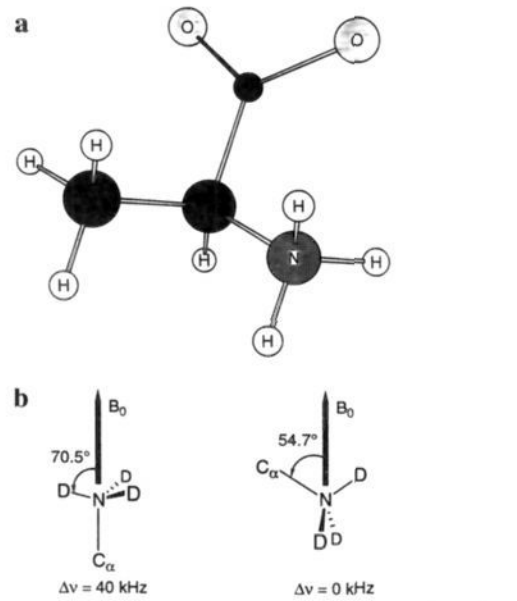

Figure 2. (a) Molecular structure of L-alanine and (b) two orientations of L-alanine in the magnetic field leading to the characteristic triplet deuterium NMR powder pattern at intermediate hopping rates.

from 3 s at higher temperatures to 10 s at lower temperatures. The resolution over the temperature range was independently monitored by observing the splitting of the −COOH protons in malonic acid; lineshapes remained consistent with minimal increases in linewidth at lower and higher temperatures (data not shown). The chemical shift scaling factor of the MREV-8 sequence was measured using a standard comb of H_2O spectra with 2 kHz frequency intervals to ±8 kHz off resonance. Spectra were collected at −4 kHz off resonance to avoid zero-frequency artifacts.

Nitrogen NMR Measurements. Magic angle spinning ^{15}N Bloch decay spectra of ^{15}N-labeled L-alanine were acquired with ^{15}N and ^{1}H Larmor frequencies of 40.3 and 397.6 MHz, respectively. The proton decoupling RF field strength was 67 kHz, corresponding to a ^{1}H 90° pulse length of 3.7 μs. Spectra were acquired under CW proton decoupling after a 5 μs 90° pulse (Figure 1c) for ^{15}N. The sample spinning rate was 4.5 kHz and repetition times during data accumulation varied from 3 s at higher temperatures to 300 s at lower temperatures.

Lineshape Simulations. ^{2}H Lineshapes. The details of the lineshape calculations are discussed elsewhere,[9] but input to the program consists of molecular geometry, quadrupolar coupling constants, η values, pulse program and processing parameters, site populations, and exchange rates. The molecular structure for L-alanine is shown in Figure 2a, and the angles between the ND bond vectors and the 3-fold C^α−N rotation axis were taken from the neutron diffraction data of Lehman *et al.*[10] In all cases the equilibrium population for each site was assumed to be one-third and the exchange rates (k) between different sites were assumed to be equal. The rigid lattice quadrupolar coupling constants (174.5, 166.9, and 145.2 kHz) and η values (0.072, 0.043, and 0.027) of the three sites were taken from zero-field quadrupole resonance data of Hunt *et al.*[11] Slight modifications to the lineshape calculation program were made to account for the different quadrupolar coupling constants of the three sites due to hydrogen bonding; modifications were not made when calculating relaxation spectra since these experiments were performed at temperatures at which one can assume the quadrupolar couplings were essentially averaged. (In this instance the average of the three quadrupolar coupling constants was used.) Each simulation provides two pieces of information: the spectral lineshape and the relative integrated intensity of the spectrum. At lower temperatures, the rate for a rotational jump of the ammonium group at a given temperature was obtained by visual comparison of the experimental and theoretical lineshapes; T_1 data were more rigorously fit via direct comparison of theoretical and experimental relaxation values for various orientations. We have corrected for finite pulse effects in the simulations,[12] but this correction is minimal given the RF field employed and spectral widths in the experiments.

^{15}N Lineshapes. Simulations of ^{15}N spectra at different jumping rates were carried out using the program NMRLAB written in FORTRAN. In NMRLAB, the spin system, consisting of a nitrogen and three protons, is defined in the input file according to the neutron diffraction structure of L-alanine.[10] Zeeman interactions, hetero- and homonuclear dipolar couplings, and chemical shift anisotropies were taken into account in order to fit the experimental results. The homo- and heteronuclear dipolar coupling constants were calculated from the crystal structure using the gyromagnetic ratios of ^{1}H and ^{15}N nuclei, yielding a ^{15}N−^{1}H dipolar coupling constant of 10.9 kHz, based on the average NH bond length, and a ^{1}H−^{1}H dipolar coupling constant of 25 kHz. The chemical shift anisotropy (CSA) tensor parameter was estimated to be about 200 Hz based on static ^{15}N spectra under decoupling and should be axially symmetric according to the molecular structure. NMRLAB can also calculate the ^{15}N spectra using various approximation methods to be discussed below and elsewhere.[13]

Results and Discussion

Quadrupole Echo Lineshapes for an $-ND_3^+$ Group. The essential features of the quadrupole echo lineshapes are similar to those for a $-CD_3$ group.[14] However, it is interesting to note that in the rigid lattice spectrum for the ammonium group, two distinct quadrupolar coupling constants may be seen due to the inequivalent environments of the deuterons arising from hydrogen bonding. As the hopping rate increases, the different quadrupolar coupling constants are motionally averaged and the spectra then more closely resemble those of the methyl group. The shapes of these spectra result from the $-ND_3^+$ undergoing

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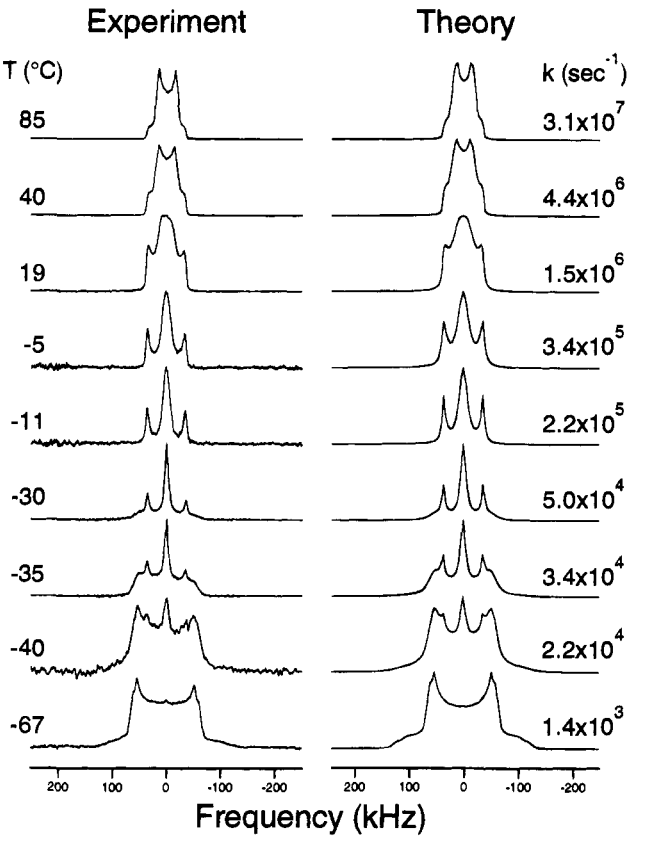

Figure 3. Experimental and simulated deuterium NMR spectra for ND_3-labeled L-alanine at temperatures and hopping rates indicated. Parameters for the simulations are given in the text.

three-site hops and can be easily understood, especially in the intermediate exchange region, by considering the two special orientations with respect to the magnetic field shown in Figure 2b. In the slow-exchange limit each deuteron in the rigid lattice yields a separate resonance line in the spectrum. A spectrum of a single crystal would have three quadrupole doublets; one for each magnetically inequivalent deuteron. In the fast hopping limit, $k \gg \omega_Q = (e^2Qq/h)$, the three ND tensors will be averaged to a single tensor with its unique axis collinear to the C^α–N bond, which is the hopping axis. This gives rise to a single quadrupole doublet for each crystalline in the fast limit spectrum. In both the rigid lattice and fast limit spectra of powders nearly axially symmetric Pake patterns are observed; however, the fast limit spectrum is a factor of three narrower due to the motional averaging. In the intermediate exchange regime, $k \approx \omega_Q$, T_2 becomes comparable to the τ value in the echo experiment for certain orientations in the powder sample. For these orientations the second pulse does not refocus the magnetization and there is a corresponding spectral intensity loss. However, for other orientations, T_2 is long since the motion does not change the orientation of an ND vector with respect to the magnetic field. These orientations give rise to prominent singularities in the lineshapes, two such orientations being illustrated in Figure 2b. The effect of these two special orientations on intermediate ^2H lineshapes is apparent in Figure 3. These spectra are similar to lineshapes previously observed in ^2H NMR studies of the methyl group in L-alanine.[14]

^2H quadrupole echo spectra of ND_3^+-labeled L-alanine were taken as a function of temperature in the range −70 to +85 °C. Figure 3 contains typical experimental and simulated lineshapes at the indicated temperatures. At −67 °C the rigid lattice $\eta \approx 0$ spectrum is obtained. This lineshape develops to the intermediate exchange spectrum, particularly in the range $-35 < T < -5$ °C, where $3.5 \times 10^4 < k < 5.5 \times 10^5$ s^{-1}. At higher temperatures (>30 °C) a fast limit Pake pattern is observed. For $30 < T < 70$ °C the lineshape remains essentially constant but the spectral intensity continues to increase with temperature. As seen in Figure 3, the simulations are in excellent agreement with the experimental results and provide a jump rate for each temperature. The simulations are based on a single correlation time motional model and their success indicates there is no significant distribution of correlation times present in ND_3-labeled L-alanine over the temperature range studied. Spectra similar to those shown in Figure 3 have been reported for the methyl group of L-alanine, although they occur at much lower temperatures.[14]

^2H Spectral Intensities. In the intermediate exchange region, the quadrupole echo spectra show frequencies arising from the molecular orientations shown in Figure 2. As discussed above, these orientations have a relatively long T_2 and lead to the triplet lineshape observed, for example, in the −12 °C spectrum. The remainder of the signal decays rapidly after the first pulse and cannot be refocused because the decay is due to the exchange process. Consequently, the intensities of the observed signals in the intermediate exchange region are only ~5% of the intensities observed in the slow and fast limit. In addition to the large intensity loss which occurs at log $k \sim 5$, there are significant intensity losses over a wide range of hopping rates. In particular, a 25% loss is observed for hopping rates between 10^4 and 10^8 s^{-1} and a 50% loss between 5×10^4 and 5×10^7 s^{-1}.

Three-site hops are particularly sensitive to intensity losses during intermediate exchange due to the high symmetry of the motion.[14] However, similar spectral intensity losses have been observed in other systems. For instance, aromatic rings in crystalline amino acids and peptides undergo 2-fold flips which transform the spectra to $\eta = 0.6$ lineshapes in the fast limit.[15] In the intermediate exchange region a minimum intensity value of ~20% is observed for this case, which is a factor of ~5 larger than the minimum value observed for three-site hops.

Anisotropic Spin–Lattice Relaxation. Anisotropic relaxation of lineshapes in T_1 experiments provides another means of determining kinetic rates of motions. A specific motional mechanism will lead to faster relaxation of certain portions of a powder lineshape than other models. In both the experimental and the simulated spectra of ND_3^+-labeled L-alanine, the parallel edges relax more quickly than the perpendicular edges. Also, T_1 values may be determined, leading to a more rigorous fit to experimental data.

Activation Energy for the $-ND_3^+$ Group. The rate constants obtained from spectra, such as those shown in Figure 3, can be used to calculate an activation energy, E_a, for the 3-fold motion of the ND_3^+ group; a plot of log k vs $1/T$ is shown in Figure 4. The errors in fitting the simulations to the data are indicated by the vertical bars and the solid line represents a least-squares fit to the results. The slope of this line yields an activation energy of 40.5 kJ/mol ($A_0 = 2.52 \times 10^{13}$). The E_a for the ammonium group of L-alanine is comparable to activation energies obtained for other $-NH_3^+$ groups. For example, in other amino acids values ranging from 32.5 to 51.7 kJ/mol have been measured.[16,17] These large activation energies for ammonium group motion in amino acids are easily understood given the hydrogen bonding present in amino acid crystallites.

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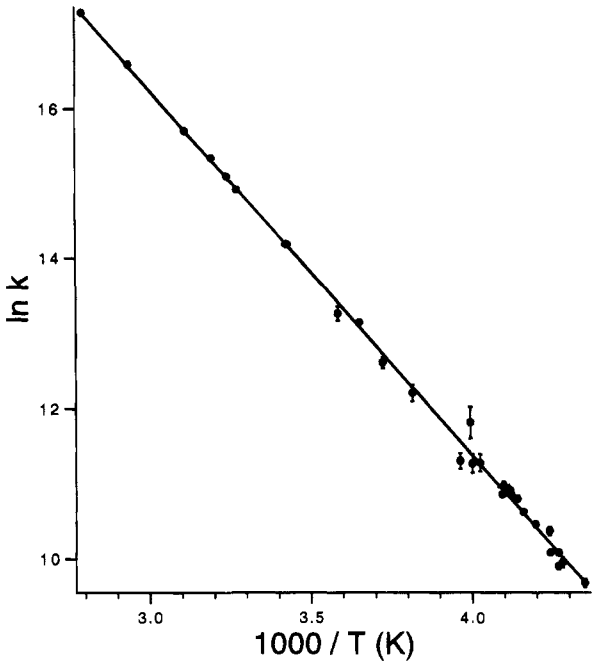

Figure 4. Arrhenius plot of hopping rates for the ammonium group of L-alanine obtained from deuterium NMR simulations. A best fit line yields an activation energy of 40.5 kJ/mol.

Finally, the activation energy measured for the ammonium group in L-alanine agrees well with the value of 38.6 kJ/mol (A_0 = 6.5 × 10^{13}) obtained from proton relaxation experiments.[17]

Molecular Motion Effects in Multiple Pulse Spectra. Interestingly, at room temperature (25 °C) the motional rate of the ammonium group in L-alanine is only 2 × 10^6 s^{-1}. This rate is close to the time scales involved in multiple pulse experiments and can explain the anomalous broadening observed in ^1H chemical shift spectra at the ammonium group resonance. As seen in Figure 5, increasing the temperature, and therefore the hopping rate, leads to significant narrowing of this resonance; lowering the temperature and decreasing the hopping rate to ~2 × 10^5 s^{-1} (on the timescale of the pulse spacings and the RF cycle time) leads to almost a complete loss of resolution, as previously predicted by Haeberlen and Waugh.[18,19]

Molecular Motion Effects in Nitrogen Solid-State MAS Spectra. ^{15}N CPMAS spectra of L-alanine measured at different sample temperatures are shown in Figure 6 (dotted lines). As the sample temperature is lowered the maximum intensity of the ^{15}N peak decreases and its linewidth increases, and near −26 °C, the ^{15}N resonance reaches a maximum width of ~1 kHz. As the sample is cooled further, the ^{15}N linewidth decreases and the spectral intensity increases.

One possible explanation for the lineshape changes with temperature is the interference between the 3-fold hopping motion of the $-NH_3^+$ group protons discussed above with the applied decoupling RF field. In order to simulate the effects of this interference on the lineshape, we assume that the intermolecular dipolar coupling can be ignored and the $-NH_3^+$ group forms an isolated four spin system in which the total spin Hamiltonian includes the Zeeman interaction, hetero- and homonuclear dipolar couplings, and chemical shift anisotropy. Transferring the total spin Hamiltonian to the rotating frame yields the zero-order average Hamiltonian

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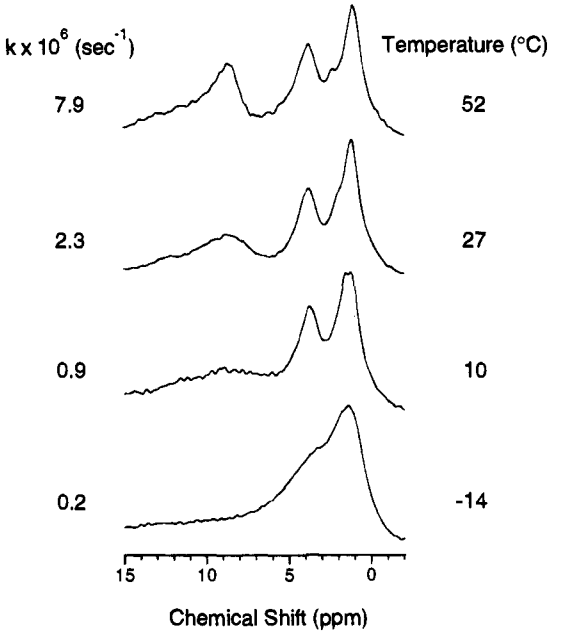

Figure 5. Multiple pulse spectra of L-alanine obtained using CRAMPS at the temperatures indicated. Estimated hopping rates for the ammonium group extracted from deuterium NMR data are also listed. Note that some broadening of the $-CH_3$ resonance (at ~1 ppm) also occurs as the hopping rate of the methyl group approaches the frequency of the decoupling field.

$$H = \sum_i (\omega_{0i}^{I} I_{iz} + \omega_{0i}^{S} S_{iz}) + H_{CS}^{I} + H_{CS}^{S} + H_{IS} + H_{II} \quad (1)$$

where

$$H_{CS}^{I} = \sum_i \omega_{CS,}^{I}(\Omega_i) I_{iz}$$

$$H_{CS}^{S} = \sum_i \omega_{CS}^{S}(\Omega_i) S_{iz}$$

$$H_{IS} = \sum_{i,j} 2\omega_{D_{ij}}^{IS}(\Omega_{ij}) I_{iz} S_{jz}$$

$$H_{II} = \sum_{i,j} 2\omega_{D_{ij}}^{II}(\Omega_{ij})(3I_{iz}I_{jz} - \mathbf{I}_i\mathbf{I}_j) \quad (2)$$

the Ω_i are Euler angles between the principal axis system of an interaction and the laboratory frame, the H_{CS} represent the chemical shift interactions, and H_{II} and H_{IS} represent the homonuclear and heteronuclear dipolar interactions, respectively. Thus, the spin system can be described by a 16 × 16 matrix in this Hilbert space. The random 3-fold hop can be represented by a permutation matrix between all the spin states in the product Zeeman basis. After the total spin Hamiltonian and the permutation matrix are transferred into Liouville space, the spin density matrix may be solved by the master equation

$$\frac{d}{dt}\varrho(t) = \hat{L}(t)\varrho(t) + \hat{\Gamma}(t)\varrho_0 \quad (3)$$

where $\hat{\Gamma}$ is the relaxation super matrix, ϱ_0 is the equilibrium density matrix, and $\hat{L}$ is the super Liouville operator given by

$$\hat{L}(t) = -i\hat{H}(t) - \hat{\Gamma} - \hat{X} \quad (4)$$

and

$$\hat{H} = H \otimes E - E \otimes H, \qquad \hat{X} = X \otimes X \quad (5)$$

where $\hat{\mathbf{X}}$ is the exchange matrix. The formal solution of eq 3, if we ignore the T_1 relaxation, may be written as

$$\varrho(t) = T\exp(\int_0^t dt'\hat{L}(t'))\varrho(0) \tag{6}$$

where T is the Dyson time ordering operator. Since the Liouvillian is time dependent and does not generally commute with itself at different times, the argument of the exponential cannot be integrated normally. To evaluate the exact evolution of the density matrix, NMRLAB divides the acquisition time into N steps. During each time step, the Liouvillian varies slightly and can be assumed to be approximately equivalent to its average. The density matrix at the end of the kth step can then be calculated by diagonalizing the time independent Liouvillian, and it is given by

$$\varrho(t_k) = \{\prod_{i=0}^{k-1}\exp[\hat{L}(t_i)\Delta t_i]\}\varrho(0) \tag{7}$$

The diagonalization of a 256 × 256 Liouvillian matrix to determine the propagator of the spin density matrix is usually very time consuming. In order to accelerate calculation of the spin density matrix, we chose a random crystallite orientation and then looked for block structure. Once the block structure of the Liouvillian was known, we needed to focus only on the blocks which related the observable operator and initial density matrix. For L-alanine, the Liouvillian of the $-NH_3^+$ group has only four such blocks, each 64 × 64. This is because the nitrogen nucleus is not involved in the chemical exchange process, and the heteronuclear dipolar coupling of the nitrogen to the protons is diagonal in the Zeeman basis. This block-diagonalized structure translates to the Zeeman basis with a spin Hamiltonian matrix comprised of 8 × 8 blocks. The chemical exchange between protons only causes evolution within individual blocks. Since our observable, I for the nitrogen nucleus, only has 8 nonzero elements in the Zeeman basis and becomes a vector with 64 elements in the Liouville space, only one of the 64 × 64 blocks of the Liouvillian matrix propagates the evolution of the spin density matrix for the observable.

For calculational purposes, we assume that the activation energy of the three-site hop is the same for protons and deuterons since the experimental data being fit were taken at relatively high temperatures. Thus, jumping rates of the protons in natural abundance L-alanine at various temperatures can be calculated from the deuterium data. With the calculated jumping rates, we have simulated ^{15}N spectra at various temperatures, and the results are shown in Figure 6 (solid lines). The maximum linewidth is found when the jumping rate is comparable to the decoupling RF field. This evidence, together with the experimental and theoretical deuterium results described above, strongly supports the argument that loss of resolution observed in various L-alanine spectra arises from the motional interference between the decoupling RF field and random 3-fold hopping of the ammonium group.

Following is a brief discussion of previous works classified as three alternative methods to simulate the ^{15}N spectra shown in Figure 6. The first derivation calculates the transverse relaxation time of the observable using classical relaxation theory; the second approach uses the random walk model; and the third approximation uses average Hamiltonian theory.

Classic Relaxation Theory. The interference between an externally applied coherent perturbation and a random molecular motion was first explored by Maricq and Waugh for the case of a spin system undergoing molecular motion while the sample

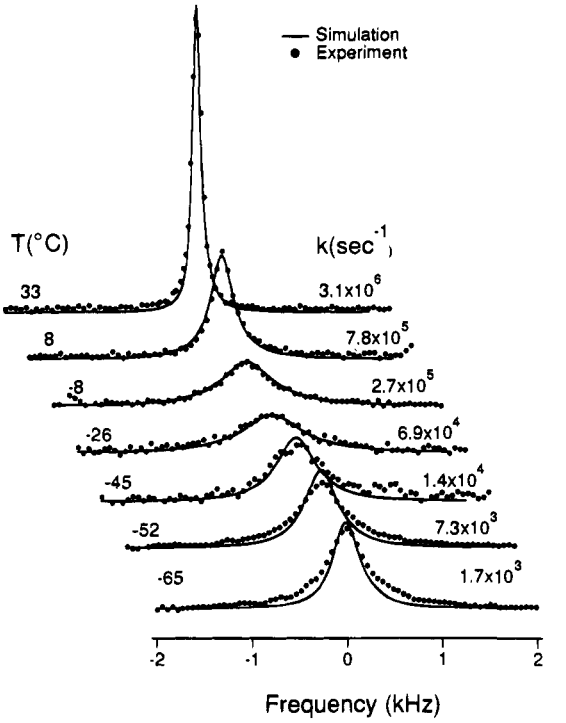

Figure 6. Comparison of experimental and simulated nitrogen NMR spectra at several temperatures and hopping rates, respectively. Simulations are exact solutions based on a 3-fold hopping model using parameters given in the text. The slight discrepancy between experiment and simulation occurring at the two lowest temperatures may be due to a departure from the magic angle or a decrease in the efficiency of decoupling.

is spinning around the magic angle.[20] Such an interference broadens the spectral lines due to a loss of efficiency in averaging the inhomogeneous and anisotropic interactions by MAS. Subsequently, this interaction was characterized as advantageous for studying slow motions in organic solids. VanderHart and co-workers first studied the interference occurring between random rotational diffusion and a continuous wave decoupling RF field using hexamethylbenzene.[21] A theoretical description of this motional interference was presented by Rothwell and Waugh, using relaxation theory with a spin Hamiltonian consisting of the Zeeman interactions, RF terms, and the heteronuclear dipolar coupling of a lone-pair IS spin system (I spin denotes a proton and S spin refers to ^{15}N or ^{13}C).[22] With this theory, the transverse relaxation time, T_2, under the high-temperature approximation is given by

$$\frac{1}{T_2} = \frac{4\omega_d^2 I(I+1)}{15} J(\omega_1) \tag{8}$$

where ω_d is the amplitude of the I−S dipolar coupling, ω_1 is the amplitude of the applied CW decoupling RF field, $J(\omega_1)$ is the spectral density function,

$$J(\omega_1) = \frac{\tau_C}{1+\omega_1^2\tau_C^2} \tag{9}$$

and τ_C is the correlation time of the random rotational diffusion. The prefactor in eq 8 is just the powder average of the I−S second moment, M_2^{IS}, as pointed out by the authors. From this

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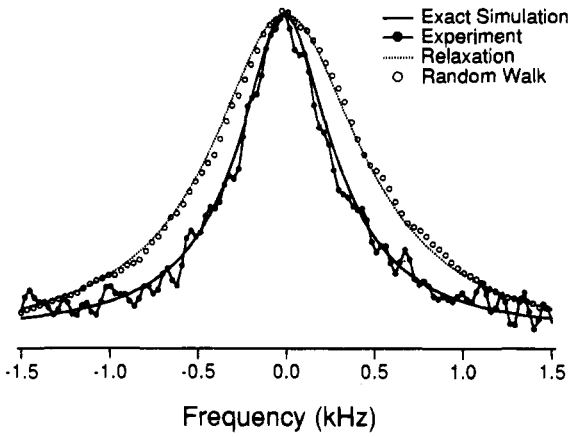

Figure 7. Comparison of nitrogen NMR spectra simulated using different approaches with experimental results obtained for L-alanine at −8 °C.

equation, the linewidth (Δ) of the affected resonance can be calculated using $\Delta = (\pi T_2)^{-1}$. To calculate the linwidth of an $I_N S$ spin system subjected to magic angle sample spinning, eq 8 has to be modified, and the result is

$$\frac{1}{T_2} = \sum_{i=1}^{N} \frac{2\omega_{d_i}^2 I(I+1)}{45}[2J(\omega_1 + \omega_r) + 2J(\omega_1 - \omega_r) + J(\omega_1 + 2\omega_r) + J(\omega_1 - 2\omega_r)] \quad (10)$$

where ω_r is the sample spinning rate and N is the number of I spins. When the sample spinning rate is small, the values of the four spectral density functions are nearly equal, and eq 10 will reduce to eq 8 for an IS spin system. In the other limit, where there is no decoupling RF field applied, even if the spinning speed is very fast, the interference between MAS and the random jumping motion results in residual linewidth, determined by eq 10, which is similar to the equation derived by Suwelack *et al.*[23]

In the derivation of eqs 8 and 10 two approximations have been made. First, only the secular terms in the master equation of the density matrix are included; since the homonuclear dipolar couplings between protons have no contribution to the transverse relaxation of the S spin, it is possible to ignore them. Second, no differentiation is made between discrete hopping and rotational diffusion. The cross-correlation terms which arise from the S spin being coupled to more than one I spin and the motion of the I spins being correlated, as in the case of the ammonium group in L-alanine, are also neglected. To include these effects, a higher order approximation has to take into account a more general treatment of the relaxation of the S spin.

When applying either eq 8 or 10 to the analysis of experimental data, one usually measures the second moment from the lineshape, which already includes the contributions from the homonuclear dipolar coupling and cross-relaxation effects. This may lead to an incorrect determination of the correlation time. In our case, since we know the crystal structure of L-alanine, the dipolar coupling constant between ^{15}N and ^{1}H may be calculated. Using a coupling of 10.9 kHz, this analysis predicts an ^{15}N linewidth of about 990 Hz at a jumping rate of 290 kHz, decoupling field of 67 kHz, and spinning speed of 4.5 kHz, corresponding to experimental data at −8 °C. However, the experimental result is 580 Hz (see Figure 7); obviously eq 10 only qualitatively describes the interference effect.

(23) Suwelack, D.; Rothwell, W. P.; Waugh, J. S. *J. Chem. Phys.* **1980**, *73*, 2559.

Random Walk Model. Another approach to predicting the effects of the interference between the decoupling and random jumping motion in a molecule on the linewidth of the S spin is to use a random walk model described by Waugh. During a decoupling cycle, all the spin packets evolve through the trajectories which depend on individual orientation and anisotropic parameters, and have the same phase at the end of each decoupling cycle if there is no interruption. However, if the molecule or a group in the molecule jumps from one configuration to another, the spin packets will evolve following different trajectories, and therefore not all spin packets will return to their original positions. This behavior is similar to interfering with echo formation by application of π pulses under MAS.[24,25] The trajectory of each spin packet is determined by the spin Hamiltonian, and the interruption of the evolution of each spin packet is also equivalent to changing the spin Hamiltonian. In the simulation, as the spin packet evolves, the spin Hamiltonian changes randomly among all possible configurations specified by two variables, one indicating the configuration of the molecule and the other denoting how long the spin packet stays at any given configuration. For a single spin packet, the time evolution of the S spin calculated by this method will be discontinuous, so an ensemble average over all spin packets is necessary; an ensemble average is equivalent to a powder average, making a reduction in computation time possible. The lineshapes of S spin nuclei calculated by the random walk method in general depend on the correlation function between sites. In our calculations a single exponential function was used, yielding results that are the same as those predicted by the classic relaxation model discussed earlier (see Figure 7). In order to obtain a reasonably smooth time evolution of the observable, many orientations have to be averaged. The number of jumps during the entire evolution depends on the jumping rate, and each jump involves at least one diagonalization of the spin Hamiltonian. When the jumping rate is very large, the number of diagonalizations increases dramatically, and the whole calculation becomes very time consuming even though this method avoids a diagonalization of a Liouvillian.

Average Hamiltonian Theory. More recently, the interference between random discrete jumps and a CW decoupling RF field was investigated by Frydman and Frydman[26] for static samples. In order to simulate the lineshape changes as sample temperature varied, they proposed an approximate method for a two-site jump model. In this approach, since the time-dependent spin Hamiltonian in the tilted rotating frame defined by the unitary transformation operator $T = \exp\{-i\omega_1 I_x t\}$ has a period of $\tau_c = 2\pi/\omega_1 t$ the effective dipolar coupling constant is positive during the first half of the decoupling cycle (relative to a positive dipolar coupling constant in the laboratory frame) and becomes negative during the second half of the decoupling cycle. The lineshape changes induced by molecular motions are different between the first and second half of the decoupling cycle; each half yields its own lineshape at a particular jumping rate. The overall lineshape will be the convolution of these two lineshapes assuming the lineshape changes due to the different splitting during each period are negligible. In other words, we can use the following average spin Hamiltonian to evaluate the density matrix of the spin during the first and the second halves of the decoupling period, respectively.

(24) Olejniczak, E. T.; Vega, S.; Griffin, R. G. *J. Chem. Phys.* **1984**, *81*, 4804−4817.

(25) Kolbert, A. C.; Raleigh, D. P.; Griffin, R. G. *J. Magn. Reson.* **1989**, *82*, 483−491.

(26) Frydman, L.; Frydman, B. *J. Chem. Phys.* **1990**, *92*, 1620.

$$H = \begin{cases} \sum_i \omega^{S}_{CS_i}(\Omega_i) S_{iz} + \sum_{i,j} \omega^{IS}_{D_{i,j}}(\Omega_{ij}) I_{iz} S_{jz} & \text{for } 0 \le t \le \dfrac{\pi}{\omega_1} \\ \sum_i \omega^{S}_{CS_i}(\Omega_i) S_{iz} - \sum_{i,j} \omega^{IS}_{D_{i,j}}(\Omega_{ij}) I_{iz} S_{jz} & \text{for } \dfrac{\pi}{\omega_1} \le t \le \dfrac{2\pi}{\omega_1} \end{cases} \quad (11)$$

The density matrix during the first half of the $n + 1$ decoupling cycle is then given by

$$\varrho[(n+1)\tau_c] = e^{0.5\hat{L}_2\tau_c}[\varrho[(n + {}^1/_2)\tau_c] - \hat{L}_2^{-1}\hat{X}\varrho_0] + \hat{L}_2^{-1}\hat{X}\varrho_0 \quad (12a)$$

and during the second half by

$$\varrho[(n + {}^1/_2)\tau_c] = e^{0.5\hat{L}_1\tau_c}[\varrho[(n\tau_c)] - \hat{L}_1^{-1}\hat{X}\varrho_0] + \hat{L}_1^{-1}\hat{X}\varrho_0 \quad (12b)$$

where $\hat{L}_1$ and $\hat{L}_2$ are super operators. In this method, the cross-correlation terms are already included in the evolution of the density matrix. However, the homonuclear dipolar coupling effects are not included in this calculation, and the model only approximately works for a 2-fold hopping process.

Conclusions

In this paper we have observed and calculated the ^2H quadrupole echo lineshapes expected for 3-fold hopping of an ammonium group. The Pake doublet observed in the slow exchange regime transforms to a triplet in the intermediate exchange region, and this in turn to a Pake spectrum of reduced width in the fast exchange limit. The shape of the intermediate exchange spectrum is due to the geometry of the methyl group and the two special orientations which occur with respect to the laboratory field. In addition to the temperature dependence of the lineshapes, we have observed spectral intensity dependencies and studied the anisotropy in the spin–lattice relaxation. We have also demonstrated that it is possible to observe the effects of hydrogen bonding on lineshapes at low temperatures and to include these effects in simulations. Simulations of all three sets of experimental data suggest that a 3-fold hopping model based on a single correlation time is an excellent representation of the dynamics of the $-NH_3^+$ group in L-alanine. The spectral lineshape simulations permit us to calculate an activation energy of 40.5 kJ/mol, in good agreement with previous measurements.

Using the deuterium results, kinetic rates at various temperatures can be calculated, and their effects on various decoupling schemes may be observed and simulated. For instance, the kinetic rate determined at room temperature provides an explanation for the broadening observed at the ammonium resonance in ^1H multiple pulse spectra of L-alanine.

In a more detailed analysis, we have observed the variation of the linewidth and intensity of the ^{15}N peak of the ammonium group in L-alanine with the sample temperature. In these spectra, the ^{15}N peak becomes broad as the sample temperature approaches −30 °C and then narrows as the temperature decreases further. This linewidth change arises from the interference between the decoupling field and the 3-fold hopping of the protons in the ammonium group. Using the hopping rates calculated based on the deuterium results, we can simulate the ^{15}N L-alanine spectra at various temperatures by solving the master equation of the density matrix in Liouville space, and the results agree well with experimental spectra. Various other approximate methods have also been considered, but cannot reproduce the experimental results. This is due to neglect of the homonuclear dipolar coupling between protons as well as the cross-relaxation effects. Further investigation of these effects are currently being done on the cyclic hexapeptide, GYGPLP, and on a metal complex, $\{W(\eta^5\text{-}C_5Me_5)Me_4\}^+$.

Acknowledgment. The authors thank L. Zheng, J. Griffiths, and K. V. Lakshmi for technical assistance and helpful discussions. J.R.L. was a recipient of a National Science Foundation Fellowship. This work was supported by the National Institutes of Health (GM-25505 and RR-00995).