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NMR Characterization of Partially Deuterated γ -Glycine

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Abstract

Mobility in γ -glycine can be measured by proton relaxation or line-shape analysis of wide-line ^2H spectra of deuterium-substituted γ -glycine. The temperature dependence of the wide-line ^2H spectra indicates an activation energy of 33 ± 2 kJ/mol, in agreement with the activation energy extracted from proton spin-lattice relaxation times. The proton spin-lattice relaxation time of the protons in partially deuterated γ -glycine is 78 sec, much longer than the 4-sec T_1 of the undeuterated material. The comparison demonstrates how effectively the amine protons act as a relaxation sink.

Key Words:

Glycine, deuteron line shape, proton relaxation, dynamics

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is an established technique for characterizing crystalline materials. In particular, NMR investigations of molecular motions in amino acids (such as glycine [1,2]) serve as model studies of the application of NMR spectroscopy for probing the dynamic and structural state of functional groups in larger biological molecules. The dynamic state is typically probed through proton relaxation measurements and/or line-shape analysis of deuterons substituted for protons.

As the simplest amino acid, glycine has been the subject of a wide range of investigations over the years, ranging from crystal structure determination [3-8] and characterization of crystal growth [9] to theoretical [10,11] studies and measurement of biochemical [12,13], spectroscopic [14-16], and physical properties [17,18], including attempts to observe it in interstellar space [19]. It is zwitterionic in the solid state and may exist as any of three polymorphs that result from different arrangements of the hydrogen bonding among molecules. The random thermal motion of the -NH_3^+ group, usually described as a free (or sometimes hindered) rotation about the $\text{C}_\alpha\text{-NH}_3$ bond, imparts the major contribution to the proton spin-lattice relaxation rate [1,2]. Due to the effects of structure on this random motion, the proton spin-lattice relaxation rate is characteristic of a particular polymorph.

Such a simple, well-characterized model system as glycine should be suitable for answering the question of whether or not replacing protons with deuterons in a chemical compound alters the motion. However, the difficulty in quantitatively answering this question for glycine from information already in the scientific literature arises from the disparate NMR methods used (e.g., whether the measurements were made on a static sample or with magic angle spinning), further complicated by which of the three polymorphs of glycine were used for the measurements. For example, as measured at ambient temperature at the same magnetic field strength, the different ^1H NMR spin-lattice relaxation times of the three polymorphs of glycine span more than two orders of magnitude [6].

In the present work, both ^1H and ^2H NMR measurements were made on the same polymorph. The amine group of γ -glycine was deuterated. The wide-line ^2H spectra were acquired as a function of temperature to investigate the motion of the -ND_3^+ . A previous study reported line-shape simulations [20] for the deuterium-substituted amine group of α -glycine to extract an activation energy for the motion of the -ND_3^+ . However, the reported model using C_{3v} symmetry (*i.e.*, equivalent deuterium sites) in the amine group fails to reproduce the observed ^2H quadrupolar asymmetry in the ambient temperature spectra in either the α or the γ polymorphs of glycine. The model used for the simulations and analyses in the present work has three inequivalent deuterium sites and produces simulations that better fit the experimental data. The temperature-dependent results were analyzed to obtain an activation energy for the motion of the deuterated amine group. This activation energy is compared with that obtained from ^1H NMR spin-lattice relaxation measurements on a static sample of fully protonated γ -glycine and with results from the literature [1,2]. In addition, the ^1H NMR spin-lattice relaxation (for the protonated α carbon) of a partially deuterated γ -glycine sample was measured to help elucidate the dominant proton relaxation mechanism.

Experimental

The ^1H NMR spin-lattice relaxation measurements and wide-line ^2H NMR data were acquired with a Bruker Avance 300 spectrometer operating at a ^1H frequency of 300.13 MHz and a ^2H frequency of 46.07 MHz. ^1H spin-lattice relaxation times were measured with an inversion-recovery sequence on a static sample. Wide-line ^2H data acquisition used a quadrupolar-echo sequence with phase cycling to suppress unwanted single- and double-quantum coherence-transfer artifacts [21]. A pulse spacing of 50 μs with a recycle delay of 1 s was used in the quadrupolar-echo sequence. With the 5-mm sample coil, the spectrometer produced 2.5- μs ^2H $\pi/2$ pulses. Solid glass rods inserted in the sample tube confined the sample to the volume of the coil. The sample temperatures were calibrated with ^{207}Pb NMR of a static lead nitrate sample, as described by Beckmann and Dybowski [22]. The γ polymorphic forms of both the fully protonated and partially deuterated polycrystalline glycine samples were confirmed with ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR and powder x-ray diffraction [16].

Results and Discussion

The wide-line ^2H quadrupolar-echo spectrum for the deuterium-substituted amine group of a polycrystalline powder sample of γ -glycine at 296 K is shown in Figure 1A. Spectral simulations of these data are also shown in Figures 1B and 1C.

Figure 1B shows the simulated spectrum for a static, randomly-oriented polycrystalline powder of spin-1 nuclei subject to the quadrupolar interaction [23]. The fit parameters yield a motionally averaged (as a result of the rapid reorientations of the $-\text{ND}_3^+$ groups at ambient temperature) quadrupolar coupling constant (QCC) of 52 ± 1.5 kHz and an asymmetry parameter of 0.274 ± 0.05 . These results for polycrystalline γ -glycine are similar to the QCC of 50.8 kHz and asymmetry parameter of 0.286 obtained by Haeberlen and co-workers [24] for a single crystal of α -glycine (although the crystal packing in the lattice is different for the two polymorphs).

The ^2H simulation of Figure 1C is from a Fortran program written by Shin-ichi Nishikiori [25] based on the principles outlined by Vega and Luz [26]. This simulation program allows analysis of intermediate-exchange spectra in terms of specific motional models (*vide infra*). The particular simulation shown here is in the fast-exchange limit ($k = 1 \times 10^8 \text{ s}^{-1}$).

The ambient-temperature ^2H spectrum of γ -glycine, as well as that of α -glycine, indicates sizable asymmetry parameters of *ca.* 0.28 even in the presence of fast motional averaging at ambient temperature. Fast three-fold rotation of the $-\text{ND}_3^+$ about the $\text{C}_\alpha\text{-ND}_3$ bond (or even fast hopping among three equivalent sites with C_{3v} symmetry) results in an average asymmetry of $\eta = 0$. The fact that the observed asymmetry at ambient temperature is nonzero gives rise to the question: how can this material exhibit a non-zero asymmetry?

The temperature dependence of the wide-line ^2H quadrupolar-echo spectrum of γ -glycine is shown in Figure 2A. At high temperatures, the spectrum remains essentially unchanged over the range from 258 K to 380 K. Below 258 K, the spectra become temperature-dependent, due to the fact that the molecular motion is no longer in the fast-

exchange limit for all molecules. It should be noted that all spectra in Figure 2A were acquired under identical conditions, with the exception of the temperature, and are plotted with the same vertical scale. The loss of intensity in the spectra is due to the incomplete refocusing of the ^2H magnetization with the quadrupolar-echo pulse sequence as the molecular motion slows from the fast-exchange limit.

At the lowest temperature achievable with this instrument (183 K), the mobility is such that the spectrum displays characteristics of the intermediate-motion regime. A similar temperature range is also reported for the experimental data for α -glycine [20], in which the spectral simulations due to the results of the molecular motion used a simple model of C_{3v} symmetry with a QCC of 152 kHz and an asymmetry η of 0.005 for the static situation. However, simulation using such a model with a single QCC (resulting in three equivalent sites) in the fast-exchange limit fails to reproduce the observed asymmetry in the experimental spectrum at ambient temperature of either polymorph.

The structure of γ -glycine determined from neutron diffraction is described [8] as "consisting of two hydrogen bonds ... to form helices around the crystallographic 3_2 screw axes. A third lateral hydrogen bond ... connects the helices, thus forming a three-dimensional network." The N-H bond distances at 83 K are 0.1052, 0.1044, and 0.1037 nanometers, with N...O distances of 0.2793, 0.2781, and 0.2942 nanometers, respectively. At 298 K the N-H bond distances are 0.1053, 0.1040, and 0.1034 nanometers, with N...O distances of 0.2809, 0.2809, and 0.2987 nanometers. In other words, the three sites are inequivalent. The inequivalence of the deuterons is also manifest in the nuclear quadrupole resonance (NQR) frequencies of γ -glycine. In the NQR study by Edmonds and Summers [27], the measurements were made at 77 K to freeze out the molecular motion of the amine group. Working at this temperature yielded deuterium QCCs of 145.3, 159.0, and 174.7 kHz and asymmetries of 0.055, 0.044, and 0.034, respectively, for the three deuterons of the $-\text{ND}_3^+$ in the static case. In an earlier NQR study of α -glycine, Hunt and McKay [28] calculated the motionally-averaged QCC and asymmetry that would be observed at sufficiently high temperatures in which " ND_3 groups in amino acids rotate rapidly about the N-C bond, so that each deuterium nucleus spends an equal amount of time in each of the three 'static' positions." For this calculation, they made the further assumptions that all asymmetries are 0, the principal axes are along the N-D bonds, and there is tetrahedral symmetry. Invoking these assumptions for a rotating $-\text{ND}_3^+$ group yields a calculated QCC of 54.2 kHz and an asymmetry of 0.314 for α -glycine. These calculations are quite similar to the actual QCC of 50.8 kHz and asymmetry parameter of 0.286 measured by Haberlen and co-workers [24] for α -glycine.

Figure 2B shows the simulated spectra in the intermediate-exchange regime for a model allowing hopping among three inequivalent sites at the corners of an equilateral triangle. These simulations correspond to the experimental spectra of Figure 2A and account for the loss of intensity in the spectra due to the incomplete refocusing of the ^2H magnetization with the quadrupolar-echo pulse sequence in this intermediate-motion regime. A difficulty arises in simulating the spectra without knowledge of QCC and η for the static situation. Since experimental data could not be obtained at a temperature low enough to be in the slow-exchange limit, the static QCCs and asymmetries for the three inequivalent sites obtained from nuclear quadrupole resonance spectra [27] at liquid-nitrogen temperatures were used for the simulations. Only the jump rate, k , is varied to fit the experimental data with regard to both line-shape and intensity. Figure 3

shows the natural logarithm of the jump rate extracted from the simulations as a function of inverse temperature. Presuming an Arrhenius-like behavior for the jump rate,

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \quad (1)$$

where E_a is the activation energy, R is 8.31451 joule/K-mol, and T is the temperature, a plot of the natural logarithm of the jump rate versus the inverse temperature yields a linear fit whose slope is equal to $\frac{-E_a}{R}$. The slope of the straight line yields a measured activation energy of 33 ± 2 kJ/mole.

^1H NMR spin-lattice relaxation results for glycine have been reported in the literature. Recasting the well-known Kubo-Tomita theory [29] for spin-lattice relaxation of spin- $\frac{1}{2}$ nuclei experiencing dipolar interactions influenced by random reorientations of either the molecules or by reorientations of molecular groups, Andrew *et al.* [1] gave the following equation for the spin-lattice relaxation rate:

$$\frac{1}{T_1} = C\tau_c \left[\frac{1}{1 + (\omega\tau_c)^2} + \frac{4}{1 + (2\omega\tau_c)^2} \right]. \quad (2)$$

Using an Arrhenius-like behavior for the jump time,

$$\tau_c = \tau_0 \exp\left(\frac{E_a}{RT}\right) \quad (3)$$

the temperature dependence of the proton spin-lattice relaxation rate could be fit to the Kubo-Tomita form with an activation energy of 28 ± 1 kJ/mole for glycine. However, the polymorph of glycine used in the study was not specified.

This highlights a difficulty regarding glycine in the scientific literature. In this study by Andrew *et al.* [1], the polymorph was not specified. While a previous proton spin-lattice relaxation study [30] with a reported activation energy of 29 kJ/mole for α -glycine suggests that the polymorph was α -glycine, Gu *et al.* [2] have measured the ^1H T_1 s for both α -glycine and γ -glycine under magic-angle-spinning conditions with a standard cross-polarization inverse-recovery pulse sequence. Their results were also analyzed with the same Kubo-Tomita model and yielded values for the activation energies of 23 ± 2 kJ/mole for α -glycine and 30 ± 3 kJ/mole for γ -glycine. These results suggest that the polymorph used by Andrew *et al.* [1] was γ -glycine, in contrast to the previous study [30]. This discrepancy may arise from whether the proton spin-lattice relaxation measurements were made on a static sample or with magic angle spinning. The results for γ -glycine from Gu *et al.* [2] compare favorably with the 33 ± 2 kJ/mole obtained for the activation energy from the wide-line ^2H measurements from a static sample given in the current work.

Gu *et al.* also point out that the constant C of equation 2 has been approximated using the "rather unrealistic assumption" that the molecule is "non-associated, *i.e.*, ignoring the

effect of additional couplings to nearby molecules." All three polymorphs of glycine exhibit hydrogen bonding to neighboring molecules. Despite this concern, the use of the Kubo-Tomita model to glycine data has typically been justified simply on the basis of how well it fit the experimental data.

The general relaxation model is based on the existence of rapid, random reorientational motions that produce fluctuating magnetic fields that induce relaxation of the amine protons. The relaxation of other nuclei occurs by spin diffusion. The fully protonated γ -glycine has a ^1H spin-lattice relaxation time of 4 s at ambient temperature, as measured at 7 Tesla. The activation energy as measured from the ^1H spin-lattice relaxation results from a static sample as a function of temperature is also 33 ± 2 kJ/mole. These results, when compared with those from the ^2H measurements, indicate that the replacement of protons with deuterons does not alter the molecular motion in γ -glycine.

The importance of the contribution of the random rotational motion of the $-\text{NH}_3^+$ to ^1H spin-lattice relaxation can be demonstrated by measuring the relaxation rate of the protons in a sample in which the amine group is deuterated. The relaxation time at ambient temperature for the methylene protons in amine-deuterated glycine is 78 s. The comparison with the much-shorter relaxation time of the fully protonated material supports the model that random molecular reorientation provides the dominant relaxation pathway. Such experimental evidence supports the use of the Kubo-Tomita model for γ -glycine in spite of concerns regarding interactions with neighboring molecules.

Similar behavior is observed [24] for the ^2H spin-lattice relaxation in a single crystal of perdeuterated α -glycine. However, the small magnetogyric ratio of the deuterium significantly reduces interaction among the deuterium nuclei by spin diffusion. The relaxation times were found to depend on orientation of the crystal axes with respect to the magnetic field, with the ^2H T_1 at room temperature of $-\text{ND}_3^+$ varying between 2.5 and 3.5 ms. The methylene ^2H T_1 also varied with orientation and could be as long as 210 s.

The importance of the contribution of the random rotational motion of the $-\text{NH}_3^+$ to the spin-lattice relaxation of nuclei other than protons in γ -glycine is also evident. The ^{15}N T_1 of the fully protonated γ -glycine at ambient temperature is only 150 ms, unexpectedly short for a rare spin nucleus with a small magnetogyric ratio in a highly crystalline solid. Upon deuteration of the amine group, this lengthens to 600 ms at ambient temperature. The increase of the spin-lattice relaxation time upon deuteration is also observed for carbon atoms in γ -glycine. The ^{13}C carbonyl T_1 increases from 55 s in the fully-protonated compound to 220 s in the partially deuterated compound. Likewise, the methylene T_1 increases from 22 s to 110 s.

Understanding segmental motion within molecules and its influence on spin dynamics is important in extracting structural information from NMR experiments. The dynamics of fully protonated and partially deuterated α -glycine have been previously studied [31] to determine ^1H - ^1H internuclear distances. An analysis of the proton spin dynamics showed how spin diffusion occurring through the homonuclear dipolar interaction within α -glycine can provide a single proton spin-lattice relaxation rate at a single temperature. It thus provides justification for the application of the Kubo-Tomita theory [29] to the proton spin-lattice relaxation data as a function of temperature. The quantitative analyses in the present study for the motion of the $-\text{NH}_3^+$ and $-\text{ND}_3^+$ groups in the fully protonated and partially deuterated molecules respectively provide further experimental support to the assumed dynamics of that previous study [31]. In particular, the activation energies

for these groups are really quite large so these are strongly hindered rotors. The dynamics are expected to be "classical" rather than "quantum" in character. In agreement with experiment, the dynamics are not expected to be strongly affected by deuteration, at least within the resolution of the spectral line-shape measurements.

Finally, the issue of sample preparation must be addressed. In the initial attempt to deuterate the -NH_3^+ group, glycine was dissolved in 98% D_2O and allowed to recrystallize undisturbed through slow evaporation. This procedure was repeated twice. While a wide-line ^2H spectrum was obtained from the resulting sample, integration of a solid-state ^1H CRAMPS [32] spectrum of the sample indicated, within the accuracy of the integration, no deuterium substitution at all! The amine-to-methylene integration ratio was 3:2, and the ^1H T_1 of the sample remained at 4 s, unchanged from that of the fully protonated sample. No changes were observed in the ^1H - ^{13}C variable-contact-time experiment. Successful substitution, as confirmed by complete loss of the amine protons from the solid-state ^1H CRAMPS spectrum, came only from repeated dissolution in 99% D_2O , freezing, and lyophilization to dryness.

Summary

^1H spin-lattice relaxation measurements of fully protonated γ -glycine yield the same activation energy within experimental error for the amine group rotation as line-shape analysis of wide-line ^2H spectra of deuterium-substituted γ -glycine. The temperature dependence of the wide-line ^2H spectra is well simulated by invoking a rotational random-jump model of the -ND_3^+ among three inequivalent sites, with an Arrhenius-like variation of the jump rate with temperature. Proton spin-lattice relaxation measurements on fully protonated γ -glycine give a T_1 of 4 sec. at ambient temperature. The proton spin-lattice relaxation time of the protons in partially deuterated γ -glycine is much longer, at 78 sec. This comparison demonstrates how effectively the amine protons act as a relaxation sink. In sample preparation, simple recrystallization from D_2O solution failed to provide complete deuteration of the amine group. Lyophilization after dissolution in D_2O was required for complete substitution.

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Figure 1. The wide-line ^2H NMR spectrum (A) of the deuterium-substituted amine group of γ -glycine is compared with simulations of (B) a static polycrystalline spin-1 quadrupolar interaction with a QCC of 52 kHz and an asymmetry parameter of 0.274 and (C) the fast exchange regime of a random-jump model with three inequivalent sites as described in the text.

Figure 2. Comparison of (A) the observed wide-line ^2H spectra (top) at 258 K, 240 K, 224 K, 207 K, 197 K and 183 K with the simulated spectra (B) having jump rates of 5.0×10^7 , 1.3×10^7 , 3.3×10^6 , 1.2×10^6 , 4.0×10^5 , and $7 \times 10^4 \text{ s}^{-1}$.

Figure 3. The natural logarithm of the jump rate versus inverse temperature of glycine-ND₃.