Two-Dimensional ^{17}O Multiple Quantum Magic-Angle Spinning NMR of Organic Solids

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Abstract: We report two-dimensional (2D) ^{17}O multiple-quantum magic-angle spinning (MQMAS) NMR spectra for four ^{17}O-labeled organic compounds: [$^{17}O_2$]-D-alanine (**1**), potassium hydrogen [$^{17}O_4$]dibenzoate (**2**), [$^{17}O_4$]-D,L-glutamic acid·HCl (**3**) and [2,4-$^{17}O_2$]uracil (**4**). The high spectral resolution observed in the 2D ^{17}O MQMAS NMR spectra allows extraction of precise ^{17}O NMR parameters for all crystallographically distinct oxygen sites. We demonstrate that rotor synchronization is important in obtaining high-quality ^{17}O MQMAS spectra for organic compounds. Several issues related to the potential of ^{17}O MQMAS NMR for large biomolecular systems are also discussed.

Introduction

Multinuclear nuclear magnetic resonance (NMR) spectroscopy is an important technique for studying molecular structure and dynamics of chemical and biological systems. Most successful NMR studies have been based on observation of ^{1}H, ^{13}C, and ^{15}N nuclei. Although oxygen is also an abundant element in organic and biological molecules, ^{17}O NMR studies are not common.[1] The scarcity of ^{17}O NMR studies arises from the fact that ^{17}O is a quadrupolar nucleus (spin $^{5}/_{2}$) with a very low natural abundance (0.037%). The major obstacle of obtaining NMR spectra for quadrupolar nuclei is the large size of the nuclear quadrupole interaction (typically of the order of 10^6 Hz). In addition, the unique nature of nuclear quadrupole interactions also makes it difficult to record NMR spectra with sufficient spectral resolution to resolve chemically or crystallographically different sites. For these reasons, the development of ^{17}O NMR spectroscopy has been quite slow in the past 50 years.

Solid-state ^{17}O NMR studies of organic compounds were pioneered by several groups in the past decade. In particular, Fiat and co-workers attempted to record solid-state ^{17}O NMR signals for crystalline amino acids.[2] Oldfield and co-workers applied solid-state ^{17}O NMR to probe hemoproteins and model compounds.[3] Ando and co-workers used solid-state ^{17}O NMR to study H-bonding interactions in polypeptides.[4] In addition, two single-crystal ^{17}O NMR studies were also reported dealing with organic molecules.[5,6] As relatively high magnetic fields are becoming available, there has been a renewed effort in exploring the potential of solid-state ^{17}O NMR in the study of organic and biological compounds.[7−11] Recent work from this laboratory has expanded considerably the realm of solid-state ^{17}O NMR for organic compounds.[12−18] We were particularly interested in the effect of intermolecular hydrogen bonding interactions on ^{17}O NMR tensors. In addition to the experimental characterization of ^{17}O NMR tensors in important functional groups, we have also performed extensive quantum chemical calculations on ^{17}O NMR properties and established a basis for further understanding ^{17}O NMR parameters.

One major limitation of the aforementioned solid-state ^{17}O NMR studies is that spectral analyses were based exclusively on observation of "low-resolution" NMR spectra obtained for either magic-angle spinning (MAS) or stationary samples. This has made it very difficult to study chemical systems with more than one type of oxygen atoms. The intrinsic difficulty of obtaining high-resolution NMR spectra for ^{17}O arises from the fact that the second-order quadrupole interaction cannot be completely averaged by the traditional MAS technique. Although new techniques such as dynamic angle spinning (DAS)[19] and double rotation (DOR)[20] were developed for achieving high-resolution for half-integer quadrupolar nuclei including ^{17}O,

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these techniques are difficult to apply to organic compounds for various reasons. To our knowledge, there has been only one preliminary ^{17}O DAS NMR study where an organic compound was examined.[21] In 1995, Frydman and co-workers[22,23] introduced a new approach known as the multiple-quantum magic-angle spinning (MQMAS) method for completely averaging second-order quadrupole interactions. This new technique immediately attracted considerable attention because it can be readily implemented on most commercial NMR spectrometers. Over the past several years, the MQMAS method has been applied to a large number of chemical systems containing common half-integer quadrupolar nuclei. The first ^{17}O MQMAS study was demonstrated by Wu et al.[24] Subsequently, several research groups have reported ^{17}O MQMAS studies for many inorganic systems where ^{17}O quadrupole coupling constants are reasonably small.[25−32] However, ^{17}O MQMAS NMR for organic compounds is still an unexplored area. In this contribution, we report for the first time ^{17}O MQMAS NMR results for four ^{17}O-enriched organic compounds, [$^{17}O_2$]-D-alanine (**1**), potassium hydrogen [$^{17}O_4$]dibenzoate (**2**), [$^{17}O_4$]-D,L-glutamic acid·HCl (**3**), and [2,4-$^{17}O_2$]uracil (**4**). The primary objective of the present study is to evaluate the feasibility of ^{17}O MQMAS NMR for studying organic compounds at a moderate field strength (11.75 T) and with a reasonable ^{17}O enrichment level (ca. 40%). In addition, this study will attempt to address the question as to whether the sensitivity of the ^{17}O MQMAS experiment is suitable for studying biological systems.

Experimental Section

Sample Synthesis. [$^{17}O_2$]-D-Alanine, [$^{17}O_4$]-D,L-glutamic acid·HCl, and [2,4-^{17}O]uracil were prepared by acid-catalyzed exchange with $H_2{}^{17}O$ (40.9 atom % ^{17}O, LOT No. IM1378-14, purchased from ISOTEC Inc., Miamisburg, OH) following the literature procedures.[33−35] For example, 150 mg of D,L-glutamic acid monohydrate was dissolved in 0.45 mL of $H_2{}^{17}O$ in a 1.0 mL glass vial. The solution was saturated with dry HCl gas, sealed, and then heated to 95−100 °C for approximately 12 h. It was noted that heating over approximately 9 days was required to exchange both O2 and O4 of uracil. Upon completion of a reaction, excessive $H_2{}^{17}O$ was recovered using a high vacuum line. Potassium hydrogen [$^{17}O_4$]dibenzoate was prepared as described previously.[12] All products were confirmed by ^1H and ^{13}C NMR. The exact level of ^{17}O enrichment in compounds **1**−**4** was not determined in this study; however, previous studies[12,34,35] have yielded estimates for the ^{17}O enrichment level in these systems: **1** (40%), **2** (50.8%), **3** (40%), and **4** (14% at O2 and 24% at O4).

Solution ^{17}O NMR. All solution ^{17}O NMR experiments were performed on Bruker Avance-400 and Avance-500 NMR spectrometers with 5-mm broadband probes. Chemical shifts were referenced to an external sample of H_2O. The solution ^{17}O NMR spectrum of [$^{17}O_2$]-D-alanine exhibits a single peak at 257 ppm at acidic pH values. KH [$^{17}O_4$]dibenzoate in aqueous solution shows a single peak at 253 ppm. The solution ^{17}O NMR spectrum of [$^{17}O_4$]-D,L-glutamic acid·HCl exhibits two peaks at 251.0 and 263.5 ppm which can be assigned to the α-COOH and γ-COOH groups, respectively. These values are in agreement with previous solution ^{17}O NMR studies.[33] The ^{17}O NMR spectrum of [2,4-^{17}O]uracil in DMSO exhibits two peaks at 329 and 247 ppm with a relative intensity ratio of 1 (O4) to 0.61 (O2). This is in good agreement with the literature values reported by Fiat and co-workers.[35]

Solid-State ^{17}O NMR. All solid-state ^{17}O NMR spectra were recorded on a Bruker Avance-500 spectrometer operating at 500.13 and 67.78 MHz for ^1H and ^{17}O nuclei, respectively. Polycrystalline samples were packed into zirconium oxide rotors (4 mm o.d.). A Bruker 4-mm MAS probe was used for ^{17}O MAS and MQMAS experiments. The sample spinning frequency was controlled to be 14 500 ± 4 Hz. The z-filter pulse sequence proposed by Amoureux et al.[36] was used: P1(ϕ1)−t_1−P2(ϕ2)−τ−P3(ϕ3)−ACQ(t_2,ϕ4) where $\phi1 = (0°)$, $\phi2 = (0, 0, 60, 60, 120, 120, 180, 180, 240, 240, 300, 300°)$, $\phi3 = (0, 180°)$, $\phi4 = (0, 180, 180, 0°)$, and $\tau = 20$ μs. The optimized excitation (P1) and conversion (P2) pulse widths were 5.5 and 2.0 μs, respectively. The radio frequency field strength at the ^{17}O frequency was approximately 80−90 kHz. The pulse width of the selective ^{17}O 90° pulse (P3) was 27 μs. The hypercomplex data method[37] was used for obtaining pure-phase 2D spectra. An external sample of H_2O (20% ^{17}O atom) was used for RF calibration and chemical shift referencing. Other experimental details are given in the figure captions.

Results and Discussion

[$^{17}O_2$]-D-Alanine. Figure 1 shows the 2D ^{17}O MQMAS spectra of compound **1** obtained without and with rotor synchronization for the t_1 increments. Because the 2D spectrum shown in Figure 1A was obtained without synchronizing the t_1 increment with the rotor period, strong spinning sidebands are present along the F_1 dimension. The advantage of this approach is that the F_1 spectral width is not limited by the sample spinning frequency. However, the presence of F_1 spinning sidebands in 2D spectra often results in low peak intensities. In contrast, the 2D spectrum shown in Figure 2B was obtained with the t_1 increment being synchronized with the rotor period, T_R (i.e., $\Delta t_1 = T_R$). Under such circumstances, the spectral width along the F_1 dimension is equal to the spinning frequency and consequently all F_1 spinning sidebands are folded onto the central bands. As previously demonstrated by Massiot,[38] the advantage of t_1 rotor synchronization is that this approach enhances the overall sensitivity of MQMAS experiments, especially for sites associated with large quadrupole coupling constants. Because the origin of F_1 spinning sidebands in 2D MQMAS spectra is quite complex,[39,40] it is often desirable to obtain sideband-free MQMAS spectra. On the other hand, as

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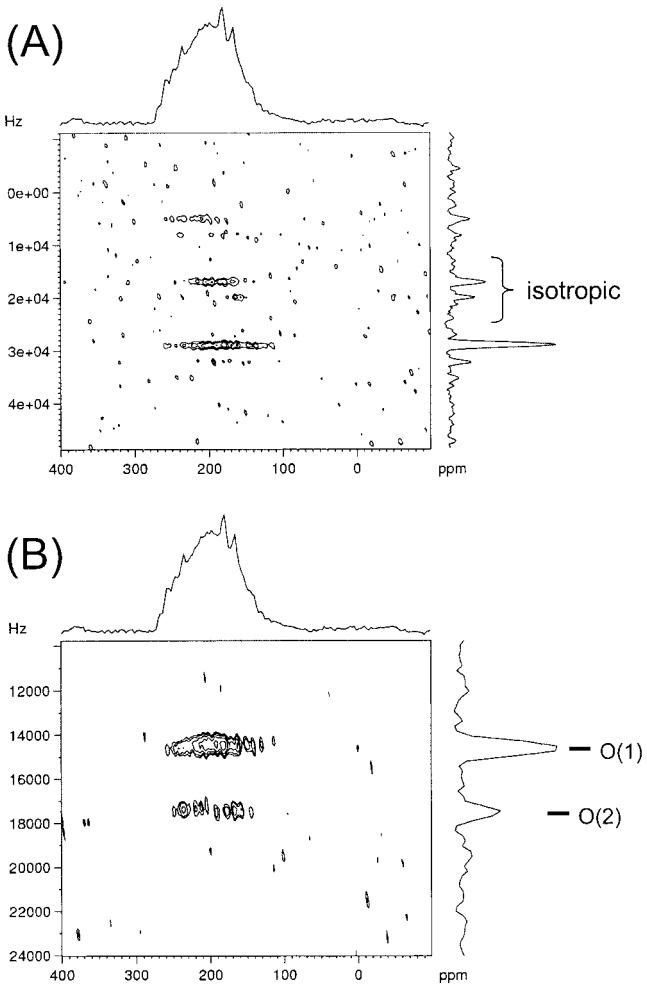

Figure 1. Contour plot of ^{17}O MQMAS spectra of [$^{17}O_2$]-D-alanine obtained without (A) and with (B) rotor synchronization. Corresponding 1D MAS spectra and the F_1 projections of the 2D spectra are shown at the top and on the side, respectively. The sample spinning frequency was 14.5 kHz. The t_1 increment was 16.67 and 68.97 μs for parts A and B, respectively. The number of t_1 increments was 84 and 42 for parts A and B, respectively. In both parts A and B, a total of 240 transients were accumulated for each t_1 increment with a recycle delay of 10 s.

the sample spinning frequency on modern NMR spectrometers can be as high as 35 kHz, it is usually not difficult to achieve a sufficiently large spectral width along the F_1 dimension. Comparison of the two NMR spectra shown in Figure 1 confirms that rotor synchronization can indeed lead to a dramatic sensitivity enhancement. In the remainder of this contribution, we will focus only on rotor-synchronized MQMAS experiments.

As also seen from Figure 1B, two isotropic peaks are observed in the ^{17}O MQMAS spectrum of compound **1**, indicating that the two oxygen atoms of the alanine molecule experience different chemical environments. Furthermore, each isotropic peak in the F_1 dimension is related to a sub-spectrum along the F_2 dimension, from which information about isotropic chemical shift (δ_{iso}), quadrupole coupling constant ($C_Q = e^2qQ/h$), and asymmetry parameter (η) can be estimated. However, it should be emphasized that, because F_2 sub-spectra often exhibit line shape distortion and do not contain quantitative information about peak intensities, they were used only as a starting point and restraints in the simulations of 1D MAS spectra. Final NMR parameter extraction was achieved by comparing spectral features (peaks and shoulders) in experimental and simulated 1D MAS spectra. Such a combined analysis of the 2D MQMAS

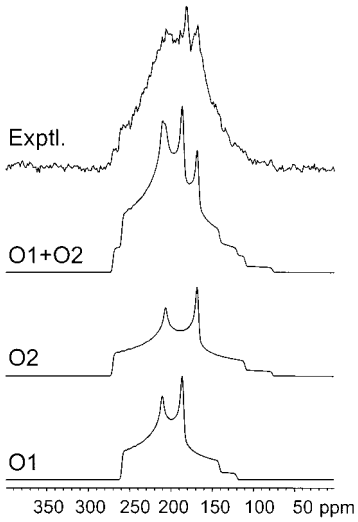

Figure 2. Experimental and simulated 1D ^{17}O MAS spectra of [$^{17}O_2$]-D-alanine. The simulated total spectrum (O1+O2) is a sum of O1 and O2 sub-spectra with a 1:1 intensity ratio. The sample spinning frequency was 14.5 kHz. A total of 512 transients were collected with a recycle time of 10 s.

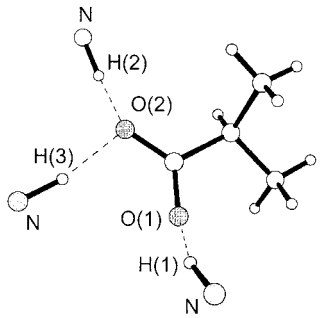

Figure 3. Molecular structure and atomic numbering for L-analine.[41] The relevant H-bond distances and angles are the following: N−H(1)···O(1), 2.853 Å, 160.9°; N−H(2)···O(2), 2.813 Å, 168.1°; N−H(3)···O(2), 2.832 Å, 163.7°.

and 1D MAS spectra yielded the following parameters for compound **1**: O1, $\delta_{iso} = 275 \pm 5$ ppm, $C_Q = 7.60 \pm 0.02$ MHz, $\eta = 0.60 \pm 0.01$; O2, $\delta_{iso} = 262 \pm 5$ ppm, $C_Q = 6.40 \pm 0.02$ MHz, $\eta = 0.65 \pm 0.01$. The observed and simulated 1D ^{17}O MAS spectra of compound **1** are shown in Figure 2. Spectral assignment can be readily achieved on the basis of the crystal structure of L-alanine.[41] As seen in Figure 3, the alanine molecule exists in its zwitterionic form and the two oxygen atoms have quite different H-bonding environments. In particular, O1 is involved in only one C═O···H−N hydrogen bond, but O2 is involved in two hydrogen bonds. The stronger H-bonding environment at O2 also results in a small but significant lengthening of the C═O(2) bond length, 1.258 Å, in comparison with C═O(1), 1.242 Å. It has been previously established that the values of both ^{17}O isotropic chemical shift and quadrupole coupling constant decrease with increasing H-bonding strength.[12−16,42] On the basis of this general trend and the aforementioned structural difference, it is straightforward to assign O1 to the oxygen atom with larger values of δ_{iso} and C_Q. It is also noted that our ^{17}O NMR parameters for compound **1** are in reasonably good agreement with the data reported by Gann et al.[21] from an analysis of ^{17}O DAS spectra. However, the MQMAS approach is clearly easier to implement than the

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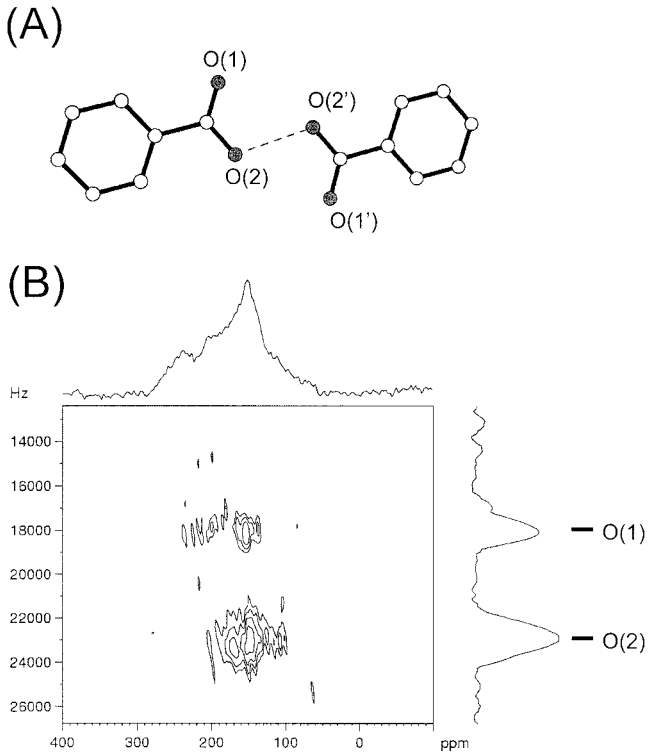

Figure 4. (A) Molecular structure and atomic numbering of potassium hydrogen dibenzoate.[43] Hydrogen atoms are not reported in the X-ray study. The O(2)···O(2′) distance is 2.51 ± 0.04 Å. (B) Contour plot of the ^{17}O MQMAS spectrum of potassium hydrogen [$^{17}O_4$]dibenzoate. Corresponding 1D MAS spectrum and the F_1 projection of the 2D spectrum are shown at the top and on the side, respectively. A total of 240 transients were accumulated for each t_1 increment with a recycle delay of 4 s. A total of 88 t_1 increments were collected.

DAS method and has also produced more accurate NMR results for this compound.

Potassium Hydrogen [$^{17}O_4$]Dibenzoate. The crystal structure of KH dibenzoate was reported by Skinner et al.[43] in an early X-ray diffraction study. As illustrated in Figure 4A, the two benzoate groups are related by an inversion center of symmetry. Although the hydrogen atom positions were not determined in the early X-ray study, the proton must lie on the midpoint between O(2) and O(2′) required by the crystallographic symmetry. Therefore, the O(2)···H···O(2′) hydrogen bond is symmetric with the distance between O(2) and O(2′) being 2.51 ± 0.04 Å.

As shown in Figure 4B, the 2D ^{17}O MQMAS spectrum of compound **2** exhibits two isotropic peaks in the F_1 dimension. A combined analysis of 2D MQMAS and 1D MAS spectra yielded the following ^{17}O NMR parameters for compound **2**: O1, $\delta_{iso} = 285 \pm 5$ ppm, $C_Q = 8.30 \pm 0.02$ MHz, $\eta = 0.15 \pm 0.01$; O2, $\delta_{iso} = 215 \pm 5$ ppm, $C_Q = 5.90 \pm 0.02$ MHz, $\eta = 0.90 \pm 0.01$. The observed and simulated 1D ^{17}O MAS NMR spectra are presented in Figure 5. Again, the above spectral assignment was based upon the H-bonding difference between O1 and O2. The ^{17}O quadrupole parameters determined for O2 are similar to those found in potassium hydrogen diacetate ($C_Q = 5.960$ MHz, $\eta = 0.980$)[44] and in potassium hydrogen diformate ($C_Q = 5.462$ MHz, $\eta = 0.833$). In these latter compounds, the O···O distance, 2.44 Å,[45] is similar to that in compound **2**. It is, however, noted that our data for compound

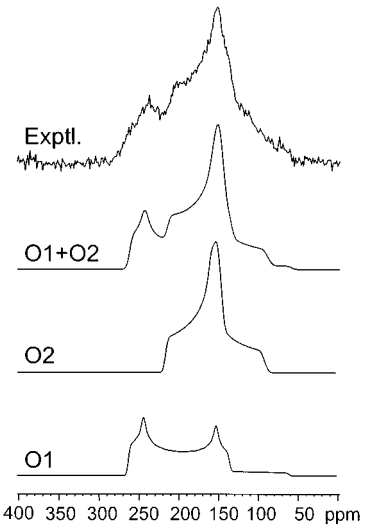

Figure 5. Experimental and simulated 1D ^{17}O MAS spectra of potassium hydrogen [$^{17}O_4$]dibenzoate. The simulated total spectrum (O1+O2) is a sum of O1 and O2 sub-spectra with a 1:1 intensity ratio. The sample spinning frequency was 14.5 kHz. A total of 128 transients were collected with a recycle time of 10 s.

2 are somewhat different from those determined for the same compound by Poplett et al.[46] in a nuclear quadrupole resonance (NQR) study ($C_Q = 6.165$ MHz, $\eta = 0.591$). Nevertheless, it is also well-known that determination of η was not accurate from NQR studies. In addition, Poplett et al.[46] were unable to determine the ^{17}O quadrupole parameters for O1, due to the lack of ^{1}H−^{17}O interactions. From this example, it is quite clear that solid-state ^{17}O NMR is more advantageous than NQR in yielding detailed information for all oxygen sites.

It is also interesting to note from Figure 4B that the isotropic peak for O2 is broader than that for O1. This is due to the strong ^{1}H−^{17}O dipolar interaction at the O2 position. Because heteronuclear ^{1}H−^{17}O dipolar interactions are amplified three times in a 3QMAS experiment, large dipolar interactions are difficult to completely remove by continuous-wave (CW) high-power proton decoupling. We recently demonstrated that insufficient CW proton decoupling can result in additional broadening or even doubling of the isotropic peaks in ^{17}O MQMAS NMR spectra.[47]

[$^{17}O_4$]-D,L-Glutamic Acid·HCl. Figure 6 shows the molecular structure and the 2D ^{17}O MQMAS spectrum of compound **3**. In the crystal lattice, both α-COOH and γ-COOH groups are in free acid form and all four oxygen atoms are crystallographically distinct.[48] Each oxygen atom of the molecule is also involved in H-bonding. In particular, O1 and O3 are involved in hydrogen bonds of the type O−H···O as donor and acceptor, respectively. O2 forms a hydrogen bond from one of the amino hydrogen atoms. O4 is associated with an O−H···Cl$^-$ hydrogen bond, which is quite different from other three-hydrogen bonds. As seen from Figure 6B, the ^{17}O MQMAS spectrum of compound **3** exhibits well-resolved isotropic peaks. Compared with the complex 1D ^{17}O MAS line shape observed for compound **3**, the resolving power of the 2D ^{17}O MQMAS approach is striking. However, only three isotropic peaks are observed in Figure 6B. Careful comparison between experimental and simulated 1D MAS spectra revealed that the sub-

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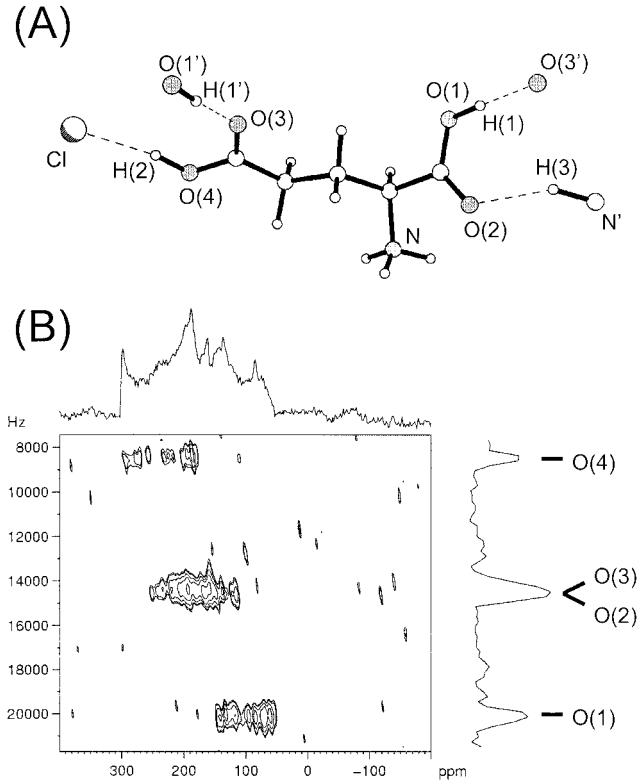

Figure 6. (A) Molecular structure and atomic numbering for L-glutamic acid·HCl.[49] The relevant H-bond distances and angles are the following: O(1)−H(1)···O(3′), 2.637 Å, 176.0°; N−H(2)···O(2), 2.904 Å, 158.6°; O(4)−H(2)···Cl, 3.043 Å, 173.4°. (B) Contour plot of the ^{17}O MQMAS spectrum of [^{17}O$_4$]-D,L-glutamic acid·HCl. Corresponding 1D MAS spectrum and F_1 projection of the 2D spectrum are shown on the top and on the side, respectively. A total of 960 transients were accumulated for each t_1 increment with a recycle delay of 5 s. A total of 28 t_1 increments were collected.

spectrum associated with the central isotropic peak must be twice as intense to achieve best agreement. This indicates that the ^{17}O NMR signals from two oxygen sites overlap severely. On the basis of this procedure, we obtained the following ^{17}O NMR parameters for compound **3**: O1, $\delta_{iso} = 170 \pm 5$ ppm, $C_Q = 7.20 \pm 0.02$ MHz, $\eta = 0.20 \pm 0.01$; O2 and O3, $\delta_{iso} = 250 \pm 5$ ppm, $C_Q = 6.80 \pm 0.02$ MHz, $\eta = 0.58 \pm 0.01$; O4, $\delta_{iso} = 320 \pm 5$ ppm, $C_Q = 8.20 \pm 0.02$ MHz, $\eta = 0.00 \pm 0.01$. The experimental and simulated 1D ^{17}O MAS spectra of compound **3** are shown in Figure 7. The observed ^{17}O NMR parameters for the two carbonyl oxygen atoms, O2 and O3, are essentially identical. It is noted that the C−O(2) and C−O(3) bond distances are practically the same, 1.221 and 1.225 Å, despite the different H-bonding environments. In contrast, the two C−O−H groups, O1 and O4, exhibit remarkably different ^{17}O NMR parameters. In particular, the value of C_Q for O4 is about 1 MHz larger than that for O1, and the ^{17}O chemical shifts for the two seemingly similar oxygen atoms differ by 150 ppm. Close inspection of the crystal structure of compound **3** indeed reveals considerable differences between the two C−O−H groups. The C−O(4) bond length, 1.315 Å, is substantially longer than that of the C−O(1) bond, 1.296 Å.[48] Furthermore, the O(4)−H(2) bond length, 0.981 Å, is significantly shorter than that of the O(1)−H(1) bond, 1.017 Å. As mentioned earlier, O4 is also involved in an unusual hydrogen bond, O−H···Cl$^-$. Despite these structural features, it still seems surprising that O4 exhibits such a large chemical shift value, which corresponds to a very deshielded environment. Because it is usually not possible to detect separate solution ^{17}O NMR signals for C═O

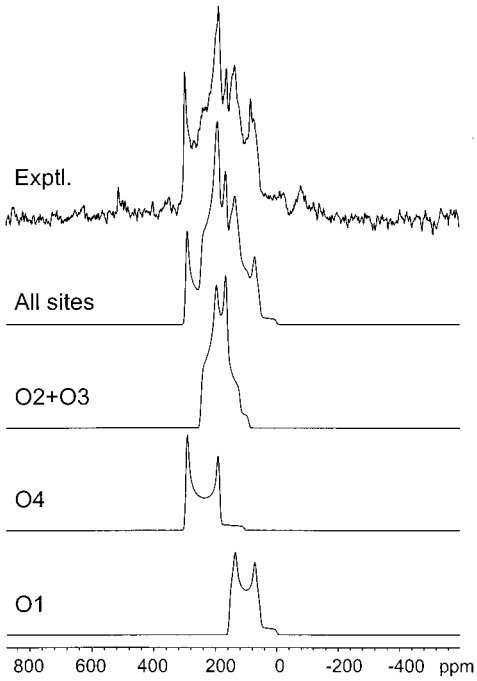

Figure 7. Experimental and simulated 1D ^{17}O MAS spectra of [^{17}O$_4$]-D,L-glutamic acid·HCl. The simulated total spectrum (all sites) is a sum of O1, O4, and O2+O3 sub-spectra. Note that the O2+O3 sub-spectrum is twice as intense as O1 and O4 sub-spectra. The sample spinning frequency was 14.5 kHz. A total of 1713 transients were collected with a recycle time of 5 s.

and C−O−H from a carboxylic acid functional group, very few data are available in the literature regarding ^{17}O chemical shifts for C−O−H groups in carboxylic acids. Therefore, the reason for the observation of such an unusual chemical shift for O4 in compound **3** is not obvious at this time. It would be of interest to see whether ab initio shielding calculations can shed light on this problem.

[2,4-^{17}O$_2$]Uracil. Uracil is one of the common nucleic acid bases. The two oxygen atoms of the pyrimidine ring are chemically different: O2 is related to a urea functional group and O4 is similar to an amide oxygen. The crystal structure of uracil indicates that O2 and O4 have quite different H-bonding environments.[49] As shown in Figure 8A, O4 is involved in two nearly linear C═O···H−N hydrogen bonds in a Y-shape fashion, meanwhile O2 is free of any direct H-bonding interaction. The two O(4)···N distances are 2.865 and 2.864 Å. As will be discussed later, these structural features have a profound effect on the observed ^{17}O NMR parameters for uracil.

The 2D ^{17}O MQMAS spectrum of compound **4** clearly shows two well-resolved peaks. Similar to the discussions described above, analyses of both the ^{17}O MQMAS and MAS spectra yield the following ^{17}O NMR parameters for compound **4**: O2, $\delta_{iso} = 240 \pm 5$ ppm, $C_Q = 7.62 \pm 0.02$ MHz, $\eta = 0.50 \pm 0.01$; O4, $\delta_{iso} = 275 \pm 5$ ppm, $C_Q = 7.85 \pm 0.02$ MHz, $\eta = 0.55 \pm 0.01$. The experimental and simulated 1D ^{17}O MAS spectra of compound **4** are shown in Figure 9. In compound **4**, the value of C_Q for the urea-type oxygen, O2, is considerably larger than that found in crystalline urea, 7.24 MHz.[16] As we noted recently, the small value of C_Q in crystalline urea is related to the very strong H-bonding interactions. The carbonyl oxygen atom of the urea molecule is hydrogen bonded to four N−H groups; such strong H-bonding interactions also lead to a considerable lengthening of the C═O bond, 1.265 Å.[50] In

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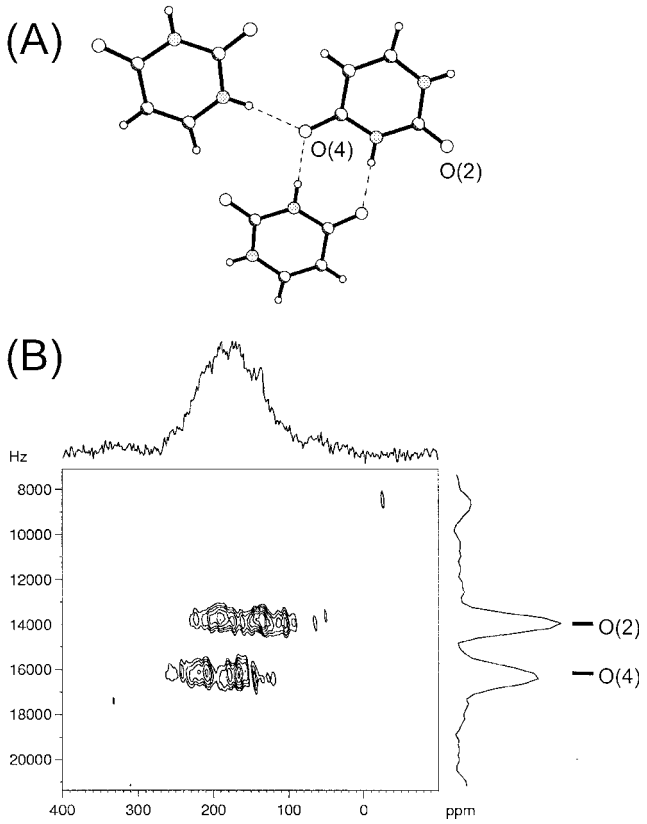

Figure 8. (A) Molecular and H-bonding structures of uracil.[50] (B) Contour plot of the ^{17}O MQMAS spectrum of [2,4-$^{17}O_2$]uracil. Corresponding 1D MAS spectrum and the F_1 projection of the 2D spectrum are shown at the top and on the side, respectively. A total of 960 transients were accumulated for each t_1 increment with a recycle delay of 5 s. A total of 24 t_1 increments were collected.

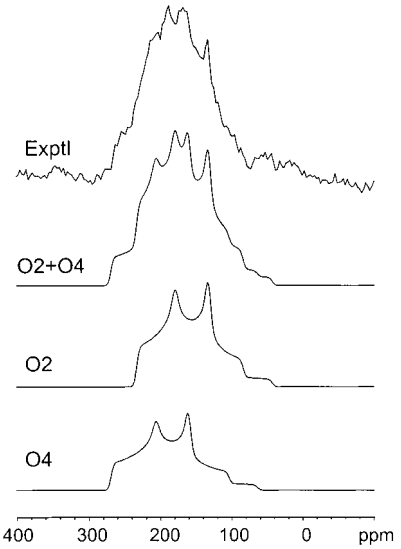

Figure 9. Experimental and simulated 1D ^{17}O MAS spectra of [2,4-$^{17}O_2$]uracil. The simulated total spectrum (O2+O4) is a sum of O2 and O4 sub-spectra with a 7:12 intensity ratio. This ratio was determined by solution ^{17}O NMR. A total of 445 transients were collected with a recycle time of 5 s.

comparison, the C═O(2) bond length in compound **4** is significantly shorter, 1.215 Å. On the other hand, the amide-type oxygen atom of compound **4**, O4, exhibits a C_Q value much smaller than those observed for secondary amides, 8.50−8.97 MHz.[15] In the secondary amides, the carbonyl oxygen is typically involved in one C═O···H−N hydrogen bond with the O···N distance varying between 2.930 and 3.112 Å. As seen from Figure 8A, O4 of the uracil molecule clearly experiences a much stronger H-bonding environment, which results in a much smaller ^{17}O quadrupole coupling constant. These general trends are perfectly consistent with the correlation between ^{17}O quadrupole parameters and H-bonding environment as we illustrated in several recent studies.[13−16,18]

It is also interesting to compare the ^{17}O chemical shifts measured for uracil in the solid state with those measured for a solution sample. The solution NMR spectrum of [2,4-^{17}O]-uracil in DMSO exhibits two peaks at 329 and 247 ppm assigned for O4 and O2, respectively. In the solid state, although the ^{17}O isotropic chemical shift for O2, 240 ppm, is essentially the same as that in solution, the ^{17}O chemical shift for O4 is shifted to the direction of shielding by 54 ppm! This large change in the ^{17}O chemical shift must be related to the strong H-bonding interaction at O4 in the solid state. As mentioned earlier, O4 is strongly hydrogen bonded to the two N−H groups of the neighboring molecules whereas O2 is free of any direct H-bonding interaction. In solution, all these intermolecular H-bonding interactions are essentially absent. Therefore, the ^{17}O chemical shift values measured in solution correspond to situations where the uracil molecules are isolated from one another. In the solid state, because strong intermolecular H-bonding interactions are present, the ^{17}O chemical shifts are profoundly changed. A similar effect was also observed for thymine.[18] The implication of our observation is that ^{17}O NMR is potentially useful for detecting base pairing in nucleic acid structures. Extensive ab initio calculations for nucleic acid bases confirmed that the ^{17}O NMR tensors are remarkably sensitive to the H-bonding environment; a detailed analysis of solid-state ^{17}O NMR parameters as a function of hydrogen bond geometry will be published elsewhere.

Resolution and Sensitivity of ^{17}O MQMAS Experiments. As is clearly seen from the above MQMAS spectra, the complete removal of second-order quadrupolar broadening has increased the spectral resolution dramatically. To make quantitative comparison, we define the MQ evolution time as t_1 and label the isotropic dimension, F_1, in frequency units (hertz) after the shearing transformation.[51] Using this convention, we can describe the peak positions along the isotropic dimension of the 3QMAS spectra for $S = {}^5/_2$ by

$$\nu_{iso}^{3QMAS} = -\nu_{iso}^{CS} - \frac{10}{17}\nu_{iso}^{(2)} \quad (1)$$

where ν_{iso}^{CS} is the frequency contribution from the chemical shift and $\nu_{iso}^{(2)}$ is the isotropic second-order quadrupole shift defined as:

$$\nu_{iso}^{(2)} = \frac{1}{500}(3 + \eta^2)\frac{{C_Q}^2}{\nu_L} \quad (2)$$

The observed line widths for the isotropic peaks in the ^{17}O MQMAS spectra were also reported in Table 1. Compared with peaks in the 1D MAS spectra, the line width of the isotropic peaks in the F_1 dimension is reduced by at least an order of magnitude. At this stage, factors that will ultimately determine the resolution limit in ^{17}O MQMAS spectra for organic compounds deserve comment. As seen from Table 1, the line

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Table 1. Experimental Solid-State ^{17}O NMR Parameters for Compounds **1**−**4**

compd		δ_{iso}/ ppm (±5)	C_Q/ MHz (±0.02)	η (±0.01)	ν_{iso}^{3QMAS}/ Hz[a] (±0.2)	$\Delta\nu_{1/2}$/ kHz[b] (±0.10)
[$^{17}O_2$]Ala (**1**)	O1	275	7.60	0.60	14.5	0.70
	O2	262	6.40	0.65	17.4	0.82
[$^{17}O_4$]PHB (**2**)	O1	285	8.30	0.15	18.0	1.17
	O2	215	5.90	0.90	23.0	1.60
[$^{17}O_4$]Glu·HCl (**3**)	O1	170	7.20	0.20	20.2	0.70
	O2	250	6.80	0.58	14.5	0.94
	O3	250	6.80	0.58	14.5	0.94
	O4	320	8.20	0.00	8.5	0.59
[$^{17}O_2$]uracil (**4**)	O2	240	7.62	0.50	16.2	0.94
	O4	275	7.85	0.55	13.8	1.12

[a] Isotropic peak positions in MQMAS spectra. [b] Full width at the half-height of the isotropic peaks.

width observed for organic solids is typically between 0.5 and 1.5 kHz. In contrast, much smaller line widths (0.1−0.2 kHz) were observed in ^{17}O MQMAS spectra of inorganic systems where $^1H-^{17}O$ dipolar interactions are weak.[24,25] It is thus believed that a main contributor to the residual line width in ^{17}O MQMAS spectra is the incomplete removal of $^1H-^{17}O$ dipolar interactions. Because new decoupling sequences such as TPPM[52] have been demonstrated to be more effective than the CW decoupling scheme, it is anticipated that the residual line width in ^{17}O MQMAS spectra can be further reduced. Other factors that may contribute to the residual line width include poor crystallinity of the sample and inaccurate setting of the magic angle. The advantage of ^{17}O MQMAS over traditional solution NMR is that the residual line width in solid-state NMR spectra is not restricted by the molecular weight of the system under observation. Another application of eq 1 is to confirm the values of δ_{iso}, C_Q, and η obtained from 1D MAS spectral analyses. This can be done simply by comparing the observed isotropic peak positions in the F_1 axis with the calculated values from eq 1 for each of the oxygen sites.

In this study, the molecular weight of the largest molecular system is on the order of 200 and the ^{17}O enrichment level is generally less than 40%. It is only natural to question whether the sensitivity of ^{17}O MQMAS experiments can be sufficiently high to make studies of biological macromolecules possible. Although the sensitivity of NMR experiments depends on many factors, it is still meaningful to use the current ^{17}O MQMAS NMR results to shed light on the feasibility of the approach for larger molecular systems. For ^{17}O MQMAS experiments, the key factors for sensitivity include (1) the level of ^{17}O enrichment of the sample, (2) the strength of the principal B_0 magnetic field, and (3) the efficiencies for both MQ generation and MQ-to-1Q conversion. Suppose that the sensitivity of ^{17}O central-transition NMR experiments depends on the applied magnetic field as $B_0^{7/2}$. With a maximum level of ^{17}O enrichment, 100%, the sensitivity of ^{17}O MQMAS experiments at 18.8 T (800 MHz for ^1H) can be increased by an order of magnitude compared with that reported in this study (40% enrichment at 11.75 T). This will make ^{17}O MQMAS experiments feasible for molecular systems of a few kDa with the methodologies used in the present study. The overall sensitivity of the MQMAS experiment can be further increased by an order of magnitude with a new data acquisition scheme utilizing a train of quadrupolar Carr−Purcell−Meiboom−Gill refocusing pulses (QCPMG).[53] This additional sensitivity enhancement may extend the MQMAS method to biomolecular systems of considerably larger sizes. Finally, several new methods[54−58] have recently been demonstrated to improve the sensitivity of MQMAS experiments by severalfold. Combining all these factors on a conservative basis leads to the conclusion that it is indeed possible to apply ^{17}O MQMAS NMR to biomolecular systems with significant molecular weights. It should be mentioned that "low resolution" solid-state ^{17}O NMR spectra have been successfully recorded for large biomolecular systems such as [$^{17}O_2$]myoglobin (ca. 17 kDa) and [$^{17}O_2$]hemoglobin (ca. 68 kDa) as reported by Oldfield and co-workers.[3]

Conclusion

We have presented high-resolution ^{17}O MQMAS NMR results for ^{17}O-enriched organic compounds ranging from free amino acids to a nucleic acid base. Using an ^{17}O enrichment level of ca. 40% and a moderate magnetic field (11.75 T), we were able to obtain high-quality ^{17}O MQMAS NMR spectra for organic compounds with ^{17}O quadrupole coupling constants greater than 8 MHz. The high-resolution ^{17}O MQMAS spectra revealed crystallographically distinct oxygen sites, which makes it possible to extract precise information about ^{17}O chemical shifts and quadrupole parameters for each of the oxygen sites. Accumulation of such site-specific NMR information will ultimately lead to a better understanding of the relationship between ^{17}O NMR parameters and molecular structure. The present study is a continuation of our recent effort in exploring the potential of solid-state ^{17}O NMR spectroscopy. Although we have examined only small organic molecules in the present study, the basic H-bonding features in the systems studied are similar to those observed in proteins (e.g., side chain conformation) and nucleic acids (e.g., base pairing). The demonstrated sensitivity of ^{17}O MQMAS experiments suggests that high-resolution ^{17}O MQMAS NMR can potentially become a practical technique for chemists to study organic and biological systems. The results of the present study are important in that they represent the first actual demonstration of ^{17}O MQMAS NMR for organic compounds. In addition, the new experimental solid-state ^{17}O NMR parameters reported in this study will be useful as testing cases for future quantum chemical computations. The challenge of ^{17}O NMR spectroscopy for biological solids remains to lie in the synthesis of ^{17}O-enriched biological molecules and in the development of new methodologies to further enhance NMR sensitivity.

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