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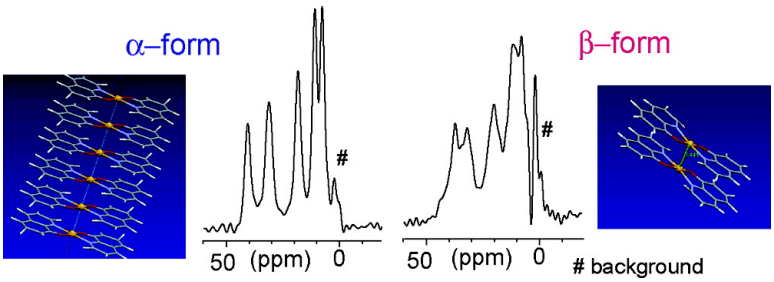
J. Am. Chem. Soc., **2005**, 127 (16), 5796-5797• DOI: 10.1021/ja042188i • Publication Date (Web): 05 April 2005



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Enhanced Sensitivity and Resolution in ^{1}H Solid-State NMR Spectroscopy of Paramagnetic Complexes under Very Fast Magic Angle Spinning

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Received December 28, 2004; E-mail: yishii@uic.edu

Paramagnetic complexes in solids have attracted increasing interest due to their diverse applications in modern material science,[1,2] bioinorganic chemistry,[3] and pharmacology.[4] Characterizing these paramagnetic complexes is essential to understand their functions and design improved systems. However, the methodologies for characterizing paramagnetic systems have been limited, compared to those for diamagnetic systems, in particular, for noncrystalline solids. Electron paramagnetic resonance (EPR) is a standard method for analyzing paramagnetic systems. However, EPR typically requires isotope labeling to obtain structural information on ligands through a hyperfine dipolar coupling. Solution NMR, a powerful tool for organic compounds, often exhibits limited resolution and sensitivity for paramagnetic materials because of paramagnetic broadening.[5] Also, solution NMR does not provide unique characteristics in solids such as morphologies, which can alter essential properties of materials and drugs.

Solid-state NMR (SSNMR) is a powerful method for structural analysis of noncrystalline solids. Among various nuclei, ^{13}C SSNMR has been most widely applied for its excellent resolution. However, the limited sensitivity of ^{13}C SSNMR has required larger amount of samples (0.1−1 mmol), compared with other analysis because of low abundance of ^{13}C. ^{1}H SSNMR is an attractive alternative to ^{13}C SSNMR, particularly for unlabeled systems and samples in limited quantities because of its high sensitivity.[6,7] In ^{1}H high-resolution SSNMR, multiple-pulse ^{1}H−^{1}H RF dipolar decoupling has been required, together with magic angle spinning (MAS) to suppress line broadening due to strong ^{1}H−^{1}H couplings.[6] On the other hand, for paramagnetic complexes, large paramagnetic shifts have inhibited resolution enhancement by multiple-pulse decoupling. Nayman et al.[8] and later Liu et al.[9] demonstrated that moderate spinning about 10 kHz improves resolution for paramagnetic systems. However, this unique idea is only effective for systems in which ^{1}H−^{1}H flip-flop is suppressed by large ^{1}H shift dispersion or motions. Therefore, few ^{1}H high-resolution NMR studies have been performed for paramagnetic systems.

Recently, our group demonstrated a new approach to obtain high-resolution ^{13}C SSNMR of paramagnetic systems using very fast MAS (VFMAS; spinning speed, $\nu_R > 20$ kHz).[10] Although MAS over 50 kHz is currently available,[11] we define *VFMAS* as above because MAS at 20 kHz or more induces crucial changes in the spin dynamics for organic solids by eliminating the majority of ^{1}H−^{1}H and ^{1}H−^{13}C dipolar couplings. Faster spinning ($\nu_R > 30$ kHz) does not qualitatively alter this spin dynamics, which forms the foundation of our approach. Although ^{1}H line narrowing by VFMAS has been shown for diamagnetic systems,[7,12] this has not been discussed for paramagnetic systems. In this study, we demonstrate that ^{1}H high-resolution SSNMR of paramagnetic systems under VFMAS exhibits excellent resolution and unparalleled sensitivity, permitting SSNMR micro analysis.

Figure 1a−c shows the spinning-speed dependence of ^{1}H MAS spectra of unlabeled Cu(DL-Ala)$_2$·H$_2$O. It is clear that the sensitivity and resolution are both excellent at $\nu_R = 24$ kHz in (a). VFMAS significantly enhanced resolution and sensitivity by removing broadening due to large anisotropic paramagnetic shifts as well as other anisotropic interactions such as ^{1}H−^{1}H dipolar couplings.[10] Compared with the spectrum in (b) at $\nu_R = 10$ kHz, the sensitivity enhancement in (a) is a factor of 12−18. It is worth pointing out that anisotropic paramagnetic shifts are generally proportional to $(S + 1)S\gamma_I/R_{IS}^3$,[5] where γ_I is the gyromagnetic ratio for the nuclear spin I, S is an electron spin number, and R_{IS} is a distance between I and the electron spin S at a paramagnetic center. Hence, a higher γ nucleus is subject to a larger anisotropic shift in Hz units ($S = {}^1/_2$ for this system). Nevertheless, most of the spinning sidebands were suppressed in (a). It is also important to point out that besides VFMAS, fast electron spin exchange by intermolecular spin couplings in solids enhances the resolution of SSNMR.[8,9] In solution NMR, molecules isolated in solvents often have long electron spin relaxation times, which lead to quenching of NMR signals.[13] The assignments given in (a) are based on 2D ^{13}C/^{1}H correlation NMR, as will be described elsewhere. The assignments agree well with those based on ^{2}D NMR of selectively ^{2}D-labeled samples.[9] Although we did not assign the signal at 20 ppm, a corresponding signal was assigned to a minor CD$_3$ species in ^{2}D NMR.[9] Figure 1a exhibiting well-resolved center bands was obtained in a total experimental time of only 18 ms because of short ^{1}H T_1 values.

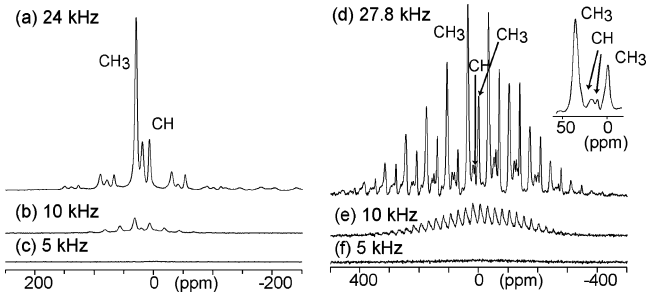

Figure 1. Spinning speed dependence of ^{1}H MAS spectra of (a−c) Cu(DL-Ala)$_2$·(H$_2$O) and (d−f) Mn(acac)$_3$. The spinning speed is indicated in the figure. The inset in (d) is the expanded center line region. The spectra were obtained at ^{1}H frequency of 400.2 MHz with 1-pulse excitation and a rotor synchronous echo with 4 scans for each spectrum. The sample amount was 17 and 14 mg for Cu(DL-Ala)$_2$ and Mn(acac)$_3$, respectively. The assignment for Cu(DL-Ala)$_2$ and Mn(acac)$_3$ was obtained from separate 2D ^{13}C/^{1}H correlation NMR experiments. The total experimental times were only (a−c) 18 ms and (d−f) 12 ms. Other experimental details are available in the Supporting Information.

Figure 1d−f shows spinning-speed dependence of ^{1}H MAS spectra of Mn(acac)$_3$ ($S = {}^5/_2$). In (f) at $\nu_R = 5$ kHz, there are no resolved signals, and only one center band is visible in (e) at 10 kHz. In contrast, the resolution and sensitivity are both significantly enhanced by VFMAS at 27.8 kHz in (d). Compared with the spectrum at 10 kHz in (e), sensitivity enhancement by a factor of 17 was observed at 27.8 kHz. Because the ^{1}H paramagnetic anisotropic shifts reach 1000 ppm, a conventional multiple-pulse

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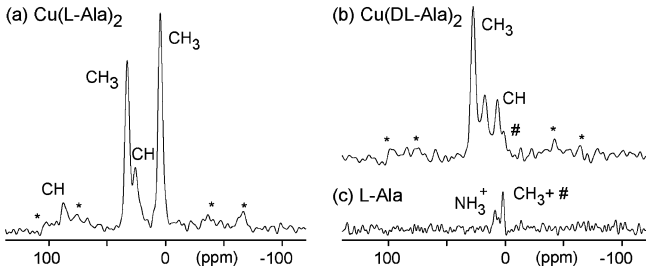

Figure 2. ^{1}H VFMAS spectra of (a) Cu(L-Ala)$_2$ and (b) Cu(DL-Ala)$_2$·H$_2$O, and (c) L-Ala obtained at ^{1}H NMR frequency of 400.2 MHz with one-pulse excitation at spinning speed of 28.57 kHz. The sample amount was (a, b) 20 nmol (5 μg) and (c) 40 nmol (4 μg). A total of (a) 38 700, (b) 26 200, and (c) 76 scans were recorded with recycle delays of (a) 3 ms, (b) 4.5 ms, and (c) 1.6 s in a common experimental time of 2 min, respectively. Background signals were suppressed by subtracting a spectrum obtained for a rotor without the samples from a spectrum obtained with the sample. Residual background signals and spinning sidebands are marked by # and *, respectively. Time-domain signals were accumulated during acquisition times of (a) 0.63 ms, (b) 0.56 ms, and (c) 0.91 ms, which were used as a part of the recycle delays in (a, b). The spectra (a, b) are scaled by a factor of $1/\sqrt{353}$ and $1/\sqrt{212}$, respectively so that all the spectra display a common noise level. Signal assignments in (a, b) were made on the basis of separate 2D ^{1}H/^{13}C correlation experiments.

or CRAMPS experiments would not be an option for this sample. Although numerous sidebands still remain even at $\nu_R = 27.8$ kHz, the four center lines in the inset of (d) are well resolved from their sidebands. The strong CH_3 signal intensities are consistent with the ratio of CH_3 and CH protons (6:1) in acac ($CH_3-CO-CH-CO-CH_3$). It is noteworthy that the high sensitivity in (d) was obtained in only 12 ms.

There has been a popular conception that sensitivity of SSNMR for paramagnetic systems is significantly lower than that of diamagnetic systems because of paramagnetic broadening. However, the sensitivity in Figure 1a, d under VFMAS appears excellent. The high sensitivity is well explained from the facts that resolution in ^{1}H SSNMR of paramagnetic systems under VFMAS is comparable to that for diamagnetic systems and that repetition rates of experiments are faster by 2−3 orders of magnitude for paramagnetic systems because of their short T_1 values (∼ms) (further analysis is available in the Supporting Information). Hence, when sidebands are sufficiently suppressed by VFMAS, the theoretical sensitivity of ^{1}H SSNMR for paramagnetic systems is greater than that for diamagnetic systems by an order of magnitude.

To confirm the expected superb sensitivity, in Figure 2, we show ^{1}H MAS SSNMR spectra for 20 nmol (5.0 μg) of unlabeled (a) Cu(L-Ala)$_2$ and (b) Cu(DL-Ala)$_2$·H$_2$O. For the most intense CH_3 signal, the signal-to-noise ratios (S/N) of 41 and 29 were obtained within 2 min for (a) and (b), respectively. Hence, analyzing several nanomoles of the samples is possible. The two compounds, which cannot be distinguished by mass spectroscopy, are clearly distinguishable in Figure 2a, b with improved resolution under VFMAS. In a control experiment for 40 nmol (3.8 μg) of L-Ala shown in Figure 2c, we obtained S/N of 3.4 for the peak at 8.2 ppm, which corresponds to NH_3^+, in a common experimental time (2 min). The other signals (CH and CH_3) are masked by a background signal. To the best of our knowledge, this is the initial example showing that SSNMR of paramagnetic systems can be more sensitive than that of corresponding diamagnetic systems by an order of magnitude.

We applied this ^{1}H VFMAS method to two crystal forms of polycrystalline Cu(II)(8-quinolinol)$_2$ [CuQ_2] to examine whether polymorphs of paramagnetic drugs or materials can be distinguished

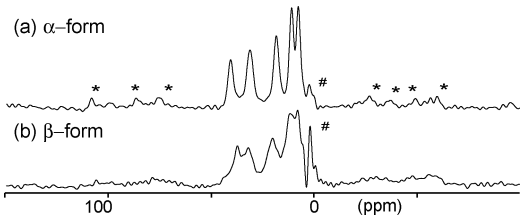

Figure 3. ^{1}H VFMAS spectra of (a) α-form and (b) β-form Cu(8-quinolinol)$_2$ obtained at ^{1}H frequency of 400.2 MHz with one-pulse excitation at spinning speed of 27.03 kHz. The sample amount was 20 nmol (7 μg) for each sample. A total of (a) 36 560 and (b) 98 304 scans were recorded with recycle delays of (a) 15 ms and (b) 5 ms, respectively. The total experimental time was 10 min each. Time-domain signals were accumulated during acquisition times of 1 ms. Each spectrum was processed with application of 400 Hz Gaussian broadening function. Background signals were removed as described in Figure 2.

in a nanomole scale by ^{1}H SSNMR. CuQ_2 is an apoptosis inducer in human cancer cells,[14] and its β-form is thermally more stable.[15] This system is also interesting as an analogue of other metal−Q complexes that function as organic light emitting diodes.[2] Figure 3 shows ^{1}H VFMAS spectra of 20 nmoles of (a) α-form and (b) β-form CuQ_2. The sensitivity is excellent after only 10 min of signal accumulation. Clearly, these spectra are distinguishable on the basis of the line positions and line widths even without signal assignments. Considering that ^{1}H SSNMR of diamagnetic systems rarely displays sufficient resolution to distinguish polymorphs, we think that the present results demonstrate the unique possibility of identifying molecular packing or supramolecular structures in paramagnetic systems by ^{1}H VFMAS.

Acknowledgment. We are grateful to Prof. Cynthia Jameson at UIC for stimulating discussion. This study was supported in part by grants from the Alzheimer's Association (NIRG 035123) and the NSF CAREER Program (CHE 449952).

Supporting Information Available: Details of experimental conditions and sample preparation. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA042188I