Inorg. Chem. **2000,** *39,* 4−5

Solid-State ^{25}Mg NMR Study of Inner-Sphere Mg^{2+} Binding Complexes

Simon Sham and Gang Wu*

Department of Chemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6

Received September 3, 1999

Magnesium is one of the most abundant metal ions in cellular organisms. Like other alkali and alkaline metal ions (Na$^+$, K$^+$, and Ca^{2+}), Mg^{2+} is involved in a wide variety of physiochemical activities necessary to sustain life.[1] For instance, magnesium serves as a cofactor responsible for the biochemical transfer of phosphate-related enzymes.[2] Mg^{2+} also occurs as an integral component in a number of non-phosphate-transferring proteins, including carbohydrate isomerases[3] and DNA-activating topoisomerases.[4] A recent study of ribozymes has suggested that the Mg^{2+} ion may play a key functional role in regulating the catalysis of this class of metalloenzymes.[5] Crystallographic studies of yeast tRNA$^{\mathrm{phe}}$ have suggested that binding of the metal ion may play a role in stabilizing the tertiary conformation of the RNA molecule.[6] Mg^{2+} has also been found to maintain the carbamate formation which activates the catalytic activities of ribulose-1,5-bisphosphate carboxylase/oxygenase ("rubisco") in carboxylation and oxygenation in green plants.[7]

Efforts to decipher the magnesium chemistry at a biomolecular level have been hampered by the lack of suitable spectroscopic techniques for Mg^{2+} due to its closed electronic structure. Application of ^{25}Mg NMR has been very limited because of the unfavorable nuclear properties of ^{25}Mg (spin $= {}^5/_2$, natural abundance $= 10.1\%$, nuclear quadrupole moment $= 0.22 \times 10^{-28}$ m^{-2}). In the liquid phase, NMR relaxation of ^{25}Mg nuclei is dominated by an efficient quadrupole mechanism and consequently leads to broad NMR signals. Nevertheless, ^{25}Mg NMR has been demonstrated to be a useful technique in studying kinetic and binding properties of Mg^{2+} in solutions.[8]

To understand the structural and catalytic roles of Mg^{2+} in biological systems requires a full knowledge of the coordination chemistry of Mg^{2+} ions at active sites.[9] In principle, solid-state ^{25}Mg NMR can provide site-specific information about the Mg^{2+} coordination environment. However, very little is known about solid-state ^{25}Mg NMR parameters in various biologically relevant binding sites; the only solid-state ^{25}Mg studies were focused on magnesium metal, alloys, and simple inorganic salts.[10] With the recent advances in solid-state NMR instrumentation, it is possible to extend solid-state NMR to biologically important low-γ metal nuclei such as ^{67}Zn.[11] We believe that solid-state ^{25}Mg NMR will also be useful for studying magnesium chemistry in a biological context. In this Communication, we report preliminary solid-state ^{25}Mg NMR results for four-inner sphere Mg^{2+} binding complexes.

The Mg compounds studied in this work are $\mathrm{Mg(H_2O)_4L_2}$, L = methylmalonate (**1**), formate (**2**), acetate (**3**), and orotate (**4**).[12] These compounds can be treated as models for the inner-sphere Mg^{2+} binding sites often found in various biological systems. In compound **1**, the Mg^{2+} ion is coordinated with six oxygen atoms in a slightly distorted octahedral geometry. Four of the six oxygen ligands are water molecules, and the other two are from the α-methylmalonate ligand.[13] Such a Mg^{2+} coordination geometry mimics those of inner-sphere binding sites where two of the six water molecules of the $[\mathrm{Mg(H_2O)_6}]^{2+}$ ion are replaced by anionic ligands. The binding of Mg^{2+} to the α-methylmalonate ligand in **1** serves as a good model for the Mg binding to β-carboxylapartatic acid (Asa), which is found in ribosomal proteins.[14,15] The ^{25}Mg nuclear quadrupole coupling constant

---

* Corresponding author. Phone: 613-533-2644. Fax: 613-533-6669. E-mail: gangwu@chem.queensu.ca.

(1) (a) Frausto da Silva, J. J. R.; Williams, R. J. P. *The Biological Chemistry of the Elements*; Clarendon Press: Oxford, 1991. (b) Cowan, J. A. *Inorganic Biochemistry: An Introduction*; VCH: New York, 1993. (c) *The Biological Chemistry of Magnesium*; Cowan, J. A., Ed.; VCH: New York, 1995.

(2) (a) Janeway, C. M. L.; Xu, X.; Murphy, J. E.; Chaidaroglou, A.; Kantrowitz, E. R. *Biochemistry* **1993**, *32*, 1601. (b) Needham, J. V.; Chen, T. Y.; Falke, J. J. *Biochemistry* **1993**, *32*, 3363.

(3) Jenkins, J.; Janin, J.; Rey, F.; Chiadmi, M.; van Tilbeurgh, H.; Lasters, I.; De Maeyer, M.; Van Belle, D.; Wodak, S. J.; Lauwereys, M.; Stanssens, P.; Mrabet, N. T.; Snauwaert, J.; Matthyssens, G.; Lambeir, A.-M. *Biochemistry* **1992**, *31*, 5449.

(4) (a) Domanico, P. L.; Tse-Dinh, Y. C.; Morgan, J. *J. Inorg. Biochem.* **1991**, *42*, 87. (b) Kim, S.; Cowan, J. A. *Inorg. Chem.* **1992**, *31*, 3495. (c) Lebeau, L.; Regnier, E.; Schultz, P.; Wang, J. C.; Mioskowski, C.; Oudet, P. *FEBS Lett.* **1990**, *267*, 38.

(5) Pyle, A. M. *Science* **1993**, *261*, 709.

(6) (a) Jack, A.; Ladner, J. E.; Rhodes, D.; Brown, R. S.; Klug. A. *J. Mol. Biol.* **1977**, *111*, 315. (b) Quigley, G. J.; Teeter, M. M.; Rich, A. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 64.

(7) Andersson, I.; Knight, S.; Schneider, G.; Lindqvist, Y.; Lundqvist, T.; Branden, C.-I.; Lorimer, G. H. *Nature* **1989**, *337*, 229.

(8) (a) Bryant, R. G. *J. Magn. Reson.* **1972**, *6*, 159. (b) Forsén, S.; Andersson, T.; Drakenberg, T.; Teleman, O.; Vogel, H. J. In *Calcium Binding Proteins*; de Bernad, B., Ed.; Elsevier: Amsterdam, 1983. (c) Shimizu, T.; Hatano, M. *Inorg. Chem.* **1985**, *24*, 2003. (d) Tsai, M.-D.; Drakenberg, T.; Thulin, E.; Forsén, S. *Biochemistry* **1987**, *26*, 3635. (e) Reid, S. S.; Cowan, J. A. *Biochemistry* **1990**, *29*, 6025. (f) Reid, S. S.; Cowan, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 673. (g) Cowan, J. A. *Inorg. Chem.* **1991**, *30*, 2740. (h) Cowan, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 675. (i) Berggren, E.; Nordenskiöld, L.; Braunlin, W. H. *Biopolymers* **1992**, *32*, 1339. (j) Wright, L.; Lerner, L. E. *Biopolymers* **1994**, *34*, 691. (k) Yan, H.; Tsai, M.-D. *Biochemistry* **1991**, *30*, 5539.

(9) (a) Black, C. B.; Huang, H.-W.; Cowan, J. A. *Coord. Chem. Rev.* **1994**, *135/136*, 165. (b) Misra, V. K.; Draper, D. E. *Biopolymers* (Nucleic Acid Sciences) **1998**, *48*, 113.

(10) (a) Dupree, R.; Smith, M. E. *J. Chem. Soc., Chem. Commun.* **1988**, 1483. (b) Bastow, T. J. *J. Phys.: Condens. Matter* **1991**, 3, 753. (c) MacKenzie, K. J. D.; Meinhold, R. H. *Thermochim. Acta* **1993**, *230*, 331. (d) MacKenzie, K. J. D.; Meinhold, R. H. *Thermochim. Acta* **1993**, *230*, 339. (e) Bleich, H. E.; Redfield, A. G. *J. Chem. Phys.* **1977**, *67*, 5040. (f) Bastow, T. J.; Smith, M. E. *J. Phys.: Condens. Matter* **1995**, *7*, 4929.

(11) (a) Wu, G. *Chem. Phys. Lett.* **1998**, *298*, 375. (b) Vosegaard, T.; Andersen, U.; Jakobsen, H. J. *J. Am. Chem. Soc.* **1999**, *121*, 1970. (c) Larsen, F. H.; Lipton, A. S.; Jakobsen, H. J.; Nielsen, N. C.; Ellis, P. D. *J. Am. Chem. Soc.* **1999**, *121*, 3783. (d) Sham, S.; Wu, G. *Can. J. Chem.* **1999**, *77*, 1782.

(12) Colorless crystals of magnesium methylmalonate tetrahydrate (**1**) were formed after slow evaporation of an ethanol solution containing 0.020 g of ^{25}MgO and 0.058 g of α-methylmalonic acid. Crystals of magnesium formate dehydrate (**2**) and magnesium diacetate tetrahydrate (**3**) were formed by slow evaporation of solutions obtained by neutralizing 0.5 M formic acid with 0.023 g of ^{25}MgO and 0.5 M acetic acid with 0.019 g of ^{25}MgO, respectively. Tetraaqua(orotato-*N,O*) magnesium 2.5 hydrate (**4**) was prepared by mixing 21 mg of enriched ^{25}MgO and 91 mg of orotic acid, i.e., equimolar, in a total 15 mL of water with gentle warming. Small needle-shaped crystals were formed after the solvent was slowly evaporated. Magnesium oxide (^{25}Mg 99.1% atom) was purchased from Trace Science International (Toronto, Ontario, Canada).

(13) Curry, M. E.; Eggleston, D. S.; Hodgson, D. J. *J. Am. Chem. Soc.* **1985**, *107*, 8234.

(14) Christy, M. R.; Barkley, R. M.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 3935.

(15) (a) Marsh, H. C.; Robertson, P., Jr.; Scott, M. E.; Koehler, K. A.; Hiskey, R. G. *J. Biol. Chem.* **1979**, *254*, 10628. (b) Prendergast, F. G.; Mann, K. G. *J. Biol. Chem.* **1977**, *252*, 840.

10.1021/ic991060c CCC: $19.00

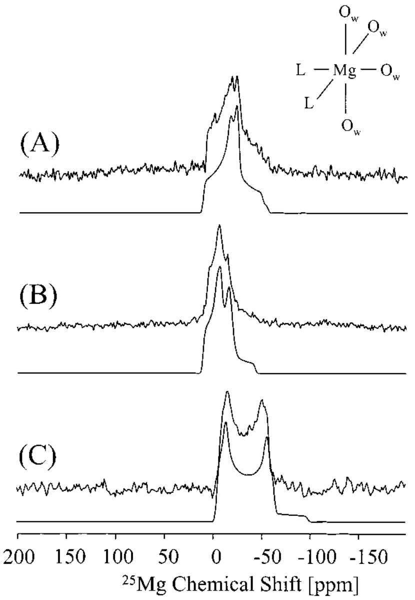

Figure 1. Experimental and simulated ^{25}Mg MAS NMR spectra of (A) magnesium methylmalonate tetrahydrate (**1**) (374 transients, 10 s recycle time, 10 kHz spinning rate), (B) magnesium formate dihydrate (**2**) (1039 transients, 5 s recycle time, 9.3 kHz spinning rate), and (C) tetraaqua-(orotato-*N*,*O*)magnesium 2.5 hydrate (**4**) (715 transients, 10 s recycle time, 8.2 kHz spinning rate). Solid-state ^{25}Mg NMR spectra were obtained on a Bruker Avance-500 spectrometer operating at 500.13 and 30.62 MHz for ^{1}H and ^{25}Mg nuclei, respectively. All ^{25}Mg chemical shifts were referenced to 3 M $MgSO_4$ (aqueous) by setting the signal of solid MgO at 26 ppm. The inset illustrates the general structure of the Mg compounds studied in this work.

(NQCC) for such a Mg site is $\chi = 1.95$ MHz with $\eta = 0.80$. The isotropic ^{25}Mg chemical shift is $\delta_{iso} = 12$ ppm. The crystal structure of **2** indicates the presence of two distinct Mg sites.[16] While one Mg site is coordinated with six formate groups, the other Mg site is bound to four water molecules and two formate groups. As seen from Figure 1B, the ^{25}Mg MAS spectrum of **2** clearly exhibits a complex line shape (vide infra). The crystal structure of **3** reveals that the Mg^{2+} ion is coordinated with the four oxygen atoms of the water molecules and two oxygen atoms of the acetate ions.[17] The following ^{25}Mg NMR parameters are obtained for **3**: $\chi = 1.90$ MHz, $\eta = 0.82$, and $\delta_{iso} = 27$ ppm.

Unlike compounds **1**−**3**, compound **4** has a Mg center coordinated with asymmetrical ligands, i.e., five oxygen ligands and one nitrogen ligand. The Mg coordination sphere in **4** is octahedral with bond length varying from 2.028 to 2.106 Å for the Mg−O bonds and 2.189 Å for the Mg−N bond.[18] Analysis of the ^{25}Mg MAS spectrum yields $\chi = 2.56$ MHz, $\eta = 0.15$, and $\delta_{iso} = 6$ ppm. To our knowledge, compound **4** represents the first example of ^{25}Mg NMR characterization of Mg complexes containing asymmetrical ligands.

Since the ^{25}Mg chemical shift range is rather small (ca. 50 ppm), spectral overlap is expected to be a common occurrence in solid-state ^{25}Mg NMR spectra if multiple Mg sites are present. As mentioned earlier, compound **2** has two distinct Mg sites, which results in a complex ^{25}Mg MAS spectrum. Therefore, it is extremely appealing to test the recently developed multiple quantum MAS (MQMAS)[19] technique for ^{25}Mg. Figure 2 shows the two-dimensional (2D) ^{25}Mg MQMAS spectrum of **2**, together with the two corresponding projections. As seen in Figure 2, two clearly resolved isotropic peaks are observed in the isotropic axis of the 2D sheared MQMAS spectrum. Compared with the ^{25}Mg MAS spectrum, the resolution of the isotropic projection of the MQMAS spectrum is increased approximately by a factor of 20. From the slice spectra, it is straightforward to extract ^{25}Mg quadrupole parameters for each of the two Mg sites: site 1, $\chi = 1.70$ MHz, $\eta = 1.0$, $\delta_{iso} = 10$ ppm; site 2, $\chi = 1.40$ MHz, $\eta = 0.80$, $\delta_{iso} = 10$ ppm. In fact, analysis of the 1D ^{25}Mg MAS spectrum shown in Figure 1B was possible only after obtaining the ^{25}Mg MQMAS result.

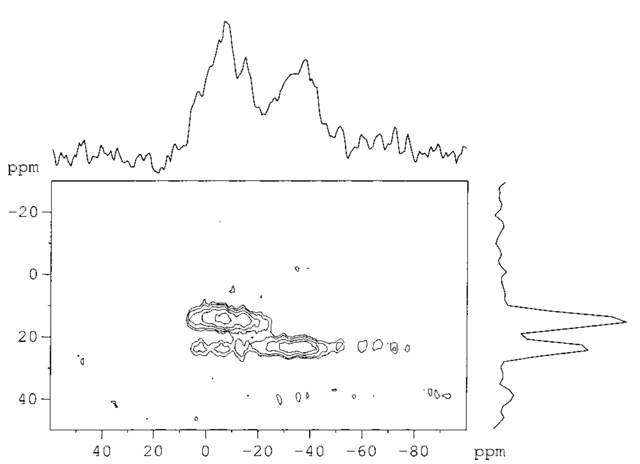

Figure 2. Two-dimensional (2D) MQMAS spectrum of magnesium formate dihydrate (**2**). The pulse sequence with a z-filter[20] was used in obtaining ^{25}Mg 3QMAS spectra. The optimized excitation and conversion pulse width was 7.5 and 2.8 μs, respectively. The selective ^{25}Mg 90° pulse for the central transition was 30 μs. The sample spinning frequency was 9 kHz. A total of 1200 transients were collected for each of the 64 t_1 increments with a recycle delay of 2 s. The 2D data was zero-filled to a size of 1024 × 128 prior to 2D shear Fourier transformation (FT).

In summary, we have presented a solid-state ^{25}Mg NMR study of magnesium complexes as models for inner-sphere Mg^{2+} coordination. We have also demonstrated the first ^{25}Mg MQMAS NMR experiment. The present study was carried out at 11.75 T where ^{25}Mg MAS NMR spectra with a reasonable signal-to-noise ratio can be obtained in an hour or so for ^{25}Mg-enriched samples. On the basis of our results, it can be concluded that Mg−DNA oligomer complexes should be accessible by solid-state ^{25}Mg NMR. As high-field NMR instruments (18.8 T or above) are becoming available, the sensitivity of solid-state ^{25}Mg NMR experiments will be drastically improved. Finally, the present solid-state ^{25}Mg NMR characterization of Mg sites has yielded benchmark values for the ^{25}Mg NQCCs in the $Mg(H_2O)_4L_2$ coordination environment, which will be useful for the interpretation of solution ^{25}Mg NMR relaxation data. We hope that the preliminary results presented in this work will encourage further solid-state ^{25}Mg NMR studies.

Acknowledgment. This work was financially supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

IC991060C

---

(16) De With, G.; Harkema, S.; Van Hummel, G. J. *Acta Crystallogr.* **1976**, *B32*, 1980.

(17) Irish, D. E.; Semmler, J.; Taylor, N. J.; Toogood, G. E. *Acta Crystallogr.* **1991**, *C47*, 2322.

(18) Mutikainen, I.; Hämäläinen, R.; Klinga, M.; Turpeinen, U. *Z. Kristallogr.* **1997**, *212*, 65.

(19) (a) Frydman, L.; Harwood, J. S. *J. Am. Chem. Soc.* **1995**, *117*, 5367. (b) Medek, A.; Harwood, J. S.; Frydman, L. *J. Am. Chem. Soc.* **1995**, *117*, 12779.

(20) Amoureux, J.-P.; Fernandez, C.; Steuernagel, S. *J. Magn. Reson. Ser. A* **1996**, *123*, 116.