Solid-State ^{25}Mg NMR of a Magnesium(II) Adensosine 5′-Triphosphate Complex

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The many significant roles that adenosine 5′-triphosphate (ATP) plays in chemical energy storage and transfer make this molecule a fundamental target of biochemical analysis. The polyanionic nature of this ligand leads to metal ions playing a significant role in the overall structure and function of the molecule.[1,2] In addition to the biological importance of understanding metal−nucleotide interactions on their own merits, these complexes serve as important models for understanding the interaction of metals with macromolecular nucleic acids. In particular the structural aspects of divalent metal ions interacting with RNA, such as magnesium-(II) binding sites within ribozymes, has become a topic of intense interest.[3,4] Yet, few spectroscopic techniques are available for the study of closed-shell diamagnetic ions such as magnesium(II). X-ray crystallography has been most useful at providing structural information about magnesium binding sites in nucleic acid and nucleotide complexes, but only within those systems that have been successfully crystallized.[2,3] This limitation has led to a growing number of studies that rely upon ion substitution. For example, Mn(II) has been used as a probe to analyze the structure of divalent metal ion binding sites in the hammerhead ribozyme via electron paramagnetic resonance (EPR).[5] Nevertheless, it would still be desirable to have a direct spectroscopic probe of EPR-silent cations such as magnesium. Recent advances in solid-state NMR have opened up new possibilities in this area, in particular with regards to the high-resolution spectroscopy of half-integer quadrupolar nuclei.[6] The advent of high-field NMR spectrometers also facilitates the study of low γ nuclei such as ^{25}Mg ($I = {}^5/_2$), as do recent developments in signal enhancement techniques.[7,8] It has recently been shown that these advances can turn ^{25}Mg NMR into a viable probe for the characterization of salts and octahedral inner-sphere coordination environments of magnesium.[9,10] In this work we demonstrate the possibility of using these solid-state NMR advances toward the resolution and assignment of magnesium binding sites within nucleic acids.

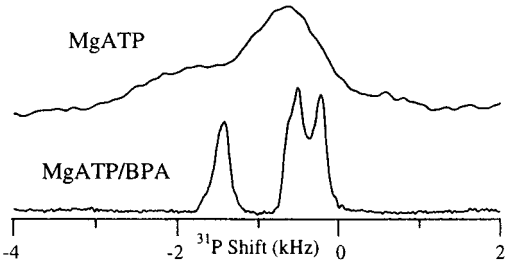

Figure 1. Comparison between the high-resolution solid-state ^{31}P NMR spectra (122.10 MHz) collected for MgATP recrystallized from H_2O (top) and for the MgATP/BPA complex. Preparations of MgATP/BPA with both natural abundance and isotopically labeled magnesium gave indistinguishable ^{31}P NMR spectra.

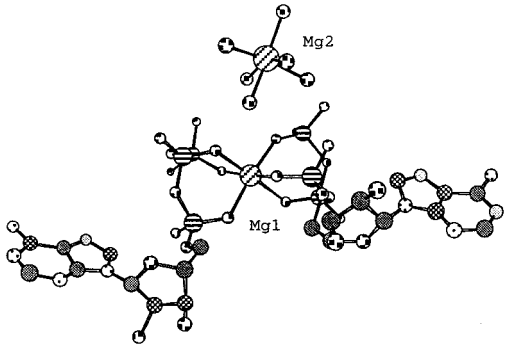

Figure 2. Illustration of the two magnesium coordination environments in MgATP/BPA, as derived from the crystal structure of Cini et al.[11]

As binary complexes of magnesium(II) and ATP are known to yield conformational mixtures,[11] this study has focused on the ternary complex of magnesium(II), adenosine 5′-triphosphate^{4-}, and bis(2-pyridyl)amine (BPA). $[Mg(H_2O)_6][HBPA]_2[Mg(HATP)_2]\cdot 12H_2O$ (for simplicity abbreviated as MgATP/BPA) was crystallized by the method of Cini et al.[11] using isotopically enriched $^{25}Mg^{2+}$ obtained by acidification of enriched (90%) ^{25}MgO. The resulting solid leads to sharp resonances in the ^{31}P cross-polarization MAS spectrum (Figure 1), an indication of high crystallinity that compares well with previously reported data for the unlabeled complex.[12]

The X-ray crystal structure of MgATP/BPA shows two distinct magnesium coordination environments: one with the ion coordinated by six phosphate oxygens coming from the pair of ATP molecules within the asymmetric unit (Mg1) and another involving magnesium coordinated by six water oxygen donors (Mg2). In both cases the magnesium coordination geometry would best be described as distorted octahedral, as is illustrated by the structure shown in Figure 2. Although the presence of two inequivalent coordination environments is not apparent from 1D NMR line shapes (data not shown), 2D triple-/single-quantum magic-angle-spinning (MQMAS) correlations clearly reveal two magnesium peaks (Figure 3). An analysis of the line shapes obtained in this experiment provides the following nuclear quadrupole and isotropic chemical shift parameters: $e^2qQ/h = 1.6$ MHz, $\eta = 1.0$, $\delta_{iso} = -2$ ppm; and $e^2qQ/h = 2.3$ MHz, $\eta = 1.0$, $\delta_{iso} = -9$ ppm. Unfortunately the nuclear quadrupole coupling parameters are too similar to be interpreted structurally in an unambiguous manner, while the small chemical shift range of magnesium makes isotropic shift data unreliable for assigning the chemical origin of the peaks. As a result of these limitations,

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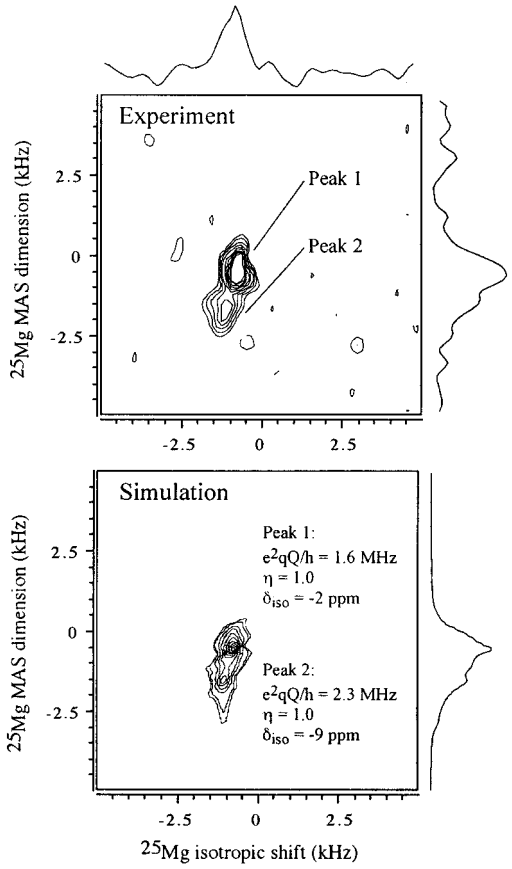

Figure 3. (Top) ^{25}Mg MQMAS NMR spectrum of MgATP/BPA, acquired on a home-built 11.7 T NMR spectrometer incorporating a Tecmag pulse programmer and a Varian 4 mm T3 MAS probe. A two-pulse sequence was used with a 7.5 μs triple quantum excitation and a 4.0 μs single quantum conversion pulse. The sample spinning frequency was set at 10.0 kHz and 24 000 transients with a 1.0 s recycle delay were recorded for each of 16 t_1 points. The spectrometer frequency was set at 30.63 MHz. (Bottom) Best fit simulations of the experimental data resulting from two equally populated sites with the parameters obtained as indicated in ref 6. Chemical shifts are reported relative to 3 M $MgSO_4$ (aq) via the resonance of solid MgO at 26 ppm.

a spectroscopic approach for distinguishing the chemical origin of the two peaks resolved by MQMAS NMR becomes highly desirable. A protocol was developed to this end that utilizes rotational-echo double-resonance (REDOR)[13] to reintroduce proton dipolar couplings that have been removed by the effects of fast MAS. Such methodology exploits the fact that Mg1 is coordinated entirely by phosphate oxygens and is expected to have few strong proton dipolar contacts, whereas Mg2 is coordinated by water ligands and thus possesses several close protons (2.5−3.5 Å) that can give rise to a substantial amount of dipolar dephasing even in the presence of fast water flipping motions. Because of the signal-to-noise limitations of MQMAS, this sequence was implemented as a 1D rather than 2D experiment,[14] with rotor synchronized ^{1}H pulses inserted within the interpulse delay of a ^{25}Mg Carr−Purcell spin-echo sequence. A comparison between the conventional spin-echo and the ^{1}H dephased spin-echo data is illustrated in Figure 4. When subtracting the data collected in the presence and absence of the ^{1}H REDOR pulses, a significant and reproducible peak is noticed. This difference spectrum reveals the approximate line shape of the signal arising from the metal site that is more strongly affected by the proton couplings. By comparing this difference spectrum to the contributions that, according to MQMAS, can be expected from each magnesium peak, it is possible to conclude which of the ^{25}Mg signals is being generated by each coordination environment. Peak one is the contribution of Mg2, the magnesium ion coordinated by waters, whereas the more weakly coupled peak two arises from Mg1 which is coordinated entirely by phosphate oxygen donors.

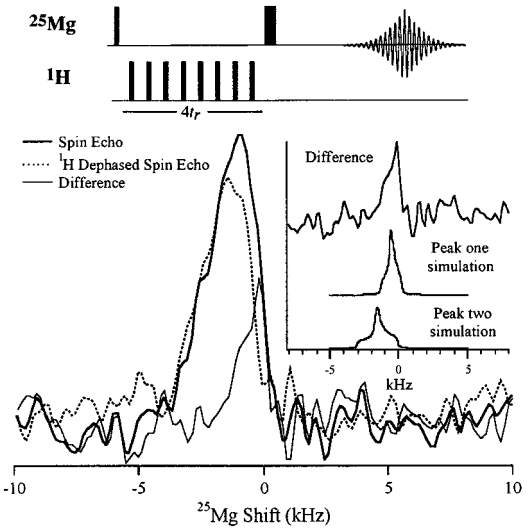

Figure 4. ^{25}Mg spin-echo data obtained utilizing the sequence shown on top with $\pi/2$ and π pulses of 4.4 μs and 8.8 μs in duration, respectively. An interpulse spacing of 400 μs allowed for the insertion of 8 ^{1}H π pulses ($xy8$ cycled), centered at $^1/_4$ and $^3/_4$ of the rotor period (t_r = 100 μs). The inset graph compares the difference spectrum with the line shapes simulated for peaks one and two using the parameters determined by MQMAS NMR.

These results show that the MQMAS experiment can reveal the presence of two magnesium sites in MgATP/BPA, and enable an estimate of their corresponding isotropic chemical shift and nuclear quadrupole coupling parameters. The selective reintroduction of dipolar couplings to nearby spins using a REDOR-type sequence then allows for the chemical assignment of the two peaks resolved by ^{25}Mg MQMAS. These experiments indicate an increase in the ^{25}Mg shielding as water substituents are replaced by phosphates, with the hexaaquo magnesium site appearing only 2 ppm away from the solution magnesium reference. Although these analyses clearly demonstrate the potential of high-resolution solid-state ^{25}Mg NMR as a tool for elucidating magnesium coordination environments in biological complexes, the question of the potential for scaling-up these experiments to larger biopolymers arises. Low-resolution studies of enriched magnesium(II) bound to DNA have previously been reported,[15] and it is reasonable to expect that the advent of higher magnetic fields coupled to ongoing improvements in the sensitivity of MQMAS[7−9,16,17] will enable high-resolution studies of systems at least an order of magnitude larger than the MgATP/BPA complex. Efforts to explore this hypothesis are currently under way.

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