Recent developments in solid-state nuclear magnetic resonance of quadrupolar nuclei and applications to biological systems

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Abstract: Recent advances in nuclear magnetic resonance (NMR) methodology and improvements in high-field NMR instrumentation have generated a new wave of research interests in the application of solid-state NMR to the study of quadrupolar nuclei. These developments now permit increasingly complex biological systems to be probed by quadrupolar NMR. In this review I describe a few recent developments in NMR studies of quadrupolar nuclei and demonstrate the potential of solid-state quadrupolar NMR in the study of biological systems. In particular, I discuss the application of solid-state NMR of $^{17}$O, $^{67}$Zn, $^{59}$Co, $^{23}$Na, and $^{39}$K nuclei with a prognosis for future work.

Key words: nuclear magnetic resonance, quadrupole, solid state, biological system.

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a powerful and versatile analytical technique that can provide site-specific information about chemical bonding, structure, and dynamics in molecular systems. NMR applications have made a major impact in a variety of disciplines ranging from materials science to molecular biology. Solid-state NMR is a branch of NMR spectroscopy that deals with solid or solidlike systems and is presently undergoing rapid expansion as a result of the significant advances in both NMR methodology and instrumentation that have occurred recently. To date most successful solid-state NMR applications to biological systems have utilized spin-1/2 nuclei such as $^{13}$C, $^{15}$N, and $^{31}$P (Griffiths and Griffin 1993; Evans 1995; Garbow and Gullion 1995). For a large number of biologically important elements, however, the only NMR active isotopes are those with nuclear spins greater than one half. Such nuclei have a nonspherical charge distribution and are known as quadrupolar nuclei, for example, $^{17}$O ($S = 5/2$), $^{23}$Na ($S = 3/2$), $^{25}$Mg ($S = 5/2$), $^{39}$K ($S = 3/2$), $^{43}$Ca ($S = 7/2$), $^{67}$Zn ($S = 5/2$), and $^{59}$Co ($S = 9/2$), to mention just a few. It should be noted that all of these examples are half-integer spins. In fact, there is another type of quadrupolar nuclei for which the spin number is an integer such as $^2$H ($S = 1$), $^6$Li ($S = 1$), $^{14}$N ($S = 1$), and $^{10}$B ($S = 3$). The integer spins have quite different NMR properties compared with those of half-integer nuclei; consequently, the techniques used to re-
more complete information about three-dimensional electronic structure. In contrast, because of rapid molecular tumbling in solutions, only averaged NMR parameters can be measured. Second, another effect of rapid molecular tumbling in solutions is to cause efficient quadrupolar relaxation leading to poor intrinsic resolution, which is a direct consequence of very short lifetimes of the quadrupole energy levels. In solids, quadrupole relaxation times are much longer, resulting in NMR spectra of high intrinsic resolution. Therefore, the dilemma of NMR studies of quadrupolar nuclei is that, although solid-state NMR spectra in principle contain more information and exhibit higher intrinsic resolution, it has been a challenge for experimentalists to devise NMR techniques that can yield such high-resolution spectra. A great deal of effort has been devoted in the last decade to developing new techniques such as dynamic-angle spinning (DAS) (Llor and Virlet 1988; Mueller et al. 1990) and double rotation (DOR) (Samoson et al. 1988; Chmelka et al. 1989; Wu et al. 1990) to overcome the difficulties encountered in obtaining high-resolution NMR spectra for quadrupolar nuclei. In the meantime, traditional techniques such as single-crystal NMR (Spiess et al. 1969; Kroeker et al. 1997), wide-line NMR (Baugh et al. 1969; Mooibroek et al. 1986; Power et al. 1990; Cheng et al. 1990), magic-angle spinning (Kundla et al. 1981; Samoson et al. 1982; Oldfield et al. 1982a, 1982b), and nutation NMR (Man 1986) continue to contribute to the studies of quadrupolar nuclei. All of these NMR techniques have severe limitations in one way or another; their direct applications to biological systems have been difficult.

Recently, Frydman and Harwood (1995) developed a new method for obtaining high-resolution NMR spectra of quadrupolar nuclei. The technique is known as multiple-quantum magic-angle spinning (MQMAS) NMR. The MQMAS method has made it possible to obtain high-resolution NMR spectra for quadrupolar nuclei under ordinary MAS conditions. The development of MQMAS may represent one of the most significant new approaches in solid-state NMR. It is an important step towards the ultimate goal of routinely obtaining solution-like NMR spectra for quadrupolar nuclei in the solid state and has consequently stirred tremendous excitement among researchers in the NMR community. It opens the door for extensive high-resolution quadrupolar NMR studies of a great variety of solids including biologically important macromolecules. Recent MQMAS results have clearly added a new dimension to solid-state NMR spectroscopy (Frydman and Amoureux 1995; Medek et al. 1995; Amoureux et al. 1996; Brown et al. 1996; Massiot 1996; Massiot et al. 1996; Rocha et al. 1996; Wu et al. 1996a, 1996b, 1997a, 1997b; Youngman et al. 1996; Brown and Winzerer 1997; Dirken et al. 1997; Duer and Stourton 1997; Frydman et al. 1997).

Partly inspired by Frydman’s beautiful experiment and partly because of recent technological advances related to high-field NMR instrumentation, a new direction of research is emerging. Solid-state NMR spectroscopy of quadrupolar nuclei is becoming a realistic and practical approach in the study of biological systems. In this review I describe a few recent developments in this rapidly expanding field, and demonstrate the potential of solid-state quadrupolar NMR in the study of biological systems. In particular, I discuss the application of solid-state, NMR of $^{17}$O, $^{67}$Zn, $^{59}$Co, $^{23}$Na, and $^{39}$K nuclei with a prognosis for future work.

Multiple-quantum magic-angle-spinning NMR

In this section I focus only on the recently developed MQMAS approach (Frydman and Harwood 1995). For other topics related to solid-state NMR of quadrupolar nuclei, readers are referred to several excellent articles (Freude and Haase 1993; Jäger 1994; Chmelka and Zwanziger 1994).

To illustrate the principles of MQMAS spectroscopy, consider a quadrupolar $S = 3/2$ spin system in a strong magnetic field. In the rotating frame, the spin Hamiltonian can be written as

$$ H = H_{CS} + H_{Q}^{(1)} + H_{Q}^{(2)} $$

where $H_{CS}$ describes the chemical shielding and $H_{Q}^{(1)}$ and $H_{Q}^{(2)}$ describe the first- and second-order quadrupole interactions, respectively. An energy level diagram for $S = 3/2$ is shown in Fig. 1. Here we are only concerned with the sym-
Fig. 2. The basic two-pulse sequence and coherence transfer pathway in MQMAS experiments

\[
\begin{align*}
H_{CS} & = \sum_{l=0,2}^{2} 2m_A a_{m}^{(1)} l Z_{m}^{l} \\
H_{Q}^{(2)} & = \sum_{l=0,2}^{2} \sum_{m} C_{lm} a_{m}^{(2)} l Z_{m}^{l} \\
\end{align*}
\]

where

\[
C_{lm}(S) = \frac{a_0}{a_0} [a_{m,-m}^{(1)} C(2, 2, l, 1, -1) + a_{m,-m}^{(2)} C(2, 2, l, 2, -2)]
\]

and

\[
\begin{align*}
a_{m,-m}^{(1)} & = m[4S(S+l) - 8m^2 - 1] \\
a_{m,-m}^{(2)} & = m[2S(S+l) - 2m^2 - 1] \\
a_0 & = \frac{e^2 aQ}{2S(S + 1)}
\end{align*}
\]

In the above equations, \(C(2, 2, l, 1, -1)\) and \(C(2, 2, l, 2, -2)\) are the Clebsch–Gordan coefficients. \(A_{m}^{S}\) and \(A_{m}^{Q}\) describe the orientation dependence of the chemical shielding and quadrupolar interactions, respectively. Under the sample rotation condition, both \(A_{m}^{S}\) and \(A_{m}^{Q}\) become time dependent and can be written using the Wigner rotation matrices as

\[
A_{m}^{CS,Q}(\tau) = \sum_{\mu=-\lambda}^{\lambda} A_{nm}^{\mu} \Omega^{SFC}(\tau) A_{m}^{S,Q}
\]

where \(\Omega^{SFC}(\tau)\) symbolizes the three Euler angles of the transformation from the laboratory frame to a sample fixed coordinate (SFC). In general, \(A_{m}^{CS,Q}(\tau)\) contains second- and fourth-rank Legendre polynomials for \(l = 2\) and \(l = 4\), respectively. Under the MAS condition, \(A_{m}^{CS,Q}\) vanishes, and eqs. 2 and 3 become

\[
H_{CS} = \sum_{m} 2m_A a_{m}^{(1)} l Z_{m}^{l} \\
H_{Q}^{(2)} = \sum_{m} |C_{lm} a_{m}^{(2)} l Z_{m}^{l}|
\]

Now one can obtain the resonance frequency for the \((m, -m)\) transition as

\[
\nu_{m,-m} = \nu_{iso}(m,-m) + \nu_{aniso}(m,-m)
\]

where

\[
\nu_{iso}(m,-m) = 2m_A a_{m}^{(1)} l Z_{m}^{l} \\
\nu_{aniso}(m,-m) = C_{4m} a_{m}^{(2)} l Z_{m}^{l}
\]

It is clear from the above equations that \(\nu_{aniso}\) is the source of the so-called second-order broadening observed in the central transition (1/2, –1/2) MAS spectra, since \(A_{m}^{(2)}\) is not averaged to zero by MAS.

Frydman and coworkers noted that \(C_{iso}\) may have an opposite sign to \(C_{4,1/2}\), which means that the evolution owing to the anisotropic part of the symmetric multiple-quantum (MQ) coherence may be reversed if the MQ coherence is transferred to the central transition (CT). In other words, the MQ and CT spectra are related by a pseudoreflection symmetry with a scaling factor, \(k = |C_{iso}/C_{4,1/2}|\), as also illustrated in Fig. 1. (For \(S = 3/2, k = 7/9\).)

The basic two-pulse pulse sequence for MQMAS experiments is shown in Fig. 2, together with the coherence transfer pathway. The first pulse, P1, is to excite the desirable MQ coherence. During the time, \(t_1\), the MQ coherence evolves with the frequency of \(\nu_{m,-m}\). The MQ coherence is then converted by the P2 pulse to the CT for detection. Since the CT evolves in an opposite sense from that of the MQ, the dephasing of the MQ coherence can be refocused after the MQ is converted to the CT coherence. Consequently, an echo will form at \(t_2 = k t_1\) and the echo intensity will be independent of the second-order quadrupolar broadenings. Most importantly, whereas the dephasing owing to the anisotropic part of the second-order quadrupole interaction, \(\nu_{aniso}(m,-m)\), is refocused at the echo maximum, information about the isotropic part of the second-order quadrupole interaction and the isotropic chemical shift is retained in the MQMAS experiment. Therefore, isotropic peaks with different peak positions will be observed in MQMAS spectra for chemically or crystallographically non-equivalent sites once the echo maxima are recorded and Fourier transformed. The coherence transfer pathway is selected by a proper phase cycling (Frydman and Harwood 1995). Of course, it is also possible to use the field-gradient technique to select the desirable coherence transfer pathway.

An example is shown in Fig. 3. For a stationary polycrystalline sample of sodium citrate trihydrate, the \(^{23}\)Na \((S = 3/2)\) NMR spectrum consists of a featureless line shape approximately 6 kHz wide. Under the MAS condition, the NMR spectrum becomes narrower but exhibits some overlapping features, from which very little useful information can be extracted. With the MQMAS technique, the NMR spectrum exhibits three isotropic peaks, indicating three crystallographically distinct sodium sites. MQMAS can also be applied to \(S = 5/2\) nuclei. Solid-state \(^{17}\)O \((S = 5/2)\) NMR
spectra of several inorganic phosphates are shown in Fig. 4. Clearly, the dramatic resolution improvement in the MQMAS spectra permits the detection of subtle differences in various oxygen sites. The conclusion derived from the $^{17}$O MQMAS spectra is consistent with that from the crystallographic symmetry (Wu et al. 1997a).

Solid-state NMR spectroscopy of quadrupolar nuclei, in particular MQMAS, is rapidly expanding. Despite its overwhelming initial success, the frontiers of solid-state quadrupolar NMR are replete with unsolved problems, many of which are related to the fundamentals of NMR. Future research on the development of new methodology for obtaining high-resolution NMR spectra for quadrupolar nuclei may be focused in the following two areas: (i) sensitivity improvement of MQMAS experiments and (ii) internuclear distance determination involving quadrupolar nuclei.

The utility of solid-state NMR spectroscopy as a tool for structure determination in biological systems has produced a new direction of research during the last decade, but successful applications have been limited to spin-1/2 systems such as those of $^{13}$C, $^{15}$N, and $^{31}$P (Griffiths and Griffin 1993; Garbow and Gullion 1995). Although there have been recent efforts to address the problem associated with quadrupolar nuclei such as the development of transfer of populations in double resonance (TRAPDOR; Grey and Veeman 1992) and rotational echo adiabatic-passage double resonance (REAPDOR; Gullion 1995), it still remains a challenge to determine internuclear distances in spin pairs involving quadrupolar nuclei (e.g., $^1$H-$^{17}$O and $^{13}$C-$^{17}$O). Research in this direction would be extremely important, since solid-state NMR potentially can pinpoint the local structure around a metal-binding pocket for noncrystalline biological samples.

**Oxygen-17 NMR**

Oxygen is a key constituent of many important functional groups in biological systems. Oxygen-17 ($S = 5/2$, natural abundance 0.037%) is the only naturally occurring, stable oxygen isotope with nonzero nuclear spin, which makes it suitable for NMR studies. Despite the enormous importance of oxygen-containing compounds in chemistry and biology, relatively few $^{17}$O NMR studies have been reported (Boykin 1991) compared with the applications of $^1$H and $^{13}$C NMR. The primary impediments of $^{17}$O NMR spectroscopy include low natural abundance of $^{17}$O nuclei and the fact that $^{17}$O is a quadrupole nucleus ($Q = -2.6 \times 10^{-30}$ m$^2$). These make $^{17}$O an extremely insensitive NMR nucleus (approximately $10^5$ times less sensitive than $^1$H). Therefore, it is not surprising that most of the $^{17}$O NMR studies so far reported have dealt only with solution samples of small molecules.

Solid-state $^{17}$O NMR applications have been rare and largely limited to studies of inorganic materials in which $^{17}$O quadrupole coupling constants are reasonably small, $e^2qQ/h < 6$ MHz (Schramm and Oldfield 1984; Walter et al. 1988; Chmelka et al. 1989; Mueller et al. 1991; Grandinetti et al. 1995; Younman et al. 1995; Bastow et al. 1996). Meanwhile, $^{17}$O NMR of organic–biological solids remains essentially an undeveloped research field. Besides an early single-crystal $^{17}$O NMR study of an organic compound, benzophenone (Scheubel et al. 1985), there have been only a few reports on systems of biological interest. Oldfield and coworkers investigated the interactions between $^{17}$O-labeled CO and O$_2$ ligands and hemoproteins and synthetic model compounds (Park et al. 1991; Oldfield et al. 1991). For the Fe-$^{17}$O$_2$ moiety, they observed remarkably large $^{17}$O chemical shift tensors but rather small quadrupole tensors for both the bridging and terminal oxygen atoms. A similar trend was observed for the CO ligand upon coordination to the Fe center. Gann et al. (1994) demonstrated the utility of cross-polarization (CP) DAS in resolving two nonequivalent oxygen atoms in $^{17}$O-labeled l-alanine. The two nonequivalent oxygen atoms are due to the different hydrogen-bonding environments. In this study, CP from $^1$H to $^{17}$O was achieved when the sample was spun about a direction parallel to the...
that with a combination of high magnetic fields (e.g., 18.8 T), fast sample spinning (e.g., >25 kHz), and NMR probes that can deliver high radiofrequency (RF) power, $^{17}$O MQMAS NMR will be feasible and useful in the study of biologically important macromolecules. The great advantage of $^{17}$O NMR spectroscopy lies in the remarkable sensitivity of $^{17}$O NMR parameters to molecular structure and hydrogen bonding effects. Future solid-state $^{17}$O NMR studies in the following areas are likely to be important.

**Oxygen-17 chemical shift tensors**

Despite numerous reported empirical correlations concerning $^{17}$O isotropic chemical shift and molecular structure, very little is known about the tensorial nature of this fundamental NMR parameter, the $^{17}$O chemical shift tensor. A knowledge of the complete $^{17}$O chemical shift tensor (rather than only its trace) is essential for understanding the relationship between $^{17}$O chemical shift and molecular structure. Furthermore, recent developments in ab initio molecular orbital (MO) shielding calculations have made calculations of $^{17}$O chemical shielding possible. However, the quality of $^{17}$O chemical shielding results calculated by the state-of-the-art MO methods is uncertain, simply because very little experimental data are available for $^{17}$O chemical shift tensors. Clearly it is necessary to carry out systematic measurements of $^{17}$O chemical shift tensors in systems with biologically relevant functional groups such as amide, carboxyl, phenol, and hydroxyl groups. It is of fundamental importance to establish correlation between $^{17}$O chemical shift tensor and molecular structure. A similar trend has emerged in solid-state $^{13}$C NMR studies of proteins (Le et al. 1995). To determine an $^{17}$O chemical shift tensor completely (i.e., both its magnitude and its orientation in the molecular frame) and to understand better this NMR parameter, both single-crystal NMR and ab initio quantum mechanical shielding calculations will be helpful.

**Hydrogen-bonded systems**

Hydrogen bonding effects are fundamental to the structure and function of many biological systems. The fact that both isotropic $^{17}$O chemical shift and quadrupole coupling are sensitive to intra- and inter-molecular hydrogen bonding has been previously observed (Boykin 1991). However, I expect that characterization of the dependence of the entire $^{17}$O chemical shift tensor on hydrogen-bonding effects will represent a promising route to delineating complete three-dimensional hydrogen-bond networks. A recent theoretical study has indicated that the $^{17}$O chemical shift tensor in a model dipeptide, Gly-Gly, is sensitive to the state of hydration, whereas only small effects are noted in the $^{13}$C and $^{15}$N chemical shift tensors (Chestnut and Phung 1993). Of particular interest is the possibility of using $^{17}$O NMR to detect the protonation state of amino acid side groups (e.g., the carboxylates) in proteins.

**Binding of small ligands to biomolecules**

Application of solution $^{17}$O NMR to the study of enzyme–substrate complexes was demonstrated some time ago (Wisner et al. 1985). However, $^{17}$O NMR spectra of enzyme–substrate complexes are often plagued by broad lines resulting from efficient quadrupole relaxation, especially when the ligand is...
tightness. Solid-state NMR is an ideal technique for studying small ligands bound to macromolecules and is not as severely limited by the molecular weight of the complex under study as is solution $^{17}$O NMR. It will be potentially important to explore the utility of $^{17}$O-labeled ligands as a reporter to probe the local chemical environment at the binding site. For example, solid-state $^{17}$O NMR studies of nucleotide–enzyme complexes will be intriguing and feasible, since $^{17}$O-labeled nucleotides can be prepared.

The extremely low natural abundance of the $^{17}$O isotope (0.037%) excludes the possibility of obtaining NMR spectra of biological systems with naturally occurring $^{17}$O nuclei. Future research will require site-specific $^{17}$O labeling. Therefore, it is necessary to develop efficient synthetic routes for introducing $^{17}$O onto important functional groups. Fortunately, many methods have been developed over the years for $^{17/18}$O labeling a variety of organic and biological compounds such as amino acids (Steinschneider et al. 1985; Kabalka and Goudgaon 1991), amino acid side chains (Eckert and Fiat 1986), polypeptides (Gilboa et al. 1984; Sakarellos et al. 1989), nucleic acid bases (Petersheim et al. 1983; Chandresekaran et al. 1985; Amantea et al. 1996), and duplex DNA (Wild et al. 1989).

The future promise of solid-state $^{17}$O NMR lies in the possibility of its becoming an additional nuclear probe for studying biological systems. This is a new and exciting frontier of considerable potential. Research along this line may provide a unique approach to the study of molecular structure and dynamics.

### Probing metal ion environments in biological systems

It has long been recognized that metal ions are vital to many biological processes. Recent discoveries about the crucial role of metal ions in gene expression, in RNA catalysis, and in anticancer drugs have had tremendous impact on a variety of problems ranging from metalloprotein and ribozyme chemistry to medicine (Lippard and Berg 1994). With only a few notable exceptions, quadrupolar metal ions tightly bound to biological macromolecules have been NMR invisible in liquid media (Forsén et al. 1987; Sanders and Tsai 1989). This is because efficient nuclear quadrupole relaxation in solutions often results in undetectably broad NMR peaks. Therefore, it is commonly accepted lore that site resolution from solution NMR spectra of quadrupolar nuclei is always elusive.

However, Vogel and coworkers recently demonstrated that for large metalloproteins (ca. 80 kDa), it is actually possible to achieve sufficient site resolution in solution NMR spectra of quadrupolar nuclei at high magnetic fields (Aramini et al. 1993a, 1993b, 1994; Aramini and Vogel 1994; Germann et al. 1994). For large metalloproteins at the so-called slow-motion regime (i.e., $\tau_D t > 1$), the line width of the central transition decreases with increasing applied magnetic field. Consequently, relatively sharp signals can be obtained at high magnetic fields (greater than 11.7 T) for metal ions tightly bound to a large protein. The approach was referred to as quadrupolar central transition (QCT) spectroscopy. It is interesting to compare QCT with solid-state NMR experiments. As mentioned earlier, in solid-state NMR of quadrupolar nuclei, one is also focused on the central transition whose line width is influenced by quadrupole interactions only to the second order. Furthermore, the second-order quadrupole broadening is inversely proportional to the applied magnetic field. Thus it is not surprising that both QCT and solid-state NMR experiments should be performed at the highest magnetic field possible. Solid-state NMR experiments may yield more information about other anisotropic nuclear spin interactions such as chemical shift anisotropy and heteronuclear dipolar interactions, which may contain valuable structural information. On the other hand, additional information may overwhelm solid-state NMR spectra, making their interpretation difficult. Nevertheless, it may prove advantageous to apply both QCT and solid-state NMR techniques in the study of large metalloproteins. In this section, I briefly discuss the present situation of solid-state metal NMR with emphasis on studies of $^{63/65}$Zn, $^{59}$Co, $^{23}$Na, and $^{39}$K nuclei.

### Zinc-67 NMR

Zinc is among the most important metal ions in biology. Zinc(II), as a diamagnetic $^{65}$Zn ion, is often referred to as being spectroscopically silent, since one cannot use EPR, UV, and visible spectroscopies to probe the nature of the Zn(II) site in an enzyme. In addition, the unfavorable NMR properties of $^{67}$Zn ($S = 5/2$, natural abundance 4.11%, $Q = 0.16 \times 10^{28} \text{ m}^{-2}$) nuclei have prevented researchers from exploiting $^{67}$Zn as a nuclear probe to biological systems. In fact, $^{67}$Zn NMR studies are scarce even for small compounds (Granger 1991). Direct NMR detection of the metal binding sites in zinc-containing proteins has entirely relied on the utility of a surrogate nuclear probe, $^{113}$Cd, which is an NMR-friendly spin-1/2 nucleus (McAteer et al. 1996). However, the question as to the validity of using cadmium to model the zinc ion environment remains partially unanswered, because the connection between zinc ion environments and $^{67}$Zn NMR parameters in native zinc metalloproteins has not yet been established.

For several zinc-containing compounds in which the cubic crystallographic symmetry at the zinc site results in negligible effects from quadrupolar interactions, $^{67}$Zn NMR parameters were reported in the solid state (Haller et al. 1980; Wu et al. 1995). In the case of $\text{K}_{2}\text{Zn(CN)}_4$, indirect spin–spin ($J$-coupling) between $^{13}$C and $^{67}$Zn nuclei is observed in the $^{67}$Zn MAS spectrum. $^{1}J_{(13\text{C}, \ 67\text{Zn})} = 88$ Hz, which appears to be the only $J$-coupling constant known involving $^{67}$Zn (Wu et al. 1995). There is only one solid-state $^{67}$Zn NMR study on compounds with noncubic symmetry. Using a spin-echo technique, Oldfield and coworkers recorded a static $^{67}$Zn NMR spectrum of zinc acetate dihydrate at 11.75 T (Kunwar et al. 1986). It is seen in Fig. 6 that the static line shape arising from the second-order quadrupolar interaction is about 50 kHz wide, indicating a large quadrupole coupling constant. As also shown in Fig. 6, the crystal structure of zinc acetate dihydrate (van Nierkerk et al. 1953) exhibits a very distorted octahedral zinc site, which is responsible for the large, asymmetric $^{67}$Zn quadrupole tensor, $e^2Q/h = 5.3$ MHz and $\eta = 0.87$. In an early solution $^{67}$Zn NMR study of the zinc(II)-insulin complex, Shimizu and Hatan: (1983) estimated the $^{67}$Zn quadrupolar coupling
constant for the zinc sites in insulin to be 1.86 MHz. However, there is serious suspicion about this estimated value, considering that each of the two zinc sites in the two-zinc insulin hexamer is coordinated by three imidazolyl nitrogen atoms and three water molecules (Adams et al. 1969). Regardless, assuming $e^2qQ/h = 1.86$ MHz and a magnetic field of 11.7 T, one would expect a line width of approximately 4 kHz in static $^{67}$Zn NMR spectra of insulin. This is significantly narrower than that observed for zinc acetate dihydrate.

The unique feature of the zinc active site in all mononuclear zinc metalloenzymes characterized to this date is an unsymmetrical tetrahedral geometry with one of the ligands being a water molecule. However, the $^{67}$Zn quadrupole coupling constant is unavailable for any complex with unsymmetrical tetrahedral coordination. Pentacoordinated zinc sites have also been found in enzymes containing trinuclear metal clusters. To assess the feasibility of using solid-state $^{67}$Zn NMR in the study of zinc metalloproteins, it is necessary to obtain fundamental $^{67}$Zn NMR parameters such as quadrupolar and chemical shift tensors in model systems of typical ion coordination geometries. To this end, single-crystal $^{67}$Zn NMR studies are underway in our laboratory. To circumvent the low sensitivity of $^{67}$Zn NMR, isotopic $^{67}$Zn enrichment may be necessary in future solid-state $^{67}$Zn NMR studies, especially for large zinc–biomolecule complexes. Given the similar NMR properties of $^{67}$Zn and $^{43}$Ca ($S = 7/2$) nuclei, it is perhaps worth mentioning that natural abundance solid-state $^{43}$Ca NMR studies of inorganic compounds were recently reported by Dupree et al. (1997).

**Cobalt-59 NMR**

Cobalt is one of the so-called ultratrace metals found in metalloenzymes. Cobalt is present in vitamin B$_{12}$ (Fig. 7) and its coenzyme, which is the first naturally occurring organometallic compound ever identified. Cobalt-59 ($S = 7/2$, natural abundance 100%) is the only stable isotope of cobalt and is also one of the most sensitive nuclei for NMR measurements. One would think naturally to apply $^{59}$Co NMR to biological systems because of the apparent interest in vitamin B$_{12}$ chemistry. However, except for an early single-crystal $^{59}$Co NMR study by Spiess et al. (1969), applications of solid-state $^{59}$Co NMR have been largely restricted to simple compounds with highly symmetric cobalt environments (Reynhardt 1974a, 1974b; Eaton et al. 1987; Chung et al. 1993; Hayashi 1996; Eichele et al. 1997). This is because both quadrupole interaction and chemical shift of $^{59}$Co nuclei are highly anisotropic, often resulting in very broad NMR lines for polycrystalline samples.

Not until very recently have researchers attempted to tackle more complicated systems with solid-state $^{59}$Co NMR. In a study of hexacoordinated cobalt porphyrin complexes, Medek et al. (1997a) reported $^{59}$Co NMR spectra for a series of polycrystalline compounds under both static and fast MAS conditions at different magnetic fields. Since Co(III) porphyrin complexes are isoelectronic to Fe(II) hemochromes, they may be used as model systems in understanding the electronic structures in the latter compounds. Cobalt-59 NMR is capable of yielding useful information about the electronic structure at the metal center. It was found that the Co(III) porphyrin complexes exhibit rather small $^{59}$Co quadrupole coupling but very large chemical shift anisotropy. Interestingly, the relation between $^{59}$Co quadrupole coupling and chemical shift tensors found by Medek et al. (1997a) deviates considerably from that previously known for simple octahedral cobalt complexes. The origin of such a discrepancy is unclear at this time.

Medek et al. (1997b) also investigated a series of naturally occurring cobalamin and synthetic cobaloximes. In particular, they obtained solid-state $^{59}$Co NMR spectra of static samples of vitamin B$_{12}$, the B$_{12}$ coenzyme, methylcobalamin, and dicyanocobyrinic acid heptamethylester. They found that the NMR parameters are sensitive to the type of ligands attached to the metal and to the crystallization history of the sample. In addition, they studied several synthetic cobaloximes of alkyl, cyanide, aquo, and nitrogenated axial ligands. All of the reported solid-state $^{59}$Co NMR spectra exhibit broad lines whose widths range between 100 and 400 kHz as a result of a combination of chemical shift anisotropy and second-order quadrupolar interactions.
As Spiess et al. (1969) demonstrated, single-crystal $^{59}\text{Co}$ NMR spectra exhibit relatively narrow lines and can be used to study systems with large anisotropic spin interactions. In addition, single-crystal NMR is the method of choice for unambiguous determination of a nuclear spin interaction tensor in the molecular frame. A beautiful example is the recent single-crystal $^{59}\text{Co}$ NMR study of vitamin B$_{12}$ by Power et al. (1998). Analysis of the single-crystal NMR spectra yields both the chemical shift and quadrupole coupling tensors of the $^{59}\text{Co}$ nucleus:

$\delta_{11} = 5075$, $\delta_{22} = 4670$, $\delta_{33} = 3901$ ppm; $e^2qQ/h = 27.3$ MHz and $\eta = 0.24$. These results are in excellent agreement with those obtained by Medek et al. (1997b) from a study of polycrystalline vitamin B$_{12}$ samples. Most importantly, the single-crystal NMR study of Power et al. (1998) also yields the orientations of the two $^{59}\text{Co}$ NMR tensors in the molecular frame. As shown in Fig. 8, the most shielded component of the chemical shift tensor, $\delta_{33}$, is found to point approximately through the center of one triangular face of the octahedral cobalt coordination. The least shielded component, $\delta_{11}$, is directed toward the phosphorus atom, whereas the $\delta_{22}$ component is in the corrin plane. The orientation of the quadrupole coupling tensor is such that the largest component of the quadrupole coupling tensor deviates by approximately 10° from the Co–N direction, whereas the other two components lie within the plane of the corrin ring. This is the first report of a single-crystal NMR study of a metal center in a biological molecule. The exciting results from these solid-state $^{59}\text{Co}$ NMR studies will surely encourage further NMR investigations of quadrupolar metal isotopes in biological systems.

**Sodium-23 and potassium-39 NMR**

Unlike divalent metal ions, monovalent ions such as alkali metal cations generally exhibit weak association to biological macromolecules. Their roles have been thought primarily to be as bulk electrolytes that stabilize surface charges on proteins and nucleic acids. However, recent findings revealed a unique structural role of potassium and sodium ions in the form of G-quartet structures in telomeric DNAs, inspiring new views about the role that alkali metals may play in biological processes (Williamson 1994). Telomeres are located at the ends of linear eukaryotic chromosomes and consist of tandem arrays of G-rich sequences. The main structural motif of such G-rich DNAs is known as the G-quartet (Williamson 1994). The G-quartet structure, as depicted in Fig. 9, consists of four guanine base pairs connected by the Hoogsteen pattern of hydrogen bonds. The remarkable stability of the G-quartet structure arises from the presence of monovalent cations, usually K$^+$ or Na$^+$. The cation-binding site in the G-quartet is thought to be at the center of a cavity formed by the eight O6 oxygen atoms from two stacking G-quartets (Sundquist and Klug 1989), similar to the K$^+$-stabilized structure of 5'-GMP (Detellier and Laszlo 1980). A recent X-ray crystallographic study of d(GGGGTTTTGGGG) (Kang et al. 1992) essentially confirmed this model of ion coordination environment; however, the crystal structure of d(TGGGGT) also revealed
some variations in ion-binding environments (Laughlan et al. 1994). Interestingly, a total of seven Na⁺ ions are found in d(TGGGGT), as shown in Fig. 10. The seven sodium ions are located between and within planes of hydrogen-bonded guanine quartets. Each Na⁺ ion, except Na(1) and Na(1’), is coordinated by eight guanine O6 atoms from the two stacking G-quartet planes. Na(1) and Na(1’) each is coordinated by four guanine O6 atoms and one water molecule at the axial position, giving rise to a square pyramidal geometry.

Another interesting aspect of ion binding to G-DNAs is the ion-binding stoichiometry. It is conceivable that alkali metal ions can bind to a G-DNA with different stoichiometries. For example, while there is only one K⁺ ion located at the center of four stacking G-quartets in d(GGGGTGGGGT) (Kang et al. 1992), two Na⁺ ions are deduced from solution ²³Na NMR study of the same DNA (Deng and Braunlin 1996). As noted earlier, there are seven Na⁺ ions interacting with the eight G-quartets in d(TGGGGT) (Laughlan et al. 1994). Recently, Hud et al. (1996) showed that there are at least two ions (either Na⁺ or K⁺) bound to the three G-quartets in d(GGGTTTTGGG). It remains unclear what factors determine the ion-binding stoichiometry in G-DNAs.

Although solution NMR of alkali metal ions was used in the early NMR applications to self-assembled systems (Detellier and Laszlo 1980), solid-state alkali metal NMR has found very little application in biological systems. This is partially because the small chemical shift range and quadrupole broadening of alkali metal nuclei often prevent one from observing separate signals from different binding sites. However, with a combination of high magnetic fields and the recently developed MQMAS technique, it may be possible to obtain solid-state NMR spectra of resolution sufficient to resolve different ion-binding sites.

Because of the striking similarity in ion coordination environment between G-DNAs and antibiotic ionophores (Dobler 1981), the latter should serve as excellent model systems for establishing a correlation between alkali metal NMR parameters and ion-binding geometry. This will be of obvious importance to further NMR studies of G-DNAs and related self-assembled systems. For instance, the Na⁺ ion in nonactin–NaSCN complex (Dobler and Phizackerley 1974) is coordinated by eight oxygen atoms as depicted in Fig. 11. An early solution NMR relaxation study of nonactin–Na⁺ complex by Saitô and Tabeta (1987) yielded an estimate for the ²³Na quadrupole coupling constant, \( e^2qQ/h \approx 0.8 \) MHz. Since Na(4) of d(TGGGGT) is equidistantly coordinated by all eight O6 atoms, it is expected that \( e^2qQ/h \approx 0.8 \) MHz for this site. It is also known that for Na⁺ ions with square pyramidal coordination geometry, ²³Na quadrupole coupling constants are \(-2.8 \) MHz (Koller et al. 1994), which should be a reasonable estimate for the Na(1) and Na(1’) sites. Several years ago, Saitô and coworkers also carried out extensive solid-state ²³Na NMR studies of sodium complexes with ionophores (Tabeta et al. 1986); unfortunately, the quality of the experimental data, obtained with techniques available at the time, not only prevented them from obtaining any useful ²³Na quadrupole parameters, but also led to

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erroneous $^{23}\text{Na}$ chemical shifts. Clearly, a reexamination of alkali metal–ionophore complexes is in order.

On the basis of the crystal structures of d(TGGGGT) and d(GGGGTTTTGGGG) described above, it is anticipated that K$^+$ binding sites in G-DNAs are generally of high symmetry, thus having a small $^{39}\text{K}$ quadrupole coupling constant, and that these K$^+$ ions may be readily accessible by solid-state $^{39}\text{K}$ NMR. It is worth noting that solid-state $^{39}\text{K}$ NMR studies have been successful in systems with more distorted ion coordination environments, $e^2qQ/h = 0.87–3.2$ MHz (Kunwar et al. 1986; Kim et al. 1996). Since similar alkali metal stabilized G-quartet structures also exist in RNAs (Kim et al. 1991; Cheong and Moore 1992), it is also possible to apply solid-state $^{39}\text{K}$ and $^{23}\text{Na}$ NMR to the study of ion-binding environments in G-rich RNAs.

Recently, a K$^+$-specific binding site was structurally characterized for the first time in a protein, dialkylglycine decarboxylase (Toney et al. 1993). Its high-resolution crystal structure revealed that the K$^+$ ion is coordinated by six oxygen atoms in octahedral geometry, similar to those found in many K$^+$-selective ionophores such as valinomycin (Hamilton et al. 1981). Interestingly, the $^{39}\text{K}$ quadrupole coupling constant in valinomycin is only 1.2 MHz (Neurohr et al. 1983), which suggests that it may be possible to use solid-state $^{39}\text{K}$ NMR to study ion binding in K$^+$-transporting proteins.

**Conclusions**

The recent invention of MQMAS spectroscopy by Frydman and coworkers has triggered a new wave of research interest in the study of quadrupolar nuclei. The examples described in this review are a survey of what is presently occurring in this research area. These initial results indicate that new areas of research are becoming available. One of the most promising aspects of solid-state NMR of quadrupolar nuclei, especially of MQMAS, is to provide a means of directly observing individual binding sites in metalloproteins consisting of multiple sites. Since most quadrupolar nuclei, especially metal ions, have been previously inaccessible in biological solids, future research in this area may provide a unique approach to the study of ion-binding structures, and ultimately to the revelation of binding specificity and functional roles (structural or catalytic) of metal cofactors in biological systems. In the meantime, since quadrupolar nuclei occupy over 70% of the NMR periodic table, effort in developing new NMR methodology for studying quadrupolar spin systems will have sig-
significant impact on many research areas in chemistry and biology. In any case, researchers should not limit themselves to only one particular type of NMR technique. As I have shown in this review, different solid-state NMR techniques ranging from single-crystal NMR and MAS to MQMAS could all be useful to the study of biological systems.

Significant advances in NMR methodology and continuing development of high-field, high-sensitivity NMR instrumentation will permit increasingly complex biological systems to be probed by quadrupolar NMR. We anticipate a rapid evolution of this new research field. At high magnetic fields, NMR of quadrupolar nuclei may possibly become as routine as NMR of spin-1/2 nuclei such as $^{13}$C or $^{15}$N. This is a truly compelling prospect, since the latter has already had a major impact on chemistry, biology, and materials science.

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