A Single-Crystal ^{59}Co NMR Study of Vitamin B_{12}

William P. Power,* Christopher W. Kirby, and Nicholas J. Taylor

Contribution from the Guelph-Waterloo Center for Graduate Work in Chemistry, Department of Chemistry, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

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Abstract: A ^{59}Co solid-state NMR study of a single crystal of vitamin B_{12} was performed at 11.75 T. The results showed that the cobalt nucleus in this biologically relevant molecule experiences a highly anisotropic chemical shift spanning 1200 ppm about the isotropic chemical shift of 4549 ppm. The large electric field gradient of the cobalt site results in a quadrupolar coupling constant of 27.31 MHz with asymmetry of 0.243. The two tensors describing the orientation dependence of these NMR interactions exhibit distinct orientations with respect to the molecule, the quadrupolar coupling lying close to the axial direction, and the chemical shift tensor tilted 41° away from this direction. This study demonstrates the importance and feasibility of single-crystal NMR to provide this orientation information in metal-centered biological assemblies, and supports the prospects for solid-state NMR of quadrupolar metal nuclei to contribute further to the elucidation of metal participation in biological systems.

Introduction

Metals play a vital role in many biological systems, occurring at sites of catalytic or structural importance in various ring-based, protein, and nucleic acid assemblies.[1,2] Numerous techniques have been used to study metal centers in such materials,[3] including X-ray absorption and diffraction, optical/ultraviolet spectroscopy, and electron paramagnetic and nuclear magnetic resonance (NMR) spectroscopy. Of these, perhaps NMR is the most versatile and widely used technique, with applications to systems containing both paramagnetic[4] and diamagnetic metal sites. Studies of these metal-centered biological materials by NMR involve predominantly ^{1}H nuclei,[5] with extensions to ^{13}C or ^{15}N,[6] to characterize the organic assembly around the metal. Direct observation of the metal nuclei themselves is generally avoided, although in many cases the nucleus is directly involved in the biological activity. In cases where NMR of metal nuclei has been used, such studies almost invariably deal with spin-1/2 isotopes,[7] such as $^{111/113}$Cd, ^{195}Pt, or ^{199}Hg. Quadrupolar nuclei (those isotopes with spin $I > 1/2$), although naturally occurring, are deemed unsuitable because of their efficient quadrupolar relaxation and concomitant broad lines; hence they are often substituted by unnatural metals possessing spin-1/2 isotopes, e.g., ^{67}Zn for ^{113}Cd or ^{199}Hg. Such metal replacements often cause a severe decrease in, or complete loss of, biological activity, thus precluding meaningful comparisons with the biologically relevant systems.

The increasing availability of high-magnetic-field ($B_o \geq 11.75$ T) NMR spectrometers makes NMR of quadrupolar nuclei increasingly accessible, particularly in solid samples, where new techniques, such as multiple-quantum magic-angle spinning (MQMAS)[8] and double rotation (DOR),[9] and new wide-line NMR capabilities permit acquisition of their spectra under high-resolution conditions. Also available from the NMR analysis of solids is the orientation dependence or anisotropic information contained in the nuclear spin interactions of chemical shielding and quadrupolar coupling.[10] Described by second-rank tensors, the orientation dependence of these interactions provides detailed descriptions of the local three-dimensional electronic environment. Chemical shielding depends largely on the electronic singlet excited states necessary for the paramagnetic electron currents at its origin. Quadrupolar coupling, through the local electric field gradient at the nucleus, depends on the ground-state electronic distribution. Together, these two tensorial interactions provide a wealth of information concerning the local structure around a metal site. Our ability to interpret NMR spectra of quadrupolar nuclei in terms of the local structure depends on an understanding of the values and orientations these tensors adopt in different environments.

Vitamin B_{12} (cyanocobalamin) and its related coenzymes constitute an important family of compounds for which elucidation of the local environment of the metal (in this case, cobalt) may lead to better understanding of the functions of these molecules in enzymatic pathways. As B_{12} and its derivatives are the only naturally occurring bio-organometallic compounds, various features of their structures have been postulated as promoting factors in the cobalt−carbon bond scission that is at the root of their biological function. Deformation of the corrin ring has been proposed to facilitate methyl transfer and generation of the alkyl radicals in which the B_{12} coenzymes are operative;[11] these deformations have been explicitly dem-

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* To whom correspondence should be addressed. Tel: (519) 888-4567, ext 3626. Fax: (519) 746-0435. E-mail: wppower@uwaterloo.ca.

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onstrated recently in B_{12} and in an imidazole-based derivative.[12] X-ray crystallography of the 27-kDa B_{12}-binding fragment of *Escherichia coli* methionine synthase has shown that the dimethylbenzimidazole “tail” of methylcobalamin is displaced by a histidine residue of the protein.[13] The changes in geometry and electronic structure at the cobalt center that accompany the actions of such mechanisms should be readily identifiable through characterization of sensitive probes of local metal environment. Specifically, the ^{59}Co chemical shielding and quadrupolar coupling nuclear spin interactions should be altered. Significantly, observation of the metal nucleus by means of NMR provides highly selective information about the metal site alone with no spectral overlap from neighboring parts of the molecule, as would occur in ^{1}H NMR or infrared spectroscopy. In the case of ^{59}Co, this information is available without isotopic enrichment or other modification of the native biological compound. Although single-crystal NMR is the method of choice described here, single crystals are not necessary for solid-state NMR spectroscopy.

With such goals in mind, we performed a solid-state ^{59}Co NMR study of a single crystal of vitamin B_{12}. Cobalt occurs naturally as one nuclear isotope, ^{59}Co, which has a nuclear spin $I = 7/2$, a relatively large nuclear quadrupole moment of 0.4×10^{-28} m^2, and a large chemical shift range of 18 000 ppm. These characteristics make ^{59}Co NMR exquisitely sensitive to the local cobalt environment. Our results provide unambiguous information concerning the orientations (with respect to the molecular frame) and magnitudes of the principal components of both the chemical shielding and quadrupolar coupling tensors for the cobalt center in this biological molecule. Our work represents the first report of a single-crystal NMR study of a metal center in a biologically relevant system. These results provide the potential to understand changes in the chemical shielding and quadrupolar coupling interactions of ^{59}Co in vitamin B_{12} and its derivatives in terms of explicit alterations of the metal environment. It also demonstrates that similar *direct* monitoring of metal sites in other diamagnetic biological metal systems involving quadrupolar metal isotopes should be possible through metal NMR of polycrystalline or single-crystal samples.

Theory

When placed in a homogeneous magnetic field, quadrupolar nuclei in a solid sample exhibit anisotropic NMR spectra that can be described in terms of contributions from several nuclear spin interactions: Z is the (dominant) Zeeman interaction, Q the nuclear quadrupolar coupling CS the chemical shielding interaction, D the (homonuclear and heteronuclear) direct dipolar coupling and J the (homonuclear and heteronuclear) indirect spin−spin (J) coupling. The only interactions relevant in this report are the first three, Z, Q, and CS; hence the frequency response can be summarized as,

$$\nu = \nu_Z + \nu_Q + \nu_{CS} \tag{1}$$

Direct and indirect coupling involving ^{59}Co can be safely neglected because of their much smaller magnitudes.

For nuclei with large quadrupolar coupling constants, the quadrupolar interaction is no longer adequately described by first-order perturbation theory, and its influence to second-order must be considered.[14] A further consequence of this feature is that the satellite transitions (those other than the central $+1/2 \leftrightarrow -1/2$ transition) are dispersed widely to either side of the Larmor frequency, ν_o, falling outside the spectral window hence generally are not observed. The NMR frequency response of the central transition ($+1/2 \leftrightarrow -1/2$) of a half-integer-spin quadrupolar nucleus (such as ^{59}Co, $I = 7/2$) in a solid powder sample under the influence of the three terms Z, Q, and CS described above is given by,

$$\nu(\theta,\phi,\vartheta,\varphi) = \nu_o [1 - (\sigma_{11} \sin^2\theta \cos^2\phi + \sigma_{22} \sin^2\theta \sin^2\phi + \sigma_{33} \cos^2\theta)] - \frac{9\chi^2}{6\nu_o} \frac{\left[I(I+1) - \frac{3}{4}\right]}{[2I(2I-1)]^2} \times [A(\varphi) \cos^4\vartheta + B(\varphi) \cos^2\vartheta + C(\varphi)] \tag{2}$$

where

$$\nu_o = \frac{\gamma B_o}{2\pi} \tag{3}$$

σ_{11}, σ_{22}, and σ_{33} are the three principal components of the chemical shielding tensor; χ is the quadrupolar coupling constant that results from the product of the nuclear quadrupole moment eQ and the electric field gradient eq:

$$\chi = \frac{eQeq}{h} \tag{4}$$

I is the nuclear spin quantum number,

$$A(\varphi) = -\frac{27}{8} - \frac{9}{4}\eta \cos 2\varphi - \frac{3}{8}\eta^2 \cos^2 2\varphi \tag{5}$$

$$B(\varphi) = -\frac{30}{8} - \frac{1}{2}\eta^2 + 2\eta \cos 2\varphi + \frac{3}{4}\eta^2 \cos^2 2\varphi \tag{6}$$

$$C(\varphi) = -\frac{3}{8} + \frac{1}{3}\eta^2 + \frac{1}{4}\eta \cos 2\varphi - \frac{3}{8}\eta^2 \cos^2 2\varphi \tag{7}$$

and η, is the asymmetry parameter of the quadrupolar coupling tensor. The angles (θ,ϕ) and (ϑ,φ) describe the orientation of the chemical shielding and quadrupolar coupling tensors, respectively, with respect to the magnetic field direction. These angles are different, because these tensors may be oriented independently at the nucleus, unless their orientations are restricted by the point symmetry at the nuclear site. Chemical shielding σ_{ii} is not directly measured in practice, because it is a scale with respect to a bare nucleus. Experimentally, it is much more convenient to measure chemical shifts δ_{ii} from a standard reference signal. Since chemical shielding decreases and chemical shifts increase with increasing frequency, chemical shifts can substituted into eq 2 as the term $(\delta_{11} \sin^2\theta \cos^2\phi + \delta_{22} \sin^2\theta \sin^2\phi + \delta_{33} \cos^2\theta)$ in place of the chemical shielding term $[1 - (\sigma_{11} \sin^2\theta \cos^2\phi + \sigma_{22} \sin^2\theta \sin^2\phi + \sigma_{33} \cos^2\theta)]$.

In a powder sample, all possible values of the orientation angles are represented in a spherical distribution, resulting in a broad anisotropic line shape in the NMR spectrum. In a single crystal, discrete values of these angles are sampled individually, resulting in a set of (relatively) narrow lines, the positions of which vary with the orientation of the crystal in the magnetic field (i.e., values of the angles). The range of frequencies over which these lines occur follows the distribution that would be observed for a powder sample. The explicit dependence of the peak positions on the orientation of the crystal can be used to determine unambiguously the magnitudes and orientations of the principal components of the chemical shift and quadrupolar coupling tensors, as first demonstrated by Volkoff.[15] To provide 15 parameters that fully describe the orientation dependence of the chemical shift and quadrupolar coupling tensors, we fit the line positions as a function of the rotation angle ρ about each of three orthogonal rotation axes, $(i, j, k) = (x, y, z)$ (and cyclic permutations), to

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$$\nu_i(\rho) = A_i + B_i \cos 2\rho + C_i \sin 2\rho + D_i \cos 4\rho + E_i \sin 4\rho \quad (8)$$

where

$$A_i = (q/8)[-11(Q_{jj}{}^2 + Q_{kk}{}^2) - 50\, Q_{jj}Q_{kk} + 28\, Q_{jk}{}^2 + 16(Q_{ki}{}^2 + Q_{ij}{}^2)] + (\delta_{jj} + \delta_{kk})\nu_o/2 \quad (9)$$

$$B_i = (3q/2)(Q_{jj}{}^2 - Q_{kk}{}^2 + 4Q_{ij}{}^2 - 4Q_{ki}{}^2)] + (\delta_{jj} - \delta_{kk})\nu_o/2 \quad (10)$$

$$Ci = -3q\, [(Q_{jj} + Q_{kk})Q_{jk} + 4Q_{ki}Q_{ij}] + \delta_{jk}\nu_o \quad (11)$$

$$D_i = (-9q/8)[(Q_{jj} - Q_{kk})^2 - 4Q_{jk}{}^2] \quad (12)$$

$$E_i = (9q/2)(Q_{jj} - Q_{kk})Q_{jk} \quad (13)$$

$$q = [3 - 4I(I + 1)]/16\nu_o \quad (14)$$

The Q_{ii} elements describe the quadrupolar coupling tensor in the goniometer reference frame, i.e., with respect to x,y,z about which the crystal rotations were performed; the δ_{ii} elements similarly describe the chemical shift tensor in this axis system. Diagonalization of these tensors yields the principal components (as eigenvalues) of these tensors and their direction cosines (as eigenvectors) in the goniometer axis system. From the expressions above, however, the parameters *A*, *B*, and *C* from each rotation evidently are functions of both the chemical shift and quadrupolar coupling interactions. Parameters *D* and *E* arise solely from the quadrupolar coupling interaction, owing to the more-complicated orientation dependence of this interaction under second-order perturbation theory. As shown by Vosegaard et al.,[16] the quadrupolar information obtained from *D* and *E* can be used to separate the influence of the two interactions from the *A*, *B*, *C* parameters, which then permits construction of the individual tensors for each interaction. We used this method to characterize the chemical shift and quadrupolar coupling tensors for ^{59}Co in vitamin B_{12}. The quadrupolar coupling constant is not given directly by this procedure; rather the quadrupolar frequency $\nu_Q = \chi/2I(2I-1)$ is obtained. To determine the magnitude of the quadrupolar coupling constant, quadrupolar frequency is multiplied by a factor of 42 for a spin $I = 7/2$ nucleus, such as ^{59}Co. Also, the relative sign of χ is unavailable, so we have assumed it has a positive value.

Experimental Section

Sample Preparation. A single crystal (2.6 mm × 1.9 mm × 1.1 mm) of vitamin B_{12} (Sigma) was obtained by slow evaporation of an aqueous solution. This crystal was mounted on a slide and coated with epoxy to prevent dehydration and consequent degradation of the single crystal. We then indexed the crystal to confirm agreement with the space group and unit cell constants in the original reported crystal structure and to determine the orientation of the cell axes with respect to the mount of the crystal. The crystallographic *a*-axis was found to lie collinear with an axis we defined as the *x*-axis of the mounted crystal within the goniometer.

NMR Spectroscopy. NMR spectra of the crystal were obtained with a Bruker AMX-500 NMR spectrometer, at a magnetic field strength of 11.75 T, which corresponds to a ^{59}Co Larmor frequency of 118.67 MHz. All ^{59}Co chemical shifts were referenced with respect to 1.0 M $K_3Co(CN)_6$ (aq) at 0 ppm. Pulses were kept short to ensure a broad spectral window of excitation, typically 0.8 to 1.0 μs (20−30° flip angles with 90° pulse width of 3.3 μs). Dwell times of 0.5 μs were used, and 1024 data points were collected during each scan. Recycle times of 50 ms were used to collect all spectra, and 153 600 to 921 600 scans were required for adequate sensitivity at each orientation of the crystal.

Single-Crystal NMR. A home-built goniometer containing the vitamin B_{12} crystal was placed within a standard Bruker single-channel wide-line solids solenoid probe. The crystal was rotated perpendicular to the magnetic field direction about each of three orthogonal goniometer axes, designated *x*, *y*, *z*. Single-crystal NMR spectra were acquired as a function of goniometer rotation in the magnetic field in 15° increments. The spectral frequencies arising from each orientation of the crystal were fitted by using Microsoft Excel97 to obtain the five parameters for each of the three rotations. The parameters were then manipulated as described in the *Theory* section.

Results

Vitamin B_{12} crystallizes from aqueous solution as a highly hydrated orthorhombic lattice with the space group $P2_12_12_1$ ($D_4{}^2$, no. 19). The unit cell dimensions agreed with those of the original report: $a = 15.92$ Å, $b = 22.32$ Å, and $c = 25.33$ Å (note that modern convention of unit cell axis assignment permutes *a* and *c* from the original literature).[17] Inclusion of water solvent molecules in the crystal lattice results in a formula for the asymmetric unit of $C_{63}H_{88}O_{14}N_{14}PCo{\cdot}22H_2O$. A more recent X-ray diffraction structure determination at 88 K on crystals obtained from a water/ethylene glycol/acetone solvent system provided a very similar structure and lattice constants: $P2_12_12_1$, $a = 15.838(7)$ Å, $b = 21.927(12)$ Å, and $c = 25.689(13)$ Å.[12] The differences between the two structures are limited largely to the included solvent structure. The vitamin B_{12} crystals tend to dehydrate and crack when left to stand after removal from the mother liquor; consequently, the crystal used for the NMR experiments was quickly dried of mother liquor and trapped within epoxy. Indexing the cell parameters after this procedure indicated that there was no degradation of the crystal within the epoxy shell.

Four molecules of vitamin B_{12} are found at general sites within the $P2_12_12_1$ unit cell; these sites are crystallographically equivalent. However, as they are related by 2-fold screw axes and not by a center of inversion, in general each site is magnetically distinct.[10] Hence four magnetically independent yet crystallographically related sites should be evident in the ^{59}Co NMR spectra of the B_{12} crystal. Special projections exist along the orthogonal cell axes *a*,*b*,*c*, where the symmetry becomes two-dimensional, *p2gg*, resulting in inversion relations between pairs of molecules along each of the crystal axes. The four general sites will exhibit chemical shift and quadrupolar coupling tensors with identical principal components, the differences being limited to their orientations with respect to the unit cell (or goniometer) axis system. The symmetry operations of the space group, when applied to any one magnetically distinct site, will generate completely the orientations of the remaining three sites.

The crystal was oriented in the goniometer such that the *x*,*y*,*z* crystal rotation axes correspond to 100, 021, and $0\bar{2}5$ directions, respectively. Typical ^{59}Co NMR spectra obtained for the single crystal of vitamin B_{12} are given in Figure 1 for rotations about the *x*-axis. In this orientation of the crystal, pairs of sites are related by a center of inversion due to special projection symmetry; this rotation axis coincides with the crystallographic *a*-axis. Hence, only two sets of peaks occur for this rotation. Similarly, special projections along the *b*- and *c*-axes relate the same pairs of molecules by inversion symmetry. During the rotation about *x* (crystallographic *a*, 100), the symmetry is such that the four nuclear sites are magnetically equivalent when the *b*- and *c*-axes, orthogonal to *a*, are oriented along the magnetic field direction, which results in complete coincidence of the

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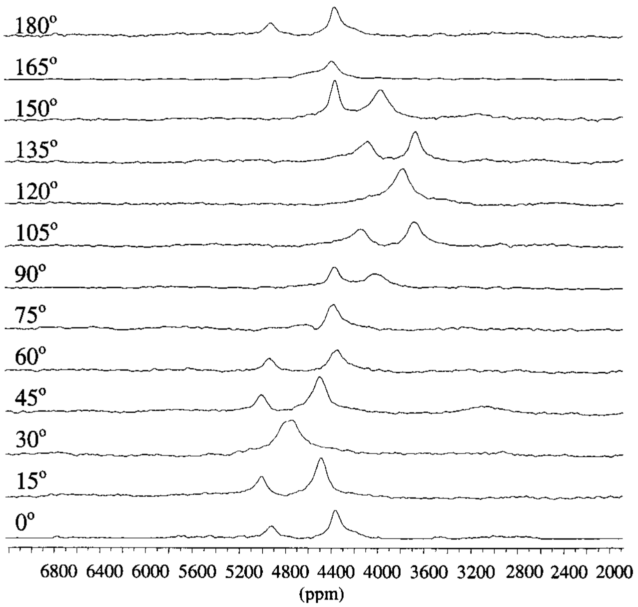


Figure 1. ^{59}Co NMR spectra of a single crystal of vitamin B$_{12}$ at 119.2 MHz (11.75 T). The spectra correspond to rotational increments about the goniometer *x*-axis.

spectra arising from the general sites. These special projections permit spectral assignment of the crystal axes to within 1°, substantially reducing any indeterminate error resulting from mounting of the crystal. The crystal orientations corresponding to these special projections are indicated in the *x*-rotation plot depicted in Figure 2. For the other two rotations, along the $0\bar{2}5$ and 021 axes of the crystal, no such symmetry restrictions occur. Fortuitously, in the *z*-rotation, two sites are very similar in orientation and their spectra overlap extensively, but not due to any symmetry constraint.

The ^{59}Co NMR signals from the single crystal under all three rotations occur within a range of ~240 kHz (2000 ppm) and have line-widths that vary from 4 to 10 kHz. The frequencies of the peaks, ν (in Hz from the chemical shift reference, 1.0 M K$_3$Co(CN)$_6$, (aq)), as a function of the rotation angle ρ about each of the three axes ($i = x, y, z$) were fit to the function given in eq 3. The data obtained from these three rotations as well as their best-fit functions are presented in Figure 2; the parameters obtained from these fits are given in Table 1. Rotations *x* and *z* were shifted by small phase angles, −6.8° and −4.7°, respectively, to correct small errors in aligning the crystal at the beginning of each set of rotations. This ensure that the complete rotation data set progressed smoothly at the 0° and 90° points from one rotation to the next, $x \rightarrow y$, $y \rightarrow z$, and $z \rightarrow x$. Note that crystal symmetry resulted in pairs of sites being related throughout rotation *x* but all sites were distinct for rotations *y* and *z*.

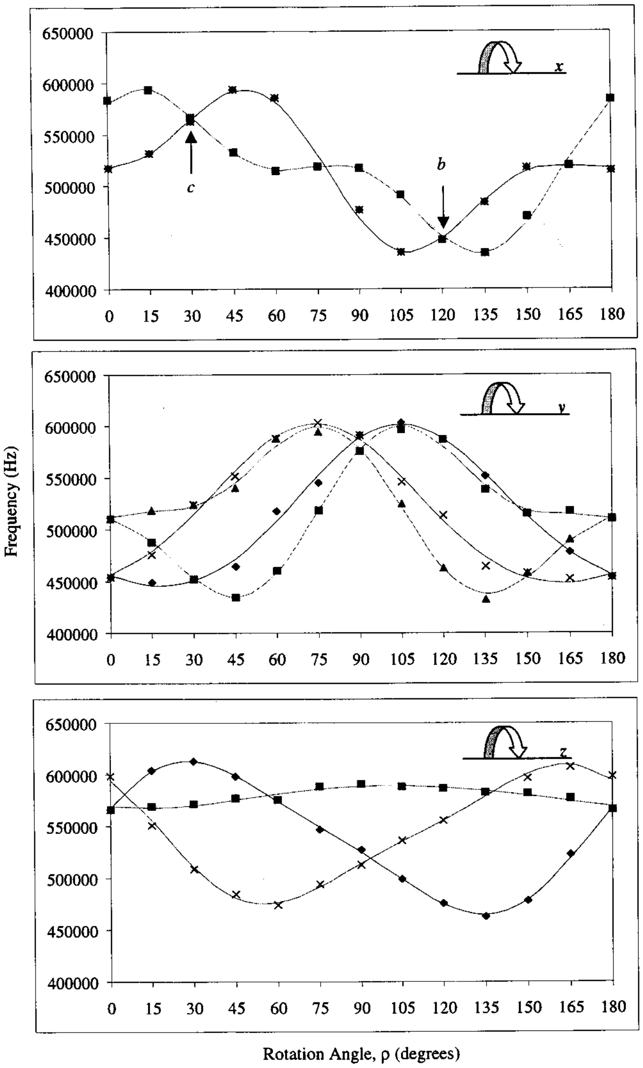


Figure 2. ^{59}Co NMR peak frequencies vs rotation angle plots for each of the three orthogonal rotations of a single crystal of vitamin B$_{12}$. The best-fit functions of eq 3 to the experimental data points are given as solid lines. Rotation *x* shows the crystal orientations at which the crystallographic *b*- and *c*-axes (special projection axes) are directed along the applied magnetic field B$_o$.

From the 15 variables obtained from the fits, tensors corresponding to the quadrupolar and chemical shift nuclear spin interactions for the cobalt nuclei were constructed with respect to the *x*,*y*,*z*-axis system of the goniometer. Diagonalization of these tensors provided their principal components (eigenvalues) in their respective principal axis systems, i.e., the values of δ_{11}, δ_{22}, and δ_{33} of the ^{59}Co chemical shift tensor and (after appropriate conversion from the quadrupolar frequency elements Q_{11}, Q_{22} and Q_{33}), the ^{59}Co quadrupolar coupling constant χ (= 42 Q_{33}) and its asymmetry η, (=[$Q_{22} - Q_{11}$]/Q_{33}). Also obtained from this procedure were the direction cosines (eigenvectors) relating the orientations of the principal axis systems to the goniometer axis system. Knowledge of the orientation of the unit cell with respect to the goniometer axes by prior indexing permitted the assignment of the principal axis systems of the quadrupolar and chemical shift tensors to discrete directions with respect to the molecular structure of vitamin B$_{12}$. Ambiguities remain, however, in the assignment of each of the NMR-characterized sites to the appropriate crystallographic site. Here, the differences in the characteristics of pairs of sites under each of the rotations proved valuable as an assignment strategy. In rotation *z*, only one of the pairs of sites that were related in rotation *x* was magnetically similar; the other pair of sites was readily resolved as two independent peaks. Inspection of the crystal structure for this orientation of the crystal revealed that one pair of sites conserved a pseudo-twofold rotation about the magnetic field direction for all crystal orientations within rotation *z*; hence these crystallographic sites could be assigned to specific sets of spectral lines by symmetry. These assignments are also given in Figure 3.

Assignment of crystallographic to spectroscopic sites permits determination of the orientation of the two NMR spectroscopic tensors (chemical shift and quadrupolar coupling) with respect to the molecular framework of vitamin B$_{12}$. The results obtained from this process are given in Table 2. Two views of these

Table 1. Best-Fit Parameters of the Experimental Data to Eq3 (values in Hz with SE from the fits given in parentheses)

	rotation *x*	rotation *y*	rotation *z*
site 1			
A	515 889 (1581)	511 134 (1481)	539 082 (576)
B	36 164 (2076)	−66 318 (2028)	32 005 (790)
C	45 426 (2195)	−40 980 (2159)	63 036 (839)
D	−31 046 (2097l)	4 763 (2028)	10 324 (794)
E	−10 528 (2175)	5 075 (2158)	6 908 (834)
site 2			
A	516 184 (1025)	510 546 (953)	579 572 (912)
B	42 884 (1409)	−32 671 (1305)	−10 433 (1252)
C	39 390 (1490)	−54 223 (1390)	−2 923 (1328)
D	34 171 (1424)	27 593 (1305)	−718 (1258)
E	−5 350 (1476)	16 492 (1390)	−798 (1322)
site 3			
A	516 184 (1025)	511 169 (908)	579 572 (912)
B	42 884 (1409)	−32 009 (1243)	−10 433 (1252)
C	39 390 (1490)	53 281 (1323)	2 923 (1328)
D	34 171 (1424)	27 619 (1243)	−718 (1258)
E	−5 350 (1476)	−15 647 (1323)	798 (1322)
site 4			
A	515 889 (1511)	512 105 (1790)	541 395 (1345)
B	36 164 (2076)	−65 170 (2451)	29 890 (1844)
C	45 426 (2195)	41 538 (2609)	−55 548 (1957)
D	−31 046 (2097)	3 693 (2451)	9 993 (1854)
E	−10 528 (2175)	−6 061 (2689)	−7 981 (1948)

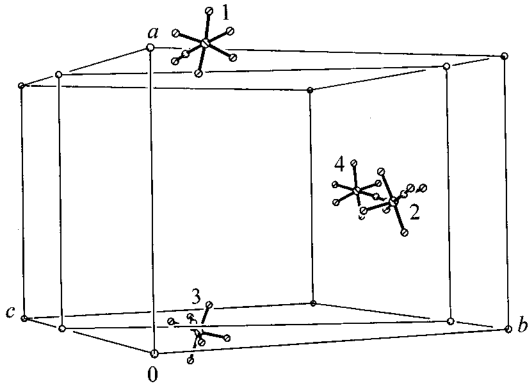
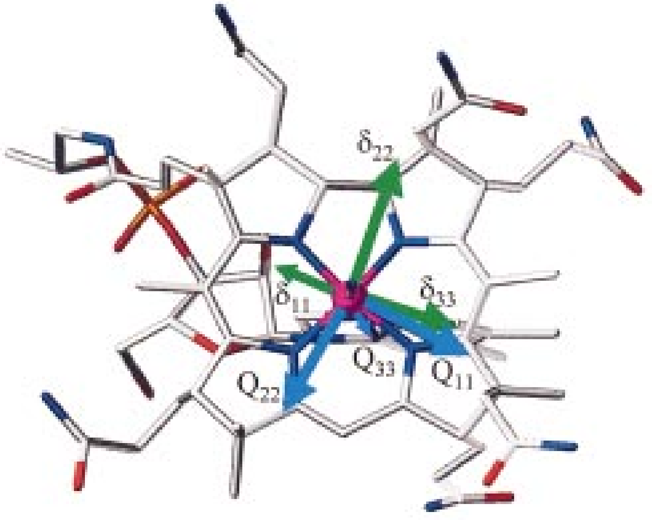


Figure 3. Views of the crystal packing of vitamin B_{12} relevant to the rotation of the crystal about the *z* ($0\bar{2}5$) direction. The molecular structure of B_{12} has been reduced to the octahedral coordination of cobalt for simplicity. Assignments of each of the NMR-characterized sites to their respective crystallographic sites are indicated. The plane perpendicular to the *z*-direction is superimposed on the unit cell, showing that two molecules are positioned such that the nitrogens of the corrin ring lie approximately in this plane.

orientations generated from the results in Table 2 are given in Figure 4. These views have been prepared to best show the orientations of the two tensors simultaneously. The top view looks down the CoCN axis of the molecule; the side view corresponds to tilting toward the top of the figure the CoCN axis of the molecule in the top view. The two tensors are centrosymmetric and also could be presented by a full inversion of each axis through the cobalt site. For chemical shielding, our analysis yields a centrosymmetric tensor, because only the symmetric part of this nonsymmetric tensor is directly observable.[18]

The quadrupolar coupling constant for ^{59}Co is quite large, 27.31 MHz. The largest component of the electric field gradient at the cobalt site lies very close (9.6°) to the Co−N(benzimidazole) or apical direction of the approximately octahedral coordination sphere of the metal. The other two minor components of the quadrupolar coupling tensor lie approximately in the plane of the corrin ring. These components are not identical, however, so the quadrupolar coupling is slightly nonaxially symmetric, with an asymmetry parameter of 0.243. This is a reflection of the nonsymmetrical nature of the corrin ring surrounding the cobalt site: two of the pyrrolidine rings are directly attached, whereas the other three inter-ring linkages have an intervening carbon. Significantly, this short linkage plays a key role in the orientation of the minor components of the quadrupolar coupling tensor. The intermediate component Q_{22} lies almost directly along the Co−C(1) vector, where C(1) is the corrin ring carbon of the short linkage on the right-hand side of both views in Figure 4. This carbon is also the point of greatest "pucker" in the corrin ring, lying well below the approximate plane (slightly bowled) formed by the other atoms.

(a) Top View



(b) Side View

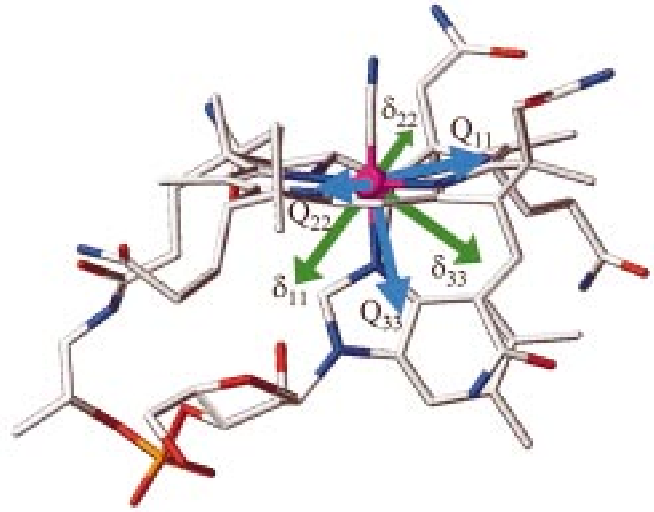


Figure 4. Molecular structure of vitamin B_{12} with the orientation of the principal components of the quadrupolar coupling (in blue) and chemical shift (in green) tensors superimposed on views from the (a) top and (b) side. The viewpoint between the two pictures differs by a 80° rotation about an axis through the corrin ring perpendicular to the direction of the views. The molecular structure has been derived from the crystallographic data of Brink-Shoemaker et al.[17] and is displayed by use of the Tripos software program *Sybyl 6.3*.

The isotropic chemical shift, obtained from one-third of the sum of the principal components, is 4549 ppm. The cobalt

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Table 2. Quadrupolar Coupling (Q_{ii}, $i = 1, 2, 3$) and Chemical Shift (δ_{ii}, $i = 1, 2, 3$) Tensors in the Crystal Axis System (*a,b,c*) for Vitamin B_{12} (crystal axes in modern convention showing the Principal Components and the Direction Cosines of Each Tensor

quadrupolar coupling, kHz			chemical shift, ppm[a]		
−404.0 (2.5)[b]	−246.3 (2.3)	650.3 (1.8)	5075 (45)	4670 (43)	3902 (42)
0.302 (0.007)	0.355 (0.005)	−0.885 (0.002)	−0.796 (0.038)	−0.117 (0.036)	−0.594 (0.060)
0.766 (0.005)	0.462 (0.007)	0.447 (0.003)	−0.384 (0.030)	−0.661 (0.052)	0.645 (0.055)
0.567 (0.003)	−0.813 (0.002)	−0.132 (0.000)	−0.467 (0.018)	0.742 (0.065)	0.481 (0.028)

[a] Reported in ppm from 1.0 M $K_3Co(CN)_6$. [b] Uncertainties derived from error propagation analysis are given in parentheses as 2 SE. The direction cosines directly relate the principal components to the orientation of the vitamin B_{12} molecule in site 1 of the $P2_12_12_1$ space group with respect to (*a,b,c*) of the crystal.

chemical shift tensor is highly anisotropic, spanning ~1200 ppm about this isotropic shift, from 5075 ppm at high frequency to 3902 ppm at low frequency. The principal components of the chemical shift tensor do not lie along any particular chemical bond of the cobalt coordination sphere. The most shielded component, δ_{33}, is directed through one triangular face of the cobalt octahedron, away from the phosphate tail of the molecule and along the plane of the benzimidazole base. This orientation also places δ_{11} directly along the vector between cobalt and the phosphorus atom of the nucleotide tail. The intermediate component, δ_{22}, lies within the plane of the corrin ring and, as found for one minor component of the quadrupolar coupling tensor, is directed within 15° of the Co−C(1) vector.

The relative orientation of the two tensors can also be expressed by using the Euler angles between them, relating the two sets of direction cosines obtained from the single-crystal analysis. This results in a relative orientation of the chemical shift tensor to the quadrupolar coupling tensor with Euler angles of $\alpha = 334.0 \pm 13.0°$, $\beta = 41.4 \pm 1.7°$, and $\gamma = 207.3 \pm 2.5°$.

Discussion

The results we have presented compare well with previous investigations of the ^{59}Co NMR properties of vitamin B_{12}. In 0.01 M aqueous (D_2O) solution, the ^{59}Co isotropic chemical shift has been reported to be 4750 ppm with a line width of 11.1 kHz;[19] a more recent investigation[20] (solvent and concentration not reported) gives a similar shift of 4650 ± 20 ppm and a line width of 28.2 kHz. The solid-state value of 4549 ppm we determined is not appreciably different from either of these values. The large line widths observed in solution are indicative of efficient quadrupolar relaxation, in accord with the large value of 27.31 MHz determined here for the quadrupolar coupling constant. As has been noted before, the concentration in aqueous solution has a significant influence on the ^{59}Co relaxation behavior of simple cobalt complexes;[21] this may explain the discrepancies in the line widths reported previously.

Comparison of our findings with recent solid-state ^{59}Co NMR results also fares well. Medek et al.[20] reported data for recrystallized vitamin B_{12} obtained from simulation of the ^{59}Co NMR spectra of a powder sample at various magnetic fields. Their estimate of the quadrupolar coupling, 26.1 ± 0.4 MHz with $\eta = 0.1 \pm 0.2$, is similar to the values in Table 2, as are their chemical shift components, which translate (from their convention) to principal components of 5105, 4845, and 4000 ppm. The Euler angles relating the orientations of the two tensors are more precisely obtained from the single-crystal analysis, which is not surprising. Their estimate of the Euler angle $\beta = 40 \pm 20°$ is in close agreement with our results. The quadrupolar−chemical shift line shapes of powder samples are particularly sensitive to the value of this angle.

The elucidation of chemical shift and quadrupolar coupling tensors for metal centers in general, and the cobalt in vitamin B_{12} in particular, appears to be a particularly convenient and insightful probe of local structure in metal-centered biological assemblies. Medek et al.[20] showed that powder samples of vitamin B_{12} in "as-purchased" and recrystallized forms exhibit significant differences in their ^{59}Co NMR powder line shapes, especially in the quadrupolar coupling constant for each form (17.7 and 26.1 MHz, respectively). As they reported, this is undoubtedly due to the effect of solvation within the crystal lattice, probably causing some deformation of the ligands on the corrin ring or axial cobalt ligands that are then transmitted electronically to the cobalt site. It is interesting that this seems to affect the quadrupolar coupling more than the chemical shift tensor. Our assignment of the orientation of the ^{59}Co quadrupolar coupling tensor places the principal component of this tensor Q_{33} along the axial ligand direction, which should make it particularly sensitive to deformations along this axis. This supports their hypothesis of axial ligand deformation as the difference between the two crystal forms. This also provides some hope that solid-state ^{59}Co NMR may contribute to the elucidation of enzyme-bound intermediate forms of B_{12} derivatives, such as the structure recently reported for *E. coli* methionine synthase.[13] Changes in axial ligands, evident from this X-ray diffraction study, should be echoed in the ^{59}Co NMR line shape of free and bound forms of the coenzymes. Characterization of the metal center by solid-state NMR also appears to be the spectroscopic avenue of choice. A recent study of ^{13}C and ^{15}N chemical shift tensors of several metal−tetraphenylporphyrin complexes showed relative invariance in the values of their principal components, although some sensitivity of the ^{15}N data to metal−nitrogen separation was reported.[22] Selective isotopic enrichment and problems arising from spectral overlap can be avoided when studying the metal directly.

The orientations of the two NMR spectroscopic tensors, chemical shift and quadrupolar coupling, for the cobalt center in vitamin B_{12} constitute the most significant result of this study. Such information, even of much simpler cobalt-centered systems, is relatively rare in the literature. The anisotropic NMR spectral properties of ^{59}Co have attracted much attention recently, through experimental characterization of chemical shift and quadrupolar coupling tensors in simple salts and complexes from solid-state NMR spectra of static powder samples[23,24] and single crystals,[25] as well as polycrystalline samples of cobalt clusters[26] and larger cobalt-centered ring-based systems.[20,27]

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These studies have shown that the chemical shift tensors of cobalt are highly anisotropic, spanning up to 2500 ppm for cobalt(III)−tetraphenylporphyrin-di-isoquinoline.[27] In the few cases where relative orientations of the two tensors have been reported, from simulation of powder line shapes[20,23,24] and the single crystal study,[25] the chemical shift and quadrupolar coupling tensors have been found to exhibit independent orientations, unless necessitated by crystallographic symmetry.[28] This is evidence of the profound fundamental differences between these two NMR spectral properties, the predominantly singlet-excited-electronic-state dependence of chemical shifts, and the ground-state dependence of quadrupolar coupling through the local electric field gradient. The significance of the characterization of these tensors, in B_{12} as well as other systems, lies in the complementary information they provide concerning the local metal environment, rather than on any common origin or underlying connection. Further studies of the behavior of these tensors and their relative orientations in a range of chemical environments will continue to shed light on their distinct sensitivities in terms of local structure.

Theoretical predictions using density functional methods have been recently reported and show promise in predicting chemical shift data for cobalt nuclei.[29,30,31] These methods will figure prominently in future studies of metal centers. It is unfortunate that the few existing reports have not included information concerning the orientation of the chemical shift tensor, although this may be due to the idealized symmetry used in some of the simple complexes studied. The ability of these methods to correctly predict quadrupolar coupling parameters still remains an open question, and an important one, considering the orientation of the quadrupolar coupling tensor determined here for vitamin B_{12} and its apparent sensitivity to crystal form reported by Medek et al.[20]

Although investigations of quadrupolar metal nuclei in biological systems by NMR have been largely avoided in the past, it is important to note the attractive features of these nuclei. As shown here, the characterization of two NMR spectroscopic interactions, and their anisotropic nature in the solid state, provide a fuller description of the local electronic environment of the metal. Although single-crystal studies such as this one are time-consuming, this is partially alleviated by efficient quadrupolar relaxation, which permit rapid signal-averaging, and the information so obtained provides complete and unambiguous information concerning tensor orientations. Significant advances in high-resolution solid-state NMR of quadrupolar nuclei,[8,9] as well as the development of cross-polarization[32−34] and other magnetization transfer techniques involving quadrupolar nuclei,[35−37] should continue to provide unique opportunities to study bioinorganic assemblies and assist in determining their precise roles and actions in many biochemical pathways.

Conclusion

The ^{59}Co chemical shift and quadrupolar coupling tensors of the cobalt site in a single crystal of vitamin B_{12} have been determined unambiguously by analysis of the ^{59}Co solid-state NMR spectra obtained as a function of crystal orientation in an 11.75 T magnetic field. This is the first example of the application of single-crystal NMR to the elucidation of the local environment of a metal in a biologically relevant compound. The results indicate that the cobalt chemical shift tensor is highly anisotropic, with principal components of $\delta_{11} = 5075 \pm 45$ ppm, $\delta_{22} = 4670 \pm 43$ ppm, and $\delta_{33} = 3902 \pm 42$ ppm. The ^{59}Co quadrupolar coupling constant is 27.31 ± 0.08 MHz, with an asymmetry parameter $\eta = 0.243 \pm 0.005$. The largest component of the quadrupolar coupling tensor lies $\sim 10°$ from the Co−N(benzimidazole) direction, with the minor components lying approximately within the plane of the corrin ring. The most shielded component of the chemical shift tensor, δ_{33}, points approximately through the center of one triangular face of the octahedral cobalt coordination and away from the nucleotide "tail," whereas the least shielded component, δ_{11}, is directed toward the phosphorus atom, and δ_{22} is approximately in the plane of the corrin ring. These results demonstrate that NMR of quadrupolar metal isotopes in biological materials is feasible, contrary to common belief, and particularly so for solid materials, where new techniques and higher magnetic fields are permitting acquisition of wide-line and high-resolution spectra.

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