Pulsed Electron Paramagnetic Resonance of High-Spin Cobalt(II) Complexes

Peter C. Kang, Gareth R. Eaton, and Sandra S. Eaton*

Department of Chemistry, University of Denver, Denver, Colorado 80208

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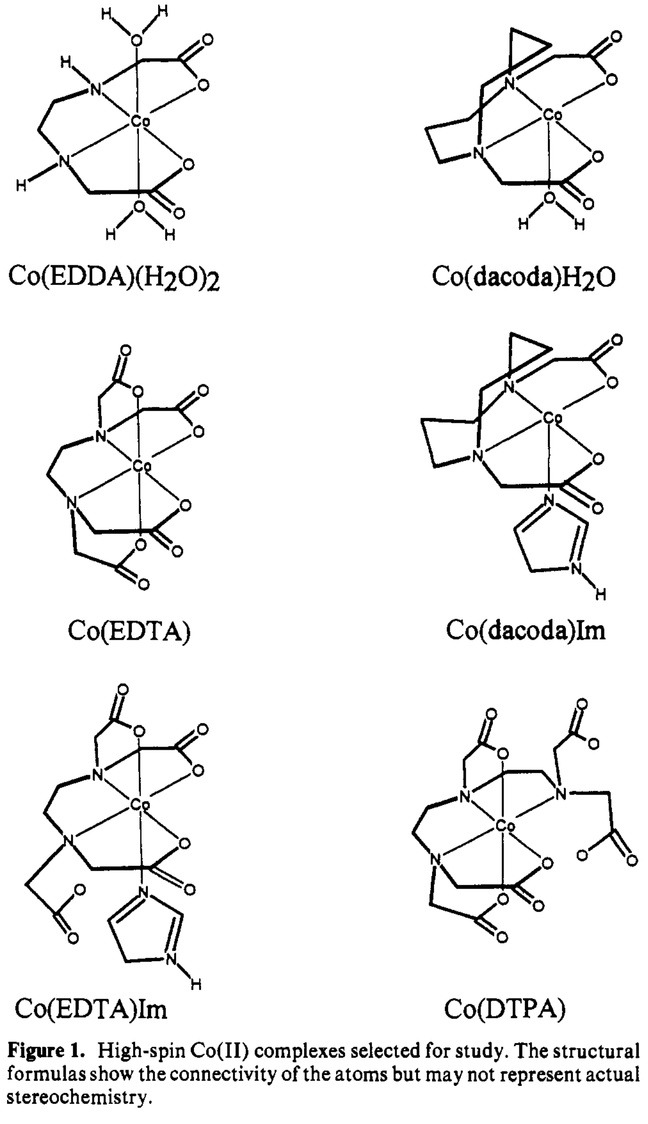
Electron paramagnetic resonance (EPR) data were obtained for $Co(H_2O)_6^{2+}$ and six Co(II) complexes with 0, 1, or 2 water molecules in the first coordination sphere. Continuous wave (CW) spectra confirmed that the complexes are high spin with zero-field splitting greater than the X-band EPR quantum. Two- and three-pulse electron spin echoes were recorded at about 6 K. The electron spin phase memory time, T_m, varied through the spectrum, with longer values observed at higher magnetic field. The electron spin lattice relaxation time, T_1, measured by saturation recovery was approximately independent of position in the spectrum but was strongly temperature dependent. In D_2O:glycerol-d_3 solution, the depth of the deuteron modulation fell into two classes: complexes with directly bound water exhibited substantially deeper modulation than complexes without directly bound water. Fourier transforms of the two-pulse electron spin echo envelope modulation in H_2O:glycerol solution reveal frequencies near twice the proton Larmor frequency that distinguish between first and second coordination sphere water. Two complexes containing imidazole bound to Co(II) exhibit characteristic nitrogen modulation frequencies in Fourier transforms of 3-pulse ESEEM data.

Introduction

Electron spin echo envelope modulation (ESEEM) is a powerful tool to examine the coordination sphere of a paramagnetic metal. It provides detailed information on electron–nuclear couplings that are too small to be resolved in the CW spectra. This technique has been applied extensively to metals with a single unpaired electron ($S = {}^1/_2$), particularly Cu(II).[1] Other $S = {}^1/_2$ metal ions that have been studied are vanadyl,[2] Ti(III),[3] low-spin Fe(III),[4] and low-spin Co(II).[4] Studies of metals with $S > {}^1/_2$ are more limited and include high-spin Fe(III),[5,6] Mn(II),[7] Gd(III)[8] and other lanthanides,[9] and Cr(III).[10] Co(II) is of substantial interest in biological systems because of the use of Co(II) to replace Zn(II) as a spectroscopic probe.[11] In these environments Co(II) typically is high spin. A report of ESEEM for aqueous Co(II) demonstrated changes in the modulation pattern due to coordination of phosphorus ligands.[9] To provide a foundation for the application of ESEEM of high-spin Co(II) to metalloenzymes we report studies of six coordination complexes. Two important questions concerning a metal ion in a biological sample are the presence of directly bound water and identification of protein ligands such as histidine imidazole. The complexes that were studied (Figure 1) have 0, 1, or 2 water molecules in the first coordination sphere, and two of the complexes have a coordinated imidazole. The data in this study demonstrate the feasibility and utility of ESEEM in elucidating water and imidazole coordination in high-spin Co(II) complexes.


Co(EDDA)(H_2O)2 Co(dacoda)H_2O

Co(EDTA) Co(dacoda)Im

Co(EDTA)Im Co(DTPA)

Figure 1. High-spin Co(II) complexes selected for study. The structural formulas show the connectivity of the atoms but may not represent actual stereochemistry.

Experimental Section

Ethylenediamine-*N,N'*-diacetic acid (H_2EDDA), ethylenediamine-*N,N',N'',N'''*-tetraacetic acid disodium salt (Na_2H_2EDTA), and dieth-

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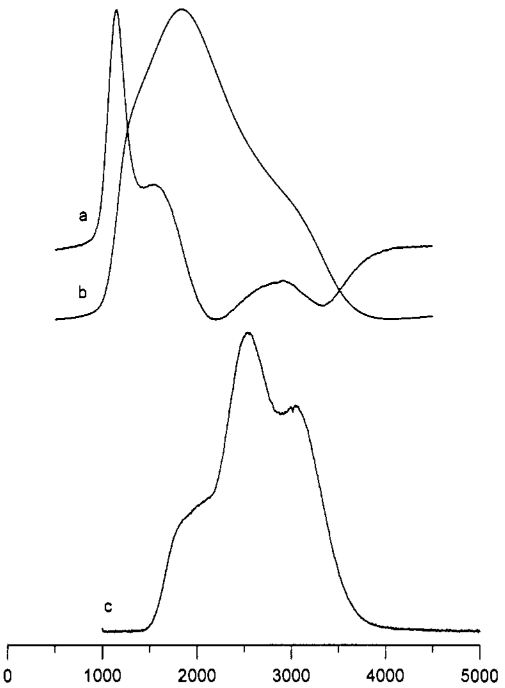

Figure 2. Spectra at X-band (9.2 GHz) of 5.0 mM Co(DTPA) in 1:1 H_2O:glycerol, pH 5.6. (a) CW spectrum at about 10 K, obtained with 0.1-mW microwave power, 4.0-G modulation amplitude at 100 kHz, scan time of 120 s, average of 2 scans. (b) First integral of spectrum shown in (a). (c) Two-pulse field-swept echo-detected spectrum at about 6.5 K, obtained with 90–τ–180–τ–echo sequence with τ = 150 ns and a 15-ns 90° pulse. The box-car digitizer was sampled 200 times at each magnetic field, and two scans were averaged.

ylenetriaminepentaacetic acid (H_5DTPA) were purchased from Aldrich Chemical or Eastman Kodak and used as received. The isotope purity of D_2O and 1,2,3-glycerol-$(OD)_3$ (glycerol-d_3) obtained from Cambridge Isotope Laboratories were 99.9% and 98%, respectively. Co(DTPA),[12] Co(EDTA),[13] Co(EDDA)$(H_2O)_2$,[14] 1,5-diazacyclooctane-*N,N'*-diacetate (dacoda),[15,16] and Co(dacoda)$(H_2O)\cdot 2H_2O$[16] were prepared by literature procedures. The imidazole (Im) adducts, Co(dacoda)Im and Co(EDTA)-Im, were prepared by addition of imidazole to Co(dacoda)(H_2O) or Co(EDTA), in 3-fold or 5-fold excess, respectively. Visible spectra indicated essentially complete conversion to the imidazole complexes at these mole ratios. Visible spectra were recorded on a Beckman DU 640 or on an OLIS-modified Cary-14.

EPR samples were prepared in 1:1 (v:v) H_2O:glycerol or D_2O:glycerol-d_3. The pH was adjusted by addition of concentrated NaOH (NaOD) or HCl (DCl). The approximation pD = pH + 0.45 was used to convert the pH meter reading to pD.[17]

Continuous wave (CW) and electron spin echo (ESE) experiments were performed on a slightly modified version[18] of the X-band (9.1 GHz) spectrometer described in the literature.[19] An Oxford ESR900 liquid-helium flow cryostat was used to obtain sample temperatures of about 10 K for CW and about 6 K for ESE. The temperature at the sample was calibrated by replacing the sample-containing tube with a tube containing a thermocouple. Since the gas flow rate has a significant impact on sample temperature, there is an uncertainty of about ±1 K in temperatures. Two-pulse ESE experiments used a 90–τ–180–τ–echo sequence. The 90 and 180° pulses were 15 and 30 ns, respectively. Three-pulse ESE experiments used a 90–τ–90–T–90–τ–echo sequence with τ set to a multiple of $1/(\nu_H)$, where ν_H is the proton Larmor frequency, to null proton modulation, and T was incremented. The microwave power required for a 90° pulse for the Co(II) complexes was about 5 dB lower than for samples with $S = {}^1/_2$, which is consistent with $S = 3/2$[20,21] for high-spin Co(II). Saturation recovery measurements of the electron spin–lattice relaxation time, T_1, were performed on the spectrometer described in the literature.[22]

Table 1. CW EPR Parameters for High-Spin Co(II) Complexes[a]

complex	g_x	g_y	g_z	lw_x	lw_y	lw_z
$Co(H_2O)_6^{2+}$	4.98	3.93	2.25	500	900	650
Co(dacoda)(H_2O)	4.97	4.10	2.25	500	960	660
Co(EDTA)Im	4.70	4.41	2.24	490	640	390
Co(EDTA)	5.12	3.94	2.16	340	850	400
Co(EDDA)$(H_2O)_2$	5.92	3.40	2.00	365	860	580
Co(DTPA)	5.70	3.47	1.98	190	540	425
Co(dacoda)Im	5.40	4.14	1.84	450	320	810

[a] Parameters for complexes in 1:1 H_2O:glycerol at about 10 K. Cobalt nuclear hyperfine was not resolved. lw is the line width in G for Gaussian line shape along the corresponding principal axis.

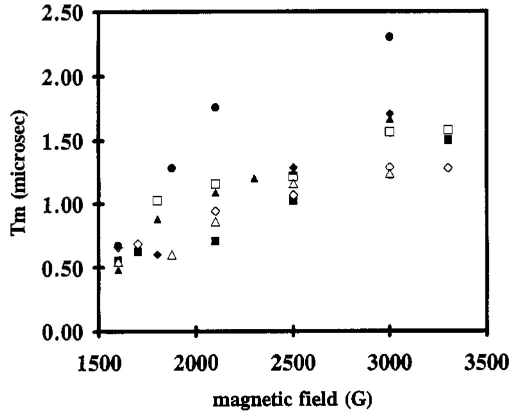

Figure 3. Dependence of the Co(II) electron spin phase memory time, T_m, on position in the X-band EPR spectrum for 5.0 mM $Co(H_2O)_6^{2+}$, pH 2.9 (■); 3.0 mM Co(EDDA)$(H_2O)_2$, pH 6.2 (□); 5.0 mM Co(dacoda)-(H_2O), pH 5.4 (◆); 5.0 mM Co(dacoda)Im, pH 8.3 (◇); 1.0 mM Co-(EDTA), pH 4.2 (▲); 5.0 mM Co(EDTA)Im, pH 4.2 (△); and 5.0 mM Co(DTPA), pH 5.2 (●).

Data were analyzed with locally-written computer programs. Electron spin phase memory relaxation times were calculated by least-squares fit to a single exponential of the form $y(t) = y(0)\exp(-(2\tau/T_m)^n)$, with n = 1 for most data sets. The ESEEM data were cosine Fourier transformed to determine the characteristic modulation frequencies. The method of Mims[23] was used to extrapolate the data into the instrument "dead-time", which was 100 ns for 2-pulse data and 100 ns + τ for the 3-pulse data. Saturation recovery data were fit to a single exponential.

Results

The continuous wave EPR spectrum of Co(DTPA) at about 10 K (Figure 2a) is representative of the spectra observed for the high-spin Co(II) complexes. The spectra exhibit three distinguishable g values (rhombic symmetry, Table 1) and are characteristic of $S = {}^3/_2$ with zero-field splitting greater than the EPR quantum (0.3 cm^{-1} at X-band). The observed transitions are within the $m_s = \pm{}^1/_2$ manifold. Cobalt hyperfine splitting was not resolved but may contribute to the large line widths of the signals (Table 1). The 2-pulse field-swept electron-spin-echo-detected spectrum of Co(DTPA) is shown in Figure 2c. In this experiment the time τ between microwave pulses was held constant at 150 ns while the magnetic field was swept. The resulting

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(21) For Kramers' ions with $S > {}^1/_2$ and zero-field splitting (ZFS) greater than the EPR quantum, it is common practice to speak in terms of effective $S = {}^1/_2$.[9] In addition, for ZFS > 0, as temperature is decreased the $m_s = \pm{}^1/_2$ levels are preferentially populated at the expense of $m_s = \pm{}^3/_2$. However the true S for high-spin Co(II) remains ${}^3/_2$ and that is reflected in the microwave power required for a 90° pulse.
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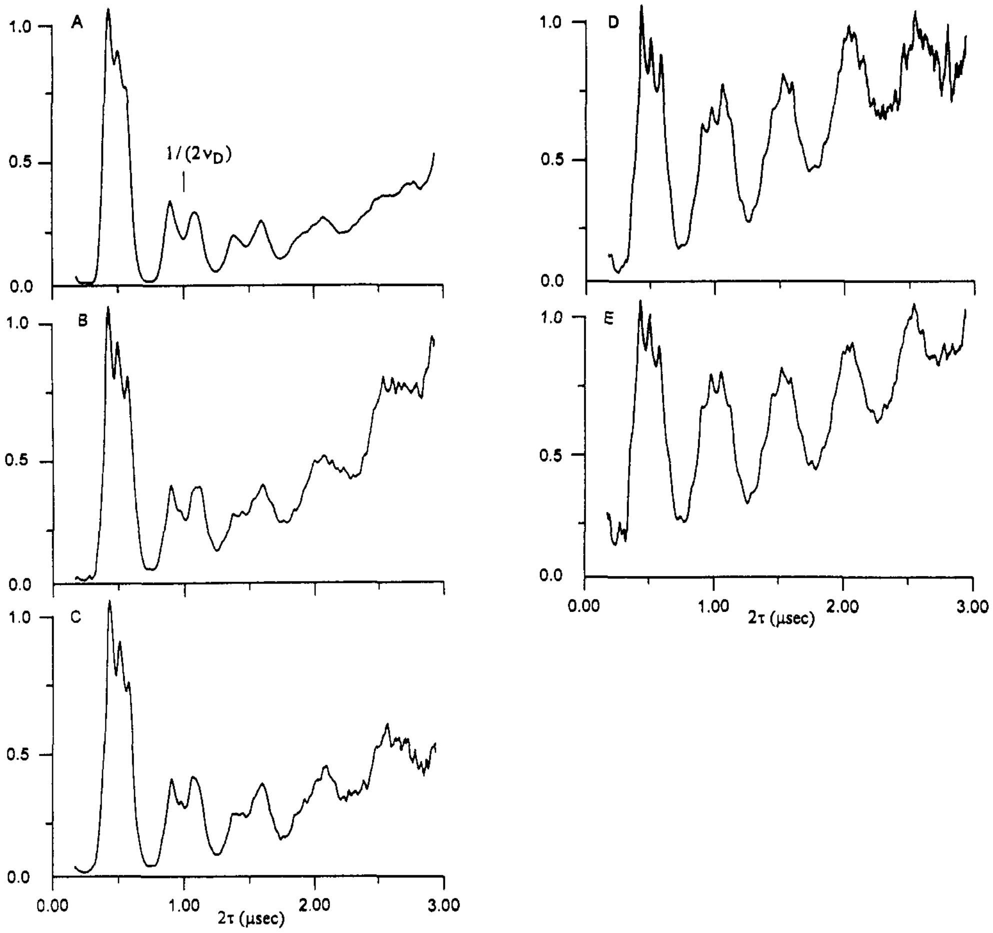

Figure 4. Ratios of 2-pulse X-band ESEEM data in D_2O:glycerol-d_3 to data in H_2O:glycerol at 3000 G and about 6 K for (A) 5.0 mM $Co(H_2O)_6^{2+}$, pH 2.9, (B) 3.0 mM $Co(EDDA)(H_2O)_2$, pH 6.2, (C) 5.0 mM Co(dacoda)(H_2O), pH 5.4, (D) 1.0 mM Co(EDTA), pH 4.2, and (E) 5.0 mM Co(DTPA), pH 5.2. The experimental data were collected for 1024 steps in τ starting at τ =100 ns, with increments of 4 ns.

spectrum is the 2-pulse echo intensity as a function of magnetic field. The spectrum in Figure 2c differs from the first integral of the CW spectrum (Figure 2b) in two important ways: (i) there are dips in the intensity of the echo-detected spectrum at about 2300 G and 2800 G; (ii) the relative intensity of the echo-detected spectrum is much lower than for the CW spectrum at all fields below about 2500 G and the signal is difficult to detect below about 1500 G. Several factors contribute to these differences. (i) Proton echo envelope modulation is deepest when $\tau = (n/2)(1/\nu_H)$, where n is an odd integer. For fixed τ = 150 ns this occurs at about 780, 2350, and 3923 G, which contributes to the decreased intensity of the echo near 2350 G. Echo intensity minima also occur for $\tau = (n/2)(1/(2\nu_H))$, n odd. For τ = 150, n = 7, this occurs at 2750 G, contributing to the decreased echo intensity at about 2800 G. (ii) Echo envelope modulation typically is deeper at lower magnetic field and is very deep for these Co(II) complexes at lower magnetic fields, which contributes to the difficulty in detecting echoes at magnetic fields below about 1500 G. (iii) As shown in Figure 3, the electron spin phase memory time, T_m, varies with position in the spectrum, with shorter values observed at lower magnetic field. For shorter values of T_m it is more difficult to detect the echo because a larger fraction of the echo intensity decays away during the instrument "dead time".

T_m decreased rapidly with increasing temperature, and electron spin echoes were difficult to detect at any magnetic field at temperatures above about 12 K. Saturation recovery measurements of the electron spin lattice relaxation time, T_1, were performed for Co(dacoda)(H_2O) and Co(DTPA). When fit to a single exponential, the values of T_1 for Co(dacoda)(H_2O) as a function of temperature were as follows: 6.5 K, 1.5 ms; 8.0 K, 43 μs; 9.8 K, 11 μs. For Co(DTPA) the values of T_1 were as follows: 6.5 K, 1.6 ms; 8.2 K, 25 μs; 10 K, 8 μs. In view of the strong temperature dependence of T_1, T_1 probably becomes less than 1 μs and comparable to T_m at about 12 K. Above about 12 K it is likely that T_m becomes approximately equal to T_1, and that the values are strongly temperature dependent, which is consistent with the difficulty in detecting an echo above about 12 K. The fits of the saturation recovery data to a single exponential exhibited deviations characteristic of the presence of a distribution of values. The distribution in relaxation times may be due to a distribution in the zero-field splitting for the Co(II) or to the temperature gradient across the sample that is present in a gas-flow cryostat. At temperatures between about 6.5 and 10 K, T_1 varied by less than about 20% when the magnetic field was varied between 1300 and 2800 G. This variation is much less than the uncertainties due to temperature fluctuations.

Two-pulse ESEEM data for the Co(II) complexes were obtained in H_2O:glycerol and D_2O:glycerol-d_3, at the same experimental parameters. The ratio of two data sets includes only the modulation that is different for the two experiments. Since proton modulation typically is much less deep than deuteron modulation, these ratios are dominated by the deuteron modulation from exchangeable protons. The ratios of data at 3000 G for a series of complexes are shown in Figure 4. The period of the modulation is $1/\nu_D$, where ν_D is the deuterium Larmor frequency at 3000 G, 1.95 MHz. The observed modulation is much deeper for $Co(H_2O)_6^{2+}$ (Figure 4a), $Co(EDDA)(H_2O)_2$ (Figure 4b), and Co(dacoda)(H_2O) (Figure 4c), which have 1 or more water molecules bound to the Co(II), than for Co(DTPA) (Figure 4e), which does not have a coordinated water molecule.[24] The ratio data for Co(EDTA) (Figure 4d) are similar to the data for Co(DTPA), which suggests that there is not a directly bound

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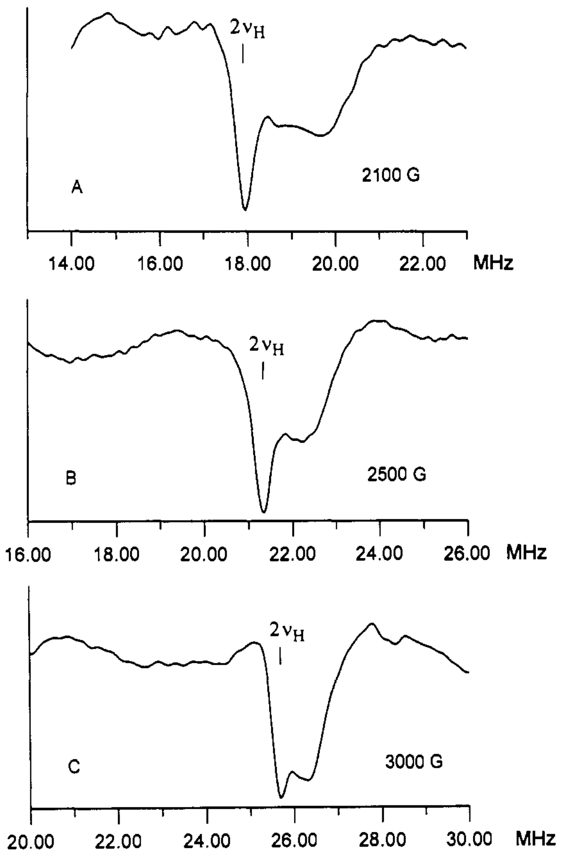

Figure 5. Cosine Fourier transforms of 2-pulse X-band ESEEM data for 5.0 mM Co(dacoda)(H_2O), pH 5.4 in 1:1 H_2O:glycerol at about 6 K, showing the region around $2\nu_H$ as a function of the magnetic field at which data were recorded: (A) 2100 G; (B) 2500 G; (C) 3000 G. The experimental data were collected for 1024 steps in τ starting at $\tau = 100$ ns, with increments of 4 ns.

water in Co(EDTA). The second feature that distinguishes the complexes with directly bound water is the observation of modulation with a period that corresponds to $^1/_2\nu_D$, which is marked on Figure 4. The deuteron modulation is deeper at lower magnetic fields, but the distinction between complexes with and without directly bound water was consistent for each of the magnetic fields examined (3400, 3000, and 2400 G).

Tsvetkov and co-workers[25] and Schweiger and co-workers[26] showed that, for Cu(II) complexes with directly bound H_2O, there are characteristic frequencies above $2\nu_H$ in the Fourier transforms of 2-pulse ESEEM data. The Fourier transforms of 2-pulse ESEEM data for Co(dacoda)H_2O at 2100, 2500, and 3000 G are shown in Figure 5 for a 10-MHz frequency range around $2\nu_H$. In addition to the peak at $2\nu_H$, there is significant intensity at higher frequency than $2\nu_H$. The peaks at $2\nu_H$ and higher frequencies have inverted phase, consistent with the assignment to "sum" peaks. The smaller shifts away from $2\nu_H$ at higher magnetic field is consistent with expectations.[25,26] The $2\nu_H$ frequency range in the Fourier transforms of 2-pulse ESEEM data at 2100 G for six additional high-spin Co(II) complexes are shown in Figure 6. The Fourier transform data for these complexes fall into two distinct sets. For $Co(H_2O)_6^{2+}$ and Co(EDDA)$(H_2O)_2$ there is intensity that is shifted by about 2 MHz, similar to what was observed for Co(dacoda)H_2O. These peaks are assigned to directly coordinated water. In the four other complexes the maximum shift at 2100 G is about 0.5 MHz. The peaks with smaller shifts may be due to second coordination sphere water, protons that are hydrogen-bonded to the first-coordination sphere ligands or ligand protons. At higher magnetic fields, all of the shifts were smaller, but the distinction between the two types of complexes was retained. In the spectrum for Co(EDDA)$(H_2O)_2$ (Figure 6B) there is an additional peak that is shifted by about 0.7 MHz that may be due to ligand protons.

ESEEM of Cu(II) complexes has been used extensively to identify coordinated histidine imidazoles in biological samples.[27] It was therefore of interest to examine the nitrogen modulation due to imidazole coordinated to high-spin Co(II). Three-pulse ESEEM was used to measure the nitrogen modulation because the decay time of the echo is longer than in the 2-pulse experiments, which permits better definition of the modulation frequencies. Data for Co(dacoda)Im showed nitrogen modulation (Figure 7) that was not present in data for Co(dacoda)(H_2O). Although the signal-to-noise ratio is poorer at 2100 G than at 3000 G due to the decrease in T_m, the nitrogen modulation is deeper at the lower magnetic field (Figure 7b). Two factors contribute to the increased depth of the modulation at lower magnetic field. (i) Echo modulation becomes deeper at lower field because the second-order terms in the Hamiltonian that give rise to the modulation are larger at lower field. (ii) At 2100 G the nitrogen isotropic hyperfine coupling (a_{iso}) appears to approximately satisfy the conditions known as "exact" cancellation: $a_{iso}/2$ = the nitrogen Larmor frequency (0.65 MHz at 2100 G), which gives a_{iso} about 1.3 MHz. For these conditions the nitrogen modulation frequencies from one nitrogen nuclear-spin manifold are close to pure quadrupole frequencies. The modulation frequencies of about 0.90, 1.58, and 2.53 MHz that are observed in the Fourier transforms of the 2100 G data (Figure 7c) support this assignment. The nitrogen frequencies observed in the Fourier transforms of 3-pulse echo ESEEM data for Co(EDTA)Im are similar to those observed for Co(dacoda)Im (Table 2).

Discussion

The g values for the Co(II) complexes are similar to those observed for other high-spin Co(II) complexes.[28] Due to the large g anisotropy, a small fraction of the electron spins are in resonance at any particular magnetic field. The microwave magnetic field, B_1, in the pulsed experiments is about 5 G, so only the spins that are in resonance within a 5-G range are excited and detected by the ESE experiments. This has two important effects on the experimental data. First, the pulsed EPR signals are much weaker than if the same molar concentration of unpaired electrons gave an EPR signal that was spread over a smaller range of magnetic fields. Second, these spins correspond to a limited range of orientations of the magnetic field relative to the molecular axis system. This orientation selection in the data at a particular magnetic field permits, in principle, the determination of the orientation dependence of the electron–nuclear couplings that give rise to the echo modulation. Analysis of the orientation dependence of the couplings for these Co(II) complexes will require data at a wider range of orientations than has been obtained to date.

The values of T_m were dependent on position in the spectrum. However, even at the lower magnetic fields, the T_m values correspond to spin packet line widths of less than 1 G, so the broad lines in the CW spectrum are not relaxation-time determined at temperatures below about 10 K. The saturation recovery data showed that the Co(II) T_1 was strongly dependent on temperature. Extrapolation of the T_1 data obtained between

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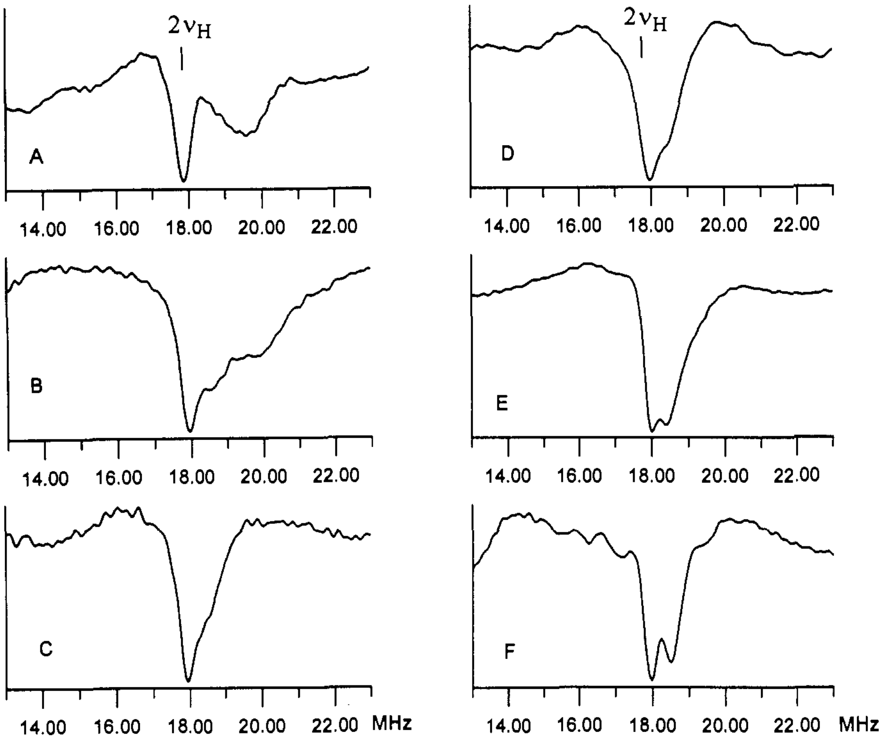

Figure 6. Cosine Fourier transforms of 2-pulse X-band ESEEM data at about 6 K and 2100 G showing the region around $2\nu_H$ for Co(II) complexes in 1:1 H_2O:glycerol solution: (A) 5.0 mM $Co(H_2O)_6^{2+}$, pH 2.9; (B) 3.0 mM $Co(EDDA)(H_2O)_2$, pH 6.2; (C) 5.0 mM Co(EDTA), pH 4.2; (D) 1.0 mM Co(EDTA)Im, pH 4.2; (E) 5.0 mM Co(DTPA), pH 5.2; (F) 5.0 mM Co(dacoda)Im, pH 8.3. The experimental data were collected for 1024 steps in τ starting at τ = 100 ns, with increments of 4 ns.

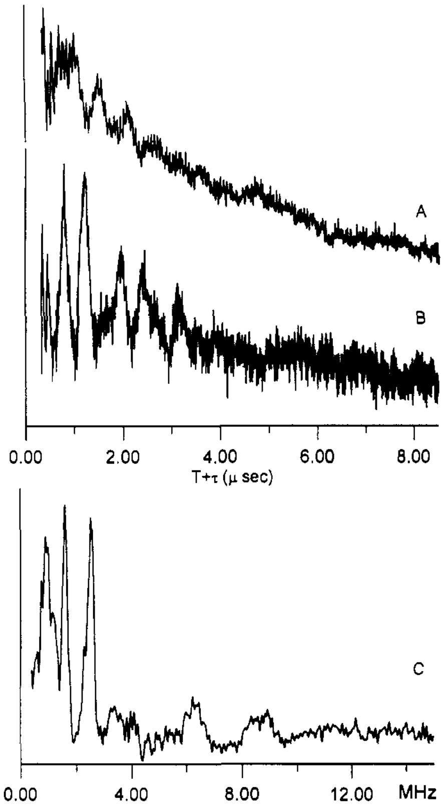

Figure 7. 3-pulse X-band ESEEM data for 5.0 mM Co(dacoda)Im in 1:1 H_2O:glycerol, pH 8.0, at about 6.5 K: (A) Experimental data at 3000 G; (B) experimental data at 2100 G; (C) cosine Fourier transform of data in (B). The experimental data were collected for 1024 steps in T, with increments of 8 ns starting at T = 100 ns, with τ = 235 ns at 3000 G and τ = 224 ns at 2100 G.

Table 2. Nitrogen Frequencies in 3-Pulse ESEEM[a]

	magnetic field (G)				
compd	1700	2100	2400	2500	3000
Co(dacoda)Im	0.67–0.91	0.74–1.13	0.75–1.22	0.79–1.23	1.17–1.29
	1.48	1.55	1.82	1.70	1.91
	2.39	2.48	2.55	2.63	2.77
	4.7 (b)	6.24 (b)		6.01 (b)	
Co(EDTA)Im		0.86–0.95			0.80–1.29
		1.58			1.85
		2.24			2.75

[a] Frequencies are reported in Mhz. (b) indicates a broad peak; a range of frequencies is given when individual frequencies are not well resolved.

6 and 10 K suggest that T_1 is very short at higher temperatures. This is consistent with our observations and that of other groups[29] that CW spectra of many high-spin Co(II) complexes are so severely relaxation broadened at 77 K that spectra are not observed.

The ratios of the 2-pulse ESEEM data in D_2O:glycerol-d_3 and H_2O:glycerol and the shifts in peaks away from $2\nu_H$ in the Fourier transforms of 2-pulse data in H_2O:glycerol clearly distinguished between complexes with and without directly bound H_2O. The data for Co(EDTA) are similar to data for complexes without a directly bound water. An X-ray crystal structure of Co(EDTA) found hexadentate EDTA.[30] However, NMR studies of Co(EDTA) in fluid solution at room temperature showed rapid isomerization between a complex with hexadentate EDTA and no coordinated H_2O and a complex with pentadentate EDTA and a coordinated water.[31] The ESEEM data indicate that in frozen 1:1 H_2O:glycerol solution the predominant form of the complex has hexadentate EDTA and no coordinated water.

Fourier transforms of the 2-pulse ESEEM data in H_2O:glycerol for $Co(H_2O)_6^{2+}$, $Co(EDDA)(H_2O)_2$, and $Co(dacoda)(H_2O)$ at 2100, 2500, and 3000 G show peaks at higher frequency than $2\nu_H$. It has been shown that $\Delta\nu = \nu_{max} - 2\nu_H$ is determined

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primarily by the anisotropic (dipolar) electron–nuclear coupling and is only weakly dependent on the isotropic contribution to the coupling.[25,26] NMR measurements of Co–H isotropic coupling constants for $Co(H_2O)_6^{2+}$ give values that are sufficiently small to permit, by analogy, analysis of $\Delta\nu$ in the present systems predominantly in terms of the dipolar contribution.[32,33] $\Delta\nu$ depends upon the orientation of the external magnetic field relative to the electron–proton interspin vector. If the subset of molecular orientations that is in resonance at a particular position in the EPR spectrum encompasses the orientations that give the largest $\Delta\nu$, then the perpendicular component of the anisotropic hyperfine interaction, $T_\perp$, is given by $T_\perp = (\Delta\nu(2\nu_H))^{1/2}$.[25b] The values of r estimated from $T_\perp$ with this assumption were 2.7 Å (2100 G), 2.7 Å (2500 G), and 2.9 Å (3000 G). If the subset of molecular orientations that give resonance at these magnetic fields does not include ones that give the largest values of ν_{max}, these values of r are overestimates of the actual interspin distance. Co(dacoda)(H_2O) is isomorphous with Ni(dacoda)(H_2O).[14] The X-ray crystal structure of Ni(dacoda)(H_2O) reported a nickel to water–oxygen bond length of 2.01 Å.[34] If the Co–O bond length is the same as the Ni–O bond length, and assuming an O–H bond length of 0.96 Å, and an H–O–H angle of 104.5°, then the Co–H distance is 2.7 Å. Thus, even with the approximate analysis of the ESEEM data, the estimated value of r is plausible.

Nitrogen modulation due to the coordinated nitrogens of EDTA, EDDA, DTPA, or dacoda were not discernible in the Fourier transforms of the ESEEM data. This may be due to the magnitude of the isotropic hyperfine coupling (a_{iso}) or to the relative magnitudes of the nitrogen quadrupole coupling and a_{iso}.[35,36] When imidazole was coordinated to the Co(II), characteristic nitrogen modulation frequencies were observed, with a_{iso} about 1.3 MHz. In Cu(II) complexes of imidazoles, the nitrogen modulation that is observed in ESEEM is due to the distal N with a_{iso} = 1.5–2.0 MHz.[37,38] Modulation due to the directly bound N typically is not observed because a_{iso} is too large.[36] The magnitude of a_{iso} for the nitrogens of a bound imidazole depends upon the orbital overlap between the orbitals containing the unpaired electron and the N orbitals. In the Cu(II) complexes for which a_{iso} is 1.5–2.0 MHz for the distal nitrogen of imidazole, the unpaired electron is in $d_{x^2-y^2}$ or d_{z^2} which overlaps efficiently with the imidazole orbitals. In the Co(II) complexes the 3 unpaired electrons occupy $d_{x^2-y^2}$, d_{z^2}, and one of the other d orbitals. Although the net overlap of these 3 orbitals with the imidazole nitrogen is expected to be less efficient than in the Cu(II) complexes, the observed a_{iso} is plausible for the distal imidazole nitrogen and seems too small for the directly bound imidazole nitrogen. The lack of discernible modulation due to the directly bound nitrogens of EDTA, EDDA, DTPA, or dacoda is consistent with the suggestion that the electron–nuclear coupling to the directly bound nitrogens is too large to observe in these ESEEM experiments.

Conclusions

Electron spin echoes were observed for Co(II) complexes at about 6 K. Complexes with and without coordinated water can be distinguished on the basis of ratios of data in D_2O and H_2O and on the basis of shifts in sum peaks to frequencies above $2\nu_H$. The predominant form of Co(EDTA) in this cryogenic system has hexadentate EDTA, in agreement with the X-ray crystal structure. Nitrogen modulation due to coordinated imidazole was observed. These results suggest that ESEEM will be an important tool to study high-spin Co(II) complexes.

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