Proton NMR Spectroscopy as a Probe of Dinuclear Copper(II) Centers

Richard C. Holz* and Julie M. Brink

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322

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Enzymes containing dinuclear Cu(II) centers play important roles in nature and, consequently, characterization of their structure and function is a problem of outstanding importance.[1,2] A fundamental and, as yet, largely unexplored issue is the determination of the structural and magnetic properties of dinuclear Cu(II) centers using NMR spectroscopy. ^{1}H NMR is a natural technique to probe these systems because only protons proximate to the paramagnetic center are affected.[3,4] However, the slow electronic relaxation typical of Cu(II) ions makes this type of study extremely difficult to execute, and correspondingly few examples exist in the literature.[5–12] In an effort to gain insight into the structures of dinuclear Cu(II) metalloprotein active sites and model complexes, we have applied one- and two-dimensional ^{1}H NMR techniques to an antiferromagnetically coupled (μ-phenoxo)(μ-hydroxo)dicopper(II) complex. Clear COSY cross-signals are observed between hyperfine shifted signals allowing the complete assignment of the ^{1}H NMR spectrum. These data, coupled with X-ray crystallographic results, indicate that a paramagnetic dipolar relaxation mechanism is the dominant proton relaxation pathway.

The previously reported (μ-phenoxo)(μ-hydroxo)dicopper(II) complex $[Cu_2(BPMP)(OH)](ClO_4)_3$ (**1**) was synthesized and crystallographically characterized.[13–15] Complex **1** exhibits several sharp, isotropically shifted ^{1}H NMR signals in acetonitrile solution at 55 °C in the 150 to −50 ppm chemical shift range (Figure 1,[25] Table 1). All of the isotropically shifted signals sharpen and shift toward the diamagnetic region as the temperature is increased. While magnetic data have not been reported for **1**, $-2J$ values for several related (μ-phenoxo)(μ-hydroxo)dicopper(II) complexes have been reported.[16–19] All of these complexes exhibit moderate to strong antiferromagnetic coupling between the Cu(II) centers with $-2J$ values greater than 100 cm^{-1}. Using the Evans susceptibility method,[20,21] the room temperature magnetic moment (μ_{eff}/Cu) of **1** was found to be 1.27 μ_B which gives the number of unpaired electrons (n/Cu) as 0.62. These data indicate that the Cu(II) ions in **1** are moderately antiferromagnetically coupled. Since the magnetic moment of the complex is small, relatively sharp isotropically shifted NMR signals are observed.[12]

Several of the isotropically shifted ^{1}H NMR signals observed for **1** can be initially assigned by inspection of their peak areas. Signals D (23.6 ppm), E (22.2 ppm), F (14.6 ppm), G (9.93 ppm), and H (5.12 ppm) integrate to 4:4:2:4:3 protons, respectively (Table 1). These data taken together with the crystallographic results suggest that signals D, E, and G arise from pyridyl protons while signals F and H are due to the *m*-phenol and the *p*-methylphenol protons, respectively. Definitive assignment of each of these signals comes from two-dimensional NMR techniques. A magnitude COSY spectrum of **1** was recorded at 25 °C and clearly shows cross-signals between resonances D and G and also between resonances E and G (Figure 2). These signals can be unequivocally assigned to the pyridine β-H (E), β'-H (D), and γ-H (G) protons, respectively.

Assignment of the remaining signals of **1** comes from T_1 values[22] and comparison of the spectrum of **1** with that of a related complex $[Cu_2(CH_3HXTA)(OH)]^{2-}$ (**2**) where the methylpyridyl ligands have been replaced by acetate moieties.[23,24] The ^{1}H NMR spectrum of **2** shows five isotropically shifted signals at 55 °C in D_2O and pH 10 (Figure 1[25]). Signals D (16.8 ppm; 7 ms) and E (9.1 ppm; 25 ms) integrate to two and three protons, respectively, and are thus assigned to the *m*-phenol and *p*-methylphenol protons, respectively. Signals B (69 ppm; ~1 ms) and C (62 ppm; ~1 ms) are assigned to the diastereotopic β-CH_2 acetate protons based upon their relative integrations, T_1 values, and the fact that they are selectively deuterated at 90 °C and pH 10. The only remaining protons in **2** unassigned are the β-CH_2 protons of the phenol–methylamine linkage. These protons can therefore be assigned to signal A (143 ppm). Comparison of the chemical shift, T_1 values, and

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(14) Abbreviations: BPMP = 2,6-bis[[bis(2-pyridylmethyl)amino]methyl]-4-methylphenol; CH_3HXTA = *N,N'*-(2-hydroxy-5-methyl-1,3-xylylene)bis(*N*-(carboxymethyl)glycine).
(15) X-ray analysis for $[Cu_2(BPMP)(OH)](ClO_4)_3$ (**1**) ($C_{33}H_{34}N_6O_{10}Cu_2Cl_2$): monoclinic space group $P2_1/c$, a = 11.843 (3) Å, b = 22.126 (7) Å, c = 14.356 (6) Å, and β = 105.58 (2)°, with V = 3623.8 (16) Å^3, ϱ_{calcd} = 1.600 g cm^{-3}, and Z = 4. A total of 4825 unique reflections out of 2θ = 50° Mo Kα were collected at −100 °C on a Seimens P4 diffractometer equipped with an LT-2a low-temperature device. The structure was solved by Patterson methods and refined anisotropically to R = 5.71%, R_w = 6.89% using SHELXTL-PLUS programs (Siemens).
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Table 1. ^{1}H NMR (300 MHz) Parameters for $[Cu_2(BPMP)(OH)](ClO_4)_2$ at 55 °C in CD_3CN

signal	assignt	chem shift (ppm)	line width [fwhm[a] (Hz)]	T_1 (ms)[b]	integration[c]	av[d] Cu−H (Å)	calcd[e] Cu−H (Å)
A	Ph−CH_2−N	130	~4000	5	~4	3.42	4.4
A′	Py α−**H**	78	1500	3	~4	3.15	4.0
B	N−CH_2−Py	69	900	4	4	3.68	4.3
C	N−CH_2−Py	56	475	2	4	3.19	3.8
D	Py β′-**H**	22.4	77	24	4	4.90	5.7
E	Py β-**H**	21.3	66	24	4	5.09	5.7
F	Ph β-**H**	14.3	40	22	2	5.66	5.6
G	Py γ-**H**	9.45	32	56	4	5.75	6.5
H	Ph−CH_3	5.06	23	103	3	7.31	
I	O**H**	−33	850	2	~1	2.54	3.4

[a] Full width at half-maximum. [b] T_1 values were obtained using the inversion-recovery method.[22] [c] Integrations are based on the area of signals H and F. [d] Crystallographically determined average distances. All Cu−H average distances are taken as the arithmetic average of equivalent protons to each copper(II) ion. [e] Distances calculated from $r_i = r_{ref}(T_{1i}/T_{1ref})^{1/6}$ assuming that signal H (r_{ref}) is purely dipolar in nature.

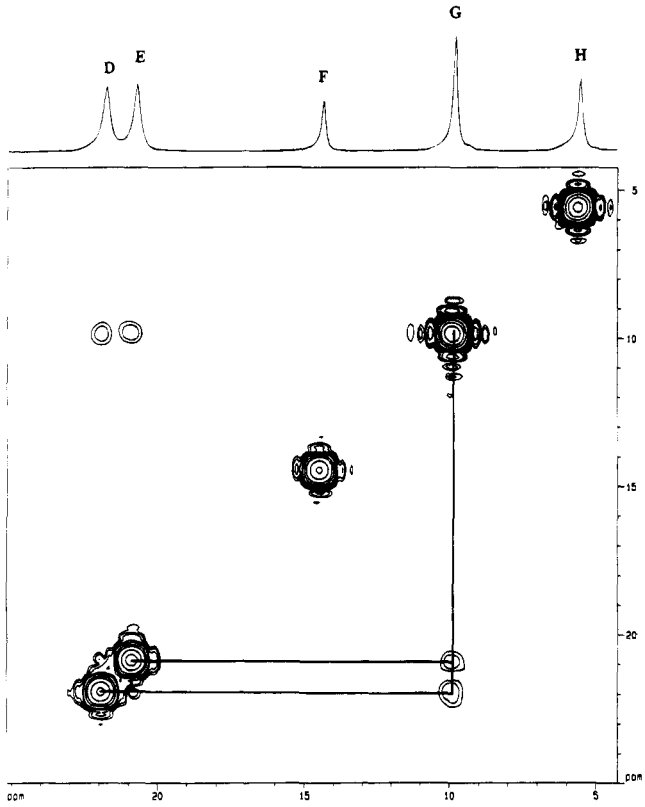

Figure 2. Magnitude ^{1}H COSY spectrum of **1** obtained at 400 MHz (Bruker ARX-400) at 25 °C in CD_3CN solution. This spectrum was obtained with an acquisition time of 15 ms and 256 data points in the F1 dimension and 512 data points in the F2 dimension. A sine bell squared weighting function and zero-filling to 1024 data points were applied prior to Fourier transformation in both dimensions.

relative integrations of signals B (69 ppm) and C (62 ppm) of complex **2** with signals B (76 ppm) and C (61 ppm) of **1** are consistent with the assignment of these signals to the diastereotopic β-CH_2 protons of the pyridylmethyl moiety. Comparison of signal A (143 ppm) of **2** with signal A (130 ppm) of **1** is consistent with this signal resulting from the β-CH_2 protons of the phenol−methylamine linkage.

Signal A′ (78 ppm) and I (−35 ppm) are the only remaining unassigned signals in the ^{1}H NMR spectrum of **1**. The only protons in **1** not assigned are the pyridine α-H protons and the μ-hydroxo OH proton. Signal I can be assigned to the μ-hydroxo OH proton since the addition of a small amount of D_2O causes this signal to disappear.[11] Moreover, the T_1 value is ~1 ms which is consistent with the short crystallographically determined Cu-H distance (2.54 Å). From X-ray diffraction results for **1** the Cu d_{z^2} orbital, which contains the unpaired electron, is directed along the Cu−O μ-hydroxo bond. Therefore, a spin polarization mechanism would cause the μ-hydroxo proton to be shielded and thus shifted upfield, consistent with its assignment to signal I. Signal A′ can be assigned to the pyridine α-H protons by default, and this assignment is consistent with T_1 values, chemical shift, and relative integrations.

Full assignment of the ^{1}H NMR spectrum of **1** combined with the crystallographic results and T_1 values allows the dominant proton relaxation pathway to be determined. Assuming a paramagnetic dipolar relaxation mechanism for antiferromagnetically coupled dinuclear Cu(II) complexes, the Cu−H distance (r) should be proportional to $T_1^{1/6}$ (Table 1).[4] Using the equation $r_i = r_{ref}(T_{1i}/T_{1ref})^{1/6}$ where r_i is the Cu−H_i distance, r_{ref} is the Cu−H_{ref} distance, T_{1i} is the relaxation time of proton i, and T_{1ref} is the relaxation time of the reference proton, distances of each proton from the Cu(II) center can be estimated. If r_{ref} is taken as the arithmetic average of equivalent protons to each Cu(II) ion for the *p*-methylphenol group (7.31 Å), the remaining distances of all of the protons in **1** can be calculated (Table 1). All of the calculated Cu−H distances are within *ca.* 20% of the Cu−H distances derived from the X-ray structure of **1** (Table 1). These data indicate that a paramagnetic dipolar relaxation mechanism dominates in antiferromagnetically coupled trigonal bypyramidal dicopper(II) complexes.

In conclusion, isotropically shifted ^{1}H NMR signals can be easily obtained for antiferromagnetically coupled dicopper(II) complexes, and both one- and two-dimensional ^{1}H NMR techniques can be performed. We are currently probing the effect of the strength of the magnetic coupling on line widths and T_1 values. The application of ^{1}H NMR techniques to dicopper(II) metalloprotein active sites is also under investigation.

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Supplementary Material Available: A thermal ellipsoid drawing, tables detailing the X-ray data collection and refinement, bond distances, bond angles, final anisotropic thermal parameters, calculated or refined H atom coordinates, and the atomic coordinates and equivalent isotropic thermal parameters for **1**, and Figure 1, showing ^{1}H NMR spectra of **1** and **2** (8 pages). Ordering information is given on any current masthead page.