Predominant Scalar Interactions in Selective Broadening of Ligand Nuclear Magnetic Resonances by Copper(II) Ions

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Abstract: Selective broadening of proton and carbon ligand resonances in NMR spectroscopy by Cu(II) is evaluated as a method for determining Cu(II) binding sites in aliphatic and aromatic nitrogen, and carboxylate ligands. For many chelating ligands such as peptides, complexes present at the very high ligand to Cu(II) molar ratios of a selective broadening experiment are different from those found at stoichiometric concentrations. At high molar ratios Cu(II) is passed rapidly from one ligand to another before peptide hydrogens undergo ionization and before six-membered or larger chelate rings can close. Measurements of both spin-lattice and transverse relaxation times indicate that for neither proton nor carbon resonances is the broadening determined predominantly by a dipolar mechanism so that interpretations based on an r^{-6} dependence are invalid. For protons the broadening is determined mainly by scalar interactions increasing in importance as the donor group is varied from carboxylate oxygen, to π bonding amines, to aliphatic amines. At least for ligands containing π bonding systems a small amount of unpaired spin density transmitted to a carbon atom, because of its nearness to the measured proton and the strong r^{-6} dependence, renders equivocal selective T_1 results. For Cu(II) bound at N3 of imidazole, only about 30% of the inverse spin lattice relaxation time of H5 is due to the direct dipolar interaction with Cu(II), while the other 70% is due to the presence of a small amount of unpaired spin density at nearby C5. Similar interactions severely compromise even selective T_1 results for determination of Cu(II) binding sites in nucleic acids.

Numerous studies have employed selective broadening by a paramagnetic ion of lines in nuclear magnetic resonance spectra to locate metal ion binding sites. Amino acids such as histidine, peptides, and nucleic acids have been studied in this fashion. The extent of paramagnetic metal ion induced line broadening is proportional to the inverse transverse relaxation time, T_{2P}^{-1}. Implicit in the identification of metal ion binding sites from a selective broadening experiment are two assumptions. In the first the transverse relaxation time in the presence of the bound paramagnetic ion is given by

$$T_{2P}^{-1} = pqT_{2M}^{-1} \quad (1)$$

where p is the ratio of molar concentrations of paramagnetic metal ion to ligand, q is the average number of ligands bound in an identical way, and T_{2M} is the transverse relaxation time of the bound ligand. In the second assumption the inverse transverse relaxation time for a nucleus on the bound ligand is given by only a dipolar term according to

$$T_{2M}^{-1} = 7a\tau_c r^{-6} \quad (2)$$

where τ_c is the correlation time modulating the dipolar interaction and a is a collection of values which are constant for a given metal ion,

$$a = \gamma_I^2 g^2 \beta^2 S(S+1)/15$$

Other assumptions made concerning the application of the Solomon-Bloembergen equations[1,2] in this paper that are true for most paramagnetic ion complexes of small ligands at 23.5 kG are that $\omega_I < \tau_c^{-1} < \omega_s > \tau_e^{-1}$. The r^{-6} dependence of eq 2 is the basis for stating that selectively broadened nuclei are nearest to the paramagnetic ion. The distance between the paramagnetic ion and the measured nucleus is denoted by r.

Both eq 1 and 2 involve severe assumptions which have seldom been verified in selective broadening experiments. Equation 1 assumes that relaxation occurs by chemical exchange in the fast exchange limit of a more general eq 3

$$T_{2P}^{-1} = pq(T_{2M}^{-1} + \tau_M \Delta\omega_M^2) \quad (3)$$

where τ_M is the lifetime of a ligand bound to the metal ion and $\Delta\omega_M$ is the chemical shift between bound and unbound ligand resonances.[2,3] Equation 3 reduces to the fast exchange limit eq 1 only when $T_{2M}^{-1} \gg \tau_M \Delta\omega_M^2$. When the reverse inequality is true, $T_{2P}^{-1} = pq\tau_M \Delta\omega_M^2$, and the line width is frequency dependent in this intermediate exchange region. In addition an increase in temperature results in exchange narrowing. Thus frequency and temperature dependence studies of line widths provide experimental tests for the dominant term in eq 3. Since each atom on a ligand has a unique $\Delta\omega_M$, it is possible that for two different atoms on the same ligand, one may have the first term on the right of eq 3 dominant and the other the second term foremost. Equation 3 also contains the restrictions that outer sphere complexation is negligible and that $\tau_M^{-1} \gg T_{2M}^{-1}, \Delta\omega_M$. When either of the last two terms exceeds τ_M^{-1}, $T_{2P}^{-1} = pq\tau_M^{-1}$, and T_{2P}^{-1} is governed by the rate of chemical exchange in this slow exchange region.[2,3] Since an increase in temperature speeds the slow chemical exchange it also broadens an NMR peak. This behavior was not observed in the results reported in this paper, and a more general equation incorporating this region is omitted here for simplicity.

Equation 2 assumes that only the dipolar term of the Solomon-Bloembergen equations dominates the transverse relaxation time. The more complete equation[1,2] also includes a scalar term,

$$T_{2M}^{-1} = 7a\tau_c r^{-6} + bA^2\tau_e \quad (4)$$

where A is the scalar or hyperfine coupling constant which is generally different for each ligand nucleus, τ_e is the correlation time modulating the scalar interactions and $b = S(S+1)/3\hbar^2$. Thus not only must the fast exchange limit eq 1 be valid but also the scalar term must be negligible in eq 4 so that it reduces to eq 2 for proper interpretation of a selective broadening result.

The advent of pulsed Fourier transform nuclear magnetic resonance spectrometers provides a generally accessible means to test the dominant term in eq 4. The corresponding Solomon-Bloembergen equation for the inverse spin-lattice relaxation time for nuclei of ligands bound to paramagnetic ions is[1,2]

$$T_{1M}^{-1} = 6a\tau_c r^{-6} \quad (5)$$

This equation contains only a dipolar term as the scalar

term of T_{1M}^{-1} is almost always negligible. Fortunately also there is no intermediate exchange region for T_{1P}^{-1} so that the complete equation corresponding to eq 3 for T_{2P}^{-1} is[2,3]

$$T_{1P}^{-1} = pqT_{1M}^{-1} \quad (6)$$

Thus measurement of T_1 relaxation times in a selective T_1 experiment allows assignment of paramagnetic ion binding sites by combination of eq 6 and 5 for dipolar, r^{-6}, interactions only according to eq 7.

$$T_{1P}^{-1} = pq6a\tau_c r^{-6} \quad (7)$$

A convenient test for whether a selective broadening experiment permits conclusions to be based on solely dipolar interactions is to evaluate the ratio T_{1P}/T_{2P}. Combination of the fast exchange limit eq 1 with eq 4, 5, and 6 yields $T_{1P}/T_{2P} = 7/6 = 1.17$ for solely dipolar interactions with the scalar term of eq 4 negligible. Greater values indicate that the fast exchange limit is not attained so that eq 3 must be employed and/or the scalar term of eq 4 is not negligible. In the fast exchange limit 50% dipolar and 50% scalar contributions to T_{2M}^{-1} yield $T_{1P}/T_{2P} = 7/3 = 2.33$. A ratio of 4 suggests a 71% scalar contribution to eq 4, and it is then better to ignore the dipolar rather than the scalar term.

The only metal ion considered in this paper is Cu(II). For complexes of Cu(II) with most ligands the inverse scalar correlation time, $\tau_e^{-1} = \tau_S^{-1} + \tau_M^{-1}$, becomes nearly equal to the inverse electron spin relaxation time, $\tau_S^{-1} \sim 10^8$ sec^{-1}, rather than to the inverse lifetime of a ligand bound to Cu(II), τ_M^{-1}. With small ligands the inverse dipolar correlation time, $\tau_c^{-1} = \tau_e^{-1} + \tau_R^{-1}$, is nearly that of the inverse rotational correlation time of the complex, $\tau_R^{-1} \sim 10^{10}$ sec^{-1}.[2,3]

This paper reports an analysis of the validity of the assumptions of fast exchange and dipolar mechanism operating in selective broadening studies with Cu(II) on a variety of carboxylate, amine, and aromatic ligands. The ligands were chosen so that peak splitting by spin-spin coupling, which might compromise the interpretation, does not occur. For each proton only a single resonance corresponding to the weighted average of unbound and bound ligand is observed. In an earlier communication we indicated that selective broadening by Cu(II) is usually not dipolar determined[4] and this paper verifies and extends the results and analysis to show that the T_{2P} relaxation is usually due to a predominantly scalar interaction.

Usually in a selective broadening experiment with a metal ion such as Cu(II) the ligand to metal ion ratio is of the order of 10^3. At such high molar ratios the predominant complex may not be the same as that existing at more nearly stoichiometric ratios. Thus in addition to the concerns about solely dipolar interactions, the selective broadening experiment may yield results that are not applicable to complexes existing at small molar ratios of ligand to metal ion. Examples are basic equimolar solutions of dipeptides and Cu(II) which are known from x-ray,[5] potentiometric, and optical studies[6,7] to form terdentate complexes with amino, ionized amide nitrogen, and carboxylate donor atoms. Selective broadening experiments with a glycyl dipeptide to Cu(II) ratio of 5000 broaden almost exclusively only the amino terminal methylene group, leaving the carboxylate terminal glycyl, or alanyl, or prolyl groups unaffected.[8] Similarly only the amino terminal methylene groups of triglycine undergo selective broadening with Cu(II)[8] even though the ligand is quadridentate in equimolar solutions.[6,7] Differences between the peptides at the two different concentration ratios may be understood by considering the rate processes involved. At high ratios, Cu(II) passes rapidly from one molecule of anionic peptide ligand to another and each is apt to be bound only in a unidentate mode through the amino group. The rate limiting process in formation of a multidentate peptide with an ionized amide hydrogen includes very slow loss of that hydrogen in the chelate ring closure step.[9] At high basic ligand to metal ion ratios amide hydrogen ionization and ring closure do not have time to occur before Cu(II) has passed onto another ligand. This conclusion is further supported by observation[8] of only amino terminal group broadening at high ratios of either glycylglycine or glycylproline to Cu(II). The latter dipeptide does not contain an ionizable amide hydrogen, and hence chelation at the amide nitrogen is not possible.[6,10] Also ruled out by the slow amide deprotonation and ring closure process is the model of Cu(II) binding to the amide nitrogen of carnosine (β-alanyl-L-histidine) at pD 11.4 in a selective broadening study[11] that has been reinterpreted.[12] However, both the original study and the reinterpretation require that selective broadening occur by a dipolar mechanism, which will be shown not to be the case for the amine and imidazole moieties of carnosine.

Experimental Section

High quality commercial ligands were prepared in D_2O solvent at concentrations of 0.1–0.5 *M*. The pD was varied by adding NaOD or DCl to the solution. Carboxylic acid ligands were about 75% ionized, amine ligands about half-neutralized, and amino acid ligands had their amine half-neutralized. Sufficient Cu(II) was added with lamda pipets from a concentrated solution of copper sulfate to obtain measurable line broadening.

The proton magnetic resonance studies were performed on a Jeol PFT-100P/EC 100 FT NMR spectrometer at 100 MHz and a Hitachi Perkin-Elmer R-20 NMR spectrometer at 60 MHz. The following two relationships were used to calculate T_{1P}^{-1} and T_{2P}^{-1}:

$$T_{1P}^{-1} = (T_{1P}^{-1})_{Cu} - (T_{1P}^{-1})_0$$

$$T_{2P}^{-1} = \pi(W_{Cu} - W_0)$$

$(T_{1P}^{-1})_{Cu}$ and $(T_{1P}^{-1})_0$ are the inverse spin-lattice relaxation time with and without added Cu(II), respectively. W_{Cu} and W_0 are the full line width in hertz at half-height with and without Cu(II), respectively. Both $(T_{1P}^{-1})_{Cu}$ and W_{Cu} were proportional to added $[Cu^{2+}]$. T_1 values were measured accurately (±10%) with the 180°-τ-90° pulse sequence. The width of the 90° pulse was usually about 25 μsec for proton and 17 μsec for carbon nuclei, and it was set with a doped sample. For the T_1 spectra, the free induction decays contained 4K data points and to improve signal to noise an exponential filter was used in the transformation.

Usually 16 different τ for proton and 10 τ for carbon were taken with the longest τ not more than 2.5 times the null point. T_1's were calculated by an external least-squares fit program that contained a plotter routine on a CDC 6400 computer. Linear first-order plots were always obtained indicative of exponential decays. The T_2 results were obtained from spectra that came from FID's of 8K data points, and no exponential filter was used in the transformation to the frequency domain. The spectra were obtained from about 100–200 pulses in order to obtain a good signal to noise ratio. In general T_1 and T_2 values were not as accurate for carbon, and protons with spin-spin couplings, due to a lower signal to noise ratio.

Results

Results for the inverse spin-lattice and transverse relaxation times for hydrogen nuclei for a series of ligands determined on a single solution in the presence of Cu(II) are listed in Table I. Representative Cu(II) molar concentrations for a single experiment are also presented, but many experiments were performed over a range of Cu(II) concentrations. Ratios of T_{1P}^{-1} or T_{2P}^{-1} to $[Cu^{2+}]/[\text{ligand}]$ reveal the sensitivity of the two inverse relaxation times to Cu(II) concentration. Thus greater broadening (T_{2P}^{-1}) occurs for unidentate amines (only about half-neutralized in these ex-

Table I. Proton Spin–Lattice and Transverse Relaxation Times in Presence of Cu(II)

Ligand	$10^4 \cdot [Cu^{2+}]/[lig]$	T_{1P}^{-1}, sec^{-1}	T_{2P}^{-1}, sec^{-1}	T_{1P}/T_{2P}
Acetate	63	6.9	48	6.9
Chloroacetate	61	2.9	3.1	1.1
Bromoacetate	76	5.6	10.5	1.9
Glycolate	46	15.0	27	1.8
Malonate	4.8	4.7	39	8.4
Succinate	93	15.6	34	2.2
Methylamine	6.8	0.23	81	350
Dimethylamine	6.5	0.22	55	250
Trimethylamine	9.8	0.037	9.6	260
1,2-Diaminoethane	2.3	0.71	104	146
Glycinate	1.7	0.84	90	108
Sarcosinate CH_2	1.0	0.50	24	49
CH_3		0.54	71	132
N,N-Dimethylglycinate CH_2	51	14.6	26	1.8
CH_3		10.8	29	2.7
L-Methionine S-CH_2	14	1.6	7.9	4.9
S-CH_3		0.26	1.9	7.3
S-Methyl-L-cysteine CH	74	0.20		
CH_2		0.19		
CH_3		0.086	2.3	27
Glycylglycinate CH_2COO^-	0.9	0.063	1.2	19
CH_2NH_2		0.16	12.9	79
Imidazole H2	3.0	1.2	25	21
H4, H5		0.71	34	48
5'-AMP H8	4.9	11.9	38	3.2
H2		3.15	16.1	5.1

Table II. Carbon-13 Spin–Lattice and Transverse Relaxation Times in Presence of Cu(II)

Ligand	$10^4[Cu^{2+}]/[lig]$	T_{1P}^{-1}, sec^{-1}	T_{2P}^{-1}, sec^{-1}	T_{1P}/T_{2P}
Acetate CH_3	0.79	0.082	50.2	615
COO^-		0.20	19.7	100
Dimethylamine	1.7	0.097	39	400
Glycinate CH_2	1.5	0.16	97	620
COO^-		0.34	31	90
Glycylglycinate $NH_2CH_2^-$	0.96	0.35	33	95
$-CONH-$		0.32	25	80
Imidazole C2	0.47	0.14	69	500
C4, C5		0.095	44	460

periments) per specified amount of Cu(II) than for the more weakly coordinating unidentate carboxylates.

For most of the ligand nuclei of Table I, T_{1P}/T_{2P} values greater than 2.3 indicate predominate scalar interactions according to eq 4 and/or contributions from the intermediate exchange limit according to eq 3. In order to distinguish between these two possibilities frequency and temperature dependence studies were performed on representative ligands. Line widths were identical at 60 and 100 MHz, and decreased with increasing temperature for the protons of acetate, methylamine, and glycinate. The lack of any frequency dependence of the line width in the Cu(II) containing solutions indicates that the fast exchange limit eq 1 is obtained in these cases. At 23.5 kG, both T_{1P}^{-1} and T_{2P}^{-1} decrease with increasing temperature for dimethylamine and imidazole and the ratio T_{1P}/T_{2P} increases. These results are consistent with a predominant scalar interaction with the temperature dependence of the rate process τ_R^{-1} greater than that of τ_S^{-1}. Due to strong chelation, the fast exchange limit may not have been achieved in the nitrilotriacetate complex reported in our earlier communication,[4] and the results for this complex should not be considered further.

T_{1P}/T_{2P} ratios of ^{13}C nuclei were determined for few selected ligands, and the results are presented in Table II. As anticipated, the ratios are very large, precluding a solely dipolar mechanism and interpretations of distance dependence based on selective broadening.

Discussion

The high T_{1P}/T_{2P} ratios for most of the ligands in Table I rule out a dipolar mechanism (eq 2) operating in the fast exchange limit (eq 1). Since the line widths are frequency independent and an increase in temperature decreases both T_{1P}^{-1} and T_{2P}^{-1}, the fast exchange limit condition (eq 1) is obeyed. Therefore, selective broadening experiments with Cu(II) do not operate by a dipolar mechanism because of an usually predominant scalar term so that in place of eq 2 the more complete eq 4 must be employed. The best way to obtain distance information in these systems is to perform selective T_1 experiments and employ eq 7.

The results of Table I demonstrate that the scalar interaction increases in importance as the donor group is varied from $-COO^-$, π bonding amines, to aliphatic amines. The increase in scalar interaction is great enough so that comparisons of Cu(II) site binding among the different donor groups by selective broadening experiments are unreliable. Thus simple selective broadening comparisons of binding to imidazole and amino groups in histidine and histidine containing peptides are likely to be incorrect. Due to the greater scalar interaction much greater broadening is caused by equivalent Cu(II) binding to amino than to imidazole groups.

Another weakness of paramagnetic metal ion binding site identification by selective broadening of resonances of nearby nuclei is that the fraction of ligand molecules complexed in a specified mode is often unknown and determination of the binding strength of the interaction requires separate experiments, usually by another technique. The results of Table I point up the fact that strongly broadened lines often result from a predominant scalar interaction which varies widely for different classes of donor atoms and also within a class. Thus broadening of a resonance line may indicate an interaction so weak that the use of the word binding is inappropriate. By themselves selective broadening experiments seldom permit differentiation of strong binding from weak interaction cases. Since the Cu(II) concentrations were adjusted to give convenient values of T_{1P}, the concentration ratios in Table I provide a qualitative measure of binding strength.

Even though an explicit distance dependence does not appear in the scalar term, selective broadening experiments may still be useful in determining sites of Cu(II) binding. Along an aliphatic chain the hyperfine constant falls off with distance, but not as predictably as the dipolar r^{-6} term. In conjugated ligands, such as imidazole and nucleic acid bases, the hyperfine constant will bear little relationship to the distance from Cu(II) but depends upon how the unpaired electron spreads through the complex. In this case, accurate molecular orbital parameters, though difficult to obtain, are required to make predictions. The best applications of selective broadening experiments would appear to be in large molecules such as peptides containing at least several amino acid residues where the sites of fleeting Cu(II) bonding may be indicated. Even then the results are limited to the high ligand to Cu(II) mole ratios of the experiment and may not apply to the complexes occurring at stoichiometric ratios.

Nearly identical T_{1P} values for the two kinds of protons in sarcosinate and dimethylglycinate indicate Cu(II) binding at the amino group as $r^6 \sim T_{1P}$. For methionine the methyl protons are 1.35 times as far from the Cu(II) as the $-SCH_2$ protons consistent with Cu(II) binding at the glyci-

nate locus and no interaction at the sulfur atom. For *S*-methylcysteine the T_{1P} values indicate more nearly equal methyl and $-SCH_2$ distances. The lack of notable selective broadening of the methyl group in methionine and its appearance with *S*-methylcysteine has been ascribed to binding of some of the latter ligand via S and N donor atoms in the Cu(II) tetragonal plane.[13] This hypothesis may be unnecessary, as Cu(II) interaction at the glycine locus and chelation at sulfur requires only a five-membered ring with *S*-methylcysteine and a six-membered ring with methionine. Five-membered chelate rings close rapidly, but six-membered rings are often found to be under steric control[14] so that the sulfur of methionine does not chelate before the Cu(II) passes on to another ligand.

The results presented in the previous section for imidazole and Cu(II) may be analyzed to test the validity of the assumptions and to provide further insight as to what may be learned from T_{1P} results. Consider the Cu(II) to be bound at N3 of an imidazole ring with a Cu–N distance of 2.00 Å commonly found in imidazole and derivatives.[5,12] From other dimensions found in imidazole rings the distance from Cu to H2 and H4 is calculated to be 3.26 Å and that to H5 to be 5.16 Å. The experimentally measured $(T_{1P}^{-1})_{H4,5} = 0.712$ sec^{-1} value is an average of $(T_{1P}^{-1})_{H4}$ and $(T_{1P}^{-1})_{H5}$. Resolution may be achieved by noting that for a single complex from eq 7

$$(T_{1P}^{-1})_4/(T_{1P}^{-1})_5 = (r_5/r_4)^6 = (5.16/3.26)^6 = 15.7$$

so that $(T_{1P}^{-1})_4 = 1.34$ and $(T_{1P}^{-1})_5 = 0.085$ sec^{-1}. Since the Cu to H2 and H4 distances are taken as equal in the single complex, the observed value of $(T_{1P}^{-1})_2 = 1.21$ sec^{-1} should be identical with that of the resolved $(T_{1P}^{-1})_4$. That they differ by only 11% supports the assumptions and analysis. For Cu(II) and protons the value of $a = 1.38 \times 10^{-44}$ m^6 sec^{-2} in eq 7. Combination with the other quantities yields, from the three protons, an average rotational correlation time of the bound paramagnetic ion, $\tau_R = 1.6 \times 10^{-11}$ sec. This value is similar to that found for other small ligand complexes. The line of reasoning presented here may be reversed to calculate relative bond distances between a paramagnetic ion and other ligand nuclei, and if a value of τ_R is assumed absolute bond distances may be estimated.

The geometric information available from analysis of the T_{1P} results for imidazole and Cu(II) is not obtainable from the T_{2P} results as the high ratio of T_{1P}/T_{2P} and the lack of any frequency dependence of the line width indicates that a scalar mechanism predominates. Since the dipolar term contributes less than 6%, combination of eq 1 and 4 yields

$$(T_{2P}^{-1})_{H2}/(T_{2P}^{-1})_{H4,5} = A_{H2}^2/A_{H4,5}^2$$

as all the other terms are constant for the two sets of protons in a single complex. From the results presented in the last section the ratio of the scalar coupling between Cu(II) and H2 to the average between Cu(II) and H4 and H5, $A_{H2}/A_{H4,5} = 0.86$. This ratio is similar to that found for a triphenyl substituted imidazole from an electron spin resonance study.[15] In order to obtain absolute values for the scalar coupling constants, a value for the electron spin relaxation time must be assumed.

As satisfactory as the preceding analysis for imidazole might seem, it is incomplete and misleading. Not all the unpaired spin density resides on the Cu(II) as a small amount is distributed about the imidazole ring. Even though the unpaired spin density at C2, C4, and C5 is small, it is nearer to H2, H4, and H5, respectively, than is the Cu(II). Since the distance dependence in eq 7 goes as r^{-6} even a small amount of spin density only 1.10 Å away may be important. If accurate molecular orbitals were known for the Cu(II) complex, unpaired spin densities about the imidazole ring could be calculated. It is possible to estimate the importance of unpaired spin density at the carbon atoms from our results by employing equations such as the following one written for H2

$$(T_{1P}^{-1})_{H2} = pq6a\tau_R(\rho_{Cu}^2 r_1^{-6} + \rho_{C2}^2 r_2^{-6})$$

where r_1 is the Cu to H2 distance, r_2 the C2 to H2 distance, and ρ is the unpaired spin density from both σ and π orbitals. Terms from the other four ring atoms of imidazole may be included in the parentheses, but their contributions are apt to be small. Two other similar equations can be written for H4 and H5. If one assumes that most of the unpaired electron resides at the Cu(II) ion ($\rho_{Cu} \approx 1$), and $(T_{1P}^{-1})_{H2} \approx (T_{1P}^{-1})_{H4}$, the following can be solved for one set of data: $\rho_{C4} = \rho_{C5} = 0.014$, and $\tau_R = 1.2 \times 10^{-11}$ sec. These numbers may only be considered estimates. They may be useful in evaluating the assumptions and determining the important interactions. The contribution of the ρ_C^2 term in the above (T_{1P}^{-1}) equations contributes about 10% for H4, and 70% for H5. Therefore, for the more distant H5, most of the contribution to T_{1P}^{-1} is not due to the direct dipolar interaction of Cu(II) according to eq 7 but rather to the dipolar interaction of only a small amount of unpaired spin density at C5.

If one employs the three (T_{2P}^{-1}) equations for the three protons of imidazole and assumes that spin density and hyperfine constant are related by the McConnell equation,[16] $A = (-)22.5\rho_c$ G, the following can also be calculated: $\rho_{C2} \approx 0.012$ and $\tau_S \approx 3 \times 10^{-9}$ sec. This approximate value of τ_S is well within the range of quoted values.[3,17] Use of the McConnell equation may not be entirely appropriate to the extent that unpaired spin density is transmitted through both the σ and π systems of imidazole.

The values estimated for τ_R, τ_S, and spin densities for protons may be verified by ascertaining whether they are in agreement with results from other nuclei. This comparison was performed with carbon-13 results on the imidazole ligand. The equation for the spin–lattice relaxation time for C2 is

$$(T_{1P}^{-1})_{C2} = pq6a'\tau_R(\rho_{Cu}^2 r_{1C}^{-6} + \rho_{C2}^2 r_{2C}^{-6})$$

where r_{1C} is the Cu to C2 distance and r_{2C} is the distance of the unpaired electron on the carbon with its nuclei, and a' has been corrected for the gyromagnetic ratio of ^{13}C. Similar equations can be written for C4 and C5. The T_{2P}^{-1} equations for carbon-13 are identical with eq 1 and 4 used for protons, except A is the hyperfine coupling constant for carbon-13 nuclei. This constant can be estimated from spin densities.[18] Using τ_R, τ_S, and spin densities from our proton results, the T_{1P}^{-1} and T_{2P}^{-1} were calculated for carbon-13 and these numbers are within a factor of two of the experimental values. This agreement is reasonable considering the number of unknowns and approximations.

A comparison of the T_{1P}/T_{2P} ratio of the two nuclei shows that ^{13}C is 24 and 10 times larger than protons for the nuclei at positions 2 and 4, 5, respectively. The following would change the T_{1P}/T_{2P} ratio of ^{13}C: (1) the smaller gyromagnetic ratio would increase it by a factor of 16; (2) the ratio would decrease as the ρ^2/r^6 term in T_{1P}^{-1} increased; and (3) the larger hyperfine constant increases the ratio as the square of its value. The first two items have large effects on the T_{1P}/T_{2P} ratio, while the last one has a small effect. The second item has a large effect due to the proximity of the unpaired electron on carbon to the carbon nucleus.

For adenosine 5′-monophosphate (AMP), the T_{1P} results of Table I suggest that more Cu(II) is bound at N7 than

has been inferred from selective broadening studies. The lack of quantitative correspondence between the T_{1P} and T_{2P} results renders suspect conclusions concerning the mode of interaction of paramagnetic ions and nucleic acid bases where only selective broadening experiments have been performed and a dipolar mechanism assumed. The T_{1P} results suggest that Cu(II) binds to both N1 and N7 of adenosine. Scalar interactions contribute to T_{2P}^{-1} and not to T_{1P}^{-1}. Table I indicates T_{1P}/T_{2P} is 1.6 times greater for H2 than H8 on AMP. Hence if equal binding were to occur at both N1 and N7 sites, H2 would exhibit greater broadening than H8, if only the effects of nearby Cu(II) are considered. However, analysis of unpaired spin densities at carbon atoms, such as those just made for imidazole also apply to nucleic acid bases, complicating greatly interpretations of selective broadening and even selective T_1 experiments. On the basis of selective broadening experiments in aqueous solutions of 5′-AMP, a binuclear structure with two stacked nucleotides and two Cu(II) has been proposed.[19] These experiments were performed with large excesses of ligand over Cu(II), 2000/1 or even greater. If all the nucleotides are stacked in pairs, the probability of two Cu(II) occurring simultaneously on one pair is one part or less in 10^6. Thus the binuclear structure proposed for stacked pairs of 5′-AMP and 3′-AMP in these solutions must be rejected. Relaxation time results for many Table I ligands with Mn(II) have also been obtained.[20]

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Photoassisted Electrolysis of Water by Ultraviolet Irradiation of an Antimony Doped Stannic Oxide Electrode

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Abstract: Products, stoichiometry, and the stability of the photoelectrode show that the n-type semiconductor Sb-SnO_2, as a single crystal, can serve as the photoreceptor in a photoelectrochemical cell to electrolyze H_2O to H_2 and O_2. The O_2 is evolved at the irradiated Sb-SnO_2 electrode, and the H_2 is evolved at the Pt electrode of the cell. Substantial photocurrents are obtained when the applied potential (+ lead to SnO_2) exceeds ~0.5 V, and light of greater energy than the 3.5 eV band gap of SnO_2 is required to observe photoeffects at 25°. Importantly, increasing the temperature results in a measurable shift to lower energy for the onset of the photoeffects. The quantum efficiency for electron flow at 254 nm at 0.0 V vs. SCE in 1.0 *M* NaOH is 0.27 ± 0.03, and the wavelength response curve and current-voltage curve show that the quantum efficiency for electron flow is near unity at higher energy excitation wavelengths and slightly higher applied potentials. The photocurrent produces H_2 with >90% efficiency. Experiments with $H_2{}^{18}O$ show that the O_2 produced is not due to decomposition of SnO_2, and additionally, stability of the SnO_2 photoelectrode has been determined by constant weight before and after prolonged irradiation.

Photoelectrochemical cells employing reduced TiO_2, an n-type semiconductor, as the photoelectrode have recently been shown[4] to sustain the photoinduced electrolysis of H_2O (reaction 1).

$$H_2O \xrightarrow{h\nu} H_2 + \tfrac{1}{2}O_2 \qquad (1)$$

Strong interest in TiO_2[4-10] stems from the fact that it does not undergo decomposition upon illumination in an aqueous electrolyte. Such decomposition is common for several semiconductor photoelectrodes that have been investigated.[11] The stability of TiO_2 has roused new hopes for the conversion of optical energy into chemical energy, and such photoassistance agents may also be used to carry out special chemical synthetic missions. In discussion[12-14] of the use of photoelectrochemical cells as solar energy conversion devices, it has been pointed out that TiO_2 is the only known semiconductor that is inert—a fact which may limit the usefulness of such systems. In this report we wish to outline our characterization of the photoassisted electrolysis of H_2O using a second inert photoelectrode system: Sb-doped SnO_2, an n-type semiconductor.