{Me_2(pip)$_2$[16]cyclidene}](PF_6)$_2$, 77424-20-5; [Ni{Me_2(OMe)$_2$[16]cyclidene}](PF_6)$_2$, 70021-28-2; [Ni{Me_2(OMe)$_2$[14]cyclidene}](PF_6)$_2$, 74465-80-8; [Ni$_2${(Me_2($NH)_2$mxyl)$_2$([16]cyclidene)$_2$}]$^{4+}$, 77827-32-8; [Ni$_2${(Me_2($NH)_2$(CH_2)$_3$)$_2$([16]cyclidene)$_2$}]$^{4+}$, 77875-37-7; [Ni{Me_2(NMe_2)$_2$[16]cyclidene}]$^{2+}$, 74466-07-2; [Ru(NH_3)$_5$(py)]$^{2+}$, 21360-09-8; [Ni$_2${(Me_2($NH)_2$mxyl)$_2$([16]cyclidene)$_2$}]$^{5+}$, 102920-30-9; [Ni$_2${(Me_2($NH)_2$mxyl)$_2$([16]cyclidene)$_2$}]$^{6+}$, 102920-31-0; [Ni$_2${(Me_2($NH)_2$$CH_2$$CH_2$)$_2$([16]cyclidene)$_2$}]$^{5+}$, 102920-32-1; [Ni$_2${($Me_2$($NH)_2$$CH_2$$CH_2$)$_2$([16]cyclidene)$_2$}]$^{6+}$, 102920-33-2; 2,7,9,15,17,22,24,30-octamethyl-3,6,10,14,18,21,25,29,32,36,39,43-dodecaazatricyclo[21.7.7.7^{8,16}]tetraconta-1,7,9,14,16,22,24,29,31,36,38,43-dodecene, 102920-34-3.

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Multifrequency ESR of Cu(II)-(His)$_n$ (His = Histidine). 1. Immobile Phase[1]

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ESR studies of Cu(histidine)$_n$ in the presence of excess histidine at two microwave frequencies were undertaken to support either the glycine-like or histamine-like complexes. Low-frequency, S-band data are well-resolved for the $M_I = -1/2$ line in the $g_\parallel$ region, and the number of lines is attributed to four approximately equivalent nitrogen donor atoms. When the pH is increased from 6.8 to 7.3, a second form is detected from the $M_I = -1/2$ line in the $g_\parallel$ region but not in other portions of the spectrum. The change in ESR parameters does not appear to be large enough to account for a change from four nitrogen donor atoms to three nitrogen donor atoms in the square-planar configuration. This small change is more consistent with a change in axial coordination or a change from four nitrogen donor atoms to an altered set of four nitrogen donor atoms. Further ESR analysis of frozen copper-histidine complexes in the presence of excess histidine where the ^{14}N isotope in the imidazole ring is substituted with ^{15}N is consistent with a structure for which four equivalent ^{15}N nitrogen donor atoms are bound to cupric ion.

Introduction

Histidine is a tridentate ligand that acts like a bidentate ligand for cupric square-planar complexes due to steric constraints.[2] Histamine-like, glycine-like, and a mixture of histamine-like and glycine-like Cu(His)$_2$ (His = histidine) complexes are possible. Additional speciation occurs in the pH range of 5-7 because formation of a Cu(H-His,His) complex has a pK of 5.63.[2] In this report, these structures, which appear to be the major species present in solution, have been studied in the presence of excess histidine in the frozen state by utilizing the ESR method.

After comparison of the ESR parameters, $g_\parallel$ and $A_\parallel$, Rotilio and Calabrese inferred that the Cu^{II}(His)$_2$ complex has the mixed histidine-like and glycine-like ligand.[3] The ESR parameters for this complex in frozen solution are between the parameters for complexes with four nitrogen donor atoms and those for complexes with two nitrogen donor atoms and similar to those for a mixed glycine-histamine-Cu(II) complex that has three nitrogen donor atoms and a single oxygen donor atom. Detection of two species from the second-derivative hyperfine structure on the high-field line for the ESR spectra of ^{63}Cu(His)$_2$ complexes in D_2O recorded at room temperature suggests that equal concentrations of two species could exist at physiological pH.[4] It is suggested that one of these species has three nitrogen donor atoms and the other four nitrogen donor atoms bound to cupric ion in a square-planar configuration.

Other techniques have been used to suggest the configuration of Cu^{II}(his)$_2$. Among these, a crystal structure determination of (L-histidinato)(D-histidinato)diaquocopper(II) tetrahydrate has been used to support a plausible structure for Cu(His)$_2$ in which bidentate amino and imidazole nitrogens from each ligand bind in a cis configuration to form the square plane.[5] For crystallographic data combined with nuclear relaxation rate analysis, it is suggested that 76% of a complex with one imidazole coordinated in the histamine-like way is in equilibrium with 24% of a complex with two imidazoles bound.[6] Multifrequency ESR analysis used in correlation with computer simulation of spectra can also be a very useful tool in determining chemical structure and molecular dynamics of copper in biological systems[7] mainly due to the existence of an optimal microwave frequency at which the superhyperfine structure is best resolved.[8,9] This work uses multifrequency ESR spectroscopy to determine whether low-frequency (S-band) ESR is consistent with or adds to the present understanding of frozen copper complexes in the presence of excess histidine.

Experimental Section

L-His from Merck and DL-His-*1,3*-$^{15}N_2$ from MSD Isotopes were used without further purification. The solutions were made in 99.75% D_2O, from Merck, and the uncorrected pD was adjusted with either DCl or NaOD. Isotopically pure ^{63}Cu (from Oak Ridge National Laboratory, Oak Ridge, TN) was used for these ESR experiments.

X-Band ESR spectra (ν = 9.1 GHz) were obtained with a Varian E-109 Century Series X-band spectrometer, and S-band spectra were obtained with a microwave bridge equipped with a loop-gap resonator operating at 3.4 GHz.[11] (The ESR facilities are located at the NIH-sponsored National Biomedical ESR Center at the Medical College of Wisconsin.) Microwave frequencies were measured with an EIP Model 331 counter, and the magnetic field was calibrated with a Radiospan MJ-11OR NMR magnetometer.

Computer programs for simulation of frozen spectra of cupric complexes were obtained from Dr. J. Pilbrow, Monash University, Clayton, Victoria, Australia. The line width is determined from the quadratic equation $W_n' = [W_n^2 + B_n M_I]^{1/2}$, where $M_I = +3/2, +1/2, -1/2$, or $-3/2$,

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Table I. ESR Parameters for $Cu(His)_n$[a]

$g_\parallel$	$g_\perp$	g_{iso}	$A_\parallel^{Cu}$	$A_\perp^{Cu}$	A_{iso}	$A_\perp^{N}$	$A_\parallel^{N}$	ref
2.220	2.034		183	19	65 ± 0.5	13	15	this work[b] (Monte Carlo treatment)
		2.119			71			5
		2.119			70			5[b]
2.23			172					3

[a] Hyperfine couplings are in gauss. [b] ESR parameters for single isotope, ^{63}Cu.

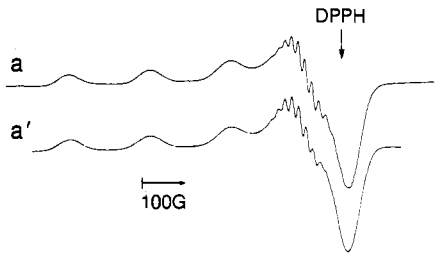

Figure 1. (a) Experimental X-band ESR spectrum of the $^{63}Cu^{II}$(L-His)$_2$ complex in D_2O solution at pD 7.3 at liquid-nitrogen temperatures (−150 °C). (a') Spectrum of the same complex simulated by using the Monte Carlo method. [L-His] is 0.1 M, and [$^{63}Cu^{2+}$] is 8 mM.

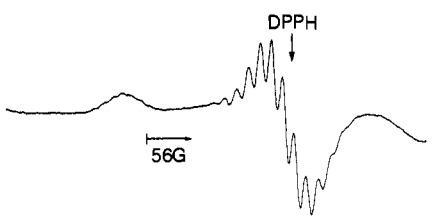

Figure 2. Well-resolved portion of the intense line composed of both $g_\parallel$ and $g_\perp$ features in the experimental S-band ESR spectrum of $^{63}Cu^{II}$(L-His) at liquid-nitrogen temperatures.

W_n and B_n are input parameters in gauss, and n is x, y, or z.

The X-band ESR spectrum for $Cu(His)_n$ was simulated by using a modified program from Dr. J. Pilbrow. In this program, simulations were based on constant frequency sampling of the spectrum, which is described in terms of the frequency domain.[11] A Monte Carlo calculation method was added to the program.[12] The Monte Carlo method randomly varied selected spectral parameters within defined limits in order to fit experimental spectra. In this study $g_\parallel$ and $A_\parallel$ were taken from the experimental data. $g_\perp$ was systematically varied to obtain the best possible overlap in the $g_\perp$ high-field region of the spectrum. $w_\perp$ (the residual line width for the perpendicular component), and $w_\parallel$ (the residual line width for the parallel component) were varied. Parameters for g and A strain were set equal to zero. Then, $A_\perp^{Cu}$, $A_\perp^{N}$, $A_\parallel^{N}$, and $w_\perp$ were fixed with use of the previous best fit and g- and A-strain parameters were varied. The best fit was obtained after 60 spectra were simulated, each spectrum requiring about 2 h of computer time (IBM-9000 with 1-megabyte memory). Further changes for the ESR parameters did not improve the quality of the fit.

Results

A scheme to optimize microwave frequency has recently been developed to determine the nitrogen donor atoms in frozen solution.[13] Both theory and experiment indicate that the optimum frequency for best resolution of the $M_I = -^1/_2$ line in the $g_\parallel$ region is about 3 GHz. The frozen-solution ESR spectra for $^{63}Cu(His)_n$ in D_2O are well-resolved for both X-band and S-band spectra in the $g_\perp$ region (Figures 1 and 2). The resolution in the $g_\perp$ region is better than that previously reported[3] because a single ^{63}Cu isotope is used to make the complex. ESR parameters from the spectra at both frequencies are consistent with previous data (Table I). The only apparent suggestion that the spectrum is not a single species is the even symmetry of the well-resolved lines in the $M_I = -^1/_2$ line in the $g_\parallel$ region (Figure 3). Given a choice of a single species with three or four approximately equivalent nitrogen donor atoms, the presence of lines marked by arrows in Figure 3 in both the experimental results and the computer simulation for four equivalent nitrogens and the absence of these lines for the simulation with three equivalent nitrogens exclude a single species with three approximately equivalent nitrogen donors. The intensity and apparently even symmetry of the $M_I = -^1/_2$ line at pD 7.3 suggests that two species exist and the concentrations of these species are about the same.

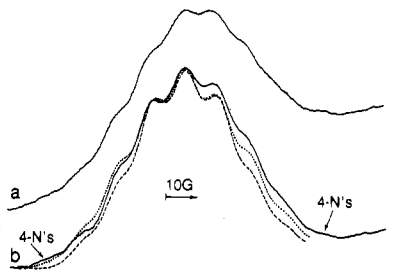

Figure 3. $M_I = -^1/_2$ line in the $g_\parallel$ region for ^{63}Cu(L-His)$_4$ in D_2O at liquid-nitrogen temperatures: (a) pD 7.3, signal averaged from 10 scans; (b), pD 6.8, and signal averaged from 50 scans. The dotted line is a computer simulation assuming four equivalent nitrogens, with the parameters from Table II, and the dashed line is a simulation assuming three equivalent nitrogen donor atoms.

If the pD is dropped to 6.8 (Figure 2), the most intense lines in the $g_\perp$ region change very little with respect to the position and resolution of the hyperfine lines obtained at pD 7.3 (data not shown). The $M_I = -^1/_2$ line in the $g_\parallel$ region at pH 6.8 not only is well-resolved but now can also be attributed to primarily a single species for which the square plane has four nitrogen donor atoms. The increased height of the high-field side of the $M_I = -^1/_2$ line in the $g_\parallel$ region may be attributed to a small amount of a second species. The number of nitrogen donor atoms is determined from the number of hyperfine atoms in the $M_I = -^1/_2$ line. The well-resolved lines can be attributed to a 1:4:10:16:19:16:10:4:1 pattern consistent with four equivalent nitrogens. Often, but not for the $Cu(His)_n$ spectrum in Figure 3, the line with the lowest intensity is lost in the noise. For this case, we have proposed a procedure[13] based on the relative intensities of the patterns for four vs. three nitrogens plus computer simulations of these patterns.[11] In this procedure the simulations are adjusted until the best possible fit of the central three lines is obtained with use of both three and four nitrogens (Figure 3). Then the simulation that agrees with the experimental data for the intensities of the lines in the wings is selected. The intensities of the lines relative to the center line are 5, 21, 53, 84, 100, 84, 53, 21, and 5% for four approximately equivalent nitrogen atoms and 14, 43, 86, 100, 86, 43, and 14% for three approximately equivalent nitrogen donor atoms. Even if the line with the lowest intensity could not be observed, a better fit of the line width for four nitrogen donor atoms is observed.

Since substitution of the ^{15}N isotope for ^{14}N in histidine is commercially available and since the ^{15}N isotope has a larger magnetic moment ($+0.19324 \times 10^4$ rad/(s G) for ^{14}N and -0.27107×10^4 rad/(s G) for ^{15}N), it became of interest to further test the scheme for determining the number of nitrogen donor atoms in copper–histidine complexes at X-band and S-band frequencies with use of histidine in which ^{15}N is substituted for ^{14}N

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Table II. ^{14}N and ^{15}N Hyperfine Coupling Constants for the $M_I = -^1/_2$ Line in the $g_\parallel$ Region

donor atoms	pattern[a]
1 ^{14}N	1-1-1
2 ^{14}N	1-2-3-2-1
3 ^{14}N	1-3-6-7-6-3-1
4 ^{14}N	1-4-10-16-19-16-10-4-1
1 ^{15}N	1--1
2 ^{15}N	1--2--1
3 ^{15}N	1--3--3--1
4 ^{15}N	1--4--6--4--1
1 ^{14}N + 1 ^{15}N	1--1--1-1--1--1
2 ^{14}N + 1 ^{15}N	1--2-1-3-2-2-3-1-2-1
1 ^{14}N + 2 ^{15}N	1--1-2--1-2-1--2-1--1
2 ^{14}N + 2 ^{15}N	1--2-2-3-4-1-2-6-2-1-4-3-2-2--1

[a] The ratio of the moment for ^{15}N to that for ^{14}N is about 1.4.

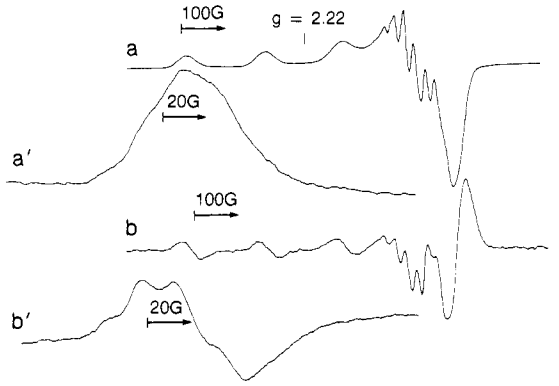

Figure 4. Experimental X-band ESR spectra of cupric ion (2 mM) in the presence of excess histidine (20 mM) for which ^{15}N is substituted for ^{14}N in the imidazole ring (histidine-*1,3*-$^{15}N_2$) at a pD of 6.3: (a) first-derivative spectrum; (a') expansion of $M_I = -^3/_2$ line in $g_\parallel$ region; (b) second-derivative spectrum; (b') expansion of $M_I = -^3/_2$ line in $g_\parallel$ region.

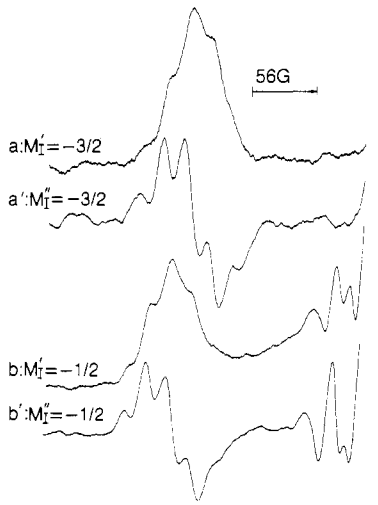

Figure 5. Expansion of $g_\parallel$ lines for S-band spectra for cupric ion in the presence of excess histidine-*1,3*-$^{15}N_2$: (a) first-derivative spectrum for $M_I = -^3/_2$ line; (a') second-derivative spectrum for $M_I = -^3/_2$ line; (b) first-derivative spectrum for $M_I = -^1/_2$ line; (b') second-derivative spectrum for $M_I = -^1/_2$ line.

in the imidazole ring. Table II compiles the hyperfine patterns for various combinations of ^{14}N and ^{15}N donor atoms. Because there is an odd number of superhyperfine lines evident in Figures 4 and 5, the number of ^{15}N nuclei must be even, either 2 or 4. The X-band ESR spectra for cupric ion in the presence of excess histidine for which ^{15}N is substituted for ^{14}N in the imidazole ring (histidine-*1,3*-$^{15}N_2$) appear to have a five-line pattern in both the $g_\perp$ region and the $M_I = -^3/_2$ line in the $g_\parallel$ region consistent with four approximately equivalent ^{15}N donor atoms (Figure 4). The 1-4-6-4-1 pattern is even clearer in the second-derivative display (Figure 4b). Thus it appears that four approximately equivalent

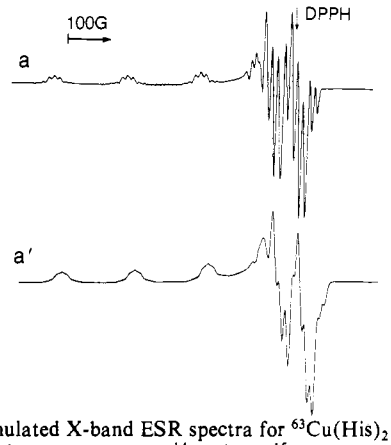

Figure 6. Simulated X-band ESR spectra for $^{63}Cu(His)_2$ assuming the four nitrogen donor atoms are 2 ^{14}N plus 2 ^{15}N: (a) line width 3.5 G; (a') line width 5.5 G.

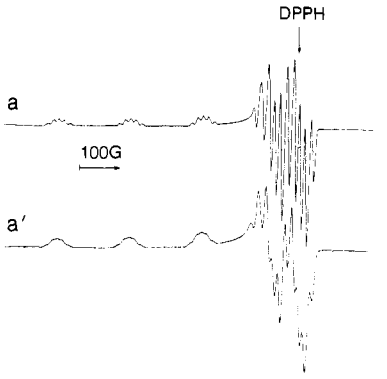

Figure 7. Simulated X-band ESR spectra for $^{63}Cu(His)_2$ assuming 1 ^{14}N, 2 ^{15}N, and 1 ^{16}O: (a) line width 3.5 G; (a') line width 5.5 G.

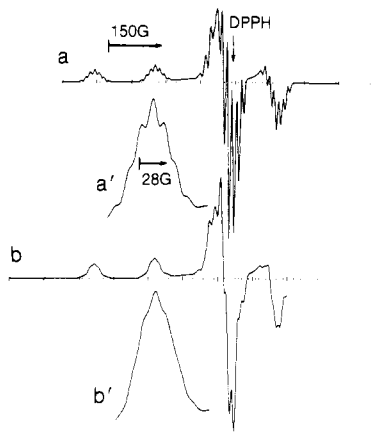

Figure 8. Simulated S-band ESR spectra for $Cu(His)_2$ assuming the four nitrogen donor atoms are 2 ^{14}N plus 2 ^{15}N: (a) line width 3.5 G; (a') expansion of $M_I = -^1/_2$ line in $g_\parallel$ region, line width 3.5 G; (b) line width 5.5 G; (c) expansion of $M_I = -^1/_2$ line in $g_\parallel$ region, line width 5.5 G.

nitrogen donor atoms from imidazole are bound to cupric ion in the presence of excess histidine in frozen solution at pD 6.3.

As predicted, S-band spectra are better resolved than X-band spectra in the $g_\parallel$ region (Figure 5). The second-derivative spectrum at low frequency is particularly well-resolved and will be the subject of a future paper in which simulations will test theory. Simulations of spectra for which 2 ^{14}N + 2 ^{15}N or 1 ^{14}N + 2 ^{15}N nitrogen donor atoms comprise the square plane may appear to resemble a five-line pattern if the resolution is poor at X-band (Table II, Figures 6 and 7), but a seven-line pattern is evident from the simulated S-band spectra for 2 ^{14}N + 2 ^{15}N if the outer lines are detected (Figures 8 and 10). The relative intensities of the pattern for 2 ^{15}N + 1 ^{14}N due to extensive overlap (about 32, 89, 100, 89, and 32%) differ substantially from the 17, 67, 100, 67, and 17% pattern for 4 ^{15}N (TableII and Figures 9 and 10). Thus,

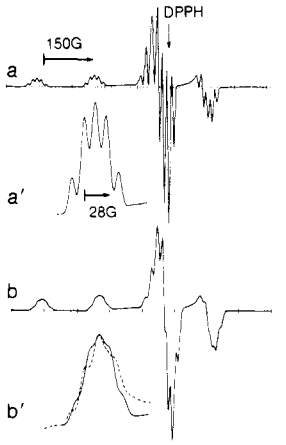

Figure 9. Simulated S-band ESR spectra for $^{63}Cu(His)_2$ assuming 1 ^{14}N, 2 ^{15}N, and one ^{16}O: (a) line width 3.5 G; (a') expansion of $M_I = -1/2$ line in $g_\parallel$ region, line width 3.5 G; (b) line width 5.5 G; (b') expansion of $M_I = -1/2$ line in $g_\parallel$ region, line width 5.5 G.

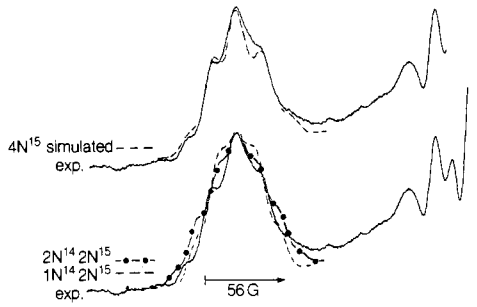

Figure 10. S-band experimental line for $M_I = -1/2$ line in $g_\parallel$ region vs. simulation assuming 4 ^{15}N (---, top spectrum), 2 ^{14}N and 2 ^{15}N (-●-, bottom spectrum), or 1 ^{14}N and 2 ^{15}N (---, bottom spectrum).

analysis of S-band spectra for cupric ion in the presence of excess histidine-*1,3*-$^{15}N_2$ confirm that four nitrogen donor atoms from the imidazole comprise the square plane under our conditions.

Discussion

Since the spectra from this work are better resolved due to use of a single isotope, ^{63}Cu, and an S-band low-frequency spectrometer,[3] the results from these data can be used to support or negate the existence of the structures in Figure 11. Initially only those species a and b are considered because thay appear to be the major forms in solution at physiological pH, even under our conditions, where excess histidine is added to prevent aggregation (results that will be submitted for publication).[2] The value for $A_\perp^{Cu}$ is the ESR parameter that is most difficult to determine. The value for $A_\parallel^{Cu}$ for $Cu(His)_4$ in frozen solution is easily determined, and A_{iso} in liquid solution at room temperature can also be precisely determined. If there are only small changes in the ESR parameters upon freezing, $A_{iso} = {}^1/_3(A_\parallel + 2A_\perp)$ and $A_\perp^{Cu}$ is less than 10 G. Simulations with the Monte Carlo treatment converged on 19 G for spectra from frozen solutions. Thus, it appears that the ESR parameters are altered after freezing the sample. Analysis of the $M_I = -1/2$ line in the $g_\parallel$ region (Figure 3) unequivocally confirms that four nitrogen donor atoms are bound in frozen solution at pD 6.8 if the coupling of the nitrogen donor atoms is approximately equivalent. This result favors structure b or c in Figure 11. That the spectrum consists of a superposition of two species at pD 7.3 in frozen solution is consistent with previous work in the liquid phase.[5] It is anticipated from Peisach–Blumberg plots that a change in donor atoms from four approximately equivalent nitrogen donor atoms to three nitrogen and one oxygen donor atom would alter the g and A values more than indicated by the spectrum in Figure 2.[16] Resolution of the $M_I = -1/2$ line in the $g_\parallel$ region favors equivalent

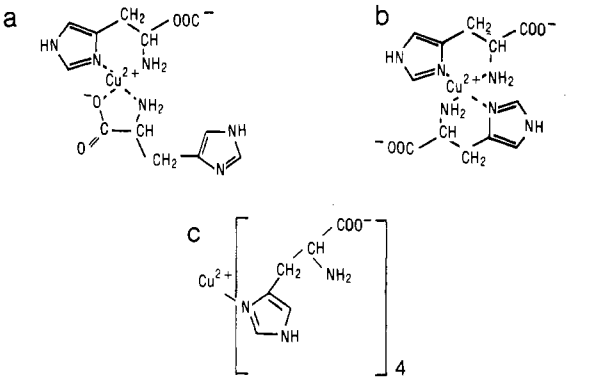

Figure 11. Tentative structures for Cu(L-His)$_n$ complexes in aqueous solution at a pH of 7.3.

amounts of two species, but the ESR parameters for these species are very similar, suggesting the perturbation is from, for example, different axial ligands like H_2O and ^-OH, from unprotonated and protonated ligand forms, or from a change in nitrogen donor atoms, i.e. from N–N–N–N to N–N′–N–N′. It is known that the pH may be altered in the absence of buffer when samples are frozen,[14,15] but the liquid phase and frozen state give similar results, that is, two forms at pD 7.3 and a single form at pD 6.8.[5]

An alternative hypothesis, which would justify an increase in the number of lines in the $g_\parallel$ region, is that the nitrogen hyperfine couplings are not approximately equivalent. To our knowledge the nitrogen hyperfine couplings are nearly isotropic. Room-temperature ESR and NMR data suggest two structures but probably not both structures a and b (Figure 11). In the former equal amounts of both species are present while in the latter the ratio of structure a to structure b is 2:1.[5,7] The best conclusion in our opinion, based on the ESR data for cupric ion in the presence of excess histidine, is that imidazole is bound to cupric ion, four equivalent nitrogen donor atoms from imidazole comprise the square plane for at least a major species in frozen solution, and the structure is pD-dependent. This conclusion for the structure of $Cu(His)_4$ in the presence of excess histidine differs from the structure determination of hydrated Cu^{II}(L-His)(D-His), potentiometric titration curves for solutions of Cu(L-His)$_2$ and Cu(L-His)(D-His), and infrared spectra of powdered crystals of Cu(L-His)(D-His)·H_2O along with the Cu^{II}(L-His)$_2$ solution spectra.[5,17] This conclusion, obtained in the presence of excess histidine in the frozen state, also differs from the structure for mixed histidine-like and glycine-like ligands obtained in the liquid state.[3] Further studies, which will be submitted for publication, show that four histidines are bound to copper in the frozen state under conditions where two histidines are bound to copper in the liquid state.

Substitution of the ^{15}N isotope for ^{14}N in the imidazole ring but not the amine nitrogen confirms that four nitrogens are bound to cupric ion in the presence of excess histidine in frozen solution at pH 6.8 and all four nitrogens are from the imidazole ring, $Cu(His)_4$ (Figure 11c). We had previously determined that four imidazole nitrogens were found to cupric ion in the presence of excess carnosine (β-alanyl-L-histidine)[18] but did not expect histidine to bind as a monodentate ligand because of the larger bidentate stability constants: $\log K_1 = 10.2$; $\log K_2 = 7.9$.[19] The conditions used may have contributed to formation of $Cu(His)_4$, for example, a pD of 6.8–7.3, excess ligand, rapid freezing, and immobilized complexes. At this time we have not studied cupric–histidine complexes as the ratio of cupric ion to histidine is decreased to 1:2 or 1:1 because aggregation occurs without the addition of glass formers and the EPR hyperfine structure is not resolved. Further studies with different solvents, possibly ethylene glycols, should overcome this problem but may prevent the formation of $Cu(His)_4$,

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which, in retrospect, provides an interesting structure for analysis of an immobilized complex.

The condition of excess ligand that prevented aggregation is pertinent for studies for which cupric ion or cupric-histidine complexes are present in complex biological media.[20] It is hypothesized that under certain conditions, that is in the presence of excess histidine, amino acid residues, upon freezing, may tend to form cupric complexes in which three or four nitrogen donor atoms are nitrogens from the imidazole ring and the amino nitrogen from histidine is released.

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Registry No. L-his, 71-00-1; $Cu(his)_2$, 13870-80-9; ^{63}Cu, 14191-84-5; ^{15}N, 14390-96-6; D, 7782-39-0.

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Mössbauer and EPR Studies of a Synthetic Analogue for the Fe_4S_4 Core of Oxidized and Reduced High-Potential Iron Proteins

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We have recently reported the synthesis and crystallographic characterization of the compound $(NBu_4)[Fe_4S_4(S\text{-}2,4,6\text{-}i\text{-}Pr_3C_6H_2)_4]$, a synthetic analogue for the $[Fe_4S_4]^{3+}$ cluster observed in oxidized high-potential iron proteins (HP). Here we report Mössbauer and EPR studies of this compound, both in polycrystalline form and in frozen solutions. Dissolved in toluene, acetonitrile, benzene, or dichloromethane, the synthetic complex displays EPR spectra very similar to those observed for oxidized HP, with $g_{av} \cong 2.06$. In polycrystalline form, however, the g values are smaller, with $g_{av} \cong 2.00$. In strong applied magnetic fields, well-resolved Mössbauer spectra are observed. The spectra are virtually identical in the polycrystalline state and in frozen toluene solution. We have analyzed the Mössbauer spectra in the framework of an $S = {}^1/_2$ spin Hamiltonian. As in HP the four iron sites occur in two equivalent pairs. For the two irons of each pair the hyperfine parameters are the same to within 5%. The set of hyperfine parameters match that of oxidized HP very well.

Introduction

More than a decade ago, it was established that the $[Fe_4S_4(S\text{-cys})_4]$ clusters of iron-sulfur proteins can exist in three different redox states, as represented by the following three-membered electron-transfer series:[1]

$$[Fe_4S_4]^{3+} \rightleftarrows [Fe_4S_4]^{2+} \rightleftarrows [Fe_4S_4]^{+}$$

$$[HP_{ox}] \rightleftarrows [HP_{red}, Fd_{ox}] \rightleftarrows [HP_{s\text{-}red}, Fd_{red}]$$

$$[Fe_4S_4(SR)_4]^{-} \rightleftarrows [Fe_4S_4(SR)_4]^{2-} \rightleftarrows [Fe_4S_4(SR)_4]^{3-}$$

In nature, the ferredoxins (Fd) utilize the $[Fe_4S_4]^{2+/+}$ core oxidation levels, while the so-called high-potential (HP) iron-sulfur proteins use the $[Fe_4S_4]^{3+/2+}$ core oxidation levels. Synthetic analogues were instrumental in the proof of the three-state hypothesis. In an extensive series of papers, Holm and his co-workers have described the synthesis and characterization of the $[Fe_4S_4(SR)_4]^{2-}$ and $[Fe_4S_4(SR)_4]^{3-}$ complexes.[2] Detailed structural and spectroscopic studies have demonstrated the congruence of these analogues with the biological $[Fe_4S_4]^{2+}$ and $[Fe_4S_4]^{+}$ clusters. We recently reported the synthesis and structural characterization of the first synthetic analogue containing the $[Fe_4S_4]^{3+}$ core.[3] That complex, $(NBu_4)[Fe_4S_4(SR_4)]$ with R = 2,4,6-*i*-$Pr_3C_6H_2$, corresponds to the oxidized form of high-potential iron-sulfur proteins (HP_{ox}).

The oxidized HP proteins have been studied by a wide range of physicochemical techniques, including Mössbauer,[4,5] EPR,[6,7] ENDOR,[8] NMR,[9] MCD,[10] and resonance Raman[11] spectroscopies as well as X-ray crystallography.[12] EPR spectroscopy has been useful in distinguishing the $[Fe_4S_4]^{3+}$ centers in HP_{ox} proteins from the EPR-active $[Fe_4S_4]^{+}$ centers of reduced ferredoxins and the $[Fe_2S_2]^{+}$ clusters of the Fe_2S_2 proteins. For several years, an EPR signal for iron-sulfur proteins with $g_{av} > 2$, which disappeared upon reduction, was assumed to be indicative of a $[Fe_4S_4]^{3+}$ center. This assumption was shown to be incorrect by the discovery that oxidized Fe_3S_n proteins and novel iron-sulfur centers in oxidized hydrogenase also display $g_{av} > 2$ EPR spectra. Mössbauer

Table I. g Values of $[Fe_4S_4]^{3+}$ Clusters in Proteins and Synthetic Complexes

	g_1	g_2	g_3	g_{av}	ref
R. gelatinosa HP	2.11	2.03	2.03	2.056	16
C. vinosum HP	2.12	2.04	2.04	2.07	6
	2.088	2.055	2.040	2.06	6
Ectothiorhodospira halophila HP	2.078	2.033	2.033	2.048	16
B center in irradiated $(NBu_4)[Fe_4S_4(SC_6H_5)_4]$	2.108	2.006	1.987	2.034	17
$(NBu_4)[Fe_4S_4(SR)_4]$ (benzene, dichloromethane, acetonitrile)	2.10	2.07	2.03	2.066	this work
$(NBu_4)[Fe_4S_4(SR)_4]$ (toluene)	2.10	2.05	2.03	2.060	this work
$(NBu_4)[Fe_4S_4(SR)_4]$ (polycrystalline)	2.065	1.97	1.96	2.000	this work

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