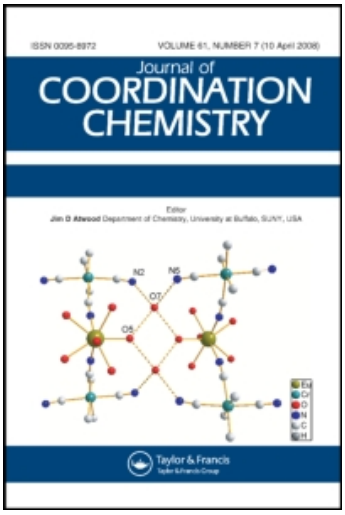

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Temperature Dependence of the EPR Spectrum of Copper(II)- carnosine

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SHORT COMMUNICATION
Temperature Dependence of the EPR Spectrum of Copper(II)-carnosine†

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EPR spectra from 77° to 300°K of Cu(II) complexed with β-alanyl-L-histidine (carnosine) were previously examined over the pH range of 4 to 11.[1] The low temperature epr spectra were anisotropic, with $g_{\perp}$ ~2.05 and $g_{\parallel}$ ~2.2. Above pH ≐ 6, the fine structure seen on the epr signals is considered characteristic of a dimeric species,[2] as was observed in the x-ray crystallographic study of Cu(II)-carnosine.[3] Room temperature spectra are more isotropic, with less resolved fine structure.

An examination of the differences in the epr spectra at various temperatures led investigators to the conclusion that the Cu(II)-carnosine dimeric species is either not detectable or does not exist in solution. Alternatively, the dimer may be only a minor component in solution that forms only upon freezing as a consequence of crystal packing in the solid state or from selective crystallization of a less soluble dimeric species.[1] To test these hypotheses we have examined the changes in the epr spectrum of Cu(II)-carnosine from 300°K down to 93°K, in 10 degree increments, in aqueous and methanol-water solvents.

The changes in the epr spectrum of Cu(II)-carnosine in aqueous media from 243° K to 213°K are shown in Figure 1. Lithium chloride (3M) was added to the aqueous samples to prevent dipolar broadening of each epr spectrum due to solute segregation as the solutions were frozen.[4] The added lithium chloride had no effect on the Cu(II) complexes being studied, as measured by a number of spectroscopic techniques.[5] The epr spectrum of Cu(II)-carnosine was unchanged from room temperature to about 243°K. The absence of four line hyperfine pattern for the monomer is due to rapid tumbling of the complex in addition to rapid ligand exchange rates. Both serve to average out the g anisotropies for this Cu complex. However, upon freezing (238°K), the seven nuclear hyperfine lines characteristic of the dimeric species were apparent (Figure 1, curve B). This fine structure became even more evident as the temperature was lowered, so that by 213°K (Figure 1, curve D) the spectrum was identical to that observed at 93°K.

The variable temperature epr study of Cu(II)-carnosine was repeated in a methanol-water (50:50 v/v) solvent system. This mixed solvent system has numerous advantages for epr spectral studies: 1) temperatures as low as 200°K can be achieved while still maintaining the system in a liquid state. 2) For the methanol to water ratio used in this study, the dielectric constant from 263°K to 223°K is approximately that of room temperature aqueous solutions.[6] 3). The H^+ activity ("pH") at low temperatures can be adjusted to be identical to the room temperature pH value.[7] From 300°K to 243°K, the epr spectrum of Cu(II)-carnosine is similar to that seen in aqueous solution with a complete absence of fine structure

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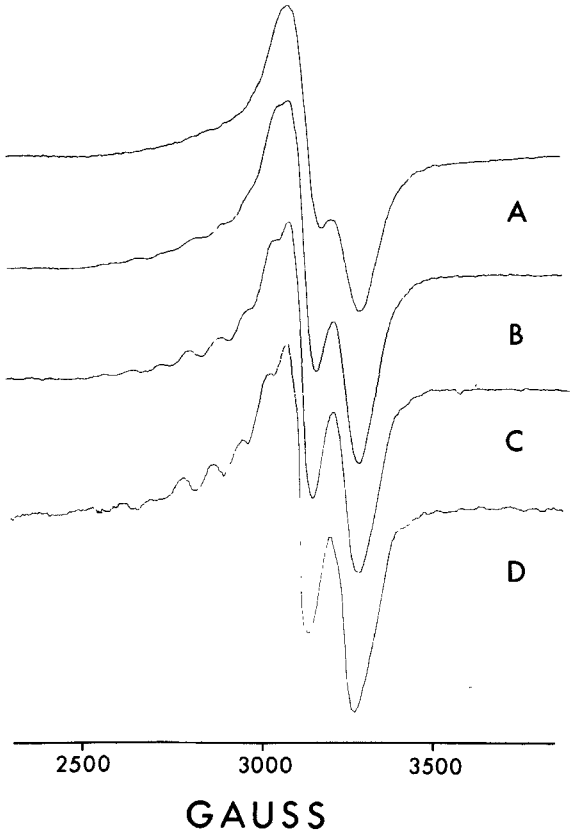

FIGURE 1 Epr Spectra of 0.01 M Cu(II)-Carnosine (1:1) at Various Temperatures in 3.0 M LiCl. A Varian E-12 Epr Spectrometer Operating at 9GHz was used with the following settings: gain = 2500, modulation = 10.0 gauss, power = 30 mW; A, temperature = 243°K, liquid state; B, temperature = 233°K, frozen solution; C, temperature = 223°K, frozen solution; D, temperature = 213°K, frozen solution.

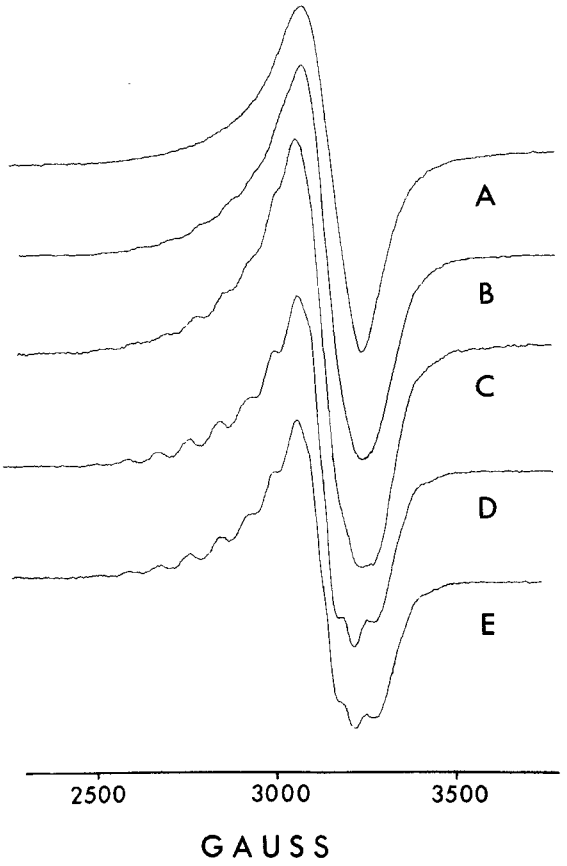

FIGURE 2 Epr Spectra of 0.01 M Cu(II)-Carnosine (1:1) at Various Temperatures in 50% Methanol-water. Gain = 2000, modulation = 10.0 gauss, power = 30 mW; A, temperature = 263°K, liquid state; B, temperature = 233°K, liquid state; C, gain = 3200, temperature = 123°K, liquid state; D, gain = 2500, temperature = 213°K, liquid state; E, gain = 2500, temperature = 203°K, liquid state.

(Figure 2, curve A). The reasons for the differences in the spectra in Figures 1A and 2A are that slightly different complexes are present. The aqueous sample was prepared in 3M LiCl and the Cu species in solution is a $Cu^{2+}(H_2O)(Cl^-)$(carnosine) complex whereas the Cl^- is absent in the methanol-water sample. As the temperature was lowered below 243°K, the seven line nuclear hyperfine pattern appeared (Figure 2, curves B–E). The hyperfine patterns appear due to formation of the dimer complex and a slowing of the rate of tumbling of the Cu complex as the temperature is lowered. This was nearly the same temperature at which the fine structure was observed in aqueous solution, however in aqueous solution it occurred because tumbling and exchange were restricted as the solution froze. In the methanol-water solvent system there was no significant change in the epr spectrum upon freezing (~200°K) since the dimer was already present. These experiments were conducted at a 1:1 ligand to Cu ratio and the top spectrum in each Figure represents the spectrum of the monomer. At different ligand to metal ratios, e.g., 4:1 or 10:1, spectra for both monomer and dimer are observed. Thus, the spectrum consists of two overlapping signals with the monomer spectrum having four hyperfine lines and the dimer spectrum having seven lines. These patterns appear in the aqueous sample only after it is frozen.

These results provide the first direct evidence for the existence of Cu(II)-carnosine dimer *in solution.*

These experiments also argue against the presence of Cu(II)-carnosine dimers resulting exclusively from packing forces in the solid state. Two alternate explanations of these data are proposed: 1) a temperature dependent equilibrium between a monomeric and dimeric Cu(II)-carnosine species, with the dimeric species being thermodynamically more stable below 233°K. 2) The line widths of the hyperfine components of the epr spectra of the dimeric species are broadened by a spin-rotational relaxation mechanism (as discussed by Kivelson and coworkers)[8,9] resulting in an apparent disappearance of the hyperfine lines above 240°K.

The appearance of an epr spectrum characteristic of dimeric species, upon freezing an aqueous solution, has been observed for a number of Cu(II) complexes.[10,11] Further analysis of the epr spectra of these systems in methanol-water solvents is necessary to test the generality of our results for Cu(II)-complexes.

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