interaction. For both alternatives above, arguments based on polarizability seem more appropriate.

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Magnetic Resonance Studies of Copper(II)–Triglycylglycine Complexes[1]

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Abstract: Cu(II)–triglycylglycine in solution (copper to peptide ratio 1:1; 5×10^{-3} *M*) has been studied in the pH range 2.5–11 by potentiometric titrations, electron paramagnetic resonance, and proton relaxation measurements. The complex formed at high pH has been investigated also in the form of a polycrystalline powder diluted with the corresponding diamagnetic Ni(II) salt. Relaxation data and epr results show that at low pH a complex is formed with the neutral peptide. This and the next complex formed at higher pH show no nitrogen hyperfine structure. The three complexes formed thereafter show nitrogen hyperfine structure due to two, three, and four nitrogens, respectively. The relative amounts of the different complexes have been estimated from epr spectra. Proton relaxation results are used to discuss the number of coordinating water molecules. Binding of at least one water molecule to the copper ion at the highest pH cannot be eliminated, in contrast to what is found in the crystal. However, a comparison between the high-pH complex in solution and in a powder yields the result that the nitrogen coordination is essentially the same.

The structure and properties of copper(II) complexes with amino acids and peptides have been extensively studied in recent years. For example, the electron paramagnetic resonance (epr) technique has been used in studies of copper complexes with glycylglycine[3] at different pH values and with some histidine-containing di-, tri-, and tetrapeptides at high pH.[4] Proton magnetic resonance has been applied by Sheinblatt[5] to the copper(II)–glycylglycine system with large excess of peptide over copper. Important information about the structure of the complexes in the solid state has been obtained from crystallographic studies, as discussed in a recent review.[6] The major aim of all these investigations has been to provide background knowledge for an understanding of the interaction of metals with proteins.

In this work we report studies on the copper complexes with triglycylglycine. This system has been studied earlier in potentiometric titrations[7,8] and with infrared spectroscopy.[8] We have followed the changes in the epr spectra and the proton relaxation rates as a function of pH in order to see what additional information about the number of complexes and their properties could be obtained.

In addition to the work on solutions we have also studied a crystalline powder of the complex at high pH. Its structure has been revealed by X-ray crystallography.[9a] The copper interacts only with four ligand atoms in a planar arrangement with no coordination in the fifth and sixth position. An interesting question concerns the extent to which structures determined in the crystalline state correspond to molecular species existing in solution. This is a particularly important problem to the protein chemist who is mainly interested in interactions occurring in aqueous solutions. Therefore, we have tried to compare results obtained from solutions and solid-state samples. For the problem at hand, it is important that the crystals of the Cu(II) and the corresponding diamagnetic Ni(II) complexes are isomorphous,[9b] so that dilute crystals of the Cu(II) complex can be made. This is a prerequisite for epr measurements on the solid compound.

Experimental Section

Materials. ^{63}Cu and ^{65}Cu were obtained from Oak Ridge National Laboratory. The monosodium salt of triglycylglycine was purchased from Fluka AG, Switzerland, and not further purified. All other reagents were analytical grade, and deionized water was used throughout.

Preparation of Crystals. Crystals of the Cu complex of triglycylglycine at high pH diluted with Ni were prepared in the following way. A solution (1 ml) containing 5×10^{-6} mole of $CuCl_2 \cdot 2H_2O$ and 5×10^{-4} mole of $NiCl_2 \cdot 6H_2O$ was warmed and mixed with 1 ml of 1 *M* NaOH. The mixture was stirred well; the precipitate was centrifuged off and washed three times with 5×10^{-3} *M* NaOH. The precipitate was added to 1 ml of a solution

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(2) (a) Department of Physical Chemistry; (b) Department of Biochemistry; (c) Carl and Thecla Lamberg Visiting Professor at the University of Göteborg.

(3) D. C. Gould and H. S. Mason in "Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press Inc., New York, N. Y., 1966, p 35.

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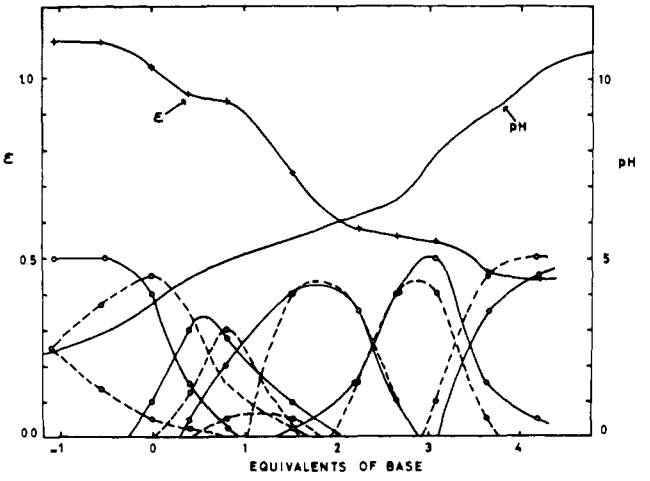

Figure 1. pH at 25°, proton relaxation enhancement (ϵ) at 60 MHz and 25°, and relative amounts of the different complexes formed (arbitrary units; *cf.* text) as a function of equivalents of base (or acid, negative values) added at 25° to a solution of 5×10^{-3} *M* Cu^{2+} and triglycylglycine containing 2 *M* $NaClO_4$. The amounts of the complexes were estimated from room-temperature epr spectra (solid lines) as well as from low-temperature spectra (dashed lines), as described in the text.

1 *M* in NaOH containing 0.1 g of the peptide. The mixture was shaken vigorously until a brown-yellow color developed, and the slight excess of hydroxide was centrifuged off. Ethanol was added dropwise until a very faint cloudiness was observed after shaking. Several more drops of ethanol were then added and the solution was left overnight for crystallization. The crystals were filtered, washed with ethanol and ether, and dried in air.

pH Measurements. The pH of all solutions was measured at 25° with a glass electrode, Radiometer Type B.

Epr Measurements. Epr spectra were obtained at 9.2–9.5 GHz with a Varian E-3 spectrometer system and at 34.3 GHz with a Varian V-4503 spectrometer equipped with a V-3401 9-in. magnet and a "Fieldial Mark 1."

At 9.2–9.5 GHz, spectra from solutions and polycrystalline powders were recorded at temperatures from 77 to 320°K. At 34.3 GHz, spectra were obtained from powder samples at room temperature and from frozen solutions at 170°K.

Proton Relaxation Rates. The measurements of the proton longitudinal relaxation rate (T_1) were made with the method of fast adiabatic passage.[10] All relaxation rates were measured at 60 MHz with a Varian DP-60 spectrometer system. A HP 3300A function generator produced the necessary low-frequency triangular field modulation. The output of the generator was connected directly to the sweep coil of the probe and to the *X* axis of the oscilloscope. The modulation amplitude was measured with the side-band technique. The signal from the receiver (V-4311) was taken from jack J302 to the *Y* axis of the oscilloscope. The ratio $\Delta t/P$ (*cf.* Conger and Selwood[10]) was obtained by measurements on the oscilloscope screen. Typical settings for measuring relaxation times about 0.7 sec are: power, 12 db below 250 mw; sweep frequency, 0.5 cps; sweep amplitude, 5500 cps.

The temperature dependence of T_1 was obtained as follows. The sample was put in hot water (~90°) or in ice water and rapidly transferred to the probe. Repeated measurements of T_1 were made as the temperature approached room temperature.

Results

Potentiometric Titration. Figure 1 gives pH as a function of the number of equivalents of base (KOH) added to a solution containing Cu^{2+} and peptide, both 5×10^{-3} *M*, and 2 *M* perchlorate. To some samples acid (HCl) was added, which in Figure 1 is represented by negative numbers of base equivalents. The results are consistent with results published earlier.[7,8]

(10) R. L. Conger and P. W. Selwood, *J. Chem. Phys.*, **20**, 383 (1952).

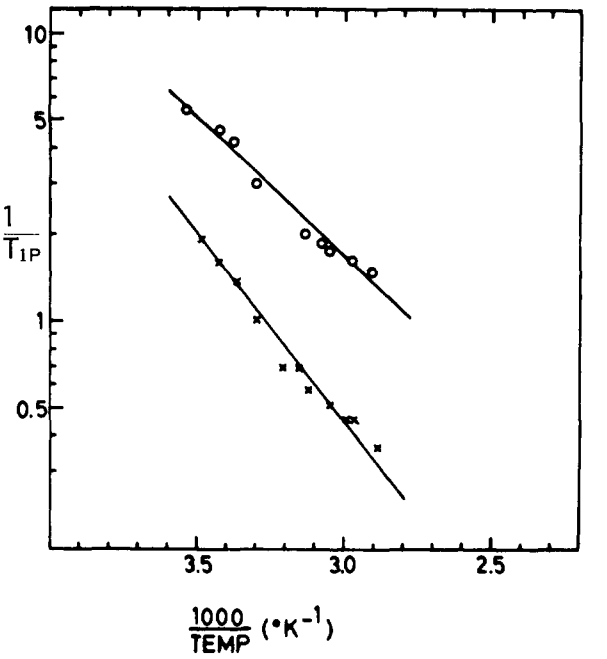

Figure 2. Temperature dependence of the relaxation rates ($1/T_{1P}$) at 60 MHz for 5×10^{-3} *M* $Cu(H_2O)_6^{2+}$, pH 5.5 (○), and for the 5×10^{-3} *M* Cu^{2+}-triglycylglycine complex, pH 11.2 (×), in a solution containing 2 *M* $NaClO_4$.

Proton Relaxation Rates. Some samples were withdrawn during the titration and examined for proton relaxation rates. The results are presented in Figure 1 as relaxation rate enhancement[11]

$$\epsilon = \frac{1/T_1^* - 1/T_1^*(0)}{1/T_1 - 1/T_1(0)} \quad (1)$$

where * indicates complexing agent present and (0) indicates no Cu^{2+} present. The relaxation rate caused by the paramagnetic ion will in what follows be designated $1/T_{1P} = 1/T_1 - 1/T_1(0)$. All samples examined contained 2 *M* perchlorate. The reproducibility of the measurements is about 3%. As the measurements are always made relative to a standard sample, the systematic errors are very small when the relaxation rate is close to that of the standard, *i.e.*, when ϵ is close to unity. Thus, the deviation of ϵ from 1 at lowest pH is thought to be significant.

The effect of temperature on the relaxation rate $1/T_{1P}$ of a 5×10^{-3} *M* Cu^{2+}–peptide solution at pH 11.5 and of $Cu(H_2O)_6^{2+}$ at the same concentration, pH 5.5, is shown in Figure 2.

A solution, pH ≈ 11, with a twofold excess of ligand gave the same value for ϵ as the solution with equivalent amounts of copper and peptide.

Room-Temperature Epr Spectra. The resolution of the epr spectra was studied as a function of sample temperature in the range 1–46° and was found to be highest at a temperature of about 20°. Addition of $NaClO_4$ up to a concentration of 2 *M* did not change the spectra. When the solvent used was a water:methanol (1:9) mixture, the lines were broadened but the positions of the lines were unchanged.

Solutions 5×10^{-3} and 5×10^{-4} *M* in both Cu and peptide gave identical spectra. A tenfold excess of

(11) J. Eisinger, R. G. Shulman, and B. M. Szymanski, *ibid.*, **36**, 1721 (1962).

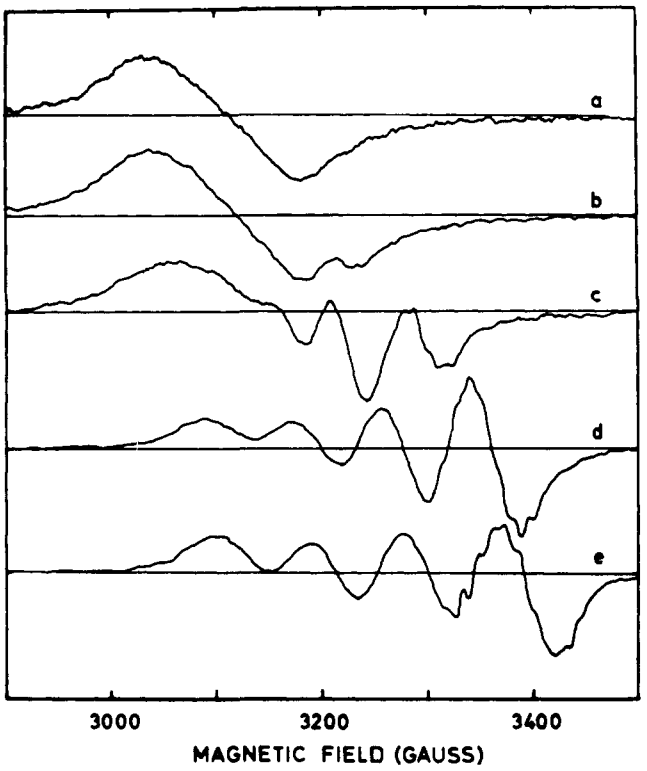

Figure 3. Room-temperature epr spectra of solutions of 5×10^{-3} M Cu^{2+} and triglycylglycine, containing 2 M $NaClO_4$, with different amounts of base added: (a) −1.1 equiv, pH 2.4; (b) 0 equiv, pH 3.8; (c) 0.8 equiv, pH 5.0; (d) 3.1 equiv, pH 7.9; and (e) 4.2 equiv, pH 10.1 (microwave frequency, 9525 MHz).

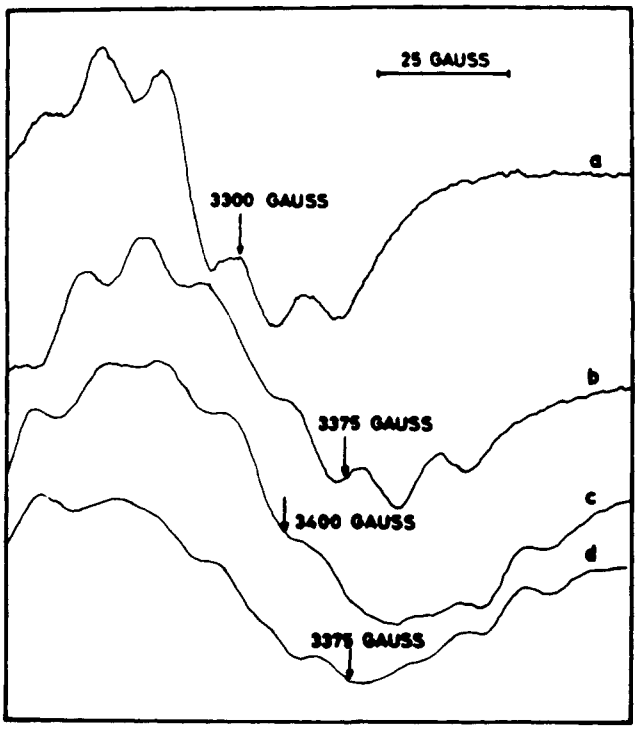

Figure 4. Details of the room-temperature epr spectra of the Cu^{2+}–triglycylglycine complexes, showing nitrogen hyperfine structure: (a–c) high-field lines of spectra in Figure 3c–e, respectively; (d) high-field ("overshoot") line of the spectrum from a polycrystalline sample of the mixed Cu^{2+}:Ni^{2+} (1:100) complex formed at the highest pH (*cf.* Figure 5g). The microwave frequency in a–c is 9525 MHz, in d, 9210 MHz. As the corresponding lines are not found at the same fields, the field at given points has been indicated for each spectrum.

peptide over Cu (5×10^{-3} M) at pH 11.2 had no effect on the spectrum.

Epr spectra were recorded of the same solutions as were used for the T_1 measurements and some representative spectra are shown in Figure 3. The g values and hyperfine constants of the various species detected are given in Table I. The spectrum of the species dominating at the lowest pH is very similar to the spectrum from a Cu^{2+} solution with no peptide present at pH 5.

Table I. Resonance Parameters for the Different $^{63}Cu^{2+}$–Triglycylglycine Complexes in Solution and for the High-pH Complex in the Diluted Powder[a]

	295°K		90°K			
Complex[b]	g	$\lvert A\rvert$, gauss	$g_{\parallel}$	$g_{\perp}$	g_0[c]	$\lvert A_{\parallel}\rvert$, gauss
$Cu(H_2O)_6^{2+}$	2.186		2.392	2.080	2.183	135
CuH_4L^{2+}	2.186		2.362	2.078	2.173	133
CuH_3L^{+}	2.160	55	2.288	2.072	2.144	150
CuH_2L	2.126	63	2.235	2.062	2.120	168
$CuHL^{-}$	2.108	83	2.202	2.047	2.099	193
CuL^{2-}	2.091	90	2.172	2.041	2.085	206
Powder of high-pH complex			2.160	2.043	2.082	205

[a] Cu^{2+}:Ni^{2+} = 1:100. [b] For definition of symbols, see text. [c] $g_0 = (g_{\parallel} + 2g_{\perp})/3$.

The details of the high-field line of spectra showing nitrogen hyperfine structure are shown in Figure 4 (together with part of the spectrum from a crystalline powder, see below).

The different complexes have spectra that in certain regions do not overlap seriously with each other. We have obtained rough estimates of the amounts of the various complexes from the amplitudes of the hyperfine line at highest fields, taking into account that the total Cu^{2+} concentration is constant (see Figure 1).

Epr of Frozen Solutions. The resolution of the epr spectrum was studied in a number of samples, all adjusted to pH 11.2 at 25°. A frozen water solution of Cu^{2+} and peptide, both 5×10^{-3} M, had a very poorly resolved spectrum (Figure 5a). Successive additions of $NaClO_4$ increased the resolution (Figure 5b,c). Excess of ligand (Figure 5d) increased the resolution somewhat. A solution in water–methanol, 1:1 (Figure 5e), had a well-resolved spectrum. Also, a more dilute water solution (5×10^{-4} M in Cu and peptide) (Figure 5f) had better resolution than the more concentrated solution (Figure 5a). The resolved parts of the spectra all had the same resonance parameters.

Some samples at various pH containing 2 M perchlorate giving resolved spectra at 77°K (*cf.* Figure 6) were warmed to about 220°K, kept at this temperature for a couple of minutes, and reexamined at 77°K. All spectra were considerably broadened. Thawing and refreezing restored the original spectra.

The same solutions as investigated by T_1 and room temperature epr measurements were studied at 90°K in the epr spectrometer. Some selected spectra are given in Figure 6. Figure 6a has one Cu^{2+} signal identical with the spectrum obtained from $Cu(H_2O)_6^{2+}$ in the pH range 2.4–4.0. Table I gives $g_{\parallel}$, $g_{\perp}$, and

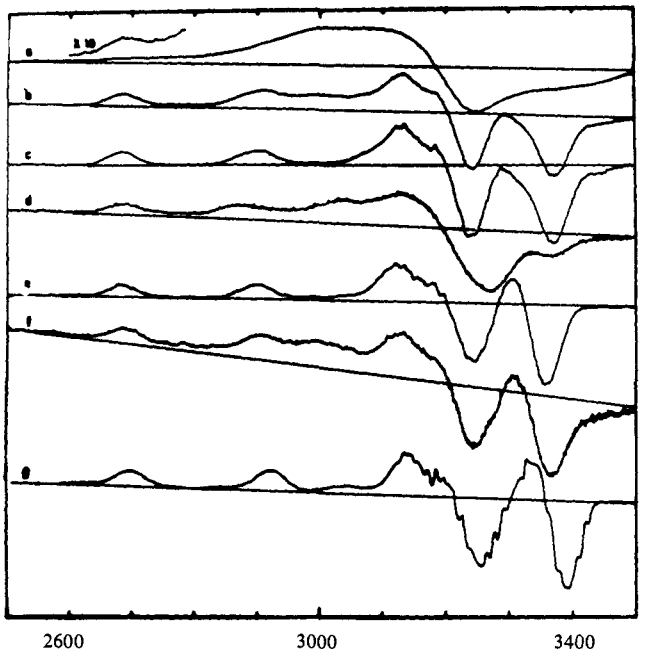

Figure 5. Epr spectra at 77°K of frozen solutions of the Cu^{2+}–triglycylglycine complex formed at the highest pH (all pH values for frozen solutions refer to measurements at 25°), with varying concentrations or additions (a–f), and of a polycrystalline sample of the corresponding mixed Cu^{2+}–Ni^{2+} (1:100) complex (g). Concentrations and additions: (a) 5×10^{-3} M Cu^{2+} and peptide; (b) as in part a with 0.2 M $NaClO_4$; (c) as in part a with 2 M $NaClO_4$; (d) 1.25×10^{-3} M Cu^{2+} and 12.5×10^{-3} M peptide; (e) 5×10^{-3} M Cu^{2+} and peptide in a water–methanol (1:1) mixture; and (f) 5×10^{-4} M Cu^{2+} and peptide (microwave frequency, ~9170 MHz).

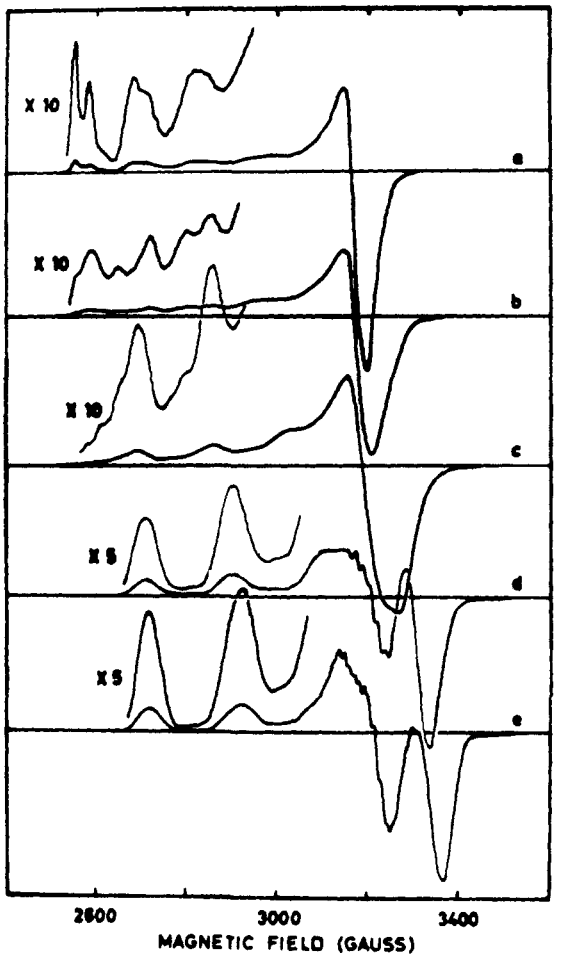

Figure 6. Epr spectra at 90°K of frozen solutions 5×10^{-3} M in Cu^{2+} and triglycylglycine, containing 2 M $NaClO_4$, with different amounts of base added: (a) −1.1 equiv, pH 2.4; (b) 0.4 equiv, pH 4.5; (c) 1.5 equiv, pH 5.6; (d) 3.1 equiv, pH 7.9; and (e) 4.2 equiv, pH 10.1 (microwave frequency, 9207 MHz).

$A_{||}$ for the signals that are thought to correspond to complexes seen in room-temperature experiments.

Spectra recorded at 34.3 GHz of a solution 5×10^{-3} M in Cu^{2+} and peptide and containing a 2 M perchlorate, pH 11.2, were typical for axial symmetry, with $g_{\perp} = 2.042$. The width of the "perpendicular" line was 140 gauss. The broadening of this line due to unresolved nitrogen hyperfine structure was estimated from room-temperature and powder spectra, and the effect of unresolved copper hyperfine structure was obtained from the measured value of $A_{||}$ and the hyperfine structure coupling of solutions. An upper limit for the broadening caused by deviation from axial symmetry could then be found giving $|g_x - g_y| < 0.005$.

As for the room-temperature spectra, rough estimates of concentrations of the different complexes have been made (see Figure 1). In this case the measurements were made on the line at lowest field as described earlier.[12]

Epr of Crystalline Powders of the High-pH Complex. The spectrum of the Cu:Ni (1:100) powder recorded at 9.2 GHz and 90°K is shown in Figure 5g. The high-field part of the same spectrum obtained at room temperature is included in Figure 4d.

The spectrum recorded at 34.3 GHz is very similar to that of the frozen solution, pH 11.2, at the same frequency.

(12) T. Vänngård in ref 5, p 213.

Discussion

Complexes at Low pH. Relaxation enhancement measurements on the solutions at the lowest pH, where the enhancement factor ϵ is 1.1 (Figure 1), indicates the presence of a complex. As ϵ for the $Cu(H_2O)_6^{2+}$ ion is 1 by definition, at least part of the Cu^{2+} present must exist in the form of another complex, probably CuH_4L^{2+} (the zwitterion form of the free peptide is throughout this paper written as H_4L). In the room-temperature epr spectrum (Figure 3a), very little effect can be seen from this complex. However, in the low-field region of the low-temperature spectrum (Figure 6a), the two species are very well resolved. The line at lowest field is due to the $Cu(H_2O)_6^{2+}$ ion, and the second line from the left originates from the first peptide complex. At pH 2.5 about 50% of the Cu^{2+} is complexed at low temperature.

The half-height widths of the low-field peak of $Cu(H_2O)_6^{2+}$ and CuH_4L^{2+} are 18 and 26 gauss, respectively, at pH 2.5. No nitrogen hyperfine structure in the peak corresponding to the peptide complex can be seen, but the line width is so large that we would not be able to see three unresolved nitrogen peaks. Thus, we cannot tell from our experiments which atom coordinates first, the carboxyl oxygen, one of the peptide nitrogens, or the amino nitrogen. In this connec-

tion it may be of interest to note that Kim and Martell[8] think that the first group is the carboxyl, whereas Gurd[7] suggests that the amino nitrogen coordinates first.

Complexes at Intermediate pH. With zero equivalents added, CuH_4L^{2+} is the dominating species (Figure 1) at least at low temperatures (*cf.* Figure 6). At the same time, the first traces of the next complex, CuH_3L^+, shows up in the room-temperature spectrum (Figure 3b). In the low-temperature spectra, however, this complex is not detected until 0.4 equiv of base is added (Figure 6b). Also, the maximum amounts are not obtained at exactly the same pH in room-temperature and low-temperature experiments. For this complex it is similarly not possible to resolve any nitrogen hyperfine structure.

The next complex, CuH_2L, has its maximum concentration both at room temperature and low temperature when about 2 equiv is added. In the room-temperature spectrum it is possible to resolve nitrogen hyperfine splitting of the high-field Cu hyperfine line (Figure 4a) into five lines.[13] This splitting is very likely caused by two nitrogens. However, the hyperfine constants of the two nitrogens might differ by as much as 20–30% and still produce a five-line spectrum, as illustrated by Lord and Blinder for solutions of DPPH.[14]

When 3 equiv of base is added, $CuHL^-$ reaches its maximum concentration. The nitrogen hyperfine structure consists of seven lines, indicating that three nitrogen nuclei interact approximately equally with the electron spin (Figure 4b).

Solutions at High pH. Upon the addition of 4 equiv of base, the final complex CuL^{2-} appears. Here the most probable interpretation of the nitrogen hyperfine structure (Figure 4c) is that four nitrogens interact with the electron spin, but that at least one of them interacts differently from the others. From crystallographic studies[9] it is known that the bond distances to the three peptide nitrogens are about equal, whereas the distance to the amino nitrogen is larger, and it might be possible to interpret the spectrum with three of the coupling constants being equal. (In the complexes CuH_2L and $CuHL^-$ the difference between the coupling constants of the coordinated nitrogens seems to be smaller. This fact does not, however, imply that the amino nitrogen coordinates only at higher pH.)

Correlation between Room-Temperature and Low-Temperature Epr Spectra. In the previous discussion of the complexes at lowest pH, we have taken advantage of the fact that low-temperature spectra sometimes are better resolved than room-temperature spectra. Also, at higher pH, around 5, 1 equiv of base (see Figure 1), a complex present in low concentration is seen in low-temperature spectra, which would be very difficult to observe at room temperature. This complex may be a Cu:peptide 1:2 compound. On the other hand, shifts in the relative amounts of the complexes do occur on freezing, particularly for the less stable complexes formed at low pH. This is not surprising as both the pH as measured at 25° and the pK of the complex formation are likely to be changed as the temperature is lowered.

If no change in the copper ligand field occurs on freezing of a given complex, the average of the g values, $g_0 = (g_{||} + 2g_\perp)/3$, from the low-temperature spectrum should be equal to the g value obtained from the room-temperature spectrum. In our case, for complexes formed at higher pH the correlation is quite good (Table I), but at lower pH significant differences appear. This again seems reasonable as the complexes at low pH are not fully chelated and would be expected to be more susceptible to deformation from the surrounding ice structure.

Proton Relaxation. Results of proton relaxation measurements usually are interpreted in terms of the Solomon–Bloembergen relation.[15] In our case, the high-frequency approximation of their expression for the relaxation rate induced by dipolar coupling simplifies to

$$\frac{1}{T_{1M}} = \text{constant} \times g^2\tau_c/r^6 \qquad (2)$$

where $1/T_{1M}$ is the relaxation rate for a proton in the neighborhood of the ion, g is the g value of the ion, and r is the distance between the paramagnetic ion and the proton. The correlation time for dipolar interaction, τ_c, is obtained from the relation

$$\frac{1}{\tau_c} = \frac{1}{\tau_r} + \frac{1}{\tau_e} + \frac{1}{\tau_h} \qquad (3)$$

where τ_r is the rotational correlation time, τ_e is the relaxation time of the electron spin, and τ_h is the residence time of the proton before being exchanged with another proton from the solvent.

The relaxation rate of protons in bulk water, $1/T_{1P}$, is[16,17]

$$\frac{1}{T_{1P}} = \frac{p}{T_{1M} + \tau_h} \qquad (4)$$

where p is the fraction of the total number of water protons in the neighborhood of the paramagnetic ions. Typical values (sec) for a low-molecular weight copper complex are: $\tau_r \approx 10^{-11}$, $\tau_e \approx 10^{-9}$, and $T_{1M} \approx 10^{-4}$. This gives a range 10^{-5}–10^{-10} sec in which τ_h can vary without affecting T_{1P}. For water protons in small complexes τ_h is usually found to have such values.

The protons of the complex may be separated into two groups, protons on water molecules in the first coordination sphere and protons on the ligand(s). It is usually assumed that the dominant contribution to $1/T_{1P}$ comes from water protons exchanging with bulk water protons. However, for the ethylenediamine–copper system exchange between free and bound ligand molecules in excess of ligand has been reported to enhance the relaxation rate.[18]

With triglycylglycine the enhancement factor for the high-pH CuL^{2-} complex is about 0.4. This comparatively high value is most likely not due to exchange of ligand molecules as a twofold excess of ligand did not change the relaxation. We will first consider the implications of the results assuming that there is no contribution from exchange of protons from the amino groups of the coordinated ligands; this is, in fact, a very reasonable assumption from considerations given below.

(13) With isotopically pure Cu the number of the lines is meaningful. In natural Cu the splitting between the two copper lines is about 10 gauss, which is of the same order as the nitrogen hyperfine splitting.

(14) N. W. Lord and S. M. Blinder, *J. Chem. Phys.*, **34**, 1693 (1961).

(15) I. Solomon and N. Bloembergen, *ibid.*, **25**, 261 (1956).

(16) Z. Luz and S. Meiboom, *ibid.*, **40**, 2686 (1964).

(17) M. Cohn in ref 5, p 101.

(18) (a) L. O. Morgan, J. Murphy, and P. F. Cox, *J. Am. Chem. Soc.*, **81**, 5043 (1959); (b) P. F. Cox and L. O. Morgan, *ibid.*, **81**, 6409 (1959).

The temperature dependence of the relaxation rate of CuL^{2-} (Figure 2) clearly shows that τ_h is not very long. Also, the activation energy for the relaxation rate of CuL^{2-} is somewhat larger than that of $Cu(H_2O)_6^{2+}$ (Figure 2) which seems consistent with the idea that the enhancement is governed by the correlation time for rotation and not by a very short τ_h.

Thus, it seems that at least one water molecule is bound to CuL^{2-}, in contrast to what is found in the solid state.[9] The value of ϵ obtained, 0.4, is also quite reasonable in this picture. As τ_h is much smaller than T_{1M}, we get

$$\epsilon = \frac{g^{*2}p^*\tau_c^* r^6}{g^2 p \tau_c r^{*6}} \qquad (5)$$

where as before starred quantities refer to the complex. The g values can be taken from Table I, and r (the Cu–proton distance in the hexaaquo ion) is estimated to be 2.8 A. The value of τ_c^*/τ_c is difficult to get but most likely it is larger than 1. Stoke's formula predicts that the ratio is proportional to ratio of the volumes of the molecules. If the peptide complex is regarded as a disk with radius 5.5 A and thickness 3.2 A, and if the $Cu(H_2O)_6^{2+}$ ion is simulated by a sphere with radius d(Cu–H) + r(H) = 3.8 A, then the ratio of their volumes is about 5:1. We find that the experimental results are then compatible with one water molecule with its oxygen 2.4 A from the Cu^{2+} ion, or with two water molecules at a distance of 2.8 A. These distances, however, are sensitive to the geometrical assumptions made in the calculation. For instance, if the ratio of the volumes were equal to 6 instead of 5, then the same calculation would lead us to find two water molecules at 2.9 A.

Since, as has already been pointed out, there is the possibility that proton exchange with coordinated amino groups does occur, our experiments do not conclusively eliminate or prove the presence of coordinated water molecules. However, comparisons with other systems make it likely that the relaxation rate is mainly determined by exchange with protons from coordinated water rather than the amino group. It has already been shown that τ_h must be equal to or smaller than 10^{-5} sec. While the exchange rate increases with pH, estimates for *free* amines and amides[19] give half-lives that are at least one order of magnitude too large even at pH 11. For example, the half-life for the exchange of the amide proton in N-methylacetamide is found to be[19] 3×10^{-4} sec at room temperature and pH 11. When the group is bound to a metal ion, the exchange rate is expected to be slower. Thus, in copper(II)–ethylenediamine complexes the exchange of protons from the amino groups contributes slightly to the relaxation rate only at temperatures higher than 70° but is completely negligible at room temperature as the activation energy is 8 kcal/mole.[18b]

The relaxation data for the other complexes are less complete. However, as pointed out above, an important observation is that the enhancement factor is greater than 1 at pH below 4. This can be interpreted as the presence of a complex for which τ_c is longer owing to an increase in the rotational correlation time. In this complex the number of coordinated water molecules must be less than 6, and presumably it is 5.

(19) A. Berger, A. Loewenstein, and S. Meiboom, *J. Am. Chem. Soc.*, **81**, 62 (1959).

With the assumption that eq 5 is valid here also, and that $r = r^*$, we get $\tau_c^* = 1.3\tau_c$. This value of τ_c^* is a lower limit and is not inconsistent with the value given above, because not all Cu^{2+} is in the complex form, but some is $Cu(H_2O)_6^{2+}$ having a lower relaxation rate.

When the number of bound water molecules is reduced by forming new complexes at higher pH, the enhancement decreases. With the assumption that τ_c^* and r^* are constant and that r^* is equal for all water molecules, p is the only parameter varying in the expression for ϵ. If one water molecule is released from the complex for every proton leaving the peptide, the enhancement curve in Figure 1 would be a straight line in the region where the water titration can be neglected. While the situation is not so simple, the general trend of the curve indicates a gradual loss of coordinated water in the stepwise complex formation. This situation is in contrast to the suggestion of Kim and Martell[8] that only one water molecule is coordinated in all the complexes CuH_3L^+ to CuL^{2-}.

Comparison between Solution and Powder at High pH. An interesting comparison can be made between the high-pH complex in solution and the corresponding sample in a powder diluted with Ni (Table I). The spectra are very similar but significantly different. This confirms the findings from the proton relaxation measurements that the structure of the molecule is not exactly the same in the crystalline state and in solution. However, comparing the nitrogen hyperfine structure in the high-field line of the room-temperature solution spectrum and that of the "overshoot" line in the powder spectrum (Figure 4c,d), one finds that as far as the nitrogen coordination is concerned this must be very much the same in solution and in crystal. The lines are not appearing at the same fields, but the comparison is justified for the following reasons. Both lines would be single lines in the absence of nitrogen hyperfine structure. This is true for the "overshoot" line because the possible splitting due to departure from axial symmetry certainly is negligible for the directions of the magnetic field relative to the molecular axes producing the "overshoot" line. These single lines in powder and solution are then duplicated by the nitrogen hyperfine structure which most likely is nearly isotropic. The "overshoot" line is not quite symmetric, but that does not disturb the comparison (*cf.* the Cu^{2+}–phthalocyanine spectrum in ref 20).[21]

Freezing Effects. The frozen solutions containing only copper and peptide (Figure 5a) gave no resolved spectrum. Similar findings for manganese have been discussed by Ross,[22] who concluded that crystallization of solvent produces high local concentration of the solute and broadening due to the interaction between the ions. Addition of various salts or alcohols was found to reduce this segregation of solute and solvent. This is also the case here (Figure 5b,c,e). The increase

(20) T. Vänngård and R. Aasa, in "Proceedings of the First International Conference on Paramagnetic Resonance," W. Low, Ed., Academic Press Inc., New York, N. Y., 1963, p 509.

(21) The spectrum of the powder also shows nitrogen hyperfine structure in the "perpendicular" direction. However, it is not correct to count the number of lines in this region and relate this to the number of nitrogens coordinating. Here the number of lines can be quite large, even in absence of nitrogen hyperfine structure, owing to copper hyperfine splitting $A_\perp$, departure from axial symmetry, and "overshoot" from one of the copper hyperfine lines.

(22) R. T. Ross, *J. Chem. Phys.*, **42**, 3919 (1965).

in resolution that we obtain by dilution further corroborates this interpretation. Note that all these solutions have the same room-temperature spectrum. On the other hand, there must be other factors influencing the resolution. The spectrum is also broadened even with perchlorate present if the samples are kept at a temperature (200°K) at which the molecules can hardly diffuse. Possibly, the structure of the ice changes at these higher temperatures and this may affect the resolution.

Proton Magnetic Resonance and Raman Spectral Studies of the Complexes Tetrakis(dimethylformamide)beryllium(II) and Acetylacetonatobis(dimethylformamide)beryllium(II) in the Solvent N,N-Dimethylformamide. Direct Determination of Solvation Numbers and Kinetics of Solvent Exchange[1]

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Abstract: At temperatures below 0°, the proton nmr signals of N,N-dimethylformamide (DMF) in the first coordination sphere of the Be(II) ion in DMF solutions can be distinguished. From the relative intensities of these signals, a primary solvation number of four was calculated for Be(II). The complex ion, $Be(DMF)_4^{2+}$, reacts with bis(acetylacetonato)beryllium(II) ($Be(acac)_2$) in DMF solutions with the formation of the mixed complex, $(DMF)_2Be(acac)^+$, which exhibits well-resolved proton nmr signals at temperatures below +5°. From the temperature dependence of the proton relaxation rates, the solvent (DMF) exchange parameters k_1 (sec^{-1} at 25°), $\Delta H^{\ddagger}$ (kcal), and $\Delta S^{\ddagger}$ (eu) were calculated for $Be(DMF)_4^{2+}$ and $(DMF)_2Be(acac)^+$, respectively: 310, 14.6, 2.6; and 22, 13.9, −6. Both complexes exhibit an intense, highly polarized Raman line at 478 cm^{-1} which is assigned as a Be–O symmetric stretching vibration of the system, Be–acac. The results are discussed briefly in terms of ligand–ligand interactions within the primary coordination sphere of Be(II).

We have initiated a general study of the coordination chemistry of the diamagnetic cations of the d^0 and d^{10} series and have focused our attention upon two main points: (1) determining the stoichiometry, structure, and lability of the primary coordination spheres of the simple solvated ions in a variety of solvents; and (2) evaluating the effects of ligands in mixed complexes upon the nature of the remaining metal ion–solvent bonds. The elucidation of these properties of diamagnetic cations is not possible using conventional experimental techniques because the complexes generally are quite labile and the cations themselves lack a property which is sufficiently sensitive to the nature of the environment that the symmetry of the primary coordination sphere can be deduced. However, the nmr techniques are particularly well suited to the study of these complexes.

The application of the nmr techniques has been discussed elsewhere.[2-10] The complexes which have been studied include, for example, $Ga(OH_2)_6^{3+}$,[9,10] $Al(OH_2)_6^{3+}$,[5] and $Be(OH_2)_4^{2+}$ in water (using ^{17}O nmr), and $Al(DMSO)_6^{3+}$ in DMSO,[7] $Al(DMF)_6^{3+}$ in DMF,[8] $Mg(CH_3OH)_{6-n}(OH_2)_n^{2+}$ in aqueous methanol,[4] $Mg(OH_2)_6^{2+}$ in aqueous acetone,[11] $Mg(CH_3OH)_6^{2+}$ in anhydrous methanol[11,12] and methanolic acetone[11] (using ^1H nmr; DMSO represents dimethyl sulfoxide and DMF represents N,N-dimethylformamide).

Experimental Section

Reagents. Eastman White Label DMF was purified as described previously.[13] Acetylacetone (2,4-pentanedione) was obtained from Matheson Coleman and Bell and was distilled immediately before use, the middle fraction being retained. Hydrated beryllium perchlorate was prepared by dissolving beryllium metal (Fisher Reagent) in 4 *M* HCl and "fuming" the resulting solution with concentrated perchloric acid until a negative test for chloride ion in solution was obtained (with a concentrated aqueous solution of silver nitrate). Then the solution was concentrated slowly by distillation until fine needles of $Be(ClO_4)_2 \cdot 4H_2O$ were deposited.[14] After the resulting mixture had been allowed to cool to room temperature, the crystals of $Be(ClO_4)_2 \cdot 4H_2O$ were separated by filtration. $Be(NO_3)_2 \cdot 4H_2O$ was obtained from Alfa Inorganics, Inc., and was used without further purification.

Preparation of Complexes. Bis(2,4-pentanedionato)beryllium(II), $Be(acac)_2$, was prepared by the method of Arch and Young.[15] The solid obtained was recrystallized from benzene and dried *in vacuo* at 25°. The white crystals have mp 108° (lit.[15] 108.5°).

(1) The authors are grateful to the National Science Foundation for funds which assisted in the purchase of the nmr spectrometers through an institutional grant.

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