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## EPR Study of Copper(II) Complexes of Poly(L-lysine)

COLEEN M. YOUNG and FREDERICK T. GREENAWAY\*

Department of Chemistry, Clark University, Worcester, Massachusetts 01610. Received June 5, 1985

The interaction of copper(II) ions with poly(L-lysine), (Lys)<sub>n</sub>, has been the subject of many investigations that have led to considerable confusion and disagreement as to the species which are present in solution. The interaction is of interest because of the observation<sup>1</sup> that (Lys)<sub>n</sub>, when bound to copper(II), catalyzes the oxidation of 3,4-dihydroxyphenylalanine, DOPA, at high pH. This has been treated as a model for DOPA oxidase, which is utilized in melanin production. The Cu(Lys)<sub>n</sub> complex behaves as an asymmetrically selective catalyst,<sup>2</sup> favoring reaction with D-DOPA over L-DOPA, particularly at high pH where (Lys)<sub>n</sub> exists in a helical form.<sup>3,4</sup> Cu(Lys)<sub>n</sub> has also been reported to model the enzyme catalase<sup>5</sup> and catalyze the decomposition of H<sub>2</sub>O<sub>2</sub> at pH between 6 and 7.

The binding of Cu(II) to (Lys)<sub>n</sub> has been previously studied by potentiometric titration,<sup>6-8</sup> absorption spectroscopy,<sup>6-9</sup> circular dichroism,<sup>6-9</sup> and NMR spectroscopy.<sup>10,11</sup> The results of these studies have been interpreted in terms of a number of models, summarized in Figure 1, which disagree with one another except for a general agreement that two types of Cu(II) complex form, one at pH < 8 and one at pH > 8. This paper reports the results of an investigation of the Cu(Lys)<sub>n</sub> system using EPR spectroscopy and resolves the discrepancies between the previous interpretations.

## Experimental Details

Poly(L-lysine) hydrobromide of molecular mass 4 000-15 000 and 70 000-150 000 was purchased from Sigma Chemical Co. and dissolved in deionized water. Copper(II) was added as a concentrated solution of the perchlorate salt, and the pH was adjusted by the addition of small volumes of concentrated HClO<sub>4</sub> or NaOH solutions. Replacement of perchlorate by chloride had no effect on the EPR spectra. Some studies were also performed with methanol as a solvent. Methanol promotes  $\alpha$ -helix formation<sup>12</sup> in (Lys)<sub>n</sub>.

EPR spectra were recorded with a Varian E-9 EPR spectrometer operating at 9.1 GHz with 100-kHz modulation. Temperatures between 110 and 300 K were achieved by means of a nitrogen-flow Dewar. The magnetic field was calibrated with a Magnion gaussmeter, and the frequency was measured on a Hewlett-Packard frequency counter. Absorption spectra were recorded on a Cary 219 UV-visible spectrophotometer.

## Results and Discussion

EPR spectra obtained of frozen solutions of Cu(Lys)<sub>n</sub> at 110 K were typical of tetragonally elongated octahedrally coordinated Cu(II) with  $g_{\parallel} > g_{\perp}$ . Anisotropic spectra were also obtained for liquid solutions at 300 K and, although of poorer resolution because of partial motional

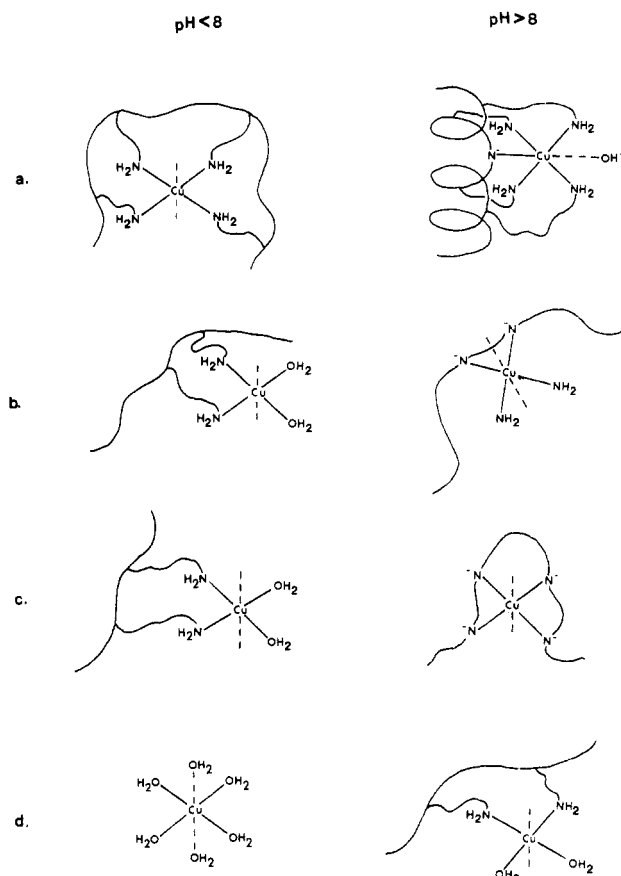


Figure 1. Structures of the copper-binding sites of (Lys)<sub>n</sub> as suggested by previous workers for pH < 8 and pH > 8: (a) ref 7; (b) ref 9; (c) ref 6; (d) ref 10 and 11.

averaging, were identifiable as arising from the same species observed in frozen solutions. Thus there is no temperature dependence of the species present in solution, and the frozen solution results can be correlated with the results obtained by other techniques using liquid solutions. Although methanol promotes  $\alpha$ -helix formation<sup>12</sup> in (Lys)<sub>n</sub>, EPR spectra obtained from frozen methanol solutions of Cu(Lys)<sub>n</sub> showed no significant differences from the spectra of frozen aqueous solutions.

EPR studies were performed at a variety of Cu:Lys ratios. At a 1:4 ratio, which is what most previous workers have used, EPR spectra were broad and baselines were poor between pH 6 and 11 owing to Cu(OH)<sub>2</sub> precipitation and, above pH 11, because of Cu(OH)<sub>4</sub><sup>2-</sup> formation. Therefore most studies were carried out at a 1:30 Cu:Lys ratio, where the copper hydroxide species did not form. Studies at other ratios were performed on a more limited basis. Although Garnier and Tosi<sup>13</sup> observed terminus binding of (Lys)<sub>n</sub> for  $n = 25$ , our spectra showed no variation with chain length, which indicates that coordination of chain termini to copper(II) is not important for chains where  $n > 25$ . Minor differences related to chain length were noted (Table I), but these do not suggest any major differences in the structures and probably reflect different degrees of intra- and interchain complexation to copper.

EPR spectra were obtained between pH 2 and 13. When solutions of (Lys)<sub>n</sub> and Cu(II) were initially mixed, a pH of about 4 resulted, and the broad EPR spectra indicated aggregation of the Cu(II) species. Slowly increasing the pH did not change the spectra. It was therefore necessary to raise the pH to 13 and reduce the pH by progressive additions of acid to fully observe all species. The same species did form at lower pH but at a much slower rate, equilibrium being attained only after several days. This

Table I  
EPR Parameters<sup>a</sup>

species	pH	$g_{\perp}$	$g_{\parallel}$	$A_{\parallel}(\text{Cu})$ , $\text{cm}^{-1}$	assigned structure
A	1-2	2.090	2.388	0.0129	$\text{Cu}(\text{H}_2\text{O})_6^{2+}$
B	2-3	2.084	2.337	0.0137	$\text{Cu}(\text{H}_2\text{O})_5(\text{RNH}_2)^{2+}$
C <sup>b</sup>	3-5	2.075	2.330	0.0145	$\text{Cu}(\text{H}_2\text{O})_4(\text{RNH}_2)_2^{2+}$
D	5-6	2.063	2.304	0.0153	$\text{Cu}(\text{H}_2\text{O})_3(\text{RNH}_2)_3^{2+}$
E	6-8	2.062	2.243	0.0171	$\text{Cu}(\text{H}_2\text{O})_2(\text{RNH}_2)_4^{2+}$
F <sup>c</sup>	8-9	2.054	2.222	0.0175	$\text{Cu}(\text{H}_2\text{O})_2(\text{RNH}_2)_3^+$ (RCONR) <sup>+</sup>
G	9-13	2.047	2.164	0.0205	$\text{Cu}(\text{H}_2\text{O})_2(\text{RNH}_2)_2^+$ (RCONR) <sub>2</sub>

<sup>a</sup> (Lys)<sub>n</sub>-Cu(II) in aqueous solution in 30:1 ratio. MW = 4000-15000. <sup>b</sup> This complex was not observed with (Lys)<sub>n</sub> of MW = 70000-150000. <sup>c</sup> With (Lys)<sub>n</sub> of MW = 70000-150000 this species had  $g_{\parallel} = 2.220$  and  $A_{\parallel}(\text{Cu}) = 0.0188 \text{ cm}^{-1}$ .

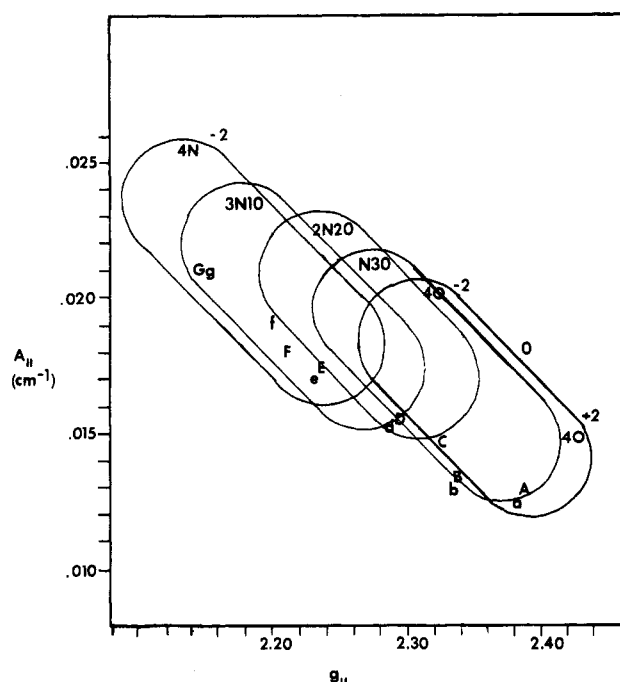


Figure 2. Plot of  $A_{\parallel}(\text{Cu})$  against  $g_{\parallel}$  showing the probable ligand environment<sup>14</sup> of the Cu(II) ion. Upper case letters refer to (Lys)<sub>n</sub> of MW = 4000-15000; lower case letters refer to (Lys)<sub>n</sub> of MW = 70000-150000. Letters refer to the proposed structures of Figure 3 and Table I.

slow rate is probably the cause of disagreement between potentiometric data obtained by titration with acid<sup>7</sup> and base.<sup>6,9</sup> Our results suggest that titration with acid from a high pH leads to a more rapid attainment of equilibrium and thus more reliable results.

Values of  $A_{\parallel}(\text{Cu})$  and  $g_{\parallel}$  are given in Table I and are plotted against one another in Figure 2 following the method of Peisach and Blumberg.<sup>14</sup> Although no nitrogen superhyperfine structure was observed, the  $A_{\parallel}/g_{\parallel}$  correlation and the widths of the parallel copper hyperfine lines that are dependent on the number of unresolved nitrogen superhyperfine lines were used to assign the ligand environments of the copper(II) ion. Because of the limited number of possible coordinating atoms in (Lys)<sub>n</sub>, we are fairly confident in assigning the structures shown in Figure 3 to the seven copper(II) complexes observed by EPR (Figure 4).

At very low pH, where the proton competes effectively with the Cu(II) for the ligand atoms, the only species present in solution is  $\text{Cu}(\text{H}_2\text{O})_6^{2+}$ . As the pH increases, the Cu(II) competes with the proton for the lone electron pair on the amine nitrogen and water molecules are suc-

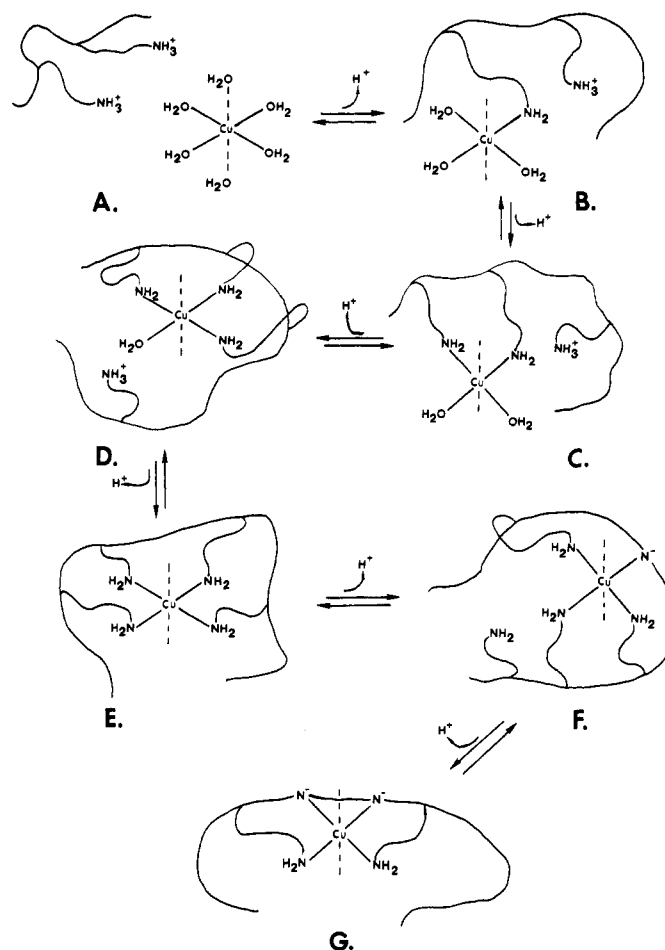


Figure 3. Diagrammatic representation of structures proposed on the basis of EPR parameters. Refer to Table I for details.

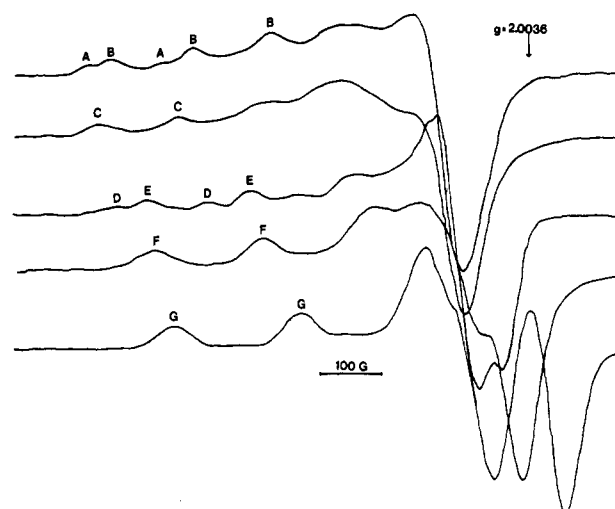
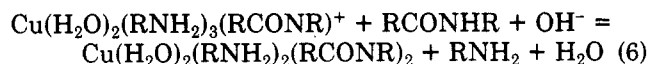
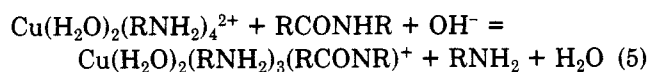
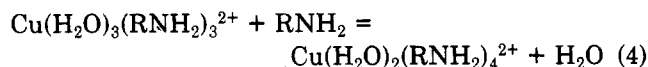
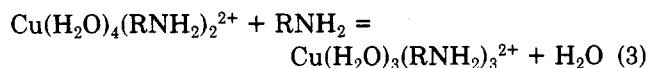
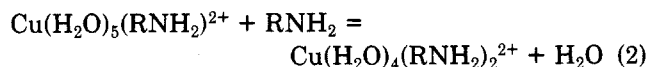
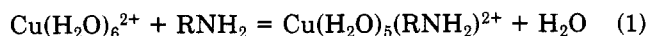


Figure 4. Representative X-band EPR spectra of 1:30 Cu:Lys frozen aqueous solutions at 110 K. The parallel copper hyperfine lines are identified with the labels of Table I.

cessively replaced by amine nitrogen atoms. Only four amines bind to the copper, in an approximately planar geometry. The axial ligands are coordinated less strongly because of the Jahn-Teller distortion common to all Cu(II) complexes. At pH > 8, two deprotonated peptide nitrogens bind to Cu(II) so that between pH 9 and 13 the major species has two amine and two deprotonated peptide nitrogens in approximately planar coordination.

The following equilibria, which are diagrammed in Figure 3, occur:



In these equilibria the axial ligands are represented as water molecules although the EPR spectra give no information about the nature of the axial ligands. Steric constraints on the polymer make it unlikely, however, that nitrogen atoms are also present at the axial sites. Our results show Cu(II) to be bound in a tetragonally elongated octahedral geometry in all compounds and not in a square-based pyramidal geometry as has been suggested<sup>8</sup> for the compounds present at pH > 10.

The peptide coordination at high pH confers dichroism to the copper d-d band. Although (Lys)<sub>n</sub> has an α-helical structure in aqueous solution at high pH, it has been found that Cu(II) destroys this structure,<sup>6</sup> and thus early explanations<sup>2</sup> of the asymmetric oxidation of DOPA cannot be correct. The binding of two peptide nitrogen atoms of the backbone at high pH does, however, confer a high degree of organization upon the (Lys)<sub>n</sub> chain which may enable D-DOPA to bind preferentially. It would be of interest to investigate the catalytic specificity of Cu(II) (D-Lys)<sub>n</sub> or (DL-Lys)<sub>n</sub>.

At neutral pH, Cu(Lys)<sub>n</sub> catalyzes the decomposition of H<sub>2</sub>O<sub>2</sub>, and it has been suggested<sup>5</sup> that the catalytic site has two replaceable ligands on the Cu(II). Our results are consistent with this structure. Below pH 8 the amine and water ligands are both quite labile and several complexes exist in equilibrium. The sensitivity of the nature of the species present in solution to pH, to the Cu:Lys ratio, and to concentration observed by others are all due to the complexity of the equilibria. Although we have not measured equilibrium constants, it is clear that they are not widely separated in magnitude. This also explains the discrepancy of the NMR results<sup>10,11</sup> with the results of other types of experiment, since the NMR data were obtained at very low Cu:Lys ratios, which shifts the equilibria 5 and 6 to the left, resulting in less peptide coordination.

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## Isomerization Reactions in the Polymerization of Alicyclic Dithiols with Aldehydes

JOSÉ L. DE LA PEÑA\* and MANUEL C. LUCIO

Colegio Universitario de Burgos, Burgos, Spain

JULIO GUZMÁN and EVARISTO RIANDE

Instituto de Plásticos y Caucho (CSIC), 28006 Madrid, Spain. Received June 12, 1985

Owing to the conformational wealth of the cyclohexane ring,<sup>1</sup> its incorporation in molecular chains may affect in a significant way the properties of the resulting polymers. Thus the alicyclic polyformal prepared from *trans*-1,4-bis(hydroxymethyl)cyclohexane and paraformaldehyde melts at higher temperature than its aliphatic counterpart. In general, the *cis* isomers of alicyclic polyesters and polyformals either are amorphous or melt at lower temperatures than the corresponding *trans* isomers.<sup>2-5</sup>

Isomerization processes in the synthesis of alicyclic polyformals were not detected.<sup>6</sup> In general, the *cis*/*trans* composition of the polymers coincides with the *cis*/*trans* content of the glycol used in the preparation of the polymers. However, isomerization interconversion in disubstituted cyclohexanes may occur. Thus, although compounds with equatorial substituents have less steric crowding and therefore are more stable than the compounds with corresponding axial substituents, *trans*-*cis* interconversion was reported for some disubstituted cyclohexanes. For example, the equilibrium of *cis*- and *trans*-4-*tert*-butylcyclohexanol, obtained by treating either isomer with an aluminum isopropoxide-isopropyl alcohol-acetone mixture, contains four parts of the *trans* isomer to one part of the *cis* isomer. It should be pointed out that in all the isomeric interconversions, the *trans* isomer is predominant. Thus the isomeric interconversion of polyesters containing cyclohexane dicarboxylate groups seems to be the same whether the polymer is prepared from *cis* or *trans* isomers.<sup>7</sup> In both cases the *cis*/*trans* ratio obtained in the polymers is 0.66.

In the present work the synthesis of alicyclic poly(thioformals) is described. The possibility of *cis*-*trans* interconversion in the disubstituted cyclohexanes is studied by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.

## Experimental Part

**Materials.** *Cis*/*trans* mixtures of 1,4-bis(hydroxymethyl)cyclohexane were used to obtain the corresponding alicyclic dithiols. The *cis*/*trans* isomer ratio was determined by <sup>1</sup>H NMR spectroscopy. Thiourea, bromhydric acid, paraformaldehyde, and propionaldehyde were used as received. Benzene and *p*-toluenesulfonic acid were purified by methods described elsewhere.

**Synthesis of *cis*/*trans*-1,4-Bis(mercaptomethyl)cyclohexane.** The preparation of this product was carried out by reaction of *cis*/*trans*-1,4-bis(bromoethyl)cyclohexane (previously obtained by reaction of the alicyclic glycol with bromhydric acid) and thiourea, followed by hydrolysis of the resulting thiouronium salt.

**Polymerization Reactions.** Two polymers were obtained by condensation polymerization of *cis*/*trans*-1,4-bis(mercaptomethyl)cyclohexane with paraformaldehyde (polymer A) and propionaldehyde (polymer B). The reactions were carried out