Copper(II) Complexes Immobilized on a Polymeric Matrix. Thermodynamics, Spectroscopy, and Molecular Modeling

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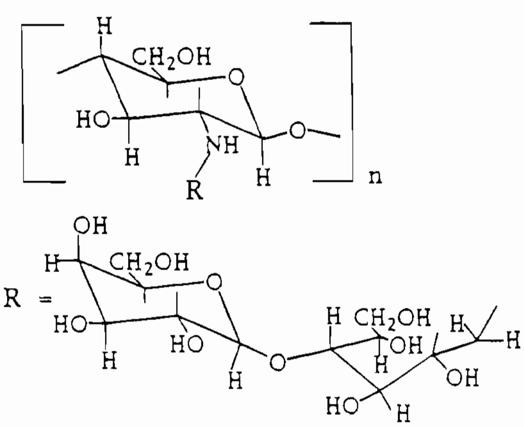
Polymer-immobilized copper(II) complexes were prepared by using deoxylactit-1-yl (**1**), 2-substituted pentanedioic acid (**2**), and 2-substituted propanoic acid (**3**) derivatives of chitosan (**4**) as polymeric ligands. The thermodynamics of formation, based on both equilibrium dialysis and microcalorimetric experiments, suggest that the functional groups in each monomeric residue are an effective site of binding for one metal ion. The enthalpies of complex formation (25 °C) are $-(35.7 \pm 2.4) \times 10^2$ and $-(46.0 \pm 7.5) \times 10^2$ J/mol for Cu(II)-**1** at pH 5.6 and 8.0, respectively, and $(13.6 \pm 2.0) \times 10^2$ J/mol for Cu(II)-**2** at pH 4.4, while the entropies of formation are around 65 $J{\cdot}mol^{-1}{\cdot}deg^{-1}$ in the former two cases and 96 $J{\cdot}mol^{-1}{\cdot}deg^{-1}$ in the latter one. ESR results (100 K) indicate that all these compounds basically have a tetragonal symmetry, but visible CD spectra suggest that the order of increasing departure from this geometry is Cu-**4** ≈ Cu-**3** < Cu-**2** ≤ Cu-**1**, the arrangement of ligands around the central metal ion being more symmetric in **3** and **4** for the lack of sterically constraining side chains. Molecular modeling of Cu(II)-**1** and Cu(II)-**2** active sites was performed by mimicking different pH conditions and using both a series of pairwise additive energy terms and a new distance-dependent dielectric function to tackle electrostatic effects that are believed to dominate metal–macromolecule interactions in solution. Hypothetical models of the metal complexes are presented and supported by experimental results, as far as the limited data allow. Implications of steric effects on the computed structures are also discussed.

Introduction

Natural and synthetic polymers have been widely used as carriers of bioactive molecules for modeling, e.g., enzyme catalysis.[1] Despite the fact that they usually show a much lower catalytic activity than a true enzyme,[2] they prove valuable in understanding some basic features of enzymic processes and in developing other types of synthetic polymers.

An important class of these model compounds is that formed by transition metal ions or complex ions anchored to a polymer. When the matrix is a polypeptide, the corresponding polymer-supported complexes are supposed to mimic the activity of metalloenzymes[3,4] because synthetic poly(α-amino acids) can reproduce a great variety of structures and properties that characterize the proteins.[3c] But even if other types of matrices are used, polymer-immobilized complexes represent realistic models of enzymic materials because of their stability within a much wider range of pH than polymer-free complex ions and the decrease in mobility of the linked species that can result in an increased activity. This is especially true for those catalysts which require the dissociation of a ligand during the reaction.

Chart 1



(1)

$= HOOC(CH_2)_2(HOOC)HC-$ (2)

$= H_3C(HOOC)HC-$ (3)

$= H-$ (4)

Novel polymeric ligands were recently synthesized by us,[5] by attaching a deoxylactyl (**1**), pentanedioic acid (**2**), or propanoic acid (**3**) moiety to the amine function of chitosan (**4**), the *N*-deacetyl derivative of chitin, the second most abundant natural polymer, as illustrated in Chart 1. We thus obtained branched-chain materials with improved solubility and increased functionality with respect to the starting polysaccharide matrix.[6,7]

We report here thermodynamic and spectroscopic results on the association complexes between the aforementioned polymers and copper(II) ions, together with some spectroscopic data

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(1) Wharton, C. W. *Int. J. Biol. Macromol.* **1979**, *1*, 3.
(2) Klotz, I. M. *Adv. Chem. Phys.* **1978**, *90*, 691. Imanishi, Y. In *Bioactive Polymeric Systems*; Gebelein, C. G.; Carraher, C. E., Jr., Eds.; Plenum Press: New York, 1985; p 435.
(3) (a) Vol'pin, M. E.; Novodarova, G. N.; Kolosova, E. M. *Inorg. Chim. Acta* **1981**, *5*, 21. (b) Lerch, K. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Dekker: New York, 1981; Vol. 13, p 143. (c) Pispisa, B. In *The Coordination Chemistry of Metalloenzymes*; Bertini, I., Drago, R. S., Luchinat, C., Eds.; Reidel: Dordrecht, The Netherlands, 1983; p 279.
(4) (a) Pispisa, B.; Barteri, M.; Farinella, M. *Inorg. Chem.* **1983**, *22*, 3166. (b) Pispisa, B.; Palleschi, A.; Barteri, M.; Nardini, S. *J. Phys. Chem.* **1985**, *89*, 1767. Pispisa, B.; Palleschi, A. *Macromolecules* **1986**, *19*, 904.
(5) (a) Chiessi, E.; Palleschi, A.; Paradossi, G.; Venanzi, M.; Pispisa, B. *J. Chem. Res., Synop.* **1991**, 248. (b) Paradossi, G.; Chiessi, E.; Venanzi, M.; Pispisa, B.; Palleschi, A. *Int. J. Biol. Macromol.* **1992**, *14*, 73.

previously shown.[8] Coordination of the polymeric ligands to Cu^{2+} ions is found to involve the donor nitrogen atom of the uncharged amines, but the ability of **1** to bind metal ions derives from additional charge interactions with OH groups in the side chain, which are responsible for a decrease in symmetry with respect to the Cu(II)-**4** complex. A similar effect is observed with polymer **2**, since the pendant carboxylates provide a more constraining environment for metal chelation than that of the side chain of **3**.

Hypothetical models of the polymer-immobilized Cu(II)-**1** and Cu(II)-**2** complexes, under conditions where different pH values are mimicked, are also presented. These computed structures were obtained by using a series of additive pairwise energy terms, similar to the potential functions previously employed by us in the simulation and energy minimization of iron(III) complex ions anchored to polypeptides[4b] and to those recently employed by Hingerty et al. for bivalent ion−macromolecule association complexes.[9] They are supported by experimental results, as far as the limited data allow, and show how both the charge state of the ionizable groups and the chirality of the carbon atom in the side chains control the stereochemical features of the Cu(II)-**2** complex.

Experimental Section

Materials. Polymeric ligands **1**−**3** were prepared from deacetylated chitosan (**4**) and characterized as already described.[5] In contrast with polymers **1** and **3**, which have a degree of substitution (ds) of 1, polymer **2** has a ds of 0.43, which corresponds approximately to two hexose rings for each bonded pentanedioic acid moiety. The ionizable groups have the following pK_a's at 25 °C, 0.01 M NaCl, and $\alpha = 0.5$. **1**: NH, 5.70. **2**: NH_2, 5.45; NH, 7.35; COOH, 2.34 (α), 3.12 (γ). **3**: NH, 7.07; COOH, 3.25. **4**: NH_2, 5.60.[5]

The complexes were obtained by mixing aqueous solutions of $CuCl_2$ and buffered polymeric material. The resulting mixtures did not show any precipitate or even opalescence, owing to the hydrolysis of free metal ions, within a wide range of $[Cu^{2+}]/[P]$ molar ratios (0.01−0.45), even at pH as high as 10. [P] denotes the concentration of the polymer referred to monomeric unit (monomol/L), as determined by weight after prolonged drying under vacuum.

All measurements were carried out on freshly prepared solutions, using doubly distilled water.

Methods. Dialysis equilibrium experiments on Cu^{2+}−polymer systems were carried out at 25.0 ± 0.1 °C in 0.01 M HEPES or PIPES buffer, depending on pH, the ionic strength being sufficient to eliminate any Donnan effect. Before use, dialysis tubings (Thomas) were carefully purified in the conventional way.[10a] Dialysis equilibrium was attained in 40 h with magnetic stirring of the external solution. At the end of each set of experiments, the concentration of bound copper ions ($[Cu^{2+}]_b$) in the "internal" solution was determined by UV absorption at 260 nm for Cu(II)-**1** and 250 nm for Cu(II)-**2** (ϵ = 5600 and 5750 $M^{-1}cm^{-1}$, respectively). The concentration of free copper ions, $[Cu^{2+}]_f$, in the "external" (polymer-free) solution was normally evaluated from the known initial concentration of Cu^{2+} ions, but it was also checked by absorbance measurements at 230 nm, after addition of ammonia to the solution, using a previously plotted calibration curve.

Isothermal flow microcalorimetric measurements were performed at 25.0 ± 0.1 °C by a LKB 2277 thermal activity monitor combined with a LKB 2277-204 measuring cylinder. Several calorimetric measurements were carried out to obtain consistency in the results, with increasing amounts of added metal ion within the range 1×10^{-5}–2×10^{-3} M and at fixed polymer concentration ($[P] = 4 \times 10^{-4}$ M). The observed heats were then corrected for dilution effects,[10b] typical dilution enthalpies being around zero for **1**, −19 J/mol for **2**, and −12 J/mol for $CuCl_2$, and normalized for polymeric ligand concentration (ΔH, J/monomol).

Cyclic voltammetry was performed at room temperature with a PAR 273 potentiostat, using working (glassy carbon, from BAS) auxiliary (Pt wire), and reference (saturated calomel) electrodes and buffered and deareated solutions, as already reported.[8b]

ESR spectra were recorded at X-band and 100 K on a Bruker ER 220D instrument with an ER 4111 VT automatic temperature controller.

Absorption spectra were recorded on a Jasco 7850 spectrophotometer, and circular dichroism (CD) spectra, on a Jasco J 600 apparatus, using appropriate quartz cells.

Table 1. Dialysis Equilibrium Data for the Formation of the Cu(II)−Polymeric Ligand Complexes[a]

polymeric ligand	pH	K, M^{-1}	β_{max}[b]
1	5.6	$(7.65 \pm 0.65) \times 10^3$	0.5
1	8.0	$(2.18 \pm 0.21) \times 10^4$	1.0
2	5.6	$(5.88 \pm 0.47) \times 10^4$	0.4
2	8.2	$(1.77 \pm 0.13) \times 10^5$	0.8

[a] 25 °C, 10^{-2} M PIPES or HEPES buffer. [b] $([Cu^{2+}]_b/[P])_{max}$, i.e. saturation value of β (see text).

Results and Discussion

Thermodynamics of Formation of the Cu(II) Complexes. The results of the dialysis equilibrium experiments (25 °C) are shown in Figure 1, where the bound ion to polymer residue molar ratio, $\beta = [Cu^{2+}]_b/[P]$, is plotted against the concentration of free copper ions ($[Cu^{2+}]_f$). All curves are concave to the free ion concentration ordinate, indicating that the binding is not cooperative. In such a case, a Langmuir isotherm applies,[10a] and approximating activity with concentration, one finds that the isotherms are governed by eq 1, where K is the intrinsic binding constant of each binding site and β_{max} the saturation value of β.

$$\Theta = (\beta/\beta_{max}) = K[Cu^{2+}]_f/(1 + K[Cu^{2+}]_f) \qquad (1)$$

A plot of β^{-1} vs $[Cu^{2+}]_f^{-1}$ must be, therefore, linear, as is indeed the case. By a least-squares best fit to the experimental data the parameters reported in Table 1 were obtained. Upon inspection of the table, we find four points worth noting. First, the constants for Cu^{2+} binding by **1** are, rather surprisingly, only about 1 order of magnitude smaller than those observed for **2**, despite the fact that polymer **1** has no negatively charged groups (Chart 1). Second, owing to the pK_a of the secondary amine in **1**, at pH 5.6 only about 50% of these groups are uncharged and hence able to coordinate copper ions. This is nicely reflected in the value of β_{max}. Third, the shape of the curve of the Cu(II)-**2** complex at high pH does not indicate the existence of two different binding sites, as one would expect owing to the degree of substitution in the polymer of about 0.43 (see Experimental Section) and the propensity of both uncharged secondary and primary amines to bind Cu^{2+} ions, even at very low complex to polymer molar ratios. The dialysis technique appears, therefore, to be insufficiently sensitive to unveil two association equilibria characterized by rather similar binding constants. Fourth, at low pH, the value of β_{max} for Cu(II)-**2** strongly decreases. As expected, the protonated chitosan-like primary amine is no more a site of binding, and the suggestion here is that the complex involving only the carboxylate groups of the pentanedioic acid moiety is now the dominant species.

(6) (a) Sanford, P. A. In *Chitin and Chitosan*; Skjak-Braek, G., Anthonsen, T., Sandford, P., Eds.; Elsevier: Amsterdam, 1989; p 51. (b) Ogawa, K.; Oka, K.; Miyanishi, T.; Hirano, S. In *Chitin, Chitosan and Related Enzymes*; Zikakis, J. P., Ed.; Academic Press: New York, 1984; p 327.

(7) Marinsky, J. A. *Coord. Chem. Rev.* **1976**, *19*, 125.

(8) (a) Chiessi, E.; Paradossi, G.; Venanzi, M.; Pispisa, B. *J. Inorg. Biochem.* **1992**, *46*, 109. (b) *Int. J. Biol. Macromol.* **1993**, *15*, 145.

(9) Hingerty, B. E.; Ritchie, R. H.; Ferrell, T. L.; Turner, J. E. *Biopolymers* **1985**, *24*, 427.

(10) (a) Pispisa, B.; Paoletti, S. *J. Phys. Chem.* **1980**, *84*, 24. (b) Pispisa, B.; Rizzo, R.; Paradossi, G.; Barteri, M. *J. Inorg. Biochem.* **1986**, *26*, 281 and references therein.

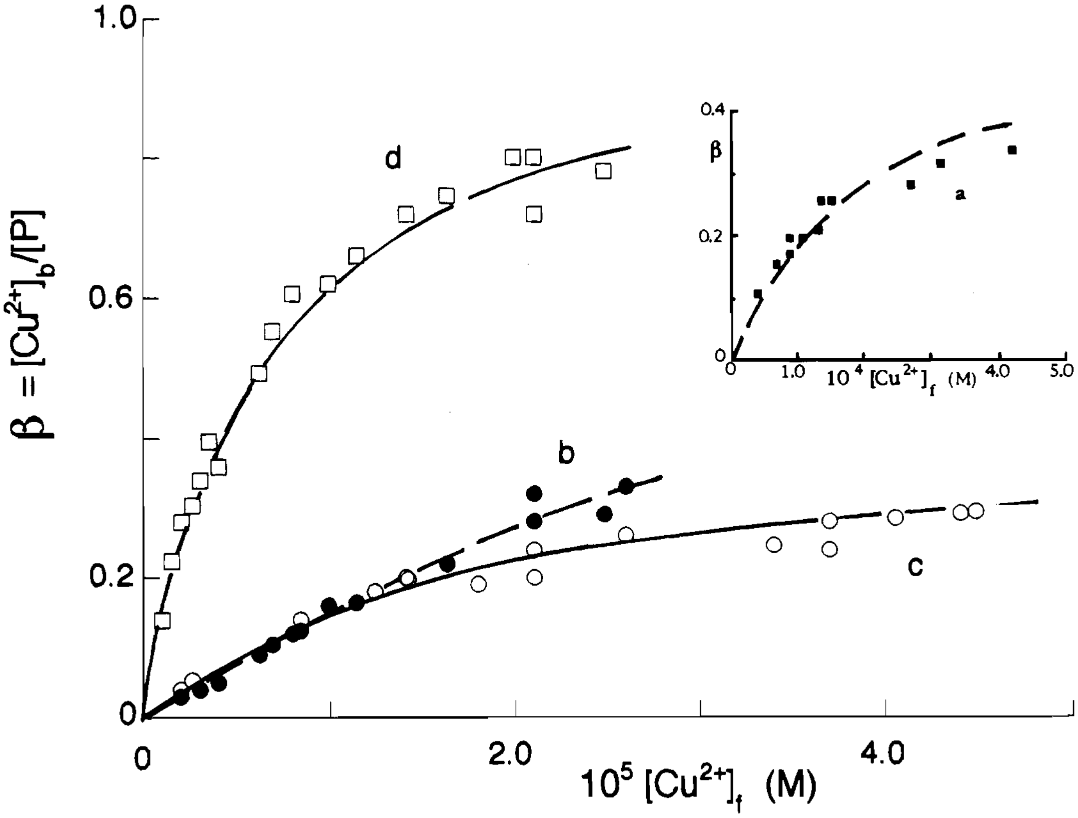


Figure 1. Binding isotherms (25 °C) of copper(II) ions on polymeric ligand **1** (full symbols) at pH 5.60 (a) and 8.00 (b) and on **2** (empty symbols) at pH 5.60 (c) and 8.20 (d), reported as bound copper(II) ion to polymer residue molar ratio, β, against free Cu^{2+} ion concentration. $[P] = 4 \times 10^{-4}$ M; 0.01 M PIPES or HEPES buffer.

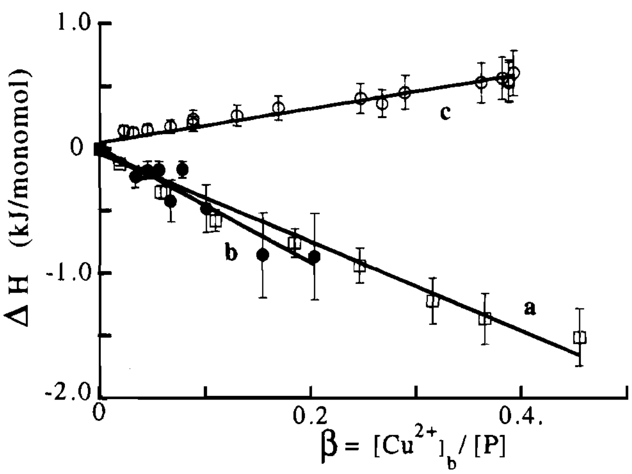


Figure 2. Enthalpy change (25 °C) as a function of the bound Cu^{2+} ion to polymer residue molar ratio for the aqueous solution of the polymeric ligand **1** (a, pH 5.7; b, pH 8.0) and **2** (c, pH 4.4). $[P] = 4 \times 10^{-4}$ M. The ordinate, ΔH, gives the enthalpy change in J/monomol of polymeric ligand, while the slope of the lines gives the enthalpy of binding, ΔH_B (J/mol of bound ions).

We next investigated the energetics of the association process. The enthalpy changes for the binding between Cu^{2+} ions and polymeric ligands **1** and **2** as a function of the bound ion to polymer residue ratio, $\beta = [Cu^{2+}]_b/[P]$, are shown in Figure 2. In the case of **1**, an exothermic effect is measured at both pH 8.0 and 5.7, as usually observed for Cu^{2+}–amine ligand interactions.[11] The enthalpy difference between the two pHs is modest, though not yet well understood because the ligands are probably the same. In the case of the polymeric ligand **2**, calorimetric measurements were carried out only in acid solution (pH ≈ 4.4) because of the insolubility of the polymer at pH 6–8. Under these conditions, only the carboxylates can coordinate the metal ion and the process is weakly endothermic, in agreement with reported enthalpies for Cu^{2+}–carboxylate interactions which are normally positive.[12a]

The thermodynamic parameters for the binding are summarized in Table 2. The main inference to be drawn from this table is that in all cases a relatively large increase in entropy is observed, very likely ascribable to the release of solvent molecules from the hydration sheaths of the interacting species. This is consistent with the observation that ΔS_B is larger for the polymeric ligand **2** than for **1**, owing to the presence of the charged carboxylic groups. The finding of a positive, relatively large binding entropy is reminiscent of the results reported for a number of simple and complex ions[10] in different polymeric solutions.

Finally, Table 2 also lists the reduction potentials of the copper(II) complexes. Typical voltammograms (vs SCE) are shown in Figure 3 for Cu(II)-**2** at both high and low pH. In the 0.1–0.4 range of $[Cu^{2+}]/[P]$ ratio explored, coordination of the polymeric ligands stabilizes the oxidized species of copper ions, mainly in alkaline solution. For instance, at pH 10 and 25 °C, the reduction potential of Cu(II)-**1** is −200 mV, as compared to −81 ± 13 mV for the polymer-free $Cu^{2+/+}$ couple (measured in the presence of precipitate), for which the calculated value is −79 mV.

Spectroscopy of the Cu(II) Complexes. In the 250–270

(11) Ciampolini, M.; Paoletti, P.; Sacconi, L. *J. Chem. Soc. London* **1961**, 2994. Anderegg, G. *Helv. Chim. Acta* **1965**, *48*, 1718. Gergely, A.; Sovago, I. *Acta Chim. Acad. Sci. Hung.* **1972**, *74*, 273.

(12) (a) McAuley, A.; Nancollas, G. H.; Torrance, K. *Inorg. Chem.* **1967**, *6*, 136. Arena, G.; Cali, R.; Rizzarelli, E.; Sammartano, S.; Barbucci, R.; Campbell, M. J. M. *J. Chem. Soc., Dalton Trans.* **1978**, 1090. Mohan, M. S.; Bancroft, D.; Abbott, E. H. *Inorg. Chem.* **1979**, *18*, 344. (b) Casella, L.; Gullotti, M. *Inorg. Chem.* **1985**, *24*, 84.

Table 2. Thermodynamic Parameters for the Binding of Copper(II) Ions by the Polymeric Ligands at 25 °C

polymeric ligand	pH	$10^{-3}(\Delta G_B)$, J·mol^{-1}	$10^{-2}(\Delta H_B)$, J·mol^{-1}	ΔS_B, J·mol^{-1}·deg^{-1}	$E_{1/2}$, mV[a]
1	5.6	−22.13 ± 2.05	−35.69 ± 2.38	63 ± 4	1.0
1	8.0	−24.73 ± 2.63	−46.02 ± 7.53	67 ± 4	−202[c]
2	4.4	−27.20 ± 2.38[b]	13.60 ± 1.97	96 ± 4	−32

[a] Half-wave potential (vs SCE), from cyclic voltammetric measurements. [b] At pH 5.6. [c] At pH 8.3.

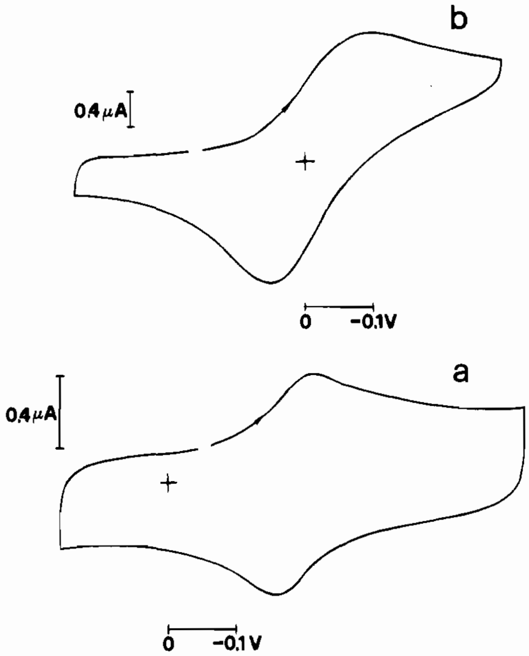


Figure 3. Room-temperature cyclic voltammograms (vs SCE) of the Cu(II)-**2** complex at pH 9.50 (a) (0.01 M CAPS buffer) and 5.50 (b) (0.01 M MES buffer). [P] = 6.7×10^{-4} M; $\beta = 0.30$; sweep rate = 0.1 V/s.

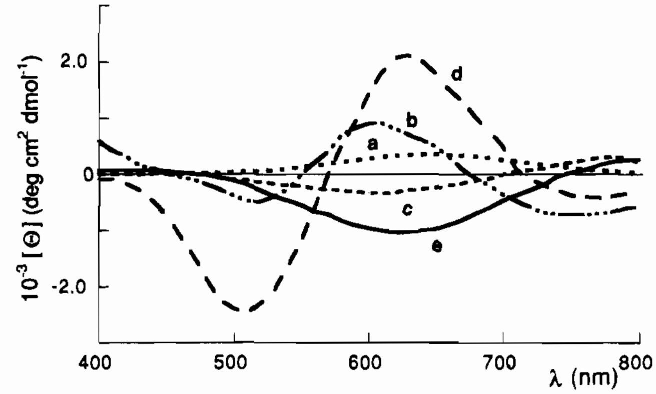
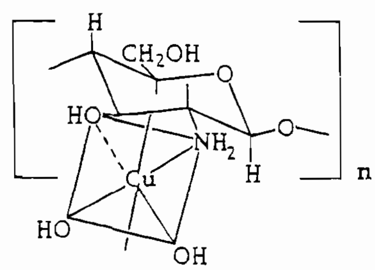


Figure 4. Visible CD spectra of Cu(II)-**4** (a), Cu(II)-**1** (b), Cu(II)-**2** (c, d), and Cu(II)-**3** (e) at pH 10.0, but curve a at pH 6.0 and curve c at pH 4.7. The molar ellipticity is normalized to the Cu^{2+} ion concentration. [P] = 2.1×10^{-4} M.

Chart 2



nm absorption region, all copper complexes exhibit a band whose intensity is pH-dependent, reflecting nitrogen atom coordination following amine deprotonation. It is therefore reasonable to assign this band to a charge-transfer (CT) Cu−N transition.[13] In the same wavelength region, all CD spectra, but those of Cu-**4**, show two bands, one around 245 nm, with positive rotational strength (R), and the other at 280−300 nm ($R < 0$). These spectra, very similar to those observed for CT transitions in some (tetraamino acid)Cu(II) complexes,[14a] are discouragingly similar in shape and wavelength for diagnostic purposes, however. We addressed this problem by recording absorption and CD spectra in the visible region.

In the case of the simplest polymeric ligand, i.e. **4**, the d−d transitions of the corresponding copper(II) complex are detected as a broad band at around 650 nm in both absorption and CD ($R > 0$) spectra (Figure 4a). Substitution of a N for an O donor in the chromophore causes no split of the visible CD band, suggesting that a D_{4h} microsymmetry is a satisfactory approximation for the complex, as schematically illustrated in Chart 2, where the secondary C_3-hydroxyl group is thought to also be involved in the coordination (broken line)[6b] and apical solvent molecules are omitted for clarity. By contrast, the broad absorption band of Cu(II)-**1** around 660 nm is split into three transitions in the CD spectrum, at 515 ($R < 0$), at 600 ($R > 0$), and around 750 ($R < 0$) nm, as illustrated in Figure 4b. The split of the visible CD bands does indicate a distinct decrease in symmetry around the cupric ion, as one would predict if the donor nitrogen atom of the uncharged secondary amine and different OH groups of the conformationally mobile lactyl moiety[5a] are involved in the coordination sphere. Owing to the complexity of the system, it does not seem possible to assign unequivocally the observed bands to the d−d transitions taking place predominantly on the Cu(II) ion. We utilize in the following the designation of the parent D_{4h} symmetry point group because this group represents the microsymmetry of the donor atoms around the metal ion. If the complex deviates appreciably from D_{4h} symmetry, the degenerate e_g orbitals may be split and transition E ($E_g \leftarrow B_{1g}$) will consist of two components.[13a] In such a case, the three d−d bands originate from transitions E (Γ_b) at 515, E (Γ_a) at 600, and B ($B_{2g} \leftarrow B_{1g}$) at 750 nm, all these transitions exhibiting comparable rotational strengths because they are magnetically allowed in the D_{4h} symmetry group.

The visible absorption spectrum of Cu(II)-**2** in acid solution (pH ≈ 4.5) shows a broad band around 720 nm that shifts to lower wavelengths at pH ≈ 10, with absorption maxima at 650 and 525 (sh) nm. This indicates coordination of nitrogen atoms as pH increases,[14b] in agreement with the ESR results shown below. According to the visible spectrum of Cu(II)-**4** discussed above, the band at 650 nm arises from the copper site of the chitosan-like primary amine, while that at 525 nm is very likely due to the other copper site. The visible CD spectrum of Cu(II)-**2** in acid solution exhibits two broad, very weak bands centered around 610 ($R < 0$) and 790 ($R > 0$) nm, as shown in Figure 4c. Under these conditions, the nitrogen atom is charged and cannot coordinate the copper ions, so that the complex

(13) (a) Tsangaris, J. M.; Martin, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 4255. (b) Wilson, E. W.; Kasperian, M. H.; Martin, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 5365.
(14) (a) Formicka-Kozlowska, G.; Kozlowska, H.; Siemion, I. Z.; Sobczyk, K.; Nawrocka, E. *J. Inorg. Biochem.* **1981**, *15*, 201. (b) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.

Table 3. ESR Parameters for Cu(II)–Polymeric Ligand Complexes[a]

complex[b]	pH		$g_{\parallel}$	$g_{\perp}$	$10^4A_{\parallel}$, cm^{-1}	$10^{-2}g_{\parallel}/A_{\parallel}$, cm
Cu(II)-**1**	8.2		2.242	2.057	190	118
Cu(II)-**2**	4.1		2.283	2.106	176	130
Cu(II)-**2**	8.5	site 1	2.243	2.057	189	119
		site 2	2.286	2.099	186	123
Cu(II)-**3**	9.0		2.270	2.083	186	122
Cu(II)-**4**	5.9		2.243	2.055	188	119

[a] $T = 100$ K, frequency 9.4 GHz, power 20 mW, $\beta = [Cu^{2+}]_0/[P] = 0.02-0.10$, $[P] = 1.2 \times 10^{-2}-5.0 \times 10^{-3}$ M. [b] Polymeric ligands as in Chart 1.

should be rather loosely bound to the chiral polymeric matrix. This implies a greater conformational mobility than the tightly bound complex at high pH, and hence a lower ellipticity, as experimentally observed (Figure 4c,d).

The visible CD spectrum of Cu(II)-**2** complex at pH ≈ 10 exhibits two rather intense bands at 510 and 630 nm and a much weaker band around 790 nm, as illustrated in Figure 4d. The split of the visible CD bands reflects again a decrease in symmetry around the cupric ion, as expected for the coordination of both the donor nitrogen atom of the uncharged secondary amine and the pendant carboxylate groups, can one of which only attain apical chelation for steric reasons. The positive Cotton effect at 630 nm may be due to both the chitosan-like copper complex and the B transition of the other Cu(II) site, which would also experience E and A ($A_{1g} \leftarrow B_{1g}$) transitions, giving rise to the negative Cotton effects around 510 and 790 nm, respectively. This latter transition has a very small rotational strength, in agreement with the fact that it is magnetically dipole forbidden in the D_{4h} symmetry group.

Finally, the d–d transitions in the CD spectrum of Cu(II)-**3** are enveloped in one negative band around 630 nm (B + E), while a very weak positive band is observed at 790 nm (Figure 4e). Interestingly enough, the ellipticity of this complex in the visible region is opposite in sign to that of Cu(II)-**4** (Figure 4a,e), despite the fact that both polymeric ligands are structurally rather simple. This is because, in contrast with Cu(II)-**4**, the Cu(II)-**3** complex exhibits a λ chirality, similar to that of copper(II)–L-amino acid compounds[12b,13b] having a negative CD band within the 600–650 nm region.

To summarize, the results of visible CD spectra suggest the following order of increasing departure from D_{4h} symmetry: Cu-**4** $\approx$ Cu-**3** < Cu-**2** $\leq$ Cu-**1**. This is the order of increasing steric constraints of the side chains carrying the chelating groups (Chart 1), implying that the smaller the steric hindrances in the polymers, the more symmetric the arrangement of ligands around the central copper ion.

We next investigated the ESR spectra of the polymer-immobilized Cu(II) complexes in frozen aqueous solution (100 K). Table 3 lists the values of $A_{\parallel}$, $g_{\parallel}$, and $g_{\perp}$, from which it appears that in all cases $g_{\parallel} > g_{\perp} > 2.04$, a finding suggestive of a tetragonally distorted octahedral, square-base pyramidal, or square-planar geometry.[15] Furthermore, all parallel values of the copper(II) hyperfine coupling constant are larger than that normally found for bis(amino acid)copper(II) complexes, suggesting a somewhat greater extent of tetragonal distortion.[15b]

The most relevant results can be summarized as follows. (1) The ESR parameters of Cu(II)-**1** are quite similar to those of Cu(II)-**4**. This is because the uncharged secondary amine of polymer **1** can act as a ligand in a fashion similar to that of the uncharged primary amine of **4**, the extended conformations of the bulky side chains leaving enough room for the approaching metal ion.[5b] (2) The Cu(II)-**2** system at pH ≥ 8 shows two metal sites, as illustrated in Figure 5b, the values of $g_{\parallel}$ and $g_{\perp}$ for site 1 being quite comparable to those for the copper–**4** complex because at these pHs the unsubstituted chitosan-like primary amine is also an effective site of binding (see Experimental Section). By contrast, at pH ≈ 4 only one metal site is populated (Figure 5c), in agreement with the fact that both primary and secondary amines are now protonated and only the pendant carboxylate groups are able to coordinate the copper ions. Under these conditions, a 4-fold coordination can be only accomplished, and $A_{\parallel}$ is seen to decrease while the ratio $g_{\parallel}/A_{\parallel}$ increases, as one would expect for distortion of the copper(II) structure from planar to tetrahedral.[16] A value of $g_{\parallel}/A_{\parallel} \geq 130 \times 10^2$ cm is in fact suggestive of a tetrahedrally distorted geometry of the copper site.[17] (3) the Cu(II)-**3** complex in alkaline solution exhibits ESR features somewhat similar to those of site 2 in Cu(II)-**2**, suggesting that the charge factor alone cannot reduce $A_{\parallel}$ to the level observed for Cu(II)-**2** complex at pH ≈ 4. This gives further support to the idea that the 4-fold-coordinated Cu(II) complex experiences a distortion from planarity. (4) The ESR parameters of Cu(II)-**4** agree with those reported[17] and are also very close to those of type 2 Cu(II) in some oxidase proteins, e.g., ceruloplasmin or ascorbate oxidase,[18] where copper is coordinated to nitrogen and oxygen ligands in tetragonal complexes.

Molecular Modeling of the Copper(II) Sites. To gather information on the geometric and steric constraints that control the formation of the copper complexes, we have undertaken molecular modeling studies by using a series of pairwise additive energy terms and a new distance-dependent dielectric constant to tackle electrostatic effects that are believed to dominate metal–macromolecule interactions in solution.[9,19]

The importance of electrostatic effects in determining the structure and function of polymeric materials is well-known, and recently it was shown that these effects can dominate many aspects of, e.g., protein behavior, such as folding and assembly processes and enzyme catalysis.[19] More recently, calculations were done on the interactions and site binding of various bivalent metal ions and biopolymers,[9] in which the dielectric function plays a crucial role[9,20a] because of the very large electrostatic energies generated by the highly charged metal ions.

The present analysis includes (1) partial atomic charges for each atom of the polymeric ligands,[21] as illustrated in Chart 3, (2) standard bond lengths and bond angles for all species considered, and (3) parameters for electrostatic, nonbonded, torsional, and polarization interactions.[4b,9,21–23a]

According to work of Hingerty,[9] the copper ion–polymeric ligand interaction energy can be written as a series of pairwise

(15) (a) Hathaway, B. J.; Billing, D. E. *Coord. Chem. Rev.* **1970**, *5*, 143. (b) Ou, C.; Powers, D. A.; Thich, J. A.; Felthouse, T. R.; Hendrickson, D. N.; Potenza, T. A.; Schugar, H. J. *Inorg. Chem.* **1979**, *17*, 34.
(16) (a) Blumberg, W. E. In *The Biochemistry of Copper*; Peisach, J., Aisen, P., Blumberg, W. E., Eds.; Academic Press: New York, 1966; p 49. (b) Yokoi, H.; Addison, A. W. *Inorg. Chem.* **1977**, *16*, 1341. Sakaguchi, U.; Addison, A. W. *J. Am. Chem. Soc.* **1977**, *99*, 5189.
(17) Sehlich, S. *Macromolecules* **1986**, *19*, 192.
(18) Reinhammar, B. Reference 3c, p 177.
(19) Perutz, M. F. *Science* **1978**, *201*, 1187.
(20) (a) Ramstein, J.; Lavery, R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7231. (b) Gabler, R. *Electrical Interactions in Molecular Biophysics*; Academic Press: New York, 1978; p 97.
(21) Pispisa, B.; Palleschi, A.; Paradossi, G. *J. Phys. Chem.* **1987**, *91*, 1546; *Biopolymers* **1989**, *28*, 319.
(22) Dalgarno, A. *Adv. Phys.* **1962**, *11*, 281.
(23) (a) Nemethy, G.; Pottle, M. S.; Scheraga, H. A. *J. Phys. Chem.* **1983**, *87*, 1883. (b) Scott, S.; Scheraga, H. *J. Chem. Phys.* **1965**, *42*, 2209; **1966**, *44*, 2054.

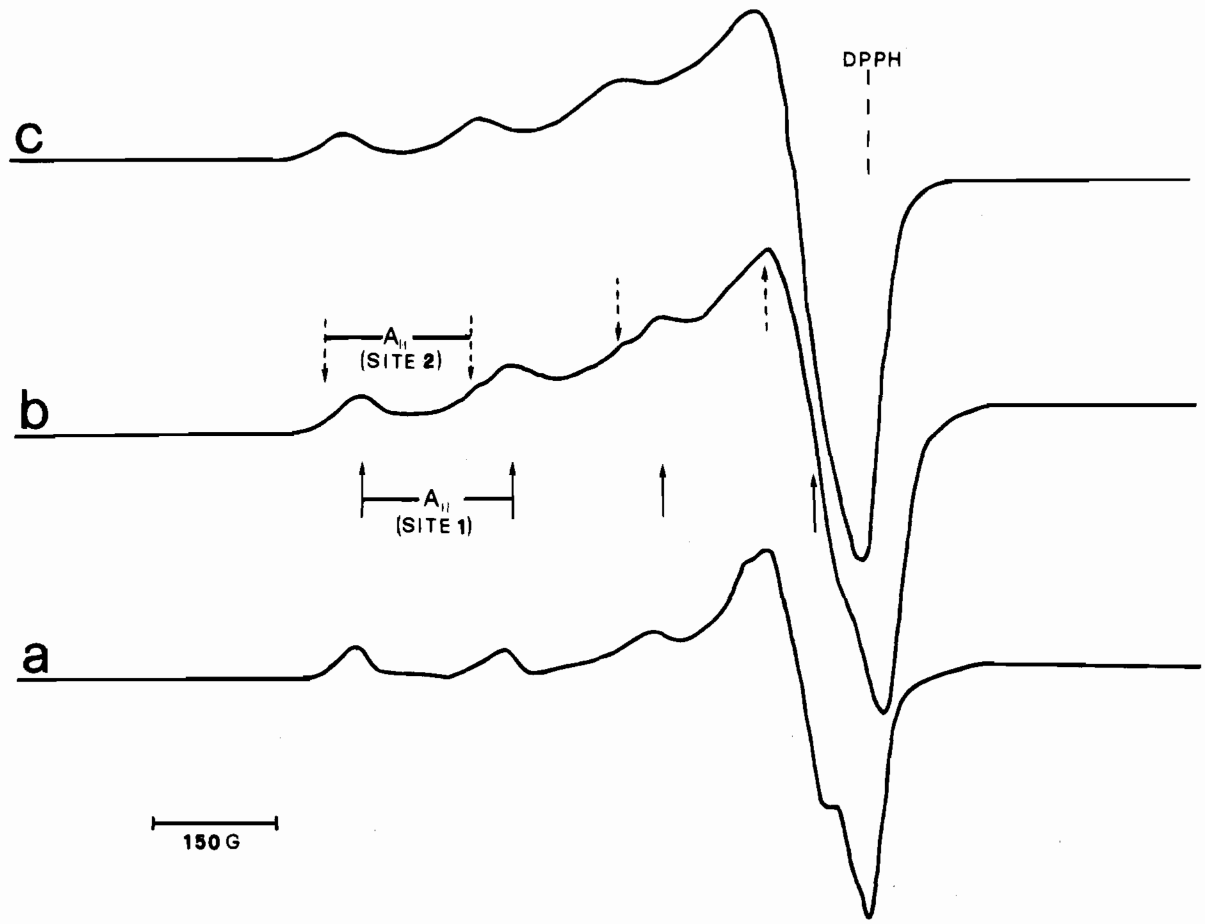


Figure 5. ESR spectra of Cu(II)-**1** at pH 8.2 (a) (β = 0.02) and Cu(II)-**2** at pH 8.0 (b) (β = 0.05) and 4.1 (c) (β = 0.10), respectively. [P] = 1.2 × 10^{-2}–5.0 × 10^{-3} M. In the case of Cu(II)-**2** in alkaline solution, the four components of the parallel orientation are indicated for sites 1 and 2 (see text). The spectra were taken at 100 K, microwave power 20 mW, and microwave frequency 9.37 GHz.

additive terms, i.e.

$$U_{\mathrm{tot}}(R_{ij}) = \mathrm{COUL} + \mathrm{TOR} + \mathrm{NB} + \mathrm{POL} \qquad (2)$$

where the various potential functions (in kJ/mol) are as follows. COUL is the electrostatic energy, calculated as a sum of interactions between atomic monopoles $q_i = z_i{\cdot}e$ and dipoles as well

$$\mathrm{COUL} = \sum_{i \neq j} \frac{1389 q_i q_j}{\epsilon(R_{ij}) R_{ij}} + \sum_{\mathrm{H\ bonds}} D R_{ij}^{-3} \qquad (3)$$

where

$$\epsilon(R_{ij}) = 78 - 77\left(\frac{R_{ij}}{2.5}\right)^2 \frac{\exp(R_{ij}/2.5)}{[\exp(R_{ij}/2.5) - 1]^2} \qquad (4)$$

is the aforementioned dielectric function,[9] R_{ij} (Å) the intercharge separation of the ij pair, and D (= −230 kJ·Å^3·mol^{-1}) the dipole−dipole parameter for evaluating hydrogen-bond interactions.[4b,21] Because of the very large Coulombic energy generated by the copper(II) ions, we had to modify the screened Coulomb potential previously used in the conformational energy calculations of the polymeric ligands.[5] The electrostatic energy with $\epsilon(R_{ij})$, as given by eq 4, is now an adequate approximation for the metal ion−macromolecule electrostatic interactions in solution,[9] because the electrostatic contribution is found to be dominated by attractive close-contact interactions, owing to the shorter ion-pairing distances found with the dielectric function of eq 4 relative to that previously used.[5]

The next energy term in eq 2 represents the distortion energy associated with the n torsion angles ζ, as given by eq 5, where E_0 = 6.3, 11.7, and 4.2 kJ/mol for the internal rotation around the N−C, C−C, and C−COO$^-$ bonds,[5,23a] respectively.

$$\mathrm{TOR} = \sum_{k=1}^{n} \frac{E_{0k}}{2}(1 + \cos 3\zeta_k) \qquad (5)$$

To evaluate the last two energy terms, copper ion−ligand interaction parameters are needed. They are based on the following information: atomic polarizability (α), effective number of outer-shell electrons (N), and van der Waals radii (R), as shown in Table 4.[22,23b] The nonbonding interaction term can be thus evaluated by a LJ 6−12 potential function, i.e.

$$\mathrm{NB} = \sum_{i \neq j} (-a R_{ij}^{-6} + b R_{ij}^{-12}) \qquad (6)$$

where

$$a = (1506 \alpha_i \alpha_j)/[(\alpha_i/N_i)^{1/2} + (\alpha_j/N_j)^{1/2}] \qquad (7)$$

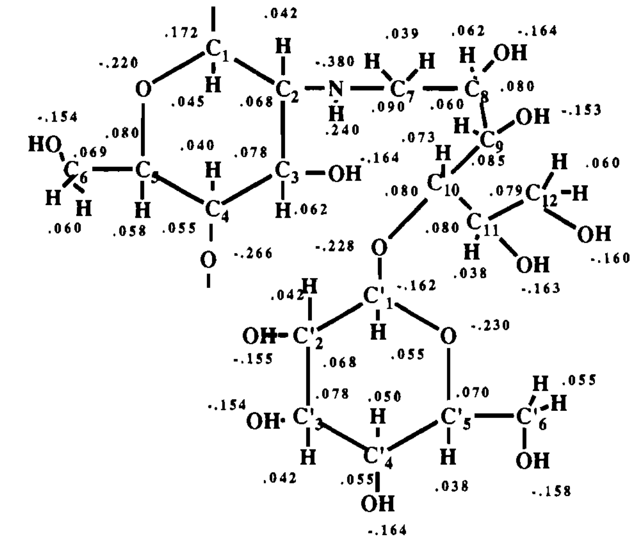
and b is calculated so that the minimum of the potential occurs at the sum of the van der Waals radii. The coefficients a and b are then recomputed with the same depth maintained for the potential well but with the sum of the van der Waals radii increased by 0.2 Å to reduce excessive electrostatic attraction.[9] The parameters so obtained are listed in Table 5.

The other potential represents the polarization energy, referring to the charge-induced dipole due to the high polarizability of the metal ions,[20b] and is given by eq 8.

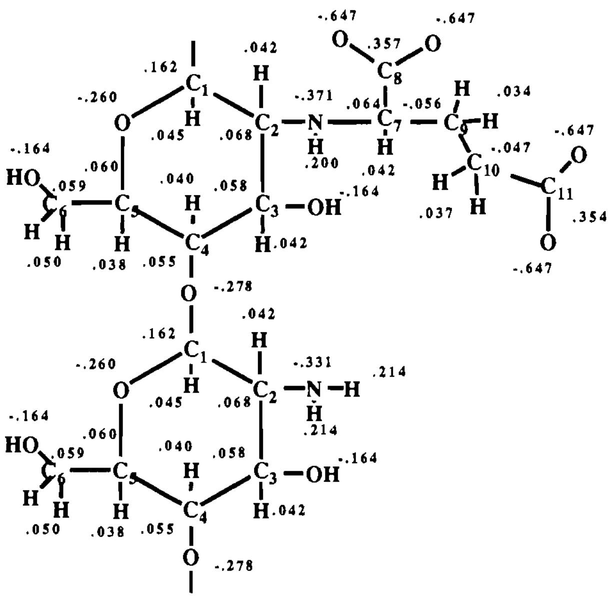
$$\mathrm{POL} = -694.5(\alpha_i q_j^2 + \alpha_j q_i^2)/\epsilon^2(R_{ij}) R_{ij}^4 \qquad (8)$$

Finally, since the polymeric ligands examined cannot fulfill

Chart 3



(1)



(2)

Table 4. Copper(II) Ion and Polymeric Ligand Parameters

ion or ligand	designation	α, Å^3 ^{a}	N^b	R, Å^c
Cu^{2+}		0.2	19.0	0.72
N	amine nitrogen	0.93	6.1	1.76
O_α	carboxylic acid (C=O) oxygen	0.84	7.0	1.56
O_β	hydroxyl or ether oxygen	0.59	7.0	1.62
O^-		3.2	7.5	1.76

^{a} Reference 22. ^{b} Effective number of outer-shell electrons.[23] ^{c} Reference 23a and: *Handbook of Chemistry and Physics*, 64th ed.; CRC Press: Boca Raton, FL, 1984.

a full coordination, the coordination sphere of the Cu(II) ions must comprise water molecules acting as ligands. For this purpose, Hingerty[9] used a charge–dipole interaction energy term,[20b] the coordinated water molecules being treated as point dipoles. Instead, we evaluated the contribution of these molecules to the total energy of the computed models as that of the other ligands, i.e. making use of eq 2 with the parameters reported in Tables 4 and 5 and partial atomic charges for H_2O that reproduce the experimental dipole moment of water.[24,25]

Table 5. Parameters for the 6–12 Nonbonded Potential of Copper(II) Ion–Ligand Pairs^a

pair^b	$10^{-2}a$, kJ·Å^6·mol^{-1}	$10^{-4}b$, kJ·Å^{12}·mol^{-1}	R_{vdW}, Å^c
$N-Cu^{2+}$	8.19	13.72	2.64
$O_\alpha-Cu^{2+}$	8.38	8.76	2.44
$O_\beta-Cu^{2+}$	6.66	8.05	2.50
O^--Cu^{2+}	18.40	30.85	2.64

^{a} Refer to eqs 6 and 7. ^{b} The subscripts denote the ligand type as defined in Table 4. ^{c} Sum of the van der Waals radii, plus 0.2 Å to reduce the Coulombic attraction (see text[9]).

Briefly, minimizations were carried out as previously described,[4b,21] by searching for the deepest minimum in the total energy in terms of both the three degrees of freedom of the Cu^{2+} ion approaching the ligands in the polymeric matrix and all torsional degrees of freedom of both backbone and side chain, starting from different directions of approach of the interacting species and assuming the glucosidic rings to be free from any distortion.[5b] However, when complex formation in alkaline solution was mimicked, the nitrogen atom was kept uncharged, and the direction of approach of the copper ion was taken as collinear to the lone pair.

Both the energy of $[Cu(H_2O)_6]^{2+}$ ($U_{Cu^{2+}}$) and that of the umperturbed polymeric ligands (U_{pol}) were used as reference energies for our calculations, the hexaaquo–Cu(II) complex being the starting species for the various polymer-immobilized Cu(II) complexes examined.[29,32] Therefore, the energy function employed here is written as eq 9, where $U_{tot}(R_{ij})$ is given by eq 2, which allowed us to achieve one of the goals of the present

(24) Besler, B. H.; Merz, K. M.; Kollman, P. A. *J. Comput. Chem.* **1990**, *11*, 431.

(25) The coordinated water molecules play an important role in the catalytic activity of the complexes examined in the air oxidation of catechol derivatives.[26] Since the reduction potential of these substrates (AH_2), for the reaction $AH^\bullet + e + H^+ \rightleftharpoons AH_2$, is generally much higher than that of the copper complexes, the oxidation reaction requires dioxygen as oxidizing species. For instance, the reduction potential of adrenaline at pH 8.50 is 92 mV (vs SCE),[27] as compared to −202 and −140 mV for Cu(II)-**1** and Cu(II)-**2**, respectively, at the same pH. Coordination of both the molecular oxygen and catecholic hydroxyl groups of the substrate to the central copper ion is thus a prerequisite for the catalysis,[26] and three easily exchangeable ligands, e.g. H_2O, are needed in the copper active site. Whether the function of the bound dioxygen in the ternary adduct is to raise the redox potential of the metal complex to the level of the substrate or to accept an electron from the substrate within the intermediate is difficult to say at present.[28]

(26) Chiessi, E.; Pispisa, B. *J. Mol. Catal.* **1994**, *87*, 177.

(27) Steenken, S.; Neta, P. *J. Phys. Chem.* **1982**, *86*, 3661. Pettit, L. D. *Pure Appl. Chem.* **1984**, *56*, 247.

(28) Harris, F.; Toppen, D. L. *Inorg. Chem.* **1978**, *17*, 74. Balla, J.; Kiss, T.; Jameson, R. F. *Inorg. Chem.* **1992**, *31*, 58 and references therein.

(29) $U_{Cu^{2+}}$ was evaluated by eq 2, but without the TOR (eq 5) and the hydrogen-bond (second term in eq 3) potentials, by using the molecular parameters of Tables 4 and 5 and partial atomic charges for H_2O.[24] From the results, $U_{Cu^{2+}} = -488.7$ kJ/mol. As expected, this value is somewhat smaller than that experimentally obtained for the $Cu^{2+}(g) \rightarrow Cu^{2+}(aq)$ process.[30a] Furthermore, the computed model of the octahedral hexaaquo–Cu(II) complex does not show any tetragonal distortion, in contrast with experimental data,[30b] but the separation distance of the six oxygen ligands from the central copper ion is 2.10 Å, in very good agreement with the experimentally determined average lengths of the six O–Cu(II) bonds.[30b] Simulation of both the plasticity and geometry of the copper(II) ion coordination sphere in polymer-free complexes may be achieved by the empirical force-field method recently described,[31] where the distance from the central metal ion of two apically placed ligand atoms is modulated by a bond-stretching parameter.

(30) (a) Marcus, Y. *Ion Solvation*; J. Wiley: Chichester, U.K., 1985; p 107. (b) Webb, M. W.; Kay, H. F.; Grimes, N. W. *Acta Crystallogr.* **1965**, *18*, 740.

(31) Sabolovic, J.; Raos, N. *Polyhedron* **1991**, *9*, 2419.

(32) U_{pol} was determined as the sum of all pairwise electrostatic, nonbonded, and hydrogen-bonding interactions, as given by eqs 3–6, but with the LJ a and b parameters previously used.[4b,21,23]

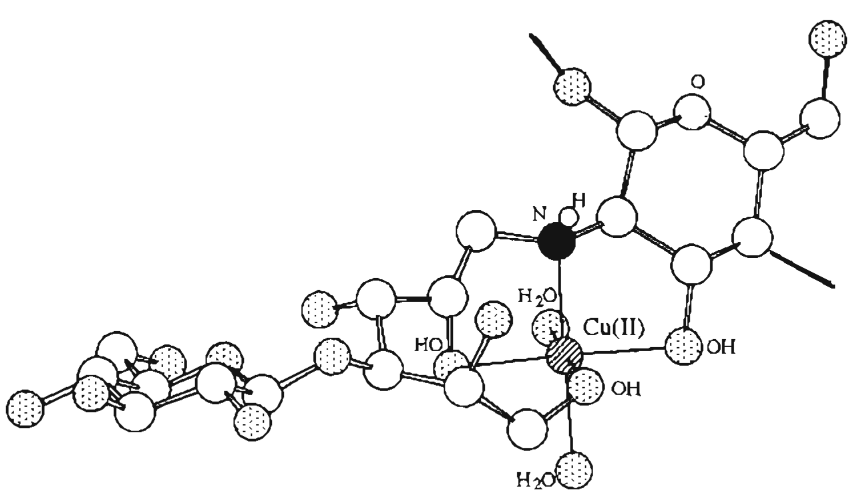


Figure 6. Computed model of Cu(II)-**1**, exhibiting a tetragonally distorted octahedral geometry (see text). The coordination sphere of the metal ion involves the donor nitrogen atom of the uncharged secondary amine (black sphere), the secondary C_3-hydroxyl group of the backbone chain (see Chart 2), two hydroxyl groups of the deoxylactyl moiety, and two water molecules. For clarity, only a short portion of the backbone chain is shown.

Table 6. Comparison between Calculated and Experimental Energies of the Copper(II) Complexes

complex[a]	secondary amine[b]	coordin no.	chirality of C atom[c]	distortion	$U_{tot}(R_{ij})$[d]	U_{pol}[e]	ΔU[f]	ΔH[g]
Cu(II)-1	uncharged	6[h]		tetragonal	−524.3	−30.1	−5.5	−4.6 ± 0.8
Cu(II)-2	charged	4[i]	*R*	tetrahedral	−595.8	−108.8	1.7	1.4 ± 0.2
	charged	4[i]	*S*	away from planarity	−593.7	−107.5	2.5	
Cu(II)-2	uncharged	6[h]	*R*	tetragonal	−661.5	−177.4	4.6	n.d.
	uncharged	6[h]	*S*	tetragonal	−630.9	−155.6	13.4	n.d.

[a] Polymeric ligands as in Chart 1. [b] Mimicking different pH conditions (see Experimental Section). [c] Absolute configuration of the chiral carbon atom in the side chain adjacent to the nitrogen atom of the polymeric ligand (**2**). [d] In kJ/mol, from eq 2. [e] in kJ/mol,[32] referring to polymer 1 or 2. [f] In kJ/mol, from eq 9, where $U_{Cu^{2+}} = -488.7$ kJ/mol.[29] [g] In kJ/mol, from microcalorimetric measurements (see Table 2). [h] The coordination sphere of Cu(II) also involves the donor N atom of the secondary amine, the structural unit being $[Cu^{II}NO_5]$. [i] Not involving the N atom of the secondary amine, the structural unit being $[Cu^{II}O_4]$.

$$\Delta U = U_{tot}(R_{ij}) - (U_{Cu^{2+}} + U_{pol}) \quad (9)$$

investigation, i.e. that of comparing the energetics of these complexes with those obtained by microcalorimetric experiments.

Correlation of Computed Models with Experimental Data. As a general observation, it is noted that both electrostatic and steric effects play an important role in determining the geometry of the computed models, depending on the structural features of the side chains. Steric effects chiefly arise from the reduced degrees of conformational freedom of the ligands, owing to the linkage to the polymeric matrix that provides a more constraining environment for metal chelation than polymer-free ligands. For example, when the absolute configuration of the chiral carbon atom in the side chain adjacent to the nitrogen atom of the polymeric ligand (**2**) is *R* and the secondary amine is uncharged (high pHs), a cagelike structure around the donor nitrogen atom forms, representing a sterically constrained binding site for the entering copper ion.[5a] A tetragonally distorted octahedral coordination is readily accomplished by minor rearrangements of the carboxylates, comprising a weaker apical chelation by one carboxylate group, in full agreement with ESR data (Table 3). Interestingly, where the absolute configuration of the chiral carbon atom in the side chain is *S*, computational results suggest that a tetragonally distorted octahedral Cu(II) complex still forms, but it is less stable than the aforementioned diastereomeric complex by some 30 kJ/mol.

Table 6 lists the deepest minimum energies of these hypothetical copper(II) complexes, while their most relevant structural features can be summarized as follows. (1) The donor nitrogen atom of the uncharged secondary amine, two hydroxyl groups of the deoxylactityl moiety, and a water molecule act as ligands in the plane containing the metal ion in the Cu(II)-**1** complex, within 2.04−2.11 Å from copper, as shown in Figure 6. Another water molecule and a hydroxyl group of the deoxylactityl moiety, at 2.25 and 2.39 Å from Cu, respectively, act as axial ligands. (2) In the case of the polymeric ligand (**2**), where the condition of low pH is mimicked, the carboxylates lie far away from the positively charged secondary amine and only a 4-fold coordination can be accomplished. As a result, two almost degenerate Cu(II)-**2** structures are obtained, depending on the chirality of the carbon atom in the side chain adjacent to the nitrogen atom. They are illustrated in Figure 7, while the corresponding energies are reported in Table 6. When the absolute configuration of this atom is *S*, the copper(II) active site exhibits a distorted square-plane geometry, where the oxygen atoms of the two carboxylates and two H_2O molecules act as ligands (Figure 7A). This because the axial positions are sterically hindered by the other two oxygen atoms of the carboxylates. The distortion of the copper site away from planarity is about 0.06 Å and that of two oxygen atoms is about 0.19 Å. Instead, when the absolute configuration is *R*, a tetrahedrally distorted complex forms, two oxygen atoms of the carboxylates and two water molecules fulfilling again the 4-fold coordination (Figure 7B).[33] Both these structures are compatible with the ESR parameters at pH ≈4 (Table 3), in that the relatively low value of $A_{\parallel}$ and a ratio $g_{\parallel}/A_{\parallel}$ of 130 × 10^2 cm are

(33) It must be noted, however, that in this case, i.e. when the chirality of the carbon atom in the side chain is *R*, other relative minima are present, with energies only slightly higher (5−10 kJ/mol) than that reported in Table 6. They correspond to copper(II) structures still characterized by a 4-fold coordination, between square-planar and tetrahedral geometries.

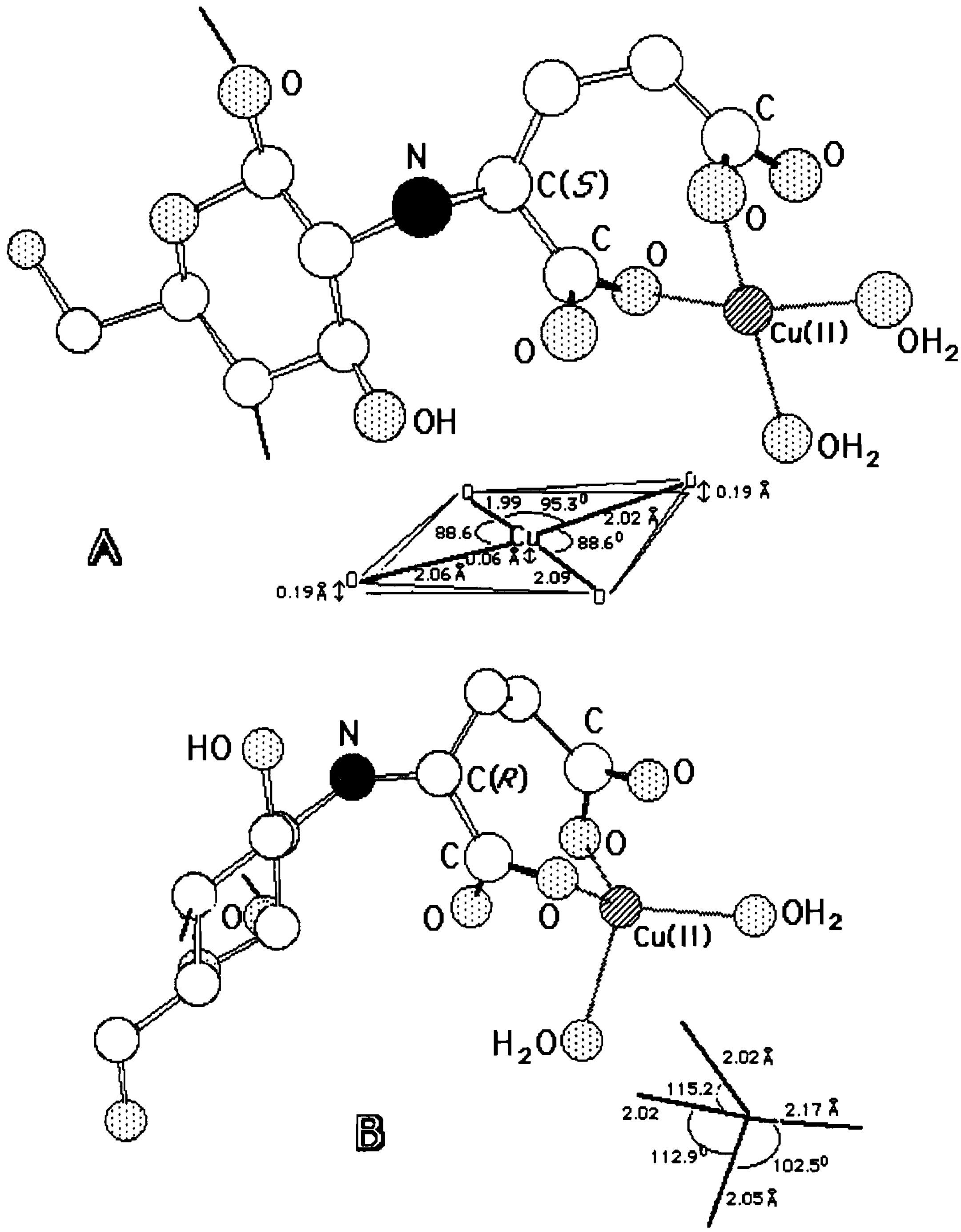


Figure 7. Computed models of Cu(II)-**2** under conditions mimicking low pH values, where the secondary amine is charged. When the absolute configuration of the chiral carbon atom in the side chain adjacent to the nitrogen is *S*, a distorted square-planar geometry is obtained (A), and when it is *R*, the complex exhibits a distorted tetrahedral structure (B). Bond lengths and bond angles are reported in the lower drawings. For clarity, only a short portion of the backbone chain is shown.

suggestive of distortion of the copper(II) coordination structure from planar to tetrahedral.[16b,17] (3) Where the secondary amine is uncharged (high pHs), tetragonally distorted Cu(II)-**2** octahedral complexes form, both the donor nitrogen atom and the secondary C_3-hydroxyl group of the backbone chain (see Chart 2) being now involved in the coordination sphere of Cu(II). They differ energetically, however, depending on the chirality of the carbon atom in the side chain. When the absolute configuration of this atom is *R*, the two apically placed ligands are a water molecule and a carboxylate oxygen, lying at 2.28 and 2.33 Å from the copper ion, respectively, while the distance of separation from Cu(II) of the N and O ligands situated in the plane ranges from 2.01 to 2.06 Å, as shown in Figure 8. When the absolute configuration of the chiral carbon atom in the side chain is *S*, the deepest relative minimum in the total energy is higher than that of the foregoing diastereomeric complex, as reported in Table 6. Accordingly, the ESR spectra of Cu(II)-**2** show the presence of one pentanedioic copper(II) site only.

So far, the stereochemistry of the computed models was successfully compared with the structural features of the complexes, as deduced by spectral data, the overall shape and coordination of the complexes being well reproduced even under different pH conditions. However, another stringent requirement for molecular modeling is the energetics. When the enthalpies of formation of the complexes are compared with the energies of the models, as given by eq 9, a surprisingly good agreement, in both magnitude and sign, is observed. This is shown in Table

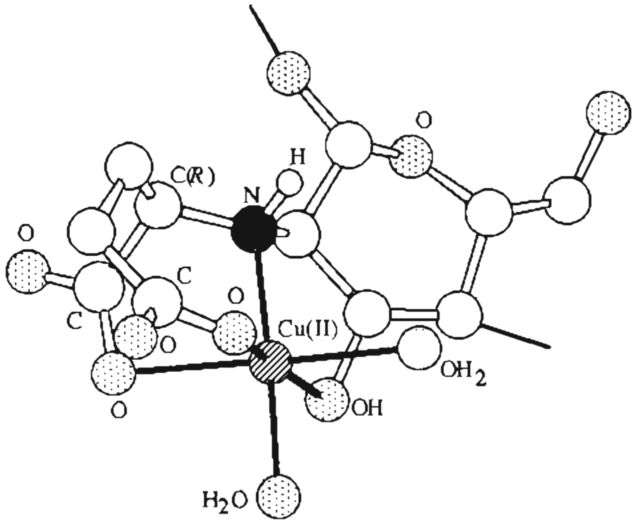


Figure 8. Computed model of the tetragonally distorted Cu(II)-**2** octahedral complex under conditions mimicking high pH values, where the donor nitrogen atom of the uncharged secondary amine (black sphere), the secondary C_3-hydroxyl group, two O^- of the carboxylates, and two water molecules are involved in the coordination of the copper ion. The chirality of the carbon atom in the side chain is *R* (see text).

6, where the coordination numbers and distortions of the complexes are also summarized. Owing to the complexity of the energy function used, eq 9, the occurrence of compensation effects cannot be excluded, though the goodness of the combined structural and energetic results makes it reasonable to consider the present hypothetical models as good representations of the actual copper(II) complexes immobilized on chitosan. They would also allow better insight into the reaction mechanism of the catalytic oxidation of suitable substrates,[25,26] as will be shown later.

Concluding Remarks

Three major conclusions are drawn from the present study. First, branched-chain analogues of linear polysaccharides, such as chitosan derivatives, represent versatile polymeric ligands for metal chelation. The corresponding polymer-immobilized copper(II) complexes are stable within a wide range of pH and are good candidates as biocompatible catalysts for the air oxidation of a number of substrates, such as catechol derivatives. Second, the thermodynamic parameters of complex formation are similar to those observed with monomeric ligands, while the stereochemical features of the complexes, as deduced from CD and EPR spectral patterns, and from computational results as well, reflects both the steric hindrances and charge density of the macromolecular ensemble. This is because the linkage to the polymeric matrix reduces the degrees of conformational freedom of the ligands, providing a more constraining environment for metal chelation than that of polymer-free ligands. Third, molecular modeling of the Cu(II) active sites leads to structures that are fully consistent with the experimental results, as far as the limited data allow. The proposed computational method appears to be of general applicability for metal centers in the domains of macromolecules, provided metal ion–ligand interaction parameters, such as atomic polarizability, effective number of outer-shell electrons, and van der Waals radii, are known.

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