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Metal Complexes of Poly(α-amino acids). A Potentiometric and Circular Dichroism Investigation of Cu(II) Complexes of Poly(L-lysine), Poly(L-ornithine), and Poly(L-diaminobutyric acid)[1]

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ABSTRACT: The conformational properties of cupric complexes of poly(L-lysine), poly(L-ornithine), and poly(L-diaminobutyric acid) have been investigated by potentiometric, visible and UV absorption, and circular dichroism (CD) techniques. The three polymers form two kinds of complexes stable at pH <8.5 (type I complexes) and at pH >8.5 (type II complexes). It has been found that in the low pH complexes of poly(L-diaminobutyric acid) at least one deprotonated amido nitrogen is coordinated to cupric ions. Type II complexes involve always amide nitrogens in the coordination sphere of Cu(II). Evidence is presented that the structure of such complexes is not compatible with the α-helical conformation of the peptide backbone.

The preparation and the catalytic properties of metal complexes of poly(α-amino acids) have been described in a number of papers.[2a] Particularly, copper complexes of amino acid polymers have been investigated in some detail both from the point of view of the catalytic activity and of the structural properties.[2b-5] Such compounds can be considered useful models in order to understand the way of action of copper-containing proteins. Addition of poly(L-histidine) ($[L\text{-His}]_n$) to several oxidation reactions was found to enhance the catalytic activity of copper toward neutral or negatively charged substrates.[3] Structural investigations on $[L\text{-His}]_n$ copper complexes revealed that two complexes are formed in aqueous solution.[3] In complex I, stable at pH 5, three imidazole nitrogens and one deprotonated amide nitrogen are coordinated to a copper ion. In complex II, formed at pH 14, four consecutive amide nitrogens have been suggested to occupy a distorted coordination square of the Cu(II) ion.[3]

Poly(L-arginine) forms three different copper complexes.[5] The first one is stable at pH <8, while the other two are formed between pH 8 and 10.5. In the last two complexes two guanido nitrogens and two peptide nitrogens have been suggested to occupy the corners of the coordination square of Cu(II).[5]

The poly(L-lysine) ($[L\text{-Lys}]_n$) copper complex has been described to behave as an asymmetrically selective catalyst for the oxidation of L-(3,4-dihydroxyphenyl)alanine (DOPA).[4] The structural properties of such a compound in aqueous solution have been recently investigated by Hatano et al.[4] Also in this case two complexes are formed. In the first one, stable at pH <8, four amino nitrogens have been suggested to occupy square-planar positions of Cu(II). In the second one, stable at pH >8, deprotonated amido nitrogens have been suggested as binding sites for Cu(II) ions.[4] Clearly, correlation between catalytic activity and complex structure is essential in order to understand the mechanism of action of such compounds, and in order to approach the problem of the way of action of copper-containing enzymes.

In view of the importance of such compounds on the effects of stereospecific catalysis and as models for copper proteins, we have reinvestigated the complex formation process between Cu(II) $[L\text{-Lys}]_n$, and the study has been extended to the copper complexes of poly(L-ornithine) ($[L\text{-Orn}]_n$) and poly(L-diaminobutyric acid) ($[L\text{-A}_2\text{bu}]_n$). The specific purpose of our work was to establish the relationship between complex structure and conformation of the polypeptide backbone.

Experimental Section

Materials. Reagent grade cupric chloride (Merck Chemical Co.) was used as obtained.

$[L\text{-Lys}]_n\cdot HCl$, $[L\text{-Orn}]_n\cdot HCl$, and $[L\text{-A}_2\text{bu}]_n\cdot HCl$ were prepared according to procedures described in the literature.[6] The intrinsic viscosities in 0.1 M KCl of the polymer samples used in the present work were the following: $[L\text{-Lys}]_n\cdot HCl$, $[\eta] = 0.24$ dL/g; $[L\text{-Orn}]_n\cdot HCl$, $[\eta] = 0.55$ dL/g; $[L\text{-A}_2\text{bu}]_n\cdot HCl$, $[\eta] = 0.67$ dL/g.

Carbonate-free potassium hydroxide was prepared from reagent grade KOH pellets (Merck Chemical Co.), according to the literature.[7]

Measurements. Potentiometric titrations were carried out at 25

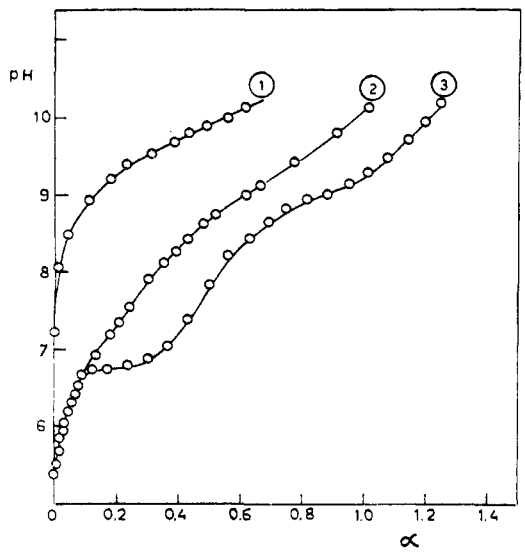


Figure 1. Potentiometric titration curves of $(Lys)_n$–Cu(II) at different Cu/peptide molar ratios: (1) Cu(II)/Lys = 0; (2) Cu(II)/Lys = 0.097; (3) Cu(II)/Lys = 0.240. In all cases $[(Lys)_n]$ was in the range 5.3 to 5.4 $\times 10^{-3}$ M residue.

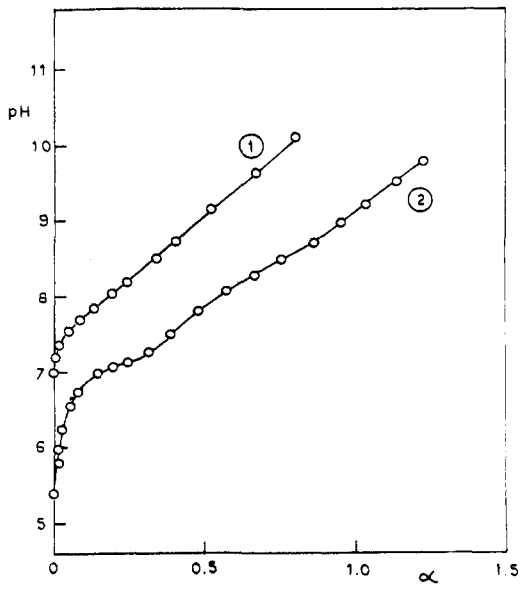


Figure 2. Potentiometric titration curves of $(Orn)_n$–Cu(II) at different Cu/peptide molar ratios: (1) Cu(II)/Orn = 0; (2) Cu(II)/Orn = 0.122. In all cases $[(Orn)_n]$ was in the range 5.5 to 5.6 $\times 10^{-3}$ M residue.

°C in 0.1 M KCl using a Metrohm Model E 540 precision potentiometer equipped with glass and calomel electrodes. Titrant addition was made using a Metrohm precision microburet Model 457 equipped with polyethylene capillary. In all titration experiments the polymer concentration was in the range 4.8×10^{-3} to 5.5×10^{-3} M residue, and the molar ratio of cupric ions to amino acid residues was in the range 0.1–0.25.

Visible and ultraviolet absorption spectra and CD spectra were recorded on samples of complex solutions during the titration, using a Cary 15 spectrophotometer and a Cary 61 dichrograph, respectively. In all spectroscopic measurements fused quartz cylindrical cells with suprasil windows were used.

Viscosity measurements were performed at 25 °C in a Ubbelohde viscometer.

Results and Discussion

Potentiometric Titrations. Examples of potentiometric titration experiments in absence and in the presence of Cu(II) ions at various molar ratios are shown in Figures 1–3. When

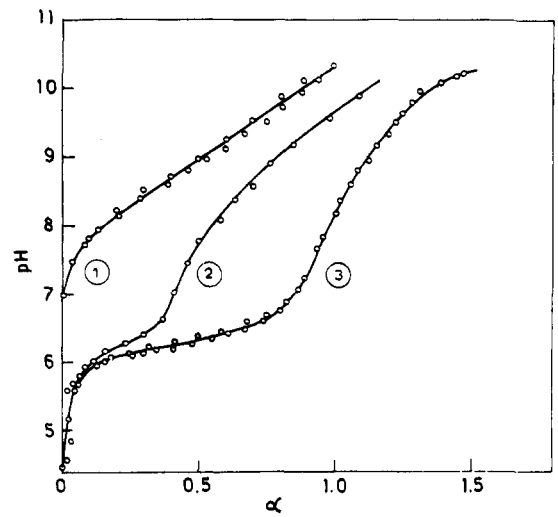


Figure 3. Potentiometric titration curves of $(A_2bu)_n$–Cu(II) at different Cu/peptide molar ratios: (1) Cu(II)/A_2bu = 0; (2) Cu(II)/A_2bu = 0.10; (3) Cu(II)/A_2bu = 0.25. In all cases $[(A_2bu)_n]$ was in the range 5.2 to 5.4 $\times 10^{-3}$ M residue.

cupric ions are present, two distinct buffered regions are observed during the titration of the three examined poly(amino acids), corresponding to the formation of two kinds of complexes. Type I complexes are formed at pH ≤8.5, while type II complexes are formed at high pH. Analysis of the potentiometric titrations data has been performed using the Bjerrum method[8] modified by Gregor,[9] in order to evaluate the number of protons displaced per mole of bound Cu(II), and consequently the number of ligands bound per cupric ion ($\bar{n}$). Let us consider first the pH region where type I complexes are formed. On assuming for the moment that side chain amino groups are the binding sites of Cu(II) ions, the molar concentration of complexed ligand moieties $[L]_b$ is given by the following equation:

$$[L]_b = \{[OH^-]_{add} - [OH^-] + [H^+]\} \times \left(1 + \frac{K_a}{[H^+]}\right) - T_L \frac{K_a}{[H^+]} \quad (1)$$

where $[OH^-]_{add}$ is the concentration of added titrant, $[OH^-]$ and $[H^+]$ are the equilibrium concentrations of hydroxyl and hydrogen ions, K_a is the acid dissociation constant of protonated side chain amino groups, and T_L is the total molar concentration of amino acid residues. From the potentiometric titration data of the three examined polymers in absence of Cu(II) it is easy to show that, in the region of formation of type I complexes, $K_a \ll [H^+]$, and eq 1 therefore reduces to:

$$[L]_b = [OH^-]_{add} - [OH^-] + [H^+] \quad (2)$$

and

$$\bar{n} = [L]_b / T_{Cu} \quad (3)$$

T_{Cu} being the total concentration of cupric ions. Analysis of the titration data in the pH region of stability of type II complex with the Bjerrum method is much more complicated. At pH >8.5 in fact acid dissociation of side chains consistently increases and the term $K_a/[H^+]$ cannot be neglected. K_a also changes with the degree of neutralization because of the polyelectrolyte effect, and, most important, deprotonated peptide nitrogens are involved in the complex formation (see the following sections), with the further complication that the acid dissociation constant of peptide protons is not known. Under these conditions a reliable determination of the formation curve of type II complexes becomes practically im-

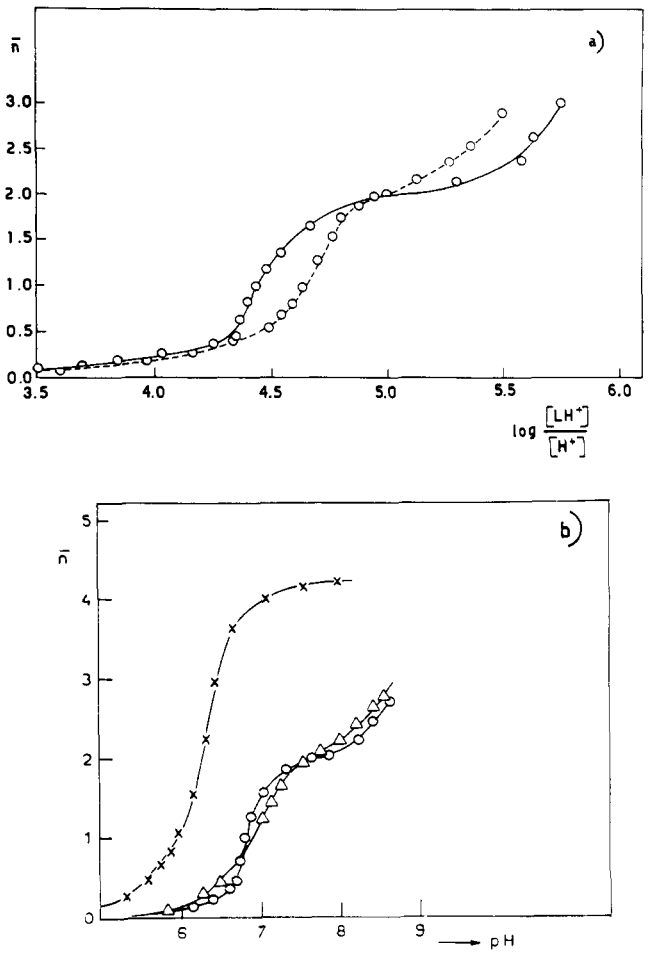


Figure 4. (a) Modified Bjerrum plots for the cupric type I complexes of $(Lys)_n$ (solid line) and $(Orn)_n$ (dashed line). (b) Plots of $\bar{n}$ vs. pH for the three type I polymeric complexes: (○) $(Lys)_n$–Cu(II); (△) $(Orn)_n$–Cu(II); (×) $(A_2bu)_n$–Cu(II).

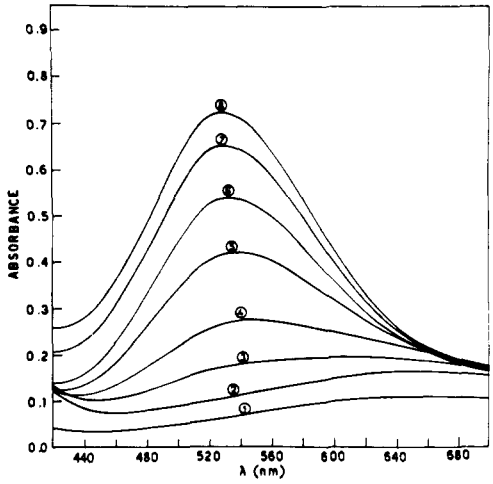


Figure 5. Visible absorption spectra of $(Lys)_n$–Cu(II) at different pH values: (1) pH 6.85; (2) pH 7.47; (3) pH = 8.37; (4) pH 8.77; (5) pH 9.22; (6) pH 9.73; (7) pH 10.18; (8) pH 10.6 $[(Lys)_n] = 5.5 \times 10^{-3}$ M residue; Cu/Lys = 0.16; cell path length = 5 cm.

possible. The formation curves of type I complexes of $[L\text{-Lys}]_n$ and $[Orn]_n$ are shown in Figure 4a.

The results indicate that two protons are displaced per mole of bound Cu(II) at pH ≃7.5 and therefore that two ligand groups are involved in the complex formation. The formation curves in fact exhibit a sigmoidal shape with an asymptot to

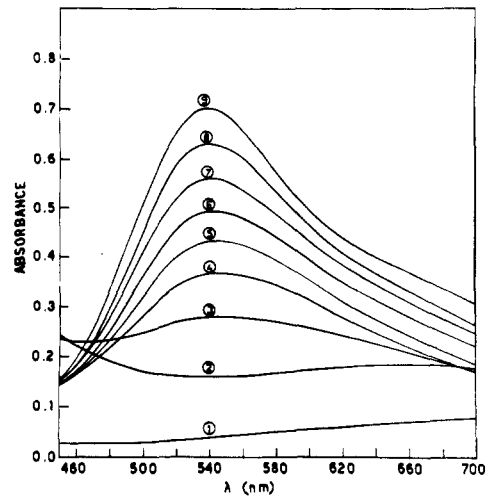


Figure 6. Visible absorption spectra of $(Orn)_n$–Cu(II) at different pH values: (1) pH 6.88; (2) pH 7.33; (3) pH 7.82; (4) pH 8.25; (5) pH 8.60; (6) pH 9.14; (7) pH 9.66; (8) pH 10.83; (9) pH >13. $[(Orn)_n] = 6.0 \times 10^{-3}$ M residue; Cu/Orn = 0.146; cell path length = 5 cm.

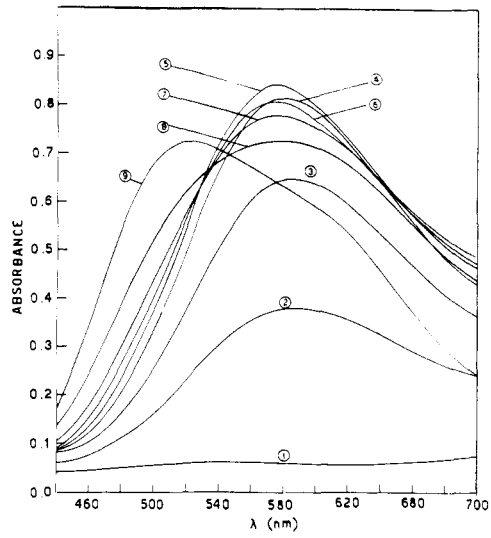


Figure 7. Visible absorption spectra of $(A_2bu)_n$–Cu(II) at different pH values: (1) pH 5.72; (2) pH 6.30; (3) pH 6.60; (4) pH 7.40; (5) pH 8.30; (6) pH 9.20; (7) pH 10.10; (8) pH 11.5; (9) pH >13. $[(A_2bu)_n] = 5.2 \times 10^{-3}$ M residue; Cu/A_2bu = 0.26; cell path length = 5 cm.

$\bar{n} = 2$. These data are inconsistent with those previously reported by Hatano et al.[4] who found that four protons are released during the formation of Type I complex of lysine.

Type I copper complex of $[L\text{-}A_2bu]_n$ behaves differently from those of $[L\text{-Lys}]_n$ and $[L\text{-Orn}]_n$. Analysis of the titration data (Figure 4b) reveals that four protons are released per mole of bound Cu(II) until pH 8.5. It will be shown in the next sections that at least one of these protons comes from deprotonation of peptide nitrogens, so that we cannot determine the complex formation function according to eq 2 and 3. Owing to the low polymer concentration used in our experiments (≃5 × 10^{-3} M residue), complex formation with Cu(II) ions should occur intramolecularly.

Visible Absorption Measurements. The results of visible absorption measurements on the copper complexes of the three poly(α-amino acids) in the pH range 5–14 are shown in Figures 5–7. Let us discuss first the absorption data relative to type I complexes formed at pH ≤8.5. The complexes of $[L\text{-Lys}]_n$ and $[L\text{-Orn}]_n$ exhibit almost identical absorption properties with a broad band at 650 nm in the region of d–d transitions. Interestingly, the absorption maximum of the low

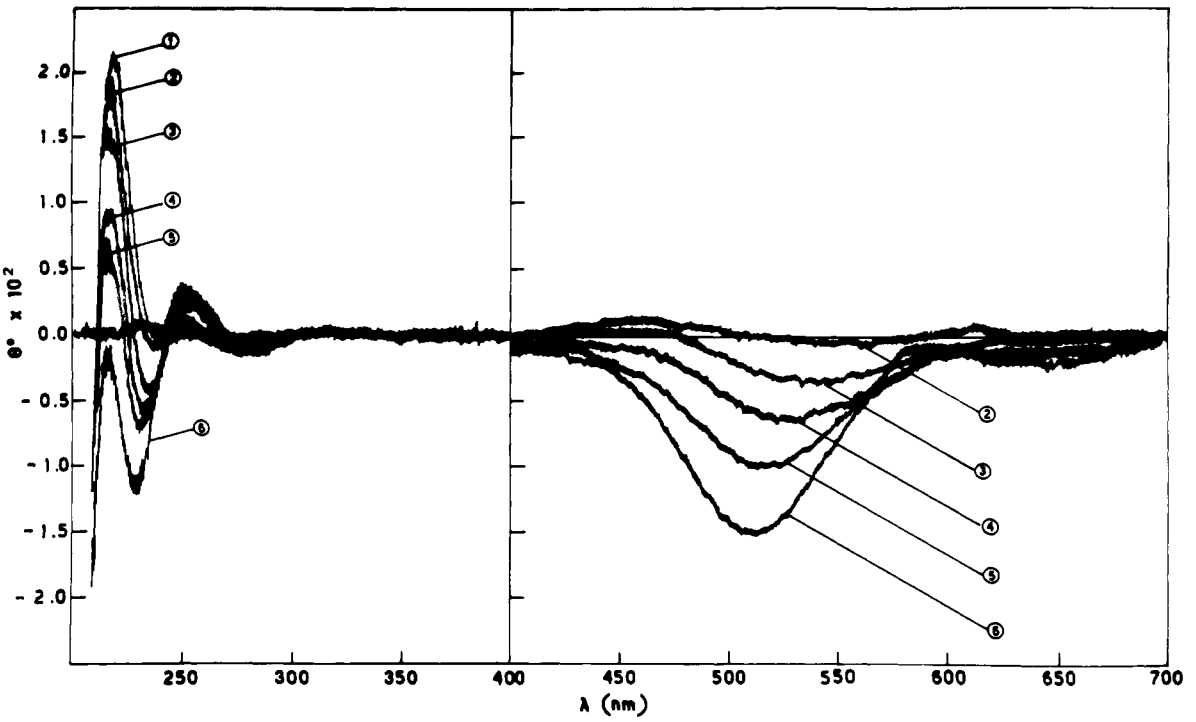


Figure 8. CD spectra of $(Lys)_n$–Cu(II) at different pH values: (1) pH 4.58; (2) pH 6.75; (3) pH 8.13; (4) pH 8.88; (5) pH 9.12; (6) pH 9.39. $[(Lys)_n] = 5.1 \times 10^{-3}$ M residue; Cu/Lys = 0.245. The cell path length was 0.1 cm in the range 210 to 400 nm and 1 cm in the range 400 to 700 nm.

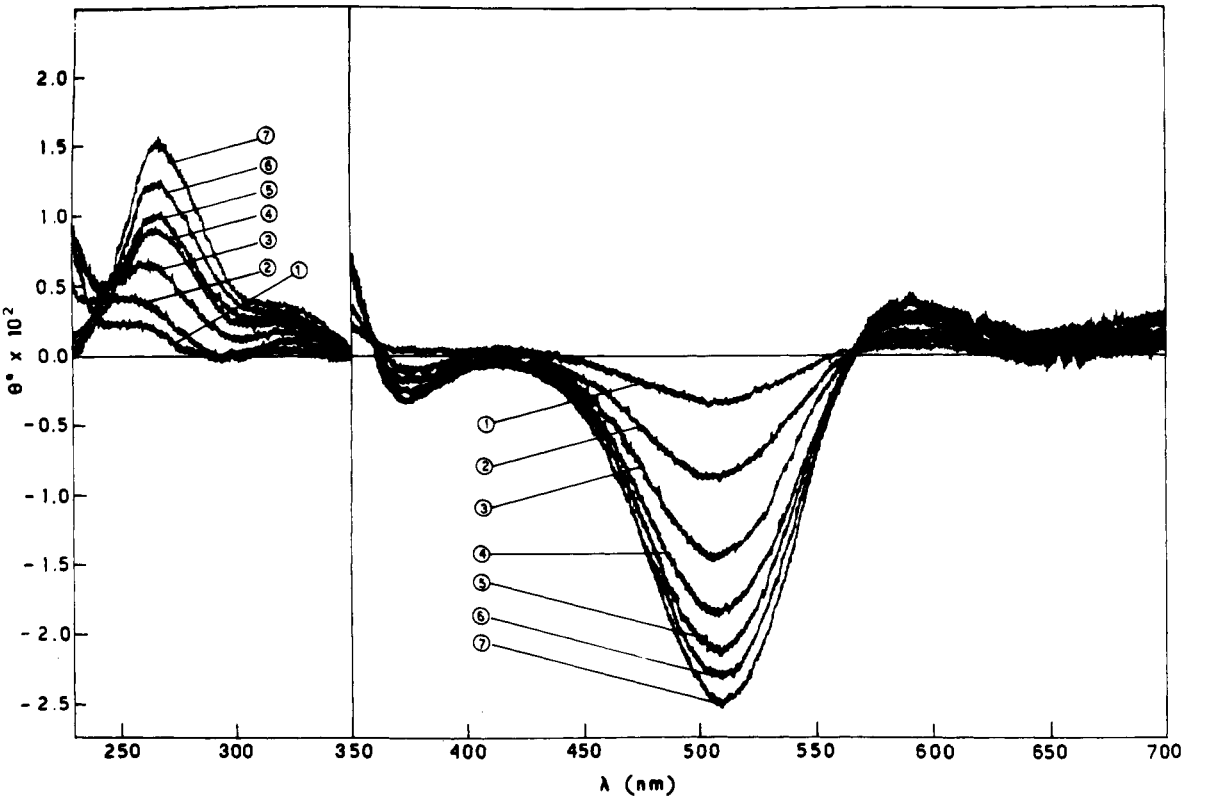


Figure 9. CD spectra of $(Orn)_n$–Cu(II) at different pH values: (1) pH 7.96; (2) pH 8.25; (3) pH 8.90; (4) pH 9.47; (5) pH 9.98; (6) pH 10.51; (7) pH 11.01. $[(Orn)_n] = 5.7 \times 10^{-3}$ M residue; Cu/Orn = 0.151. The cell path length was 0.1 cm in the range 230 to 350 nm and 1 cm in the range 350 to 700 nm.

pH copper complex of $[L\text{-}A_2bu]_n$ is blue shifted down to ~585 nm with respect to that of the homologous complexes of $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$. In general visible absorption bands below 600 nm have been observed in copper complexes of peptides when deprotonated amide nitrogens are involved in the complex formation.[10] Our absorption data therefore suggest that, differently from $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$, the $[L\text{-}A_2bu]_n$ type I copper complex formed at low pH contains amido nitrogens in the square planar coordination positions of the metal ion.

On increasing the pH above 8.5 type II complexes start to form. In the case of $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$ complex formation

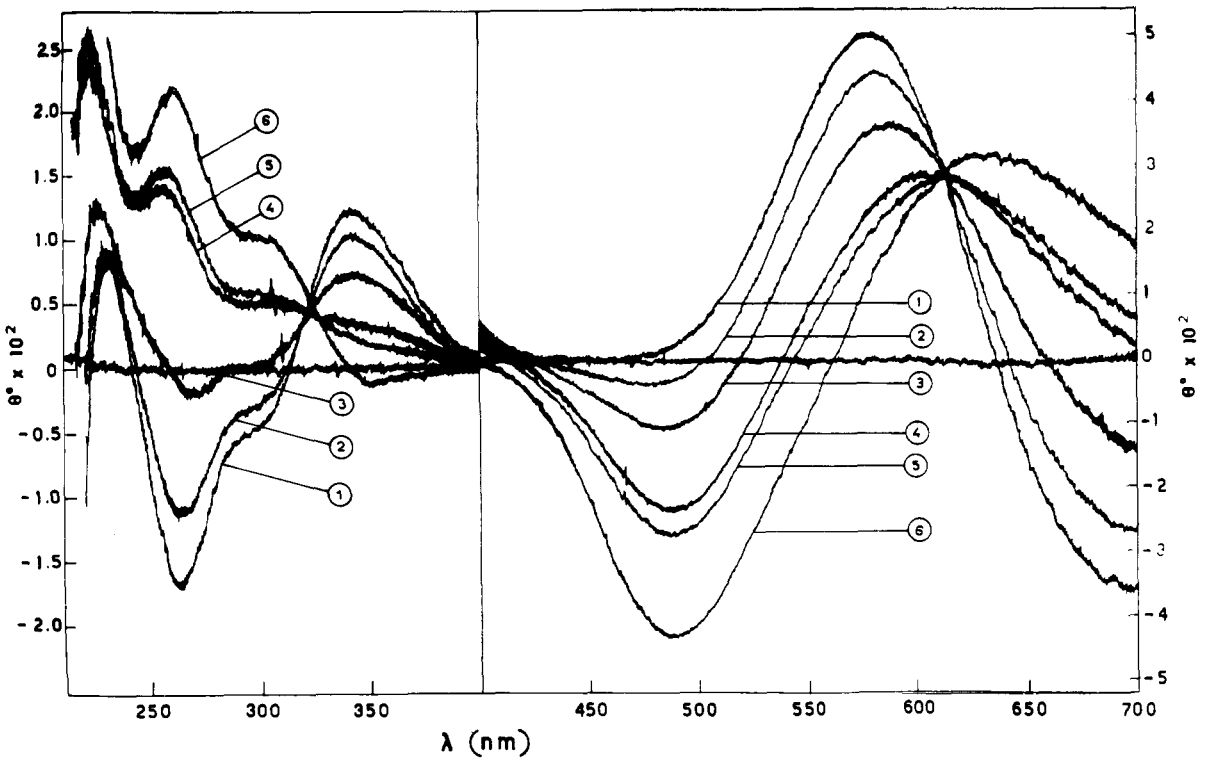
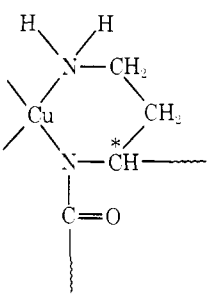


Figure 10. CD spectra of $(A_2bu)_n$–Cu(II) at different pH values: (1) pH 8.29; (2) pH 9.31; (3) pH 10.18; (4) pH 11.67; (5) pH 12.09; (6) pH >13. $[(A_2bu)_n] = 4.7 \times 10^{-3}$ M residue; $Cu/A_2bu = 0.26$. The cell path length was 0.1 cm in the range 210 to 400 nm and 1 cm in the range 400 to 700 nm.

causes a blue shift of the visible band to 528 and 538 nm, respectively. Taking into account the above mentioned literature data the blue shift of the visible band is consequent to substitution of amino nitrogens with amido nitrogens in the coordination sphere of Cu(II). These spectra are qualitatively similar to that of $[L\text{-His}]_n$–Cu(II) complex at pH 14, where four amide nitrogens have been suggested to occupy square planar coordination positions of the central metal ion.[2b] In the case of type II Cu(II) complex of $[L\text{-}A_2bu]_n$ a small shift of the visible band is observed on increasing the pH value above 8.5. Only at very high pH (~1 N NaOH) a second band appears at ~520 nm (Figure 7).

Circular Dichroism Measurements in Water. The results of CD absorption measurements on the copper complexes of the three examined polymers in the visible and far-UV spectral regions in the pH range 5–14 are shown in Figures 8–10.

In the pH range of stability of type I complexes (pH ≤8.5) of $[L\text{-Lys}]_n$ and $[L\text{-Orn}]_n$ vanishingly small optical rotation is observed both in the d–d and charge-transfer transition region (Figures 8 and 9). In the peptide absorption region the CD patterns are typical of the random coil form. These results are consistent with those previously reported by Hatano et al.[4] for the low pH copper complex of $[L\text{-Lys}]_n$. Interestingly, the corresponding complex of $[L\text{-}A_2bu]_n$ exhibits substantial optical rotation in the regions both of d–d and charge-transfer transitions (Figure 10). Two strong bands opposite in sign are observed at 700 nm (negative) and at 580 nm (positive). In the near-UV, the CD pattern is rather complicated with bands at 340 nm (positive), 300 nm (negative), and 265 nm (strong and negative). Again the CD properties in the far-UV peptide transition region are those typical for the random coil conformation. The strong optical activity of the low pH $[L\text{-}A_2bu]_n$ copper complex must therefore be ascribed to asymmetric induction from the chiral centers of single amino acid residues. This result is in favor of the hypothesis that in the case of diaminobutyric acid polymer deprotonated amido nitrogens are coordinated to Cu(II) ions. In fact when side chain amino nitrogens only are coordinated to the metal ion, very small optical rotation is observed, owing to the large distance of the asymmetric α-carbon from the coordination sphere of Cu(II). This indeed is the case of type I complexes of $[L\text{-Lys}]_n$ and $[L\text{-Orn}]_n$. When at least one deprotonated peptide nitrogen occupies one of the square planar coordination positions of Cu(II) the chiral center is much closer and induced optical rotation in the transitions region of the complex therefore appears. The reason why in the low pH region amido nitrogens are coordinated to copper ions only in the case of $[L\text{-}A_2bu]_n$ probably rests on the fact that a side chain amino group and an adjacent peptide nitrogen can form a stable hexatomic chelate ring with two square planar coordination positions of Cu(II):



Since four protons are displaced per mole of Cu(II) until pH 8.5, structures involving three amino nitrogens and one amido nitrogen and two amido and two amino nitrogens can be postulated, the third possibility of three peptide and one amino nitrogens being ruled out by the visible absorption characteristics. Actually, from our data it is not possible to resolve this ambiguity.

The CD properties of complex I of $[L\text{-}A_2bu]_n$ are very similar to those of Cu(II)–$[L\text{-His}]_n$ complex formed at pH 5.[3] Also in the latter case in fact, remarkable optical activity has been observed in the visible absorption region, with bands having

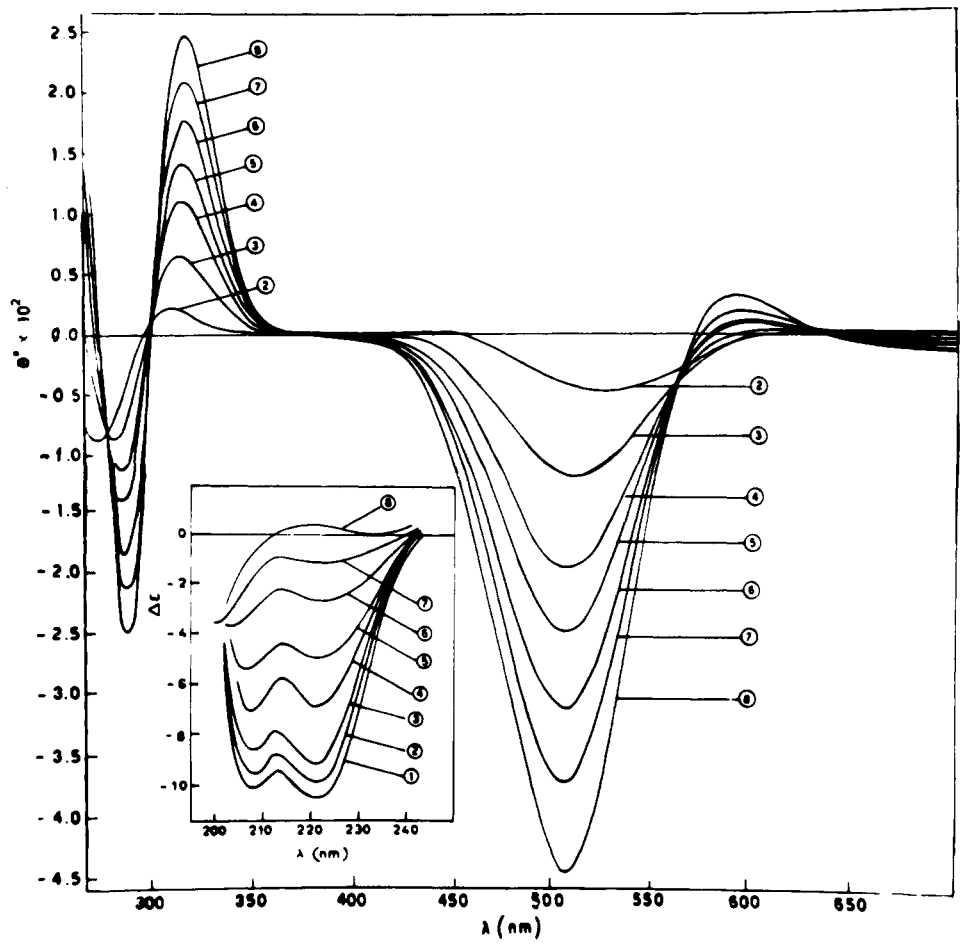


Figure 11. CD spectra of $(Lys)_n$–Cu(II) at pH 11.0 and at different Cu/Lys molar ratios: (1) Cu/Lys = 0; (2) Cu/Lys = 0.016; (3) Cu/Lys = 0.033; (4) Cu/Lys = 0.057; (5) Cu/Lys = 0.082; (6) Cu/Lys = 0.106; (7) Cu/Lys = 0.131; (8) Cu/Lys = 0.164. $[(Lys)_n] = 5.4 \times 10^{-3}$ M residue. The cell path length was 1 cm in the range 250 to 700 nm.

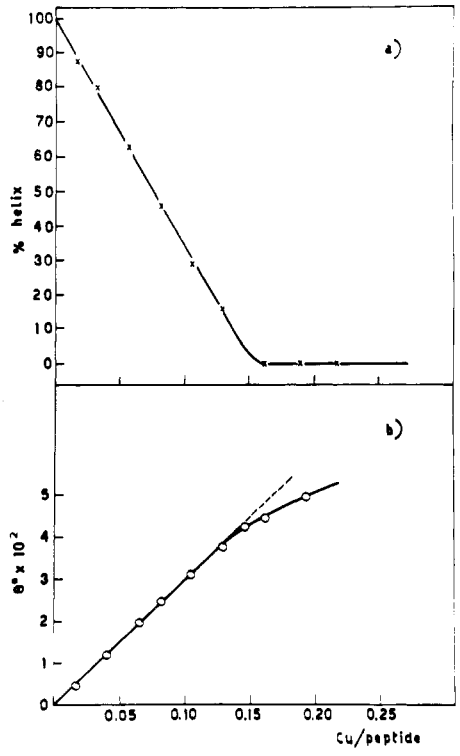
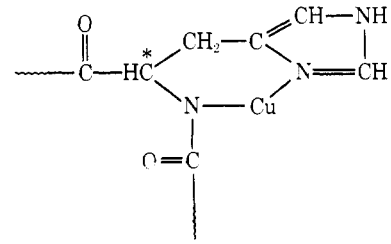


Figure 12. (a) Plot of percent helix vs. Cu/peptide ratio for the type II $(Lys)_n$–Cu(II) complex. (b) Ellipticity of the type II $(Lys)_n$–Cu(II) complex at 510 nm vs. Cu/peptide ratio.

about the same sign, position, and intensity as those of complex I of $[L\text{-}A_2bu]_n$. Also for the $[L\text{-}His]_n$ complex the possibility of forming hexatomic chelate rings could account for the suggested coordination of peptide nitrogens:[3]



Turning now to the results obtained on type II complexes formed at high pH, in all three cases strong optical activity is observed both in the visible and UV region. Our data on $[L\text{-}Lys]_n$ are consistent with those reported by others.[4] Comparison of the CD patterns of Figures 8–10 reveals that type II complexes are different from one another. The complexes of $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$ exhibit quite similar CD properties in the visible absorption region (negative band at 510 nm and positive peak at 585 nm) but exhibit substantial differences in the charge transfer transition region. On the other hand the CD pattern in the visible absorption region of the $[L\text{-}A_2bu]_n$ complex is different from those of $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$, while the CD pattern in the UV is qualitatively similar to that of the $[L\text{-}Orn]_n$ complex (Figures 8–10). These differences among the CD properties of the polymeric complexes can be due to a different number of deprotonated amido nitrogens

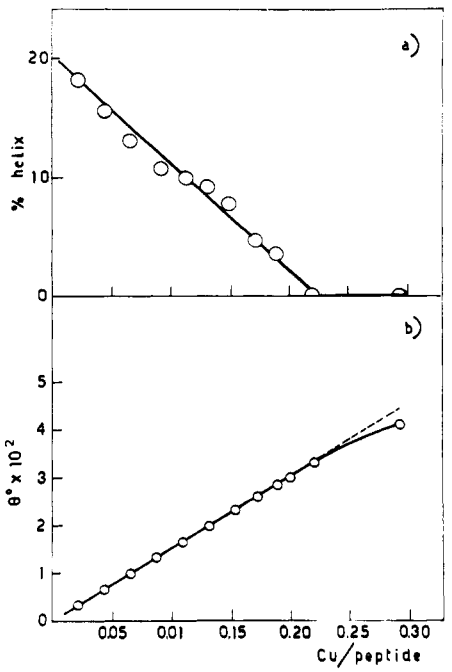


Figure 13. (a) Plot of percent helix vs. Cu/peptide ratio for the type II $(Orn)_n$–Cu(II) complex. (b) Ellipticity of the type II $(Orn)_n$–Cu(II) complex at 510 nm vs. Cu/peptide ratio.

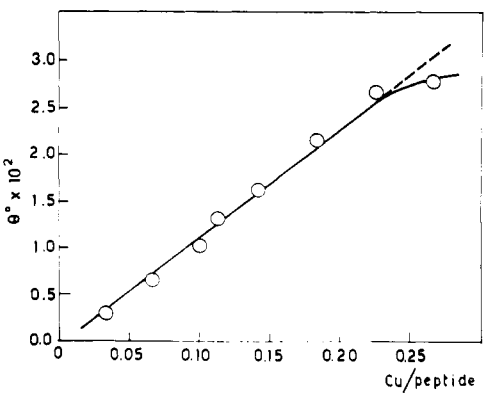


Figure 14. Ellipticity of the type II $(A_2bu)_n$–Cu(II) complex at 600 nm vs. Cu/peptide ratio.

coordinated to the central ion at the planar positions, and/or to different geometries in the three cases. Coordination of peptide nitrogens at apical positions, as suggested by others,[4] is rather improbable, since in no case has it been found in solution and in the crystal structure of copper complexes of oligopeptides.[11,12]

The CD properties of complex II of $[L\text{-}A_2bu]_n$ deserve a further comment. The spectrum is characterized by a positive band at 635 nm, followed by a negative one at 490 nm of almost identical rotatory strength (Figure 10). Below 400 nm the CD pattern is almost the mirror image of complex I. The entire series of CD spectra recorded in the pH range 8.5–14 shows two isodichroic points at 613 and 322 nm. This result is consistent with the presence of a two-component equilibrium system, with additive contributions from each component (complex I and complex II) to the total optical activity.

Again we note that the CD spectrum of complex II of $[L\text{-}A_2bu]_n$ is almost identical with that of the corresponding complex of $[L\text{-His}]_n$ formed at pH 14.[3]

As previously mentioned $[L\text{-}A_2bu]_n$ assumes the random coil conformation in the entire range of examined pH, and no asymmetric induction on the copper complex is possible from a helical backbone. The same could not be true for the high pH complexes of $[L\text{-Lys}]_n$ and $[L\text{-Orn}]_n$, since both polymers are known to undergo a coil–helix transition at pH values higher than 11.[13] For both these cases the optical activity of type II complexes could be due, at least in part, to asymmetric induction from the helical backbone. In order to explore this possibility we must first answer the question whether the structure of type II Cu(II) complexes is compatible with the α-helical conformation of the peptide backbone or if binding of Cu(II) ions causes disruption of such a structure. To clarify this point the following experiment has been performed. To a solution of $[L\text{-Lys}]_n$ at constant pH 11 (where the polymer is completely in the α-helical form), increasing amounts of Cu(II) ions were added and the CD spectrum was recorded after each addition of copper. The results, reported in Figure 11, clearly show that the dichroic bands at 222 and 208 nm typical of the α-helical form decrease on increasing the extent of complex formation. This is even more evident in Figure 12 where the helix content, as determined by the amplitude of the 222-nm band, is plotted in the function of the Cu/peptide ratio. On the same figure the amount of formed complex, as determined by the amplitude of the 510-nm CD band, in the function of the Cu/peptide ratio, is also reported for comparison. The disruption of the α-helical structure appears not to be cooperative in character, being linearly proportional to the extent of complex formation. The helix content drops to zero when the Cu/peptide ratio is of the order of 0.16. Interestingly, above this value the plot relative to the amount of formed complex shows a curvature, resembling a saturation curve for the binding process. Unfortunately no experiment can be performed at Cu/peptide ratios >0.25, since complex precipitation occurs, so that we have been unable to determine completely the saturation curve. Substantially identical results have been obtained with $[L\text{-Orn}]_n$ which is partially in the helical form at pH 11 (Figure 13). In the case of $[L\text{-}A_2bu]_n$, we have determined only the extent of complex II formed in the function of the Cu/peptide molar ratio (Figure 14), since the polymer is always in the random coil conformation in the entire range of examined pH. Curvature in this case starts to appear at a Cu/peptide ratio close to 0.22. From these experiments the general conclusion therefore follows that the structure of type II complexes is not compatible with the α-helical conformation of the peptide backbone and that the binding process causes disruption of the helical structure. Previously proposed models[4] based on coordination of Cu(II) ions to helical $[L\text{-Lys}]_n$ are therefore inconsistent.

Circular Dichroism Measurements in Water–Methanol Mixtures. It is known from the literature[14] that $[L\text{-Lys}]_n$ assumes the right-handed α-helical conformation in water–methanol mixtures containing more than 90% methanol. Most important the α-helix is stable at apparent pH values lower than 8, where the side chain groups are completely protonated. We have found that also $[L\text{-Orn}]_n$ and $[L\text{-}A_2bu]_n$ at pH_{app} <8 assume the helical form in the same solvent mixture. This property allowed us to investigate the effect of formation of type I complexes, stable in the pH region below 8.5, on the conformation of the polypeptide backbone. The experiments were performed in the following way. To the polymer solutions in a water–methanol mixture containing 90% methanol (v/v), adjusted at apparent pH 7.55, increasing amounts of cupric ions were added. The helix content in the function of the copper over amino acid ratio was determined by CD measurements in the peptide absorption region. The extent of complex formation was monitored by visible absorption measurements in the case of $[L\text{-Lys}]_n$ and $[L\text{–Orn}]_n$, the corresponding type I complexes being optically inactive. In the case of $[L\text{-}A_2bu]_n$ the formation of the optically active complex I was followed by CD measurements in the visible.

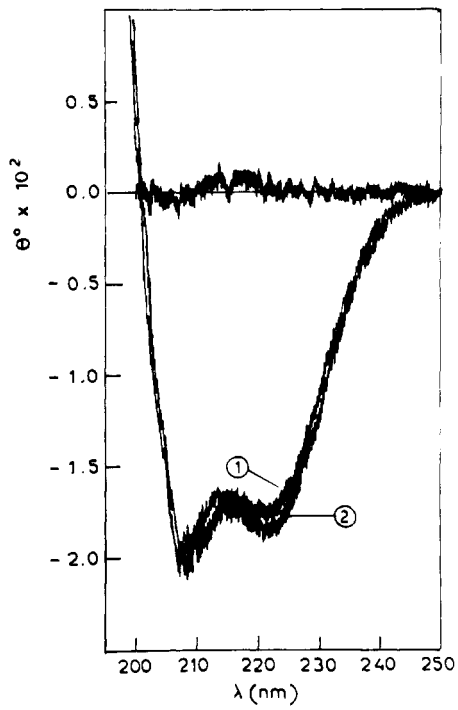


Figure 15. CD spectra of $(Orn)_n$–Cu(II) in 90:10 v/v methanol–water at apparent pH 7.55: (1) Cu/Orn = 0; (2) Cu/Orn = 0.184. $[(Orn)_n]$ = 4.88×10^{-3} M residue; cell path length = 0.01 cm.

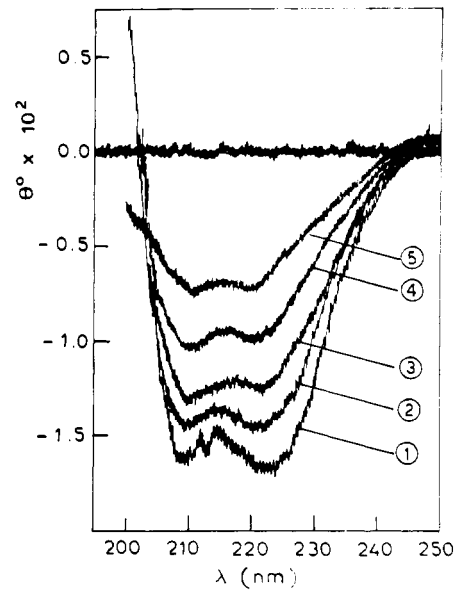


Figure 16. CD spectra of $(A_2bu)_n$–Cu(II) in 90:10 v/v methanol–water at different apparent pH values: (1) pH 6.16; (2) pH 6.85; (3) pH 7.55; (4) pH 9.13; (5) pH 10.6. $[(A_2bu)_n] = 5.3 \times 10^{-3}$ M residue; Cu/A_2bu = 0.17; cell path length = 0.01 cm.

The results relative to $[L\text{-}Orn]_n$ are shown in Figure 15. Formation of complex I does not change appreciably the helix content of the polymer backbone. The same has been found also for $[L\text{-}Lys]_n$. Complex I of $[L\text{-}A_2bu]$ behaves in a different way. In this case in fact complex formation causes a decrease of the helix content (Figure 16).

Conclusions

The most direct conclusion of the present paper is the incompatibility of the α-helical conformation of the three examined polymers with the structure of type II complexes where amide nitrogens are coordinated at the square planar positions of Cu(II). These conclusions apply to intramolecular complexes only, formed under our experimental conditions. It cannot in fact be excluded, even if rather unlikely in our opinion, that in more concentrated polymer solutions cupric ions could provoke aggregation of helices, acting as polymerizing agent as in the case of insulin[15] and serum albumin.[16] The denaturation of the α-helical structure consequent to binding of cupric ions at high pH is consistent with data obtained by other investigators on various systems including proteins.[17,18] *R*-Nase for instance is known to bind more than 29 cupric ions at pH 11, with complete denaturation of the native form.[18] Similarly, binding studies on myoglobin in the solid state and in solution indicate that, when one Cu(II) ion is bound to the protein, its native form is retained. Subsequent binding leads to denaturation.[17]

At low pH we presented evidence that the structure of type I complexes is incompatible with the α-helical backbone only in the case of $[L\text{-}A_2bu]$, that is only when amide nitrogens are involved in complex formation. Coordination of peptide nitrogens at low pH is not an unusual result since these groups have been recognized as important binding sites for Cu(II) at physiological pH values in oligopeptides,[11,12] polypeptides,[3] and proteins.[19,20]

A final observation concerns the absence of appreciable optical activity in type I complexes of $[L\text{-}Lys]_n$ and $[L\text{-}Orn]_n$ even in water–methanol solvent mixtures where the polypeptide chain is in the α-helical conformation. There is therefore no asymmetric induction from the α-helix on the cupric complexes. Optical activity appears only when amido nitrogens are coordinated to Cu(II) ions and the chiral centers of single amino acid residues are close to the coordination sphere of the metal.

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