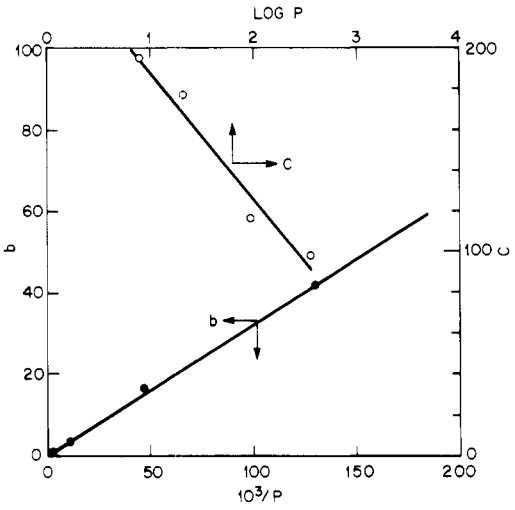

Figure 6. Effect of PS molecular weight on interaction parameter and morphological contribution.

for the magnitude of b. The vanishing value of χ for very high molecular weight PS agrees with the results of ref 9 and 10.

We now address the significance of the parameter C. In computing the various terms in eq 11, we have found that the morphological effect exceeds the free energy of mixing by a substantial margin in all cases. Although C decreases with increasing molecular weight of PS, so does b. Thus, the importance of the morphological term becomes more pronounced as molecular weight increases. When the molecular weight of PS reaches 37 000, the melting point depression is almost entirely attributable to morphological effects.

It remains to be shown that the magnitude of C calculated by us is consistent with reasonable values of σ_e, ζ^0, and ζ. For this purpose we have carried out the following computation using: $\sigma_e = 89$ erg cm^{-2} equal to the value for polyethylene,[20] ~ 40 Å^2 as the cross-sectional area of a PPO segment, and $\zeta^0 = 25$ segments. Thus, $2\sigma_e/\zeta^0$ is estimated to be 410 cal mol^{-1}. If ζ is reduced from 25 to 20, $[2\sigma_e/\zeta - (2\sigma_e/\zeta)^0] = 102$; for $\zeta = 17$, the latter quantity becomes 193. Therefore, modest changes in crystal thickness suffice to account for our C values.

At present, we do not understand fully the dependence of C on molecular weight. But we notice in passing an approximately linear relationship between C and log P (Figure 6).

Conclusion

We have devised a procedure to calculate, from melting point measurements, the interaction parameter between a crystalline and an amorphous polymer. The procedure takes into account the effect of morphological changes on melting point depression. The calculated values of χ for PPO–PS agree with earlier findings. The morphological contributions obtained from our analysis also seem to be reasonable in magnitude.

Acknowledgment. The authors wish to thank Mrs. M. Y. Hellman for molecular weight determinations. Encouraging discussions with Drs. E. Helfand and S. Matsuoka are gratefully acknowledged. One of us is indebted to the National Science Foundation for support of this research.

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Metal Complexes of Poly(α-amino acids). Conformational Aspects of the Interaction between Cupric Ions and Poly(L-histidine)[1]

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ABSTRACT: The interaction between cupric ions and poly(L-histidine) under various experimental conditions has been investigated by potentiometric, visible absorption, and circular dichroism techniques. In addition to the low- and high-pH forms described by Levitzki et al.[2] a new complex has been detected, which is formed with poly(L-histidine) in the ordered structure. Moreover, at very low Cu/peptide ratios, a species is observed, the optical rotatory properties of which are time dependent and slowly transform into the CD pattern typical of the low-pH complex. From the spectroscopic properties, a structure has been proposed for the new complexes.

In 1967 Pecht and co-workers first reported that copper complexes of poly(L-histidine) [(L-His)$_n$] exhibit oxidase activity which is about two orders of magnitude higher than that of the Cu(II) aquo complex.[3] In a subsequent work investigations have been carried out in the attempt to correlate catalytic activity with stereochemistry of the

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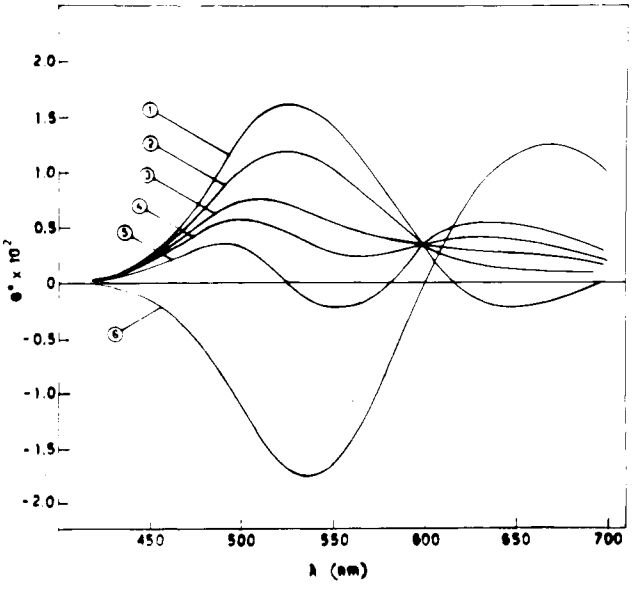


Figure 1. CD properties of the Cu(II)–(L-His)$_n$ system above pH 5 in the visible region. Cu/His = 0.12: (1) pH 6.05; (2) pH 7.07; (3) pH 7.60; (4) pH 7.88; (5) pH 8.0; (6) pH >12.

complexes. Levitzki et al.[2] have been able to detect two different complexes of Cu(II) with (L-His)$_n$. Complex I is formed at pH <5 and involves at least one deprotonated peptide nitrogen at the square planar coordination positions of copper. At strongly alkaline pH values a biuret type complex is formed, in which four consecutive peptide nitrogens have been suggested to occupy the square planar coordination sites of the metal ion.[2]

It is presently well established that (L-His)$_n$ undergoes a disorder–order transition at pH >5, and that the ordered form is that of a β-pleated sheet.[5] In order to investigate the relationships between complex formation and conformational properties of the peptide backbone, in the present paper, we have examined the conformational aspects of the interaction between (L-His)$_n$ and Cu(II) ions under various experimental conditions.

Experimental Section

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

(L-His)$_n$ was prepared according to procedures described in the literature.[4,5] The intrinsic viscosity of the polymer in 1 N hydrochloric acid was 0.22 dL/g.

Carbonate-free potassium hydroxyde was prepared from reagent grade KOH pellets (Merck Chemical Co.), according to the literature.[6]

Measurements. Potentiometric measurements were carried out at 25 °C using a Metrohm Model E 540 precision potentiometer equipped with glass and calomel electrodes. Titrant addition was made using a Metrohm precision microburet Model E 457 equipped with polyethylene capillary. In all experiments the polymer concentration was in the range 4.8×10^{-3}–5.8×10^{-3} M residue, and the molar ratio of cupric ions to amino acid residues was in the range 0.05–0.50.

Visible absorption spectra and CD spectra were recorded 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

The CD spectra of (L-His)$_n$ at pH values above 5.5 in the presence of Cu(II) ions (Cu/peptide molar ratio = 0.12) are shown in Figure 1. Between pH 3 and 5 (spectra not reported in the figure) we have observed the formation of complex I already described by Levitzki et al.[2] Our CD

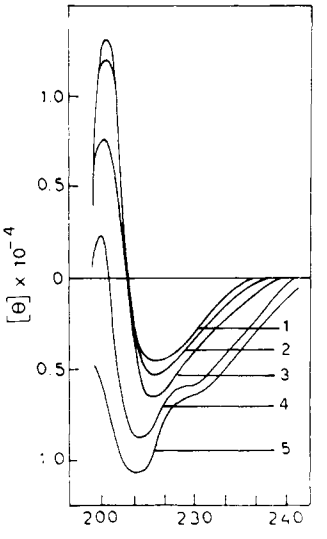
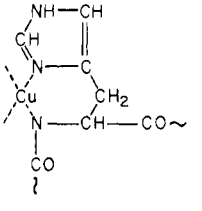


Figure 2. CD properties of the Cu(II)–(L-His)$_n$ system in the peptide absorption region. Cu/His = 0.12: (1) pH 7.04; (2) pH 6.56; (3) pH 6.06; (4) pH 5.54; (5) pH 4.47.

and potentiometric titration data are in a fairly good agreement with those published by the above-mentioned authors. The structure proposed for complex I of (L-His)$_n$ involves three imidazole nitrogens of residues 1, 2, and 4, and one peptide nitrogen of residue 3, in the classical square-planar geometry. The relevant optical activity observed in the metal d–d transitions of the copper–histidine chelate could also be explained by a structure in which one imidazole nitrogen and the adjacent deprotonated peptide nitrogen occupy two square-planar coordination positions of copper, forming a stable, hexaatomic ring containing the asymmetric center.



Above pH 5 our CD results give evidence for the formation of a new, previously undetected, complex which has been called complex II. A new CD pattern is obtained characterized by two positive bands at 630 and 490 nm and by a negative band at 550 nm. Finally, the presence of a rather well-defined isodichroic point at ~600 nm is indicative of a two-component equilibrium system. Under the experimental conditions at which the results reported in Figure 1 have been obtained, complex II precipitates at pH ≥8.0.

It has to be emphasized that the complex tends to precipitate even at lower pH values, when allowed to stand for long times. At strongly alkaline pH values all material redissolves, due to the formation of the biuret type complex, which we now call complex III, already described by Levitzki et al.[2] In Figure 1 the CD pattern of complex III is also shown for comparison.

It is well known from the literature that (L-His)$_n$ above pH 5 undergoes a coil-β transition.[5,7] The formation of complex II at pH >5 must be therefore related in some way to the conformational change of the polymer. In order to clarify this point we have recorded CD spectra in the UV region at various decreasing pH values starting from 7.04 at constant Cu/peptide ratio 0.12 (Figure 2). The polymer is in the β conformation at pH 7.04. The minimum at 217 nm and the maximum at 203 nm are in quantitative

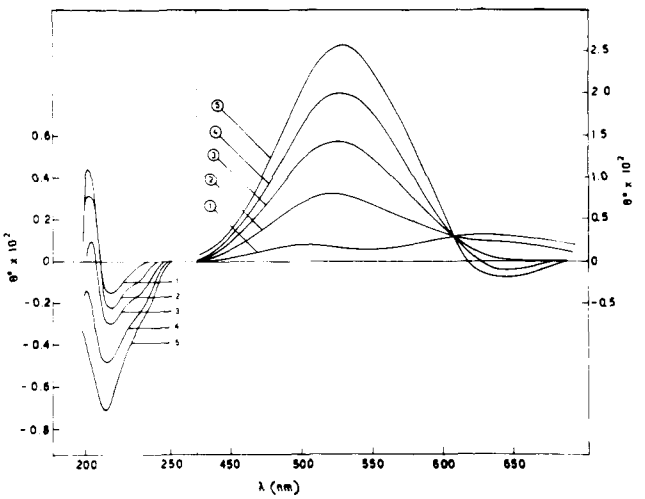


Figure 3. CD spectra of the Cu(II)-(L-His)$_n$ system at pH 6.0 and at different Cu/His molar ratios: (1) Cu/His = 0.06; (2) Cu/His = 0.106; (3) Cu/His = 0.128; (4) Cu/His = 0.150; (5) Cu/His = 0.195. [(L-His)$_n$] = 5.8 × 10^{-3} M residue.

agreement with literature data on the β form of (L-His)$_n$.[5] In this connection we should however point out that, in the peptide absorption region, partial overlapping of charge-transfer bands of the complex with peptide and imidazole bands could complicate the interpretation of the CD spectrum in terms of conformation. However, using low Cu/peptide ratios, as in the experiment shown in Figure 2, the possible contribution from CT bands of the complex is a minor one, and the assignement of the polymer conformation is reasonably safe.

On decreasing the pH below 7.04, we observed the progressive formation of complex I and the parallel decrease of the content of β structure (Figure 2). The two equilibrium components, indicated by the isodichroic point at ~600 nm (Figure 1), are complex II, where cupric ions are coordinated to (L-His)$_n$ in the β conformation, and complex I, where cupric ions are coordinated to a random polypeptide chain. From the above results it follows that the structure of complex II of (L-His)$_n$ is compatible with the β structure.

Actually we have shown that the stability of complex II of (L-His)$_n$ with cupric ions depends upon at least three intercorrelated factors, namely pH, polypeptide conformation, and Cu/peptide molar ratio. If Cu(II) ions are added to a polymer solution at constant pH 6, until a Cu/peptide ratio of 0.05–0.06, we observe first that complex II is predominantly formed, and the polymer is almost entirely in the β-pleated sheet conformation (Figure 3). If more cupric ions are added to such a solution we obtain the progressive decrease of the content of β structure and the parallel formation of the CD pattern typical of complex I. It follows that complex I causes disruption of the ordered structure of the polypeptide backbone. At Cu/peptide ratios >0.3 the formation of complex II can be achieved only by increasing the pH above 7, but, under these experimental conditions, simultaneous precipitation of the complex occurs. However it appears that increasing amounts of Cu(II) ions shift the coil-β transition of (L-His)$_n$ and the consequent formation of complex II toward higher pH values.

The above results give a clear example of a strict relationship between the way of coordination of cupric ions and the conformational change of the polypeptide matrix. As previously mentioned, coordination of cupric ions in complex I involves at least one deprotonated peptide nitrogen. In complex II, whose structure is compatible with

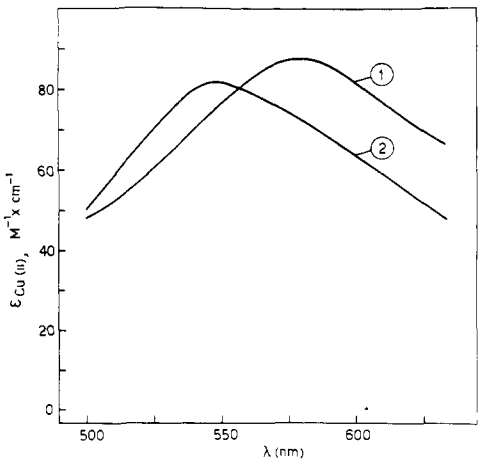


Figure 4. Visible absorption spectra of the Cu(II)-(L-His)$_n$ system: (1) pH 7.59, Cu/His = 0.17; (2) pH 5.0, Cu/His = 0.1 (from ref 2).

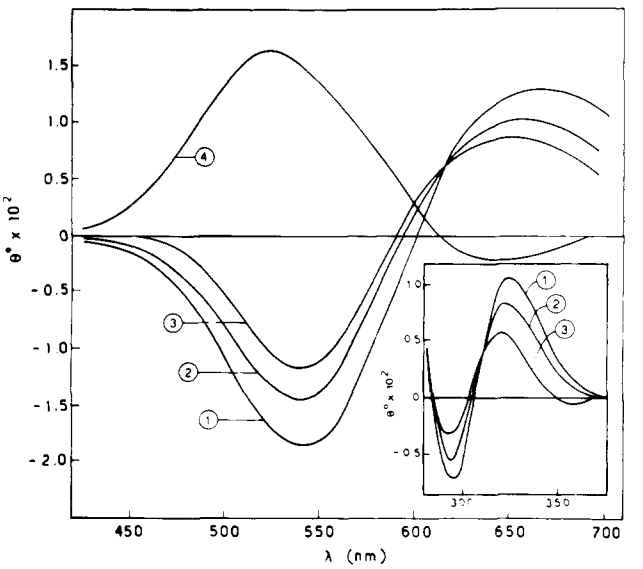


Figure 5. CD spectra of the Cu(II)-(L-His)$_n$ system in the high-pH region. Cu/His = 0.12, [(L-His)$_n$] = 5.3 × 10^{-3} M residue: (1) pH 12.45; (2) pH 11.15; (3) pH 10.5; (4) spectrum of complex I (pH 5.0) reported for comparison.

the polymer β form, the weak rotational strength and the position of the visible absorption maximum (Figure 4), which is red shifted by about 40 nm with respect to that of complex I,[2] strongly suggest that imidazole nitrogens only are coordinated to Cu(II) ions.[8]

As reported by Levitzki et al.,[2] at strongly alkaline pH values the biuret-type copper complex of (L-His)$_n$ is formed (complex III), which involves only deprotonated amide nitrogens at the square-planar coordination positions of the metal ion. On decreasing the pH from 12.5 a new series of CD spectra have been obtained, which reflect the equilibrium between complex III and complex II (Figure 5). Below pH 10.5 extensive precipitation of the complex prevents reliable measurements.

As a summary the CD spectra, on a molar ellipticity basis, of the three cupric complexes of (L-His)$_n$ are collected in Figure 6.

During experiments carried out at very low Cu/peptide molar ratios, we became aware of a kinetic effect in the formation of complex I. If Cu(II) ions are added to a polymer solution fixed at pH 4.5, until a Cu/peptide ratio of 0.05, a CD pattern completely different from that of complex I is obtained immediately after addition of the metal ions (Figure 7). However, the CD spectrum is time

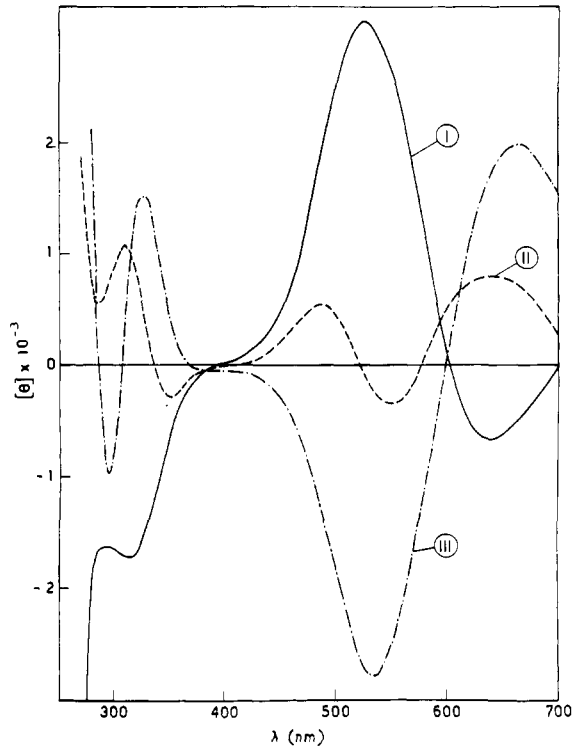


Figure 6. CD spectra of complexes I, II, and III. The molar ellipticities are given per bound Cu(II).

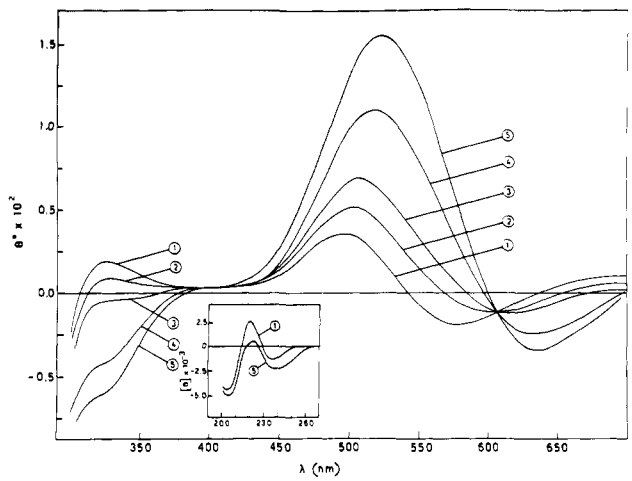


Figure 7. CD spectra of the Cu(II)–(L-His)$_n$ system at pH 4.5 recorded at different times from Cu(II) addition. [(L-His)$_n$] = 5.2 × 10^{-3} M residue, Cu/His = 0.05: (1) t = 0 min; (2) t = 45 min; (3) t = 190 min; (4) t = 1150 min; (5) t = 6900 min.

dependent, and slowly transforms into the CD pattern characteristic of complex I. Again there is an isodichroic point at ~600 nm, but at a *negative* ellipticity value. The CD spectral properties in the far-UV region reveal that the polymer is always in the random coil conformation. These results reflect the equilibrium between two species in both of which Cu(II) ions are bound to a random polypeptide chain. One of the two equilibrium components is obviously complex I. The second equilibrium component has optical rotatory properties similar, but not identical, to those of complex II formed at pH >5. The position of the bands is slightly red shifted with respect to the CD spectrum of complex II, and this causes the occurrence of the isodichroic point at negative ellipticity values, as shown in Figure 7.

Significantly the rate of formation of complex I from the initial complex obtained immediately after addition of Cu(II) is pH dependent, being faster on increasing pH above 4.

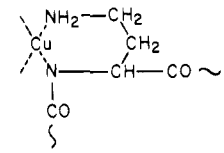
The kinetic effect on the formation of complex I has been observed only when the molar ratio Cu/peptide was of the order of 0.05 or lower. Using higher copper contents the predominant formation of complex I was observed.

The weak rotational strength and the CD and absorption properties very similar to complex II lead us to conclude that in this labile complex also imidazole nitrogens only are probably coordinated to Cu(II). The slightly different CD pattern observed in the latter case with respect to complex II can be explained by the fact that Cu(II) ions are now coordinated to imidazole nitrogens in a random polypeptide chain, while in complex II coordination occurs to imidazole nitrogens of a polymeric matrix in the β conformation.

Discussion and Conclusions

The results presented in this work give evidence for the effect of peptide conformation on the binding of Cu(II) ions to (L-His)$_n$. Clearly, the structure of complex I, which involves one deprotonated amide nitrogen, is not compatible with the β-pleated sheet conformation. This result is in line with those obtained with copper complexes of poly(L-lysine), in which it has been shown that coordination of Cu(II) to deprotonated peptide nitrogens causes disruption of the α-helical and β structure.[9-11]

In complex I, stable at pH <5, the presence of imidazole nitrogens favors deprotonation of peptide nitrogens, consistent with what is observed in histidine containing oligopeptides.[12-14] In this connection it is worth recalling the CD results obtained on the Cu(II) complex of poly(L-diaminobutyric acid) at pH >8.0. Also in that case a structure has been proposed in which the asymmetric α-carbon atom is part of a stable hexa-atomic chelate ring, with one amino and one deprotonated peptide nitrogen coordinated at two of the square-planar positions of copper:[9,10]



The structure similarities between this complex and complex I of (L-His)$_n$ are evident and are reflected in their almost identical CD properties (Figure 8). In both cases Cu(II) ions are bound to a random polypeptide chain. These results strongly support the conclusion that histidine and diaminobutyric acid residues could be equivalent as ligands for Cu(II) ions.[9,10]

A new complex has been characterized, in which Cu(II) ions are bound to (L-His)$_n$ in the ordered structure. It is interesting to observe that, while complex I contains 3 imidazole and 1 deprotonated peptide nitrogen donors,[2] at higher pH the most basic donor is removed from coordination, as indicated by the absorption and circular dichroism properties of complex II. The conclusion therefore follows that deprotonation and coordination of peptide nitrogens at physiological pH is no more favorable when the conformational transition from the coil to the β structure occurs.

The kinetic effect observed at low Cu/peptide ratios in the formation of complex I of (L-His)$_n$ suggests an explanation for the recent results obtained by Wasylishen and Cohen[15] on the influence of Cu(II) ions on proton nuclear relaxation rates of (L-His)$_n$. These authors observed a sharp maximum of the relaxation rates of the C_2 imidazole protons between pH 3.5 and 4.0. In this pH

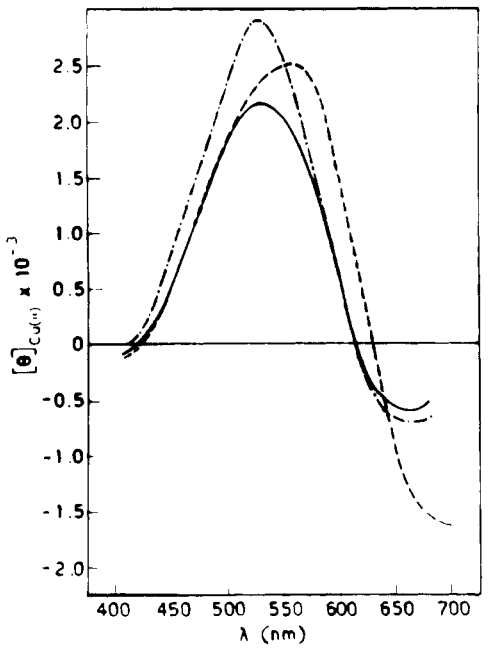


Figure 8. CD spectra of the low-pH cupric complexes of (L-His)$_n$ and poly(L-diaminobutyric acid) in the visible region: (—) (L-His)$_n$–Cu(II) (from ref 2); (-·-) (L-His)$_n$–Cu(II) (our measurements); (- - -) (L-diaminobutyric acid)$_n$–Cu(II) (from ref 9).

range and at the very low Cu/peptide ratio used by Wasylishen and Cohen, instead of complex I a complex in which imidazole nitrogens only are coordinated to Cu(II) is formed immediately after addition of the metal ions. Significantly, between pH 3.5 and 4.0, the transformation of this complex into complex I is very slow, so that the above NMR measurements very probably refer to the former. At pH ~5 the rate of formation of complex I is much faster, so that the decreased relaxation effect of Cu(II) on (L-His)$_n$ above pH 3.5 can be explained by the presence of increasing amounts of complex I in the mixture with consequent slower exchange of cupric ions which are bound to deprotonated peptide nitrogens.

Acknowledgment. The authors are indebted to Mrs. Clara Benvegnu' for her skillful technical assistance.

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Mechanophotochemistry

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ABSTRACT: A model is presented for materials that deform due to exposure to light. This model is based on the conclusions drawn from mechanochemistry that chemically induced ionizations cause changes in the dimensions of porous polyelectrolytic gels. The mechanophotochemical properties of poly[*p*-(*N*,*N*-dimethylamino)-*N*-γ-D-glutamanilide], that displays up to 35% dilation in each dimension when exposed to light, are presented as proof of the correctness of the model.

Dilation and contraction of synthetic fibers due to changes in the state of ionization of polyelectrolytic gels have been observed as early as 1950.[1] The authors noticed that expansion of polyacrylic acid gels could be induced by suspension in alkali solutions, while restoration of the original size took place when the solution was titrated with acid. Very spectacular were the experiments on threads of poly(vinyl phosphate).[2] On addition of strong alkali the threads elongated to about three times their original length, while subsequent addition of acid caused rapid reversion to the original length. A proposal was put forward to utilize this phenomenon for conversion for chemical into mechanical energy.[3] Continued research in the field of "mechanochemistry" produced deeper understanding of the phenomenon[4,5,6] and many more materials were reported to be suitable for conversion of chemical energy into mechanical motion.[4] Two distinguishable contractile and elastic mechanisms govern the phenomenon. One of these is restricted to amorphous polymers and the other to crystalline polymers. In the former the initial and the deformed states are both amorphous, while in the latter the polymer undergoes a crystal to amorphous transition (or vice versa). Excellent reviews describing these mechanisms in great detail have appeared in the literature.[5,7]

When a polymer having a random network structure is elongated, the chain's end-to-end vector both increases in length and aligns itself in the direction of the elongation. Simultaneously, the number of configurations that the chains could have assumed prior to the application of the

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