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Complex formation between copper(II) and poly-N^5-(3-hydroxypropyl)-L-glutamine

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Summary

The complex formed between copper(II) and a polymeric ligand derived from poly-N^5-(3-hydroxypropyl)-L-glutamine (PHPG) has been investigated by circular dichroism. It was found that the conformational changes of PHPG occurred by forming complexes with Cu(II) according to the Cu/HPG molar ratios and different pH values. The helical structure was induced in PHPG by bound Cu(II) at Cu/peptide ratios $< 1/16$, while the structure changed from α-helix to the random coil and β-form at Cu/peptide ratios $> 1/16$. It was also found that complex formation between Cu(II) and PHPG occurred above pH 9.5.

Introduction

Poly(α-amino acids)-metal ion complexes have been investigated from the point of view of both the catalytic activity and the conformation of macromolecular complexes(1, 2,3,4,5). Such compounds can be considered as useful models for understanding the way of action of copper-containing proteins.

It is known that all water soluble poly(α-amino acids) such as poly(L-glutamic acid)(6),poly(L-lysine)(7,8),poly (L-ornithine)(8),poly(L-diaminobutyric acid)(8),poly(L-arginine)(9),poly(L-histidine)(3),poly(L-alanine)(10) and poly (L-glycine)(11),with the only exception of poly(L-proline) (8),bind copper ions in aqueous solution. Optical spectroscopic studies showed changes in the configuration of Cu(II) bound by ligands and the conformational transition of poly (α-amino acids) induced by bound Cu(II). But very few has been known on the interaction between Cu(II) and poly-N^5-(3-hydroxypropyl)-L-glutamine(PHPG). PHPG is freely soluble in water without having any ionizable functional groups.

In this paper,we wish to report the conformational changes of PHPG after the complex formation with Cu(II).

Experimental

Materials: Reagent grade cupric(II) chloride dihydrate was used as obtained. PHPG was prepared according to the procedure described in the literature(12). The molecular weight ($\bar{M}v$=40000) was estimated from the [η] vs. MW plots of Lupu-Lotan et al.(12) for PHPG in water.

Preparation of the polymer-Cu(II) solutions: Typically, a sample solution at the appropriate pH and mixing ratio was prepared by adding appropriate amounts of $CuCl_2$ and NaOH(or HCl) solutions, in this order, to PHPG solution. The ratio of [Cu]/[HPG] was varied by adjusting the amount of Cu(II). The final concentration of the polymer residue was maintained constant at 5×10^{-4}M throughout.

Measurements of the CD spectra and pH: CD spectra of the complex solution in a quartz cell with a path length of 10.0 mm were recorded in a JASCO J-500A Recording Spectropolarimeter at room temperature. The ellipticity, [θ] values for the complex, was calculated on the basis of the molar residue concentration of PHPG. The pH of the solutions was determined on a Hitachi -Horiba Model N-5 pH meter.

Results and discussion

Fig. 1 shows the CD spectra of Cu(II)-PHPG complex at various [Cu]/[HPG] ratios. The CD spectrum of PHPG alone is also shown in Fig. 1. As is well known, PHPG takes the mixture of random coil and α-helical conformations(12)(helicity: ca. 13 mole-%). Ellipticity [θ] at 222 nm is frequently used to estimate the α-helix content, because $[\theta]_{222}$ in deg·cm^2·dmol^{-1} is nearly zero for random coil and -4·10000 for α-helical conformation, resp. The α-helical content of PHPG in the Cu(II)-PHPG complex is estimated ca. 26 mole-% at [Cu]/[HPG]=1/92 and ca. 1 mole-% at 1/4. Induction of the helix by bound Cu(II) is evident from the CD spectra of PHPG-Cu(II) solutions at [Cu]/[HPG]=1/64, 1/80 and 1/92 while transition of the helix is shown at [Cu]/[HPG]=1/4, 1/8 and 1/16. Interestingly, α-helix of PHPG is supported by binding of a small amount of Cu(II), but α-helix structure of PHPG is changed into the random coil and β-form(13) at high [Cu]/[HPG] ratios.

Comparison of the CD patterns in the complex according to the amount of Cu(II) shows that there are two types of complexes. The complex of Cu(II)-PHPG exhibits the mixture of random coil and α-helical form of PHPG when [Cu]/[HPG] ratio is smaller than 1/16. On the other hand, the trough at 222 nm for the α-helix decreases and is shifted to the longer wavelength when the ratio is larger than 1/16, which may indicate that some of helical form of the PHPG undergo a conformational transition to random coil and β-form(β_2-form) by bounding a large amount of Cu(II). It has not been clarified so far, however, how much the β-structure is formed. It can be regarded from these differences that the former complex may be formed within a molecule, while the latter complex may mostly be formed between molecules(14). The complex formation between molecules causes the α-helix of the PHPG to the random coil and β_2-structure(13) in the complex.

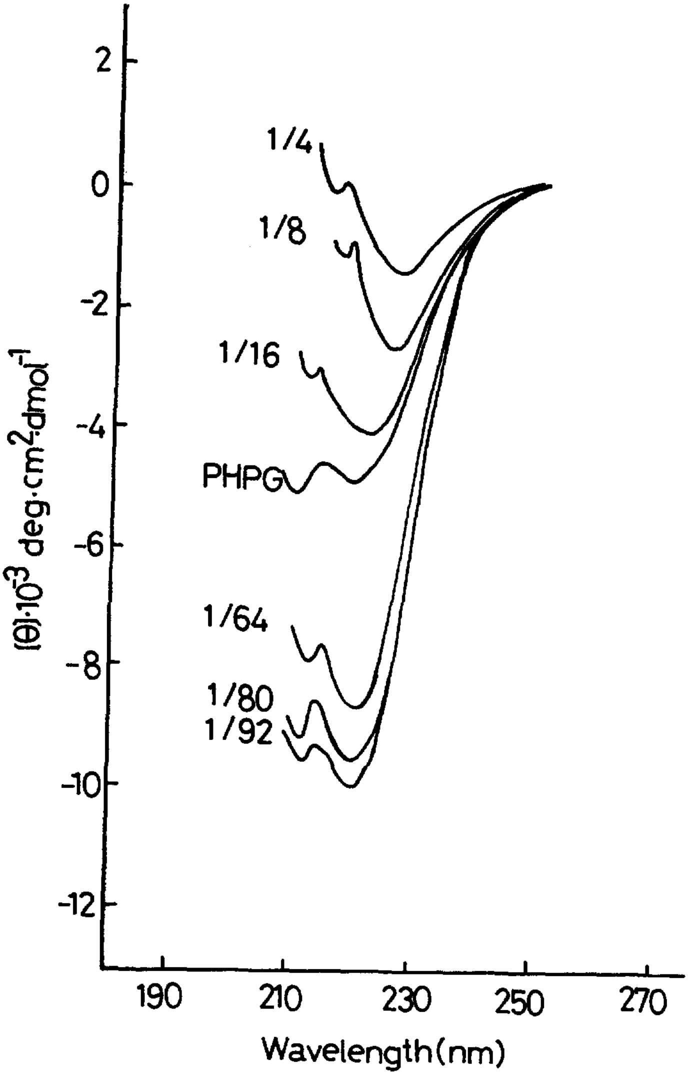

Fig. 1 CD spectra of Cu(II)-PHPG complex at various [Cu]/[HPG] ratios and pH=10.0. [HPG]=5×10^{-4}M

Therefore the ellipticity $[\theta]$ at 222nm decreases and the large negative trough is shifted to the longer wavelength with increasing Cu(II).

Ellipticity of the Cu(II)-PHPG complex is plotted as the function of the Cu/peptide ratio in Fig.2. The induction of the α-helical structure in the complex appears when the Cu/peptide ratio is below 6.25×10^{-2}, and above this value, binding of Cu(II) ions causes transition of such a structure.

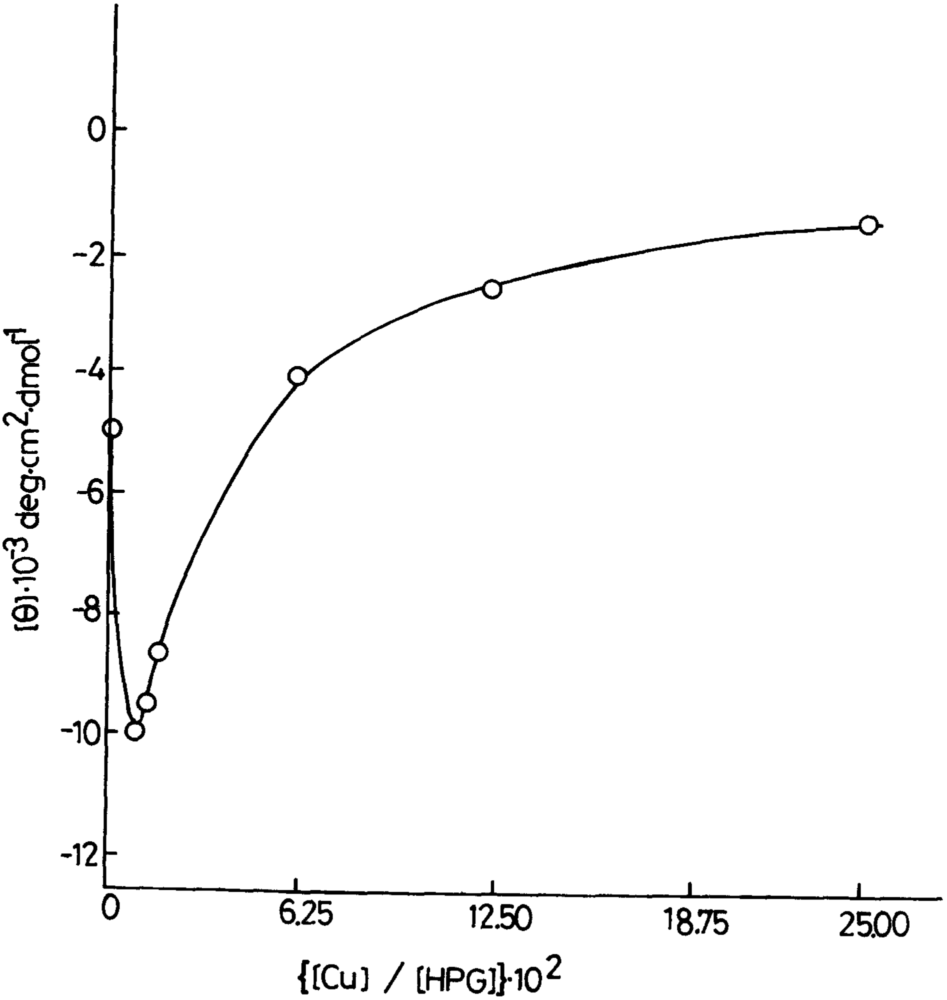

Fig.2 $[\Theta]_{max}$ values of Cu(II)-PHPG complex versus [Cu]/[HPG] ratio and at pH=10.0. [HPG]=5×10^{-4}M

Fig.3 shows the CD spectra of Cu(II)-PHPG complex at various pH. The ellipticity of the complex is the same as that of PHPG alone below pH 8.4,but it decreases with the increase of pH above pH 9.5. The CD spectra of PHPG itself is almost same over the pH range from 4 to 11(not shown in Figure). This may indicate that the conformation of PHPG is independent of pH of the solution. Also,this result may be related to the hydroxy group of PHPG which is not ionized at above pH. It may be regarded from these results that the complex formation between Cu(II) and PHPG does not occur at pH values below 8.4,but occurs above 9.5. And the conformational transition of PHPG in the complex occurred from the α-helix to both of the random coil and β-form with increasing

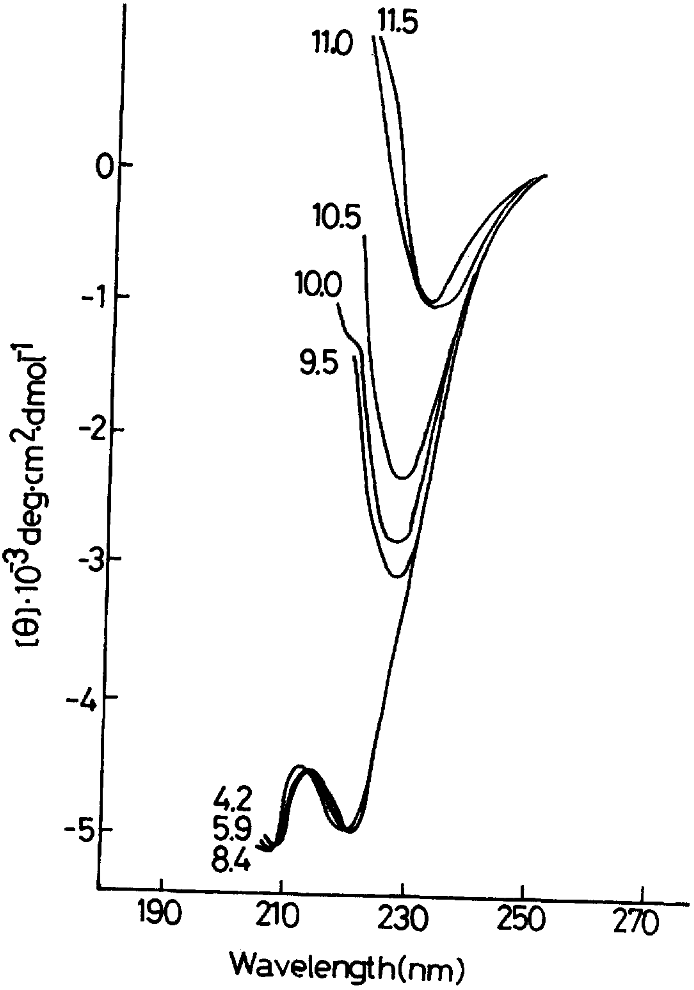

Fig.3 CD spectra of Cu(II)-PHPG complex as a function of pH. (Cu)/(HPG)=1/8,(HPG)=5×10^{-4}M

of pH. Hojo and his coworkers reported that the hydroxy group of poly(vinyl alcohol) coordinated to cupric ions above pH 6.5 (15). But our results suggest that the hydroxy group of PHPG coordinates to cupric ions above pH 9.5.

Ellipticity of the Cu(II)-PHPG complex is plotted as the function of various pH values in Fig.4. The ellipticity of the complex is almost same as that of PHPG below pH 8.4, but it decreases with increasing pH when pH is higher than 9.5.

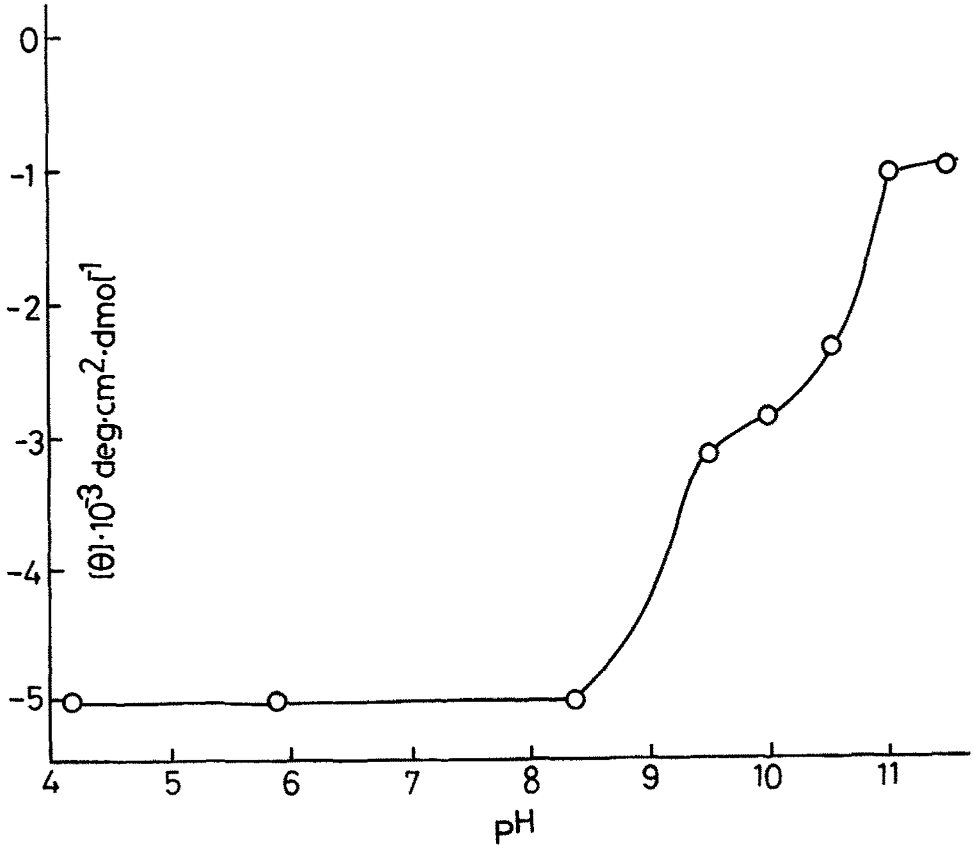

Fig.4 $[\theta]_{max}$ values of Cu(II)-PHPG complex versus pH. $[Cu]/[HPG]=1/8$, $[HPG]=5\times10^{-4}$M

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