Bull. Chem. Soc. Jpn., *57*, *1699—1700 (1984)*

Induced Circular Dichroism in the Complexes of Histidine Polypeptides with Methyl Orange. Effect of Distance between Histidine Residues

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(Received December 22, 1983)

Synopsis. The induced circular dichroism (CD) spectra of the sequential histidine polypeptide-azo dye complexes were measured. The induced CD arose from the dye bound to the polypeptide in the random coil conformation. The intensity of the induced CD decreased with increasing distance between the intramolecular histidine residues.

The induced optical activities of complexes of anionic poly(L-glutamic acid) (PLG) with cationic dyes were first reported by Stryer and Blout.[1,2] Induced optical activities have been reported mostly for the PLG complexes. Lately, however, the complexes of cationic poly(L-lysine)-,[3,4] poly(alkyl-L-lysine)s-,[5,6] and poly(L-arginine)-azo dyes[7,8] have been reported to exhibit optical activity. In the course of our study of the induced CD of biopolymers,[4-8] we wish to report here on the induced CD due to the interaction between the cationic poly(L-histidine) (PLH) and an azo dye.

Experimental

The four histidine polypeptides, PLH (I, $[\eta]^{25}_{\mathrm{DCA}}=0.18$), poly(His-Gly) (II, $[\eta]^{25}_{\mathrm{DCA}}=0.19$), poly(His-$\beta$Ala-Gly) (III, $[\eta]^{25}_{\mathrm{DCA}}=0.20$), and poly(His-Gly$_3$) (IV, $[\eta]^{25}_{\mathrm{DCA}}=0.17$) were synthesized according to the racemization-free diphenyl phosphoryl azide method (dichloroacetic acid was abbreviated as DCA).[9,10] 4-(4-dimethylaminophenylazo)benzenesulfonic acid sodium salt (Methyl Orange, MO) was guaranteed reagents (Wako). The mixtures were prepared by adding the dye solution to the polypeptide solutions; the pH values of the solutions were then brought up to the desired pH by the addition of NaOH or HCl. The final concentrations of histidine residue in polypeptides and MO were 10^{-4} mol residue dm^{-3} and 2×10^{-5} mol dm^{-3}, respectively. The absorption and CD spectra were measured with JASCO UVIDEC-1 and CD J-40A spectrometers, respectively. The molar ellipticity $[\theta]$ (degree cm^2 dmol^{-1}) and molar extinction coefficient ε (dm^3 mol^{-1} cm^{-1}) were expressed on the basis of the dye concentration.

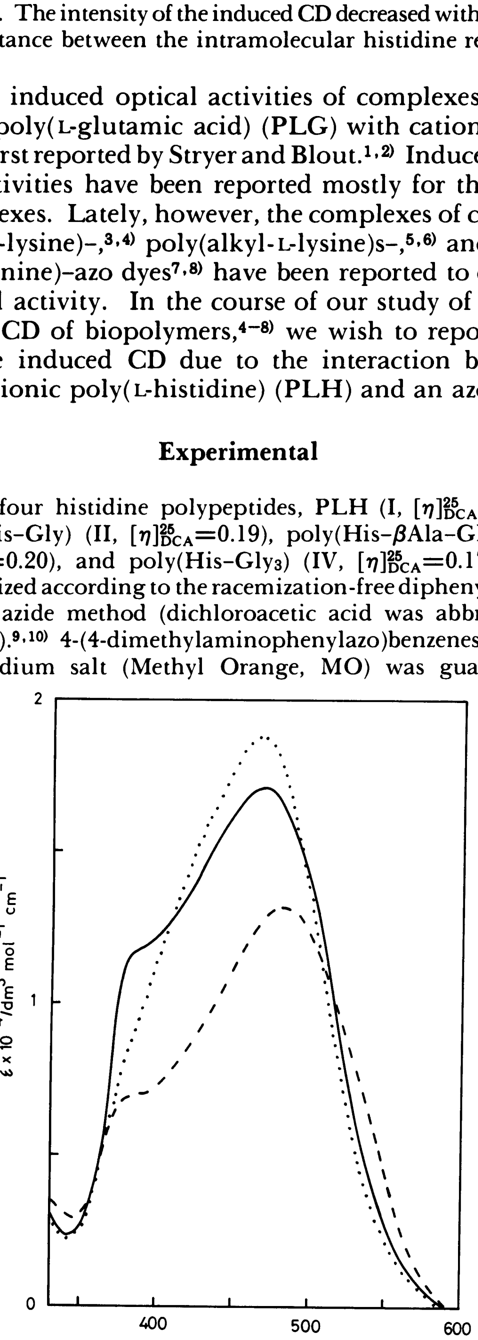

Fig. 1. Absorption spectra of the PLH (I)-MO complex in water at R/D=5: (·······) at pH 4.4, (——) at pH 4.1, and (------) at pH 3.7.

Results and Discussion

Since PLH is not soluble in water above pH 6,[11-13] the absorption and CD spectra of MO with and without polypeptides were measured over the pH range 3 to 6. Upon adding PLH (I), the position of the peak and the band shape of MO in the visible absorption spectrum were changed and the PLH (I)-dye complex exhibited the induced CD. Figure 1 shows the absorption spectra of the PLH (I)-MO complex in water as a function of pH at residue/dye (R/D)=5. At pH 4.6, the visible absorption spectrum showed a peak at 467 nm. As the pH of the solution was lowered from 4.6 to 3.1, the peak at 467 nm shifted to 506 nm. Between pH 4.3 and 3.2 an additional shoulder was observed at around 370 nm. The blue-shifted shoulder (or peak) suggests a nearly parallel orientation of the MO molecules.[3,14] At low PLH (I) concentration, R/D<2, the shoulder at 370 nm was not clear. Figure 2 shows the induced CD spectra of the PLH (I)-MO complex in

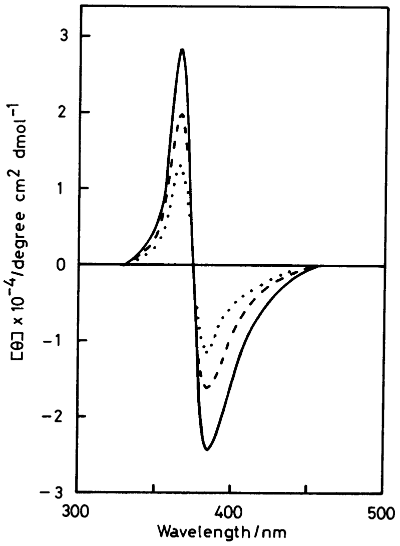

Fig. 2. Induced CD spectra of the PLH (I)-MO complex in water at R/D=5: (·······) at pH 4.4, (——) at pH 4.1, and (------) at pH 3.7.

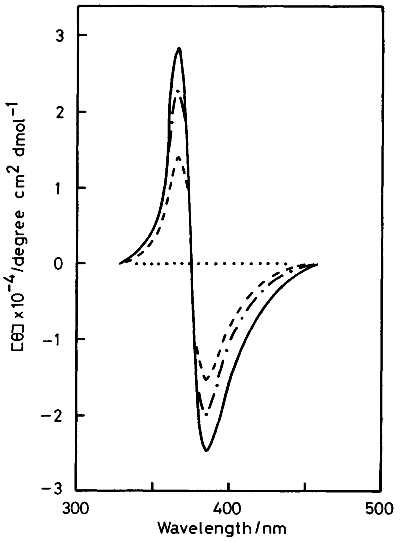

Fig. 3. Induced CD spectra of the PLH (I)-MO complex at various NaCl concentrations (at pH 4.1 and R/D=5): (——) 0 mM, (–·–·–) 0.5 mM, (------) 2 mM, and (······) 5 mM.

water as a function of pH at R/D=5. pH 4.1 was optimal for the induced CD of the PLH (I)-MO system, and the spectra at pH 3.7 and 4.4 showed smaller CD values. Two induced CD extrema were observed at 385 nm with $[\theta]=-25200$ and at 366 nm with $[\theta]=28800$ at pH 4.1. The PLH (I)-MO complex at pH 4.1 showed the characteristic CD bands at 222 nm with $[\theta]=2000$ and at 203 nm with $[\theta]=-4200$ (based on the histidine residue). These ellipticity values suggest that the degree of ionization of the imidazole side chains is about 50% and the conformation of the complex is a random coil.[13]

Figure 3 shows the change in the induced CD spectra of the PLH (I)-MO complex in an aqueous solution with various sodium chloride concentrations at pH 4.1 and R/D=5. With increasing concentration of sodium chloride from 0 to 0.005 mol dm^{-3}, the intensity of the induced CD became weaker. No induced CD was observed in 0.005 mol dm^{-3} sodium chloride.

The main features of the optical activities arise from the dipole-dipole couplings of the chromophores with each another. The intensity of the split Cotton effect is inversely proportional to the square of the interchromophoric distance and is changed by the dihedral angle between the transition moments.[15] In an electrostatic interaction systems, the dihedral angle between the transition moments is difficult to examine but the interchromophoric distance can be changed by sequential polypeptides. Figure 4 shows the induced CD values at the extrema of four sequential polypeptide-MO complexes as a function of distance between C_α of the histidine residues. Since the random coil conformations of the four sequential polypeptide-MO complexes do not allow the exact estimation of the distance between chromophores, we tentatively used the numbers of backbone atoms between histidine residues as abscissa. Clearly, an increased distance decreased the intensity of the induced CD. Beyond the bold approximation of the distances between the nearest intramolecular histidine residues by the CPK model examinations, the observed values of the induced CD of sequential polypeptides decreased sharply.

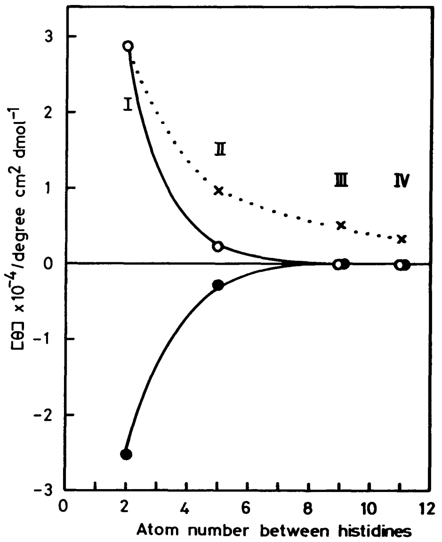

Fig. 4. Induced CD values at the extrema of the sequential polypeptide MO-complexes as a function of distance between histidine residues in water at pH 4.1 and R/D=5: ○ at 366 nm and ● at 385 nm. Samples I—IV, see text. The estimated induced CD values (X and dotted line) were calculated from the CPK model examinations, assuming the stretched backbone conformation and the nearest distances between histidine residues.

These results are the first observed induced CD of PLH-MO complexes and demonstrate that the intensity of the induced CD of the polypeptide-dye complex is dependent on the distance between the intramolecularly interacting chromophores.

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