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# Induced Circular Dichroism in the Complex of Poly(L-arginine) Hydrochloride with Azo Dyes

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**Synopsis.** The induced circular dichroism (CD) spectra of the poly(L-arginine) hydrochloride–azo dye complexes were measured. The induced CD arose from the azo dyes bound to the polypeptide in the α-helical conformation. The configuration of the transition dipole moments of dye was examined by the exciton chirality method.

The induced optical activities of complexes of synthetic poly(L-glutamic acid) (PLG) with cationic dyes, such as Acridine Orange (AO) and pseudoisocyanine, in the pH range from 4.2 to 6.5 were first reported by Stryer and Blout.[1,2] Induced optical activities have been reported mostly on the PLG complexes. Lately, however, the complexes of poly(*S*-carboxymethyl-L-cysteine)-AO[3] and poly(L-lysine) (PLL)–azo dyes[4–6] have been reported to induce optical activity.

The first complete synthesis of poly(L-arginine) (PLA) by the *N*-carboxy anhydride method and a conformational study of the polypeptide have been reported from this laboratory.[7,8] In the course of our study of the induced CD of biopolymers, we wish to report our findings on the induced CD due to the interaction between the cationic PLA and anionic azo dyes.

## Experimental

The average degree of polymerization of the PLA hydrochloride (HCl) used was 70. The three analogous azo dyes, *p*-(4-aminophenylazo)benzenesulfonic acid sodium salt (AA), *p*-(4-dimethylaminophenylazo)benzenesulfonic acid sodium salt (Methyl Orange, MO) and *p*-(4-diethylaminophenylazo)-benzenesulfonic acid sodium salt (Ethyl Orange, EO), were guaranteed reagents (Wako). The mixtures were prepared by adding the dye solutions to the PLA solution; 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 the dyes were $2\times10^{-5}$ M, and the ratio of polypeptide residues to dye molecules (R/D) was less than 5. The absorption and the CD spectra were measured with JASCO UVIDEC-1 and CD J-40A instruments respectively. The ellipticity, $[\theta]$ (degree cm$^2$ dmol$^{-1}$), or molar extinction coefficient, $\varepsilon$ (l mol$^{-1}$ cm$^{-1}$), was expressed on the basis of the dye concentration.

## Results and Discussion

The CD and absorption spectra of the three azo dyes with and without polypeptide (R/D=0—5) were measured in the pH range from 2.0 to 13.6. Figure 1 shows the induced CD and absorption spectra of the PLA HCl–MO system at pH 8.7 and 10.3 for R/D=1. MO showed a single visible absorption band at 467 nm with $\varepsilon_{467}=25600$ (and $\varepsilon_{378}=8100$) at pH 7. When a complex was formed with PLA HCl, the spectrum showed an additional peak at 378 nm. The blue-shifted band at 378 nm has been considered to reflect a nearly parallel orientation of the MO molecules.[4,9] The values at R/D=1 were $\varepsilon_{467}=11400$ and $\varepsilon_{378}=12400$ at pH 8.7, and $\varepsilon_{467}=14600$ and $\varepsilon_{378}=12600$ at pH 10.3. EO showed similar changes in the absorption spectra, but no change was found in AA.

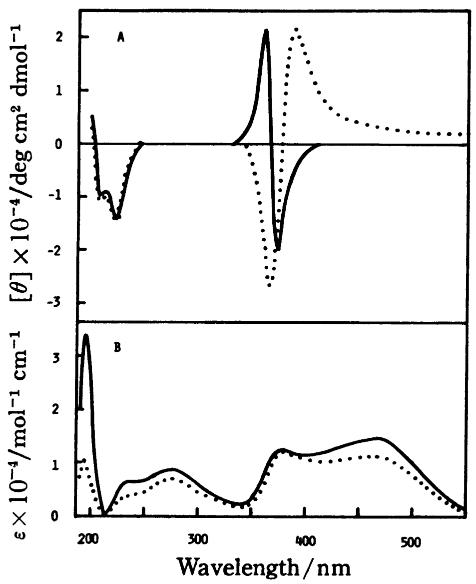

Fig. 1. Induced CD (A) and absorption (B) spectra of the PLA HCl–MO complex at R/D=1: (······) at pH 8.7 and (——) at pH 10.3.

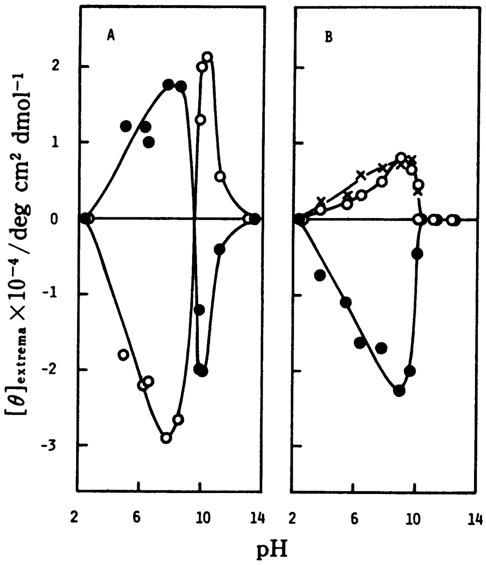

Fig. 2. Induced CD values at extrema of the PLA HCl–dye complexes as a function of pH at R/D=1. (A) PLA HCl–MO complex: ○ at 370 nm and ● at 390 nm; (B) PLA HCl–EO complex: ○ at 378 nm, ● at 400 nm, and × at 450 nm.

Figure 2 shows the induced CD values at the extrema of the PLA HCl–azo dye complexes as a function of the pH at R/D=1. The PLA HCl–MO complex showed a

Table 1. Characteristic of CD in the PLA HCl–dye complexes

| Dye | R/D | pH | $\varepsilon_{max}$/nm | | Induced CD $[\theta]_{extrrema}$/nm | | |
|---|---|---|---|---|---|---|---|
| MO | 0.5 | 8.1 | 19100(467) | 10900(378) | | 3400(390) | −4700(370) |
| | | 10.3 | 18500(467) | 8800(378)[a] | | −4200(374) | 2400(361) |
| | 1 | 8.7 | 11400(467) | 12400(378) | | 21400(390) | −26300(370) |
| | | 10.3 | 14600(467) | 12600(378) | | −19800(374) | 21500(361) |
| | 5 | 8.0 | 9000(467) | 18800(378) | | 35400(390) | −47200(370) |
| | | 10.4 | 11400(467) | 10700(378) | | −22100(374) | 28800(361) |
| EO | 1 | 9.3 | 17600(477) | 13000(400) | 7400(450) | −22600(400) | 8400(378) |
| | 5 | 9.0 | 13900(477) | 17800(400) | 9100(450) | −17900(400) | 2500(378) |

a) Shoulder.

positive CD band at 390 nm and a negative CD band at 370 nm below pH 9.6, while it showed a negative CD band at 374 nm and a positive CD band at 361 nm above pH 9.6. On the other hand, the PLA HCl–EO complex induced three CD extrema, at 450 and 378 nm (positive) and at 400 nm (negative), in the pH range of 4—10.5 (Table 1).

Both the PLA HCl–MO and EO complexes showed $[\theta]_{222}$ values of −13000—−16000 at R/D=1 in the pH range of 4—10. The positions and magnitudes of the extrema suggest that the two PLA HCl complexes are mostly in a helical conformation, since the n-$\pi^*$ peptide transitions of PLA have been reported to have the ellipticity values of $[\theta]_{222}=-19000$ for a helical structure[8] and of $[\theta]_{217}=3000$—3900 for a random-coil structure.[10,11]

Many rules correlating the signs of ellipticity with the absolute configuration have been proposed including the validity of the exciton chirality method for aromatic compounds[12–15] and for polypeptide–dye complexes.[4,5] The sign of the longer wavelength CD extremum was in agreement with the sense of the handedness of the interacting transition dipole moments.[12] Since the transition of bound MO in the visible wavelength region was determined to be long-axis polarized by the stretched polyvinyl alcohol film method,[16] the CD spectra of the PLA HCl–MO complex were examined by means of the exciton chirality method. The interacting transition dipole moments of dimeric dye can be assumed to have the clockwise *R*-chirality for the PLA HCl–MO complex below pH 9.6 and the *S*-chirality for the same complex above pH 10, while the PLA HCl–EO complex showed a multiple induced CD band in the pH range of 4—10.5. When the pH was raised, the *R*-to-*S* configurational inversion of the PLA HCl–MO complex was found at pH 9.8. The reason is not clear at present, since no significant conformational differences between the two PLA HCl–MO and EO complexes were found. It is, however, conceivable that the delicate interaction of the polarized transition dipole moments affects the dihedral angle or the $C_2$ rotation axis of the dimeric MO molecules[17,18] accompanying the helix-to-coil conformational transition of the PLA HCl–MO complex around pH 9.8.

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