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Induced Circular Dichroism in the Complexes of Sequential Lysine Polypeptides with Methyl Orange. On Intermolecular Aggregation of the Complexes and Distance between Lysine Residues

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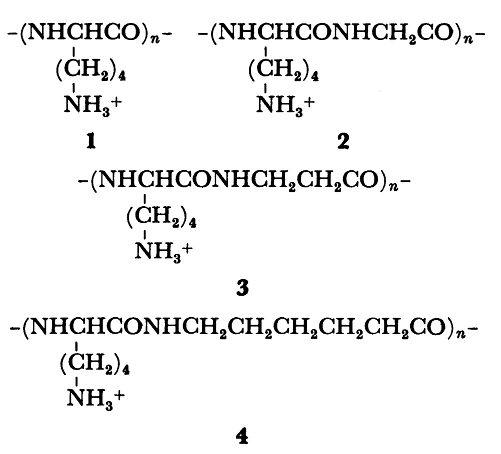
Synopsis. Aggregation of the sequential poly(Lys HCl-X) (X: Gly, βAla, and εAhx)-Methyl Orange (MO) complexes was studied by means of a circular dichroism (CD) measurement. The intensity of the induced CD was affected by the concentration of the complexes and time after preparing the complex solutions. When MO molecules which are electrostatically bound to lysine residues are separated from each other, the aggregation of the complexes takes place to a lesser degree.

The origin of induced optical activities arises from dipole–dipole interactions between two transition dipoles of the chromophores under chiral environmental conditions.[1,2] Recently, several groups reported the results on an induced CD due to chiral interaction between cationic poly(Lys)[3] and anionic azo dyes.[4–8] The structural isomer εpoly(Lys) has also been examined from the standpoint of binding affinity to small molecules such as dyes and metals,[9] and of the induced CD properties on complexation with MO.[10] The εpoly(Lys)–MO complex caused an interchain aggregation. The differences in the results between poly(Lys) and εpoly(Lys) have mainly been attributed to the intramolecular distance between the ammonium groups, to which anionic azo dyes interacted electrostatically. To clarify the effect of distance between chromophores, sequential polypeptides containing functional groups may be one of the most suitable systems. In this Note a series of sequential lysine polypeptides are described in order to add some knowledge regarding the induced CD of cationic biopolymer–dye complexes.



$$-(\mathrm{NH}\begin{array}[t]{c}\mathrm{CH}\\|\\(\mathrm{CH_2})_4\\|\\\mathrm{NH_3^+}\end{array}\mathrm{CO})_n-$$

1

$$-(\mathrm{NH}\begin{array}[t]{c}\mathrm{CH}\\|\\(\mathrm{CH_2})_4\\|\\\mathrm{NH_3^+}\end{array}\mathrm{CONHCH_2CO})_n-$$

2

$$-(\mathrm{NH}\begin{array}[t]{c}\mathrm{CH}\\|\\(\mathrm{CH_2})_4\\|\\\mathrm{NH_3^+}\end{array}\mathrm{CONHCH_2CH_2CO})_n-$$

3

$$-(\mathrm{NH}\begin{array}[t]{c}\mathrm{CH}\\|\\(\mathrm{CH_2})_4\\|\\\mathrm{NH_3^+}\end{array}\mathrm{CONHCH_2CH_2CH_2CH_2CH_2CO})_n-$$

4

Scheme 1.

Experimental

Using lysyl-X dipeptides as precursors, three sequential lysine polypeptides, poly(Lys HCl-Gly) (2, degree of polymerization as total amino acid residues, DP,=40), poly(Lys HCl-βAla) (3, DP=60), and poly(Lys HCl-εAhx) (4, DP=90) were synthesized according to the racemization-free diphenyl phosphor azidate method.[11] Poly(Lys HBr) (1, DP=60) was purchased from Sigma and converted to poly(Lys HCl).[12] The chemical structures of the four polypeptides are shown in Scheme 1. Sodium 4-(4-dimethylaminophenylazo)benzenesulfonate (Methyl Orange, MO) was a guaranteed reagent from Wako. The mixtures were prepared by adding a dye solution to the polypeptide solutions. The final concentrations of lysine residues in polypeptides and MO were 1—7×10^{-4} mol residue dm^{-3} and 2—14×10^{-5} mol dm^{-3}, respectively, at residue/dye ratio (R/D)=5. The CD spectra were measured with a Jasco CD J-40A spectrometer. The molar ellipticity $[\theta]$ (degree cm^2 dmol^{-1}) was expressed on the basis of the dye concentration.

Results and Discussion

The induced CD of the poly(Lys)–azo dye complexes has been studied[4–8] and the poly(Lys HCl)–MO complex used here (DP=60) exhibited intensities of $[\theta]_{371}=-25000$ and $[\theta]_{359}=25000$ at pH 6 and R/D=5. The induced CD spectra of the sequential lysine polypeptide–MO complexes were recorded by changing the concentration of the dye and keeping the R/D ratio constant at 5. Figure 1 shows the time dependence of the induced CD spectra of the poly(Lys HCl-Gly)–MO complex in water at pH 6 and dye concentration 10^{-4} mol dm^{-3}. Immediately after preparing the mixed solution, the complex exhibited no induced CD. After 1 h, a weak split-type induced CD spectrum with extrema at 371 nm (negative) and 359 nm (positive) was observed and their intensities at the extrema were found to increase on standing without stirring. After 23 h, the intensities were −16600 (371 nm) and 16100 (359 nm), and still increasing. Figure 2 shows the concentration and time dependence of the induced CD of the poly(Lys HCl-Gly)–MO complex in water at pH 6 (R/D=5). When the dye concentrations were less than 4×10^{-5} mol dm^{-3}, the system did not exhibit induced CD, even after 3 d. On the other hand, when the dye concentrations were greater than 6×10^{-5} mol

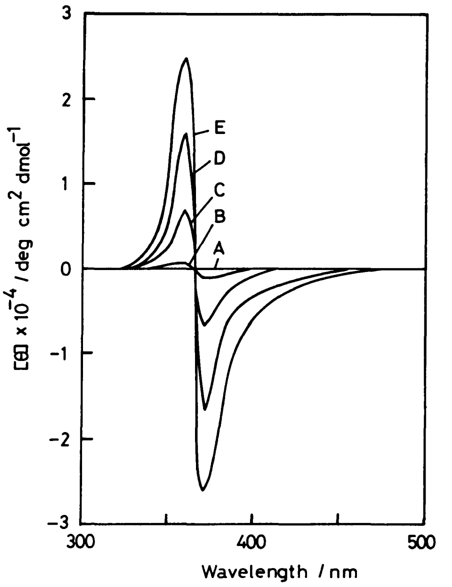


Fig. 1. Time dependence of the induced CD spectra of the poly(Lys HCl-Gly)-MO complex in water at pH 6 and R/D=5; MO, 10^{-4} mol dm^{-3}. A at 0 h, B after 1 h, C after 9 h, D after 23 h, and E after 47 h.

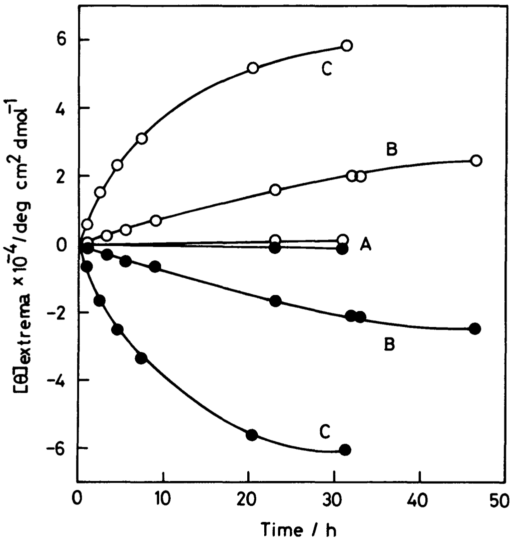


Fig. 2. Concentration and time dependences of the induced CD intensities of the poly(Lys HCl-Gly)-MO complex in water at pH 6 and R/D=5; ○ at 359 nm and ● at 371 nm.
Concentration of MO (mol dm^{-3}); A 6×10^{-5}, B 10^{-4}, and C 1.4×10^{-4}.

Table 1. Induced CD of MO Complexes with Sequential Lysine Polypeptides in Water at pH 6 and R/D=5

Polypeptide	Circular Dichroism[a]	
	Wavelength	[θ]
	nm	deg cm^2 dmol^{-1}
Poly(Lys HCl)	371	−25000
	364	0
	359	25000
Poly(Lys HCl-Gly)	371	−16600
	364	0
	359	16100
Poly(Lys HCl-βAla)	435	−8300
	412	0
	397	12100
Poly(Lys HCl-εAhx)	424	−7300
	405	0
	388	8200

a) MO 10^{-4} mol dm^{-3} and after 23 h.

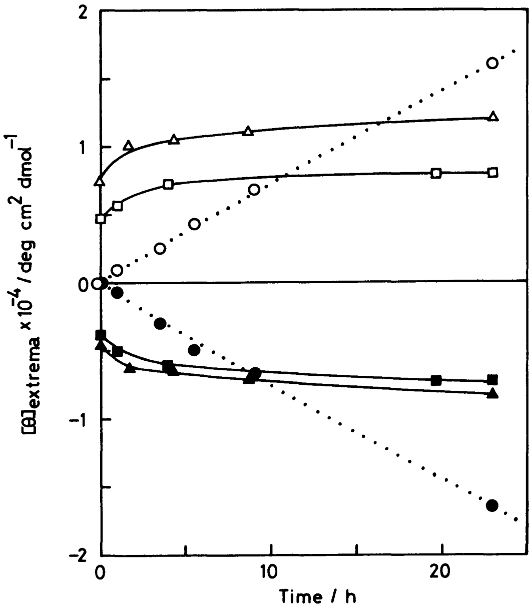


Fig. 3. Time dependence of the induced CD intensities of the poly(Lys HCl-X)-MO complexes in water at pH 6 and R/D=5; MO, 10^{-4} mol dm^{-3}. Poly(Lys HCl-βAla), △ at 397 nm and ▲ at 435 nm; poly(Lys HCl-εAhx), □ at 388 nm and ■ at 424 nm. Poly(Lys HCl-Gly) (○ at 359 nm and ● at 371 nm) was partly reproduced from Fig. 2 (curve B).

dm^{-3}, the intensities of the induced CD were continued to increase after 2 d. This suggests that an intermolecular aggregation of the complex occurs. As listed in Table 1, the poly(Lys HCl-βAla)-MO complex exhibited the induced CD with the extrema at 435 nm (negative) and at 397 nm (positive), and poly(Lys HCl-εAhx)-MO complex at 424 nm (negative) and at 388 nm (positive). The induced CD spectra of the poly(Lys HCl-βAla)-MO and poly(Lys HCl-εAhx)-MO complexes, which have one and four more backbone atoms than poly(Lys HCl-Gly)-MO complex, exhibited smaller intensities and a little time dependence as depicted in Fig. 3. This suggests that, when MO molecules that are electrostatically bound to lysine residues are separated from each other, the intra- and intermolecular interactions are

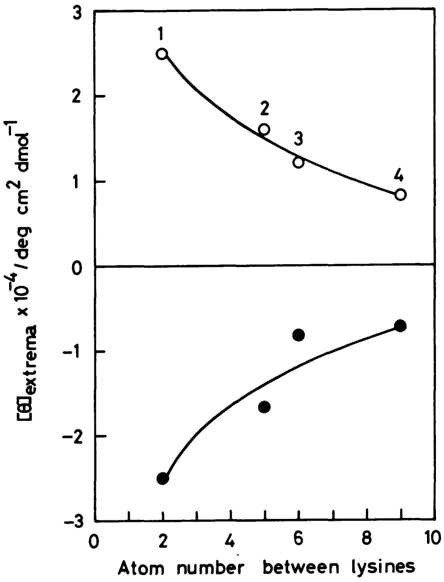


Fig. 4. Induced CD intensities at the extrema of the sequential polypeptide–MO complexes as a function of distance between lysine residues in water at pH 6 and R/D=5; MO, 10^{-4} mol dm^{-3}. After 23 h; samples 1—4, see text.

weaker.

At the beginning of the present study we expected to change the intramolecular interchromophoric distance using sequential polypeptides, although the problem was more complicated than anticipated. We tentatively plotted the induced CD intensities after 23 h at the extrema of four sequential polypeptide–MO complexes including poly(Lys HCl)–MO complex as a function of distance between C_α of the lysine residues in Fig. 4. Since the random coil conformations of the four sequential polypeptide-MO complexes did not allow an exact estimation of the distance between chromophores, the numbers of backbone atoms between lysine residues were plotted as abscissa. Boldly, it could be concluded that an increased distance between the N^ε-ammonium groups of lysine residues decreased the intensity of the induced CD (see also Fig. 3). The results in Fig. 4 are fundamentally similar to the results of the induced CD of the sequential poly(His)–MO complexes reported by us.[13] Since the DPs (40—90) of the sequential poly(Lys HCl-X) samples used here may be too low to investigate the intramolecular dye–dye interaction, very high DP samples with the same Lys HCl-X sequences might give additional information regarding the induced CD of the sequential lysine polypeptides.

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