

Induced Circular Dichroism and Mode of Binding of Acridine Orange Adsorbed on β -Form Poly(S-carboxyethyl-L-cysteine) in Aqueous Solutions

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Synopsis

The absorption spectra and circular dichroism (CD) have been measured for aqueous solutions of acridine orange of a constant concentration, $[D] = 5 \times 10^{-5}M$, mixed with poly(S-carboxyethyl-L-cysteine) in various mixing ratios, $[P]/[D]$, ranging from 330 to 11, at different pH. The absorption spectra of the dye-polymer solutions are hypochromic, and the main band is located at 470 nm, accompanying a shoulder at 500 nm. At alkaline pH, no CD is induced in the visible region. At neutral and acidic pH, where the polymer is in the β -conformation, CD is induced in the visible and near-uv regions. A pair of CD bands is located at the region around 450 nm, when the pH is around the neutrality, while it appears at the region around 500 nm at acidic pH. Thus, the optically active species of bound dye changes from dimer to monomer on lowering the pH. These species form dissymmetric arrays along a polypeptide chain. The fraction of bound dye forming dissymmetric sequences is not high, but most of bound dye is adsorbed randomly on the ionized carboxyl groups of polypeptide chain and gives rise to hypochromism only. A dissymmetric structure of dye-polymer complexes is presented, in which the polymer has the β -conformation and the dye cations, either dimeric or monomeric, bind to its side chains, in such a way that the longer axes of molecular planes of bound dye form a two-fold, right-handed helix along the extended polypeptide chain. A zeroth-order calculation of CD based on the coupled oscillator model leads to the result that each dissymmetric array of dye consists, on the average, of two dimeric or monomeric cations. This low number of bound cations in a dissymmetric array and the large fraction of randomly adsorbed dye suggest that the hydrophobic interaction of dye with the polymer is strong, so that dye cations are adsorbed sparsely on both sides of the extended polypeptide chain.

INTRODUCTION

The induced circular dichroism (CD) of dye bound to biopolymers is a unique characteristic reflecting the conformation of biopolymers and the mode of binding of dye. The induced optical activity was discovered two decades ago for aqueous solutions of some cationic dyes mixed with α -helical poly(L-glutamic acid) [poly(Glu)].^{1,2} Since then, the induced CD of complexes of various dyes bound to poly(Glu), either helical or randomly coiled, has been investigated by many workers.³⁻⁷ We have proposed a structure of acridine orange- α -helical poly(Glu) complex that explains the observed CD and absorption spectra.⁵

Poly(S-carboxymethyl-L-cysteine) (poly[Cys(CH₂COOH)]) forms the

β -conformation in aqueous solutions of low pH, and the induced CD of acridine dyes bound to β -form poly[Cys(CH₂COOH)] was investigated in great detail.⁸⁻¹⁰ The induced CD of acridine orange bound to β -form poly[Cys(CH₂COOH)] is quite different from that of the case of α -helical poly(Glu), reflecting the difference in structure between the β -form and α -helix. We have presented a structure of acridine orange- β -form poly[Cys(CH₂COOH)] complex that can be derived from the induced CD and absorption spectra.^{8,10}

For both α -helical poly(Glu) and β -form poly[Cys(CH₂COOH)], acridine orange binds to their ionized carboxyl groups, and the long axes of molecular planes of bound dimeric dye cations follow a right-handed helix of 1.8 and 2 folds, respectively. For these polypeptides the modes of binding of dye are identical with each other, and the induced optical activity reflects the main-chain conformation of polypeptide chains.

In this paper we investigate acridine orange- β -form poly(*S*-carboxyethyl-L-cysteine) complexes, of which the induced optical activity is different from that of acridine orange- β -form poly[Cys(CH₂COOH)]. Poly(*S*-carboxyethyl-L-cysteine) (poly[Cys((CH₂)₂COOH)]) can assume the β -conformation in aqueous solutions at neutral and acidic pH.¹¹ However, this polymer has characteristics distinct from its lower side-chain homolog in two respects.¹² The intrinsic CD of the two polypeptides in water differs in its magnitude, especially at acidic pH: the residue ellipticity at 200 nm of the β -conformation of poly[Cys((CH₂)₂COOH)] has a value about a half that of poly[Cys(CH₂COOH)]. The β -form poly[Cys((CH₂)₂COOH)] has greater stability than the β -form poly[Cys(CH₂COOH)] with respect to the electrostatic repulsion: the pH-induced coil-to- β transition of poly[Cys((CH₂)₂COOH)] occurs at pH 7.3, about 2 pH units higher than that of poly[Cys(CH₂COOH)] does, in spite of the fact that their carboxyl groups have p*K* values which differ by only 1 pH unit.

In this work the absorption spectra and CD are measured for aqueous solutions of acridine orange mixed with poly[Cys((CH₂)₂COOH)] in different polymer residue-to-dye mixing ratios, $[P]/[D]$, and the effect of complex formation on the spectroscopic properties is examined at different pH values, with reference to the polypeptide conformation. We confine ourselves to the dye-polymer solutions of relatively high $[P]/[D]$ ratios, 330-11. Thus, the induced CD is observed only at neutral and acidic pH, where poly[Cys((CH₂)₂COOH)] is in the β -conformation.

EXPERIMENTAL

Materials

A sample of poly[Cys((CH₂)₂COOH)] with a code number K917 was used throughout the present work; its properties were described previously.¹² The polymer sample has an intrinsic viscosity, 0.310 dl g⁻¹, in 0.2*M* NaCl

at pH from 6.9 to 9.5. Acridine orange was purified from a commercial material purchased from the Sigma Chemicals Co., Inc., as previously described.^{8,13}

Preparation of Dye-Polymer Solutions

Stock solutions of acridine orange had a concentration of $5.0 \times 10^{-4}M$, and the final concentration of acridine orange in the dye-polymer solutions was always adjusted to $5.0 \times 10^{-5}M$.

Stock solutions of poly[Cys((CH₂)₂COOH)] were prepared by suspending the polymer sample in distilled water or 0.2M NaCl solution, dissolving at pH 9.5 by the addition of 0.1N NaOH, and then back-titrating the solution down to pH 7.4 with 0.1N HCl. The polymer concentration was about 0.5 g dl⁻¹ or $2.5 \times 10^{-2}N$.

To prepare a series of dye-polymer solutions of different $[P]/[D]$ ratios at constant pH or of constant $[P]/[D]$ at different pH, an aliquot of the acridine orange solution was added to the stock solution of the polymer, and the dye-polymer solution was diluted with water or 0.2M NaCl solution. The pH was adjusted by adding 0.01N NaOH or 0.01N HCl.

Apparatus

The pH of solutions was measured by a glass electrode on a Hitachi-Horiba model F-5 pH meter. The pH of the dye-polymer solutions was examined before and after each spectroscopic measurement; it was confirmed that the pH values were in agreement with each other within 0.05.

The absorption spectra were recorded on a Jasco ORD/UV 5 spectrophotometer, using a quartz cell with a path length of 2 mm. The CD was measured on a Jasco J-20 circular dichrometer, using a quartz cell 1 cm thick. This instrument was calibrated by means of aqueous solutions of (+)-10-camphor sulfonic acid. The wavelengths covered in these spectroscopic measurements ranged from 600 to 260 nm.

All measurements were performed at room temperature ($25 \pm 2^\circ C$).

Absorption spectra and CD were expressed by the molar extinction coefficient, ϵ_D , and the molar ellipticity, $[\theta_D]$, both based on the molar concentration of total added dye.

The absorption spectra of the dye-polymer solutions had a background contribution, especially in the near uv region, which arose from the tail of the polymer absorption bands in the far-uv region. Furthermore, the dye-polymer solution was generally more turbid, shifting the background curve upward, as the pH was lower than about 6 and the $[P]/[D]$ ratio or the polymer concentration was higher. Consequently, the background curves were appropriately subtracted from the recorded spectra. These corrected absorption spectra should represent the structure of the dye-polymer complexes.

RESULTS

[P]/[D] Dependence

The absorption spectra of free acridine orange in water consist of two visible bands, one at 492 nm and the other at 470 nm, as shown in Fig. 1, and these bands are assigned to the monomeric and dimeric dye cations, respectively.¹³⁻¹⁸ At the dye concentration, $[D] = 5 \times 10^{-5}M$, acridine orange is mostly in the monomeric form and partly in the dimeric form. The uv band at 270 nm is not influenced by the dimerization equilibrium very much.

Figure 1 shows the absorption spectra of the dye-polymer solutions of different $[P]/[D]$ ratios at pH 7.6. The absorption band of dye is now at 470 nm and strong hypochromism occurs. These changes indicate that the dye is strongly adsorbed on the polymer and the bound dye is mostly dimeric, in contrast to the free monomeric dye. At this alkaline pH, where the side-chain carboxyl groups of the polymer are completely ionized, the absorption spectra do not change appreciably with the molar mixing ratio, $[P]/[D]$. Thus, almost all the dye is adsorbed on the polymer at alkaline pH. However, these alkaline dye-polymer solutions do not show any CD in the visible region. Thus, the dimeric dye cations bound to the randomly coiled polymer are subject to hypochromism alone. The uv band at 270 nm also undergoes the hypochromic effect.

When the pH of solution is lowered and made neutral or acidic, the absorption spectra become dependent on $[P]/[D]$, giving a stronger hypochromic effect at lower $[P]/[D]$. This effect is caused by the strong interaction between bound dye cations at lower polymer concentrations, in addition to the binding of dye to the polymer. The absorption spectra in the visible region are illustrated in Fig. 2 for the dye-polymer solutions of different $[P]/[D]$ at pH 5.6. A similar dependence of hypochromism on $[P]/[D]$ is observed in the near-uv region.

The degree of hypochromism is very high for $[P]/[D]$ as low as 11. Nevertheless, the wavelength of the main band remains at 470 nm for all $[P]/[D]$ ratios. This means that the dimer is the highest aggregate species

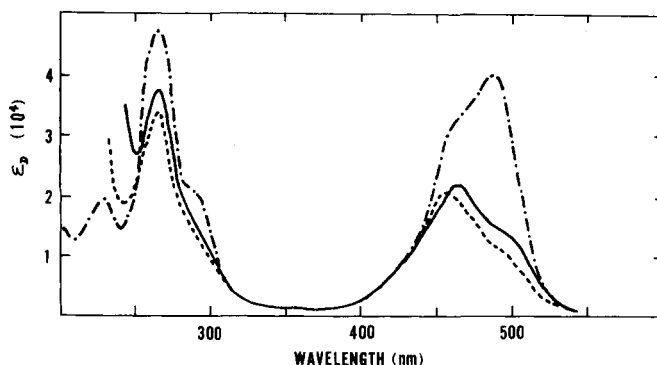


Fig. 1. Absorption spectra of acridine orange-poly[Cys((CH₂)₂COOH)] solutions in water at pH 7.6 with $[D] = 5 \times 10^{-5}M$: (---) free dye, (-.-) $[P]/[D]$ -42, (—) $[P]/[D]$ -167.

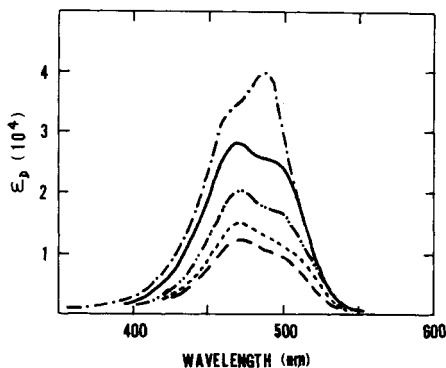


Fig. 2. Absorption spectra of acridine orange-poly[Cys((CH₂)₂COOH)] solutions in water at pH 5.6 with $[D] = 5 \times 10^{-5}M$: (---), free dye, (--) [P]/[D]-11, (···) [P]/[D]-33, (-·-·) [P]/[D]-55, (—) [P]/[D]-165.

of dye formed on the polymer; many dimeric cations are adsorbed on the polymer, without forming a stacked sequence. This is different from the results for acridine orange-poly[Cys(CH₂COOH)] solutions, for which the main visible band is at 458–460 nm. The presence of the shoulder band at 500 nm indicates that some of bound dye is monomeric, which is absent from the acridine orange-poly[Cys(CH₂COOH)] solutions. The shift of the monomer band from 492 to 500 nm is caused by the effect of the binding of monomeric dye, as was observed for DNA,^{19–21} polynucleotides,^{19,22} and polysaccharide.¹⁹

Around neutral pH, where the polymer is partly in the β -conformation, the CD is induced in the visible and near-uv regions, exhibiting positive bands at 470 and 270 nm and a negative band at 440 nm. This is somewhat similar to that of acridine orange-poly[Cys(CH₂COOH)] solutions at acidic pH.⁸ However, the magnitudes of molar ellipticity are much smaller.

At lower pH, where the polymer assumes the β -conformation, CD of a different kind is induced, in spite of almost the same absorption spectra. Figure 3 shows the CD of the dye-polymer solutions of different [P]/[D] at pH 5.6. At low [P]/[D] such as 11, a positive CD band appears at 510 nm, together with a shoulder around 480 nm, and a negative band is at 452 nm. In the uv region a positive band appears at 270 nm. At high [P]/[D] ratios the positive bands at 510 and 270 nm become strong and the negative band disappears. A positive shoulder appears around 297 nm.

The enhancement of induced CD with increasing [P]/[D] ratio is not necessarily consistent with the corresponding increase in absorption, if hypochromism were all related to the amount of bound dye. As noted above, part of the observed hypochromism must be attributed to the strong interaction between bound dye cations. Then the observed results may be interpreted as the change in mode of interaction of bound dye. That is, as [P]/[D] is increased, the interaction between bound dye cations diminishes and the dye cations can interact more directly with the polymer to form a greater number of dissymmetric sequences.

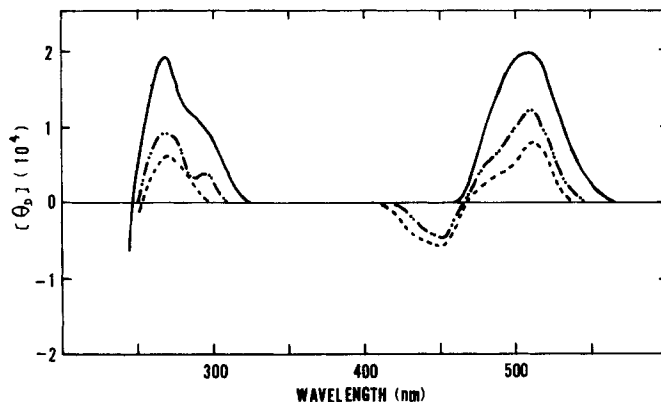


Fig. 3. CD of acridine orange-poly[Cys((CH₂)₂COOH)] solutions in water at pH 5.6 with [D] = 5 × 10⁻⁵M: (---) [P]/[D]-11, (-·-·-) [P]/[D]-55, (—) [P]/[D]-165.

pH Dependence

Figure 4 shows the absorption spectra of the dye-polymer solutions of [P]/[D]-110 at different pH. The dye-polymer solution develops visible turbidity at pH 5.4, and it separates precipitates at pH lower than 4.5. When the [P]/[D] is kept at 110, the hypochromism is the strongest at alkaline pH and the absorption increases with lowering pH. This effect would be specific for the ratio of [P]/[D]-110 or higher and would simply reflect the amount of bound dye: the number of bound dye cations decreases with decreasing number of ionized carboxyl groups upon lowering pH.

The main band is located at 463 nm when the pH is around neutrality. This indicates that bound dye is mainly dimeric at neutral pH. With lower pH, however, the band at 500 nm becomes stronger relative to that at 470 nm, indicating that the fraction of bound monomeric dye somewhat increases at acidic pH.

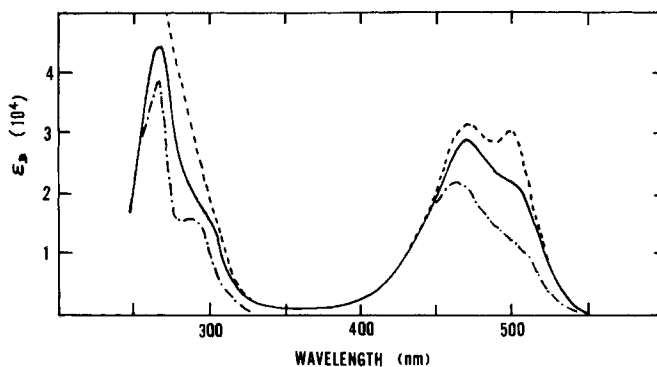


Fig. 4. Absorption spectra of acridine orange-poly[Cys((CH₂)₂COOH)] solutions of [P]/[D]-110 in water with [D] = 5 × 10⁻⁵M: (---) pH 7.27, (—) pH 6.32, (-·-·-) pH 5.18.

The CD of the dye-polymer solutions of $[P]/[D]$ -110 at different pH is shown in Fig. 5. No CD is induced at pH higher than 7.5 in the visible region. At pH 7.27 a positive CD band appears at 480 nm having a positive shoulder around 500 nm, and a negative band at 440 nm accompanies it. On lowering the pH, the positive CD band at 510 nm becomes strong, and the negative band shifts to the red. At pH 6.32 the positive band at 510 nm is the strongest, and a positive shoulder band at 490 nm follows it.

Figure 6 shows the location of visible CD bands and the values of their molar ellipticity at different pH. It clearly shows the dimer-to-monomer replacement of optically active species of bound dye occurring around pH 7. This is in accord with the corresponding replacement of the absorption bands described above. The two pairs of positive and negative CD bands in the visible region, one at 510 and 470 nm and the other at 480 and 440 nm, can be assigned to the dimeric and monomeric species of bound dye, respectively, both arranged dissymmetrically on the extended and hydrogen-bonded polypeptide chains.

Around neutral pH the bound dimeric dye is optically active and gives a conservative pair of CD bands, but at acidic pH the monomeric dye becomes the main dissymmetric species of bound dye and even shows non-conservative CD. However, the optical activity of bound dye is not very high. Most of dye cations are randomly bound to the polymer, and only some, either dimeric or monomeric, are arranged dissymmetrically. This can also be seen in the low values of the dissymmetry factor, $[\theta_D]/\epsilon_D$, as listed in Table I, which are compared with those for acridine orange poly-[Cys(CH₂COOH)] solutions.

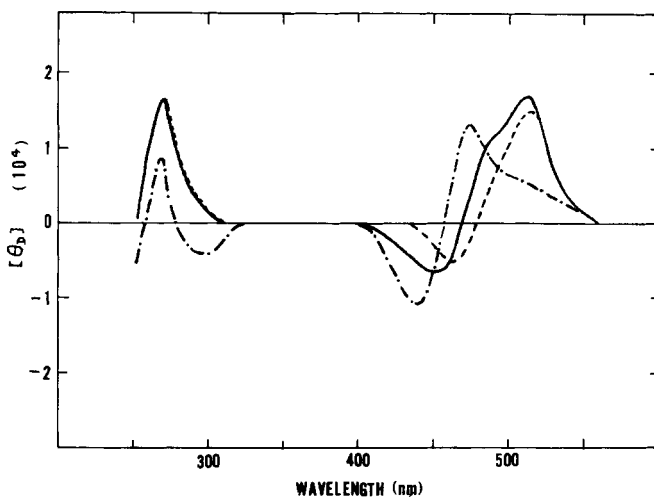


Fig. 5. CD of acridine orange-poly[Cys((CH₂)₂COOH)] solutions of $[P]/[D]$ -110 in water with $[D] = 5 \times 10^{-5}M$: (---) pH 7.27, (—) pH 6.32, (-·-·) pH 5.18.

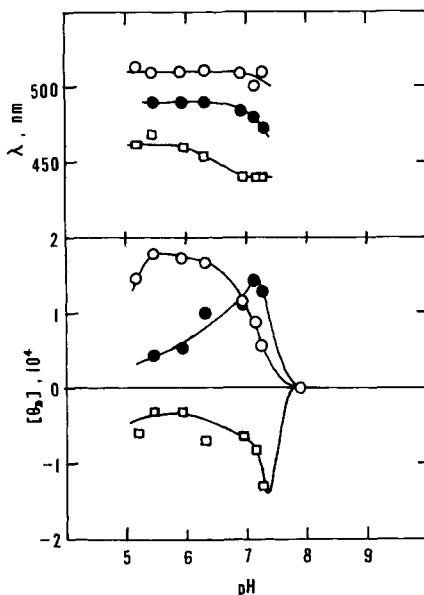


Fig. 6. pH dependence of wavelengths and molar ellipticities of CD bands for the solutions of $[P]/[D]$ -110 in water. Top, wavelengths; bottom, molar ellipticities.

Effect of Added NaCl

Figure 7 shows the absorption spectra and CD of the dye-polymer solutions of $[P]/[D]$ -100 in 0.2M NaCl. The absorption spectra are almost independent of pH, in the range of pH from 7.37 to 5.82. The hypochromism is caused by the presence of the polymer, and the main band is located at 470 nm, as in the case of no added salt. The main species of bound dye is dimeric even in the presence of added NaCl. The shoulder band at 490–500 nm indicates the presence of bound monomeric dye. The absorption spectra at acidic and neutral pH are strengthened by added NaCl, which means that the interaction between bound dye is suppressed electrostatically.

The CD of the dye-polymer solutions is exhibited at all pH regions from 7.37 to 5.82, but it differs markedly, depending on whether the pH is above or below 6.25. Above pH 6.25, the main CD bands appear at 465 and 435 nm, in the form of a conservative pair. Below pH 6.25, a conservative CD pair occurs at 484 and 463 nm. This can also be seen in Fig. 8, in which the peak positions and magnitudes of CD bands are plotted against pH. Around neutral pH dimeric dye is dominantly optically active, and at acidic pH monomeric dye is optically active. In spite of the almost identical absorption spectra, the optically active species of bound dye changes from dimer to monomer on going from neutral to acidic pH, as was also observed in the case of no added salt. The optical activity of bound dye is again weak, as can be seen from Table I.

TABLE I
 Absorption and CD of the Dye-Polymer Solutions

Polymer	Conditions	λ (nm)	ϵ_D	λ (nm)	$[\theta_D]$	$[\theta_D]/\epsilon_D$
Water Poly[Cys(CH ₂ COOH)]	pH 4.37 [P]/[D]-167	458	30,000	470	90,000	3.0
				437	-20,000	
Poly[Cys((CH ₂) ₂ COOH)]	pH 7.15 [P]/[D]-110	500	12,500	500	9000	
				480	14,500	0.71
				461	20,500	
Poly[Cys((CH ₂) ₂ COOH)]	pH 5.45 [P]/[D]-110	500	30,000	510	18,000	0.60
				490	4200	
				470	32,000	
				468	-3000	
0.2M NaCl Poly[Cys(CH ₂ COOH)]	pH 4.68 [P]/[D]-167	458	31,000	470	50,000	1.6
				437	-15,000	
Poly[Cys((CH ₂) ₂ COOH)]	pH 6.72 [P]/[D]-100	490	18,500	500	5000	
				465	9500	0.39
				460	24,500	
Poly[Cys((CH ₂) ₂ COOH)]	pH 5.82 [P]/[D]-100	500	20,000	500	9600	0.48
				484	5000	
				464	26,000	
				463	-8500	

DISCUSSION

Dissymmetric Structure of the Dye-Polymer Complexes

Acridine orange binds to the polymer mostly in the dimeric form and partly in the monomeric form. On the random-coil polymer the bound dye cations are arranged randomly. Around neutral pH, where the polymer partially takes on the β -conformation, some of the bound dimeric dye becomes optically active. At acidic pH, where the β -conformation is more complete, some of the bound monomeric dye becomes optically active, while bound dimeric dye is less active.

The induced CD of dimeric and of monomeric dye is similar, in that it consists of a conservative pair of positive and negative CD bands at the absorption bands. At the most acidic pH, and especially at higher [P]/[D] ratios, even the nonconservative CD of monomeric dye is manifest.

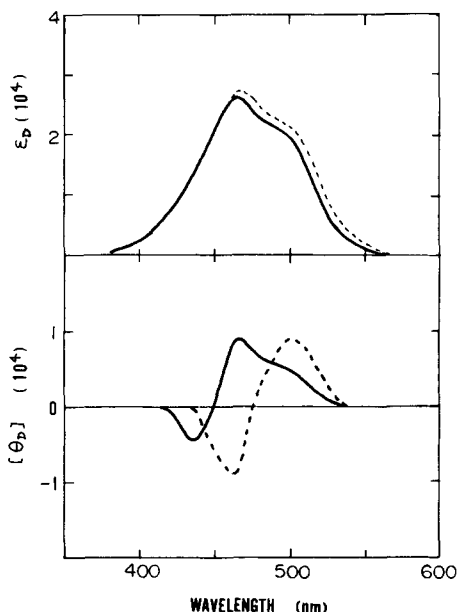


Fig. 7. Absorption spectra and CD of acridine orange-poly[Cys((CH₂)₂COOH)] solutions of $[P]/[D]$ -100 in 0.2M NaCl with $[D] = 5 \times 10^{-5}M$: (—) pH 7.37 and 6.72; (---) pH 5.82.

Thus, we can consider two kinds of similar dissymmetric structures of the dye-polymer complexes, when the polymer is in the β -conformation: one has dissymmetric arrays of bound dimeric dye cations and the other has similar arrays of monomeric cations.

In the β -structure, the polypeptide chains are extended and hydrogen-bonded to form a sheet, and the dye cations should be electrostatically bound to the ionized carboxyl groups of the polymer. In the dissymmetric array, the dye cations should bind to the ionized carboxyl groups in a sequence, forming an alternating series. They should be tilted from the direction of extended polypeptide chain in such a way that bound dye cations form a twofold helix of a definite sense around an extended polypeptide chain.

To the visible absorption band at 492 nm and the uv band at 270 nm are assigned transition electric moments, both parallel to the longer axis of the molecular plane of acridine orange.²³ A dimeric dye cation has an antiparallel stacking structure of two monomeric cations,¹³ so that its transition moment for the absorption bands at 470 and 270 nm should have the same direction as in the monomeric cation.

Considering the molecular structure of acridine dye and antiparallel extended polypeptide chains, we can propose a dissymmetric structure of the dimeric dye-polymer complex, in which the dye molecular planes stand on the side-chain direction and are vertical to the plane of main chain and hydrogen bonds or of the β -sheet, with their longer axes slightly tilted from perpendicular to the main-chain direction. Then, the transition moments

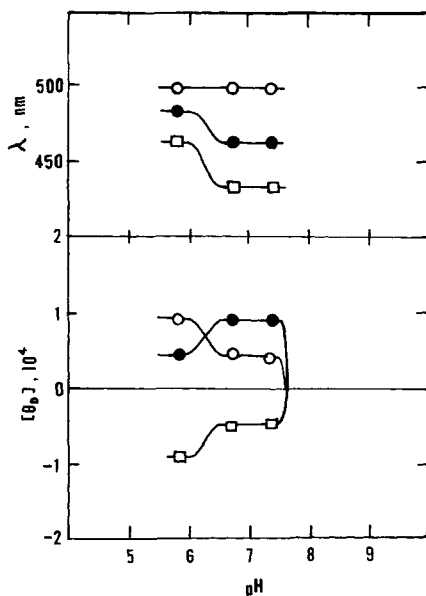


Fig. 8. pH dependence of wavelengths and molar ellipticities of CD bands for the solutions of $[P]/[D]$ -100 in 0.2M NaCl with $[D] = 5 \times 10^{-5}M$. Top, wavelengths; bottom, molar ellipticities.

of bound dye should form a twofold helix along an extended polypeptide chain, as illustrated schematically in Fig. 9. A similar structure was proposed for the complexes of acridine dyes formed with β -form poly[Cys-(CH₂COOH)].⁸⁻¹⁰

Since the observed CD has similar features, the monomeric dye cations also bind to the polymer in a similar way, forming a twofold helix of their transition moments along a polypeptide chain. In this case, the dye molecular planes might not be vertical to the plane of main chain and hydrogen bonds, or of the β -sheet, but they would lie more flatly on the plane, possibly due to hydrophobic interaction.

In these structures of dye-polymer complexes, as in the dye-poly[Cys-(CH₂COOH)] complex, the carboxyl groups of side chains of adjacent chains will play an important role in giving the bound cations a definite tilt. A possible configuration of carboxyl groups on the side chains is illustrated in Fig. 9.

Dissymmetry and CD of the Arrays of Bound Dye Cations

For a degenerate system consisting of a linear array of N identical chromophores, which may be either dimeric or monomeric cations in the present dye-polymer complexes, the molar ellipticity per chromophore for the transition, O-E, can be expressed by²⁴

$$[\theta(\nu)]_E = \sum_{K=1}^N [\theta(\nu)]_{EK} = \frac{48\pi^2 N_A}{Nhc} \nu \sum_{K=1}^N R_{EK} f(\nu - \nu_{EK}) \quad (1)$$

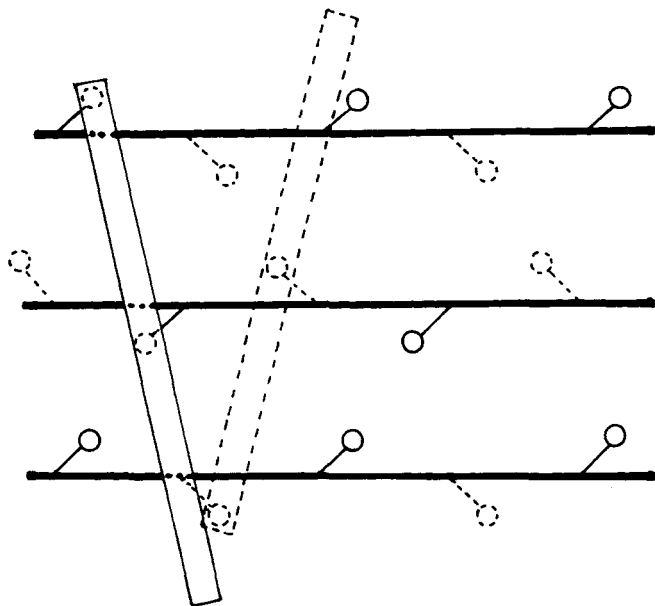


Fig. 9. Dissymmetric array of transition electric moments (longer axes of molecular planes) of dye cations bound to antiparallel extended polypeptide chains. Twofold, right-handed helix: $\mu_{0e,\parallel}\mu_{0e,t} > 0$. \circ , Carboxyl group on the side chain.

where N_A is Avogadro's number, h the Planck constant, and c the light velocity; and $f(\nu - \nu_{EK})$ is the normalized band shape at a frequency ν . The frequency, ν_{EK} , and the rotatory strength, R_{EK} , for the K th exciton level of an excited state, E , can be calculated by the perturbation theory. The frequency is given by

$$\nu_{EK} = \nu_{E0} + \frac{1}{h} \sum_{k=1}^N \sum_{l=1}^N C_{keK} C_{leK} V_{k0e,l0e} \quad (2)$$

where ν_{E0} is the unperturbed frequency, C_{keK}, C_{leK} is the exciton coefficient of the correct zeroth wave function, and $V_{k0e,l0e}$ is the interaction potential between transition electric moments of k th and l th chromophores. Here, E stands for the visible, A , and the near-uv, B , transitions.

We consider a linear alternating array or the twofold helix consisting of chromophores, each having a transition moment, μ_{0e} , at the radius (the distance from the helix axis), a , whose parallel and tangential components are given by $\mu_{0e,\parallel} = \mu_{0e} \cos \theta$ and $\mu_{0e,t} = \mu_{0e} \sin \theta$, where θ is the angle between the helix axis and the transition moment. Then, the rotatory strength is given by²⁵

$$R_{EK} = \frac{2\pi\nu_{E0}}{c} \left(\sum_{k=1}^N \sum_{l=1}^N C_{keK} C_{leK} \right) a \mu_{0e,\parallel} \mu_{0e,t} \quad (3)$$

For semiquantitative discussion on constructing a possible structure of

the dissymmetric complexes, we use a neighbor approximation for the exciton coefficient.^{26,27} Then, we have

$$C_{keK} = \left(\frac{2}{N+1} \right)^{1/2} \sin \frac{\pi k K}{N+1} \quad (4)$$

The dipole strengths have significant values only at the two exciton levels, $K = 1$ and N , which prove to be parallel and perpendicularly polarized, respectively. The frequencies are given by

$$\nu_{E\parallel} = \nu_{E1} = \nu_{E0} + \frac{2}{h(N+1)} \sum_{k=1}^N \sum_{l=1}^N V_{k0e,l0e} \sin \frac{\pi k}{N+1} \sin \frac{\pi l}{N+1} \quad (5a)$$

$$\nu_{E\perp} = \nu_{EN} = \nu_{E0} + \frac{2}{h(N+1)} \sum_{k=1}^N \sum_{l=1}^N (-1)^{k+l} V_{k0e,l0e} \times \sin \frac{\pi k}{N+1} \sin \frac{\pi l}{N+1} \quad (5b)$$

The rotatory strengths also have significant magnitudes only at these two frequencies:

$$R_{E\parallel} = R_{E1} = \frac{\pi \nu_{E0}}{c} \frac{2}{N+1} a \mu_{0e,\parallel} \mu_{0e,t} \cot^2 \frac{\pi}{2(N+1)} \quad (6a)$$

$$R_{E\perp} = R_{EN} = - \frac{\pi \nu_{E0}}{c} \frac{2}{N+1} a \mu_{0e,\parallel} \mu_{0e,t} \cot^2 \frac{\pi}{2(N+1)} \quad (6b)$$

Then, we have a conservative pair of positive and negative CD bands represented by

$$[\theta(\nu)]_E = \frac{48\pi^2 N_A}{Nhc} \nu \{ R_{E\parallel} f(\nu - \nu_{E\parallel}) + R_{E\perp} f(\nu - \nu_{E\perp}) \} \quad (7)$$

The sign of the conservative pair of CD bands is determined by the sign of interaction potential, $V_{k0e,l0e}$, and the direction of transition moments or the sense of polarization helix, i.e., the sign of $\mu_{0e,\parallel} \mu_{0e,t}$. The difference in the two active frequencies is

$$\nu_{E\parallel} - \nu_{E\perp} = \frac{4}{h(N+1)} \sum_{k+l:\text{odd}} V_{k0e,l0e} \sin \frac{\pi k}{N+1} \sin \frac{\pi l}{N+1} \quad (8)$$

If the interaction between the transition moments on the opposite side of a chain is attractive, i.e., $V_{k0e,l0e} < 0$, then we have $\nu_{E\parallel} < \nu_{E\perp}$; that is, the parallel polarized band appears at a longer-wavelength side than the perpendicular band. We have proposed structures of the complexes, especially, for the case of dimeric dye cations, in which the transition electric moment should be almost perpendicular to the helix axis. In these structures, $V_{k0e,l0e}$ is negative for odd values of $k+l$. Then, the dipole strength for the parallel band should have a negligible magnitude, and only the perpendicular band can contribute to the absorption spectra. This result seems to be coincident with the observed absorption spectra.

The signs of rotatory strengths are determined by the sense of the po-

larization helix, i.e., the sign of $\mu_{0e,\parallel}\mu_{0e,t}$. If the longer axes of dye molecules form a right-handed helix, i.e., $\mu_{0e,\parallel}\mu_{0e,t} > 0$, then the parallel polarized band should be associated with the positive CD band, and the perpendicular band with the negative CD band. Then, in the case of $V_{k0e,t0e} < 0$ for odd values of $k + l$, the positive CD band should appear at a longer-wavelength side than the negative CD band. This is in agreement with the conservative CD pairs observed for the dye-polymer solutions. Thus, the dissymmetric dye-polymer complexes should have such structures as illustrated in Fig. 9; the polarization helix is right-handed.

Calculated CD and Low Dissymmetry of Bound Dye

The CD of the dye-polymer complexes was calculated numerically over the visible and near-uv regions by means of Eqs. (1)–(3), using the values of parameters obtained from the observed absorption spectra or their assumed values. The magnitude of dipole strength is related to the molar extinction coefficient by

$$\mu_{0e}^2 = i \frac{6909hc}{8\pi^3 N_A} \int \frac{\epsilon_D}{\nu} d\nu \quad (9)$$

For the dimeric dye-polymer complex, the absorption spectra of the solutions of $[P]/[D]$ 110 at pH 7.27 were integrated over the visible band of dimer, assuming a symmetrical shape, and the transition moment, μ_{0a} , was calculated by putting $i = 2$ in Eq. (9), since the dimeric dye cation is a chromophore. The transition moment for the uv band, μ_{0b} , was calculated simply by postulating that the value of ratio, μ_{0b}/μ_{0a} , is equal to that of the acridine orange-poly[Cys(CH₂COOH)] complex previously observed.¹⁰

For the monomeric dye-polymer complex, we do not have any appropriate absorption spectra, and, consequently, the values of transition moments for the two bands were obtained by integrating the monomer spectra for the free dye of concentration, $[D] = 5 \times 10^{-5}M$, in water, assuming symmetrical shapes and putting $i = 1$ in Eq. (9).

These estimated values of the transition moments are listed in Table II, together with the unperturbed wavelengths, whose values were again taken from those of the dye-poly[Cys(CH₂COOH)] solutions.

TABLE II
Values of Optical Parameters

Chromophore	<i>E</i>	$\lambda_{E0} = \frac{c}{\nu_{E0}}$ (nm)	μ_{0e} (D)	$\frac{\theta_E}{c}$ (cm ⁻¹)
Dimer	A	458	7.73	1725
	B	261	8.67	3400
Monomer	A	492	7.55	1725
	B	261	8.43	3400

The geometry of the complexes was based on the β -structure of anti-parallel chains having side chains of appropriate length. The transition dipoles were put on a radius, $a = 7.0 \text{ \AA}$, and the repeat distance in the main-chain direction was put as $2b = 7.0 \text{ \AA}$. The transition dipoles were tilted from perpendicular to the main-chain direction by $90^\circ - \theta = 10^\circ$.

The values of exciton coefficients were numerically calculated by the perturbation theory. It was found that the rotatory strength has values nearly equal to those in the neighbor approximation, except in the case of $N = 3$. Thus, we calculated the CD spectra by means of Eq.(7). The band shape was assumed to be Gaussian:

$$f(\nu - \nu_{EK}) = \frac{1}{\theta_E \sqrt{\pi}} \exp\left(-\frac{(\nu - \nu_{EK})^2}{\theta_E^2}\right) \quad (10)$$

The values of half-width, θ_E , were again assumed to be identical with those for the dye-poly[Cys(CH₂COOH)] solutions.

In Fig. 10 the CD calculated for the dimeric dye-polymer complexes of different N values and expressed on the basis of mole of monomeric dye are shown, together with those observed for the solution with $[P]/[D]$ -110 at pH 7.15. Similarly, Fig. 11 compares the calculated CD for the monomeric dye-polymer complexes of different N values with those observed for the solutions of $[P]/[D]$ -110 at pH 5.45.

For both complexes the observed CD has magnitudes even smaller than those calculated for $N = 2$. Since the observed CD is expressed on the basis of total concentration of added dye, which is mostly bound randomly and partly bound dissymmetrically, and also partly free, the actual or effective magnitudes of observed CD should be larger than those recorded here. However, even considering this difference, the average number of chromophoric dye cations, either dimeric or monomeric, would be very small, at most 2. Such a short dissymmetric sequence of bound dye cations

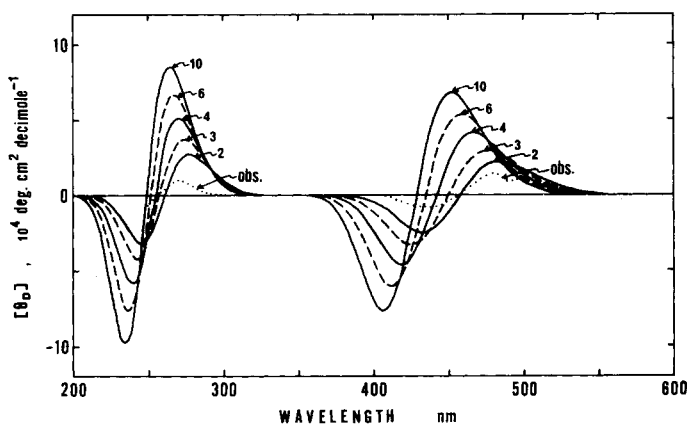


Fig. 10. Calculated CD of acridine orange- β -form polypeptide complexes consisting of N dimeric dye cations: the observed CD for acridine orange-poly[Cys((CH₂)₂COOH)] solutions of $[P]/[D]$ -110 in water at pH 7.15 (---).

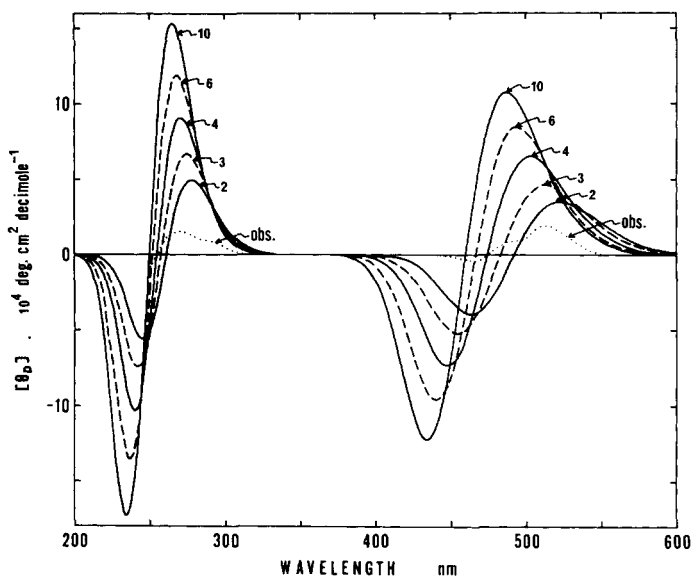


Fig. 11. Calculated CD of acridine orange- β -form polypeptide complexes consisting of N monomeric dye cations: the observed CD for acridine orange-poly[Cys((CH₂)₂COOH)] solutions of $[P]/[D]$ -110 in water at pH 5.45 (· · ·).

is characteristic of the present polymer, which can be compared with the average number, 4, of dimeric dye cations bound in a sequence on poly-[Cys(CH₂COOH)].¹⁰

This result means that a dimeric or monomeric cation is adsorbed on each side of an extended polypeptide chain, isolated from the other without stacking together. In spite of the strong stacking tendency of the dye, as revealed even when it is bound to various biopolymers,^{13-16,19-22} the bound dye cations are more stable on the polypeptide chain when adsorbed sparsely rather than when bound stacking together. Such isolated adsorption of dye cations on the polymer can be attributed mainly to the strong hydrophobic interaction of the dye with the polymer, which is caused by the presence of an additional methylene group on the side chain, as compared with poly[Cys(CH₂COOH)].

A recent work²⁸ claimed that an inherently dissymmetric dye, 2-*p*-toluidinonaphthalene-6-sulfonate, was induced the CD at its absorption bands when it was adsorbed on β -form poly(L-lysine) at pH 10.8. In that case, a single molecule bound to poly(L-lysine) can acquire molecular dissymmetry by the axial chirality.

In the present dye-polymer complexes, however, it is necessary to have dissymmetric electronic coupling among two or more bound dye cations. Such coupling can occur even for these isolated bound dye cations, since there is a good probability that two dye cations will be adsorbed at the consecutive ionized carboxyl groups, which are on the opposite sides of a chain. This corresponds to the case of $N = 2$.

There is another reason for the weak CD; the β -conformation is incomplete and some random-coil parts are involved. Then the fraction of dye bound in dissymmetric sequences should be low as compared with the case of more complete formation of the β -conformation as in poly[Cys-(CH₂COOH)]. This situation will be especially true in the case of the dimeric dye-polymer complex, which is formed at neutral pH.

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