

Calculated Circular Dichroism of Acridine Orange Bound to Random-Coil Polypeptide

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ABSTRACT: A theory of circular dichroism for the amide transition of polypeptide in random-coil conformation has been developed on the basis of the exciton theory of optical activity and the Monte-Carlo approach to the treatment of statistical systems. This theory has further been extended in such a way as to be applicable for electronic transitions of chromophores bound to the side chains of the random-coil polypeptides. Circular dichroism is calculated for some binding models of acridine orange—random-coil polypeptide complexes. The results are compared with those observed, and it is found that the longer axes of the bound dye molecules are almost parallel or perpendicular to a polypeptide chain, depending on whether ionization of the polypeptide is low or high.

KEY WORDS Circular Dichroism / Acridine Orange / Random-Coil Polypeptide / Poly(*S*-carboxymethyl-L-cysteine) / Poly(L-glutamic acid) /

Recently we reported that various types of circular dichroism (CD) are induced on acridine orange bound to poly(*S*-carboxymethyl-L-cysteine) in solution.¹ These are observed at different pH and [P]/[D] (polymer residue/added dye) mixing ratios, and by polymers of different molecular weights. The observed CD is characteristic of the bonding species of the dye, dimeric dye or monomeric dye, as well as of the conformation of the polymer and can be classified into several types on the basis of the number and sign of the CD bands.

Type I CD is induced on an aggregate of dimeric dyes bound to a polymer in the β -conformation and has a pair of bands at 466 and 433 nm. A model for the binding mode has been presented and discussed,^{2,3} in which the dimeric dye molecules attach to ionized carboxyl groups and stack together to form linear arrays on both sides of an extended polypeptide chain.

Types II and III CD have a pair of bands at 470 and 420 nm and additional bands at longer wavelength. These bands are opposite sign to

each other for these two types. Types II' and III' CD have bands at 540 and 425 nm with opposite sign to each other. While Types II and II' CD are exhibited by the solutions of random-coil polymers with low ionization, Types III and III' CD are characteristic of highly ionized random-coil polymers and of solutions of low [P]/[D] ratios.

The acridine orange—poly(L-glutamic acid) mixture also induces Types III and III' CD at low [P]/[D] ratios and alkaline pH.^{4,5} The CD spectra similar to Types II and III were recently reported for an acridine orange—glycosaminoglycan mixture by Salter, *et al.*,⁶ whose designation accidentally coincided with our's except for Type I.

Several theoretical investigations⁷⁻¹⁰ have attempted to evaluate the optical activity for the amide π — π^* and n — π^* transitions of polypeptide in random-coil conformation. However, these have relied on different approximations and methods and, consequently, have yielded inconsistent results. In the present work, we will apply a method of calculation to the optical

activity of random-coil system, which was developed by means of an exciton theory of optical activity^{11,12} and a Monte-Carlo approach for the treatment of statistical systems. Then we shall extend it to the case of the electronic transitions of dye chromophores bound to side chains of the random-coil polymer and discuss the binding models of acridine orange—random-coil polymer complexes which induce Types II, III, II', and III' CD.

CALCULATION

Binding Mode of Acridine Orange to the Random-Coil Polypeptide

In solutions for which Type II CD is induced, carboxyl groups of the polymer are less ionized and adsorb dimeric dyes.¹ The polymer chain should be rather compact and the bound dyes on it should form a short sequence. The polymer in the solutions inducing Type III CD is fully ionized and most of its carboxyl groups should be occupied by the dimeric cations.¹ The high charge density and bulky bound dye should cause the polymer chain to extend, owing to electrostatic repulsion and steric hindrance of bound dyes. In Types II' and III' complexes, the binding unit is the monomeric dye, instead of the dimeric dye in Types II and III complexes.

Polarization of Absorption Bands

According to Zanker,^{13,14} the 492 nm absorption band of acridine orange can be assigned as the transition for the ¹L_b state and its polarization is along the longer axis of the dye molecular plane. The 470 nm band in the dimeric form, belonging to the same electronic transition as ¹L_b, should have the same polarization as the monomeric form, when Zanker's anti-parallel dimer model¹⁵ is assumed. The electrically forbidden ¹L_a transition of the monomeric dye may appear around 540 nm with a very small extinction coefficient, at the time the dye aggregates.¹³

The 260 nm band is assigned as the transition for the ¹B_b state,¹⁶ whose polarization is along the longer axis of molecular plane,¹⁷ while the 290 nm band, assigned as the ¹B_a transition,¹⁶ has a polarization parallel to the shorter axis, like the 540 nm band.¹⁷ Assignment of the band at 230 nm is not definite. Table I summarizes

Table I. Electronic parameters for the visible and ultraviolet transitions

Bound species	c/ν_{E0} , nm	μ_{0e} , D	Θ_E/c , cm ⁻¹	State	Polarization direction
Dimer	540	1.00	1725	¹ L _a	Shorter axis
	458 ^a	9.18	1725	¹ L _b	Longer axis
	294	2.40 ^b	—	¹ B _a	Shorter axis
	261	10.29	—	¹ B _b	Longer axis
	230	2.50 ^b	—	—	—
Monomer	492	7.55	1725	¹ L _b	Longer axis
	294	1.98	—	¹ B _a	Shorter axis
	261	8.43	—	¹ B _b	Longer axis
	230	2.06	—	—	—

^a Dimeric band of free acridine orange in 470 nm shifts to blue in the presence of polypeptide.

^b These were calculated as $\mu_{0e}(\text{dimer}) = \{\mu_{01B_b}(\text{dimer}) / \mu_{01B_b}(\text{monomer})\} \mu_{0e}(\text{monomer}) = (10.29/8.43) \mu_{0e}(\text{monomer})$.

these assignments.

We will focus our attention on calculation of conservative CD for the ¹L_b transition, since a pair of CD bands is observed on both sides of its absorption band. The ¹B_b transition should make the largest contribution to the nonconservative term of CD for the ¹L_b transition, owing to its large transition dipole moment. For the electrically allowed ¹L_a, ¹L_b, ¹B_a, and ¹B_b transitions, their magnetic moments are assumed to be small.

Theory and Method

The spatial configuration of the L-polypeptide chain is determined by the pairs of rotational angles, φ around the N—C^α bond and ψ around the C^α—C' bond. We have adopted the convention in which bond rotation angles φ and ψ are zero in the planar trans conformation.¹⁸ The transformation matrix from the Cartesian reference frame of the residue $k+1$ to that of the residue k is represented by T_k and is formulated as a function of φ_k and ψ_k associated with the residue k .¹⁸ We consider that an array of dye formed along a random-coil polypeptide chain is composed of N dye units attached to side chains of N successive residues, numbered 1 to N , on a polymer chain. Thus the dye chromophores form an exciton, and an excited state E splits into N exciton levels.

Let column vectors μ_{k0e} and μ_{l0e} be the electric

transition dipole moments of the k -th and l -th chromophores, respectively, for the transition 0—e. The rotatory strength for the K -th exciton level of an electrically allowed transition 0—E is, to the zeroth order perturbation, expressed by,

$$R_{EK} = -\frac{\pi\nu_{E0}}{c} \sum_{k=1}^N \sum_{l>k}^N C_{keK} C_{leK} \times \{ \mathbf{r}_{kl} \cdot [\boldsymbol{\mu}_{k0e} \times \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0e}] \} \quad (1)$$

where c is the light velocity, ν_{E0} the excitation frequency of a chromophore in the static field of the rest of the array, and C_{keK} and C_{leK} the exciton coefficients. \mathbf{r}_{kl} is the distance vector from the position of the transition dipole of the k -th chromophore to that of the l -th one. $\mathbf{T}_k^{(l-k)}$ means the serial product of $l-k$ factors, the first of which is \mathbf{T}_k . The frequency for the K -th level of a transition 0—E, ν_{EK} , is shifted from the unperturbed frequency ν_{E0} by the perturbation energy term E'_{EK}/h , where h is Planck's constant. The exciton coefficients and the perturbation energies are obtained by the orthonormal conditions and by solving a set of applicable linear equations.^{3,12} The interaction potential $V_{k0e,l0e}$ between electric dipoles of the k -th and l -th chromophores, both for the transition 0—e, is described by the dipole approximation in the form

$$V_{k0e,l0e} = \{ (\mathbf{r}_{kl} \cdot \mathbf{r}_{kl}) (\boldsymbol{\mu}_{k0e} \cdot \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0e}) - 3(\boldsymbol{\mu}_{k0e} \cdot \mathbf{r}_{kl}) (\mathbf{r}_{kl} \cdot \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0e}) \} (\mathbf{r}_{kl} \cdot \mathbf{r}_{kl})^{-5/2} \quad (2)$$

where $V_{k0e,k0e} = 0$. The interaction potential matrix is symmetric. The molar ellipticity per chromophore associated with the excited state E, at the frequency ν , is written as

$$[\theta]_E = \frac{48\pi^2 N_A}{hcN} \nu \sum_{K=1}^N R_{EK} f(\nu - \nu_{EK}) \quad (3)$$

where N_A is Avogadro's number. $f(\nu - \nu_{EK})$ is the shape function of a CD band associated with the level EK and is expressed as a Gaussian function with a half width θ_E .

The first order CD of a transition 0—E arising from its electronic coupling with another electrically allowed transition 0—F is calculated by

$$\sum_{K=1}^N R_{EK} = - \sum_{K=1}^N R_{FK} = \frac{2\pi}{hc} \frac{\nu_{E0}\nu_{F0}}{\nu_{F0}^2 - \nu_{E0}^2}$$

$$\times \sum_{k=1}^N \sum_{l>k}^N \{ (\mathbf{r}_{kl} \cdot [\boldsymbol{\mu}_{k0e} \times \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0f}]) V_{k0e,l0f} + (\mathbf{r}_{kl} \cdot [\boldsymbol{\mu}_{k0f} \times \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0e}]) V_{k0f,l0e} \} \quad (4)$$

$$[\theta]_E = \frac{48\pi^2 N_A}{hcN} \nu f(\nu - \nu_{E0}) \sum_{K=1}^N R_{EK} \quad (5)$$

The interaction potentials $V_{k0e,l0f}$ and $V_{k0f,l0e}$ between different transitions 0—e and 0—f have forms identical with eq 2, except for $\boldsymbol{\mu}_{l0f}$ in place of $\boldsymbol{\mu}_{l0e}$ or for $\boldsymbol{\mu}_{k0f}$ in place of $\boldsymbol{\mu}_{k0e}$. If the two excited states E and F have an identical polarization direction, eq 4 reduces to

$$\sum_{K=1}^N R_{EK} = - \sum_{K=1}^N R_{FK} = \frac{4\pi}{hc} \frac{\nu_{E0}\nu_{F0}}{\nu_{F0}^2 - \nu_{E0}^2} \left(\frac{\mu_{0f}}{\mu_{0e}} \right)^2 \times \sum_{k=1}^N \sum_{l>k}^N (\mathbf{r}_{kl} \cdot [\boldsymbol{\mu}_{k0e} \times \mathbf{T}_k^{(l-k)} \boldsymbol{\mu}_{l0e}]) V_{k0e,l0e} \quad (6)$$

where μ_{0e} and μ_{0f} are the magnitudes of the dipole moments associated with the transitions 0—e and 0—f.

The hypothetical virtual bonds which join successive α -carbon atoms each other are represented by the bond vectors $\mathbf{l}_{u,k}$ with a suffix k numbered from 1 to N . The X_k axis of the coordinate system affixed to bond k is taken in the direction of bond k . The Y_k axis is in the plane of bonds $k-1$ and k with its positive direction chosen to render its projection on X_{k-1} positive. The direction of the Z_k axis completes a right-handed Cartesian coordinate system.

Then, $\mathbf{l}_{u,k} = l_u \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}$. The virtual bond length l_u is taken as 3.80 Å.¹⁸

Let \mathbf{r}_k and \mathbf{r}_l be the position column vectors for the transition moment in the k -th or l -th chromophore. Then,

$$\mathbf{r}_{kl} = \sum_{i=1}^{l-k} \mathbf{T}_k^{(i)} \mathbf{l}_{u,k} + \mathbf{T}_k^{(l-k)} \mathbf{r}_l - \mathbf{r}_k \quad (7)$$

It may be postulated that the direction of side chain is from C^α to C^β and the transition moment of dye chromophore is situated on its extension. When l_c represents the distance between C^α and the position of the transition moment, \mathbf{r}_k is calculated to be

$$r_k = \begin{bmatrix} r_{k,X} \\ r_{k,Y} \\ r_{k,Z} \end{bmatrix} = l_c \begin{bmatrix} \cos \xi & \sin \xi & 0 \\ -\sin \xi & \cos \xi & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \cos \theta^\beta \\ \sin \theta^\beta \cos(\varphi_k - 120) \\ \sin \theta^\beta \sin(\varphi_k - 120) \end{bmatrix} \quad (8)$$

for an L-polypeptide. The angle ξ between the virtual bond and the single bond N—C $^\alpha$ and the supplementary angle θ^β between N—C $^\alpha$ and C $^\alpha$ —C $^\beta$ are given as 13.2° and 70.52°, respectively.¹⁸

We define the new coordinate system which describes the direction of electric transition dipole moment of the k -th dye chromophore. The x_k axis is taken to be parallel to the direction

$$\mu_{k0c} = \begin{bmatrix} a & b & ac \\ \sqrt{a^2+b^2+c^2} & \sqrt{a^2+b^2} & \sqrt{a^2+b^2+c^2}\sqrt{a^2+b^2} \\ b & a & bc \\ \sqrt{a^2+b^2+c^2} & \sqrt{a^2+b^2} & \sqrt{a^2+b^2+c^2}\sqrt{a^2+b^2} \\ c & 0 & \sqrt{a^2+b^2} \\ \sqrt{a^2+b^2+c^2} & & \sqrt{a^2+b^2+c^2} \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & d & e \\ 0 & e & d \end{bmatrix} \begin{bmatrix} \mu_{0e} \sin \theta \cos \phi \\ \mu_{0e} \sin \theta \sin \phi \\ \mu_{0e} \cos \theta \end{bmatrix} \quad (9)$$

$$\begin{bmatrix} a \\ b \\ c \end{bmatrix} = (T_k + E)l_{u,k}$$

$$d = -\frac{1}{\sqrt{a^2+b^2}}(br_{k,X} - ar_{k,Y})$$

$$e = -\frac{1}{\sqrt{a^2+b^2+c^2}\sqrt{a^2+b^2}}\{acr_{k,X} + bcr_{k,Y} - (a^2+b^2)r_{k,Z}\}$$

where E is a unit matrix.

The five different regions of the angles φ and ψ were chosen. They were designated by Zubkov, *et al.*⁹ as $\alpha_1(80^\circ \leq \varphi \leq 140^\circ, 100^\circ \leq \psi \leq 140^\circ)$, $\beta_2(0^\circ \leq \varphi \leq 120^\circ, -90^\circ \leq \psi \leq 0^\circ)$, $\beta_3(30^\circ \leq \varphi \leq 90^\circ, -90^\circ \leq \psi \leq -30^\circ)$, $1(0^\circ \leq \varphi \leq 150^\circ, -210^\circ \leq \psi \leq 0^\circ)$, and $2(0^\circ \leq \varphi \leq 120^\circ, -60^\circ \leq \psi \leq 0^\circ)$, and each of these was assumed to be a rectangular well. Angles were taken at 10° intervals. The 50 random chains consisting of 10 residues ($M=50, N=10$) were generated by choosing the pairs of angles φ and ψ at random from the allowed regions. The CD of 50 chains was averaged with the statistical weight of unity for all chains. The molar ellipticity calculated on the basis of mole of dimeric dye unit had to be divided by a

factor 2 for comparison with those observed, because the observed value is based on the mole of monomeric dye.

If all carboxyl groups were occupied by the dye molecules, two dye molecules bound close to each other should undergo steric hindrance. Hence, when a dye with an excluded volume overlaps with the other dye, one of them must be removed; it was assumed that such dye can not bind to the carboxyl group with a large numbering. Thus the number of dyes bound to the N successive amino acid residues becomes less than N and it was designated as N' . In the present case, the dye molecule has 9.0 Å in length, 3.0 Å in width and 3.0 Å thick. Its

thickness increases to 5.0 Å, when it dimerizes.

Parameters

The electronic parameters are given in Table I. The magnitudes of the electric transition dipole moments for monomer species were estimated graphically by the previously reported method³ from the observed absorption spectrum of free acridine orange at $[D]=5.0 \times 10^{-5} M$ and pH 6.3.² Corresponding magnitudes for dimer species were calculated from the observed spectrum of dye—poly(*S*-carboxymethyl-L-cysteine) mixture for $[P]/[D]$ 167 at $[D]=5.0 \times 10^{-5} M$ and pH 7.2.² The half width of the visible bands was taken as 1725 cm^{-1} , which was the same as that adopted previously.³

The distance l_0 between C^α and the position of transition moment of acridine orange was assumed to be 5.0 Å, approximately equal to the distance for the planar trans conformation of the side chain. The longer axis of each adsorbed dye molecule, *i.e.*, the 1L_b electric transition moment, was put at $\phi_l=0^\circ$, where the suffix *l* refers to the longer axis of dye molecule.

For the system of dye— β form poly(*S*-carboxymethyl-L-cysteine), *i.e.*, for the two-fold helix, it is found that the parameters $\theta_1=10^\circ$, $\varphi=41^\circ$ and $\psi=315^\circ$ ¹⁹ correspond to the parameters adopted in the helical parameter method.³ The CD bands calculated for $N=10$ by both methods were in relatively close agreement with each other.

RESULTS

Amide $\pi-\pi^*$ Transition of Random-Coil Polypeptide Chain

The CD calculation of the random-coil polypeptide chain was carried out for the electrically allowed amide $\pi-\pi^*$ ($c/\nu_{E0}=190 \text{ nm}$) transition by the method given in Appendix. We attempted four kinds of computations for population-averaged CD spectra. In the first case, pairs of rotational angles φ_k and ψ_k were selected at random from the chosen region $\{\varphi, \psi\}$ and a chain composed of N residues was generated. In this way M chains were constructed. The CD was calculated for each of them and its statistical average with equal weight was computed. In the second, the CD of the chains

generated in the same way as above was averaged with the statistical weight $\exp\{-\sum_{k=1}^{N-1} E(\varphi_k, \psi_k)/RT\}$, where E is the conformational energy, R is gas constant and T is the temperature. In the third, the angles φ_k and ψ_k were selected from the chosen region $\{\varphi, \psi\}$ with the weight given by conformational energy $\exp\{-E(\varphi, \psi)/RT\}$ and the CD was averaged with an equal weight for all chains.

We chose the region of energy lower than 6 kcal mol^{-1} from the conformational energy map given by Brant, *et al.*²⁰ The temperature T was 293 K. The convergence for $N=10$ was reached at a value for M much less than 50. The final results of all methods above gave similar spectra, having a positive CD band around 198 nm and a negative CD band around 180 nm. The calculation by the first method yielded a pair of bands with a molar ellipticity of 8,000 and $-9,000$ at most, which were much smaller than the observed values.²¹ The molar ellipticity calculated by the second method was smaller than that found by the first. For the third method, the calculated molar ellipticity was 27,000 at a longer wavelength side and $-31,000$ at a shorter wavelength side, in agreement with the observed values. In the 900 pairs of rotational angles φ and ψ selected by the third method, 81% was generated from the region with the lowest energy value of $E=0 \text{ kcal mol}^{-1}$, in spite of the fact that the region of $E=0 \text{ kcal mol}^{-1}$ occupies, at most, 17% of all the region.

In the last method, the φ and ψ were generated from the region of $10^\circ \leq \varphi \leq 110^\circ$ and $280^\circ \leq \psi \leq 350^\circ$. An equal weight was assigned for the chosen region $\{\varphi, \psi\}$, although its region consisted of 75% of the region of $E=0 \text{ kcal mol}^{-1}$ and 25% of the region of $E=1 \text{ kcal mol}^{-1}$. The averaged CD was calculated with an equal weight for all chains. The result was almost the same as that by the third method and therefore this method is used for the CD calculation of acridine orange—random-coil polymer mixture, for simplicity.

The 1L_b Transition of Acridine Orange Bound to Random-Coil Polypeptide Chain

In Table II, the average end-to-end distance between C_0^α and C_N^α , $\langle R_{0N}^2 \rangle^{1/2}$, at $N=10$ is shown

for each region. The end-to-end distances were in the following order: $\beta_3 > 2 > \beta_2\alpha_1 (P_\alpha=0.21) > 1 > \beta_2\alpha_1 (P_\alpha=0.57) > \alpha_1$, where P_α is a probability

Table II. End-to-end distance of random-coil polypeptide and $\langle N' \rangle$ value for dimeric dyes bound to polypeptides ($M=50$, $N=10$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$)

Region	$\langle R_{0,N}^2 \rangle^{1/2}$, \AA	$\langle N' \rangle$	
		$\theta_1=90^\circ$	$\theta_1=0^\circ$
β_3	31.29	6.3	8.4
2	30.40	7.5	8.8
$\beta_2\alpha_1 (P_\alpha=0.21)$	22.30	7.0	7.7
1	20.41	6.9	7.3
$\beta_2\alpha_1 (P_\alpha=0.57)$	12.91	6.0	6.6
α_1	9.30	5.5	6.1

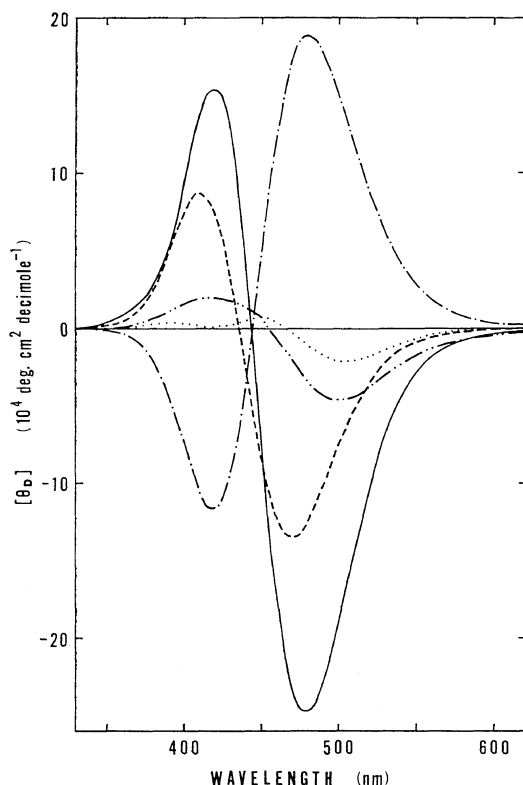


Figure 1. The θ_1 dependence of the CD of the 1L_b transition calculated for dimeric dye bound to polypeptides generated from the α_1 region: —, 0° ; ····, 45° ; - - - - , 90° ; — · — · , 115° ; — — — , 135° ; — · — · — · , 135° ; $M=50$, $N=10$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$.

of the α region and $P_\beta=1-P_\alpha$. It may be noted that the end-to-end distance for the β_3 region is shorter than that of the antiparallel β -form and that the distance for the $\beta_2\alpha_1 (P_\alpha=0.57)$ region is slightly shorter than that of the α -helix.

The shorter axis of the dye was put parallel to the y axis, that is, $\theta_s=90^\circ$ and $\phi_s=90^\circ$, where the suffix s refers to the shorter axis of dye molecule. The $\langle N' \rangle$ values for dimeric dyes bound to random-coil polypeptides are shown

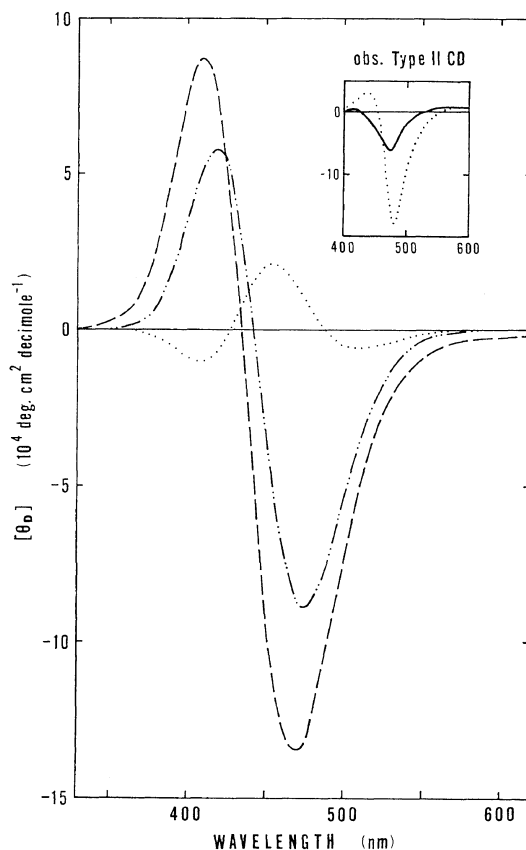


Figure 2. CD of the 1L_b transition calculated for dimeric dye bound to polypeptides generated from the various regions: ····, region 1; — · — · — ·, region $\beta_2\alpha_1 (P_\alpha=0.57)$; — — —, region α_1 ; $M=50$, $N=10$, $\theta_1=135^\circ$; $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$. Insert: —, the observed CD for the acridine orange—poly(*S*-carboxymethyl-L-cysteine) system of [P]/[D] 2 at [D]= $4.94 \times 10^{-5} M$ and pH 6.81¹; ····, the observed CD for the acridine orange—low-molecular-weight poly(*S*-carboxymethyl-L-cysteine) (E515) system of [P]/[D] 167 at [D]= $5.2 \times 10^{-5} M$ and pH 4.71.²

in Table II. As predicted and shown practically, the steric hindrance of bound dyes occurs very frequently on the more compact polypeptide chain conformations and also more for $\theta_1=90^\circ$ than for $\theta_1=0^\circ$.

The zeroth-order CD for the 1L_b transition of dimeric dye bound to a polypeptide was evaluated by eq 1 to 3 and yielded a conservative pair of ellipticity bands, the rotatory strengths of which sum to zero. The first-order nonconservative calculation for the 1L_b transition arising from the interaction with the 1B_b transition was carried out by means of eq 5 and 6. Figure 1 shows that, for the α_1 region generating the more compact polypeptide chains, the angle $\theta_1=90-135^\circ$ results in a conservative pair of CD bands with a negative band around 500 to 470 nm and a positive band around 420 to 410 nm, accompanied by a negative nonconservative band. The calculated results at $\theta_1=135^\circ$ for some regions are given in Figure 2. The α_1 and $\beta_2\alpha_1(P_\alpha=0.57)$ regions yield a pair of CD bands with the same sign as the Type II CD, while the other four regions cannot reproduce

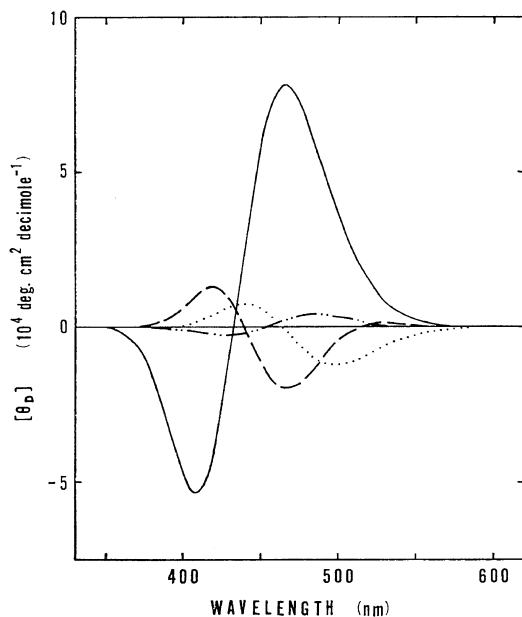


Figure 3. The θ_1 dependence of the CD of the 1L_b transition calculated for dimeric dye bound to polypeptides generated from the β_3 region: —, 0° ; ····, 45° ; -·-·, 90° ; ----, 135° ; $M=50$, $N=10$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$.

the Type II CD.

For the β_3 region generating more extended polypeptide chains, $\theta_1=0^\circ$ gives rise to a pair of CD bands, having a positive band at the longer wavelength side, and a positive non-conservative CD, as seen in Figure 3. The CD bands calculated at $\theta_1=0^\circ$ for the regions other than the 1 region are also consistent, in their sign and position, with the Type III CD, as well as the β_3 region. This is shown in Figure 4. However, among them, the α_1 and $\beta_2\alpha_1(P_\alpha=0.57)$ regions are not likely for the Type III model, because they generate compact polypeptide chains.

The calculation assuming the bound species to be monomeric dye was also carried out and the results are shown in Figures 5 and 6. Figure

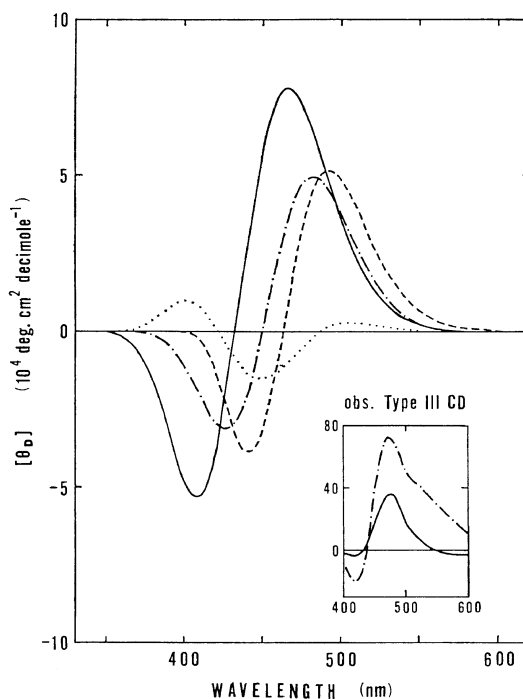


Figure 4. CD of the 1L_b transition calculated for dimeric dye bound to polypeptides generated from the various regions: —, region β_3 ; ----, region 2; -·-·, region $\beta_2\alpha_1(P_\alpha=0.21)$; ····, region 1; $M=50$, $N=10$, $\theta_1=0^\circ$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$. Insert: —, the observed CD for the acridine orange—poly(*S*-carboxymethyl-L-cysteine) system of [P]/[D] 2 at [D]= $4.94 \times 10^{-5} M$ and pH 7.43¹; ----, the observed CD for the acridine orange—poly(L-glutamic acid) system of [P]/[D] 1 at [D]= $2 \times 10^{-5} M$ and pH 9.1.⁴

5 gives the calculated CD at $\theta_1=135^\circ$ for the α_1 and $\beta_2\alpha_1(P_\alpha=0.57)$ regions. A negative band around 520 to 505 nm and a positive one around 450 to 435 nm appear as a conservative CD, together with a negative nonconservative band. The signs of the bands are found to be consistent with the Type II' CD. The calculation at $\theta_1=0^\circ$ for the β_3 , $\beta_2\alpha_1(P_\alpha=0.21)$ and 2 regions shown in Figure 6 gives rise to a positive band

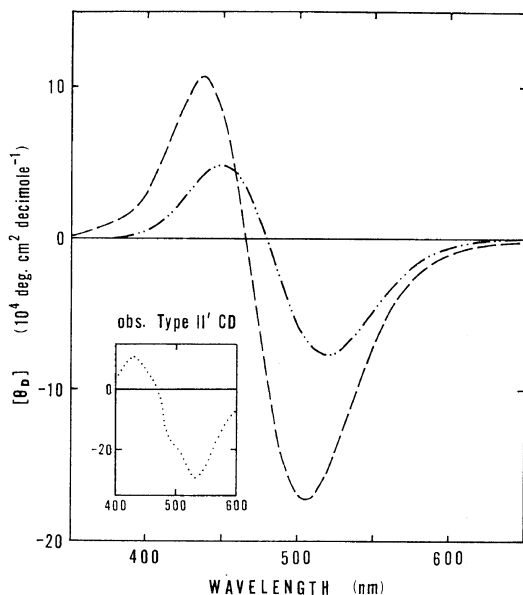


Figure 5. CD of the 1L_b transition calculated for monomeric dye bound to polypeptides generated from the various regions: \cdots , region $\beta_2\alpha_1$ ($P_\alpha=0.57$); \cdots , region α_1 ; $M=50$, $N=10$, $\theta_1=135^\circ$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$. Insert: \cdots , the observed CD for the acridine orange—low-molecular-weight poly(*S*-carboxymethyl-L-cysteine) (E515) system of $[P]/[D]$ 0.8 at $[D]=4.94 \times 10^{-5} M$ and pH 4.84.¹

in the longer wavelength side and a negative band in the shorter wavelength side. The nonconservative CD is positive. The signs of bands are in agreement with those observed in the Type III' CD.

The nonconservative CD of the 1L_b transition, arising from the interaction with the other

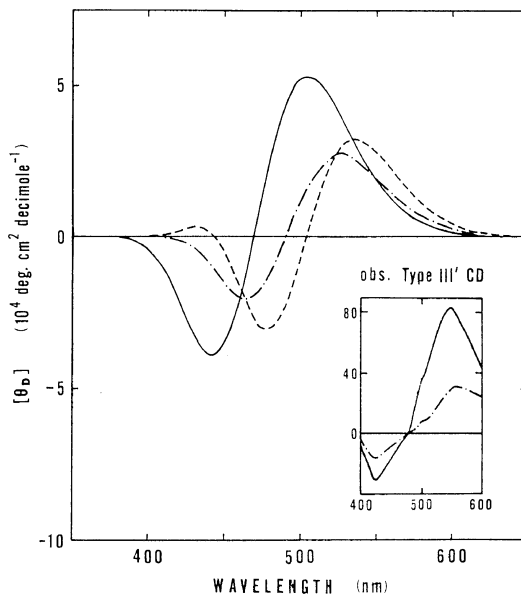


Figure 6. CD of the 1L_b transition calculated for monomeric dye bound to polypeptides generated from the various regions: \cdots , region β_3 ; \cdots , region 2; \cdots , $\beta_2\alpha_1(P_\alpha=0.21)$; $M=50$, $N=10$, $\theta_1=0^\circ$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$. Insert: \cdots , the observed CD for the acridine orange—poly(*S*-carboxymethyl-L-cysteine) system of $[P]/[D]$ 0.9 at $[D]=4.94 \times 10^{-5} M$ and pH 6.26;¹ \cdots , the observed CD for the acridine orange—poly(*L*-glutamic acid) system of $[P]/[D]$ 0.8 at $[D]=4.94 \times 10^{-5} M$ and pH 7.00.⁵

Table III. The calculated CD for the 1L_b transition of dimeric dyes bound to random-coil polypeptides ($M=50$, $N=10$, $\phi_1=0^\circ$, $\theta_s=90^\circ$, $\phi_s=90^\circ$, and $l_c=5.0 \text{ \AA}$)

Region	θ_1 , deg	Conservative CD		Nonconservative CD			
		c/ν , nm	$[\theta]_E$	c/ν , nm	$[\theta]_E$		
				1B_b	1L_a	1B_R	
α_1	90	440	38,300	455	-32,200	-1,500	3,100
		505	-38,700				
β_3	0	410	-56,700	455	30,800	700	-1,500
		470	49,600				

electrically allowed transitions besides the 1B_b state, was obtained according to eq 4 to 6. The results for the two systems of the α_1 region at $\theta_1=90^\circ$ and of the β_3 region at $\theta_1=0^\circ$ are given in Table III. The contribution from the 1L_a and 1B_a states was very small, as predicted from their small dipole moments, in comparison with that from the 1B_b state.

The dependence of calculated CD values on the parameter N was examined for the β_3 region at $\theta_1=0^\circ$. For the Type III complex, the sequence of chromophores can be longer. However, the calculated molar ellipticity at $N=20$ was not large as compared with that at $N=10$, because the available number N' of chromophores decreased owing to steric hindrance.

We have given CD values averaged over $M=50$. In some cases, convergence was not always reached, but these should not be far from their asymptotes.

The 1L_a Transition of Acridine Orange Bound to Random-Coil Polypeptide Chain

In the visible region, there is an electronic transition of the 1L_a state of acridine orange, besides 1L_b state. In order to explain the additional CD bands observed at the longer wavelength side than the 1L_b bands in Types II and III CD,¹ the CD of the 1L_a transition should be considered. As the dipole moment of the 1L_a transition is very small, conservative rotational strength should be small, according to eq 1. However, from eq 4, the nonconservative term caused by the interaction with the 1L_b transition

may have a significant magnitude, because the unperturbed frequency of the 1L_a state at 540 nm is close to that of 1L_b at 458 nm.

For the two typical cases of dimeric dye—random-coil polypeptide model, the nonconservative CD of the 1L_a transition due to the interaction with the other transitions was evaluated by the eq 4 and 5, and these results are shown in Table IV. The shorter axis of dye molecular plane was tilted from $\theta_s=90^\circ$ and $\phi_s=90^\circ$. For the α_1 region at $\theta_1=90^\circ$, the nonconservative CD by interaction with the 1L_b transition was positive for the tilt angle $\theta_s=90-135^\circ$. For the β_3 region at $\theta_1=0^\circ$, it was negative for the tilt angle $\phi_s=90-135^\circ$. These signs are coincident with the observed additional band at the longest wavelength side, apart from their calculated smaller magnitudes. The contributions from the 1B_a and 1B_b transitions are smaller than those from the 1L_b transition, as would be expected.

DISCUSSION

Myer and Foss²² and Eyring, *et al.*,²³ reported that the Cotton effect in the visible region was induced for acridine orange—poly(L-glutamic acid) complex even under a condition where the structure of poly(L-glutamic acid) is a random-coil. Sato and Hatano⁴ claimed that, in the cases where $[P]/[D]$ is around unity, a complex having an ordered structure can be formed even in the neutral and alkaline pH region and proposed a binding model for Type III CD. In

Table IV. The nonconservative contributions for the 1L_a transition of dimeric dyes bound to random-coil polypeptides ($M=50$, $N=10$, and $l_c=5.0 \text{ \AA}$)

Region	Longer axis		Shorter axis		Rotatory strength, $10^{-38} \text{ erg} \cdot \text{cm}^3$			CD, $10^4 \text{ deg} \cdot \text{cm}^2 \text{ decimol}^{-1}$		
	θ_1	ϕ_1	θ_s	ϕ_s	1L_b	1B_a	1B_b	1L_b	1B_a	1B_b
α_1	90	0	0	90	-0.0166	0.0008	-0.0044	-723	33	-189
			45	90	-0.0516	0.0011	-0.0135	-2245	47	-589
			90	90	0.0288	0.0002	0.0076	1252	9	328
			135	90	0.0602	-0.0009	0.0158	2619	-37	687
β_3	0	0	90	0	0.0002	-0.0000	0.0000	7	0	2
			90	45	0.0044	-0.0003	0.0012	190	-11	50
			90	90	-0.0140	0.0002	-0.0037	-607	6	-159
			90	130	-0.0242	0.0003	-0.0064	-1,054	11	-276

consideration of the induced CD designated as Types II, III, II', and III', however, we can say that a polymer in dye—polymer complex remains the random-coil form, on the basis of our experimental evidence.¹ Incidentally, Salter, *et al.*,⁶ supposed that there was no evidence to support a completely helical structure for the glycosaminoglycan, when it adsorbs dyes and induces the so-called Types II and III CD.

The observed CD spectra¹ of Types II, III, II', and III' are shown in the inserts of Figures 2, 4, 5, and 6, and are compared with one calculated. A pair of 1L_b bands in the visible region of Types II and II' CD were successfully obtained by the CD calculated for the binding model, where dyes are bound to the more compact polypeptide chain generated from the α_1 and/or $\beta_2\alpha_1$ ($P_\alpha=0.57$) region and the longer axes of dyes are arranged on it with a tilt of $\theta_1=90-135^\circ$; that is, they are tilted slightly from the direction along the polypeptide chain. A pair of 1L_b bands of Types III and III' CD were reproduced by a binding model of dye on the more extended polypeptide chain generated from the β_3 , 2 and/or $\beta_2\alpha_1$ ($P_\alpha=0.21$) region, where the longer axes of dye have a tilt of $\theta_1=0^\circ$; *i.e.*, they were almost perpendicular to the polymer chain.

The binding of the dye to the polymer is

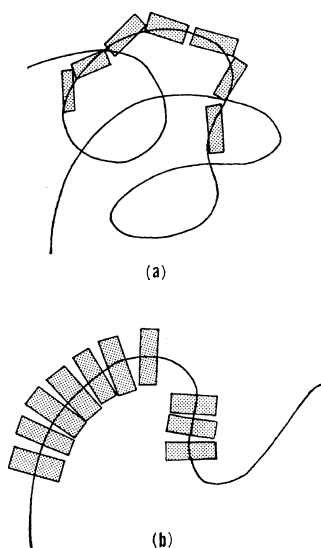


Figure 7. Two models proposed for the acridine orange—random-coil polypeptide complex.

primarily electrostatic; one of the nitrogen atoms of the dye binds to an ionized carboxyl group of the polymer. In order to fix the dye rigidly on the polymer, either of the other nitrogen atoms must approach the other carboxyl groups to form a hydrogen bond. Then the longer axis of the molecular plane of dye should favorably lie along the polymer chain, as illustrated in Figure 7(a). This is considered as a model for Types II and II' complexes. For the more extended random-coil polymer, it should be possible for dyes bound to nearby residues to stack together and hence the molecular planes of the dye become almost perpendicular to the polypeptide chain, as shown in Figure 7(b). This is a model of Types III and III' complexes.

As the observed CD is more nonconservative than the calculated CD based on the conservative 1L_b and its nonconservative coupling with 1B_b , other contributions must be taken into consideration for calculation. However, consideration of the nonconservative CD arising from the coupling with the 1L_a or 1B_a state brought about merely a small increase in the magnitude of the 1L_b bands and was not sufficient to reproduce the observed nonconservative values.

Types II and III CD have additional bands at the longer wavelength side, besides a pair of 1L_b bands, in three ways;¹ one has a broad and weak band around 600 to 540 nm, one has a shoulder around 540 to 510 nm and one has both of them. Their difference seems to originate from the intensity of both bands, because the two additional bands have opposite signs to each other. The band around 540 to 510 nm may be assigned to the contamination of Type II' or III' CD, because this band grows into a strong band around 600 to 540 nm of Type II' or III' CD with changing the pH.¹ The 1L_a transition can be assigned to the band around 600 to 540 nm. The calculated nonconservative CD for the 1L_a transition was too small in magnitude to coincide with the observed ones.

The observed molar ellipticity for Types III and III' CD is larger than one calculated. It very much increases beyond the magnitude of CD drawn in Figures 2, 4, 5, and 6, when the $[P]/[D]$ value decreases.¹ Thus, the deviation from the calculated CD becomes larger. A change in value of parameter to $N=20$ does not

lessen the disagreement above. In order to reduce the discrepancy in our calculation, we will have to consider some theoretical improvement of the CD calculation. Salter, *et al.*,⁶ reported that the aggregate of acridine orange—glycosaminoglycan complexes exhibits CD bands with large magnitude, and designated as Type II or III. If their interpretation is correct, the existence of a long-range ordering of dye—polymer complexes within the aggregate must be taken into account for our calculation.

The sign of the CD bands induced on the dye bound to the polymer having a regular structure can be determined mainly by the sign of the strongest interaction potential between the pair of chromophores.^{2,3,5} However, in the dye—random-coil polymer complex, this is not the case. The sign of most of interaction potentials was found to be negative (attractive) at $\theta_1=90^\circ$ for all the regions. Nevertheless, the sign of CD bands is not definite but depends on a dimension of the polypeptide, extended or compact.

Our method of calculation was similar to that by Zubkov, *et al.*⁹ We adopted the regions of rotational angles φ and ψ which Zubkov, *et al.* used for the calculation of the amide $\pi-\pi^*$ CD of random-coil polypeptide. It may be noted that the visible CD of dye—random-coil polymer complex is very sensitive to the selection of the region, while the amide $\pi-\pi^*$ CD changes only in the magnitude at every region, even when the selected region differs. For the CD calculation of $\pi-\pi^*$ transition of random-coil polypeptide, the nearest neighbor approximation holds well, while the CD calculation for the transition in the visible region of acridine-orange—random-coil polymer mixture can not be carried out by the nearest neighbor approximation, as expected from the chromophore position far away from the main chain and as elucidated by the calculated potential values.

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APPENDIX

Amide $\pi-\pi^*$ Transition of Random-Coil Poly-

peptide

The calculation of the CD associated with amide $\pi-\pi^*$ transition of random coil polypeptide was carried out by eq 1, 3, and 7. When the monopole approximation is used in representing the $\pi-\pi^*$ electric transition moment, the dipole moment μ_{k0e} is given by $\mu_{k0e} = \sum_{\sigma} \rho_{k0e,\sigma} \mathbf{r}_{k\sigma}$, where $\rho_{k0e,\sigma}$ is the monopole charge associated with the transition 0—e at the position σ in the k -th residue and $\mathbf{r}_{k\sigma}$ is the vector of the position σ . \mathbf{r}_k is taken to be the center of the transition monopole charges. The interaction potential in the monopole approximation is

$$V_{k0e,l0e} = \sum_{\sigma} \sum_{\tau} \rho_{k0e,\sigma} \rho_{l0e,\tau} (\mathbf{r}_{k\sigma,l\tau} \cdot \mathbf{r}_{k\sigma,l\tau})^{-1/2} \quad (\text{A.1})$$

where $V_{k0e,k0e} = 0$. $\mathbf{r}_{k\sigma,l\tau}$ is the distance vector between the position σ in the k -th residue and the position τ in the l -th one. It is written by eq 7, in which \mathbf{r}_k is replaced by $\mathbf{r}_{k\sigma}$ and \mathbf{r}_l by $\mathbf{r}_{l\tau}$. The values of the monopole parameter and the other optical parameters were adopted from Tonoco, *et al.*^{24,25}

The CD calculation for the amide $\pi-\pi^*$ transition of α -helical polypeptide was done with $\varphi=122^\circ$ and $\psi=133^\circ$.¹⁸ The calculated CD for $N=20$ were found to agree fairly well with those computed by the helical parameter method.⁵

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