

## Calculated Circular Dichroism of Acridine Orange Bound to $\beta$ -Form Poly(*S*-carboxymethyl-L-cysteine)

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**ABSTRACT:** A zeroth order theory of circular dichroism has been reviewed, which was previously developed for a linear alternating array or a two-fold helix of chromophores, and it has been further extended to the first order approximation for a system, in which each chromophore has only two excited states with mutually parallel polarizations. These theories are applied to circular dichroism associated with the visible and the near ultraviolet transitions of the acridine orange— $\beta$ -form poly(*S*-carboxymethyl-L-cysteine) complex. Results predicted by the zeroth order theory are confirmed in this system. Essentially two exciton levels of each excited state are optically active, and they have rotatory strengths of equal magnitude but of opposite signs. Circular dichroism calculated to the first order is compared with the observed one, and it is shown that, on the average, up to eight acridine orange molecules can stack together to form a linear alternating array. Assumptions inherent in the theory and in its application to the present system are discussed.

**KEY WORDS** Acridine Orange /  $\beta$ -Form Poly(*S*-carboxymethyl-L-cysteine) / Linear Alternating Array / Circular Dichroism / Ellipticity Band / Electrically Allowed Transition /

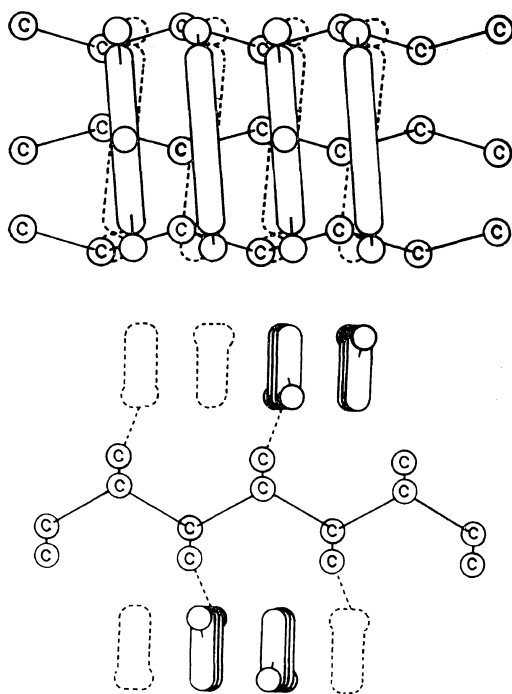
Recently,<sup>1</sup> we have reported that a symmetric dye, acridine orange, acquires optical activity when it is mixed with  $\beta$ -form poly(*S*-carboxymethyl-L-cysteine) in solution, at high polymer residue-to-dye mixing ratios, and presented a possible model for the optically active aggregates of the dye formed on the  $\beta$ -form polypeptide chain. The model consists of a linear alternating stack of dimeric dye molecules, to which our previous treatment of conservative optical activity<sup>2</sup> can be readily applied. We could explain the number and sign of observed ellipticity bands by simple inspection of this model.<sup>1</sup>

In the present paper we will describe in some detail the calculation of the induced optical activity of acridine orange— $\beta$ -form poly(*S*-carboxymethyl-L-cysteine) complex, based on the previous theory<sup>2</sup> and its extension, by assuming a possible geometry for the model. Calculations are first carried out to the zeroth order approximation of wave functions, which lead to characteristically paired ellipticity bands in circular dichroism spectra, and then they are further refined to the first order approximation, in

order to explain their nonconservative nature.

### MODEL FOR THE DYE-POLYMER COMPLEX

The model which we have proposed for the complex of acridine orange with  $\beta$ -form poly(*S*-carboxymethyl-L-cysteine) is schematically drawn in Figure 1. It was deduced from the absorption characteristics of acridine orange together with the Pauling—Corey pleated sheet structure of the  $\beta$ -form polypeptide. Acridine orange has two strong absorption bands, one in the visible and the other in the near ultraviolet, both of which have polarizations along the longer axis of dye molecular plane. Only dimer components of those two bands are concerned with absorption and circular dichroism when the dye is mixed with the  $\beta$ -form polymer. Consequently, dimeric dye molecules are adsorbed on the charged side chains of an extended polypeptide chain, forming linear stacks, and they must be on both sides of the chain, with the longer axis of each dye molecular plane slightly tilted from perpendicular to the chain direction. The direction of tilt of the plane



**Figure 1.** Model proposed for the complex of acridine dye formed with  $\beta$ -form poly(*S*-carboxymethyl-L-cysteine): upper, top view of a  $\beta$ -sheet; lower, side view of a  $\beta$ -sheet. The pleated sheet structure of polypeptide is assumed to be of anti-parallel chains type.

and the interaction potential among adsorbed dye molecules determine the sign of induced ellipticity bands.

### THEORY OF CIRCULAR DICHROISM

We will briefly review our previous treatment of conservative circular dichroism for a linear alternating array of  $N$  chromophores,<sup>2</sup> and then extend it for a system with particular electronic properties, to introduce nonconservative nature into the spectra.

For an electrically allowed transition, 0—E, each of the chromophores forming a linear alternating stack is fixed at a distance,  $a$ , from the center line of the array and has  $a$  transition electric dipole moment,  $\mu_{k0e}$ , with tangential, parallel and radial components,  $\mu_{0e,t}$ ,  $\mu_{0e,\parallel}$  and  $\mu_{0e,r}$ , respectively. Here the directions of those components are taken along the chain direction ( $\parallel$ ) common to all chromophores, toward the

chromophore position form the center line ( $r$ ), and perpendicular to both ( $t$ ), in such a way that they ( $t$ ,  $\parallel$ ,  $r$ ) form a right-handed  $xyz$  coordinate system at each chromophore. The directions,  $t$  and  $r$ , depend on the position,  $k$ , numbered along the chain, or whether  $k$  is even or odd.

Rotatory strength for the  $K$ -th exciton level of an excited state,  $E$ , of the array is given by

$$R_{EK} = (2\pi\nu_{E0}/c) \left( \sum_{k=1}^N \sum_{\substack{l \neq k \\ k+l: \text{odd}}}^N C_{keK} C_{leK} \right) a \mu_{0e,t} \mu_{0e,\parallel} \quad (1)$$

where  $c$  is the velocity of light and  $\nu_{E0}$  is the excitation frequency of a chromophore in the static field of the rest of the array. The frequency of transition to the  $K$ -th level is related to the unperturbed frequency,  $\nu_{E0}$ , by

$$\nu_{EK} = \nu_{E0} + E'_{EK}/h \quad (2)$$

where  $h$  is Planck's constant. The perturbation energy,  $E'_{EK}$ , can be determined by a secular equation and is also expressed by

$$E'_{EK} = \sum_{k=1}^N \sum_{\substack{l \neq k}}^N C_{keK} C_{leK} V_{k0e,l0e} \quad (3)$$

The interaction potential,  $V_{k0e,l0e}$ , between electric dipoles of the  $k$ -th and  $l$ -th chromophores, both for the transition 0—e, can be represented by the dipole approximation:

$$V_{k0e,l0e} = \left\{ \mu_{0e,\parallel}^2 + (-1)^{k+l} \mu_{0e,\perp}^2 - \frac{3\{-2[1-(-1)^{k+l}]a^2\mu_{0e,r}^2 + (l-k)^2b^2\mu_{0e,\parallel}^2\}}{2[1-(-1)^{k+l}]a^2 + (l-k)^2b^2} \right\} / \{2[1-(-1)^{k+l}]a^2 + (l-k)^2b^2\}^{3/2} \quad (4)$$

where  $\mu_{0e,\perp} = \sqrt{\mu_{0e,t}^2 + \mu_{0e,r}^2}$  and  $b$  is the repeat distance of chromophores along the center line. The exciton coefficients,  $C_{keK}$ , can be determined by a set of equations

$$\sum_{k=1}^N C_{keK} (V_{k0e,l0e} - \delta_{k,l} E'_{EK}) = 0, \quad l=1, 2, \dots, N \quad (5)$$

and

$$\sum_{K=1}^N C_{keK} C_{leK} = \delta_{k,l} \quad (6)$$

where  $\delta_{k,l}$  is 1 for  $k=l$  and 0 for  $k \neq l$ .

If the shape of a circular dichroic band associated with the  $K$ -th level is represented by

$f(\nu - \nu_{EK})$ , the molar ellipticity per chromophore arising from the excited state, E, can be expressed by

$$[\theta]_E = (48\pi^2 N_A / hcN) \nu \sum_{K=1}^N R_{EK} f(\nu - \nu_{EK}) \quad (7)$$

at a frequency,  $\nu$ , where  $N_A$  is Avogadro's number. For a linear alternating stack, only two exciton levels, polarized parallel and perpendicularly, have large rotatory strengths of equal magnitude but of opposite signs, and, therefore, eq 7 can be approximated by

$$[\theta]_E = (48\pi^2 N_A / hcN) \nu \{ R_{E\parallel} f(\nu - \nu_{E\parallel}) + R_{E\perp} f(\nu - \nu_{E\perp}) \} \quad (8)$$

with  $R_{E\parallel} = -R_{E\perp}$ . Alternatively eq 7 can be approximated by

$$[\theta]_E = (48\pi^2 N_A / hcN) \nu \left\{ \left( \sum_{K=1}^N R_{EK} \right) f(\nu - \nu_{EO}) - \left( \sum_{K=1}^N (\nu_{EK} - \nu_{EO}) R_{EK} \right) \frac{\partial f(\nu - \nu_{EO})}{\partial \nu} \right\} \quad (9)$$

where

$$\sum_{K=1}^N (\nu_{EK} - \nu_{EO}) R_{EK} = (2\pi \nu_{EO} / hc) a \mu_{0e, \tau} \mu_{0e, \parallel} \sum_{k=1}^N \sum_{\substack{l \neq k \\ k+l: \text{odd}}}^N V_{k0e, l0e} \quad (10)$$

The total rotatory strength associated with an excited state, A, arises mainly from the interactions of a chromophore excited to the state, a, with the other chromophores excited to the other states, b, and this was calculated by Tinoco.<sup>3</sup> For an electrically allowed transition, 0—A, Tinoco's equation may be simplified as

$$\sum_{K=1}^N R_{AK} = \frac{2\pi}{hc} \sum_{b \neq a} \frac{\nu_{AO} \nu_{BO}}{\nu_{BO} - \nu_{AO}} \sum_{k=1}^N \sum_{\substack{l \neq k \\ k+l: \text{odd}}}^N ((\mathbf{R}_l - \mathbf{R}_k) \cdot [\boldsymbol{\mu}_{k0a} \times \boldsymbol{\mu}_{l0b}]) V_{k0a, l0b} \quad (11)$$

where  $\mathbf{R}_l - \mathbf{R}_k$  is the vector from the  $k$ -th to  $l$ -th chromophore. If only two excited states, A and a particular B, are taken into account that have an identical polarization direction, eq 11 reduces to a form independent of the nature of approximation for interaction. In the dipole approximation, the interaction potential,  $V_{k0a, l0b}$ , has a form identical with eq 4, except for  $\mu_{0a, d} \mu_{0b, d}$  in place of  $\mu_{0e, d}^2$ , where  $d = (t, \parallel) / \text{or } r$ , and for  $\mu_{0a, t} \mu_{0b, t} + \mu_{0a, r} \mu_{0b, r}$  in place of

$\mu_{0e, \perp}^2$ . Then eq 11 is expressed by

$$\sum_{K=1}^N R_{AK} = -\frac{4\pi}{hc} \frac{\nu_{AO} \nu_{BO}}{\nu_{BO} - \nu_{AO}} a (\mu_{0a, \tau} \mu_{0b, \parallel} + \mu_{0a, \parallel} \mu_{0b, \tau}) \sum_{k=1}^N \sum_{\substack{l \neq k \\ k+l: \text{odd}}}^N V_{k0a, l0b} \quad (12)$$

and the two transitions, 0—A and 0—B, form a conservative pair of total rotatory strengths:

$$\sum_{K=1}^N R_{AK} + \sum_{K=1}^N R_{BK} = 0 \quad (13)$$

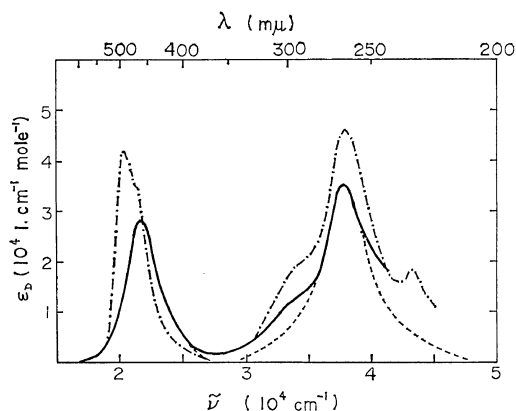
Spectral shape may be represented by a Gaussian function with a half-width,  $\theta_E$ :

$$f(\nu - \nu_{EK}) = (1/\theta_E \sqrt{\pi}) \exp [-(\nu - \nu_{EK})^2 / \theta_E^2] \quad (14)$$

#### PARAMETERS FOR CALCULATION

Dimeric molecules of adsorbed dye were assumed to be of antiparallel type such as Zanker suggested,<sup>4</sup> and each of these was considered here as a chromophore. Since the observed molar extinction coefficient and molar ellipticity are based on the mole of monomeric dye but the theoretical equations are derived on the basis of mole of chromophore, *i.e.*, of dimeric dye here, the calculated values must be divided by 2 to be compared with those observed.

Distances of the acridine orange chromophores from the center line,  $a$ , were assumed to be 6.00 Å, and the repeat distance of chromophores along the center line,  $b$ , was taken as 3.50 Å to reconcile with the Pauling—Corey pleated sheet structures. The radial direction would not be very different from being perpendicular to the  $\beta$ -sheet, owing to the steric hindrance of bound dye with the  $\beta$ -sheet. The longer axis of each adsorbed dye molecule, *i.e.*, its transition electric moment, was put perpendicular to the radial direction, that is, parallel to the plane of the pleated sheet ( $\mu_{0e, r} = 0$ ), and its angle of tilt ( $\cos^{-1} (\mu_{0e, \perp} / |\mu_{0e}|)$ ) from perpendicular to the chain direction was fixed at  $10^\circ$ , in such a way that  $\mu_{0e, \tau} \mu_{0e, \parallel} > 0$ , that is, the locus of extension of transition dipole vectors along the surface of the circumscribed cylinder forms a right-handed helix. The direction of tilt would be determined by the configuration of side chains of the  $\beta$ -form polypeptide. Since  $a > b$ , the second nearest neighbor interaction,  $V_{10e, 30e}$ , should be



**Figure 2.** Observed absorption spectra of acridine orange:  $[D]=5.0 \times 10^{-5}M$  and pH 4.3 to 7.0; ----, free; —, bound to poly(*S*-carboxymethyl-L-cysteine) at  $[P]/[D]$  167 (obtained by correcting for polymer absorption in the ultraviolet region); - · - ·, Assumed band shape for bound dimeric dye.

the strongest in this system, which is another extreme case different from that previously treated.<sup>2</sup> The interactions between the chromophores on the opposite sides of a sheet, *i.e.*, for odd  $k+l$  values are clearly negative in the assumed model.

Figure 2 shows the absorption spectra of acridine orange bound to the  $\beta$ -form polymer, which are independent of polymer conformation, either  $\beta$ -form or random coil, as well as of polymer-to-dye mixing molar ratio,  $[P]/[D]$ , larger than about 10. Both visible (0—a) and near ultraviolet (0—b) transitions of the dye have a common polarization direction,<sup>4,5</sup> and, therefore, their dimeric components of bound dye are also polarized in an identical direction. By extracting those components from each band of the absorption spectrum (for the random coil system), as shown in Figure 2, magnitudes of electric moment for both transitions were graphically estimated by

$$|\mu_{0e}|^2 = 2(6909 hc/8\pi^3 N_A) \int (\epsilon_D/\tilde{\nu}) d\tilde{\nu} \quad (15)$$

where  $\tilde{\nu}$  is the wave number and  $\epsilon_D$  is the molar extinction coefficient of bound dye, which was obtained from the observed spectrum, by correcting for the polymer absorption in the ultraviolet region. The factor 2 was introduced for conversion from monomer to dimer basis.

**Table I.** Electronic transitions of a bound dimer of acridine orange

Transition	$\lambda_{E0} = \frac{c}{\nu_{E0}}, m\mu$	$ \mu_{0e} , D$	$\frac{\Theta_E}{c}, cm^{-1}$
0—A	458	$\sqrt{2} \times 6.49$	1725
0—B	261	$\sqrt{2} \times 7.28$	3400

Mixing of small parallel components was ignored. The half-width,  $\Theta_E$ , was obtained from wave numbers at  $1/e$  of each peak height. Electronic properties of the chromophore thus obtained are listed in Table I.

Calculations were performed on the Facom 230-60 of the Kyoto and Nagoya Universities, and the average number of chromophores forming a sequence of alternating stack,  $N$ , was varied from 2 to 10.

## RESULTS

As was predicted previously,<sup>2</sup> zeroth order rotatory strengths calculated by eq 1 have significant magnitudes only at two exciton levels for each transition, and all the other levels are substantially optically inactive, less than  $1/10$  in magnitude of the two. The two optically active levels are polarized parallel and perpendicularly, respectively. Table II gives calculated location and magnitude of the two rotatory strengths. The perpendicularly polarized level is at the shortest wavelength and has a negative rotatory strength, while the parallel polarized level is at

**Table II.** Zeroth order spectral strengths associated with the two exciton levels<sup>a</sup>

(A) Visible transition (0—A)

$N$	$\lambda_{AK} = \frac{c}{\nu_{AK}}, m\mu$	$\Delta\tilde{\nu}_A, cm^{-1b}$	$\frac{D_{AK}}{N}, D^{2c}$	$\frac{R_{AK}}{N}, DBM$
2	462.36	411	2.54	6.40
	453.73		81.73	-6.40
3	459.49	1267	12.51	2.77
	243.42		71.26	-2.77
4	442.46	719	2.54	6.39
	428.82		81.70	-6.40
5	440.02	1004	4.97	5.23
	421.41		78.21	-5.23
6	434.03	921	2.47	6.22
	417.34		80.05	-6.27
10	427.03	1146	2.19	5.52
	407.11		77.53	-6.07

## Calculation of Induced Circular Dichroism

## (B) Ultraviolet transition (0—B)

$N$	$\lambda_{BK} = \frac{c}{\nu_{BK}}, m\mu$	$\Delta\nu_B, cm^{-1}^b$	$\frac{D_{BK}}{N}, D^{2c}$	$\frac{R_{BK}}{N}, DBM$
2	262.77	517	3.19	14.10
	259.25		102.69	-14.10
3	261.61	1592	15.72	6.11
	251.15		90.16	-6.11
4	254.60	904	3.19	14.09
	248.87		102.65	-14.10
5	253.58	1261	6.25	11.54
	245.72		98.27	-11.54
6	251.07	1158	3.10	13.71
	243.98		100.58	-13.82
10	248.11	1439	2.76	12.17
	239.56		97.41	-13.38

<sup>a</sup>  $K$  represents either the parallel or perpendicularly polarized level.

<sup>b</sup>  $\Delta\nu_E = (\nu_{E\perp} - \nu_{E//})/c$  with  $E=A$  or  $B$ .

<sup>c</sup> Dipole strength per chromophore, see eq 29 of ref 2.

**Table III.** Total spectral strength associated with each transition

## (A) Visible transition (0—A)

$N$	$\frac{\sum (\nu_{AK} - \nu_{AO}) R_{AK}}{N}, 10^{-48} c.g.s.$	$\frac{\sum R_{AK}}{N}, DBM$
2	-814	1.00
3	-1085	1.36
4	-1424	1.79
5	-1627	2.04
6	-1821	2.29
10	-2268	2.85

## (B) Ultraviolet transition (0—B)

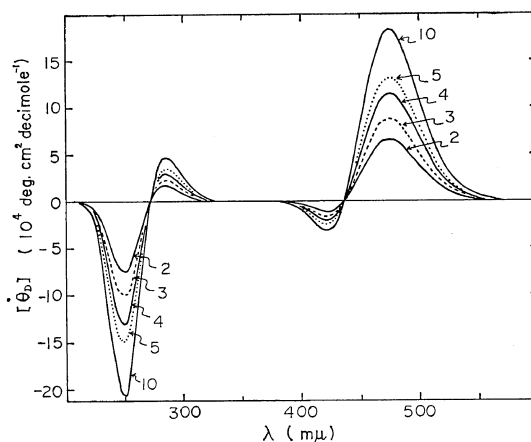
$N$	$\frac{\sum (\nu_{BK} - \nu_{BO}) R_{BK}}{N}, 10^{-48} c.g.s.$	$\frac{\sum R_{BK}}{N}, DBM$
2	-2255	-1.00
3	-3006	-1.36
4	-3944	-1.79
5	-4506	-2.04
6	-5043	-2.29
10	-6282	-2.85

the second shortest wavelength and has a positive rotatory strength of equal magnitude. All the other exciton levels are at longer wavelengths. The magnitude of the two rotatory strengths hardly depends on the number of chromophores in a sequence, but their location does depend on it.

First order calculations were carried out by means of eq 9. The total rotatory oscillator strength term, given by eq 10, determines the sign and occurrence of characteristic paired ellipticity bands, and the total rotatory strength, eq 12, confers a nonconservative contribution on those bands. Calculated values for those two strengths are given in Table III. The total rotatory oscillator strength term is negative, and, therefore, a positive ellipticity band occurs at the longer wavelength side at each transition and a negative band at the shorter wavelength side. Total rotatory strength is positive for the visible (0—A) transition, and, as required by eq 13, it is negative for the near ultraviolet (0—B) transition. Both total rotatory and rotatory oscillator strengths increase in magnitude with number of chromophores in a sequence, suggesting a dependence of the resulting circular dichroism spectra.

Molar ellipticity, based on the mole of monomeric dye, was calculated by eq 9, assuming a spectral shape given by eq 14, with the observed half-width. Figure 3 shows calculated circular dichroism spectra for different numbers of chromophores in a sequence.

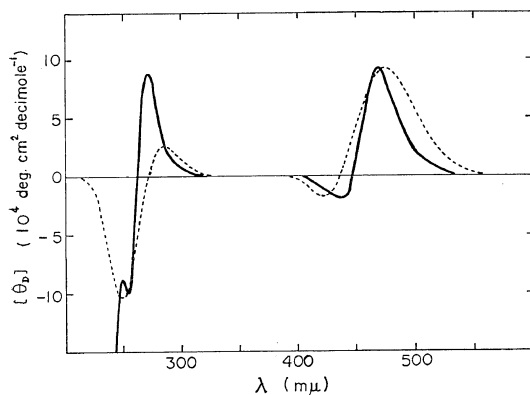
The calculated spectra can be compared with those observed for acridine orange bound to the polymer at the most acid pH, where the poly-



**Figure 3.** Calculated circular dichroism of acridine orange bound to  $\beta$ -form poly(*S*-carboxymethyl-L-cysteine);  $a, 6.60^\circ \text{ \AA}$ ; tilt angle,  $10^\circ$ . Figures represent the number of dimeric dye molecules in a linear alternating array.

mer would have the  $\beta$ -structure in solution. Figure 4 illustrates the spectrum calculated for the number of chromophores in a sequence, 4, and that observed for the complex of  $[P]/[D]=167$  at pH 4.37. The agreement is rather good at least, in the visible region. This suggests that optically active aggregates, each composing of, on the average, up to eight dye molecules in a sequence, are formed on the  $\beta$ -form polypeptide and circular dichroism is induced. That is, two dimer molecules of acridine orange can stack at each side of a polypeptide chain. Binding of dye to the opposite side of a stack could be caused by the tendency for an even distribution of charged sites over a polypeptide chain or a pleated sheet. Discrepancies seen at the ultraviolet bands can be ascribed to the electronic coupling of the ultraviolet transition with the other ultraviolet transitions, which was ignored in eq 12 and were considered to have only a small effect on the visible transition.

In a recent work,<sup>6</sup> we have observed that two diaminoacridines, proflavine and acridine yellow, are also induced circular dichroism when they are bound to  $\beta$ -form poly(*S*-carboxymethyl-L-cysteine). Sign and relative location of observed ellipticity bands for those two systems are common to those for the acridine orange system, and consequently, the model for the complex and the mechanism for induction of the circular dichroism should be similar. However, magni-



**Figure 4.** Comparison of observed circular dichroism with calculated circular dichroism: —, observed for a complex of  $[P]/[D]$  167 at pH 4.37; ---, calculated for a complex with  $N=4$ .

tude of ellipticity band relative to molar extinction coefficient was found to be smaller in the two diaminoacridine systems than in the acridine orange system. For example, values of  $[\theta_D(\nu_{A+})]/\epsilon_D(\nu_{AO})$  with  $\nu_{A+}$  for the observed frequency of positive ellipticity band were in the ratio, 9:1:3 for acridine orange, proflavine and acridine yellow systems. As compared with the number 4, obtained for the acridine orange system, the average number of dimeric dye in a sequence should be less than 2 for the two diaminoacridine systems, that is, many of proflavine and acridine yellow dimers are bound to the polymer in an isolated form and are essentially optically inactive.<sup>6</sup>

## DISCUSSION

We will now discuss some assumptions inherent to the previous calculations. First, the magnetic moment terms were all ignored in the expressions for rotatory strength, as the two transitions in question were electrically allowed and sufficiently strong. Acridine orange is certainly bound even to random coil poly(*S*-carboxymethyl-L-cysteine), as exhibited in the observed absorption spectra, independent of pH, and as expected from higher ionization of the polymer, but it remains optically inactive. This means that no optically active aggregates of bound dye is formed but dimeric dye molecules are bound randomly and separately on a randomly coiled polypeptide. The asymmetric potential acting on each chromophore, exerted by the other dye and peptide groups, would be so weak on the randomly coiled polypeptide that the one-electron rotatory power of those isolated bound dyes would be small. However, each chromophore in a linear stack of dye should be exerted a stronger asymmetric potential of the two-fold helix of the dye and peptide, and its electric moment could be tilted from perpendicular to its magnetic moment so that the one-electron rotatory power could be induced. Rotatory power induced by this mechanism can be evaluated by the method of Schellman and Oriel and others,<sup>7-9</sup> for example. The effect of salt on the induced circular dichroism, as was observed and reported previously,<sup>1</sup> would be an indication that this mechanism is operative in

this system, because the interaction potential should be influenced by the surrounding ionic atmosphere, which could alter direction of transition electric moment.

Next, it can be seen in Figure 2 that hypochromism occurs in the two absorption bands located at 458 and 261  $m\mu$ , when acridine orange binds to the polypeptide. However, the absorption spectrum of dye bound on the randomly coiled polypeptide is scarcely distinguishable from that on the  $\beta$ -form polypeptide. Thus the origin of the observed hypochromism cannot be attributed to the perturbation arising from the regular arrangement of bound dye, but the electrostatic interactions of dye chromophore with other dye and peptide, which have been neglected in eq 3 but should be included in the perturbation,  $E'_{EK}$ , must play a main role in reducing absorption intensity upon binding of dye. Those interactions have been incorporated into  $\nu_{EO}$  in the present calculations.<sup>2</sup>

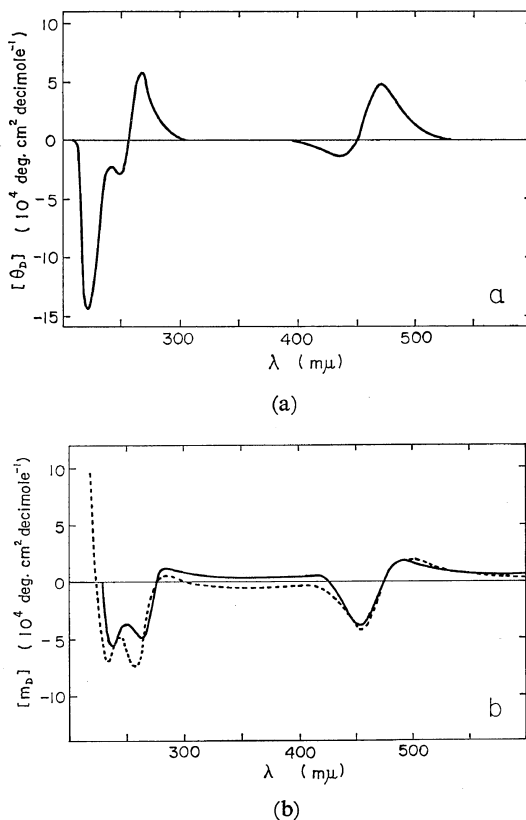
On the other hand, it cannot be observed that there are any changes in absorption intensity at the two bands, when bound dye molecules rearrange themselves from a randomly oriented array to a linearly stacked array, according to the conformational change of polypeptide from random coil to  $\beta$ -form. If the conservation law, eq 13, were to hold for the two transitions centered at 458 and 261  $m\mu$ , a change in absorption intensity at 458  $m\mu$ , which, in principle, should be present in accordance with eq 12, must be compensated by that at 261  $m\mu$ . As can be seen in Figure 4, however, it seems unlikely that observed circular dichroism spectra conform with eq 13, since the ultraviolet transition appears to be associated with a positive total rotatory strength as is the visible transition. Thus the excitations of chromophores at 458 and 261  $m\mu$  must have some interactions with those at the other ultraviolet transitions, in addition to their mutual interactions as considered in eq 12 and 13. Such an electronic coupling could occur with the transitions of dye at 290 and 230  $m\mu$ , as Figure 2 shows their presence, and even with the  $N-V_1$  transition of peptide. It should have larger influence on the transition at 261  $m\mu$ , which is located closer to the interacting transitions than that at 458  $m\mu$ , and thus could

alter the calculated value of the total rotatory strength associated with the former transition.

Finally, apart from the circular dichroism calculations, it seems worthwhile to examine how rotatory dispersion is influenced by the far ultraviolet transitions. As is well known, molar ellipticity,  $[\theta_D]$ , can be converted into molar rotation,  $[m_D]$ , by means of the Kronig—Kramers transform

$$[m_D]_{\lambda} = \frac{2}{\pi} \int_0^{\infty} \frac{\lambda' [\theta_D]_{\lambda'}}{\lambda^2 - \lambda'^2} d\lambda' \quad (16)$$

where  $\lambda$  and  $\lambda'$  are wavelengths. This transformation has been applied for an observed spectrum of circular dichroism given in Figure 5a, and the calculated rotatory dispersion curve is compared with the observed curve in Figure 5b. The integration has been performed at



**Figure 5.** (a) Observed circular dichroism for a complex of  $[P]/[D]=21$  at pH 4.57. (b) Corresponding rotatory dispersion: —, observed; ---, Kronig—Kramers transform of (a).

2.5 m $\mu$  intervals over the region from 535 to 210 m $\mu$ , but the points for  $\lambda' = \lambda$  have been omitted. It is clear that the effect of far ultraviolet ellipticity bands, neither observed nor included here, is primarily to shift smooth background rotation, but the general features of the observed Cotton effects can be well reproduced in the observed region.

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