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Induced Optical Activity of Acridine Orange Bound to Poly-S-carboxymethyl-L-cysteine

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Synopsis

The absorption and rotatory properties of acridine orange-poly-S-carboxymethyl-L-cysteine system in water and in 0.2 M NaCl have been measured at different pH and polymer-to-dye mixing ratios. The absorption spectra indicate that the dyes are bound to the polymer in dimeric or highly aggregated forms. At neutral pH where the polymer is randomly coiled, no optical activity is induced on the absorption bands of bound acridine orange. At acid pH where the polymer has the β-conformation, a pair of positive and negative circular dichroic bands occur at each of the absorption bands, centered around 458 and 261 mμ. The signs of those bands are opposite to those found for α-helical poly-L-glutamic acid. A model for the binding of dye to the β-form polymer is presented, in which dimeric dyes are attached to ionized carboxyl groups and stack one another to form linear arrays on both sides of an extended polypeptide chain. The observed circular dichroism spectra can be explained by the Tinoco's exciton mechanism, based on this model. Low molecular weight poly-S-carboxymethyl-L-cysteine induces quite a different circular dichroism on bound acridine orange.

INTRODUCTION

Some symmetric dyes are known to exhibit optical activity at their characteristic absorption bands when they are bound to helical macromolecules in aqueous solution. The extrinsic Cotton effects were first discovered in the optical rotatory dispersion for aqueous solutions of cationic dyes such as acridine orange added to α-helical poly-L-glutamic acid.[1,2] Later, this anomalous rotatory dispersion has been widely found for systems of acridine dyes bound to anionic macromolecules including DNA[3–5] and mucopolysaccharides.[6,7] This phenomenon was at first considered to be characteristic of helical conformation of macromolecules to which dyes bind, but later works have shown that it occurs even when dyes are bound to "randomly coiled" macromolecules.[5,8–10] The induced optical activity can also be observed by circular dichroism measurements,[9–15] in which circular dichroic bands appear at the region of absorption bands where anomalous dispersion occurs.

Poly-S-carboxymethyl-L-cysteine is known to undergo a conformational transition in aqueous solutions by changing its ionization state of side chain carboxyl groups. The pH-induced transition was observed around pH 5 by means of optical rotation, viscosity and infrared spectra[16,17] and further by

the measurements of ultraviolet rotatory properties[18,19] and hydrogen ion titration.[20] The polymer is in the β-conformation at acid pH, while it is in the randomly coiled form at neutral pH. The transition pH slightly depends on the ionic strength of solution; the higher the ionic strength, the more acid the transition pH.

In the present work it is found that the Cotton effects or circular dichroism are induced in the region of absorption bands of acridine orange, when the dye is added to poly-S-carboxymethyl-L-cysteine in solution, specifically at acid pH where the polymer has the β-conformation. The induced rotatory properties of the acridine orange-β-form poly-S-carboxymethyl-L-cysteine system are distinct from those of acridine orange-α-helical poly-L-glutamic acid system, and they are characteristic of the β-conformation in the present system. The behavior of the induced optical activity is examined in relation to the conformational change through the effects of pH, ionic strength and polymer-to-dye mixing ratio, the last being expressed by the molar ratio of polymer residue-to-total added dye, [P]/[D]. With reference to the absorption spectra and the molecular structures, a possible model for the binding of dye to the polymer is put forward, which makes dye chromophores optically active. Signs of circular dichroism curve can be explained by this model. The work is further extended to the polymer sample of lower molecular weight.

EXPERIMENTAL

Materials

Poly-S-carboxymethyl-L-cysteine samples were the same as previously described.[17] The intrinsic viscosities of the three samples, E602, E527 and E515, in 0.2 M NaCl at pH 7, 25°C were 0.230, 0.089 and 0.041 dl. g^{-1}, respectively. Most measurements were made on the sample E602, and the effect of polymer molecular weight was examined with the other two samples.

Acridine orange was purified according to the method of Zanker.[21] Commercial salt (Chroma-Geselschaft, Schmid & Co.) was dissolved in 50% ethanol, filtered off and then converted to the neutral form with 0.1 N NaOH. The base was extracted with chloroform, and the chloroform layer was passed through an alumina column (30 × 3 cm) to remove dark brown impurities. After the eluate was evaporated up, the residue was dissolved in ethanol and neutralized with an equivalent of 0.1 N HCl. After removing solvent *in vacuo*, dark red needle was recrystallized from ethanol-ethyl ether. The purified acridine orange gave a single spot on silica gel chromatogram. $\epsilon_{496} = 72{,}800$ in chloroform.

Preparation of Solutions

Stock solutions of poly-S-carboxymethyl-L-cysteine were prepared by suspending the polymer sample in distilled water, followed by addition of 1 N

NaOH to complete solution. They had the concentration, about 2 g dl^{-1}, and pH 6.7. Stock solutions of acridine orange in distilled water had the concentration, 8×10^{-4} M, and pH 6.9.

The absorption and rotatory properties of the dye-polymer system at different pH, both in the presence and absence of salt, were determined at a constant polymer residue-to-dye molar ratio, $P/D = 167$. The stock solution of the polymer was diluted with distilled water or with 0.2 M NaCl, and the pH of solution was adjusted to a desired value either with 0.1 N HCl or 0.1 N NaOH. The polymer solution gelled at pH lower than about 4.2. To this solution was added the stock solution of dye to give a polymer concentration, 0.13 g dl^{-1}, and a dye concentration, 5.0×10^{-5} M. At pH lower than 3.8 in water and 4.3 in 0.2 M NaCl, precipitation occurred.

The effect of P/D value on the absorption and rotatory properties was determined at pH 4.5 in water. The P/D value was altered from 3 to 1000. To cover the whole range of P/D values, four series of mixed solutions were prepared, each having a constant polymer concentration, 3.2×10^{-2} N, 8.6×10^{-3} N, 1.4×10^{-3} N or 3.5×10^{-4} N. To the stock solution of polymer or to a solution diluted with distilled water, the acridine orange solution was added to give an appropriate P/D value. The mixture was adjusted to pH 4.5 with 0.1 N HCl and diluted with distilled water to give a definite polymer concentration.

The spectra of all the mixed solutions were determined within a few hours after their preparation, and their pH was measured before and after the spectral determination to assure the difference to be within 0.05. Polymer solutions without added dye of the same pH were also examined for comparison.

Apparatus

The pH of solutions was measured with a Horiba Model P pH-meter of potentiometric type with a standard buffer, pH 6.88.

The absorption spectra, rotatory dispersion and circular dichroism were measured on a JASCO ORD UV/5 Spectropolarimeter with the CD attachment over the wavelength region from 600 to 220 mμ at room temperature (22°C). The instrument for rotatory dispersion was calibrated with an aqueous solution of sucrose, according to Samejima and Yang.[22] The circular dichroism attachment was calibrated with an aqueous solution of d,10-camphor sulfonic acid (recrystallized from ethyl acetate) using a value for the molar circular dichroism, $\epsilon_L - \epsilon_R = 2.20$ at 291 mμ.[23]

The rotation and dichroism at different pH and ionic strengths were determined with a 10 mm quartz cell, and the absorption spectra were measured with a 5 mm cell. For the examination of the effect of P/D, the cell path length was chosen to give optical density lower than 2 and changed at 10, 5, 2 and 1 mm.

Almost all data presented below are reduced to the molar basis of total dye concentration and are expressed by the molar extinction coefficient, ϵ_D, the molar rotation, $[m_D]$, and the molar ellipticity, $[\theta_D] = 3300\ (\epsilon_L - \epsilon_R)$, respectively.

RESULTS

Absorption Spectra

Figure 1 shows the absorption spectra of acridine orange-poly-S-carboxymethyl-L-cysteine systems in water at different pH, where the polymer is supposed to be in the random coil and in the β-form, respectively. The spectra of free acridine orange, also given in Figure 1, are substantially independent of pH in this region. While free acridine orange has a main peak at 492 mμ with a weaker peak around 470 mμ, the presence of the polymer alters the intensity relation of those two peaks. The main peak in the visible region is at 458 mμ, with a more symmetrical band shape, and the overall intensity of the band is reduced by 30% or more. The spectra of the mixed system are slightly dependent on pH, especially at the transition pH region.

The ultraviolet bands of the dye-polymer system are overshadowed by the stronger peptide bands at shorter wavelength region, having higher absorption as P/D is higher. The spectra of the dye alone in the mixed system are obtained by subtracting the contribution of the polymer spectra from the observed dye-polymer spectra. This has been done at pH 4.37, P/D = 167, and the result is shown in Figure 1. The spectra have a main peak at 261 mμ and a shoulder at 290 mμ, and their intensity is reduced by about 30%, as compared with those of free dye.

The visible absorption spectra of the dye-polymer system at pH 4.5 are almost independent of P/D mixing ratio, except for at very low ratios. At very low P/D values the peak shifts to the blue and its intensity is also weaker.

In contrast to those in water, the absorption spectra of acridine orange-polymer system in 0.2 M NaCl are strongly dependent on pH, as shown in Figure 2. The spectra remain identical at acid pH, irrespective of the

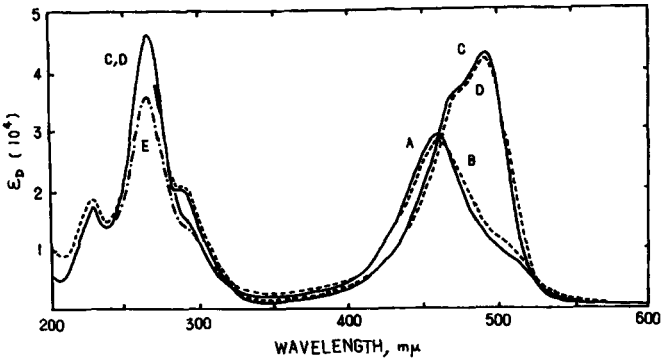

Fig. 1. Absorption spectra of acridine orange-poly-S-carboxymethyl-L-cysteine system of P/D = 167 in water, curve A, ——, pH 4.37 and curve B, - - -, pH 7.41, and of free acridine orange in water, curve C, ——, pH 4.43 and curve D, - - -, pH 6.31. [D] = 5.0×10^{-5} M. Curve E, -·-·-·-, represents the spectra of bound dye obtained by correcting for the spectra of existing polymer.

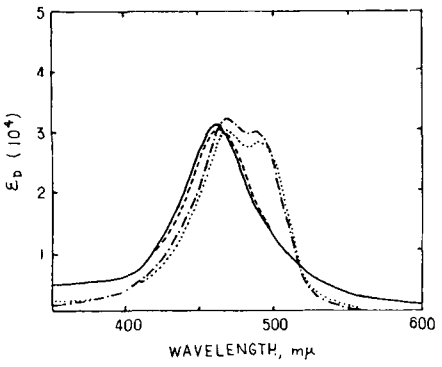

Fig. 2. Absorption spectra of acridine orange-poly-S-carboxymethyl-L-cysteine system of P/D = 167 in 0.2 M NaCl. ——, pH 4.67; - - - -, pH 4.73; —·—·—, pH 5.89; ·····, pH 7.21.

presence of salt, but they are strongly influenced by the ionic strength of solution at neutral pH. At pH 7 to 5 the 490 mμ band has an appreciable intensity even in the presence of polymer.

Optical Rotatory Dispersion

Figure 3 gives the optical rotatory dispersion of acridine orange-poly-S-carboxymethyl-L-cysteine systems in water at different pH. At neutral pH, the dispersion is normal and the rotation is negative over the wave-

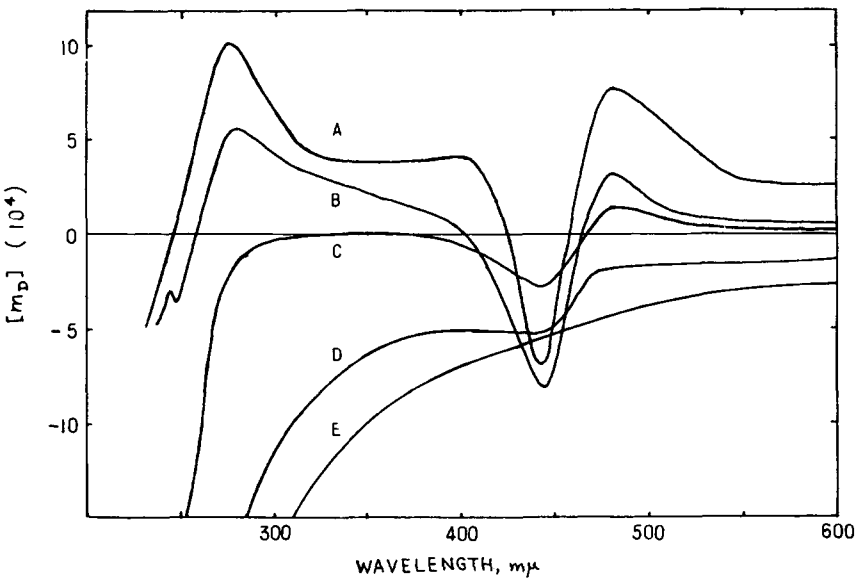

Fig. 3. Rotatory dispersion of acridine orange-poly-S-carboxymethyl-L-cysteine system of P/D = 167 in water. Curve A, pH 3.86; B, pH 4.19; C, pH 4.67; D, pH 5.02; E, pH 7.41 and 8.31.

length region examined, but, with lowering pH, the dextrorotation generally increases and Cotton effects appear at two regions. The peaks of the Cotton effects are at 490 and 288 mμ and their troughs are at 443 and 248 mμ. The signs of the Cotton effects are both positive in appearance,[24] but their unsymmetrical shapes suggest that more than one Cotton effects are associated with each of those regions. As the pH of solution is lowered, the amplitudes of the Cotton effects increase. The general increase of the background dextrorotation with lowering pH is in accord with that observed for the solution of polymer without added dye,[17] and this can be considered to be an indication of the coil-to-β transition of the polymer. It is shown that the residue rotation of the polymer in the dye-polymer system is identical with that of the polymer solution, if the rotation is measured at a wavelength, say 360 mμ, at which both Cotton effects would have the least influence on the rotation. This means that the presence of acridine orange does not alter the transition pH of the polymer.

These Cotton effects occur at pH 4.5, irrespective of P/D ratio, but their amplitudes are generally larger as the ratio is lower. At the lowest P/D, 3, the anomalous dispersion occurs at slightly shorter wavelengths.

Circular Dichroism

Circular dichroism of the dye-polymer system in water is shown in Figure 4. It is clear that at least two dichroic bands are associated with each of the two dye absorption bands, 458 and 261 mμ, at pH lower than 6. Both bands consist of a pair of positive and negative dichroic bands, the positive

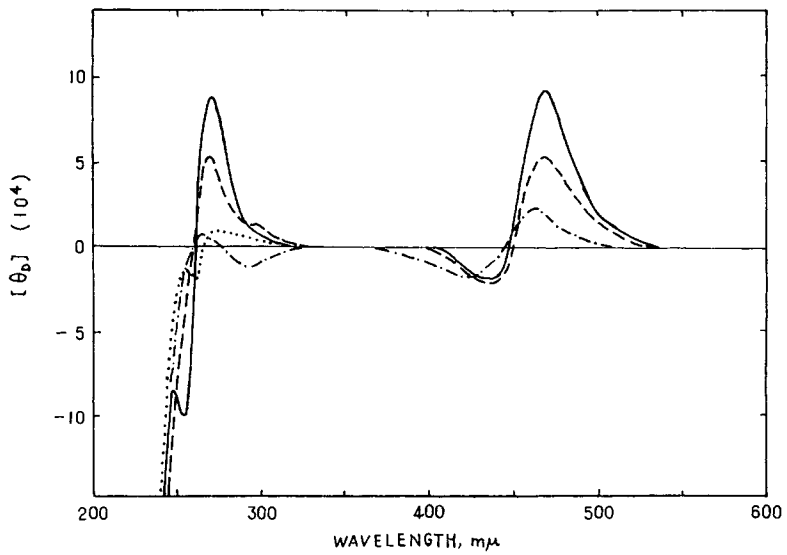

Fig. 4. Circular dichroism of acridine orange-poly-S-carboxymethyl-L-cysteine system of P/D = 167 in water. ——, pH 4.37; - - - -, pH 4.67; -·-·-·-·-, pH 5.45; ·····, pH 7.41.

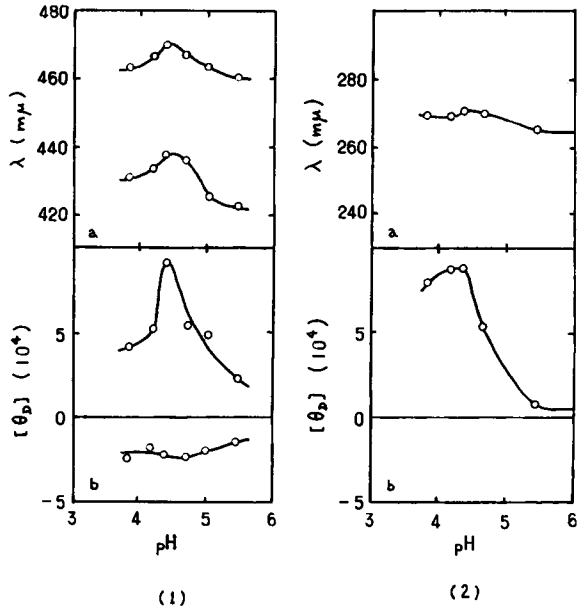

Fig. 5. Variation of circular dichroic bands with pH. (1) visible bands; (2) ultraviolet bands. a, wavelength; b, molar ellipticity.

one at longer wavelength side. At acid pH lower than 5, where the polymer has the β-conformation, the visible paired bands are at 470 and 437 mμ, and the ultraviolet paired bands are at 270 and 254 mμ. The magnitude of visible positive band is much dependent on pH, as is also seen in Figure 5. The ultraviolet dichroic bands at wavelength shorter than 260 mμ is overshadowed by stronger negative bands of polypeptide at 225 mμ.[18,19] However, this effect was largely reduced for systems of low P/D ratios, and the position of negative dichroic band could be isolated at 254 mμ.

The magnitudes of dichroic bands of the system at pH 4.5, where the system shows the largest dichroism, are not only dependent on P/D value but also on polymer concentration, as shown in Figure 6, while their wavelengths remain scarcely changed. For $P/D = 3$ all the bands shift to the blue and an additional negative band appears at 290 mμ.

At neutral pH, there are no visible dichroic bands but small dichroic bands at 270 and 260 mμ. As the pH of solution is lowered to less than 6, the pair of dichroic bands appear in the visible region as well as in the ultraviolet region. At the transition pH region all the bands shift to the blue and a negative band appears at 290 mμ.

Addition of salt to the system lowers the transition pH slightly, but red precipitates separate at pH 4.8. At both sides of this pH, solutions are homogeneous and circular dichroism is observed. Although the absorption spectra are largely influenced by the presence of salt, no visible dichroic band appears at neutral pH. At acid pH lower than 4.8, the doublet of di-

chroic bands appear at each of the absorption bands, as in the case of the system without added salt. However, the magnitude of the positive band in the ultraviolet is twice as large as that in the visible. These are shown in Figure 7.

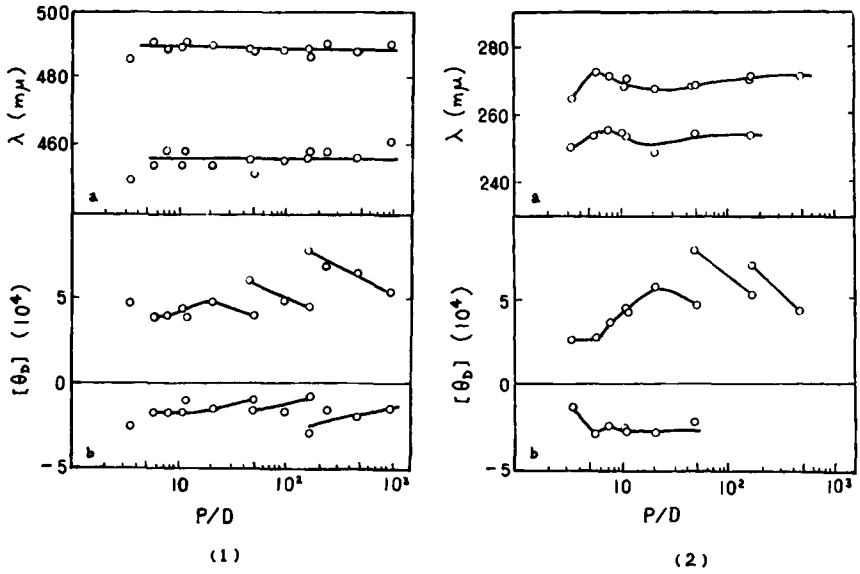

Fig. 6. Dependence of circular dichroic bands on P/D at pH 4.5. (1) visible bands; (2) ultraviolet bands. a, wavelength; b, molar ellipticity.

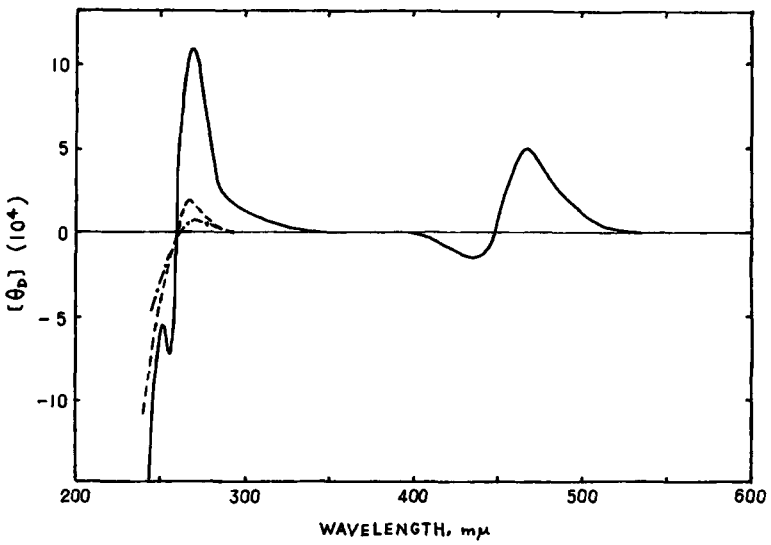

Fig. 7. Circular dichroism of acridine orange-poly-S-carboxymethyl-L-cysteine system of P/D = 167 in 0.2 M NaCl. ——, pH 4.68; - - - -, pH 5.89; -·-·-·-, pH 7.21.

It is noted that the circular dichroism of the acridine orange-β-form poly-S-carboxymethyl-L-cysteine complex appears particularly simple as compared with that of acridine orange-other macromolecule systems. The α-helical poly-L-glutamic acid induces on bound acridine orange an additional positive circular dichroic band at a longer wavelength side of the pair of dichroic bands, and the signs of the paired bands are opposite to those of the β-form poly-S-carboxymethyl-L-cysteine system.

DISCUSSION

Mode of Binding of Acridine Orange to the Polymer

Zanker[21] has shown that acridine orange molecules reversibly aggregate into dimer and higher aggregates in aqueous solutions, with increasing total dye concentration. In the range of 10^{-6} to 10^{-4} M, monomers and dimers are in mutual equilibrium, and the main absorption peak is at 492 mμ at low concentrations, while it is at 470 mμ at high concentrations. These bands can be assigned to the monomeric and dimeric forms of dye, respectively. Beyond 10^{-3} M, higher aggregates are formed, the absorption peak shifting to the more blue down to 450 mμ. A plausible model for the dimeric acridine orange has been suggested by Zanker,[21] in which two dye molecules stack together in an antiparallel way.

In the acridine orange-polymer systems, except for those in 0.2 M NaCl at neutral pH, the main peak is at 458 mμ with almost symmetrical band shape. The spectra have largely changed by the presence of polymer and they are scarcely influenced by the P/D ratio. These suggest that almost all the dye molecules exist in dimeric form or in highly aggregated form and are bound to the polymer.

In the presence of salt at neutral pH, monomeric dyes are also present together with higher aggregates of dye, but again in this case no circular dichroic band is associated with the monomer band. Monomeric dyes would not be bound to the polymer, or even if they were bound, no regular arrangement of monomeric dyes would form in such a way as to induce asymmetry on the bound dyes.

The binding of dye to the polymer may be primarily electrostatic, the central nitrogen atoms on dye cation being attracted and brought close to the ionized carboxyl groups of the polymer. At neutral pH where the polymer is fully ionized and randomly coiled, the binding would be strongly subject to the effect of added salt. At acid pH the polymer is only partially ionized[20] and the electrostatic effect of the polymer is reduced. Thus the binding is less susceptible to the ionic strength at acid pH.

In order to present a possible model for the binding of the dye to the β-form polymer, it will be postulated that the pleated sheet structure such as Pauling and Corey proposed exists in aqueous solution. In this structure the extended polypeptide chains are hydrogen bonded to one another through peptide bonds and form a two-dimensional sheet-like structure.

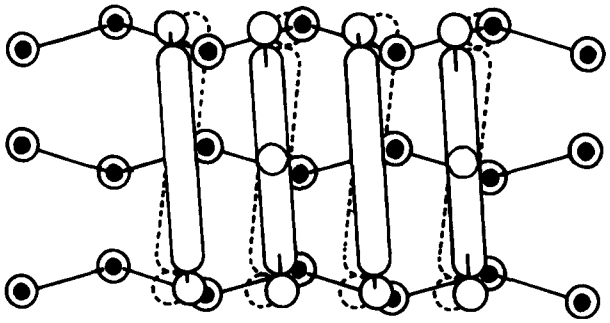

Fig. 8. Schematic drawing of the model proposed for the acridine orange-β-form polymer complex. Polypeptide chains are assumed to be of an antiparallel type. (Top view of a β-sheet).

The sheet may stack one another, but it will be reasonable to assume that the sheet is the fundamental structure for the dye binding as well as for the induced optical activity. Side chain carboxyl groups of the polymer in the pleated sheet structure are situated on each side of the sheet with repeat distances, 6.5 to 7.0 A along the chain direction and 4.5 or 9.0 A along the hydrogen bonding direction. Since acridine orange molecule is about 3.2 A thick, a linear array of stacked dimeric dyes can best form on a single extended polypeptide chain, each central nitrogen atom on every other dye cations being close to each side chain carboxyl group, and the molecular planes of dye being almost perpendicular to the chain direction. Formation of such a stacked linear array of dye molecules is consistent with the strong stacking tendency of acridine orange.[25,26] To make the arrangement of dye chromophores asymmetric for optical activity to be induced, such linear arrays must occur on both sides of a sheet, or most likely, of a single polypeptide chain, and the molecular planes of dye must be inclined from perpendicular to the chain direction. From the assignment of polarization directions of absorption bands given below, it follows that the shorter axis of dye molecular plane will remain perpendicular but its longer axis should have a small component parallel to the chain direction. Dimension of a dye molecule in its longer axis direction extends over three polypeptide chains, and in this lateral direction no regular arrangement of bound dyes can be expected to form. All these considerations lead to the structure of the acridine orange-β-form complex, as illustrated in Figure 8.

At neutral pH where the polymer is randomly coiled, the dye-polymer complex is also formed, but the stacking interaction among bound dimeric dyes is weaker, and no regular array of bound dyes can be formed. Thus no circular dichroism is observed in the visible region. The weak dichroic bands in the ultraviolet region would be induced by electronic coupling of the dye transition with shorter wavelength transitions of peptide chromophores.

Polarization of Absorption Bands

Zanker's extensive work[27] and Mason's molecular orbital calculation[9] on acridine orange have shown that the absorption band at 492 mμ is definitely due to the transition to the ^1L_a state and its polarization is, therefore, along the longer axis of molecular plane. Jakobi and Kuhn[28] have confirmed this experimentally. The 470 mμ band of dimeric form, which is the same electronic transition but of a different vibrational level,[29] should have the same polarization as the monomeric form, if Zanker's antiparallel dimer model is assumed.

The assignment of the ultraviolet absorption bands seems less definitive. From the fluorescence depolarization experiment, however, Zanker[27] assigned to the 290 and 270 mμ bands the transitions to the ^1B_a and ^1M_a states, respectively, but Mason[9] has given another assignment to the former band, which has a polarization along the shorter axis of the dye molecular plane. In accordance with Zanker,[27] Jakobi and Kuhn[28] have given a polarization parallel to the longer axis to the 290 mμ band as well as to the 270 mμ band. In the dimeric dye such as Zanker's model, the polarization of those bands must be kept in the same direction.

When the acridine orange dimers form a regular linear array on an extended polypeptide chain, as shown in Figure 8, each of those dimer absorption bands splits into two exciton levels, one polarized parallel to the chain direction and the other perpendicular to it.[30-32] All the other exciton levels do not contribute to the absorption.

In the present dye-β-form complex, only a single peak is observed at each of the absorption bands, and, even if two peaks exist, one of them would be so weak that it might be concealed in the tail of the stronger absorption band. In the proposed model for the complex, which has the dye molecular plane almost perpendicular to the chain direction, a significant contribution of the perpendicular component emerges in the absorption but there is a very weak contribution of the parallel component. Since the 458 and 261 mμ bands have a polarization along the longer axis of the dye molecular plane and are both optically active, it is required that their polarization direction or the longer axis of the dye molecules must be inclined from perpendicular to the chain direction. Since no other transitions show observable optical activity, at least when the polymer is in the β-form, the shorter axis of the dye would be vertical to the chain direction, or presumably to the plane of sheet. These considerations lead to the inclination of bound dye molecules, as shown in Figure 8.

Optical Activity of the Bound Acridine Orange

The observed circular dichroism spectra of the dye-β-form complex have a pair of positive and negative dichroic bands at each of the absorption bands, 458 and 261 mμ. These dichroic bands arise from contributions to the circular dichroism of many exciton levels of a degenerate system, which are "nonuniformly" distributed around the absorption bands.[33] The unsym-

metrical shape of the circular dichroism curve comes from a nonconservative contribution of the exciton levels to the rotatory strengths, including the electronic interaction of the transition with other transitions.[34]

In an N-fold degenerate system, an excited state, E, consists of N exciton levels, the K-*th* level of which has a frequency, ν_{EK}, and a rotatory strength, R_{EK}. The molar ellipticity associated with the K-*th* level, at a frequency ν, can be written as

$$[\theta]_{EK} = \frac{48\pi^2 N_A}{hc} \nu R_{EK} f(\nu - \nu_{EK}) \tag{1}$$

where h is the Planck constant, c is the light velocity and N_A is the Avogadro number. The spectral shape function, $f(\nu - \nu_{EK})$, positive and normalized, is assumed to be independent of K.

According to Tinoco,[33-35] circular dichroism associated with the transition, 0-E, consists of a sum of those N circular dichroic bands and is given by

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

where ν_{EO} is some definite frequency at the circular dichroic bands. While the first term gives a normal circular dichroic band with the rotatory strength, ΣR_{EK}, the second term yields a pair of bands with opposite sign but with almost equal magnitude, determined by the rotatory oscillator strength, $\Sigma(\nu_{EK} - \nu_{EO})R_{EK}$.

In the present dye-β-form complex the signs of the experimental circular dichroism curve lead to

$$\sum_{K=1}^{N} (\nu_{EK} - \nu_{EO}) R_{EK} < 0 \tag{3}$$

for both visible (E: 458 mμ) and ultraviolet (E: 261 mμ) bands. The nonconservative circular dichroism observed in the visible region requires

$$\sum_{K=1}^{N} R_{EK} > 0 \text{ for E: 458 m}\mu \tag{4}$$

Since no optical activity is induced on the dye-random coil complex, at least in the visible and near ultraviolet regions, it can be similarly assumed that the transition magnetic moment term in the expression of rotatory strength will also be small and negligible for the dye-β-form complex. This is consistent with the fact that no circular dichroism is associated with weak absorption bands such as the 290 mμ band for the dye-β-form complex.

Based on the proposed model for the dye-β-form complex in Figure 8, the rotatory oscillator strength[34] can be calculated, according to the method of Tinoco, Woody and Bradley.[36] If the components of electric dipole mo-

ment of the transition, 0-E, are taken to be $\mu_{E/\!/}$ along the chain direction (y), μ_{Er} along the radial or probably the side chain direction (z) and μ_{Et} along the direction (x) perpendicular to both, as in the right-handed xyz coordinate system, it follows that

$$\sum_{K=1}^{N} (\nu_{EK} - \nu_{EO})R_{EK} = \frac{2\pi\nu_{EO}}{hc} a\mu_{E/\!/}\mu_{Et} \sum_{\substack{k=1 \\ k+l:\text{odd}}}^{N} \sum_{l=1}^{N}{}' V_{kE,lE} \tag{5}$$

where a is the distance of a chromophore from the center line of the extended polypeptide chain. $V_{kE,lE}$ represents the interaction potential of the electric moments, both for the transition 0-E, of *k-th* and *l-th* chromophores, numbered along the chain. Equation 5 indicates that only the interactions of chromophores with those on the other side of the chain or sheet contribute to optical activity. It also shows that the transition electric moment must have a parallel component. Numerical calculation, using assumed geometry for the model, e.g., a = 6 A, and the angle of inclination of the dye plane from perpendicular to the chain direction = 5 to 15°, gives the result that all the above dipole-dipole interactions are attractive for odd values of $k + l$. This is in agreement with the observed result (Eq. (3)) if the dye molecular planes are tilted in such a way they form right-handed helical arrays ($\mu_{E/\!/}\mu_{Et} > 0$) as shown in Figure 8.

For the calculation of the total rotatory strength[34] it is necessary to know the nature of far ultraviolet transitions as well as those in the observed region. To an approximation, only the two transitions (458 and 261 mμ) that are optically active are taken into account here and all the other transitions (including all the peptide transitions and the other dye transitions) are omitted. Thus only the mutual interaction between the above two transitions are included in the calculation. This would be permissible in the discussion only concerning the sign of nonconservative contribution to the paired band, especially in the visible region far apart from the other bands. This approximation simplifies the calculation, and the fact that the two transitions have the same polarization direction leads to

$$\sum_{K=1}^{N} R_{EK} = \mp \frac{4\pi}{hc} \frac{\nu_{AO}\nu_{BO}}{\nu_{BO}^2 - \nu_{AO}^2} a(\mu_{A/\!/}\mu_{Bt} + \mu_{At}\mu_{B/\!/}) \sum_{\substack{k=1 \\ k+l:\text{ odd}}}^{N} \sum_{l=1}^{N}{}' V_{kA,lB}$$

$$\begin{aligned} &- \text{ for E = A: } 458 \text{ m}\mu \\ &+ \text{ for E = B: } 261 \text{ m}\mu \end{aligned} \tag{6}$$

where $V_{kA,lB}$ is the interaction of the *k-th* electric moment for the transition 0-A with the *l-th* moment for 0-B. Numerical calculation for the proposed model of the complex gives negative values for all the interactions with odd $k + l$ values. This leads to a positive rotatory strength for 0-A or visible and a negative strength for 0-B or ultraviolet, if the helical sense of dye aggregate is right-handed as shown in Figure 8. The former result is in accord with the observed one (Eq. (4)).

Induced Circular Dichroism of the Low Molecular Weight Polymer

As was previously shown,[17,18,20] the three polymer samples of different molecular weights exhibited different behavior in the coil-β transition. The two higher molecular weight polymers (E602 and E527) are subject to a clear coil-β transition by changing pH, but the lowest molecular weight polymer (E515) behaves quite differently and no transition occurs. It was suggested that the low molecular weight polymer cannot assume the β-conformation as the high molecular weight polymers can, but probably it remains randomly coiled even at acid pH.

Circular dichroism of the acridine orange-low molecular weight polymer (E515) system in water is shown in Figure 9. At neutral pH no circular dichroism is induced in the visible region, as in the cases of higher molecular weight polymers, but at acid pH strong circular dichroic bands are manifest. Absorption spectra are substantially identical with those given in Figure 1, but circular dichroic spectra at acid pH are quite different from those in Figure 4. Pairs of dichroic bands appear at about the same wavelengths as for the high molecular weight polymers, but they have opposite signs. Furthermore, at the longer wavelength side of each pair, an additional negative band occurs as a weaker shoulder. The mode of binding and the structure of the complex for the low molecular weight polymer must be different from those which higher molecular weight polymers form. The polymer conformation would be different from either β-form or random coil, and optical activity could be conferred on dyes bound to terminal carboxyl groups of the polymer as well as to its side chain carboxyl groups.

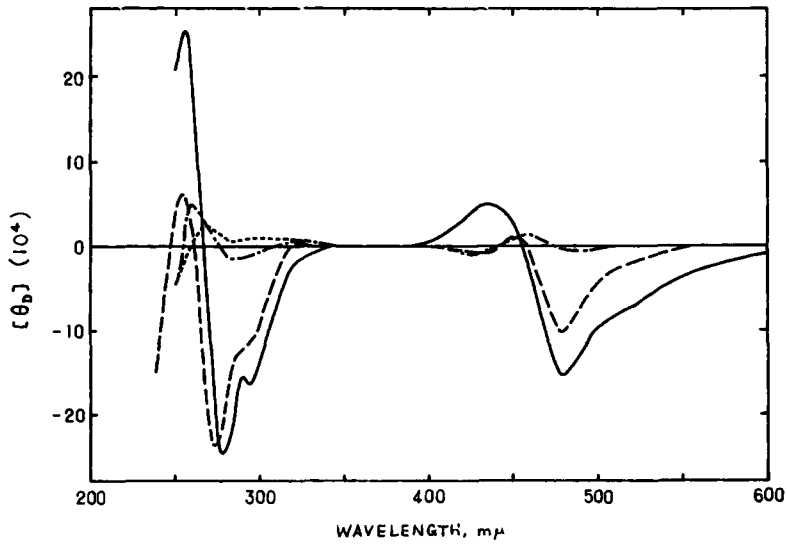

Fig. 9. Circular dichroism of acridine orange-low molecular weight poly-S-carboxymethyl-L-cysteine (E515) system in water. P/D = 167, [D] = 5.2 × 10^{-5} M; ——, pH 4.57; - - - -, pH 4.87; —·—·—, pH 5.52; ·····, pH 7.41.

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