

ture for 2 hr. and was then heated on the steam-bath for 15 min. The product consisted of 0.265 g. (80%) of a semi-solid which was recrystallized from hexane to yield a product, m.p. 113–115°. Further recrystallization gave 0.165 g. (50%) of XVIIb as colorless needles, m.p. 115–

115.5° (lit.¹⁸ 115.5°). A mixed m.p. with a sample of XVIIb prepared by a different route¹⁸ showed no depression in m.p.²⁰

Anal. Calcd. for C₁₈H₂₄O₄: C, 71.02; H, 7.95. Found: C, 71.03; H, 8.16.

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON 15, MASS., AND THE DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH, ARGONNE NATIONAL LABORATORY, LEMONT, ILLINOIS]¹

Optical Rotatory Dispersion of Dyes Bound to Macromolecules. Cationic Dyes: Polyglutamic Acid Complexes^{2,3}

BY LUBERT STRYER AND E. R. BLOUT

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The optical rotatory dispersions of complexes of cationic dyes with synthetic high molecular weight poly- α -glutamic acids have been measured. The dyes used were Acridine Orange, pseudoisocyanine and a substituted toluidine-thiazole dye, [*p*-(5-acetyl-4-methyl-2-thiazolyloxy)-*N*- β -aminoethyl-*N*-ethyl-*m*-toluidine]. Each of these dyes forms a complex with polyglutamic acid over the pH range 4.2 to 6.5. At pH's above 5.5 polyglutamic acid is in the random conformation and the dye:polypeptide complexes show optical rotatory dispersion due to the polypeptide alone. At pH's below 5.1 polyglutamic acid is in the α -helical conformation and the dye:polypeptide complexes show anomalous rotatory dispersion in the dye absorption band (the Cotton effect). The magnitude of the observed Cotton effects is very high; in some cases the molar rotation is in the range 100,000 to 1,000,000. The inflection points of the induced Cotton effects are in good agreement with the absorption maxima of the dyes which leads to the conclusion that the dye chromophores acquire optical rotatory power upon binding to the helical polypeptides. For a single absorption band the signs of the induced Cotton effect are opposite for poly- α ,L-glutamic acid and poly- α ,D-glutamic acid. It is therefore concluded that helices of opposite screw-sense (L and D) show Cotton effects of opposite sign. Two models are suggested to explain the observed Cotton effects in dye:helical polypeptide complexes. Possible uses of this method in the elucidation of the structure of macromolecules of unknown conformation are mentioned.

Introduction

Optical rotation has been used in recent years to study the conformations of numerous macromolecules of biological interest.⁴ Since helical structures are dissymmetric and contribute to the optical rotation of a macromolecule,⁵ they may be characterized by this method. For example, the thermodynamic stability⁶ and proportion of helix⁷ present in some proteins has been estimated. In addition, the activation⁸ and denaturation^{9a} of enzymes have been studied by this method. These are significant contributions to our understanding of the macromolecular conformations of synthetic polypeptides and proteins.

Another approach to the determination of the conformations of macromolecules is the study of the optical rotation within the intrinsic absorption

bands of the macromolecules. However, most work to date has been limited for technical reasons to wave lengths outside these ultraviolet absorption bands.^{9b} In these spectral regions where absorption is low the sum of several optical rotatory contributions has been measured.

A more feasible approach, presented in this paper, involves the measurement of the optical rotatory dispersion throughout the absorption bands of extrinsic chromophores (dyes) bound to synthetic polypeptides. We have reported previously that some symmetric (non-optically active) dye molecules bound to the helical conformation of a polypeptide show an induced anomalous optical rotatory dispersion (Cotton effect) in the absorption band of the bound dye, while no such effect was observed in complexes of dye and the random conformation of the polypeptide.¹⁰

This paper deals with the optical rotatory dispersion of complexes of three different types of cationic dyes with polyglutamic acid. Induced Cotton effects in the absorption bands of the bound dye have been studied with respect to the helix content of the polypeptide, the relative screw-sense of the helix and some of the conditions which alter the mode of binding. These findings with a model system of known conformation may provide a basis for using this method in studying macromolecules of unknown structure.

Experimental

Polypeptides.—Two synthetic polypeptides were used in this study. The sodium salts of poly- α ,L-glutamic acid (L-PGA), sample R4273-112, and of poly- α ,D-glutamic acid (D-PGA), sample GF-5-219, were prepared in a manner

(1) A portion of this work was performed under the auspices of the U. S. Atomic Energy Commission, while L. S. was in residence at the Argonne National Laboratory.

(2) This paper is Polypeptides XXXIII. For the preceding paper in this series see T. Miyazawa and E. R. Blout, *THIS JOURNAL*, **83**, 712 (1961). Alternate address of E. R. Blout, Research Division, Polaroid Corporation, Cambridge 39, Mass.

(3) This work was supported in part by U. S. Public Health Service Grant #A2558.

(4) See for example (a) E. R. Blout, "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Company, New York, N. Y., 1960, p. 238; (b) J. R. Fresco, *Trans. N. Y. Acad. Sci.*, **21**, 653 (1959).

(5) (a) C. Cohen, *Nature*, **175**, 129 (1955); (b) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

(6) (a) J. A. Schellman, *Compt. rend. trav. Lab. Carlsberg, ser. chim.*, **30**, nos. 21–26 (1958); (b) C. Schellman and J. A. Schellman, *ibid.*, **30**, no. 27 (1958); (c) W. F. Harrington and J. A. Schellman, *ibid.*, **30**, no. 3 (1956).

(7) P. Doty, *Rev. Modern Phys.*, **31**, 107 (1959).

(8) K. U. Linderström-Lang and J. A. Schellman, *The Enzymes*, **1**, 477 (1959), 2nd Ed., Academic Press, Inc., New York, N. Y.

(9) (a) For references, see W. Kauzmann, *Adv. Protein Chem.*, **14**, 1 (1959); (b) But see N. S. Simmons and E. R. Blout, *Biophysical Journal*, **55** (1960).

(10) (a) E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci. U. S. A.*, **45**, 1591 (1959); (b) L. Stryer and E. R. Blout, *Abstracts of the Biophysical Society, 4th Annual Meeting*, February, 1960.

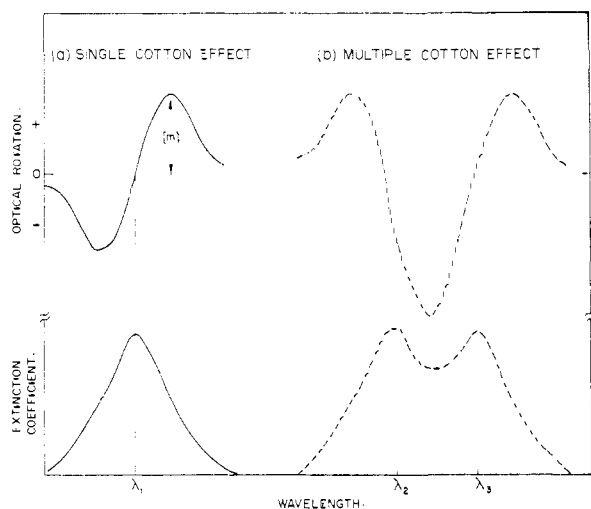
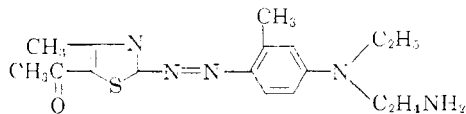


Fig. 1.—Idealized representation of optical rotatory dispersion in the absorption bands of optically active chromophores: (a) a single Cotton effect, characterized by a magnitude $[m]$, a positive sign, and an inflection point at λ_1 , is shown to correspond with the isolated absorption band whose absorption maximum is at λ_1 ; (b) a multiple Cotton effect is shown for two optically active chromophores at wave lengths near one another; the multiple Cotton effect is composed of a negative Cotton effect at λ_2 and a positive Cotton effect at λ_3 .

previously described.^{14b} The L-PGA has an intrinsic viscosity of 1.12 at pH 7.3 in 0.2 M NaCl from which the weight average molecular weight of 51,000 is estimated. The D-PGA has an intrinsic viscosity of 1.29 at pH 7.3 in 0.2 M NaCl from which a weight average molecular weight of 58,000 is estimated.

Dyes.—Three different types of cationic dyes with a single positive charge in the pH range of this study were used. Acridine Orange (AO), 3,6-dimethylamino-acridine, was a commercial preparation (National Aniline) used after two-fold recrystallization from methanol. The commercial preparation is the zinc chloride salt of the dye, but essentially identical results were obtained with a zinc-free sample of Acridine Orange, kindly given to us by Dr. D. Freifelder of the Massachusetts Institute of Technology. Pseudoisocyanine (PIC), 1,1'-diethyl-2,2'-cyanine iodide, was obtained commercially (Light), while the more water-soluble tosyl salt of the dye was synthesized for us by A. Rosenoff of Polaroid Corporation. The substituted toluidine-thiazole dye (TT), *p*-(5-acetyl-4-methyl-2-thiazolylazo)-*N*- β -aminoethyl-*N*-ethyl-*m*-toluidine



was synthesized by Dr. S. Kasman of Polaroid Corporation.

Preparation of Dye: Polypeptide Complexes.—Aqueous solutions of the sodium salt of the polypeptides were acidified by slow addition of 0.1 N HCl with vigorous stirring to avoid precipitation. When the polypeptide solution had a pH between 4.2 and 4.5, it was filtered through sintered glass. Dye solution and, when desired, salt solution, were then added slowly and brought to the appropriate pH by addition of 1 N NaOH; a Beckman pH meter was used. In some cases, upon standing, a minor amount of precipitation occurred. The solutions were shielded from bright light in order to avoid photosensitized reactions.

A complex of the micellar form of pseudoisocyanine and polyglutamic acid was prepared in a different manner. The polymerized form of pseudoisocyanine was obtained by salting-out the dye (1 ml. of 11.3 mg./ml. in methanol) upon addition of 4 M NaCl (15 ml.); this preparation exhibited the brilliant fluorescence and birefringence characteristic of the micellar state of the dye.¹¹ The micellar PIC:PGA com-

plex was formed by adding 1 ml. of micellar dye to 5 ml. of 0.014% L-PGA in 3.2 M NaCl, with a final pH of 4.7. Exact concentrations could not be determined because some precipitation occurred in the preparation of the micellar dye and also in the complex after several days. The rotatory dispersion and absorption spectra were obtained soon after preparation of the complexes. The rotation readings at each wave length were constant during the period of measurement.

Absorption Spectra.—Absorption spectra were measured in 1 cm. cells in a Cary Model 11 recording spectrophotometer. Both absorption spectra and rotatory dispersion work were carried out at $24 \pm 1^\circ$. Extinction coefficients are expressed in cm^2/mM .

Optical Rotation.—Optical rotations were measured with a Rudolph photoelectric polarimeter Model 200. Accurate readings in the dye absorption bands with an optical density as high as 2.5 required a high intensity light source covering a large spectral region. A General Electric AH-6 water-cooled, high pressure mercury arc was used for this purpose. A Beckman DU monochromator was used to obtain essentially monochromatic radiation. Since the sign and magnitude of rotation change quite abruptly with wave length in the absorption band, it is important to obtain high spectral purity. This can be estimated from the slit-width and the dispersion of the monochromator. The maximum half-intensity band width for the Acridine Orange complexes was $3 \text{ m}\mu$, which is satisfactory for accurate rotation measurement. On the other hand, for the toluidine-thiazole complexes it was $16 \text{ m}\mu$, which is of the same order of magnitude as the observed Cotton effect for those complexes. The maximum half-width for the pseudoisocyanine complexes was $5 \text{ m}\mu$, which is adequate for good resolution, except for the Cotton effect corresponding to the very narrow J band at $574 \text{ m}\mu$, whose half-width is approximately $5 \text{ m}\mu$. Spectral impurity leads to a diminution and broadening of the observed Cotton effect.

Cell paths of 1, 5 and 10 cm., were used for the complexes, while a 20 cm. path was used for polypeptide solutions without dye. The 1 and 5 cm. cells had fused quartz end-plates with a maximum birefringence about $\pm 0.005^\circ$, and thus care was required in the positioning of the cells. A symmetrical angle of 2° was used for all the measurements. We estimate that our maximum uncertainty for a reading at a particular wave length is $\pm 0.003^\circ$, while the internal consistency of a set of readings at various wave lengths once the cell has been positioned is about $\pm 0.001^\circ$.

The data (see Figs.) are expressed in terms of both observed rotation, α , and the molar rotation, $[m]$; the former providing an index of the reliability of a set of observations and the latter showing the magnitude of the optical rotation in terms of the contribution per chromophoric group. The molar rotation is defined as

$$[m] = [\alpha] \frac{\text{mol. wt.}}{100}$$

$$[\alpha] = 100.\alpha/l.c$$

where $[\alpha]$ = specific rotation (degrees), mol. wt. = gram molecular weight of the dye, α = observed rotation (degrees), l = cell path (decimeters), c = concentration of dye in grams/100 ml. solution. The units of $[m]$ are degrees mole⁻¹ decimeter⁻¹ ml. The total concentration of dye, rather than that bound to sites inducing the optical activity, has been used to calculate $[m]$; therefore the reported molar rotations are smaller than the true molar rotations.

Results

The anomalous optical rotatory dispersion observed in the absorption band of an optically active chromophore is termed the Cotton effect.^{12,13} A Cotton effect is characterized by its sign, magnitude half-width and wave length of the inflection point (Fig. 1a). When the Cotton effect corresponds to an isolated absorption band, the inflection point is

(11) E. E. Jelley, *Nature*, **138**, 1009 (1936).

(12) For example, see W. Kuhn, *Ann. Revs. Phys. Chem.*, **9**, 417 (1958).

(13) (a) T. M. Lowry, "Optical Rotatory Power," Longmans, Green and Co., London, 1935; (b) C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

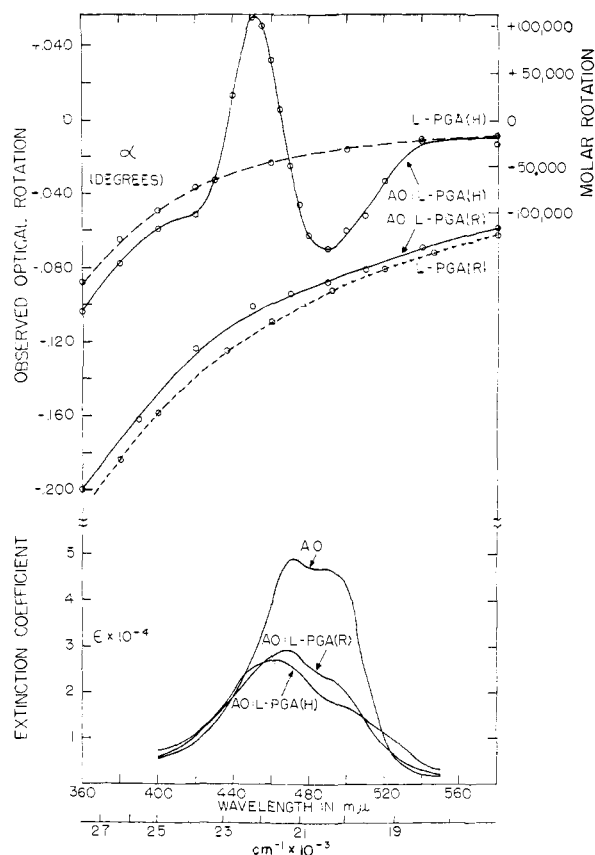


Fig. 2.—Optical rotatory dispersions and absorption spectra of complexes of acridine orange and poly-L-glutamic acid: the absorption spectrum of unbound acridine orange (AO) markedly changes upon binding to either the random conformation of poly-L-glutamic acid (AO:L-PGA(R)) or the helical conformation (AO:L-PGA(H)); only the complex of acridine orange and the helical conformation of L-PGA (AO:L-PGA(H)) exhibits a Cotton effect (1 cm. path cell, see Table I).

the wave length at which the partial rotation is zero and coincides with the absorption peak of the optically active chromophore. The sign of the Cotton effect is, by convention, positive if the partial rotation is positive at wave lengths longer than the inflection point and negative if the partial rotation is negative.¹⁸ The magnitude of the Cotton effect is the absolute value of the maximum molar rotation on either side of the inflection point. When there are two optically active chromophores in the same wave length region, multiple Cotton effects (Fig. 1b) are observed. The parameters of each Cotton effect can be obtained by resolution of the multiple effect into the partial rotations of each chromophore. Optically inactive chromophores, such as those of the unbound dyes in this study, show no Cotton effect or optical rotation in their absorption bands.

The optical rotatory dispersion has been studied for each of the dyes bound to both random and helical conformations of polyglutamic acid. It has been shown by optical rotation, viscometry and infrared dichroism that poly- α -L-glutamic acid (L-PGA), a water-soluble synthetic polypeptide, undergoes a reversible change in conformation with pH .¹⁴

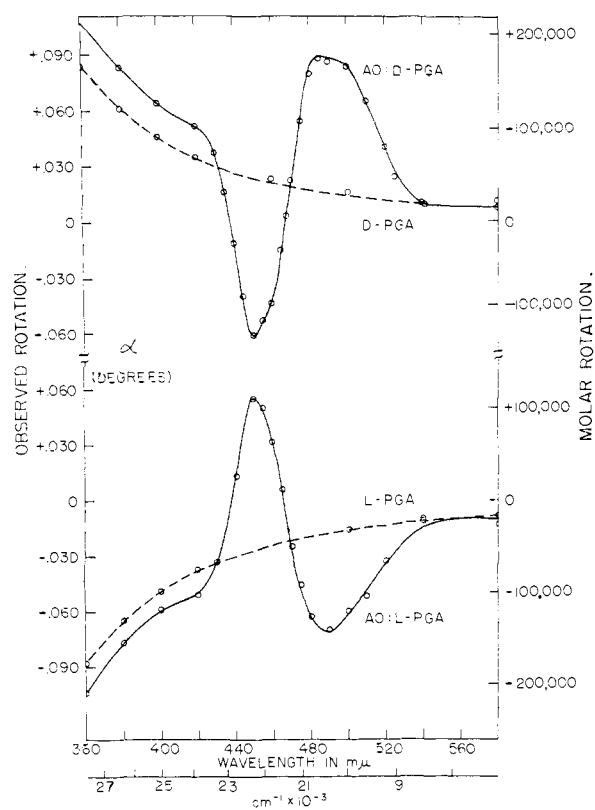


Fig. 3.—Optical rotatory dispersions of complexes of acridine orange and helical poly-L-glutamic acid (AO:L-PGA), and of acridine orange and the polypeptide of opposite screw-sense of helix, poly-D-glutamic acid (AO:D-PGA) (1 cm. cell path, see Table I).

At lower pH 's, L-PGA is in the α -helical¹⁵ conformation, while at higher pH 's it exists in the random coil; the transition pH depends upon the salt concentration of the solution. In water, the helical structure loses its stability around pH 5.5 and becomes essentially random at pH 6.1, while in 0.2 M NaCl the transition takes place between pH 5.0 and 5.6.

A. Acridine Orange.—Acridine Orange (AO) forms complexes with both the random and α -helical conformations of polyglutamic acid, as shown by the large spectral shift observed upon binding (Fig. 2).

The optical rotatory properties of the AO:random L-PGA complex are almost identical with those of the random L-PGA without dye (Fig. 2). However, the AO:helical L-PGA complex displays a markedly anomalous optical rotatory dispersion, with the Cotton effect having an inflection point which closely corresponds with the absorption maximum of the bound dye (Table I).

The complex of acridine orange with the helical polypeptide of opposite screw-sense, D-PGA, gives a Cotton effect with about the same inflection point and magnitude as the AO:L-PGA complex but of opposite sign (Fig. 3).

(14) (a) P. Doty, A. Wada, J. T. Yang and E. R. Blout, *J. Poly. Sci.*, **23**, 851 (1957); (b) M. Idelson and E. R. Blout, *THIS JOURNAL*, **80**, 4631 (1958).

(15) The X-ray diffraction work of C. Johnson shows that polyglutamic acid in the solid state is in the α -helical conformation: Ph.D. Thesis, Massachusetts Institute of Technology, 1959.

TABLE I
CHARACTERISTICS OF COTTON EFFECTS IN THE ABSORPTION BANDS OF SEVERAL CATIONIC DYES BOUND TO α -HELICAL POLY-GLUTAMIC ACID

Dye (mole/l.)	Polypeptide (g./100 ml.)	Glutamate/ dye residue (mole ratio)	Electro- lyte	pH	Absorp- tion max. bound dye ($m\mu$)	Cotton effect		Approx. magnitude ^a [m]	Ref. to fig.	
						Inflection point ($m\mu$)	Sign			
Acridine orange ($5 \times 10^{-5} M$)	L-PGA (0.72%)	960	None	4.5	465	468	Negative	150,000	2, 3	
	D-PGA (0.68%)	900	None	4.5	465	468	Positive	150,000	3	
	L-PGA (0.77%)	1020	0.18 <i>M</i>	NaH_2PO_4	4.3	477	468	Negative	150,000	5
						499	510	Positive	300,000	
D-PGA (0.67%)	880	0.18 <i>M</i>	NaH_2PO_4	4.2	477	468	Positive	100,000	5	
					499	510	Negative	200,000		
Toluidine-thiazole dye ^b ($1.2 \times 10^{-4} M$)	L-PGA (0.44%)	242	None	4.5	480	485	Positive	15,000	6	
	D-PGA (0.33%)	183	None	4.5	479	477	Negative	24,000	6	
Pseudoisocyanine (soluble form) ($4.5 \times 10^{-5} M$)	L-PGA (0.66%)	977	None	4.6	493	487	Negative	70,000		
					529	538	Positive	140,000		
Pseudoisocyanine (micellar form)	L-PGA (0.012%)	3 to 9	2.7 <i>M</i> $NaCl$	4.6	496	503	Negative	See text	7	
					525	535	Negative			
					574	574	Positive			
(less than $2.6 \times 10^{-4} M$, see text)	D-PGA (0.012%)	3 to 9	2.7 <i>M</i> $NaCl$	4.6	496	503	Positive	See text	7	
					525	536	Positive			
					574	574	Negative			

^a See Experimental section for the definition of [m]. ^b See Experimental section for the chemical definition of the toluidine-thiazole dye.

TABLE II
COMPARISON OF THE SHORT WAVE LENGTH AND LONG WAVE LENGTH COTTON EFFECTS OF ACRIDINE ORANGE BOUND TO α -HELICAL POLYGLUTAMIC ACID

	Short wave length Cotton effect	Long wave length Cotton effect
Wave length of absorption maximum	Varies from 455 to 470 $m\mu$ depending on the concentration of acridine orange and polyglutamic acid	498 $m\mu$ for the complexes which exhibit the largest magnitude of the long wave length Cotton effect
Inflection point of the Cotton effect	468 $m\mu$ ($\pm 2 m\mu$)	510 $m\mu$ ($\pm 2 m\mu$)
Sign of the Cotton effect for the L-PGA complex	Negative	Positive
Magnitude of the Cotton effect as a function of pH	Constant from pH 4.3 to 4.9. Diminishes at pH 's higher than 4.9 in a manner that closely parallels the helix-coil transition. Reaches zero at pH 6.2 where polyglutamic acid is no longer helical	Greatest magnitude at pH 4.3, and diminishes with increasing pH . Reaches zero at pH 4.9
Magnitude of the Cotton effect as a function of the ionic strength of the solution	Does not vary with ionic strength in the range studied (up to 0.18)	Magnitude is zero in 0.018 <i>M</i> NaH_2PO_4 and increases with ionic strength to its maximum in 0.18 <i>M</i> NaH_2PO_4 . Sodium chloride, sodium borate and sodium sulfate act in a similar manner
Magnitude of the Cotton effect as a function of the polypeptide/dye residue ratio	Does not vary between ratios of 10 to 10,000 glutamate residues/dye	Magnitude is zero at a glutamate residue/dye ratio of 41 and increases to a maximum value at a ratio of ~ 500

AO:helical PGA complexes may exhibit either one or two Cotton effects, depending on the conditions of binding. While the shorter wave length Cotton effect (468 $m\mu$) is observed whenever acridine orange binds to helical PGA, the longer wave length (510 $m\mu$) Cotton effect is seen only under conditions of high ionic strength, low pH and high glutamate/dye residue ratio (Table II). These two Cotton effects differ in sign and magnitude, and their superposition results in a multiple Cotton effect (Fig. 4). The data in Fig. 5 show the multiple Cotton effects observed with AO and that the sign of each Cotton

effect is opposite for dye complexes with helical polypeptides of opposite screw-sense.

The absorption spectrum of the complexes exhibiting only the shorter wave length Cotton effect is different from that of the complexes exhibiting both Cotton effects (Table II). The magnitude of [m] is of the order of 100,000 for the shorter wave length Cotton effect, while it is about 200,000 for the fully developed longer wave length Cotton effect.

B. Toluidine-Thiazole Dye.—The toluidine-thiazole dye (TT) forms complexes with both

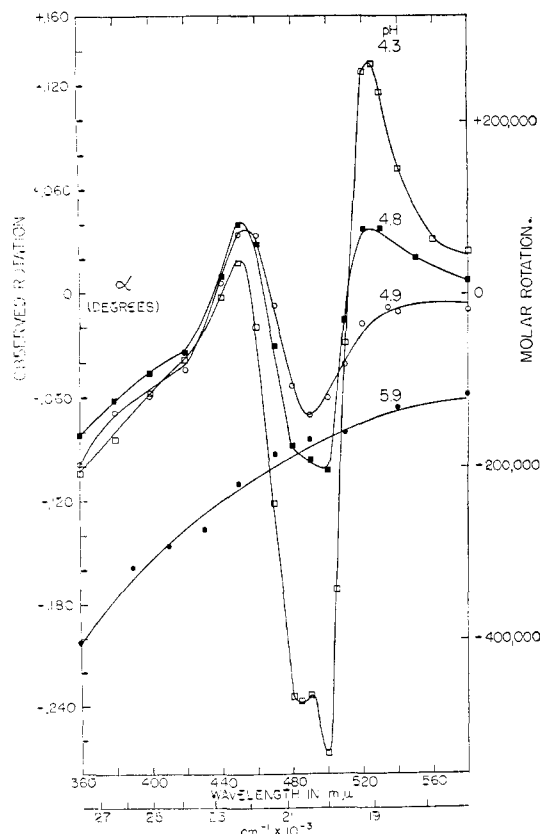


Fig. 4.—Optical rotatory dispersions of complexes of acridine orange and helical poly-L-glutamic acid in solutions of high ionic strength ($0.18 M NaH_2PO_4$) at pH 4.3, 4.8, 4.9 and 5.9. The magnitude of the shorter wave length Cotton effect ($468 m\mu$) is nearly constant between pH 4.3 and 4.9, while the magnitude of the longer wave length Cotton effect ($510 m\mu$) decreases from its maximum at pH 4.3 to zero at pH 4.9. No Cotton effect is observed at pH 5.9, where polyglutamic acid is in the random conformation (1 cm. cell; L-PGA, 0.77%; AO, $5 \times 10^{-4} M$).

the random and α -helical conformations of polyglutamic acid. Upon binding, the absorption maximum of the dye shifts to shorter wave lengths (529 to $480 m\mu$) and the maximum extinction coefficient is diminished (3.4×10^4 to 1.4×10^4) (Fig. 6).

As with Acridine Orange:PGA complexes, a Cotton effect is observed with the TT:helical PGA complexes but not in the TT:random PGA complexes (Table I). The Cotton effect has its inflection point near the absorption maximum of the bound dye, and the magnitude of $[m]$ is of the order of 20,000. The sign of the Cotton effect of TT:L-PGA is positive, while that of the dye bound to the helical polypeptide of opposite screw-sense is negative (Fig. 6).

C. Pseudoisocyanine.—Two types of complexes of pseudoisocyanine (PIC) and PGA were studied. In solutions of low ionic strength, pseudoisocyanine is in true solution, while in saturated salt solutions, the dye exists in micellar form.¹¹

The soluble PIC:PGA complex has two absorption peaks in the visible spectrum, and a multiple Cotton effect is observed for the complex with

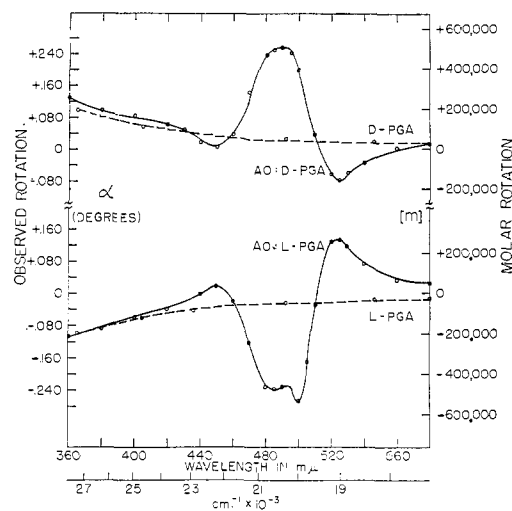


Fig. 5.—Optical rotatory dispersions of complexes of acridine orange and helical poly-L-glutamic acid (AO:L-PGA) and of acridine orange and the opposite screw-sense of helix, poly-D-glutamic acid (AO:D-PGA), in a solution of high ionic strength ($0.18 M NaH_2PO_4$) (1 cm. cell, see Table I).

helical PGA. The two component Cotton effects have inflection points which show good correspondence with the absorption peaks (Table I) of the bound dye.

The micellar PIC:PGA complex is of interest, since there is a longer wave length absorption band of very narrow half-width (J band) in addition to the two absorption bands of the soluble PIC:PGA complex. The positions of these bands are unaltered when the micellar pseudoisocyanine complexes with polyglutamic acid.

The optical rotatory dispersions of the micellar PIC:helical PGA complexes (Fig. 7) are a composite of three individual Cotton effects with inflection points which agree well with the absorption maxima (Table I). The sign is negative for the two shorter wave length Cotton effects and positive for the long wave length absorption band. The micellar PIC:D-PGA complex shows Cotton effects of opposite sign.

The magnitude of the long wave length ($574 m\mu$) Cotton effect is very large but is not known exactly because of the uncertainty of the micellar dye concentration resulting from precipitation in the course of preparation. An estimate of the actual concentration was made spectrophotometrically. The limits of the magnitude of this long wave length Cotton effect then can be calculated to be $1,000,000 \pm 500,000$ with the higher estimate more likely. No Cotton effect is observed with micellar PIC:random PGA complexes.

Discussion

These studies show that certain symmetric dye molecules exhibit anomalous optical rotatory dispersion (Cotton effect) in their absorption bands upon binding to helical polyglutamic acid, while the unbound dyes and the dyes bound to random polyglutamic acid do not show this effect. The molar rotations of these induced Cotton effects are exceptionally large. The inflection points of the induced Cotton effects show good agreement with

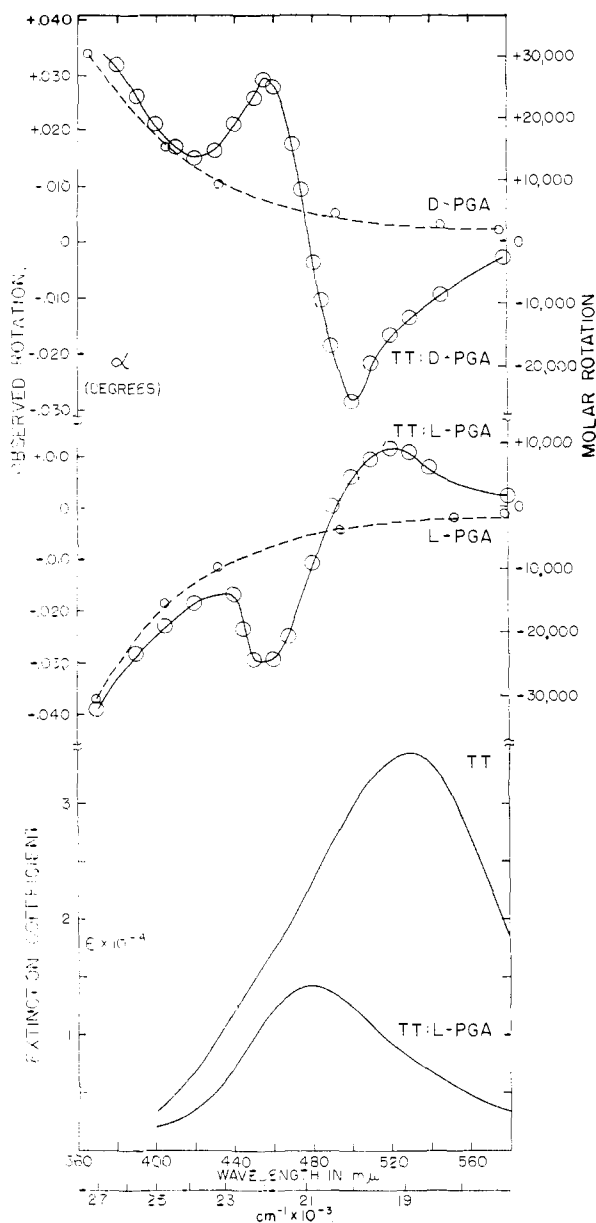


Fig. 6.—Optical rotatory dispersions and absorption spectra of complexes of the toluidine-thiazole dye and helical polyglutamic acid: TT, unbound dye; TT:L-PGA, toluidine-thiazole dye:helical poly-L-glutamic acid complex; TT:D-PGA, toluidine-thiazole dye:helical poly-D-glutamic acid complex (1 cm. cell, see Table I).

the absorption peaks of the bound dye, and thus it is established that the dye chromophore acquires optical rotatory power upon binding to a helical polypeptide. For a single absorption band, the sign of the induced Cotton effect is opposite for polyglutamic acid helices of opposite screw-sense, *i.e.*, containing either L or D residues.

The acquired optical rotatory power of the bound dye molecule can be of only two types: configurational or conformational. "Configurational-induced" optical rotatory power¹⁶ could arise from

(16) This type of induced optical rotatory dispersion has been extensively studied for the interaction of the symmetric ketone chromophore and asymmetric steroid molecules. See C. Djerassi, "Optical

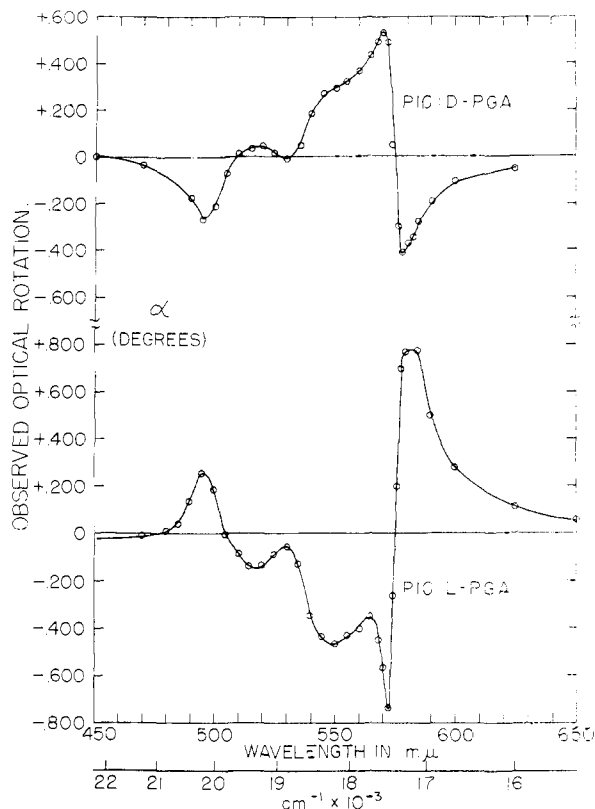


Fig. 7.—Optical rotatory dispersions of complexes of m-cellar pseudoisocyanine and helical poly-L-glutamic acid (PIC:L-PGA) and of helical poly-D-glutamic acid (PIC:D-PGA) (1 cm. cell, see Table I).

the interaction of the symmetric dye chromophore with the local asymmetric environment of the α -carbon atom of the polypeptide. On the other hand, "conformationally-induced" optical rotatory power might result from the interaction among several symmetric dye chromophores which have been oriented in a specific fashion to one another by binding to the asymmetric polypeptide.¹⁷ Our data are insufficient to evaluate these two types unequivocally, but nevertheless certain inferences can be made, and three schematic models are offered to facilitate discussion (Fig. 8).

"Configurational-induced" optical rotatory power could arise from the type of bonding shown in model I, but there is no experimental support for this arrangement. The data favor the conformational type of induced optical rotatory power, in which the dye aggregate is the asymmetric unit, and two models of this type (II and III) are shown in Fig. 8. In model II, dye molecules are bound in a repetitive fashion to form a dye "super-helix" of a single screw-sense which is superimposed on the polypeptide α -helix. A different type of dye helix is

Rotatory Dispersion," McGraw-Hill Book Company, New York, N. Y., 1960.

(17) This type of conformational dissymmetry was lucidly described by Pasteur a century ago: "Imagine a spiral stair whose steps are cubes, or any other objects with superposable images. Destroy the stair and the dissymmetry will have vanished. The dissymmetry of the stair was simply the result of the mode of arrangement of the component steps," L. Pasteur, "Collected Works," Vol. 1, Masson, Paris, 1922, p. 332.

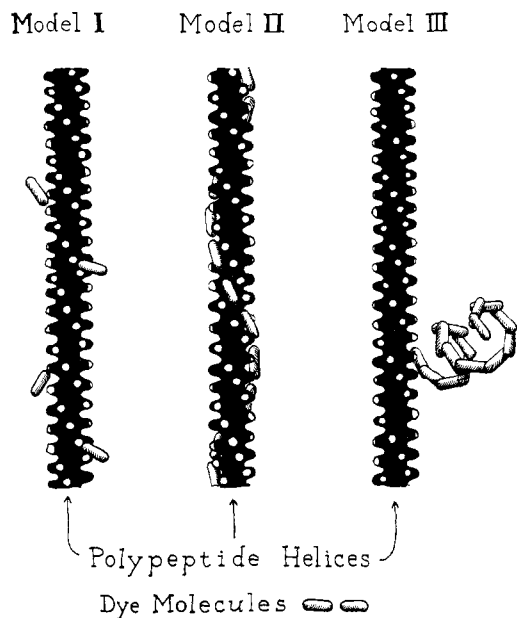


Fig. 8.—Suggested modes of interaction between dye and polypeptide that could give rise to the induced Cotton effects in the absorption bands of the bound dye; model I, unaggregated dye interacts near the asymmetric α -carbon atom of the peptide residue; model II, dye end-to-end aggregate giving rise to dye super-helix; model III, tangential dye helix.

shown in model III, in which the asymmetric polypeptide acts as a sterically determined "seeding center" to favor one of the screw senses of a dye helix which forms tangential to the polypeptide α -helix. In this model, the helical structure of the dye cluster is an intrinsic feature of the dye-dye interaction, but in the absence of an asymmetric "seed," equal amounts of left- and right-handed dye helix are formed.

Models II and III are supported by the observation that all dyes which exhibit these induced Cotton effects are known to aggregate in water solutions.^{18,19} The absorption spectra of these dye: polyglutamic acid complexes in low-ionic strength solutions are similar to those observed for solutions of unbound aggregated dye.^{18,20} This also has been observed in dye complexes of several other polyanions and has been thought to indicate dye-dye interaction on the polyanion surface.²¹

Furthermore, recent X-ray diffraction studies have indicated that several dyes,²² including pseudoisocyanine,²³ aggregate in a helical fashion when concentrated in water solution. Such a helical

(18) For Acridine Orange, see V. Zanker, *Z. physik. Chem.*, **199**, 15 (1952); for rhodamine, see V. L. Levchine and E. G. Baranova, *J. Chim. Phys.*, **55**, 869 (1958); for pseudoisocyanine, see G. Scheibe, *Kolloid-Z.*, **82**, 1 (1938).

(19) It should also be noted that all cationic dyes that we have studied that fail to aggregate also fail to exhibit induced Cotton effects.

(20) We have observed a shift of the absorption maximum from 528 to 478 $m\mu$ of the toluidine-thiazole dye upon increasing the concentration of the dye in water solution.

(21) D. F. Bradley and M. K. Wolf, *Proc. Natl. Acad. Sci. U. S.*, **45**, 944 (1959).

(22) (a) A. Rich and D. M. Blow, *Nature*, **182**, 423 (1958); (b) D. M. Blow and A. Rich, *This Journal*, **82**, 3566, 3752 (1960).

(23) A. Rich and M. Kasha, unpublished data. We wish to thank Professors Kasha and Rich for making this information available to us.

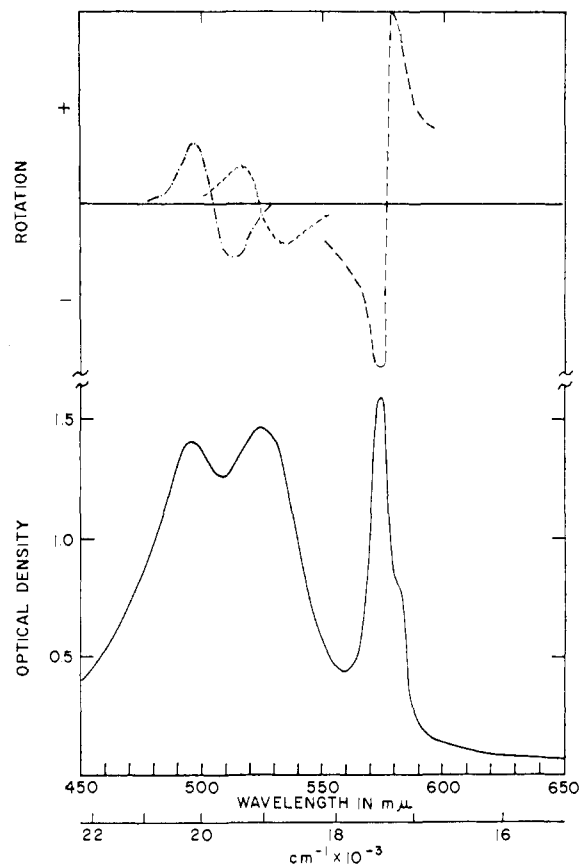


Fig. 9.—The relationship of the absorption bands (lower curve) of the micellar pseudoisocyanine:helical poly-L-glutamic acid complex to the resolved Cotton effects (upper curves) (cf. Fig. 7). The dichroism of the absorption bands corresponds with the signs of the Cotton effects.

pseudoisocyanine micelle has no optical rotation since it is a racemic mixture of both screw-senses of helix. However, in the presence of helical polyglutamic acid, it seems likely that the formation of one of the screw-senses of "tangential-dye helix" is favored, and this right- or left-handed pseudoisocyanine helix then exhibits optical rotatory power.

Further evidence for the postulate that the micellar pseudoisocyanine:polyglutamic acid complexes exist as "tangential-dye helices" is suggested by correlating the signs of the observed Cotton effects and the dichroism of the micellar dye. It has been observed that in micellar pseudoisocyanine the dichroism is *perpendicular* to the dye fiber axis for the two shorter wave length absorption bands in the visible, while it is *parallel* for the longer wave length J band.²⁴ The signs of the Cotton effects of these complexes correspond with the dichroism, in that the two shorter wave length Cotton effects have a sign opposite that of the long wave length Cotton effect in the J band (Fig. 9).

Apart from micellar pseudoisocyanine there is no information on the dichroism of the dyes studied; hence interpretation of the signs of their Cotton effects is difficult. However, there is a common pattern in the anomalous optical rotatory dispersions of the complexes of Acridine Orange, Rhoda-

(24) W. Hoppe, *Kolloid-Z.*, **109**, 21 (1944).

mine B,²⁵ Rhodamine 6G^{10a} and the soluble form of pseudoisocyanine. When bound to helical poly-L-glutamic acid, a negative Cotton effect is observed at the short wave length visible absorption band, and a positive Cotton effect at the longer wave length absorption band. At this time we have not determined whether this difference in the sign of the Cotton effects arises from dye molecules bound in an identical manner but possessing different dichroisms for the two bands or from dye molecules bound in two different ways as in models II and III.

The wave lengths of the inflection points of these Cotton effects, as well as their signs, may reveal some features of the interactions between these dyes and polypeptides that result in induced anomalous optical rotatory dispersion. The complexes of acridine orange and helical polyglutamic acid (Table II) show Cotton effects with inflection points at 468 and 510 $m\mu$. The optically active dye complexes have absorption bands displaced to wave lengths both shorter and longer than that of the unaggregated free dye at 492 $m\mu$. If these shifts result from dye-dye interactions, then certain inferences as to the geometry of these aggregates can be made on the basis of the recently developed exciton theory for dye aggregates.²⁶ Long wave length shifts are thought to indicate a head to tail arrangement of the dye transition moments, while short wave length shifts are thought to arise from a planar stacking arrangement of dyes. Thus, it is possible that in the acridine orange:helical polyglutamic acid complexes, the shorter wave length Cotton effect reflects the model III interaction ("tangential dye-helix" in a more card-pack like arrangement), while the longer wave length Cotton effect results from the model II interaction ("tangential-dye super-helix" in which a head to tail arrangement of dye transition moments is possible).

There are some additional findings that are of theoretical interest. The molar rotations, $[m]$, of these induced Cotton effects are very large (of the order of 1,000,000 for one of the complexes) which may be related to the suggested helical arrangement

(25) Unpublished data.

(26) (a) E. G. McRae and M. Kasha, *J. Chem. Phys.*, **28**, 721 (1958); (b) M. Kasha, *Rev. Modern Phys.*, **31**, 162 (1959).

of the dye chromophores.²⁷ Another feature of the micellar pseudoisocyanine:helicalpolyglutamic acid complexes is the very narrow half-width of the Cotton effect at 574 $m\mu$ corresponding to the J band. This seems to be the first example of anomalous optical rotatory dispersion in a J band. We note that these complexes may be a convenient system for the theoretical evaluation of the optical rotatory power of helices, since only one or two absorption bands are involved in some of the complexes.

Finally, the dye:macromolecule rotatory dispersion method may prove to be a powerful tool in the elucidation of the conformation of several types of macromolecules. A study of the optical rotatory dispersion of complexes of dyes and other macromolecules has begun and Cotton effects have been observed in the absorption bands of dyes bound to tobacco mosaic virus and desoxyribonucleic acid.²⁸ The existence of Cotton effects in dye:macromolecule complexes could point to the presence of helical regions, while the sign might reveal the relative sense of the helix. The broadest application of this method in elucidating the structure of macromolecules will require a more precise knowledge of the mode of binding and transition moments of the bound chromophores.

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(27) Another molecule possessing an exceptionally large molar rotation is hexahelicene; unpublished data of A. Moscovitz. It is possible that such large molar rotations may be found only in systems with helical dissymmetry.

(28) N. S. Simmons, L. Stryer and E. R. Blout, to be published.