

*ANOMALOUS OPTICAL ROTATORY DISPERSION OF
DYE: POLYPEPTIDE COMPLEXES* †*

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The optical rotatory dispersions of various anionic azo dye:serum albumin complexes outside the region of the dye absorption maxima have been reported to be significantly different from those of serum albumin solutions without dye. Since the dyes alone possessed almost no optical rotatory activity, the observed differences were thought to be the result of structural changes occurring in the serum albumin molecule upon the binding of dye.¹

To investigate the various factors influencing the optical rotation of dye:protein complexes, we have initiated a study of the optical rotatory dispersion of complexes of dyes and high molecular weight water-soluble polypeptides of known structure and conformation. In this communication we describe the results with two cationic dyes and poly- α ,L-glutamic acid (L-PGA).

Experimental.—Commercial preparations of dyes were used: Acriflavine Neutral (National Aniline) and Rhodamine 6G (E. I. du Pont). The sodium salt of poly- α ,L-glutamic acid, sample R4273-112, prepared in the manner previously described² was used to prepare the free acid (L-PGA). This sample has an intrinsic viscosity of 1.12 at pH 7.3 in 0.2 M NaCl from which a weight average molecular weight of 51,000 is estimated. A Rudolph spectropolarimeter with a mercury arc was used, and readings were taken at 23°C ($\pm 2^\circ$). 1-mm, 1-cm, and 10-cm cells were used. The dye:PGA solutions were prepared and adjusted to pHs between 4.5 and 4.9 by slow addition of 0.1 N and 1 N HCl with constant stirring. The clear solutions were immediately used and were protected from light before measurements were made.

Results.—The dyes alone showed no optical rotation. The optical rotatory dispersion of L-PGA alone in its helical conformation at pH 4.8 is shown in Figure 1 alone with that of the Acriflavine:L-PGA complex. This complex and the other (Table 1) show markedly anomalous optical rotatory dispersions in the wavelength regions corresponding to the dye absorption bands.

TABLE 1
ANOMALOUS OPTICAL ROTATION IN THE ABSORPTION BAND OF CATIONIC DYES BOUND TO
POLYPEPTIDE

Dye:L-PGA Complex and pH	Dye Concentration	PGA Carboxyl Concentration	Carboxyl/ Dye Ratio	Inflection Point of Anomalous Rotatory Dispersion	Absorption Maximum of Bound Dye
Acriflavine pH 4.9	$3.3 \times 10^{-4} M$	$6.1 \times 10^{-2} M$	185	458 m μ	457 m μ
Rhodamine 6G pH 4.6	$2.5 \times 10^{-5} M$	$6.6 \times 10^{-2} M$	2640	491, 502, 533 m μ	496 and 529 m μ
	$5.0 \times 10^{-5} M$	$6.6 \times 10^{-2} M$	1320	492, 506, 537 m μ	498 and 530 m μ

The observed anomalous rotatory dispersion of the dye:PGA complexes is the Cotton effect³ (circular dichroism). The Cotton effect observed in these complexes indicates that the chromophoric group of the dye has acquired asymmetry, since a

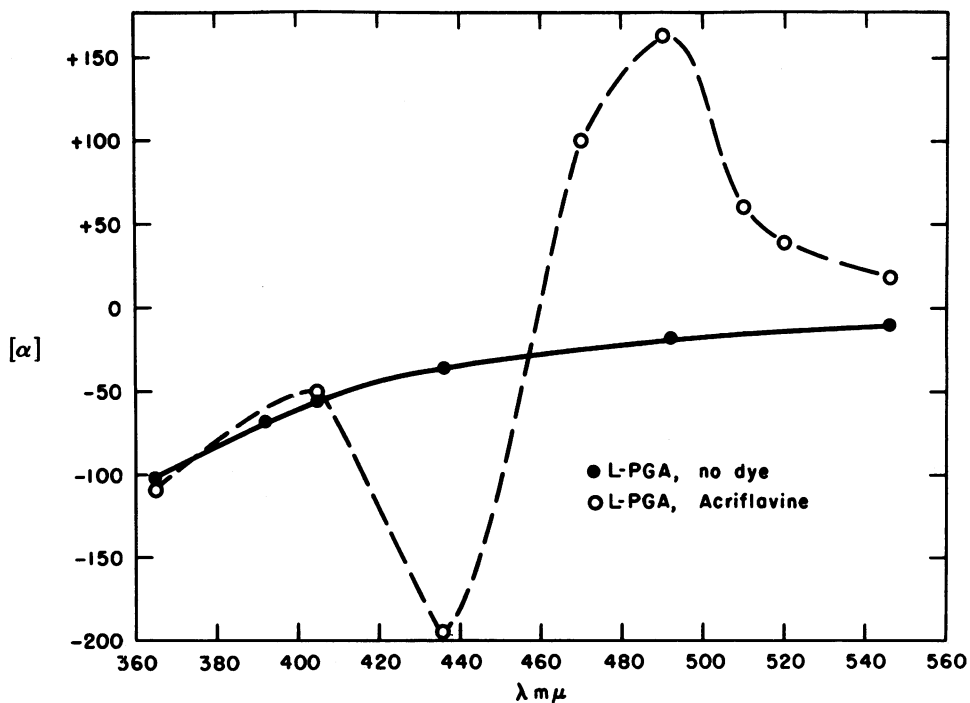


FIG. 1.—Optical rotatory dispersion of an Acriflavine:L-PGA complex at pH 4.9. Concentrations are Acriflavine, $3.3 \times 10^{-4} M$; PGA carboxyl, $6.1 \times 10^{-2} M$. $[\alpha]$ for both figures has been calculated on the basis of PGA concentrations.

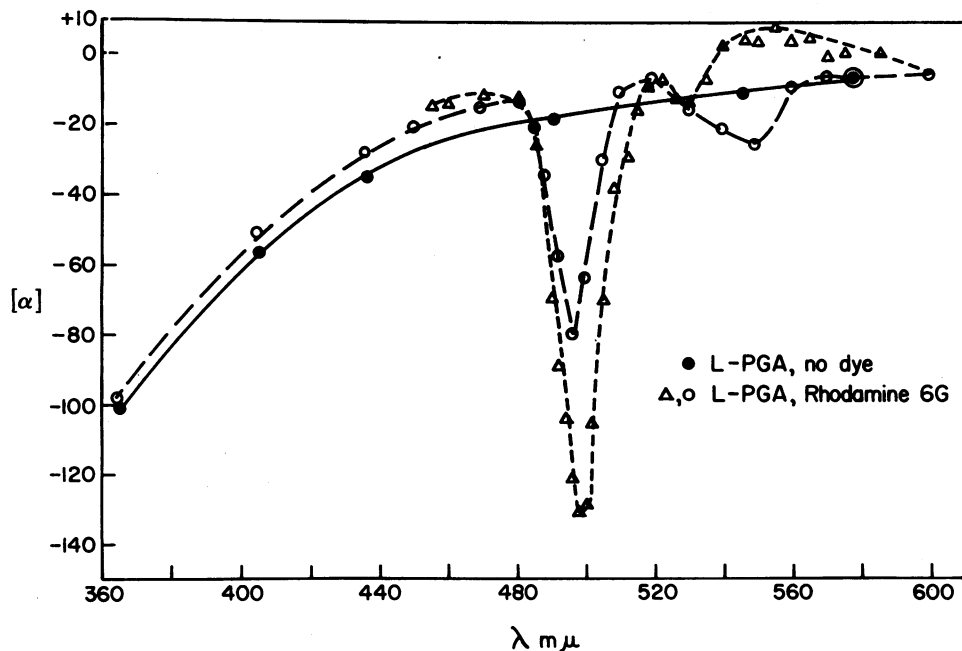


FIG. 2.—Optical rotatory dispersion of Rhodamine 6G:L-PGA complexes at pH 4.6. Concentrations are Rhodamine 6G, $-\circ-\circ-$, $2.5 \times 10^{-5} M$ (and $-\Delta-\Delta-$, $5 \times 10^{-5} M$; PGA carboxyl, $6.6 \times 10^{-2} M$.

symmetrical dye molecule does not show this effect.⁴ This finding is of considerable interest; we believe that this is the first report that the chromophoric group of a symmetrical molecule may show this effect upon binding to an asymmetric helical macromolecule.

The inflection points in the optical rotatory curves indicate the absorbing moiety responsible for the anomaly inasmuch as these inflections show good agreement with the absorption maxima of the dyes (Table 1). Rhodamine 6G:L-PGA solutions exhibit several inflection points in its dispersion curves (Fig. 2). Two of these are near absorption maxima observed at 496 and 529 m μ .

In contrast to results obtained when the L-PGA is in the helical conformation at low pH, no anomalous dispersion is exhibited by the dye:PGA complexes at pHs above 6, where the PGA is in a random conformation.^{2, 5} L-PGA in the random conformation strongly binds the dyes, as shown by equilibrium dialysis and spectral shift studies, and thus the difference appears to be related to the helix content. The magnitude of the Cotton effect of Acriflavine:L-PGA as a function of pH shows good agreement with the helix content as determined from $[\alpha]_{546}$.

It should be noted that a large Cotton effect is observed with low dye concentrations. $[\alpha]$ for all figures has been calculated on the basis of PGA concentration. However, on the basis of dye concentrations, the observed anomalous rotations would be of the order of 20,000. The general nature of this effect has been confirmed and extended by experiments with several other cationic dyes.⁶

In summary, cationic dyes and helical PGA interact and apparently create an asymmetry in the chromophoric groups of the formerly symmetrical dye as evidenced by the observed Cotton effect. The dependence of the observed circular dichroism on the helix content of the polypeptide may make this a useful method in estimating the helix content of other polypeptides and proteins. In order to further study this effect experiments are underway with polypeptides of the opposite sense of twist of the helix as well as investigations of dye interactions with other types of helical macromolecules. The results reported here thus demonstrate that interpretations of optical rotatory data must take into account the possibility that an optically inactive substance may contribute to the optical rotation upon binding to an asymmetrical substance, and indicate that this phenomenon may be utilized as a tool in the study of interactions between small molecules and macromolecules.

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¹ Markus, G., and F. Karush, *J. Am. Chem. Soc.*, **80**, 89 (1958); Winkler, M. H., and G. Markus, *ibid.*, **81**, 1873 (1959).

² Blout, E. R., and M. Idelson, *J. Am. Chem. Soc.*, **78**, 497 (1956); Idelson, M., and E. R. Blout, *ibid.*, **80**, 4631 (1958).

³ For a review and previous references see Kuhn, W., *Ann. Rev. Phys. Chem.*, **9**, 417 (1958).

⁴ Partington, J. R., "An Advanced Treatise on Physical Chemistry," in *Physical Chemical Optics*, **4** (London, Longmans, 1953).

⁵ Doty, P., A. Wada, J. T. Yang, and E. R. Blout, *J. Polymer Sci.*, **23**, 851 (1957).

⁶ Observations of L. Stryer at Argonne National Laboratory.