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HELICAL SENSE OF POLYPEPTIDES AND PROTEINS

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IN this paper no attempt will be made to give a general review of the rotatory properties of polypeptides and proteins, since such an account has appeared recently.[1] Instead, some work will be summarized, most of it as yet unpublished, which relates to the determination of one aspect of the detailed macromolecular conformations of such molecules. It is now evident, as a result of recent investigations,[2] that the optical rotatory dispersion serves to distinguish the helical conformation of a synthetic polypeptide from random conformations. Since the helical conformation[3] (Fig. 1) appears to be a basic structural unit not only of synthetic polypeptides but also of proteins[4] it is important to be able to determine the sense of helix. Helices are dissymmetric and as such can be either right-handed or left-handed. The work reported here describes two methods (and some results) for determining the relative sense of helical polypeptides through the use of optical rotatory dispersion measurements and has been carried out in most part by Norland, Stryer and Fasman in our laboratory.

Although the equation (1) proposed by Moffitt[5] now is recognized

$$[\alpha]_\lambda = \left(\frac{100}{M_0}\frac{\eta^2+2}{3}\right)\left[a_0\frac{\lambda_0^2}{\lambda^2-\lambda_0^2} + b_0\frac{\lambda_0^4}{(\lambda^2-\lambda_0^2)^2}\right] \qquad (1)$$

to be without sound theoretical basis,[6] it does describe the optical rotatory dispersion of a number of helical polypeptides. For example, poly-γ-benzyl-L-glutamate has been shown to obey equation (1) from 265 to 600 mμ and to yield b_0 values of *minus* 660°.[7] We have extended these measurements below the absorption band due to the benzyl group and find the equation is still fitted down to 240 mμ.[8] It was therefore with some surprise that we observed several years ago that poly-β-benzyl-L-aspartate showed rotatory dispersive properties which fitted the Moffit equation but which gave b_0 values of *plus* 630°.[9] Since polybenzyl-aspartate can be considered as a lower homolog of polybenzyl-glutamate we were unprepared to observe any such radical change in the rotatory dispersive properties. However, these results have been confirmed by repeated

[1] C. Djerassi, *Optical Rotatory Dispersion* Chap. 17 by E. R. Blout, p. 238. McGraw-Hill, New York (1960).
[2] W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.* **42**, 596 (1956); J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[3] L. Pauling and R. B. Corey, *J. Amer. Chem. Soc.* **72**, 5349 (1950).
[4] C. Cohen and A. Szent-Gyorgyi, *J. Amer. Chem. Soc.* **79**, 248 (1957); J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957); J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim.* **30**, Nos. 21-26 (1958); G. C. Schellman and J. A. Schellman, *Ibid.* **30**, No. 27 (1958); B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 57, 70 (1958).
[5] W. Moffitt, *J. Chem. Phys.* **25**, 467 (1956); *Idem., Proc. Natl. Acad. Sci.* **42**, 736 (1956).
[6] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci.* **43**, 723 (1957).
[7] P. Doty, *Proceedings of the IVth International Congress of Biochemistry, Vienna* Vol. IX, p. 8. Pergamon Press, London (1959).
[8] R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).
[9] E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.* **80**, 1259 (1958); E. R. Blout, *Proceedings of the IVth International Congress of Biochemistry, Vienna* Vol. IX, p. 37. Pergamon Press, London (1959).

syntheses and by hydrolysis of the polymers to the resulting L-amino acids to assure that no racemization had taken place during the synthesis.[8]

The observation that poly-β-benzyl-L-aspartate had a b_0 value equal in magnitude but opposite in sign to that of poly-γ-benzyl-L-glutamate immediately suggested the possibility that the aspartate polypeptide preferred to form the helix of opposite sense to that of the glutamate, but alternative explanations could account for these changes

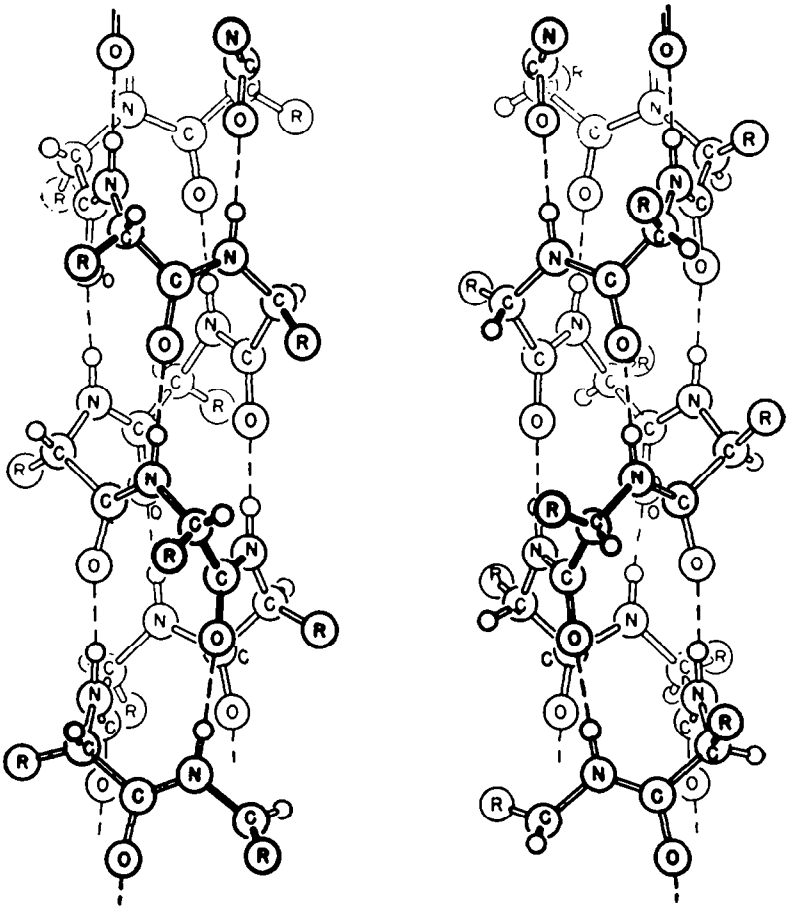


FIG. 1. A drawing showing two possible forms of the α helix; the one on the left is a left-handed helix, the one on the right is a right-handed helix. In both, the amino-acid residues have the L configuration. [Reproduced by permission of the Istituto Lombardo, Milano, from R. B. Corey and L. Pauling, *Rend. ist. lombardo sci.* P 1, **89**, 10 (1955).]

in properties. The problem we set ourselves was to determine the relative sense of helix of polybenzyl-L-glutamate and polybenzyl-L-aspartate. This we believe we have done by studying the optical properties of a series of copolymers of benzyl-L-aspartate with benzyl-L-glutamate and a series of copolymers of benzyl-D-aspartate with benzyl-L-glutamate.

If the sense of helix of poly-γ-benzyl-L-glutamate is opposite to that of poly-β-benzyl-L-aspartate then copolymers of D-aspartate with L-glutamate should show a regular progression of optical properties whereas those composed of L-aspartate and

L-glutamate residues should show a nonregular progression of optical properties because of the change of sense of helix at some point in the copolymer series. Fig. 2 shows the specific rotations of these two series of copolymers in a solvent (chloroform) which favors the helical conformation. It is apparent that the optical rotation in the copolymer series L-benzyl-aspartate:L-benzyl glutamate is non-linear and markedly

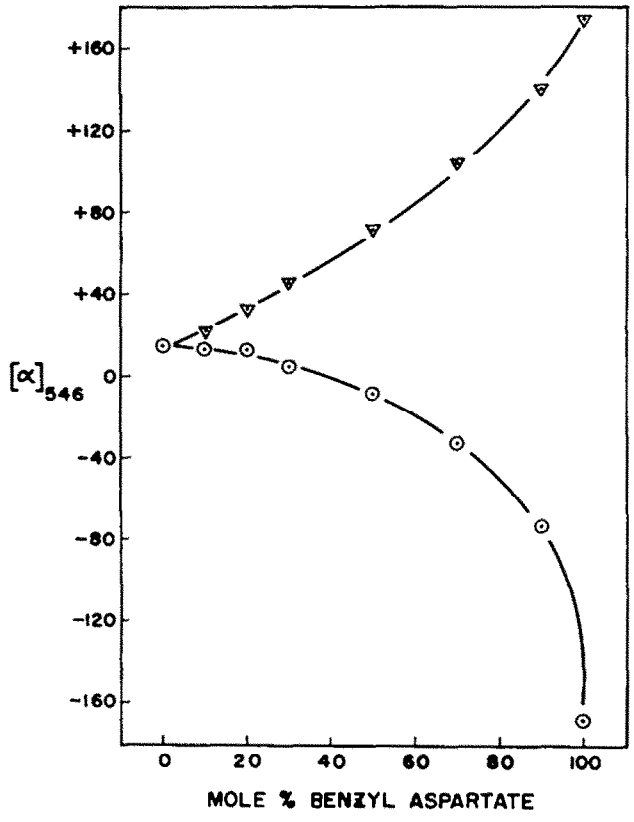


FIG. 2. The specific rotation, $[\alpha]_{546}$, of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ○— ○—○, and of co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in chloroform solutons. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

dependent on the mole percent benzyl-aspartate in the copolymers. On the other hand in the copolymer series D-benzyl-aspartate:L-benzyl-glutamate the optical rotation varies in a regular manner with the mole percent benzyl aspartate.

When the same copolymers are dissolved in a strongly hydrogen-bonding solvent, which destroys the helical conformation, both series of copolymers showed linear variation of $[\alpha]_{546}$ with the mole percent benzyl-aspartate as seen in Fig. 3.

When the optical rotatory dispersions of the copolymers in the helical conformation (chloroform solution) are fitted to equation (1) the coefficient of the second term, b_0, is found to vary in the manner shown by the optical rotation, i.e., there is marked dependence upon mole percent benzyl-aspartate in the L-benzyl-aspartate:L-benzyl-glutamate copolymer series and a relatively linear dependence in the D-benzyl-aspartate:

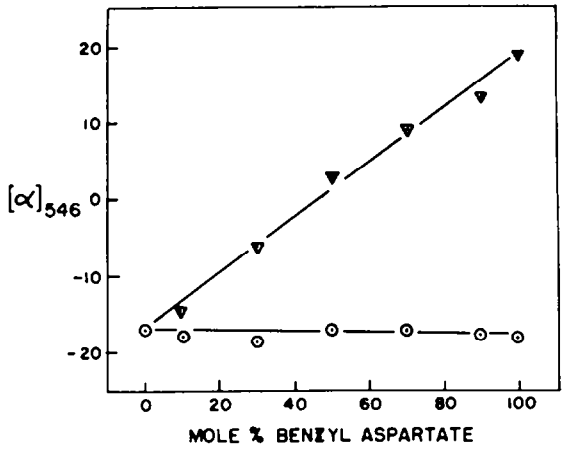


FIG. 3. The specific rotation, $[\alpha]_{546}$, of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ○—○—○, and of co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in dichloroacetic acid solutions. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

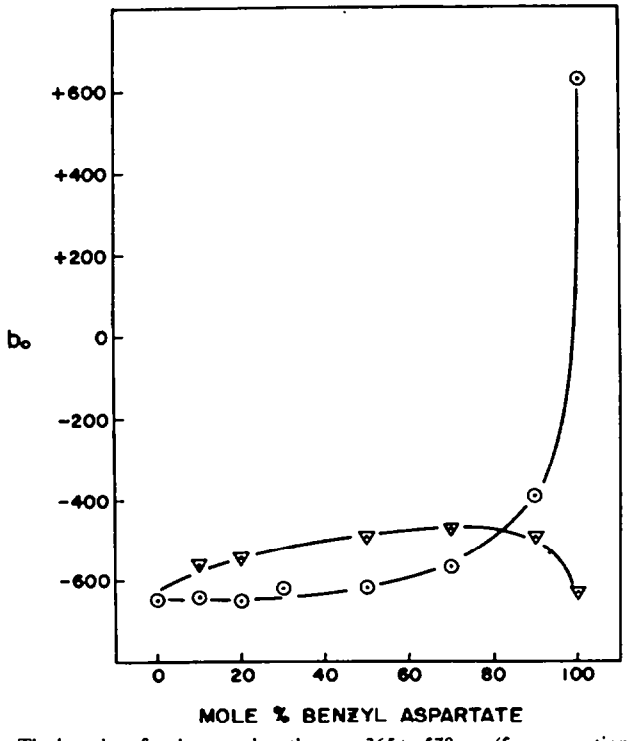


FIG. 4. The b_o values for the wave length range 365 to 578 mμ (from equation (1) assuming λ_o = 212 mμ) of co-polymers of β-benzyl-L-aspartate:γ-benzyl-L-glutamate ○—○—○, and co-polymers of β-benzyl-D-aspartate:γ-benzyl-L-glutamate ▽—▽—▽ in chloroform solutions. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

L-benzyl-glutamate series. These data are shown in Fig. 4. From previous studies on copolypeptides of D- and L-amino acids,[10-12] it might be argued that if L-polybenzyl-aspartate and L-polybenzl-glutamate exist as helices of the opposite sense of twist, the optical rotation and b_0 of *random* copolymers of these polypeptides should change markedly over a relatively narrow range of composition representing a region of transition from one sense of helix to the other. Furthermore, since it has been demonstrated through titration[8] and deuterium exchange[13] studies that the L-polybenzyl-aspartate helix is less stable than the L-polybenzyl-glutamate helix the transition should occur at less than 50 mole percent L-benzyl-glutamate. On the other hand the optical rotation and b_0 of D-benzyl-aspartate:L-benzyl-glutamate copolymers in a helical conformation should show a regular dependence on composition which would be evidence that no change of helix sense occurs in this series. As can be seen from the figures, this is indeed the case and therefore we conclude that there is a change of helical sense in the L:L copolymer series while no change of sense or twist occurs in the D:L copolymer series.

Further evidence for the thesis that the opposite sense of twist exists in L-polybenzyl aspartate than does in L-polybenzyl glutamate is obtained from ultraviolet rotatory dispersion data.[8] The data for two polypeptides over the wavelength range 240–340 mμ are shown in Fig. 5. The dispersion of L-polybenzyl aspartate is anomalous while that of L-polybenzyl glutamate in this region is not anomalous but is complex.

When the optical rotatory dispersion data from 600 to 240 mμ for these polymers was plotted in the manner suggested by Moffitt it was found (as mentioned above and as can be seen in Fig. 6) that polybenzyl-L-glutamate fits the Moffitt equation to yield a b_0 value of *minus* 660°. Polybenzyl-L-aspartate fits the Moffitt equation to wavelengths as short as 265 mμ with a b_0 approximately equal to *plus* 700°, but at shorter wavelengths deviates markedly. It has been suggested that this deviation is due to the onset of a positive Cotton effect which could arise from an interaction between the asymmetric α-carbon atom and the ester chromophore which absorbs in the region 220–230 mμ. The suggestion is supported by the fact that the specific rotation at 240 mμ of polybenzyl-L-aspartate is much more positive than that of polybenzyl-L-glutamate is negative. If it is assumed that the rotation of polybenzyl-L-glutamate is due only to the peptide helix and the residue contributions (and this seems a reasonable assumption since no anomaly is observed) then the increased dextrorotation and the anomaly observed in polybenzyl-L-aspartate around 240 mμ must arise from the approach to another absorption band, presumably that of the ester group. The existence of a Cotton effect would imply that the rotation of the ester group in polybenzyl-L-aspartate is restricted whereas where no Cotton effect is observed, as in polybenzyl-glutamate, the ester group (located here on the γ-carbon) would have not have restricted rotation. Indeed it would be desirable to be able to measure to wave-lengths shorter than 240 mμ in order to settle this point. It must be emphasized however that although the ester groups in polybenzyl-L-aspartate may have restricted rotation, which in turn causes the complex rotation below 265 mμ, the presence of oriented

[10] P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.* **43**, 213 (1957).
[11] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* A **242**, 325 (1957).
[12] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957).
[13] E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature, Lond.* **183**, 1736 (1959).

chromophores in the side chain cannot explain all the observed optical effects. To explain the rotatory effects noted above it is necessary to invoke a change of helical sense in L-polybenzyl-aspartate compared to L-polybenzyl-glutamate.

This argument is supported by ultraviolet rotatory measurements on copolymers of D-aspartate and L-aspartate with L-glutamate and some data are shown in Figure 7.

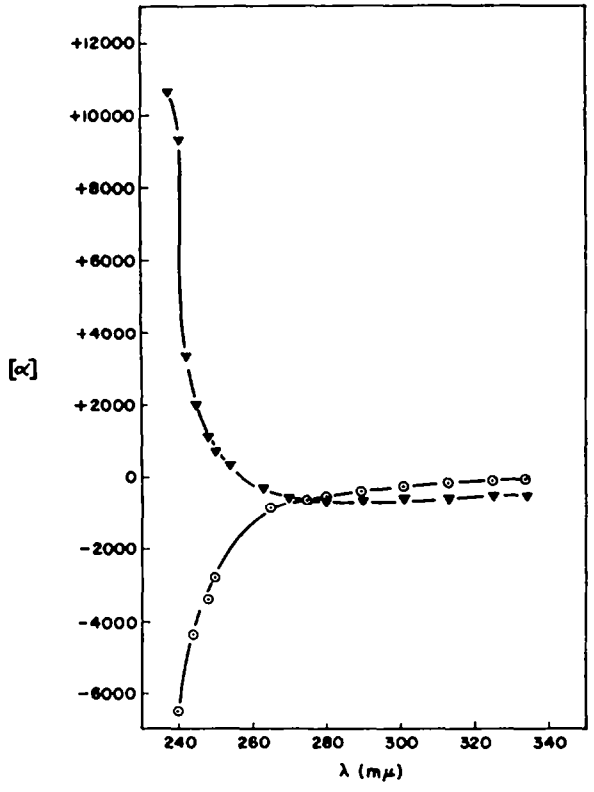


FIG. 5. The ultraviolet rotatory dispersions of poly-β-benzyl-L-aspartate ▽—▽—▽ and poly-γ-benzyl-L-glutamate in chloroform solutions. The specific rotations, $[\alpha]_\lambda$, are plotted as a function of wave length in mμ. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

The anomaly observed and noted above for L-polybenzyl-aspartate is completely lost upon incorporation of 10 mole percent L-polybenzyl glutamate whereas the incorporation of 10 mole percent L-benzyl-glutamate in D-polybenzyl-aspartate does not result in the loss of the anomaly observed with D-polybenzyl-aspartate. In other words it can be concluded from these data that the incorporation of 10 mole percent L-benzyl glutamate in D-polybenzyl-aspartate does not affect the helical sense whereas the incorporation of 10 mole percent L-benzyl-glutamate in L-polybenzyl-aspartate involves a structural change—that is a change in the sense of the helix. Thus these ultraviolet rotatory dispersions offer additional support for the conclusion that L-polybenzyl-aspartate and L-polybenzyl glutamate exist as helices of the opposite sense.

The fact that polybenzyl-L-aspartate may exist with a sense of helix opposite to that observed with poly-γ-benzyl-L-glutamate is not only interesting in itself but may have

some important implications for protein structures. Since our knowledge of helix content and sense of helix of proteins is meagre it is obvious that all methods should be used which may aid in these structural problems. It is possible that the direct observation of the optical rotatory dispersive properties of proteins is such a tool, but from the results now available[1,2,4] it does not appear that the method has sufficient precision to

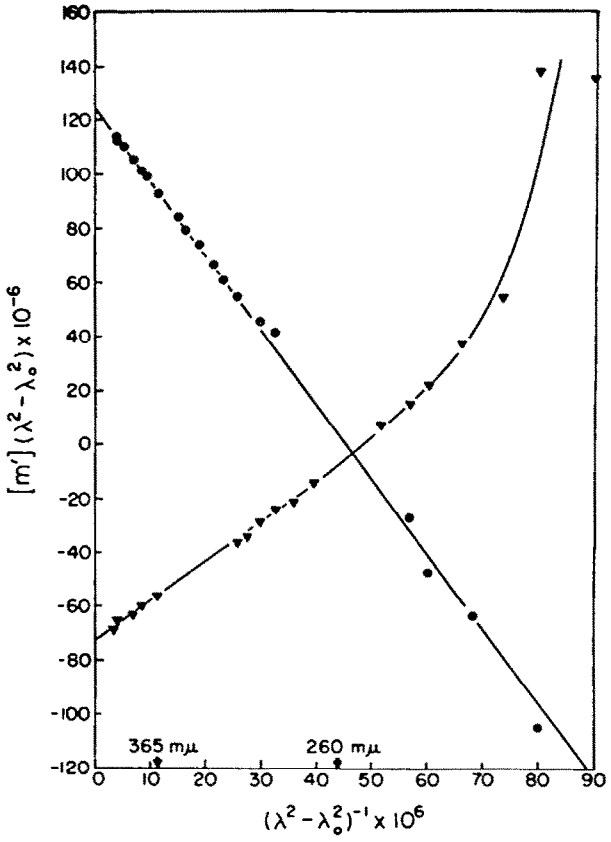


FIG. 6. Moffitt plot (from equation (1) using $\lambda_0 = 212$ mμ) of the optical rotatory dispersion of poly-β-benzyl-L-aspartate ▽—▽—▽ and poly-γ-benzyl-L-glutamate ○—○—○ in chloroform solutions over the wave length range 237 to 578 mμ. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

yield the desired data. As another approach to obtaining information about the relative helix sense in polypeptides and proteins I wish to describe an effect recently observed[14] by Lubert Stryer which is showing considerable promise.

It is well-known that proteins and other macromolecules bind dyes and we have been examining the binding of dyes to solutions of synthetic polypeptides. If basic dyes, such as Acriflavine or Acridine Orange, are added to solutions of polyglutamic acid, it is found that the polyglutamic acid binds the basic dyes over the pH range 4–7. At pH 7 polyglutamic acid exists mainly as the charged polyion and because of its charge this polypeptide attains random conformations at this pH. Measurements of the optical rotatory dispersion of basic dyes bound to polyglutamic acid in the random conformation show rotatory dispersions identical with that of the polypeptide

alone. Since the dyes contain no asymmetric center this finding is not surprising. However, if the optical rotatory dispersion is measured on solutions of the dye:polypeptide complexes when the polypeptide is in a helical conformation, rotatory dispersion of the type shown in Fig. 8 is observed.

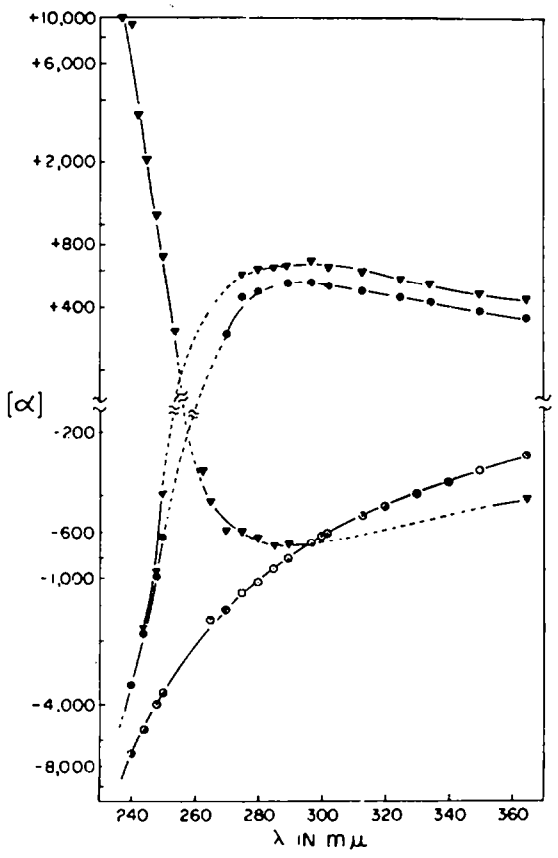


FIG. 7. Ultraviolet rotatory dispersions in chloroform solutions: ▽—▽—▽ poly-β-benzyl-L-aspartate; ○—○—○ 9:1 co-polymer of β-benzyl-L-aspartate:γ-benzyl-L-glutamate; ▼—▼—▼ poly-β-benzyl-D-aspartate; ●—●—● 9:1 co-polymer of β-benzyl-D-aspartate:γ-benzyl-L-glutamate. [Reproduced by permission of the American Chemical Society from R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.* **82**, 2268 (1960).]

When the polypeptide is in the helical conformation, markedly anomalous rotatory dispersions are observed in the wavelength regions corresponding to the dye absorption band. The observed anomalous rotatory dispersion of the dye:helical polyglutamic acid complex is the Cotton effect. The observation of the Cotton effect under these conditions indicates that the chromophoric group of the dye has acquired asymmetry, and the location of the inflection point in the rotatory dispersion curve indicates that the dye is the absorbing moiety responsible for the anomaly. Measurements of these inflection points show good agreement with the absorption maximum of the dye. Data on a number of dye:polypeptide complexes are shown in Table 1 (from Refs. 14 and 15).

[14] E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).
[15] L. S. Stryer and E. R. Blout, *J. Amer. Chem. Soc.* **83**, 1411 (1961).

We have recently investigated this phenomenon in some detail since the magnitude of the Cotton effect with dye:polypeptide complexes may serve as a useful measure of the helix content of polypeptides and proteins. To do this we have measured the Cotton effect of Acridine Orange:polyglutamic acid complexes as a function of pH over the range where polyglutamic acid changes from helix to random conformation. From preliminary data it appears that the dye:helical polypeptide Cotton effect is a good measure of the helix content in this system.

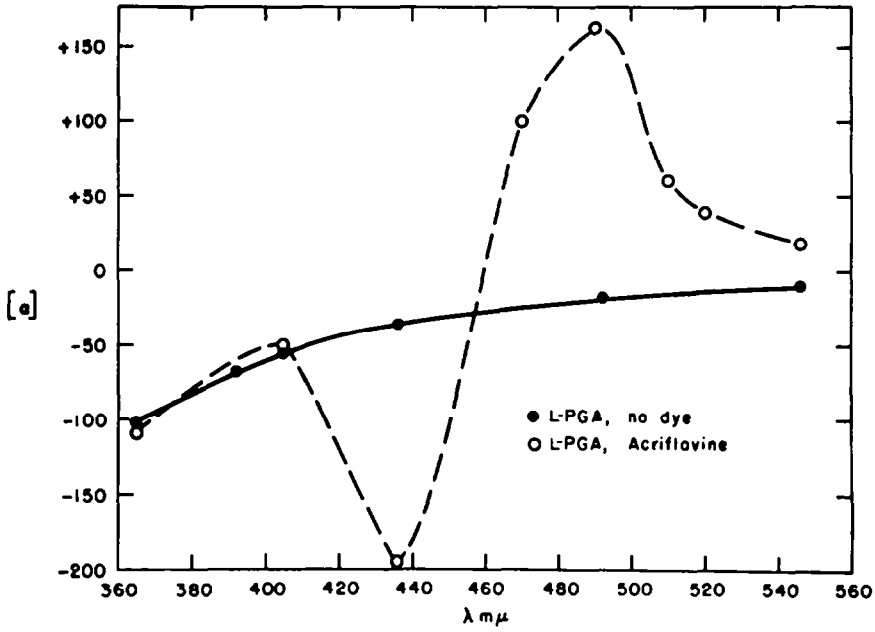
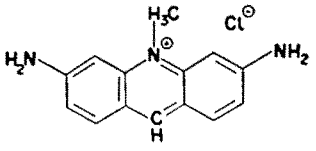
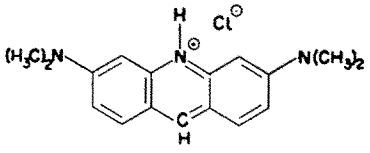
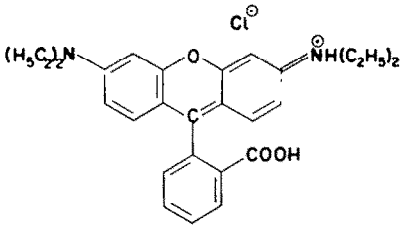
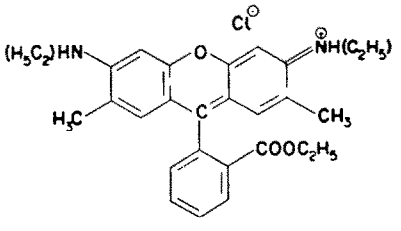
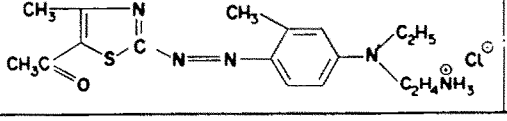


FIG. 8. – – – – – – The rotatory dispersion of an acriflavine:polyglutamic acid complex at pH 4·9. ———— The rotatory dispersion of L-polyglutamic acid at the same pH. [Reproduced by permission of the National Academy of Sciences, Washington, D.C. from E. R. Blout and L. S. Stryer, *Proc. Natl. Acad. Sci.* **45**, 1591 (1959).]

It should be noted that a large Cotton effect can be obtained with various dye: residue ratios (see Table). In fact, definite and measurable Cotton effects have been obtained where the ratio of glutamic acid residues to Acridine Orange had a range between 20 to 1 and 4500 to 1.[15]

Since Acridine Orange shows a negative Cotton effect with L-polyglutamic acid and since L-polyglutamic acid molecules presumably have only one sense of twist, it was of interest to inquire what the effect of a polypeptide substrate of the opposite sense of twist would be on the rotatory dispersion of a dye:polypeptide complex. To examine this we chose the enantiomer of poly-L-glutamic acid, namely poly-D-glutamic acid. Whatever the absolute sense of twist of poly-L-glutamic acid, poly-D-glutamic should have the opposite sense of twist. The results of the optical rotatory dispersion measurements are shown in Fig. 9.[15] As can be seen the Acridine Orange:L-polyglutamic acid complex shows a *negative* Cotton effect whereas the Acridine Orange:D-polyglutamic acid shows a *positive* Cotton effect. Thus the Cotton effects observed with dye:

TABLE 1. SPECTRAL DATA ON THE BINDING OF SEVERAL BASIC DYES TO POLY-L-GLUTAMIC ACID

Dye	Dye structure	pH	Carboxyl: dye ratio	Inflection point of Cotton effect (in mμ)	Absorptio maxima of d (in mμ)
riflavine		4·9	185	458	457
ridine Orange		4·5	915 2435	470 ~ 475	467 and shoulder a 497
odamine B		4·7	1476	558 and 588	556 and shoulder at 585
odamine 6 G		4·6	2640 1320	491, 502, 533 492, 506, 537	496, 529 498, 530
iazole Magenta–T		4·5	190	480	478

polypeptide complexes may serve as a simple and effective method for the determination of the relative helix sense of polypeptides. Such measurements have now been extended to a variety of other dye types.[15]

Finally, encouraged by the above results with polypeptides, we have begun investigations of the rotatory dispersion of dyes bound to proteins. One protein which has

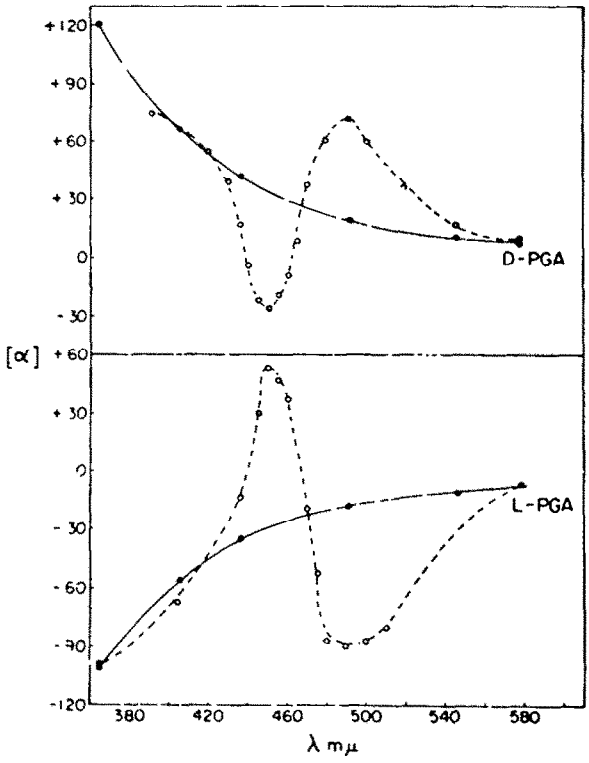


FIG. 9. - - - - - - The optical rotatory dispersion of acridine orange:D-polyglutamic acid complex (top figure), acridine orange:L-polyglutamic acid complex (bottom figure), at pH 4-7. ———— The rotatory dispersions of D- and L-polyglutamic acid respectively at the same pH. [Reproduced by permission of the American Chemical Society from L. S. Stryer and E. R. Blout, *J. Amer. Chem. Soc.* **83**, 1411 (1961).]

been studied is tobacco mosaic virus (TMV) and these experiments are being carried out in collaboration with Simmons[16]. When Acridine Orange is bound to TMV the rotatory data are anomalous and a Cotton effect is observed. If these data are compared with those obtained with Acridine Orange and polyglutamic acid in the presence of phosphate,[15] it is evident that the Cotton effect observed at 510 mμ has the same sign as that observed with the Acridine Orange:L-polyglutamic acid complex under the same conditions. Therefore the preliminary conclusion can be drawn that the sense of helix in TMV is the same as that in L-polyglutamic acid.

Acknowledgement—Much of the work reported here was supported in part by the Office of the Surgeon General, Department of the Army and Grant A2558 from the National Institutes of Health.

[16] N. Simmons, L. Stryer and E. R. Blout, to be published.