

The Ultraviolet Circular Dichroism of Polypeptides¹

G. Holzwarth² and P. Doty

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Measurements of circular dichroism upon α -helical polypeptides clearly reveal three rotatory bands between 185 and 245 $m\mu$. Since the frequencies of these bands correlate well with those observed in the absorption spectra of these molecules, the three rotatory bands are assigned to the $n_1-\pi^-$ transition at 222 $m\mu$, the parallel-polarized $\pi^0-\pi^-$ exciton transition at 206 $m\mu$, and the perpendicularly polarized $\pi^0-\pi^-$ exciton transition at 190 $m\mu$. From absorption and circular dichroic spectra, estimates of both oscillator and rotational strengths have been made. The observed rotational strengths are -22×10^{-40} , -29×10^{-40} , and $+81 \times 10^{-40}$ erg cm.³ rad, respectively. The values agree in sign and order of magnitude with theoretical predictions. Application of the Kronig-Kramers transform to the circular dichroic data shows good agreement between calculated and observed optical rotatory dispersion in the Cotton-effect region. At higher wave lengths, the transform indicates that the parameters of the Moffitt equation are largely a result of the peptide transitions between 180 and 245 $m\mu$. Parallel studies upon disordered polypeptides reveal a region of weak positive circular dichroism from 210 to 235 $m\mu$, contiguous with a region of strong negative dichroism centered near 200 $m\mu$. Although the detailed origins of the optical activity of the disordered polymer are not known, the observed circular dichroism probably arises from the amide $n_1-\pi^-$ and $\pi^0-\pi^-$ transitions. The circular dichroism of metmyoglobin also reveals the three peptide rotatory bands characteristic of synthetic helical polypeptides. This demonstrates that the electronic structure and, hence, the conformation of the natural and synthetic polymers are largely identical.

Introduction

Optical rotatory dispersion between 300 and 600 $m\mu$ has been shown to be a sensitive indicator of the conformation of α -helical and randomly disordered polypeptides.³ The primary dependence of the rotatory dispersion upon molecular conformation rather than upon side-chain constitution suggests that the electronic transitions of the peptide group must be the dominant source of the rotatory power of these molecules. However, dispersion measurements in this spectral region do not reveal which electronic transitions of the peptide group are important to the optical activity. In order to determine the rotational strengths of the individual electronic transitions, the measurements of optical activity must be extended into the spectral regions of the peptide absorption bands. Recently, determinations of both circular dichroism⁴⁻⁶ and

rotatory dispersion⁷⁻¹¹ have been reported for the spectral region of the amide $\pi^0-\pi^-$ and $n_1-\pi^-$ transitions,¹²⁻¹⁴ but these measurements have not yet allowed an unambiguous assessment of the relative roles of these electronic transitions. This was attributed to difficulties of interpretation arising in part from a lack of experimental information on the magnitude and location of the $n_1-\pi^-$ absorption band and in part from the relatively poor precision of the circular dichroic data.

In order to resolve these difficulties, an earlier investigation¹⁵ of the polarized absorption spectra of polypeptide films in the region of the $n_1-\pi^-$ transition has been refined, and an instrument has been constructed which provides circular dichroism curves of considerably greater precision between 185 and 250 $m\mu$ than those previously reported.⁴ This has led to new observations on both α -helical and randomly disordered polypeptides. Before presenting these results, however, it may be useful to review briefly the theoretical expectations for the rotatory power arising from the known electronic transitions of polypeptides.

The importance of resonance coupling of electric dipole transition moments to the optical activity of helical molecules was first noted by Moffitt.¹⁶ He showed that strong rotatory bands of equal intensity but opposite sign, which one may term $[R]_{\nu_{\parallel}}$ and $[R]_{\nu_{\perp}}$, should be associated with the parallel-polarized and perpendicularly polarized exciton absorption bands¹⁷ originating from each strong electronic transition such as the 190- $m\mu$ $\pi^0-\pi^-$ band in polypeptides. It was later shown¹⁸ that Moffitt neglected terms as important as $[R]_{\nu_{\parallel}}$ and $[R]_{\nu_{\perp}}$ because the Born-von Karman boundary conditions used were not applicable to cal-

(5) J. Brahm and G. Spach, *Nature*, **200**, 72 (1963). The measurements reported in this paper are all too large in absolute value by a factor of 10 (J. Brahm, private communication).

(6) M. Grosjean and M. Tari, *Compt. rend.*, **258**, 2034 (1964).

(7) N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer, and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

(8) E. R. Blout, I. Schmier, and N. S. Simmons, *ibid.*, **84**, 3193 (1962).

(9) J. T. Yang and T. Samejima, *J. Biol. Chem.*, **238**, 3262 (1963).

(10) B. Jirgensons, *Makromol. Chem.*, **72**, 119 (1964).

(11) E. Shechter and E. R. Blout, *Proc. Natl. Acad. Sci. U. S.*, **51**, 695 (1964).

(12) The orbital designation is that of H. D. Hunt and W. T. Simpson, *J. Am. Chem. Soc.*, **75**, 4540 (1953).

(13) J. S. Ham and J. R. Platt, *J. Chem. Phys.*, **20**, 335 (1952), and references therein.

(14) D. L. Peterson and W. T. Simpson, *J. Am. Chem. Soc.*, **79**, 2375 (1957).

(15) W. B. Gratzler, G. M. Holzwarth, and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1785 (1961).

(16) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956); *Proc. Natl. Acad. Sci. U. S.*, **42**, 736 (1956).

(17) The existence of two bands arising from exciton splitting was predicted by Moffitt.¹⁶ Evidence for the applicability of this theory to the α -helix was provided by K. Rosenheck and P. Doty (*Proc. Natl. Acad. Sci. U. S.*, **47**, 1775 (1961)), by I. Tinoco, A. Halpern, and W. T. Simpson (in "Polyamino Acids, Polypeptides, and Proteins," M. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 147), and by Gratzler, Holzwarth, and Doty.¹⁵

(18) W. Moffitt, D. D. Fitts, and J. G. Kirkwood, *Proc. Natl. Acad. Sci. U. S.*, **43**, 723 (1957).

(1) Taken in part from the dissertation of G. H., Harvard University, May 1964.

(2) Institute of Molecular Biophysics, Florida State University, Tallahassee, Fla.

(3) See P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961), for a review.

(4) G. Holzwarth, W. B. Gratzler, and P. Doty, *J. Am. Chem. Soc.*, **84**, 3194 (1962).

culations of rotatory power. It should be noted in passing that these additional terms were recently described by Mason^{19,20} as being "significant only as an end-correction," but this claim is mistaken.²¹ More relevant is the evaluation of these terms by Tinoco and co-workers,²²⁻²⁵ who show that for infinitely long helices these terms lead to the prediction of another pair of rotatory bands. These bands, which are of equal strength but opposite sign, are located at frequencies $\nu_{\perp} + \Gamma/\sqrt{2}$ and $\nu_{\perp} - \Gamma/\sqrt{2}$, where ν_{\perp} is the frequency of the perpendicularly polarized absorption band of the helix and Γ is a "damping factor" whose value is difficult to predict. However, Γ may be estimated empirically from the absorption spectrum as the full band width between the frequencies of half-intensity. These additional rotatory bands may be termed $[R]_{\Gamma}^{+}$ and $[R]_{\Gamma}^{-}$, respectively. Thus, the exciton theory predicts that each strong electronic transition in the monomer unit should in any helical arrangement give rise to a total of four rotatory bands, if the monomer moments are skewed with respect to the helix axis.

The rotatory bands that arise in specifically α -helical polypeptides from the 190-m μ peptide π^0 - π^{-} transition have been theoretically treated by Woody.²⁵ His numerical calculations indicate that the four rotatory bands should occur at 185, 189, 193, and 195 m μ , assuming a damping factor of 2500 cm.⁻¹. In addition, the n_1 - π^{-} transition of the peptide group, located near 225 m μ , was predicted to be optically active in these molecules.^{25, 26} Theoretically, therefore, α -helices should exhibit as many as five rotatory bands between 185 and 230 m μ , provided no other transitions are concealed in this spectral region.

In testing these expectations against observations, one finds that the experimental measurements to date, both on the optical rotatory dispersion and on the circular dichroism of α -helical polypeptides, are not sufficiently precise to permit either an unambiguous determination of the relative roles of the n_1 - π^{-} and π^0 - π^{-} transitions or a verification of the four separate π^0 - π^{-} rotatory bands predicted. As a result, the applicability of the exciton theory of rotatory power remains in doubt, and divergent theoretical analyses of the electronic origins of the rotatory dispersion of polypeptides remain untested. In this situation it has not been possible, for example, to decide between Schellman and Oriel,²⁶ who argue that the n_1 - π^{-} transition is a major source of rotatory power, and Tinoco and co-workers, who assign greater importance to the π^0 - π^{-} bands than to the n_1 - π^{-} transition.^{23, 25} The purpose of the present work has been to resolve the roles of the n_1 - π^{-} and π^0 - π^{-} transitions and to provide firm evidence for at least two π^0 - π^{-} bands.

(19) S. F. Mason, *Nature*, **199**, 139 (1963).

(20) S. F. Mason, *Quart. Rev.* (London), **17**, 20 (1963).

(21) See the Appendix to this article, where Mason's analysis is examined in some detail. Tinoco (private communication and footnote 22c) has also found these errors in Mason's work.

(22) (a) I. Tinoco, Jr., *Advan. Chem. Phys.*, **4**, 113 (1961); (b) *Radiation Res.*, **20**, 133 (1963); (c) *J. Am. Chem. Soc.*, **86**, 297 (1964).

(23) I. Tinoco, Jr., R. W. Woody, and D. F. Bradley, *J. Chem. Phys.*, **38**, 1317 (1963).

(24) D. F. Bradley, I. Tinoco, Jr., and R. W. Woody, *Biopolymers*, **1**, 239 (1963).

(25) R. W. Woody, Dissertation, University of California, Berkeley, Calif., 1962.

(26) J. A. Schellman and P. Oriel, *J. Chem. Phys.*, **37**, 2114 (1962).

Experimental

Instrumental. Polarized absorption spectra were measured on a Beckman DK-2A recording spectrophotometer adapted in a manner previously described.¹⁵ Circular dichroism measurements were obtained by two different methods, both of which involved modifications of the DK-2A spectrophotometer. The first method, termed method I, utilized a quartz polarizing prism and quartz retardation plate to produce circularly polarized light in the sample beam of the spectrophotometer. When identical solutions were placed in the sample and reference beams, the instrument measured directly the difference in transmittance of the sample toward circularly polarized and unpolarized light. No absolute calibration of instrument gain was required in this method. The second method for measurement of circular dichroism, termed method II, involved an adaptation of the electro-optic method of Grosjean and Legrand²⁷ to the Beckman DK-2A spectrophotometer. Calibration of this method was performed using data obtained by method I. This calibration was tested against published values of the circular dichroism of testosterone.²⁸ The band intensity obtained by method II fell within the limits of error of the published value.

The accuracy of the circular dichroic measurements is estimated to be $\pm 10\%$ for wave lengths greater than 200 m μ and $\pm 20\%$ for wave lengths less than 200 m μ , although the precision of measurements obtained by method II is considerably better than these figures. Adherence of the measured dichroism to Beer's law verified that stray light is not significant in the DK-2A at the cell absorbances and wave lengths used here. Measurements obtained by methods I and II are compared in Figure 4 below. It should be noted that instrumental errors for both methods may cause diminished but not increased absolute values of the measured circular dichroism.²⁹ A detailed description of both methods for the measurement of circular dichroism is being published elsewhere.³⁰

Materials. Poly-L-glutamic acid, poly-L-lysine, and copoly-L-Glu⁴²Lys²⁸Ala³⁰, purchased from Pilot Chemical Corp., had average molecular weights of 84,000, 324,000, and 74,000, respectively.³¹ The fluoride salts of cationic side chains were prepared by dialysis against sodium fluoride. Poly- γ -methyl-L-glutamate³² of molecular weight 600,000 was lyophilized from trifluoroacetic acid. Metmyoglobin was prepared from

(27) M. Grosjean and M. Legrand, *Compt. rend.*, **251**, 2150 (1960).

(28) L. Velluz and M. Legrand, *Angew. Chem.*, **73**, 603 (1961).

(29) Any artifacts would probably arise from excessive spectral band widths or slit dimensions. With method I, excessive band widths have the effect of averaging over peaks and troughs of the oscillations in transmission and thus would lead to measured dichroism of lower absolute value than the true value. With method II, oversize slit dimensions can lead to similar artifacts arising from depolarization of the light beam. Such depolarization can arise if a nonparallel light beam passes through a naturally birefringent crystal such as the KHzPO₄ used in the electro-optic plate (see, for example, F. A. Jenkins and H. E. White, "Fundamentals of Optics," 2nd Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 537). These points are discussed further in ref. 30.

(30) G. Holzwarth, *Rev. Sci. Instr.*, submitted for publication.

(31) E. Friedman, Dissertation, Harvard University, Cambridge, Mass., 1962; E. Friedman, T. J. Gill, and P. Doty, *J. Am. Chem. Soc.*, **83**, 4050 (1961); T. J. Gill, H. W. Kunz, E. Friedman, and P. Doty, *J. Biol. Chem.*, **238**, 108 (1963).

(32) Synthesized by R. Karlson. We wish to thank Mr. Karlson for the samples.

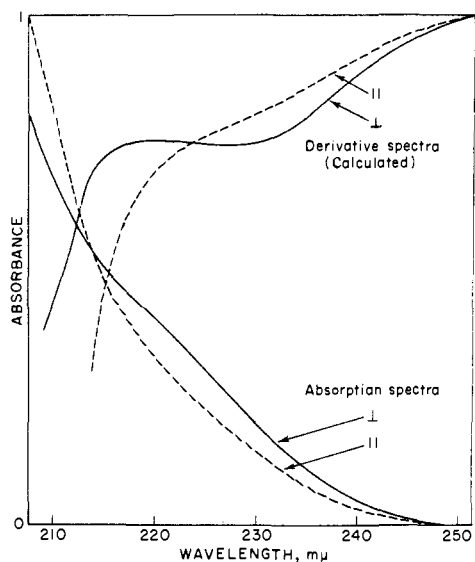


Figure 1. The polarized absorption spectra of poly- γ -methyl-L-glutamate films: lower curves, obtained by averaging the normalized measurements upon four different films; upper curves, derivative spectra calculated from the observed absorption spectra (no scattering correction has been applied).

sperm whale muscle.³³ Water was doubly distilled. Trifluoroethanol was obtained from Eastman Distillation Products and used without further purification.

The polymer concentrations were in all instances determined spectrophotometrically. The residue molar extinction coefficients at the indicated wave lengths used in these determinations were as follows: poly-L-glutamic acid, helix, $\epsilon(190)$ 4200³⁴; poly-L-glutamic acid, disordered chain, $\epsilon(200)$ 5660³⁴; poly-L-lysine, helix, $\epsilon(190)$ 4400³⁴; poly-L-lysine, disordered chain, $\epsilon(190)$ 7100³⁴; poly- γ -methyl-L-glutamate, helix, in trifluoroethanol, $\epsilon(190)$ 3200³⁵; copoly-L-Glu⁴²Lys²⁸-Ala³⁰, helix, $\epsilon(190)$ 4300³⁶; sperm whale metmyoglobin in 0.02 *M* phosphate buffer, pH 7.1, $\epsilon(280)$ 218.³⁷

All measurements of spectral properties below 210 $m\mu$ were made in a 1-mm. cell. Total cell absorbances were kept below 1.4. For measurements between 180 and 200 $m\mu$, nitrogen flushing was employed to remove oxygen from the light path.

Polarized Absorption Spectra. Before presenting the principal results of this work, which deal with circular dichroism, it will be useful to indicate briefly new measurements of the ultraviolet spectra of oriented films of polypeptide helices. In our previous study of this problem,¹⁵ it was concluded that there were three bands in the accessible region; these were centered at 191, 206, and 222 $m\mu$ and were polarized perpendicular, parallel, and perpendicular, respectively. The 191- and 206- $m\mu$ bands were assigned to the two allowed $\pi^0-\pi^-$ resonance excitation interaction (exciton) bands predicted by Moffitt,¹⁶ whereas the 222- $m\mu$ band was

(33) The protein was obtained from Dr. J. C. Kendrew and recrystallized by P. Urnes: see P. Urnes, Dissertation, Harvard University, Cambridge, Mass., 1963.

(34) K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci. U. S. A.*, **47**, 1775 (1961).

(35) M. Goodman, I. Listowsky, Y. Masuda, and F. Boardman, *Biopolymers*, **1**, 33 (1963).

(36) The extinction coefficient used is the mean of the coefficients of helical poly-L-glutamic acid and helical poly-L-lysine.³⁴

(37) See P. Urnes, Dissertation, Harvard University, Cambridge, Mass., 1963.

assigned to the $n_1-\pi^-$ transition. However, the observations of the 222- $m\mu$ band were much less satisfactory than for the others, and in one published result (Figure 3 of ref. 15) its perpendicular polarization was by no means apparent. We have therefore obtained a number of spectra in the 210- to 245- $m\mu$ region in order to establish the polarization of this band.

These spectral studies revealed a region of predominantly perpendicular polarization from 215 to 245 $m\mu$, thereby clearly showing the presence of a weak absorption band centered at about 222 $m\mu$ in addition to the two strong bands. This point is best conveyed by presenting a spectrum in this region obtained by averaging the results obtained on four oriented poly- γ -methyl-L-glutamate films. Such an average should serve as a more reliable index to the spectra than any single film might, because of variations in the scattering, thickness, and per cent orientation of the films produced. This composite spectrum is shown in the lower portion of Figure 1. The perpendicular polarization and the shoulder near 222 $m\mu$ are clearly evident, and the original result is therefore confirmed.³⁸ The ratio of perpendicular to parallel absorbance is only 1.5 near 222 $m\mu$ in Figure 1, although values as high as 2 were observed in individual spectra.

The location of the band producing the perpendicularly polarized shoulder between 215 and 245 $m\mu$ can be made more precise by resorting to derivative spectra.³⁹ These are plotted in the upper part of Figure 1. A well-defined minimum is seen at 227 $m\mu$, and a less clear maximum occurs at about 216 $m\mu$. The inflection point is not well defined but the midpoint between the two extremes is 222 $m\mu$. Hence this wave length assignment can now be used with more confidence in its precision; it is presumably correct to within ± 2 $m\mu$. It is the overlapping $\pi^0-\pi^-$ bands at lower wave length that prevent a more precise location.

Circular Dichroism. As our earlier work has shown,⁴ the circular dichroism spectra of α -helical and disordered poly-L-glutamic acid differ profoundly. This is evident in more recent results shown in Figure 2. The improved precision now available shows that the α -helical polymer exhibits not one but two negative dichroic peaks; these are located at 222 and 209 $m\mu$, clearly separated by a distinct notch at 215 $m\mu$. There is a single positive dichroic band with peak at 191 $m\mu$. These findings for the α -helix contrast with those for disordered polyglutamic acid, which reveal a region of weak positive dichroism between 210 and 235 $m\mu$ contiguous with a region of strong negative dichroism marked by an extremum at 202 $m\mu$. The new observations on polyglutamic acid differ from those reported previously⁴; the earlier, less precise data did not show either the 215- $m\mu$ notch in the dichroism of the helix or the weakly positive dichroism of the disordered

(38) The ambiguity in one of the previously published spectra, Figure 3 in ref. 15, can now be explained. In this spectrum the parallel absorbance was greater than the perpendicular one in the region of the $n_1-\pi^-$ transition, unlike Figure 1 above. From this, one might conclude that the dichroism was parallel, but this was not done because (1) the 222- $m\mu$ band was much more clearly evident in the perpendicular spectrum, and (2) other spectra did not show this kind of inversion. We now believe that this displacement arose in this one case because the film thickness in the parallel spectrum exceeded that in the perpendicular case, owing to an instrumental artifact which allowed the sampled area to change upon rotation of the sample. Improvements in the apparatus eliminated this problem and invariably showed perpendicular dichroism in this spectral region.

(39) A. T. Giese and C. S. French, *Appl. Spectry.*, **9**, 78 (1955).

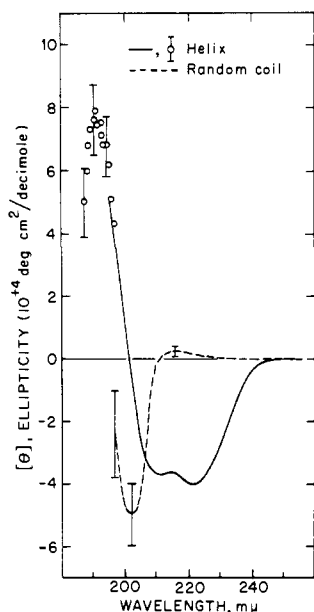


Figure 2. The circular dichroism of poly-L-glutamic acid in 0.1 *M* NaF. Helix (pH 4.3): the circles indicate method I whereas the solid line indicates method II. Random coil (pH 7.6): method I.

chain. These observations are a measure of the instrumental improvement that has been achieved.

In order to ensure that the results for polyglutamic acid depicted in Figure 2 were typical of α -helical and disordered polymers rather than a manifestation of the carboxylic side chains, measurements were performed upon poly-L-lysine, a polypeptide also known to undergo a helix-coil transition upon ionization.⁴⁰ The circular dichroism of the two conformations of this molecule are shown in Figure 3. Like poly-L-glutamic acid, poly-L-lysine exhibits in the helical conformation two negative extrema, at 207 and 220 $m\mu$, as well as a region of positive dichroism near 190 $m\mu$. The data for randomly disordered poly-L-lysine show positive dichroism near 220 $m\mu$ and strong negative dichroism below 210 $m\mu$. Although the data below 200 $m\mu$ were found to be less reproducible for polylysine than for polyglutamic acid, it is clear that the two polymers exhibit closely similar changes in dichroism upon conformational alteration. Consequently, it is reasonable to conclude that the observed curves reflect the properties of the amide groups and their mutual interactions.

Inasmuch as the circular dichroic measurements were performed by two different methods, it was deemed important to compare measurements made by the two techniques. Such a comparison is presented in Figure 4, which shows the optical activity of an α -helical polymer in an organic solvent. It is seen that methods I and II are in good agreement above 200 $m\mu$, although method II is more precise and clearly reveals the two negative peaks only crudely discernible in the measurements by method I. Below 200 $m\mu$, method I appears to give larger values of the circular dichroism than method II; it is difficult to be sure of the origins of this discrepancy, but inasmuch as artifacts are generally expected to produce absolute values of measured dichroism smaller than the true values,²⁹ it is probable

(40) J. Applequist and P. Doty in "Polyamino Acids, Polypeptides, and Proteins," ref. 17, p. 161.

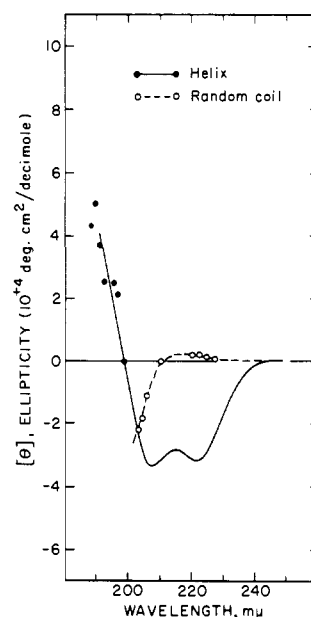


Figure 3. The circular dichroism of poly-L-lysine in 0.1 *M* NaF. Helix (pH 10.6-10.8): filled circles, method I; solid line, method II. Random coil (pH 6.7): method I.

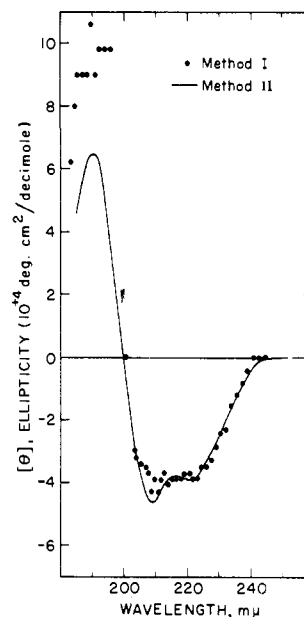


Figure 4. The circular dichroism of α -helical poly- γ -methyl-L-glutamate in trifluoroethanol, as recorded by methods I and II.

that the measurements obtained by method I, although less precise, are more accurate below 200 $m\mu$. Within this discussion of instrumental accuracy, the discrepancies between the circular dichroic measurements reported here and the measurements reported by Brahm and Spach⁵ should be examined. When their data for helical polyglutamic acid are reduced in absolute magnitude by a factor of 10, good agreement is observed with our data from about 225 to 245 $m\mu$. Between 210 and 225 $m\mu$, however, our data show circular dichroism of considerably greater absolute magnitude than their results suggest. The discrepancies are all in the direction expected if their measurements are adversely affected either by stray light or by bire-

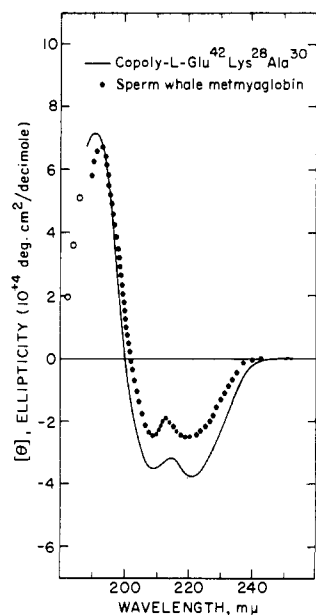


Figure 5. The circular dichroism of α -helical copoly-L-Glu⁴²-Lys²⁸Ala³⁰ at pH 3.1 in 0.1 M NaF, and the circular dichroism of sperm whale metmyoglobin at pH 7.0 in 0.02 M phosphate buffer. Both curves were recorded by method II. The open circles are the extrapolated values of the dichroism of the synthetic polymer used for the calculation of the Kronig-Kramers transform shown in Figure 6.

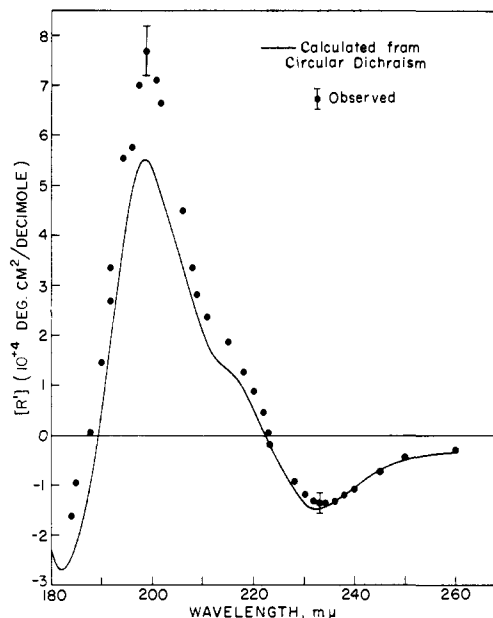


Figure 6. The optical rotatory dispersion of α -helical polypeptides, as calculated from circular dichroic data and as observed: the calculated curve is based upon data shown in Figure 5 for poly-L-Glu⁴²Lys²⁸Ala³⁰; the observed data shown were obtained upon poly-L-glutamic acid in water at pH 4.3 by Blout, Schmier, and Simmons.⁸

fringence depolarization.²⁹ Inasmuch as our measurements by two different methods agree, whereas their measurements disagree in the direction predicted for artifacts, we believe that their measurements for wave lengths shorter than 225 $m\mu$ should be disregarded.

In order to strengthen the connection between the conformation-dependent optical activity of synthetic polypeptides and the optical activity of proteins, the

circular dichroism of a water-soluble copolymer of glutamic acid, lysine, and alanine, and of the protein myoglobin, were measured. At low pH, the synthetic polymer is completely helical,³¹ and its circular dichroism curve, as shown in Figure 5, is in excellent accord with that of other helical polymers shown in Figures 2-4. These data may be compared to those of the largely helical protein metmyoglobin. The circular dichroism of this natural polypeptide, also shown in Figure 5, reveals clearly the two negative dichroic peaks at about 208 and 220 $m\mu$, as well as the positive dichroic band near 190 $m\mu$. Thus these two features of the circular dichroism are basic characteristics of the α -helix, either in synthetic polypeptides or in proteins.

Analysis and Discussion

The absorption and circular dichroic spectra which have been presented can be utilized in two different ways. First, by applying a Kronig-Kramers transform to the circular dichroic data, the rotatory dispersion arising from the observed dichroic bands can be calculated.^{41,42} Comparison of such calculated dispersion data to the observed rotatory dispersion can then provide a measure of the reliability of both types of measurement and, in addition, should show the contributions of all the bands detected in the circular dichroism to the rotatory dispersion. However, the second and more important application of the circular dichroic and absorption spectra is in determining the spectral locations, rotational strengths, and oscillator strengths of separate electronic transitions. With such experimental parameters in hand, a critical evaluation of our theoretical understanding of the optical properties of α -helices may be possible.

Kronig-Kramers Transforms of the Circular Dichroic Data. Electronic optical rotatory dispersion and circular dichroic curves have been shown⁴¹ to obey dispersion relations of the Kronig-Kramers type, subject only to two easily satisfied conditions. The Kronig-Kramers transform for the calculation of the optical rotatory dispersion function $[\phi'(\lambda)]_K$ arising from the partial circular dichroism $[\theta'(\lambda)]_K$ of the K th band may be written⁴²

$$[\phi'(\lambda)]_K = (2/\pi) \int_0^{\infty} [\theta'(\lambda')]_K (\lambda' / [\lambda^2 - \lambda'^2]) d\lambda' \quad (I)$$

The prime (') in the expressions $[\phi'(\lambda)]$ and $[\theta'(\lambda)]$ indicates that these quantities are assigned their vacuum values; observed data $[\phi(\lambda)]$ and $[\theta(\lambda)]$ are usually related to the vacuum parameters by the Lorentz factor. For example, $[\theta'(\lambda)] = 3[\theta(\lambda)]/[n(\lambda)^2 + 2]$, where n is the refractive index of the solvent. Thus from circular dichroism such as that shown in Figures 2-5, the optical rotatory dispersion arising from the band(s) may be determined. For complex circular dichroism curves, however, the accurate evaluation of the integral (I) is feasible only by digital computer. In the IBM 1620 computer program used here, the integral was approximated by a summation over intervals of 1 $m\mu$, and the point at $\lambda = \lambda'$ was eliminated.

(41) W. Moffitt and A. Moscovitz, *J. Chem. Phys.*, **30**, 648 (1959).

(42) A. Moscovitz in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 150.

Using this procedure, the optical rotatory dispersion arising from the circular dichroism of copoly-L-Glu⁴²Lys²⁸Ala³⁰ as shown in Figure 5 was determined. It is important to note that no assumptions have been made in this transformation from circular dichroic data to rotatory dispersion, either in the number of bands or in their shapes. The only assumption involved is the minor one in the extrapolation between 175 and 185 m μ of the positive circular dichroic band evident at 190 m μ ; the extrapolation adopted is shown in Figure 5. The calculated optical rotatory dispersion is shown in Figure 6 together with observed data for the similar helical polymer poly-L-glutamic acid. A detailed comparison shows that the agreement between calculated and observed rotatory dispersion curves is excellent as to the location of peaks, troughs, cross-over points; and inflection points, although the magnitude of the calculated curve at the 198-m μ rotatory dispersion peak seems to be about 30% lower than the observed curve. This discrepancy may arise from a steeply rising positive background term, from an underestimation of the magnitude of the 190-m μ rotatory band; or, perhaps, from errors in the dichroic or rotatory data. It is the improvement in the precision of the circular dichroic measurements and the development of a computer program for the exact evaluation of the Kronig-Kramers transform that has led to the considerably better fit to the observed dispersion data than was reported earlier.⁴ In particular, the shoulder in the dispersion curves is now clearly reproduced. This agreement between calculated and observed dispersion curves, which are here based upon quite different experimental techniques, argues strongly for the reliability of both sets of measurements.

Comparisons between calculated and observed rotatory dispersion over the spectral range from 300 to 600 m μ are most conveniently carried out in terms of the parameters λ_0 , a_0 , and b_0 of the Moffitt equation.^{3, 43} These parameters can be determined rapidly and with accuracy by means of standard statistical procedures using a digital computer.⁴⁴ The only variable in the computed values arises from the extrapolation of circular dichroism between 175 and 190 m μ . We have applied two linearly decreasing extrapolations from the observed value of $[\theta]$ at 190 m μ to values: (a) $[\theta] = 0$ at 180 m μ , and (b) $[\theta] = 3.5 \times 10^4$ deg. cm.² decimole⁻¹ at 180 m μ . In both extrapolations $[\theta]$ was set equal to zero for wave lengths shorter than 180 m μ . The true values of the circular dichroism should fall between these extreme values if the 190-m μ rotatory band is symmetrical in shape. From these extrapolations one finds that λ_0 for poly-L-glutamic acid falls between 209 and 212 m μ ; this is in excellent agreement with the observed values, which fall between 210 and 213 m μ .⁴³⁻⁴⁵ The value of a_0 derived from transforms of the circular dichroic curves lies in the range -290 to -800° , depending strongly on the choice of the extrapolation, if λ_0 is set at 212 m μ . From rotatory dispersion one finds that a_0 is about -150° , again using $\lambda_0 = 212$ m μ . The agreement in the value of b_0

(43) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **42**, 596 (1956).

(44) M. Sogami, W. J. Leonard, Jr., and J. F. Foster, *Arch. Biochem. Biophys.*, **100**, 260 (1963); W. J. Leonard, Jr., and J. F. Foster, *J. Mol. Biol.*, **7**, 590 (1963).

(45) P. Urnes, private communication.

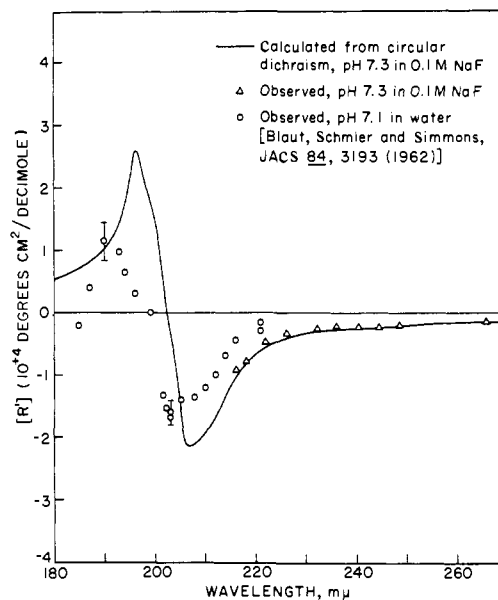


Figure 7. The optical rotatory dispersion of randomly disordered poly-L-glutamic acid, as calculated from circular dichroism and as measured. The calculated curve is based upon data shown in Figure 2. The observed data were obtained under the following conditions: Δ , pH 7.1 in 0.1 M NaF; \circ , pH 7.1 in water. The data in water were reported by Blout, Schmier, and Simmons.⁸

is considerably better⁴⁶; the value of b_0 derived from transforms of the circular dichroic data falls between -465 and -582° , which may be compared to observed values of -600 to -650° . The close agreement in b_0 allows one to say that at least 70 to 90% of the observed b_0 value for helical polypeptides appears to arise from the peptide rotatory bands between 180 and 245 m μ . This observation shows that the dominant contribution to the rotatory dispersion of helical polypeptides does indeed arise from electronic transitions of the peptide group. It was Moffitt and Yang⁴³ who originally suggested that the complex nature of the rotatory dispersion of helical polypeptides probably arises from contiguous regions of circular dichroism of nearly equal intensity but opposite sign peculiar to the peptide backbone. The circular dichroic curves reported here, and the agreement in the calculated and observed parameters b_0 and λ_0 , largely support this suggestion.

The circular dichroism of randomly disordered polymers may be analyzed in a manner parallel to that of the helix. By application of the Kronig-Kramers transform to the circular dichroic data of poly-L-glutamic acid shown in Figure 2, the optical rotatory dispersion arising from the observed dichroic bands can be obtained; this is shown in Figure 7, together with observed data obtained in part under somewhat different solvent conditions. The position of the strong negative band is confirmed by both types of measurement, although the rotational strengths of the bands

(46) It is not surprising that the values of a_0 obtained by transformation from circular dichroism, and the values of a_0 obtained from rotatory dispersion, differ more strongly than do the values of the parameter b_0 , because the contributions of rotatory bands to the first term of the Moffitt equation vary as $(\lambda^2 - \lambda_0^2)^{-1}$, whereas the contributions to the second, or b_0 , term vary as $(\lambda^2 - \lambda_0^2)^{-2}$. Thus it is to be expected that transitions below 180 m μ , which are not taken into account here, will make larger partial contributions to a_0 than to b_0 in the observed rotatory dispersion curves.

differ by about a factor of two. Furthermore, the weak positive dichroic region near 220 m μ accurately reproduces the flattened region between 230 and 240 m μ observed in experimental dispersion curves.^{4,7,8}

For randomly coiled polypeptides, a comparison between the calculated and observed rotatory dispersion between 300 and 600 m μ is conveniently carried out in terms of the Drude equation.³ The characteristic parameters R and λ_c have the values -12×10^{-40} erg cm.³ rad and 202 m μ from circular dichroism; the values from the observed dispersion between 300 and 600 m μ are -6×10^{-40} and 204 m μ when correction is made for the dispersion of the Lorentz factor. The close agreement in the parameters suggests that the rotatory dispersion in the visible spectrum reflects largely the rotatory bands of the peptide group which occur between 190 and 230 m μ .

In summary, Kronig-Kramers transformations of the circular dichroic curves, as discussed in the paragraphs above, have provided mutual support for the reliability of the circular dichroic and optical rotatory dispersion data. At the same time, they have provided evidence that the rotatory bands between 180 and 245 m μ are the dominant source of the dispersion curves in the visible spectrum. It is then of interest to attempt an assignment of the observed data to particular electronic transitions.

Estimation of Oscillator and Rotational Strengths. Comparison of Theory and Experiment. Complex circular dichroic curves such as those shown in Figures 2-5 are not of themselves adequate for a unique assessment of the locations, shapes, and magnitudes of their component rotatory bands because such bands, being signed, may largely cancel one another in regions where they overlap. However, the shapes and spectral locations of circular dichroic and absorption bands have been shown both experimentally and theoretically to be closely related to one another.^{41,47} Consequently, it may be possible, through a simultaneous analysis of both the absorption and circular dichroic spectra, to resolve the experimentally observed spectra into a unique set of rotatory and absorption bands assignable to particular electronic transitions. Once such a resolution has been made, theoretical and experimental rotational strengths may be compared.

The theoretical investigations⁴¹ indicate that the relationship of circular dichroic to absorption band shape and location will depend on the type of electronic transition involved. Observed bands are expected to be related in one of the following three ways.

(a) The absorption and dichroism may simply be proportional to one another. This is Moffitt and Moscovitz's Case I⁴¹; it is expected to hold for all electric-dipole-allowed transitions. The partial circular dichroism $[\theta]_K$ and partial absorbance ϵ_K for the K th electronic transition are then related by an expression of the form $[\theta(\lambda)] = c_K \epsilon_K(\lambda)$, where c_K is a positive or negative constant. The bands $[R]_{\nu_{\parallel}}$ and $[R]_{\nu_{\perp}}$ in the α -helix are presumed to fall into this case.

(b) The absorption and dichroic bands may be of similar shape but the band centers may be shifted slightly relative to one another. This is Moffitt and Moscovitz's Case II⁴¹; it is expected for magnetic-

dipole-allowed, electric-dipole-forbidden transitions such as the $n_{\Gamma}-\pi_{\Gamma}^{-}$ transitions in amides.^{48, 49}

(c) Finally, we come to a case theoretically predicted by Tinoco and co-workers^{22, 23, 25} to arise in the perpendicularly polarized absorption bands of helical polymers. In this instance, Case III, the absorption band, with a single peak, is associated with circular dichroic bands $[R]_{\Gamma^{+}}$ and $[R]_{\Gamma^{-}}$ exhibiting positive and negative dichroic peaks antisymmetric about the absorption maximum. In this situation, the absorption and circular dichroism are obviously not proportional to one another throughout the band. According to Woody's analysis,²⁵ the 189-m μ perpendicularly polarized absorption band of the α -helix is expected to exhibit Case III dichroic bands $[R]_{\Gamma^{-}}$ and $[R]_{\Gamma^{+}}$ superposed on a Case I band, $[R]_{\nu_{\perp}}$.

A. The α -Helix. Inspection of the absorption and circular dichroic spectra of the α -helical polymers suggests that both can be fitted by three bands centered at 222, 206, and about 190 m μ .⁵⁰ The 206-m μ band is expected to fall into Moffitt and Moscovitz's Case I. A comparison of the slopes of the absorption and circular dichroic curves from 230 to 245 m μ shows excellent agreement; this observation suggests that the 222-m μ absorption and circular dichroic bands are fortuitously also of identical shape and location.

The third rotatory band near 190 m μ is predicted to consist of a sum of Case I band $[R]_{\nu_{\perp}}$ and one limb, $[R]_{\Gamma^{-}}$, of the Case III bands. Since these two rotatory bands are of the same sign but are centered about frequencies ν_{\perp} and $\nu_{\perp} - \Gamma/\sqrt{2}$, the dichroic band is expected to display maximum values at somewhat longer wave lengths than the maximum of the perpendicularly polarized absorption band.

Inasmuch as the 222- and 206-m μ circular dichroism and absorption bands are of identical shape and location, and, in addition, the 190-m μ circular dichroic band contains a strong Case I contribution, ϵ and $[\theta(\lambda)]$ are related by the expressions

$$\epsilon(\lambda) = \sum_{K=1}^3 \epsilon_K(\lambda) \quad (\text{II})$$

and

$$[\theta(\lambda)] = \sum_{K=1}^3 c_K \epsilon_K(\lambda) \quad (\text{III})$$

These three bands should then collectively account for the magnitude and shapes of both the absorption and optical activity. As noted earlier, neither the absorption spectrum nor the circular dichroic curve alone allows a *unique* subdivision of the observed curves. However, the requirement that the bands simultaneously satisfy relations II and III severely restricts the freedom available in subdividing the bands.

The procedure adopted to find the band shapes was as follows. First, the absorption spectrum was subdivided according to relation II into three roughly symmetrical bands centered at about 189, 206, and 222

(48) See M. Kasha in "Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961, p. 31.

(49) See also Schellman and Oriol, ref. 26.

(50) J. P. Carver, E. Shechter, and E. R. Blout have informed us that they have developed a computer program for determining the number of parameters of Gaussian circular dichroic bands required to generate an observed rotatory dispersion curve. They find that three bands between 190 and 230 m μ , plus background terms, give a very good fit to the observed optical rotatory dispersion of α -helical polymers.

(47) See, for example, W. Kuhn, *Ann. Rev. Phys. Chem.*, **9**, 417 (1958), and references therein.

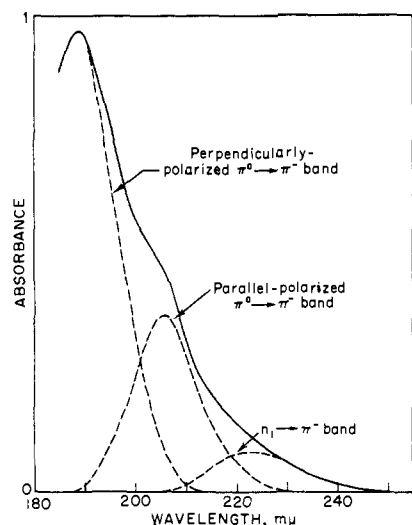


Figure 8. Absorption spectrum of α -helical poly- γ -methyl-L-glutamate in trifluoroethanol, showing the proposed resolution into parallel and perpendicularly polarized π^0 - π^- exciton band and an n_1 - π^- band.

$m\mu$. The intensity of the 222- $m\mu$ band was largely fixed by assigning to it all absorbance for wave lengths greater than 230 $m\mu$. This procedure was required by the observations, noted earlier, that the circular dichroism and absorbance were proportional from 227 to 245 $m\mu$ and that the absorbance in this spectral range was therefore associated predominantly with the 222- $m\mu$ absorption band. Appropriate values of the parameters c_K were then selected such that, using relation III, the observed circular dichroic curve was approximated as closely as possible. Inspection of the deviations between the observed and the fitted circular dichroic curves then suggested alterations in the original subdivision of the absorption spectrum, and the entire cycle was repeated until, by successive approximations, the circular dichroism calculated from relation III agreed closely with the observed data.

The final resolution of the absorption spectrum, showing the shapes and sizes of the three bands, is shown in Figure 8. All three bands are seen to make significant contributions to the absorbance. The component circular dichroic bands, and the sum of the three component bands, as obtained from relation III using the absorption bands shown in Figure 8, are displayed in Figure 9. Also shown in Figure 9 is the observed circular dichroism; the agreement between the fitted and the observed data is excellent above 200 $m\mu$, supporting the hypothesis that the absorption and circular dichroism are proportional to one another at the 206- and 222- $m\mu$ bands. The agreement in the spectral region of positive circular dichroism is less satisfactory and suggests that the actual circular dichroic band may be shifted to the red of the absorption band, as expected. This point is further discussed below.

From the resolution of the absorption and circular dichroic spectra shown in Figures 8 and 9, the experimental oscillator and rotational strengths can be readily evaluated. The experimental band intensities can then be compared to the theoretical predictions discussed above. In the paragraphs which follow, we shall consider, in turn (a) the oscillator strengths of the π^0 - π^- bands, (b) the absorptive properties of the

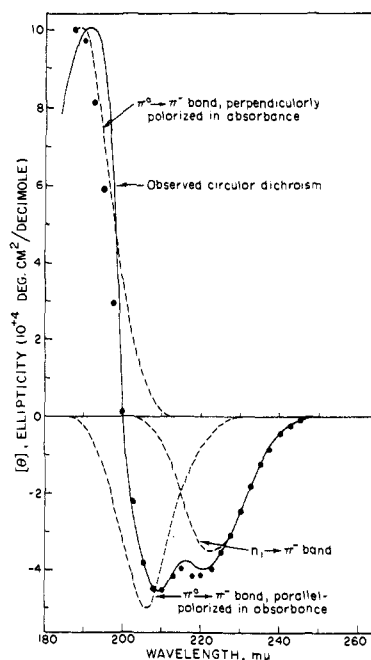


Figure 9. The circular dichroism of poly- γ -methyl-L-glutamate in trifluoroethanol, showing the proposed resolution of the observed data into three rotatory bands associated with observed bands in the absorption spectrum. The solid line is the measured circular dichroism (see Figure 4). The shape and intensity of each of the component rotatory bands has been obtained from one of the component absorption bands shown in Figure 8 by application of the relation, $[\theta(\lambda)]_K = c_K \epsilon_K(\lambda)$. The filled circles indicate the sum of the three component rotatory bands shown.

n_1 - π^- band, (c) the rotational strength of the n_1 - π^- band, and (d) the rotational strengths and spectral locations of the π^0 - π^- bands.

The observed oscillator strengths of the 189- and 206- $m\mu$ π^0 - π^- bands are found to be 0.10 and 0.03; these intensities may be compared to predicted values, 0.16 and 0.09.⁵¹ The two strong π^0 - π^- exciton bands are thus in roughly the expected intensity ratio and, furthermore, their energy separation, about 4000 cm^{-1} , compares favorably with the calculated value, about 2700 cm^{-1} , as noted earlier.⁵²

The observed oscillator strength of the n_1 - π^- transition, 0.007, is substantially larger than the calculated intensity,⁵³ which is only 0.0001. However, this discrepancy is hardly surprising inasmuch as vibrational effects and highly probable n -electron delocalization are entirely neglected in the calculations. Furthermore, it should be noted that the observed perpendicular polarization of the n_1 - π^- band is consistent with the vibrationally induced polarization of the similar n - π^*

(51) See Tinoco, Halpern, and Simpson, ref. 17. The theoretical values are based upon a subdivision of the peptide π^0 - π^- oscillator strength observed in the random coil. Correction for the hypochromicity of this transition would diminish the predictions for the intensities of both bands by an identical factor and thus would not affect the relative band intensities.

(52) See ref. 15 and 17. The experimental energy separation between the π^0 - π^- bands is about 4000 cm^{-1} and not 2700 cm^{-1} as given in ref. 15. The error arose from an arithmetic mistake and not from different data.

(53) The value used here is a corrected value calculated by Schellman and Oriol.²⁶ Their published value of the electric-dipole transition moment of the n_1 - π^- band in α -helical polypeptides is five times too large because of an error in the calculation (J. A. Schellman, private communication).

band in formaldehyde.⁵⁴ The observed polarization might at first sight appear to be in disagreement with the theoretical predictions of Schellman and Oriol.²⁶ However, the dichroic ratios are such as to be entirely consistent with a parallel-polarized $n_1-\pi^-$ oscillator strength of 0.002, which is considerably larger than the theoretically predicted value. Thus, although the observed oscillator strength and polarization do not support the theoretical calculations, they do not necessarily invalidate them.

The rotational strengths of the circular dichroic bands of poly- γ -methyl-L-glutamate may be readily obtained from the data of Figure 9 by suitable integration over each partial rotatory band.⁴¹ It is clear from Figures 2-5 that other α -helical polymers would yield rotational strengths differing by 20% at most from those of the methyl glutamate polymer. The observed rotational strength of the $n_1-\pi^-$ band, -22×10^{-40} erg cm.³ rad, is of the predicted sign and roughly also of the predicted magnitude,^{25,53} -3.4×10^{-40} . A correction for solvent polarizability has not been applied to the data because the dispersion of the refractive index of the solvent is not known. Inasmuch as such corrections would always decrease the absolute value of an observed rotatory band, however, their application would improve the agreement to the calculated $n_1-\pi^-$ rotational strength. In view of the approximations made in the amide wave functions and in the estimation of the electrostatic field of the helix in the process of the calculations, this agreement can be regarded as satisfactory. It is therefore probable that the source of the optical activity of the $n_1-\pi^-$ transition has been correctly identified.

Proceeding from the $n_1-\pi^-$ transition to the $\pi^0-\pi^-$ bands, one finds that the observed rotational strength of the 206-m μ band is -29×10^{-40} ; the calculated rotational strength^{16, 25,54a} is -126×10^{-40} . Although there is agreement as to both the sign and the order of magnitude of the band, the differences in band intensity are greater than might be expected from a semiempirical theory which does not require the use of accurate wave functions. The origin of the discrepancy is not known, but the neglect of vibrational effects upon the electronic interactions may well prove significant in the calculations.

Turning from the optical activity of the parallel-polarized $\pi^0-\pi^-$ transition to that of the perpendicularly-polarized 190-m μ transition, one finds that the observed rotational strength is $+81 \times 10^{-40}$; this value may be compared to the theoretically predicted value,²⁵ $+242 \times 10^{-40}$. The theoretical value arises from the two bands $[R]_{\nu_{\perp}}$ and $[R]_{\Gamma^-}$ which have intensities $+126 \times 10^{-40}$ and $+116 \times 10^{-40}$, respectively. The strength of the band $[R]_{\Gamma^-}$ depends strongly upon

(54) See G. W. Robinson and V. E. Di Giorgio, *Can. J. Chem.*, **36**, 31 (1958), and references therein.

(54a) NOTE ADDED IN PROOF. J. A. Schellman (private communication) has pointed out to us an additional source of rotatory power in α -helical polypeptides. By general arguments based upon perturbation theory, he has shown that if a magnetically-allowed transition acquires electric-dipole transition moment by configuration interaction, then the opposite must also occur. Thus, if the magnetically-allowed $n_1-\pi^-$ transition acquires a rotatory strength of -22×10^{-40} from the $\pi^0-\pi^-$ band, then the parallel-polarized $\pi^0-\pi^-$ band must acquire positive rotatory power by the same mechanism. Consequently, one should expect the observed 206-m μ rotatory band to arise from negative rotatory power arising from exciton coupling and positive rotatory power arising from static coupling. The theoretically expected rotatory intensity should then be increased from -126×10^{-40} to approximately -104×10^{-40} .

the selected value of the "damping factor" Γ . As with the 206-m μ band, theory and experiment are in accord as to the sign and order of magnitude of the band, but the quantitative agreement is poor.

Because the 190-m μ band is expected to arise from two rotatory bands $[R]_{\nu_{\perp}}$ and $[R]_{\Gamma^-}$, it would be significant if the observed band exhibited a red shift relative to the absorption band with a maximum near this wave length. Such a red shift would be good evidence for the band $[R]_{\Gamma^-}$ predicted by the Moffitt-Fitts-Kirkwood-Tinoco-Woody theory. Some of the circular dichroism curves shown in Figures 2-5 do appear to show a maximum value to the red of the absorption maximum, which occurs at about 189 m μ for these helical polymers, but it must be realized that all artifacts in the circular dichroic measurements would be such as to show a false red shift in the peak of the circular dichroic bands.²⁹ Consequently, it is felt that the present data provide suggestive but not conclusive evidence for the band $[R]_{\Gamma^-}$. Further evidence favoring the presence of this band is the observed difference in absolute intensity of the 190- and 206-m μ rotatory bands. Such a difference is not predicted by Moffitt's earlier theory,¹⁶ which neglected the band $[R]_{\Gamma^-}$, but is a feature of the more recent theory. However, convincing evidence for the bands $[R]_{\Gamma^+}$ and $[R]_{\Gamma^-}$ must await more precise measurements below 200 m μ , or further penetration into the ultraviolet region of the band $[R]_{\Gamma^+}$, or measurements upon oriented but isolated helical molecules.

In conclusion, then, the new measurements provide strong evidence for the involvement of both the $n_1-\pi^-$ and the $\pi^0-\pi^-$ transitions in the optical activity of α -helical polypeptides. Although the measurements of circular dichroism are not sufficiently precise for a clear determination of the presence and magnitude of the Moffitt-Fitts-Kirkwood-Tinoco-Woody bands $[R]_{\Gamma^+}$ and $[R]_{\Gamma^-}$, the two strong $\pi^0-\pi^-$ rotatory bands which are observed are in agreement as to both sign and order of magnitude with the predictions of the exciton theory. The experimental findings and theoretical predictions for both the absorption spectra and the circular dichroic spectra of α -helical polypeptides are summarized in Table I.

B. The Randomly Disordered Chain. The absorption spectrum of disordered polypeptides shows only a single strong band with a peak at 191 m μ ³⁴ which is presumably the $\pi^0-\pi^-$ band. The weak $n_1-\pi^-$ transition must also be present,¹³ but is thought to be masked by the stronger neighboring band. The circular dichroic spectra reveal regions of weakly positive dichroism near 220 m μ and strong negative dichroism about 200 m μ . It is not clear whether the observed dichroism results from two strong overlapping bands of opposite sign near 200 m μ , or whether there is a single strong negative band (strength -19×10^{-40}) at 200 m μ and a weak positive band, of strength $+0.8 \times 10^{-40}$, at 220 m μ . If there is indeed a band near 220 m μ , then this is most probably the $n_1-\pi^-$ band.¹¹ In either case no ready explanation for the 200-m μ band is at hand. Possibly this band may arise from coupling of $\pi^0-\pi^-$ moments; this is not unexpected in view of evidence that supposedly disordered polymers have some net secondary structure.⁵⁵ Again,

(55) See A. Katchalski, *Biophys. J.*, **4**, No. 1, part 2, 9 (1964); see

Table I. Ultraviolet Optical Properties of the α -Helix

Transition	Absorption bands				Rotatory bands			
	Wave length, $m\mu$		Oscillator strength		Wave length, $m\mu$		Rotational strength, 10^{-40} erg cm. ³ rad	
	Theory ^a	Exptl.	Theory ^b	Exptl.	Theory ^c	Exptl.	Theory ^d	Exptl.
$n_1-\pi^-$	210-230	222	0.0001	0.007	210-230	222	-3.4	-22
$\pi^0-\pi^-$, parallel polarized in absorbance	198	206	0.09	0.03	198	206	-126	-29
$\pi^0-\pi^-$, perpendicularly polarized in absorbance	188	189	0.16	0.10	191	190	+242	+81
$\pi^0-\pi^-$, rotatory band only					185	...	-115	...

^a For the $n_1-\pi^-$ band, see Ham and Platt¹³; for the $\pi^0-\pi^-$ bands, see Moffitt¹⁶ and Tinoco, Halpern, and Simpson.¹⁷ ^b For the $n_1-\pi^-$ band, see Schellman and Oriol⁵³; for the $\pi^0-\pi^-$ bands, see Moffitt¹⁶ and Tinoco, Halpern, and Simpson.¹⁷ ^c For the $n_1-\pi^-$ band, see Ham and Platt¹³; for the $\pi^0-\pi^-$ bands, see Moffitt¹⁶ and Woody.²⁵ ^d For the $n_1-\pi^-$ band, see Schellman and Oriol⁵³ and Woody²⁵; for the $\pi^0-\pi^-$ bands, see Moffitt¹⁶ and Woody.²⁵

the optical activity of the disordered chain could arise from hitherto unsuspected electronic transitions. It is clear that further experimental work on randomly disordered polymers is necessary.

C. Metmyoglobin. The similarity of the circular dichroism of metmyoglobin to that of the helical synthetic polymers, as shown in Figure 5, has been noted above. This similarity is significant for three reasons. First, it suggests an identity of rotatory band energies and relative band intensities. This argues strongly that the natural and synthetic polymers are largely in an identical conformation. Second, the integrated intensity of the negative dichroic band of myoglobin is between 65 and 80% of that of the completely helical polymers shown in Figures 2-5. The intensity of the circular dichroic bands, like the appropriate parameters of an optical rotatory dispersion curve,³ should serve as an index of helix content in polypeptides and proteins of mixed conformation. Thus, the circular dichroic measurements of myoglobin suggest that 65 to 80% of the peptide groups in this protein are in a helical conformation. This helix content is consistent with that observed in myoglobin crystals; X-ray analysis shows that 77% of the myoglobin peptide residues are in the α -helical fold.⁵⁶ Furthermore, the absence between 190 and 245 $m\mu$ of major rotatory bands arising from the heme group and side chains supports the more precise measurements of helix content of metmyoglobin which are based upon rotatory dispersion measurements between 240 and 300 $m\mu$.⁵⁷ Third, the circular dichroism of myoglobin sheds some light upon the question of whether helical polypeptides in dilute solution adopt the α -helical structure or the 3_{10} conformation. The evidence favoring the α -helical structure in dilute solution⁵⁸ has recently been questioned on the basis of small angle X-ray measurements.⁵⁹ Inasmuch as it is highly unlikely that the myoglobin molecule changes from 77% α -helical to a similar percentage of the 3_{10} helix upon dissolving, the similarity of the myoglobin measure-

ments to the measurements upon helical synthetic polymers provides further evidence that in the solvents utilized here the synthetic polymers must also be in the α -helical conformation.

The investigations reported in the present work may now be summarized briefly. More precise measurements of the circular dichroism of α -helical polypeptides have revealed fine structure in the curves between 185 and 245 $m\mu$. The circular dichroic data provide a basis for resolving the absorption and circular dichroic curves into three separate peptide electronic bands, namely an $n_1-\pi^-$ band near 222 $m\mu$ and two $\pi^0-\pi^-$ peptide exciton bands at 206 and about 190 $m\mu$. Oscillator and rotational strengths obtained from the data are in rough accord with the predictions of the exciton theory for the $\pi^0-\pi^-$ bands and with calculations for the $n_1-\pi^-$ transition as well.

The optical rotatory dispersion arising from the observed circular dichroic bands reveals that the characteristic parameters of the rotatory dispersion in the visible are largely determined by the peptide transitions here investigated.

Parallel circular dichroic studies on the disordered polypeptide chain reveal quite different optical properties from those observed for helical polymers. The circular dichroism of metmyoglobin closely agrees to that observed for the helical polypeptides. This finding underlines the common, conformation-dependent electronic origin of the optical properties of the natural and synthetic polymers.

Acknowledgments. It is a pleasure to acknowledge the indispensable contributions to this work of W. B. Gratzer, P. Urnes, and H. Auer, all of this laboratory. Communications with Drs. I. Tinoco, Jr., A. Moscovitz, A. Hansen, R. W. Woody, and J. A. Schellman have been helpful in illuminating several theoretical points. Financial support was provided by Grant No. GB-1328 from the National Science Foundation as well as by stipends from both the National Science Foundation and National Institutes of Health to G. H.

Appendix

In the discussion of the theory of the rotatory power of helical macromolecules above, it was noted that Mason^{19,20} has demoted to "end-corrections" the additional rotatory terms found by Moffitt, Fitts, and Kirkwood¹⁸ (hereafter designated as MFK) in their critique of Moffitt's theory.¹⁶ MFK, however, specifically

also J. A. Schellman and C. Schellman in "The Proteins," 2nd Ed., H. Neurath, Ed., Academic Press, New York, N. Y., 1964.

(56) J. C. Kendrew, H. C. Watson, B. E. Strandberg, R. E. Dickerson, D. C. Phillips, and V. C. Shore, *Nature*, **190**, 666 (1961).

(57) See ref. 37 and references contained therein.

(58) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956); see also P. Doty and W. B. Gratzer in "Polyamino Acids, Polypeptides, and Proteins," ref. 17, p. 111, and references therein.

(59) See V. Luzzati, M. Cesari, G. Spach, F. Masson, and J. M. Vincent, *ibid.*, p. 121.

asserted that the additional terms were important even for infinite helical polymers. Examination of the work of the authors listed above has revealed two errors in Mason's work. These are as follows.

(a) Mason utilizes only the exciton wave functions of Moffitt's original work,¹⁶ terming these wave functions the "symmetric" and "antisymmetric coupling modes." MFK showed, however, that these wave functions are inadequate in first order for calculations of rotatory power.

(b) Mason's calculations for the rotatory power of coupled dipoles (eq. 4-7 in ref. 19), although correct in themselves, lead to mistaken conclusions that some terms cancel one another. For the symmetric coupling mode, the rotational strength $R^s_{m,m+n}$ of two dipoles at loci m and $m+n$ in a right-handed helix is

$$R^s_{m,m+n} = 4dq_vq_t \sin^2(\alpha_n/2) - nZ(q_r^2 - q_t^2) \sin(\alpha_n) \quad (\text{IV})$$

Here d is the helix radius, q_r , q_t , and q_v are the radial, tangential, and vertical components of the electric dipole transition moment, Z is the pitch of the helix, and $\alpha_n = 2\pi n/P$, where P is the number of dipoles per turn of the helix. In contrast to Mason's conclusion, both the first and second terms in (IV) are even functions of n , so that summations of the form ($R^s_{m,m+n} + R^s_{m,m-n}$) do not lead to cancellations of terms.

It may be noted that a summation of paired terms of the form ($R^s_{m,m+n} + R^s_{m,m-n}$) occurs in the article of MFK in going from their eq. 20 to their eq. 21. Their vector analysis, which is correct, also shows that a cancellation of terms does not occur.

Magnetic Circular Dichroism and Magnetic Optical Rotatory Dispersion

John G. Foss and Michael E. McCarville

Contribution from the Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa. Received March 2, 1964

The relative merits of magnetic circular dichroism and magnetic optical rotatory dispersion are discussed and some examples of dichroism spectra are presented. These spectra appear to be of two classes which are designated as (1) Zeeman splitting and (2) perturbation. Possible applications of magnetic circular dichroism are considered briefly.

A recent paper of Shashoua¹ describes some measurements of the optical rotatory dispersion of samples in a magnetic field (MORD), *i.e.*, the dispersion of the Faraday effect. These dispersion measurements were made through absorption bands most of which exhibited a magnetic Cotton effect; *i.e.*, there was a characteristic anomaly similar to that observed in the absorption bands of naturally optically active molecules. Associated with this anomaly is a "magnetic circular dichroism" (MCD), and the object of this paper is to report some measurements of MCD and comment on their relationships to the work of Shashoua.

The experiments were made using a commercial Jouan circular dichrometer which is capable of measuring differences in the absorbance of right- and left-handed light of about 10^{-4} . The sample was mounted between iron pole pieces attached to a permanent magnet and with a gap of approximately 2 mm.; the average field was 8.1 kgauss (as determined by Faraday rotation measurements on water at 436 m μ). For all measurements reported, the samples used were 1 mm. thick.

Basically there appear to be two general types of MCD spectra. These are the spectra obtained (1) by a longitudinal Zeeman splitting and (2) by a magnetic perturbation giving a small difference in the absorption

of right- and left-handed light. Splittings can be recognized by characteristic positive and negative peaks (located approximately under the steepest portions of the absorption band) with a cross-over point at the same wave length as the center of the ordinary absorption band. By analogy with the convention adopted for optical rotatory dispersion (ORD) for naturally active compounds,² the splitting will be called positive if the molecular extinction coefficient of left-handed light is greater than that of right-handed light ($\epsilon_L > \epsilon_R$) for the peak with the longer wave length. It can easily be shown that positive splitting will lead to a characteristic three-peaked MORD curve with positive, negative, and positive peaks on going from longer to short wave lengths. This type of MORD curve is called type III by Shashoua. A negative splitting will give a similar curve, but will be reflected through the wave length axis. MCD spectra with a "perturbation origin" consist of a single peak centered at approximately the same wave length as the ordinary band. If $\epsilon_L > \epsilon_R$, the associated MORD curve has the appearance of the ordinary positive anomalous dispersion curve; *i.e.*, on going from long to shorter wave length, the curve is first positive and then negative. This is referred to as type I by Shashoua, and the corresponding negative dispersion is called type II.

It can easily be shown that splittings are most readily detected in absorption bands with very narrow band widths. For this reason, and because compounds with varying degrees of paramagnetism could be studied, the MCD spectra of several rare earths were measured. As an example, Figure 1 shows the splittings obtained with

(1) V. E. Shashoua, *J. Am. Chem. Soc.*, **86**, 2109 (1964).

(2) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.