

## Electric Dichroism Studies. Poly- $\gamma$ -benzylglutamate and Poly- $\beta$ -benzylaspartate

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Received September 9, 1969*

**Abstract:** The technique of electric-field induced dichroism has been explored to determine structural parameters of the side chain of the polypeptide, poly- $\gamma$ -benzyl-L-glutamate (PBLG). This technique is the most direct method available for obtaining structural parameters of rigid dipolar molecules in solution. The reduced dichroism, defined as  $\Delta\epsilon/\epsilon^0 = (\epsilon_{\parallel} - \epsilon_{\perp})/\epsilon^0$ , has been measured as a function of field strength and wavelength in several solvents. In 1,2-dichloroethane at 258 nm, the observed value of  $\Delta\epsilon/\epsilon^0$  is 0.09 at electric fields sufficient to completely orient the polymer molecules. This value indicates that the electronic transition moment for the 258-nm absorption band of known orientation in the phenyl group makes an angle of  $53.5^\circ$  with the major molecular axis. With this information a configuration of the benzylglutamate side chain of PBLG is proposed which is, in fact, in accord with infrared data from solid films. Measurements on poly- $\beta$ -benzyl-L-aspartate indicate that either the side chains are randomly oriented or are so oriented as to reduce the overall dipole moment of the polymer to too small a value to permit meaningful measurements. A highly resolved vibrational structure is revealed in the dichroism spectra. The analysis of this structure reveals the power of electric-field induced dichroism for obtaining optical as well as structural information.

Electric dichroism is one of the most direct methods for the examination of the structures of large molecules in solution. Combined with electric birefringence studies which provide information about the orientation of the molecular dipole moment with respect to the overall molecular shape, it is intrinsically capable of giving detailed structural information. Flow dichroism, the only comparable procedure, is less sensitive, requiring higher concentrations with all their concomitant drawbacks, and, in addition, suffers from turbulence instabilities at velocity gradients sufficiently high to make the desired measurements. Both dichroism methods require the appearance of electromagnetic transitions in accessible regions of the spectrum. These transitions are polarized along fixed directions in the chromophores and therefore can give rise to linear dichroism related to their orientation with respect to the applied electric field direction and to their direction in the molecule. Symmetry considerations frequently limit the possible transitions both in electronic and vibrational spectra. In the vibrational (infrared) region, the many available chromophoric groupings could provide a rich harvest of structural information. Thus, such studies in the infrared would be invaluable, but solvent absorption in that spectral region presents additional although not insuperable difficulties.<sup>1</sup> The present study on poly- $\gamma$ -benzylglutamates and poly- $\beta$ -benzylaspartate takes advantage of the 258-nm absorption band of the benzyl group. This band, which is largely a single electronic transition polarized in the plane of the ring, is revealed in these measurements to give rise to a highly structured dichroism spectrum arising from components polarized in directions perpendicular to that of the main source of intensity. It is, in fact, a demonstration that electric-field induced dichroism is capable of revealing spectroscopic features which for large molecules hitherto have

been available, if at all, only from polarized spectra of single crystals.

Electric dichroism also offers the possibility of studying conformational equilibria which cannot usually be observed in the solid state. While the X-ray determination of solid structures is capable of giving precise structural information, well-ordered crystals, frequently difficult to obtain, are required. There has been some concern over whether physical measurements made on polypeptides in the solid state accurately reflect the structure of these molecules in solution. While we do not particularly subscribe to this concern so far as it reflects the peptide helix backbone, it is far less certain that side chain conformations remain unchanged between solid and solution.

In solvents such as 1,2-dichloroethane (EDC), chloroform, or dioxane, poly- $\gamma$ -benzyl-L-glutamate (PBLG) exists mainly in helical form,<sup>2-4</sup> while in strong acids such as dichloroacetic acid (DCA), it exists as a random coil.<sup>3</sup> The helical form of the polymer is probably that of a right-handed  $\alpha$ -helix with some degree of flexibility.<sup>5</sup> Superimposed on this relatively rigid regular helical backbone are the positions of the side chains. Evidence from infrared studies on PBLG films<sup>6</sup> and other studies using X-ray techniques<sup>7</sup> indicate that the benzyl groups are oriented in dry polymer films.

Some solution studies have been done on the configuration of the benzyl groups of PBLG and on their contribution to the stability of the helix, using optical rotatory dispersion,<sup>8</sup> dielectric properties,<sup>9</sup> and flow

(1) G. Spach, *C. R. Acad. Sci., Paris*, **249**, 667 (1959).

(2) P. Doty and J. T. Yang, *J. Amer. Chem. Soc.*, **78**, 498 (1956).  
 (3) P. Doty, J. H. Bradbury, and A. M. Holtzer, *ibid.*, **78**, 947 (1956).  
 (4) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).  
 (5) G. Spach, L. Freund, M. Daune, and H. Benoit, *J. Mol. Biol.*, **7**, 468 (1963).  
 (6) M. Tsuboi, *J. Polym. Sci.*, **59**, 139 (1962).  
 (7) Y. Mitsui, Y. Iitaka, and M. Tsuboi, *J. Mol. Biol.*, **24**, 15 (1967).  
 (8) R. D. B. Fraser, B. S. Harrap, R. Ledger, T. P. Macrae, F. H. C. Stewart, and E. Suzuki, *Biopolymers*, **5**, 797 (1967).  
 (9) A. Wada in "Polyamino Acids, Polypeptides, and Proteins,"

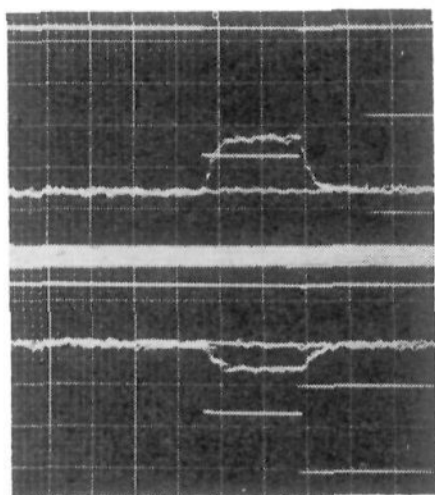


Figure 1. Oscilloscope traces for a typical electric dichroism experiment. Incident light is polarized parallel (top) or perpendicular (bottom) to the external field direction. See text for an explanation of the traces.

dichroism methods.<sup>10</sup> These studies suggest that the side chains have a specific orientation in the benzyl glutamate polymer in solution as well.

The benzyl side chains do appear to have an important effect on the conformation of the polymer. For example, about 70% DCA (volume %) in an organic solvent is required to force the helix to coil transition of PBLG, while poly- $\gamma$ -methyl-L-glutamate undergoes the transition in about 60% DCA, despite the fact that the less polar methyl groups are expected to be less solvated by the DCA than the benzyl groups, making the methyl polymer more stable on these grounds.<sup>11</sup> This is generally interpreted as providing evidence that the benzyl groups stabilize the  $\alpha$ -helix. Poly- $\beta$ -benzyl-L-aspartate (PBLA) (with one less methylene on the side chain) exists in a left- rather than a right-handed helix<sup>12,13</sup> and goes into the coil form at less than 10% DCA.

To understand the importance of the benzyl side chain in maintaining the  $\alpha$ -helix in solution, it is imperative that the orientation of the benzyl groups with respect to the helix backbone be determined. Electric dichroism offers the possibility of directly measuring this orientation and the present work is directed at making this measurement. The benzyl groups contain a chromophore which is easily accessible; the allowed transition for monosubstituted benzenes at 258 nm lies in the plane of the ring, perpendicular to the ring axis (*vide infra*), with no other interfering absorption bands in this region. Also PBLG is known to have a large dipole moment,<sup>9</sup> making it possible to obtain good orientation by a pulsed electric field of reasonable magnitude. The pulsed external fields result in a differential absorption of incident radiation polarized in mutually perpendicular directions. In the measurements to be described below, these directions are parallel to the direction of the electric field and perpendicular to the field as well as to the direction of the incident radiation. The orienting

force is the torque exerted by the field on the permanent or induced dipoles in the molecules. If the helices can be well oriented in solution, electric dichroism can be a sensitive probe for future studies of conformational influences<sup>14</sup> and allows a direct comparison between results of structural studies on the solid and on the solution.

## Experimental Section

Polymers studied included PBLG, molecular weight about 300,000, and PBLA, molecular weight about 180,000, which were obtained from Pilot Chemicals, Inc. (Watertown, Mass.) and purified by fractional precipitation. The polymers PBLG, molecular weights about 530,000 and 190,000, and poly- $\gamma$ -benzyl-D-glutamate (PBDG), molecular weight about 100,000, were synthesized. These three polymers are probably reasonably polydisperse. Fractionated samples of the 190,000 molecular weight PBLG and the PBDG were used for some of the measurements indicated below. The molecular weight of the PBDG fraction was estimated at 136,000. The molecular weights,  $M_w$ , of the polymers were determined by viscosity measurements carried out in DCA at 25.0° using the method of Blout and Karlson<sup>15</sup> and the relationship between molecular weight and intrinsic viscosity as worked out for PBLG by Doty, *et al.*<sup>3,16</sup>

The partial specific volume of PBLG was measured in a pycnometer with a capillary at 25.0° in dioxane. For a 0.1% solution,  $\bar{v} = 0.798 \pm 0.007$ .

Solvents were Matheson Coleman and Bell spectroquality. Dichloroacetic acid was vacuum distilled before use.

Solutions were prepared immediately before use and refrigerated between measurements to minimize concentration change due to solvent evaporation. Over a period of several weeks a given solution did not appear to undergo any change as judged from uv absorption spectra and electric dichroism data. In the solvents used, at the concentrations used, aggregation as described by Powers and Peticolas<sup>17</sup> did not affect the electric dichroism results. Dimerization, however, does occur in dioxane as discussed below. All measurements were made after the disappearance of all turbidity from the freshly prepared solutions.

Absorption data were taken on a Cary Model 14 spectrometer using matched 1-cm cells.

**Description of Apparatus.** The electric dichroism apparatus used will be described elsewhere.<sup>18</sup> The cell was made from a cylindrical Kel-F block with an H-shaped opening in the center. Highly polished stainless steel electrodes were fitted into the upright parts of the H, with an electrode gap of 1.22 mm, making contact with leads to the high-voltage pulse generator. Strain-free high-purity quartz windows (Opticell) were placed on either side of the opening in the block to form a sample compartment having a path length of 9.956 mm. The cell was filled from the top using a syringe. The cell was maintained at 25° by placing it in a blackened brass block through which coolant was circulated. In order to avoid heating the solution, short, single square-wave electric field pulses of 1 to 2 msec duration were used. In Figure 1, the pulse is shown in an oscilloscope photograph as an alternated negative signal (measured through a precision high voltage divider network) approximately 3.1 cm in height. The rise (and fall) time of the pulse is too rapid to appear in these photographs. Because of the thermal and diffusional resistance to orientation, the rise and fall (exponential) of the dichroism signal when the field is pulsed are readily observed.

## Analysis of Data

Figure 1 shows typical oscilloscope photographs. The pulse voltage was determined directly from the photograph by measuring the pulse height which was photographed simultaneously with the dichroism. The

M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, pp 131-146.

(10) A. Wada, "Seitaikobunshi I," Yoshioka Shōten, Kyoto, Japan, 1965, p 44.

(11) G. D. Fasman in "Poly- $\alpha$ -Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 11.

(12) R. H. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2268 (1960).

(13) E. M. Bradbury, A. R. Downie, A. Elliott, and W. E. Hanby, *Proc. Roy. Soc., Ser. A*, **259**, 110 (1960).

(14) T. C. Troxell and H. A. Scheraga, *Biochem. Biophys. Res. Commun.*, **35**, 913 (1969).

(15) E. R. Blout and R. H. Karlson, *J. Amer. Chem. Soc.*, **78**, 941 (1956).

(16) It has not been established that this relationship holds for PBLA, but it is at present the most convenient method for estimating molecular weights.

(17) J. C. Powers, Jr., and W. L. Peticolas in "Ordered Fluids and Liquid Crystals," Advances in Chemistry Series, No. 63, American Chemical Society, Washington, D. C., 1967, p 217.

(18) K. Yamaoka and E. Charney, manuscript in preparation.

signal heights with the light beam polarized perpendicular and parallel to the orienting field were similarly determined. The dark current signal and the light intensity in the absence of the field were measured each time just prior to pulsing and are shown as the relatively sharp lines in the right third of the photograph. The values of the experimental parameters were obtained by multiplying these measurements by the appropriate sensitivity factors determined from the oscilloscope amplification settings and, in the case of the electric field strength, from the values of the voltage divider described previously.

The reduced dichroism at any wavelength is calculated from these measurements by means of the expression

$$\frac{\Delta\epsilon}{\epsilon^0} = \frac{1}{A} \log \frac{1 + \frac{\Delta I_{\perp}}{I_{0\perp}}}{1 + \frac{\Delta I_{\parallel}}{I_{0\parallel}}} \quad (1)$$

where  $\Delta I_{\perp}$  and  $\Delta I_{\parallel}$  are changes in light intensity (the signal) of components polarized perpendicular and parallel, respectively, to the electric field direction and, similarly,  $I_{0\perp}$  and  $I_{0\parallel}$  are the light intensities in the absence of the field.  $A$  is the absorbance determined for the same path length and concentration as used in the dichroism experiment. Thus  $\Delta\epsilon = (\epsilon_{\parallel} - \epsilon_{\perp})$  is the differential molar extinction parallel and perpendicular to the field, and  $\epsilon^0$  is the isotropic molar extinction which was determined from the absorption spectrum using comparable slit widths.

According to the theory developed for rigid molecules the electric dichroism should show a linear dependence on the square of the field strength.<sup>18,19</sup> However, for highly polar or anisotropically polarizable polymers, the relation is predicted to be linear only for low field strength. For a molecule whose nuclear and electronic structure can be represented by a rigid ellipsoid of revolution with a nonzero dipole moment in the direction of the major axis of the ellipsoid, the relation at low field strengths for this reduced dichroism at absorption band maxima takes the approximate form

$$\frac{\Delta\epsilon}{\epsilon^0} = \frac{\beta^2}{5}(3 \cos^2 \alpha - 1) \quad (2)$$

where  $\beta = \mu_3 E/kT$ ,  $\mu_3$  is the dipole moment along the major axis,  $E$  is the applied field strength,  $kT$  is the Boltzmann thermal energy at the temperature,  $T$ , and  $\alpha$  is the angle between the transition moment and dipole moment  $\mu_3$ . At high field strengths where the molecules are substantially completely oriented, the relation is approximately

$$\frac{\Delta\epsilon}{\epsilon^0} = \frac{3}{2}(3 \cos^2 \alpha - 1) \quad (3)$$

and thus independent of field strength. Under this condition  $\alpha$  is uniquely determined. Nonquadratic dependence on field strength occurs before saturating fields, where the dichroism is independent of field strength, are attained.<sup>20</sup>

(19) V. W. Liptay and J. Czekalla, *Z. Elektrochem.*, **65**, 721 (1961).

(20) Cf. ref 18. See also the treatment of electric birefringence in which the orientation factor has a related behavior: C. T. O'Konski, K. Yoshioka, and W. H. Orttung, *J. Phys. Chem.*, **63**, 1558 (1959).

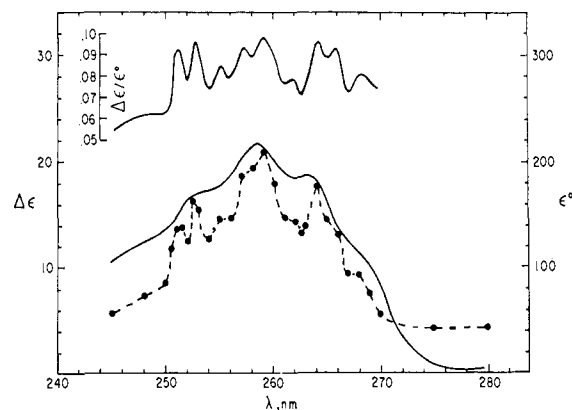


Figure 2. Absorption spectrum (—) and wavelength dependence of electric dichroism (---) for PBLG in EDC in the region of the 258-nm absorption band. The insert shows the dispersion of the reduced dichroism in the same region.

## Results

Figure 1 shows the dichroism signals as two noisy pulses with nearly exponential rise and decay times, the upper signal taken with the polarization direction parallel, and the lower signal with the polarization perpendicular, to the electric field direction. The field strengths in both cases (shown as square pulses approximately 3 cm from the upper traces) are the same, as are the incident light intensities (determinable from the approximately 2.2-cm distances between the sharp traces on the right-hand side of the photographs). The upper (parallel) signal is larger than the lower (perpendicular) signal and the dichroism is therefore positive.

The absorption spectrum for PBLG and the dispersion of the electric dichroism in EDC are plotted in Figure 2, as well as the reduced dichroism through the absorption band. Similar results were obtained for PBDG, while no measurable dichroism was observed with PBLA. Dichroism is measurable up to 285 nm. (A solution of poly- $\gamma$ -methyl-L-glutamate in  $\alpha$ -helical form but without the benzyl chromophore showed no measurable dichroism above 270 nm.)

Measurements of the field strength dependence of the electric dichroism were made at the peak of the 258-nm absorption band. In all cases, the dichroism increases with increasing field strength until fields approaching 15,000 V/cm are reached. Above this, for the polymers with larger effective dipole moments, the molecules are substantially completely oriented, further increases in values of field strength producing little or no additional dichroism, and the fields are termed "saturating."

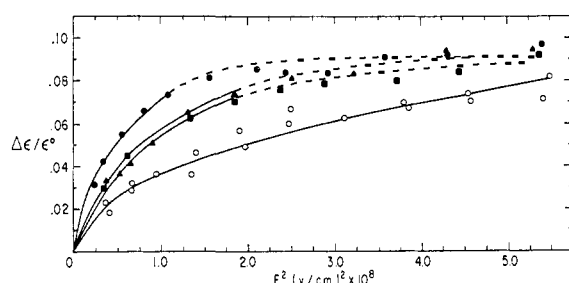
Data in EDC are shown in Figure 3, taken at an absorbance of about 0.7, which illustrate the dependence of the dichroism on the square of the field strength. The points are experimental, while the curves are least-squares fits of the data which yielded the coefficients given in Table I. Included in Table I are data obtained in dichloromethane (DCM) and in dioxane as well. Most of the data are approximated quite well by equations which are linear in  $E$ . In dioxane, the quadratic component decreases as the molecular weight increases, until, for the 530,000 molecular weight sample, the reduced dichroism shows

**Table I.** The Maximum Values of the Reduced Dichroism and the Least-Squares Coefficients and Standard Deviations,  $\sigma$ , for the Relation  $\Delta\epsilon/\epsilon^0 = AE^2 + bE + c$ ;  $\lambda = 258$  nm

Polymer	$\Delta\epsilon/\epsilon^0_{\max}$	$a \times 10^{11}$	$b \times 10^3$	$c \times 10^3$	$\sigma \times 10^3$
PBDG 100,000 EDC	0.083	0	3.35	2.90	4.68
PBLG <i>ca.</i> 300,000 <sup>a</sup> EDC	0.093	0	5.28	0.509	1.76
PBLG 300,000 EDC	0.095	0	5.56	-1.59	1.99
PBLG 530,000 EDC	0.098	0	7.30	-1.42	2.62
PBDG 100,000 DCM	0.028	0	1.22	0.351	0.798
PBDG 136,000 DCM	0.039	1.86	1.13	0.0294	1.52
PBLG 190,000 DCM	0.055	0	2.28	-1.85	3.22
PBLG 300,000 DCM	0.053	0	2.28	0.677	1.19
PBLG 530,000 DCM	0.052	0	2.46	-0.904	1.74
PBDG 100,000 dioxane	0.059	2.46	1.64	0.226	2.53
PBLG 190,000 dioxane	0.079	1.74	2.52	2.64	5.63
PBLG 300,000 dioxane	0.076	0.727	2.90	-0.953	2.16
PBLG 530,000 dioxane	0.052	0	2.10	0.245	1.19

<sup>a</sup> This sample was the high molecular weight fraction of the 190,000 molecular weight PBLG. Viscosity measurements were not performed on this fraction; thus, the molecular weight is estimated.

only a linear dependence on field strength. The data obtained at saturating fields where the dichroism is independent of the magnitude of the field, were not included in the least-squares calculations. As a result calculated curves do not follow the data at high field strengths, and, in Figure 3, dashed curves are drawn.



**Figure 3.** Field strength dependence of electric dichroism in EDC of PBLG, ●,  $M_w = 530,000$ ; ▲,  $M_w = 300,000$ ; ■,  $M_w$  *ca.* 300,000; and PBDG, ○. The solid lines are least-squares fits, neglecting the effects of saturation, assuming a linear dependence of  $\Delta\epsilon/\epsilon^0$  on  $E$ .

Experiments were carried out in DCM, EDC, and dioxane. Other work indicates that the polypeptide exists in DCM in the same form as it does in EDC.<sup>21</sup> However, no saturation is observed in DCM, indicating that the effective dipole moment of PBLG is less in this solvent than in EDC. The behavior in dioxane differs from that in the other two solvents. Again saturation is not reached, indicating a lower effective dipole moment. Also, the dichroism is not proportional to the molecular weight of the polymer.

Equation 3 relates the angle  $\alpha$  between the transition moment and the direction of the rigid dipole axis to the reduced dichroism  $\Delta\epsilon/\epsilon^0$ .<sup>18,19,22</sup> For the value of  $\Delta\epsilon/\epsilon^0$  observed at 258 nm for nearly completely oriented PBLG at high field strength in EDC, namely  $\Delta\epsilon/\epsilon^0 = 0.09$ , this angle  $\alpha$  is about  $53.5^\circ$ .<sup>18,19,22</sup> In this range of  $\Delta\epsilon/\epsilon^0$  ( $0.09 \pm 0.04$ ),  $\alpha$  is a relatively insensitive function of  $\Delta\epsilon/\epsilon^0$  so that low values of  $\Delta\epsilon/\epsilon^0$  resulting from incomplete orientation do not cause large errors in  $\alpha$ .

Table II gives the results of concentration studies in EDC. The results, and those for the other polymers

(21) J. Y. Cassim and E. W. Taylor, *Biophys. J.*, **5**, 553 (1965).

(22) W. Kuhn, H. Dührkop, and H. Martin, *Z. Phys. Chem.*, **B45**, 121 (1940).

**Table II.** The Concentration Dependence of the Reduced Dichroism of PBLG in EDC

Concentration, $M \times 10^3$	$\Delta\epsilon/\epsilon^0$
10.0	$0.076 \pm 0.003$
8.06	$0.076 \pm 0.005$
6.43	$0.082 \pm 0.007$
5.15	$0.080 \pm 0.002$
2.06	$0.075 \pm 0.001$

in EDC and in DCM as well, suggest that at the concentrations used, no aggregation occurred in these solvents. In an attempt to further examine the possibility that association phenomena may affect the dichroism results, viscometric studies in DCM and sedimentation studies in dioxane were performed. Viscometry in DCM gave essentially identical results to similar experiments conducted in 0.5% formamide-DCM, *i.e.*,  $[\eta]_{25^\circ}$  PBLG ( $M_w = 300,000$ ) = 4.5 in formamide-DCM, 4.2 in DCM, as compared to 6.4 in dimethylformamide where no aggregation occurs.<sup>3</sup> The Huggins' constant,  $k'$ , for the relation

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c$$

where  $k'$  is less than unity for nonaggregated systems, is 1.4 in DCM, 1.24 in formamide-DCM, and about 0.8 in dimethylformamide.<sup>3</sup> The addition of 0.5% formamide to a PBLG solution in DCM should make association negligible.<sup>3</sup> Equilibrium ultracentrifugation was performed in dioxane. (We wish to thank Mrs. Bethel Stannard, Dr. Dan Inners, and Dr. Gary Felsenfeld for these.) Dioxane was the only solvent practical for such a study of the three used in this investigation since EDC has about the same density as PBLG and DCM appears to be too volatile for prolonged experiments. The results of these experiments as determined from the slopes of the resulting plots of  $\ln c$  against the square of the cell radius are given in Table III. The plot of the sample of

**Table III.** Molecular Weights Determined from Sedimentation Equilibrium Studies on PBLG in Dioxane

Concentration, $M \times 10^3$	$M_w$
4.16	$3.2 \pm 0.1 \times 10^5$
2.68	$5.2 \pm 1.1 \times 10^5$
1.54	$6.2 \pm 0.1 \times 10^5$

intermediate concentration was highly curved and this is reflected in the uncertainty in the value of the molecular weight. The sample used for these experiments was that of 300,000 molecular weight as determined by viscometric measurements in DCA.

All dichroism experiments were performed with freshly prepared solutions in a period over which no changes in dichroism were observed with time. Solutions in EDC older than a few weeks were observed to give different and sometimes erratic results.

## Discussion

From the electric dichroism data, the determination of the orientation of the electronic transition moment directions with respect to fixed axes in the polymer is straightforward under the assumptions already discussed, namely that of rigid behavior of the polymer molecule and the attainment of substantially complete orientation. The structural conclusions, however, must rely on a determination or an assumption of the direction of these transitions with respect to the structural parameters of the chromophore itself, which in this case is a monosubstituted benzene. In many cases symmetry considerations alone are sufficient to limit the possible directions of these transitions within the chromophore, and polarized absorption spectra of single crystals or vapor phase studies of the ultraviolet absorption spectra of simple molecules containing the chromophore may be used to determine these directions experimentally. If we are prepared to ignore the latent possibility that the helical field of the polymer will perturb these orientations in the chromophore these studies may be carried over when the chromophore is located as it is here in a more complex molecule. In this case, as we have noted earlier, the 258-nm transition, which in the accepted Schönflies symmetry notation is known as  ${}^1B_2$ , is an electric-dipole allowed electronic transition perpendicular to the axis of the phenyl group running through the substituted carbon and the carbon atom opposite and in the plane of the ring.<sup>23</sup> In the  $C_{2v}$  symmetry of the isolated monosubstituted benzene there are, however, electric-dipole transition moments which are allowed in both directions perpendicular to this axis. These have overall symmetry  $B_1$  and  $A_1$  where the former is perpendicular to the plane of the ring, and the latter is in the plane but perpendicular to the transition which is allowed to a state of symmetry  $B_2$ . In order, however, for any absorption to appear in these directions, there must be vibronic interaction with other states. If all the vibrational components of the upper electronic state are totally symmetric vibrations of type  $A_1$ , by the rules of group theory all the intensity arises from the  ${}^1B_2$  transition described above and the electric field-induced reduced dichroism should be independent of wavelength. However, if vibronic interactions contribute some intensity (in general, one can show theoretically that vibronic contributions will be relatively small compared to those of electronically allowed transitions<sup>24</sup>), and if the vibrational components can be spectroscopically resolved, then the reduced dichroism will vary with the wavelength, changes taking place when and if an antisymmetric vibration gives rise to

components of overall symmetry  $A_1$  or  $B_1$ . Indeed if magnetic dipole transitions are also permitted these may show up with extremely low intensity in absorption and more significantly in optical activity. Horwitz, Strickland, and Billups<sup>25</sup> have recently analyzed the low-temperature absorption and circular dichroism spectra of phenylalanine derivatives in these terms.

Since we do not intend to use the information for the structural investigation which is the main point of this research, we report and discuss here only briefly the rich electric dichroism spectra of PBLG illustrated in Figure 2. Comparison of the dichroism,  $\Delta\epsilon$ , with the isotropic absorption,  $\epsilon_0$ , measured with the same spectral resolution reveals considerably more highly resolved structure in the dichroism, as expected from an *oriented* system in which there are components polarized in different directions. The almost surprisingly highly resolved dichroism spectrum is indeed indicative of a most advantageous feature of electric dichroism spectra, namely that the orientation in the electric field creates a uniaxial system so that measurements in two directions perpendicular to the field (*i.e.*, parallel and perpendicular to the unique axis) contain all the optical absorption information. A preliminary analysis of the highly resolved reduced dichroism is given in Table IV, where a comparison is also made with the analysis of the CD and absorption data of substituted phenylalanines by Horwitz, Strickland, and Billups.<sup>25</sup> It is apparent that carrying over their analysis, which in turn depends on an interpretation of the ultraviolet vapor spectrum of toluene,<sup>23</sup> we can identify four separate progressions in the  $\Delta\epsilon/\epsilon_0$  spectrum and in addition assign three features not identified by them. Two of the progressions, the vibrational progressions involving only the totally symmetric upper-state vibration of about  $930\text{ cm}^{-1}$  and the other involving one quantum of the totally symmetric  $750\text{-cm}^{-1}$  vibration with one or more quanta of the  $930\text{-cm}^{-1}$  vibration, thus in all cases transitions to states of the  ${}^1B_2$  symmetry responsible for most of the intensity, give maxima in  $\Delta\epsilon/\epsilon_0$ . Two others, involving one quanta of the upper state antisymmetric vibrations at about  $520\text{ cm}^{-1}$  and  $180\text{ cm}^{-1}$  leading to components with overall symmetry  $A_1$  and  $B_1$ , respectively, appear as minima in the reduced dichroism. The fact that none of these minima are large enough to develop negative values of the electric dichroism is indicative of the fact that the vibronic intensities are small and the band system is not completely resolvable. Thus the polarization of the band fluctuates near the maximum value which arises from the  ${}^1B_2$  electronically allowed transition and this provides the data for the structural conclusions drawn here. No shifts in frequency for the overall band nor for the vibrational components are observed, indicating that the applied electric field does not seriously perturb the electronic or nuclear structure of the molecule.

The direction of orientation of the molecules with respect to the direction of the electric field has previously been determined by electric birefringence measurements to be with the major axis of polarizability parallel to the electric field.<sup>26</sup> If the molecule exists as a relatively rigid helical rod, this requires that

(23) N. Ginsburg, W. W. Robertson, and F. A. Matsen, *J. Chem. Phys.*, **14**, 511 (1946).

(24) G. Herzberg and E. Teller, *Z. Phys. Chem.*, **B21**, 410 (1933).

(25) J. Horwitz, E. H. Strickland, and C. Billups, *J. Amer. Chem. Soc.*, **91**, 184 (1969).

(26) Kiwamu Yamaoka, Ph.D. Thesis, University of California, Berkeley, Calif., 1964.

Table IV

Observed extrema <sup>a</sup> in dichroism curve	Corresponding features in phenylalanine <sup>b</sup> derivatives	Frequency <sup>c</sup> assignment	Predicted <sup>d</sup> symmetry	Observed <sup>e</sup> symmetry
268.3	268.2	0	B <sub>2</sub>	B <sub>2</sub>
267.1	267.2	0 + 180	B <sub>1</sub>	B <sub>1</sub>
265.8	<i>c</i>	0 + 2 (180)	B <sub>2</sub>	B <sub>2</sub>
265.1	265.0	0 + 520	A <sub>1</sub>	A <sub>1</sub>
264.0	<i>c</i>	0 + 750 <sup>f</sup>	B <sub>2</sub>	B <sub>2</sub>
262.7	<i>d</i>			
261.8	261.6	0 + 930	B <sub>2</sub>	B <sub>2</sub>
261.1	260.5	0 + 180 + 930	B <sub>1</sub>	B <sub>1</sub>
259.0	<i>d</i>			
258.0	258.2	0 + 520 + 930	A <sub>1</sub>	A <sub>1</sub>
257.2	256.7	0 + 750 + 930 <sup>f</sup>	B <sub>2</sub>	B <sub>2</sub>
255.9	<i>d</i>			
255.1	255.4	0 + 2 (930)	B <sub>2</sub>	B <sub>2</sub>
254.0	<i>c</i>	0 + 180 + 2 (930)	B <sub>1</sub>	B <sub>1</sub>
252.7	<i>d</i>			
252.0	252.3	0 + 520 + 2 (930)	A <sub>1</sub>	A <sub>1</sub>
251.1	250.7	0 + 750 + 2 (930) <sup>f</sup>	B <sub>2</sub>	B <sub>2</sub>

<sup>a</sup> These data are estimated to be accurate to  $\pm 0.25$  nm. Extrema in italics are minima. <sup>b</sup> The values tabulated here are 0.5 nm greater than those given in ref 25 since these authors state that their data, which are taken from spectra at 77°K, indicate that a blue shift of approximately 0.5 nm occurred in their 77°K glasses as compared to their room temperature spectra. <sup>c</sup> Not assigned by Horwitz, Strickland, and Billups. <sup>d</sup> Unassigned. <sup>e</sup> Tabulated upper state vibrational levels are in  $\text{cm}^{-1}$ . <sup>f</sup> We have identified, for ease of comparison, the progression in this frequency with the value  $750 \text{ cm}^{-1}$  used by Horwitz, Strickland, and Billups<sup>25</sup> and Ginsburg, Robertson, and Matsen.<sup>23</sup> However, as the former authors point out, it would be quite fortuitous if the benzyl group vibrations in their phenylalanine derivatives were precisely the same as those of toluene vapor. In the case of the 180-, 520-, and  $930\text{-cm}^{-1}$  vibrations our data are, within experimental error, sufficiently close to the toluene data to accept these as approximate values. However, our data for the progression involving the peaks at 264.0, 257.2, and 251.1 nm seem to be far better fit by a frequency of about  $680 \text{ cm}^{-1}$ , which is quite reasonably within the range through which this vibration could fluctuate between toluene and the benzyl group of PBLG. <sup>g</sup> The predicted symmetry is based on assignments of the corresponding features in the CD and absorption spectra of phenylalanine derivatives in ref 25. These in turn depend on analogies with the toluene vapor spectra of ref 23. In the case of the features of 265.8, 264.0, and 254.0, which were not assigned in ref 25, the predictions are based directly on frequency intervals observed in the toluene spectra. <sup>h</sup> The observed symmetry is based on the assignment using frequency differences from the 0-0 band and the nature of the dichroism extrema, *i.e.*, maxima or minima.

the helix axis be oriented parallel to the field direction. In these nonaqueous media the main orienting force is the torque exerted on the permanent dipoles of the peptide units which are directed approximately parallel to the helix axis.<sup>9,27</sup> Except for field induced dipoles, the other orienting element is the dipole of the side chain ester carbonyl.

The limiting effect of the side chain would be to direct all the carbonyl dipoles oppositely to that of the peptide units and so effectively cancel part of the 4-5 D per peptide of the helix backbone,<sup>28</sup> or to direct the side chain dipole in the same direction as that of the peptide units. The former situation would result in a relatively low effective overall moment, a small orienting force, and, in the limiting case of exact cancellation, no dichroism except for that produced by the orientation due to dipoles induced by the action of the field on the anisotropic electron distribution in the molecule. Electrostatic considerations would tend to favor this antiparallel alignment of permanent dipoles, but since dipole interaction falls off rapidly with distance, other side chain orienting forces, chiefly induced dipole and dispersion interactions, can come into play.

The experimental value of  $\alpha$  of  $53.5^\circ$  can be compared with that obtained by Tsuboi from infrared studies on films of PBLG.<sup>6</sup> He suggests an  $\alpha$  of  $57^\circ$ . Since the sign of the dichroism changes at  $\alpha = 54.7^\circ$ , Tsuboi thus sees perpendicular (or negative) dichroism

(27) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci. U. S.*, **37**, 235 (1951).

(28) The dipole moment of the  $\alpha$ -helix backbone is discussed in ref 9, but is not known with great accuracy. For PBLG, the dipole moment per residue, which is the vector sum of all the moments, has been obtained from birefringence and dielectric measurements, is solvent dependent, and is about 3.4-3.6 D for the helix form in EDC.

in contrast to the parallel (or positive) dichroism observed in the present work, though both values are small. Shirai observed a sign change in the electric dichroism of PBLG in tetrahydrofuran at 263 nm as the temperature was changed from  $18^\circ$  to  $5^\circ$ .<sup>29</sup> These results suggest that the side chains are in a temperature dependent equilibrium and their orientation differs slightly between solid state and solution.

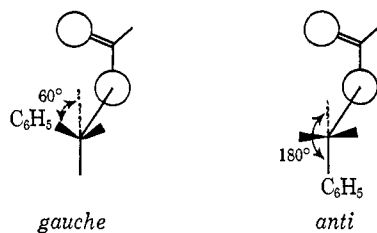
The electric dichroism results are compatible with those found by Wada<sup>10</sup> from flow dichroism studies, but because, as we have noted previously, complete orientation is very difficult to achieve by flow dichroism, Wada's value of  $\Delta\epsilon/\epsilon^0$  of only 0.05 at the band maximum is somewhat smaller than the 0.09 value found in these experiments.

The benzyl glutamate side chain consists of a sequential grouping of two alkyl carbons attached to the  $\alpha$ -carbon, the ester oxygen, a fourth methylene group (the benzyl carbon), and the phenyl ring. If the  $\alpha$ -helical backbone is assumed to be rigid, there are several possibilities for the orientation of the side chains: (1) free rotation about all bonds in the side chain (space-filling models indicate that steric hindrance effectively prevents this; this arrangement would show no electric dichroism because the transition moment vector is randomly oriented with respect to polarized light even when the polymer is oriented in the field, and thus, this possibility can be eliminated since electric dichroism is observed), (2) restricted but not prohibited rotation around bonds in the side chain (it is difficult to predict what the dichroism would be in

(29) M. Shirai, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, p 131.

this case as there are a large number of possibilities for the position of the atoms around the few bonds, even with restricted rotation), (3) little or no rotation around bonds in the side chain (the range of available conformations can be limited on heuristic grounds presented below to support the last of these possibilities, but we wish to be careful to point out that while these considerations are supported by our data, the heuristic argument is strictly that, and cannot in turn be used to support the data).

There is ample evidence<sup>6,30-33</sup> that the most stable conformation of the three-carbon alkyl sequence and the ester oxygen is one in which the alkyl bonds are in the *anti* conformation (*i.e.*, the  $\sigma$  bonds of the methylene carbons and the  $\sigma\pi$  bond to the carbonyl oxygen are sequentially opposed) and the bonds to the ester carbonyl are in the *cis* arrangement.<sup>30</sup> Steric considerations, however, do not place quite such strong restrictions on the possible conformations about the O-C<sub>benzyl</sub> and the C<sub>benzyl</sub>-C<sub>ring</sub> bonds. The angle of rotation about the former between the O-C<sub>carbonyl</sub> bond and the C<sub>benzyl</sub>-C<sub>ring</sub> bond is energetically stable at 60° (*gauche*) or 180° (*anti*).<sup>6,32</sup> These positions are illustrated below. If the ring is *anti*, it can rotate



freely about its axis. However, if it is *gauche* with respect to the O-C<sub>carbonyl</sub> bond, rotation is severely restricted because of steric hindrance between the *ortho* hydrogen atoms and the carbonyl group. This hindrance also inhibits somewhat interchange between the *gauche* and *anti* conformations.

Tsuboi,<sup>6</sup> on the basis of his film dichroism studies and from X-ray evidence on the radius of the PBLG helix cylinder,<sup>34</sup> favors the 60° position of the ring. The loss of entropy due to the steric restrictions on this orientation may be more than compensated for by orbital interactions between the carbonyl oxygen and the phenyl group. In this case the position of the ring and thus of the transition moment vector are relatively fixed. The observed angle  $\alpha$  of 53.5° is compatible with this configuration. In this position, the helix backbone is well shielded, which could account in part for the great stability of PBLG to strong organic acids. The *gauche* conformation is incorporated into the proposed structure shown in Figure 4.

If the ring were in the *anti* position, and thus free to rotate about its axis, the conformational freedom would permit a corresponding freedom in the allowed values of  $\alpha$ . Under these conditions the dichroism would be expected to be rather smaller than is observed.

(30) M. O'Gorman, W. Shand, and V. Schomaker, *J. Amer. Chem. Soc.*, **72**, 4222 (1950).

(31) S. Mizushima, "Structure of Molecules and Internal Rotation," Academic Press, New York, N. Y., 1954.

(32) M. Tsuboi, T. Shimanouchi, and S. Mizushima, *J. Amer. Chem. Soc.*, **81**, 1406 (1959).

(33) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).

(34) C. H. Bamford, W. E. Hanby, and F. Happey, *Proc. Roy. Soc., Ser. A*, **205**, 30 (1951).

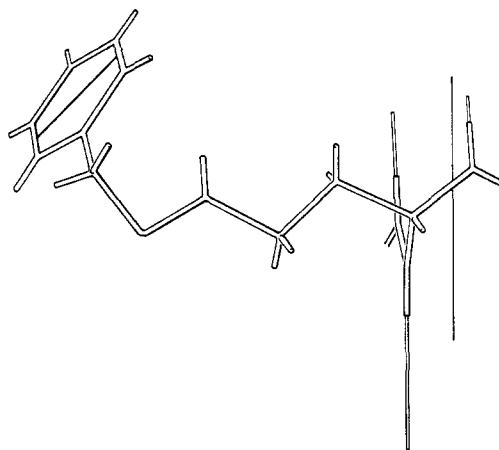


Figure 4. Proposed conformation of PBLG showing the positions of the side chains and the orientation of the electronic transition moment.

Figure 3 shows that as the molecular weight of PBLG becomes larger, the dichroism increases at low field strengths. The same trend was observed in DCM. A somewhat different pattern was observed in dioxane, as Table I suggests. A PBLG sample having a molecular weight of 530,000 showed a lower dichroism than PBDG with  $M_w = 100,000$ . This may be an indication of extreme flexibility or of molecular association of the antiparallel type, so that the dipole moment is decreased. This type of association does indeed exist in dioxane solutions of higher concentrations.<sup>17</sup> Viscosity studies of molecules of various molecular weights have indicated that the rigidity of the  $\alpha$  helix falls off at molecular weights above 250,000-300,000<sup>35,5</sup> and other evidence indicated that the hydrodynamic persistence length of PBLG may be considerably smaller.<sup>35,36</sup> In a previous communication<sup>37</sup> flexibility was proposed as an explanation for the lack of a quadratic dependence on field strength for the electric dichroism. If this is true for PBLG, then the molecule is relatively flexible even in EDC and DCM, and in dioxane the onset of flexibility can be monitored as the molecular weight increases by the decrease in the quadratic coefficient. It should be pointed out that the flexibility of the molecule may have an effect on  $\Delta\epsilon$ , making the correlation between the orientation of the transition moment vector and the reduced dichroism,  $\Delta\epsilon/\epsilon^0$ , subject to some uncertainty. The sedimentation data (Table III) indicate that PBLG exists as dimers in dioxane at the concentrations used in the field strength dependence studies in this solvent. The dimeric association of PBLG in dioxane apparently results in a stiffer molecule as evidenced by the quadratic dependence of the samples of molecular weight 190,000 and 300,000. The same is apparently true for the sample of PBDG of low molecular weight ( $M_w = 100,000$ ) where an examination of the data of Table III shows that in dioxane the quadratic coefficient in the

(35) P. Moha, G. Weill, and H. Benoît, *J. Chim. Phys.*, **61**, 1240 (1964).

(36) V. N. Tsvetkov, Yu. V. Mitin, I. N. Shtennikova, V. R. Glushenkova, G. V. Tarasova, V. S. Skazka, and N. A. Nikitin, *Vysokomol. Soedin.*, **7**, 1098 (1965); V. N. Tsvetkov, I. N. Shtennikova, E. I. Ryumtsev, and G. I. Okhrimenko, *ibid.*, **7**, 1104 (1965); V. N. Tsvetkov, I. N. Shtennikova, E. I. Ryumtsev, and V. S. Skazka, *ibid.*, **7**, 1111 (1965).

(37) J. B. Milstien and E. Charney, *Macromolecules*, **2**, 678 (1969).

field strength dependence is  $2.46 \times 10^{11}$ , while in EDC and DCM the quadratic coefficient is nil within the limits of the least-squares fit of the data.

Other workers have assigned a conformation intermediate between that of a rectilinear rod and a Gaussian coil for high molecular weight PBLG in EDC (with 1% DMF added to prevent aggregation) from viscosity, sedimentation, and birefringence experiments.<sup>36</sup> The electric dichroism experiments neither confirm nor support this interpretation, but the field strength behavior at both high and low fields and the comparison with the results of dichroism studies on poly-(butyl isocyanates)<sup>37</sup> are in accord with an interpretation that the polymer remains helical but becomes increasingly flexible as the molecular weight increases.

Since a measurable dichroism signal was observed with a PBDG sample having a molecular weight of half that of the PBLA, the fact that PBLA in DCM gave no dichroism signal must have an origin in its configuration and dipole moment. The birefringence of PBLA has been observed to be less than one-fifth that of PBLG in the same solvent,<sup>26</sup> despite considerable evidence that it exists in a helical (not necessarily  $\alpha$ -helix) configuration. It would appear therefore that the absence of a measurable dichroism in the aspartate polymer must be at least partly due to its small dipole moment. Since the groups which make substantial contributions to the moments of both the glutamate and the aspartate polymers are identical, the secondary structure of the latter must be different from that of the former. A variety of possibilities exists<sup>38</sup> and the lack of a positive experimental result prohibits us from distinguishing among these possibilities. It would be of interest to determine whether in addition to the difference in dipole moments and thus in molecular orientation in the applied field, the dichroism is still further decreased by a fortuitous configuration in which the angle  $\alpha$  is about  $54.7^\circ$  for which  $(3 \cos^2 \alpha - 1)$  is zero, but this determination must await the construction of a still more sensitive apparatus.

The power of the electric dichroism approach is

(38) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, and W. E. Hanby, *J. Mol. Biol.*, **5**, 230 (1962).

pointed up by its ability to detect subtle differences in conformation in different solvents which are not detected in previously used solution techniques such as optical rotatory dispersion,<sup>21</sup> and which obviously cannot be studied in the solid state.

### Summary

The electric dichroism of solutions of PBLG, PBDG, and PBLA has been measured. A significant positive dichroism is observed for the two former polymers and little or no dichroism for the latter. The observed dichroism at field strengths sufficient to virtually completely orient PBLG exhibits a highly structured spectral dependence which has been analyzed as vibrational substructure arising from the allowed electronic state of overall symmetry  ${}^1B_2$  and from vibronic states of symmetry  ${}^1A_1$  and  ${}^1B_1$ . The fact that this structure is so much more highly resolved in the dichroism measurements than in isotropic absorption and thus can be vibrationally analyzed indicates that the benzyl moiety is highly oriented in the polymer. The magnitude of the dichroism arising from the  ${}^1B_2$  transition permits the determination of the orientation of the transition moment which lies in the plane of the phenyl ring. The polar angle which this moment makes with the polymer axis is  $53.5^\circ$ . This angle is compatible with that determined from infrared investigations of a solid film. The dependence of the electric dichroism on the orienting field strength in EDC and DCM is largely linear even at low field strengths. This indicates that even at molecular weights of 100,000, the lowest molecular weight polymer measured, the poly- $\gamma$ -benzylglutamate molecule is flexible. In dioxane, the quadratic component of the field strength is sufficiently large so that it may be used to measure at least semiquantitatively the degree of flexibility of the polymer in that solvent. In EDC and DCM the dichroism of both the D and L forms of poly- $\gamma$ -benzylglutamate increases with molecular weight at field strengths insufficient to cause complete orientation indicative of the fact that the flexibility does not destroy the rodlike characteristics. The absence of a measurable dichroism for PBLA prevents the assignment of its conformation.