Electric Dichroism and Polymer Conformation. II. Theory of Electric Dichroism, and Measurements on Poly(n-butyl isocyanate)[1]

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ABSTRACT: The theory of electric dichroism (ED), with the permanent dipoles of helical macromolecules being oriented by a static external electric field, is discussed. The effects on the ED of orientation due to an anisotropic polarizability, flexibility of the backbone and side chain, aggregation, and polydispersity in the degree of polymerization (DP) are also considered. This theory, and a new method for measuring ED, are applied here in a study of poly(n-butyl isocyanate) in nonpolar solvents over a range of DP from 200 to 2400. The ED results show that the polymer behaves as a rigid rod up to DP $\sim$ 600, with an onset of flexibility at higher DP. The ED spectrum down to 192 mμ shows only positive dichroism which arises from a weakly dichroic band at <203 mμ and a stronger one at 249 mμ. The directions of polarization with respect to the helix axis, determined for these bands, are 53 and 44° for the <203- and the 249-mμ bands, respectively. Consistent with the nonplanarity of the >N—C=O group, the ED data indicate that there is very little electron delocalization over the whole macromolecule. Also, there appears to be no strong or intermediate strength exciton coupling observable in the spectral region studied. The directions of polarizations could be primarily along the C=O bond (for the <203-mμ transition) and primarily in the plane of the N—C=O group and perpendicular to the C=O bond (for the 249-mμ transition); however, it is not possible to make a definite assignment of these polarization directions at this time.

In preceding papers[4,5] we pointed out that the electric dichroism (ED) produced by placing a polymer solution in a static electric field depends on the permanent dipole moment, $\boldsymbol{\mu}$, and on the electric dipole transition moment, $\boldsymbol{\mu}_{0i}$, for the electronic transition, $0 \rightarrow i$, between the ground and ith excited states, respectively, and that ED can provide information about $\boldsymbol{\mu}$, orientation and mobility of chromophores, polarization of transitions, flexibility, and aggregation. In this paper we relate the observed ED to molecular properties, and study the ED of poly(n-butyl isocyanate) (PBIC).

Several ED investigations, using various techniques, have already been reported. Czekalla,[6] Labhart,[7] Liptay,[8] and Kuball and Singer[9] studied the static electric field (E) dependence of the absorption in small molecules. The high fields (of the order of 10^5 V/cm) required to study small molecules induce shifts in absorption frequency and changes in $\boldsymbol{\mu}_{0i}$ as well as dichroism. Pulsed fields have also been used,[10-12] the primary advantage being the applicability to conducting solutions and the ability to study rotational diffusion; however, because of the short observation time of the signal, the sensitivity of the pulsed-field technique is necessarily low. On the other hand, the method described in the previous paper[5] uses a (lower) static electric field for nonconducting polymer solutions and possesses not only the inherent increased sensitivity of a static method but also an increase by a factor of about 33 in the sensitivity (compared to the usual direct measurement of the separate absorbances of two mutually perpendicularly polarized light beams) because the dichroism is measured in terms of a rotation of linearly polarized light. In our method, the Cary Model 60 spectropolarimeter is used to measure the electric dichroic rotation, the only additional apparatus required being an ED cell and an apparatus for generating and measuring the high voltage.

While our primary interest in this series of investigations is in α-helical poly(amino acids), it appeared worthwhile to consider initially the fairly rigid polymer PBIC, which has the repeating unit $-N(CH_2CH_2CH_2CH_3)-CO-$. The backbone of this polymer differs from that of a poly(amino acid) by the absence of the C^α atom and the hydrogen-bonding H atom on the N atom. Since the peptide C–N bond in poly(amino acids) has partial double bond character, the possibility of extended electronic delocalization in PBIC exists, which might result in interesting electronic and spectral properties in the spectral region accessible to the Cary Model 60 spectropolarimeter. Further, there are no electronic transitions in the butyl side chain in this same spectral region which could interfere with the spectrum of the backbone. The butyl side chain must also shield the highly polar backbone from the medium, since PBIC is soluble in nonpolar and slightly polar solvents.[13] Its solubility in nonconducting solvents permits the application of our static electric field method to study the ED of this polymer.

Sedimentation, diffusion, light scattering, viscosity, and dielectric measurements[14-17] indicate that PBIC (and its

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n-hexyl homolog) has an extended rigid structure. The dielectric measurements also reveal a significant residue dipole moment of 1.13 D[17] parallel to the rod axis, with no detectable dipole moment in the perpendicular direction. Flow and electric birefringence studies[18] and light-scattering measurements in electric fields[19] also support the conclusion of high rigidity and a large $\boldsymbol{\mu}$. Because of this large permanent dipole moment, significant electric-field-induced orientation is readily attainable for ED measurements. X-Ray studies[20] show that PBIC is helical, with a 1.94-Å translation and 135° rotation per residue. In the same study,[20] it was found that the ultraviolet absorption spectrum of an oriented film is essentially identical with that of a cyclohexane solution of the polymer. This observation, indicating that the structure is the same in the solid phase and in solution, is supported by osmotic pressure and light-scattering studies,[21] from which it was concluded that the monomer translation along the helix axis is between 1.8 and 2.1 Å. Considering the above evidence,[17-21] it appears likely that, in solvents of low dielectric constant, PBIC is a mixture of right- and left-handed helices which are derived from the cis-trans planar form, shown in Figure 1,[22] by small rotations around the C-N bonds in order to relieve the steric interference of the *n*-butyl side chains and the carbonyl group. The rigidity arises, on the one hand, from the relatively high potential barrier to rotation from the planar amide form and, on the other hand, from severe steric interference.

As with all polymers, the flexibility increases with increasing degree of polymerization (DP). From light-scattering measurements,[21] a persistence length of 500-600 Å (*i.e.*, 250-300 residues) is computed, while dipole moment measurements[17] suggest that there is a persistence of 800 monomer units. On the basis of the appearance of a linear term in the electric-field dependence of ED, Milstein and Charney[23] concluded that the onset of flexibility occurs at a DP of ~1000. From all of these measurements, it is seen that PBIC is quite rigid, similar to α-helical poly(α-amino acids).[24-26]

From the ED studies of PBIC reported here, we obtain information about its flexibility and about the polarization properties of its ultraviolet electronic transitions. In order to analyze and interpret the ED data, we first discuss some aspects of the theory of ED as it applies to rigid rodlike polymers in static electric fields. The ED technique will be applied to poly(α-amino acids) in papers III[27] and IV.[28]

I. Theory of ED in a Static Field

(A) Dipole Orientation of Monodispersed Particles. When a system of rigid monodispersed molecules with a permanent dipole moment $\boldsymbol{\mu}$ is placed in a static electric field, the molecules become preferentially oriented with their dipole moments in the direction of the field, with a specific distribution of orientations about the field direction. The resulting ED will depend on the magnitudes of $\boldsymbol{\mu}$ and $\boldsymbol{\mu}_{0i}$ and on the relative orientation of $\boldsymbol{\mu}_{0i}$ to $\boldsymbol{\mu}$. If $(\mu_{0i})_{\parallel}$ and $(\mu_{0i})_{\perp}$ are the components of $\boldsymbol{\mu}_{0i}$ parallel and perpendicular to **E**, respectively, and both components are perpendicular to **k**, the light-propagation vector, the contribution of the given molecule to the ED is $(\mu_{0i})_{\parallel}{}^2 - (\mu_{0i})_{\perp}{}^2$, since the extinction coefficient depends on the square of the transition moment. An expression for the observed ED,[10,29] $\epsilon_{\parallel} - \epsilon_{\perp}$, is derived by averaging $(\mu_{0i})_{\parallel}{}^2 - (\mu_{0i})_{\perp}{}^2$ over all orientations (Figure 2), weighted by the Boltzmann factor, in which the interaction energy U is

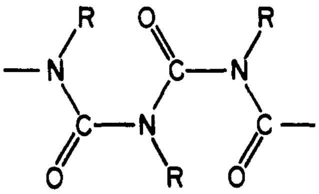

Figure 1. Alternating cis-trans planar form of PBIC.[22]

$$U = -\mu E \cos\gamma \tag{1}$$

The result is

$$\langle(\mu_{0i})_{\parallel}{}^2 - (\mu_{0i})_{\perp}{}^2\rangle = \mu_{0i}{}^2 f(x) g(\xi_{0i}) \tag{2}$$

where the orientation factor is

$$f(x) = 1 - (3/x)\coth x + 3/x^2;\ x = \mu E/kT \tag{3a}$$

$$= \frac{1}{15}\left[x^2 - \frac{2}{21}x^4 + \frac{1}{105}x^6 - \ldots\right] \text{for } x < \pi \tag{3b}$$

$$f(\infty) = 1$$

and

$$g(\xi_{0i}) = (3\cos^2\xi_{0i} - 1)/2 \tag{4}$$

$\langle\ldots\rangle$ indicates the average, k is the Boltzmann constant, T is the absolute temperature, and ξ_{0i} is defined in the legend of Figure 2. If $\epsilon_{E=0}$ is the molar extinction coefficient in the absence of the electric field, then the observed ED is

$$\frac{\epsilon_{\parallel} - \epsilon_{\perp}}{\epsilon_{E=0}} = \frac{\langle(\mu_{0i})_{\parallel}{}^2 - (\mu_{0i})_{\perp}{}^2\rangle}{\langle\mu_{0i}{}^2\rangle_{E=0}} \tag{5}$$

since

$$\epsilon_a \propto \langle(\mu_{0i})_a{}^2\rangle \tag{6}$$

where ϵ_a is the molar extinction coefficient along a principal axis of the *medium*. Since

$$\langle\mu_{0i}{}^2\rangle_{E=0} = \mu_{0i}{}^2/3 \tag{7}$$

for an isotropic medium (because the light is polarized in only one direction, whereas the $\boldsymbol{\mu}_{0i}$'s are isotropically distributed), the combination of eq 2, 5, and 7 leads to

$$\epsilon_{\parallel} - \epsilon_{\perp} = 3f(x)g(\xi_{0i})\epsilon_{E=0} \tag{8}$$

for a single transition $0 \rightarrow i$. Parenthetically, it might be noted that, for a dichroic medium in an electric field

$$\epsilon_{E=0} = (\epsilon_{\parallel} + 2\epsilon_{\perp})/3 \tag{9}$$

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According to eq 22 of paper I,[5] the relation between the linear dichroic rotation at 45°, $(\alpha_E)_{45°}$, and $\epsilon_\parallel - \epsilon_\perp$ is

$$(\alpha_E)_{45°} = 3.3 \times 10^4(\epsilon_\parallel - \epsilon_\perp)lc \quad (10)$$

where $(\alpha_E)_{45°}$ is in millidegrees, and l and c are path length and molar concentration, respectively. Thus, eq 8 and 10 yield

$$(\alpha_E)_{45°} = 9.9 \times 10^4 f(x)g(\xi_{0i})\text{abs}_{E=0} \quad (11)$$

where the isotropic absorbance is

$$\text{abs}_{E=0} = \epsilon_{E=0}lc = -\log T \quad (12)$$

with T being the transmittance.

According to eq 3b and 11, for a weakly oriented system, $(\alpha_E)_{45°}$ has the form

$$(\alpha_E)_{45°} = A_0E^2 + A_1E^4 + A_2E^6 \quad (13)$$

where E is expressed in statvolts per centimeter, and μ and g are related to the coefficients by the following equations

$$\mu = 3.24kT(-A_1/A_0)^{1/2} \quad (14)$$

$$g = 1.515 \times 10^{-4}(kT/\mu)^2(A_0/\text{abs}_{E=0}) \quad (15)$$

According to eq 4, g varies between +1 at $\xi = 0°$ and -0.5 at $\xi = 90°$ and is zero at $\xi = 54.7°$ (or, alternatively, $g = 0$ for a random distribution of $\boldsymbol{\mu}_{0i}$'s relative to $\boldsymbol{\mu}$). From eq 4 we see that

$$|g(90°)| = g(0°)/2 \quad (16)$$

This behavior arises because rotation around the long axis of the rod does not change the energy of its interaction with the electric field. For example, in a completely oriented sample, the components of the $\boldsymbol{\mu}_{0i}$'s perpendicular to $\boldsymbol{\mu}$ are isotropically distributed in the plane perpendicular to $\boldsymbol{\mu}$, while only those components perpendicular to **k** can contribute to $\epsilon_\perp$. On the other hand, the components of the $\boldsymbol{\mu}_{0i}$'s parallel to $\boldsymbol{\mu}$ lie along one axis, and therefore they all contribute to $\epsilon_\parallel$. Further, for a rigid helical molecule, $\boldsymbol{\mu}$ lies along the helix axis, since the components of $\boldsymbol{\mu}$ which are perpendicular to the helix axis (from all monomer units of the polymer) cancel; in this case, ξ_{0i} is also the angle between $\boldsymbol{\mu}_{0i}$ and the direction of the helix axis.

In a dielectric, the internal electric field, $\mathbf{E}_{\text{int}}$, in a cavity formed by removing the solute molecule, is the orienting field rather than **E**. The difference between $\mathbf{E}_{\text{int}}$ and **E** depends on the dielectric constant, D, of the solvent and on the geometry of the cavity. For a homogeneous applied field, the internal field of an ellipsoidal cavity is uniform, and has the components[30]

$$\begin{aligned}(E_{\text{int}})_1 &= a_1E_1 = a_1E\cos\gamma \\ (E_{\text{int}})_2 &= a_2E_2 \\ (E_{\text{int}})_3 &= a_3E_3\end{aligned} \quad (17)$$

where 1, 2, and 3 are the directions of the principal axes of the ellipsoidal cavity and γ is the angle between E and the major axis of the ellipisod, analogous to the same quantities for a helical molecule shown in Figure 2. If $\boldsymbol{\mu}_0$, the dipole moment of the molecule *in vacuo*, lies along one of the principal axes (*e.g.*, the 1 axis), then eq 1 must be replaced by

$$U = -\mu_0a_1E\cos\gamma \quad (18)$$

so that x of eq 3a becomes

$$x = \mu E/kT = \mu_0a_1E/kT \quad (19)$$

i.e.

$$\mu = \mu_0a_1 \quad (20)$$

where μ is now an apparent dipole moment. For a spherical cavity, a_1 has the simple form

$$a_1 = 3D/(1 + 2D) \quad (21)$$

according to Onsager.[31] If the cavity is a very thin prolate ellipsoid, then[30] $a_1 \rightarrow 1$, so that $\mu = \mu_0$. Thus, the cavity field has no effect on $f(x)$ for a long thin cylinder (which is equivalent to a very thin prolate ellipsoid) if μ_0 is parallel to the axis of the cylinder (the case of interest here). However, because of the existence of a reaction field, μ may differ from μ_0, and this effect is independent of the application of an external field. The field due to the dipole $\boldsymbol{\mu}_0$ at a few ångströms from the solute molecule is extremely high (~10^7 V/cm).[32] Consequently, there is a preferential orientation of the solvent molecules by the electric field of the dipole $\boldsymbol{\mu}_0$ (the field of the dipole $\boldsymbol{\mu}_0$ also induces a dipole moment in the solvent molecules[8]). If there is no external electric field, and if the solute molecule is removed from the cavity, the average field in the cavity is zero. If we now insert the solute into the cavity, allow the solvent molecules to orient, and then again remove the solute (but this time freezing the solvent molecules in their preferentially average oriented positions), then there will exist a very large field, the reaction field $\mathbf{E}_R$, in the cavity arising from the orientation of the neighboring solvent dipoles (induced dipoles also contribute a large field) caused by the field of $\boldsymbol{\mu}_0$. $\mathbf{E}_R$ is parallel to $\boldsymbol{\mu}_0$ and its magnitude depends on that of $\boldsymbol{\mu}_0$, on D, and on the cavity size and geometry.[8] The field $\mathbf{E}_R$ will induce an additional dipole moment in the solute, which is parallel to $\boldsymbol{\mu}_0$. Thus, even if the solvent does not induce a conformational change in the solute, the dipole moment of the latter will depend on the nature of the solvent. For an array of dipoles arranged along the cylinder axis of a long thin cylindrical cavity of radius 6.0 Å, Wada[33] computed μ/μ_0 to be 1.2 for $D = 2$ (*e.g.*, in cyclohexane) and 1.45 for $D = 10$ (*e.g.*, in ethylene dichloride). Thus, the departure of μ from μ_0 would be expected to be greater the greater is the dielectric constant of the solvent. Finally, it should be noted that μ may differ from μ_0 if *bound* dipolar solvent molecules contribute to the dipole moment of the solute. Fortunately, g may be obtained (from eq 15) without the necessity of evaluating μ_0; all that is required is μ.

(B) Effect of Anisotropic Polarizability on the Orientation. In the derivation of eq 11, we assumed that orientation was due only to the interaction of the permanent dipole moment with the electric field, and have thus far neglected the contribution from the anisotropic polarizability.[10,29,34] If α_1, α_2, and α_3 are the polarizabilities along the principal axes (Figure 2), and α_2 is assumed to be equal to α_3, then this contribution to the interaction energy is[29]

$$U = -(1/2)(\alpha_1 - \alpha_2)E^2\cos^2\gamma \quad (22)$$

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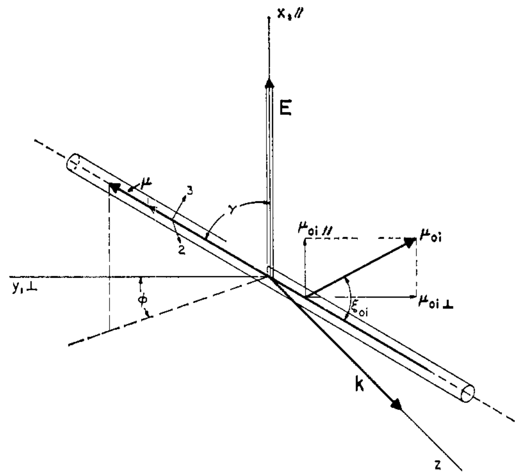

Figure 2. Representation of a molecule in an electric field E. The right-handed set of axes, x, y, z, is chosen so that x is in the direction of the electric field and z is in the direction of k. γ and ϕ are the polar and azimuthal angles, respectively, representing the orientation of the molecule in the given coordinate system. Since the molecule is assumed to be a rigid helix, $\boldsymbol{\mu}$ lies along the helix axis. However, $\boldsymbol{\mu}_{0i}$ need not intersect the helix axis. ξ_{0i} is the angle between $\boldsymbol{\mu}_{0i}$ and a line parallel to $\boldsymbol{\mu}$. The directions of the principal polarizabilities of the molecule are represented by 1, 2, and 3.

For weakly oriented samples, the combined effect of the dipole moment and the polarizability leads to the following modified form of eq 3b[29]

$$f = \frac{1}{15}\left[\frac{\alpha_1 - \alpha_2}{kT} + \left(\frac{\mu}{kT}\right)^2\right]E^2 + \frac{2}{315}\left[\frac{1}{2}\left(\frac{\alpha_1 - \alpha_2}{kT}\right)^2 + \left(\frac{\mu}{kT}\right)^2\left(\frac{\alpha_1 - \alpha_2}{kT}\right) - \left(\frac{\mu}{kT}\right)^4\right]E^4 + \ldots \quad (23)$$

For a helix with a DP of 300, with $\mu = 2$ D/residue and $\alpha_1 - \alpha_2 = 2 \times 10^{-23}$ cm^3/residue, $(\alpha_1 - \alpha_2)/kT < 0.001 \cdot (\mu/kT)^2$ at 300°K. Thus, the contribution to the orientation from the anisotropic polarizability is negligible. However, in conducting solutions, the anisotropic polarizability of the ion atmosphere could be quite large. In this case, eq 14 and 15 would give erroneously low values of μ and high values of g, because of the effect of $(\alpha_1 - \alpha_2)$ on A_0 and A_1 of eq 13. But this problem is of no concern to us, since our experiments are restricted to nonconducting solutions (in a static field, which would rid the solution of spurious ionic impurities) and nondissociating solutes.[35–37]

(C) Overlapping Transitions. Absorption spectra are often quite complex. In a single electronic band, sometimes vibronic transitions within the band can have different ξ values. Also, several electronic bands often overlap. Additional complexities in the spectra of polymers arise from the interaction between chromophores, leading to hypo- and hyperchromism and exciton splitting. When transitions of different ξ values appear at a given wavelength, λ, then g will be an average over these, *viz.*

$$\langle g_\lambda \rangle = \left(\sum_i g(\xi_{0i})[\epsilon_{0i,E=0}]_\lambda\right)/\epsilon_{\lambda,E=0} \quad (24)$$

Since $g(\xi_{0i})$ may be positive or negative, such averaging tends to decrease g. For the same reason, an ED spectrum is useful in resolving a complicated absorption spectrum into its component transitions, in the same way that a CD spectrum helps in resolving an absorption spectrum.

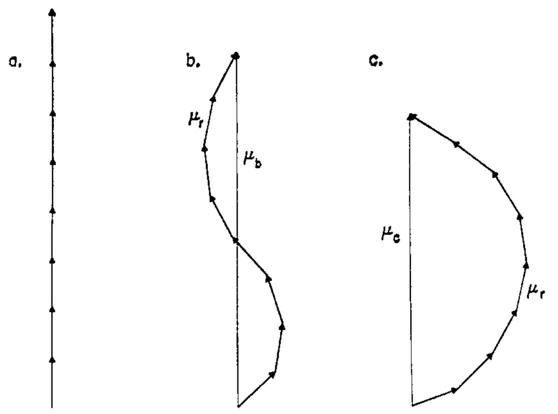

Figure 3. Illustration of the effect of slight polymer chain flexibility on $\boldsymbol{\mu}$ and g, with eight residues shown. $\boldsymbol{\mu}_{0i}$ is assumed to be parallel to $\boldsymbol{\mu}_r$ and to the backbone: (a) rigid rod, $\boldsymbol{\mu}_a = 8\boldsymbol{\mu}_r$ and $g = 1$; (b) $\boldsymbol{\mu}_b$ arbitrarily taken as $0.90\boldsymbol{\mu}_a$, $g = 0.70$, which is an average value for the arbitrarily selected ξ's; (c) $\boldsymbol{\mu}_c$ taken as $0.74\boldsymbol{\mu}_a$, $g = 0.38$.

(D) Effect of Backbone Flexibility. Even rigid polymers like PBIC exhibit backbone flexibility if the chain length is great enough. Examples of the effect of flexible backbones on $\boldsymbol{\mu}$ and g are illustrated in Figure 3, where $\boldsymbol{\mu}_r$ is the dipole moment in each residue. In examples b and c, the flexible cases, the net dipole moments are 0.90 and 0.74, respectively, of the value for the rigid molecule in case a. However, g differs more dramatically, being 0.70 and 0.38 for a case where $\boldsymbol{\mu}_{0i}$ is assumed to be parallel to the helix axis. Thus, qualitatively, we see that ED is much more sensitive to backbone flexibility than is an ordinary dipole moment measurement. To obtain a quantitative estimate of the effect of such flexibility on ED, one must take an average, as follows. For each conformational species, k, in an ensemble of flexible molecules, g_k is computed (as in the examples in Figure 3); then the average value $\langle fg \rangle$ will be given by

$$\langle fg \rangle = \sum_k g_k f_k q_k \quad (25)$$

where q_k is the probability of occurrence of conformational species k.

For a weakly oriented sample, eq 25 still leads to a dependence of $(\alpha_E)_{45°}$ on E as obtained in eq 13, but with coefficients A_0, A_1, and A_2 which reflect the appropriate averaging. For the limiting case of a freely jointed chain, the ED is reduced dramatically below its value for a rigid rod, but not to zero.

When the backbone is flexible, the internal field problem is more complicated, and conceivably there could arise a deviation from the form of eq 13. However, no such devia-

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(35) Even for nonelectrolyte solutes such as poly(γ-benzyl L-glutamate) (PBLG), the contribution from possibly charged α-amino and α-carboxyl groups to μ must be considered. In nonpolar solvents, such "end charges" would probably consist of ion pairs rather than full charges; the contribution of such ion pairs at the ends to μ is, of course, much smaller than that which would arise if these groups were fully charged. Furthermore, in PBLG, the α-carboxyl end group would be uncharged in a medium of low dielectric constant,[36] leaving at most an ion pair at the α-amino end.[37] Finally, if the polymer were prepared by primary or secondary amine-initiated polymerization of an N-carboxyanhydride, the amine would be covalently bound to the α-carboxyl group, thereby precluding the existence of a charged α-carboxyl group.

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TABLE I

AVERAGE VALUES OF g AND μ FOR A FREELY JOINTED CHAIN OF TWO MONOMER UNITS, WITH $\boldsymbol{\mu}_r$ AND $\boldsymbol{\mu}_{0i}$ ALONG THE BACKBONE

Method of measurement	Av value[f] of g	Av value[f] of μ
ED at very high field[a]	0.25	
ED at low field, together with independent measurement of dielectric constant[b]	0.50	
ED at moderate fields[c]	0.30	
Dielectric constant[d]		$2^{1/2}\mu_r$
ED at moderate fields[e]		$1.82\mu_r$

[a] At very high fields, we assume that $f \rightarrow 1$ (and also that the field does not change the distribution of conformations). Under these conditions, the observed value of $(\alpha_E)_{45°}$ gives $\langle g\rangle$ directly, according to eq 11 and 25. [b] At low field we obtain A_0, from which $\langle g\mu^2\rangle$ is calculated using eq 3b, 11, 13, and 25. Then, using the value of $\langle\mu^2\rangle$ from the dielectric constant measurement, an average value of g is calculated as $\langle g\mu^2\rangle/\langle\mu^2\rangle$. [c] At moderate fields, we obtain both A_0 and A_1. According to eq 3b, 11, 13, and 25, this gives the average value of g as $\langle g\mu^2\rangle^2/\langle g\mu^4\rangle$. [d] By conventional procedures, this gives $\langle\mu^2\rangle^{1/2}$ directly. [e] From the values of A_0 and A_1, we obtain the average value of μ as $[\langle g\mu^4\rangle/g\mu^2\rangle]^{1/2}$, according to eq 3b, 11, 13, and 25. [f] The averages $\langle g\rangle$, $\langle g\mu^2\rangle$, $\langle g\mu^4\rangle$, and $\langle\mu^2\rangle$ were calculated from

$$\langle g^m\mu^n\rangle = \int_0^\pi [(g_1 + g_2)/2]^m\mu^n \sin\theta\, d\theta \Bigg/ \int_0^\pi \sin\theta\, d\theta$$

where $m = 0, 1$; $n = 0, 2, 4$; $g_1 = g_2 = [3\cos^2(\theta/2) - 1]/2$; $\mu = 2\mu_r\cos(\theta/2)$; and $\theta = (180 - \text{bond angle})$, the bond angle being the angle between residue dipole moments in this case.

tion is observed,[27,28,38] even in the case of PBIC in a range of DP where the molecule exhibits flexibility.

The effect of backbone flexibility on ED can be illustrated more quantitatively by considering a freely jointed chain consisting of only two monomer units, with $\boldsymbol{\mu}_r$ and $\boldsymbol{\mu}_{0i}$ assumed to be parallel to each other (and both lying along the rodlike backbone of each residue). For a rigid rod, the values of g and μ would be expected to be 1.0 and $2\mu_r$, respectively. However, by averaging over all conformations of the freely jointed chain (see Table I), the values of g and μ are lower than those expected for a rigid rod; the lowerings would be expected to be very much greater for a high-DP polymer (for the same reason that the root-mean-square end-to-end distance of a polymer is reduced below the value for a rigid rod, more dramatically for high DP than for low DP). In addition, however, we find that the average values of g and μ vary, depending on how they are measured; this behavior arises when the magnitude of the net dipole moment varies from conformation to conformation, and the flexibility thus introduces a heterogeneity in the dipole moment. However, in the molecular weight region in which a polymer shows only slight flexibility, then the function $W(\mu_r)$, which describes the probability of occurrence of a particular magnitude of the net dipole moment on a residue basis, will be sharply peaked only slightly below the value for the completely rigid polymer; in this case, the average values of μ and g will be independent of the method of measurement, since there would be essentially no heterogeneity in the dipole moment.

(E) Effect of Aggregation. If the molecule exists as an aggregate in the given solvent (independent of the field strength), we can also observe the same effects (including the internal field problem) as discussed in section ID. However, for PBIC, there is no aggregation[17] at the concentrations used in our ED measurements.

Besides aggregation, there are two types of concentration-dependent polymer–polymer interactions, which may affect the observed ED. These are hydrodynamic interactions in which the rotating macromolecules collide with each other and electrostatic interactions arising from the interactions of neighboring dipoles. While the simple foregoing theory does not take such interactions into account, they can be reduced or eliminated by carrying out the measurements at very low concentration, as is possible with our method;[5] in the range of concentrations of our measurements, Bur and Roberts[17] observed little dependence of the relaxation time for rotation on concentration.

It should be noted that flexibility, aggregation, and backbone and side-chain conformations may themselves depend on the field strength. However, since the external fields used in the measurements are much lower than local electric fields (10^7 V/cm),[32] these effects are rarely observed. Nevertheless, Gregson, *et al.*,[39] reported a field-induced aggregation of PBLG, and Schwarz and Seelig[40] observed a significant effect of the electric field on the helix content of PBLG.

(F) Effect of Side-Chain Mobility. In an α helix such as poly(γ-benzyl L-glutamate) (PBLG), the backbone is quite rigid, but the side chains may be mobile. Since the absorption process occurs much more rapidly than internal rotation of the side chain, the observed ED represents an average over the set of equilibrium conformations, *i.e.*, ED measures a steady-state conformational property in contrast to a method such as fluorescence depolarization which measures a dynamic conformational property.

For an isolated electronic transition in a side-chain chromophore in a helical macromolecule containing no side-chain dipole moment

$$\langle g\rangle = \sum_j g_j p_j \qquad (26)$$

where g_j is $g(\xi_{0i})$ in the side chain when it is in the jth conformation, which has the probability p_j of occurring. Because of the strong dependence of g on ξ (see eq 4), mobility of the side chains tends to yield low values of $\langle g\rangle$; since the average value of $(3\cos^2\xi - 1)$ is zero for the chromophores of a completely flexible side chain, $\langle g\rangle$ would be zero in such a case.

If the side chain contains a dipole moment, μ_r(sc), then mobility of the side chain will lead to a distribution of values of μ_{tot}, and different methods of measurement will lead to different average values $\langle g\rangle$ and $\langle\mu\rangle$, as in the case of the flexible backbone (see Table I). However, as demonstrated below, a unique average value of g is obtained at high DP, *i.e.*, for DP $\gtrsim$ 50. Consider the extreme case of completely flexible side chains, in which μ_r(sc) can take on all orientations with equal probability. In this case, the distribution function $W(\mu_r)$ will be symmetric around $\mu_r(\text{bb}_\parallel)$, the component of the residue backbone dipole moment parallel to the helix axis (to avoid the necessity of having to treat an asymmetric distribution function, we assume that $\mu_r(\text{sc}) \leq \mu_r(\text{bb}_\parallel)$; the final conclusions are the same, at sufficiently high DP, if $\mu_r(\text{bb}_\parallel) \neq 0$). By averaging over this distribution, it is found that $\langle\mu_r^2\rangle^{1/2} > \mu_r(\text{bb}_\parallel)$ if the DP is low, since the half-width of $W(\mu_r)$ is comparable to $\mu_r(\text{bb}_\parallel)$. However, as the DP be-

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(38) See section III of this paper.

(39) M. Gregson, G. P. Jones, and M. Davies, *Chem. Phys. Lett.*, **6**, 215 (1970).

(40) G. Schwarz and J. Seelig, *Biopolymers*, **6**, 1263 (1968).

TABLE II
PROPERTIES OF PBIC SAMPLES[a] INVESTIGATED

Sample	M_w[b] × 10^{-4}	$\langle\mu^2\rangle_B$ × 10^{-6}, D^2	Log τ	$(W/2)$,[c] decades	M_w/M_n[d]	$[DP_z/DP_w]^{1/2}$[e]	$\langle\mu_2\rangle_{obsd}$,[f] D
1	2.0	0.051	−6.40	1.75		1.063	339
2	3.5	0.153	−5.70	1.60		1.049	579
3	5.1	0.313	−5.35	1.70		1.058	834
4	6.2	0.496	−4.8	1.90	1.19	1.072	1065
5	9.1	0.852	−4.6			1.063	1384
6	23.5	2.47	−3.50	1.75	1.17	1.063	2357

[a] Samples were a gift from Dr. A. J. Bur, who also gave us all of these data[17,44,45] except those in the last two columns. [b] M_w = weight-average molecular weight; uncertainty 5%. [c] Error = ±0.05 decade. [d] M_w/M_n is from light-scattering and osmotic pressure measurements; uncertainty is 10%. [e] Estimated $[DP_z/DP_w]^{1/2}$; see section III. [f] Calculated from $\langle\mu^2\rangle_B$; see section III.

comes large, the half-width of $W(\mu_r)$ becomes $\ll \mu_r(bb_{\|})$, so that $W(\mu_r)$ becomes sharply peaked at $\mu_r(bb_{\|})$. This occurs because a greater proportion of the macromolecules have freely jointed side chains distributed so that the side-chain dipole moments average to approximately zero. As expected from the fact that $W(\mu_r) \ll \mu_r(bb_{\|})$, it is found that $\langle\mu_r^2\rangle^{1/2} \simeq \mu_r(bb_{\|})$ for large DP, *i.e.*, at DP $\gtrsim$ 50.[33] In other words, all macromolecules in the system have essentially the same net dipole moment. As a result, the average values of g and μ will be independent of the method of measurement, and the average value of μ will be (DP)[$\mu_r(bb_{\|})$]. By a similar analysis of a polymer with side chains of intermediate flexibility, the same results would be obtained, except that the average value of μ would be (DP)[$\mu_r(bb_{\|}) + \langle\mu_r(sc)\rangle$]. Thus, at a high enough DP, one will obtain a unique value of g, which is represented by the average given in eq 26.

While the above simple theoretical considerations show that $W(\mu_r)$ becomes a narrow, sharply peaked function at high DP, there is also some experimental evidence to support this idea. Tsvetkov, *et al.*,[41] have measured the electrical birefringence (EB) of PBLG of DP ~ 420 (which most likely has flexible side chains[27]) in ethylene dichloride (plus 1% dimethylformamide) as a function of the frequency of the orienting electric field, in the range from 0 to 150 kHz. They found that the low-frequency value of the EB fell essentially to zero at high frequency. The low-frequency EB arises mainly from orientation due to $\mu_{\|}$, whereas, at high frequency where the rodlike molecule can no longer follow the alternation of the field, the contribution from $\mu_{\|}$ vanishes and the EB from orientation due to $\mu_{\perp}$ would be observed. Thus, we can conclude that, for high-DP PBLG, $\langle\mu_{\perp}^2\rangle^{1/2} \ll \langle\mu_{\|}^2\rangle^{1/2}$. We would expect to find many molecules with significant values of $\mu_{\perp}$, if $W(\mu_r)$ were a broad function, and *vice versa* for a narrow function $W(\mu_r)$. Thus, the EB experiment indicates that $W(\mu_r)$ is narrow, as deduced theoretically.

(G) Effect of Polydispersity. Since the dipole moment $\boldsymbol{\mu}$ depends on the DP, molecular weight polydispersity will affect the dependence of the orientation factor $f(x)$ on E. In order to determine this effect, f must be averaged over the molecular weight distribution.

$$f = \sum_l h_l f_l \tag{27}$$

where h_l is the residue mole fraction of molecular weight species l. If we consider a sample of a monodispersed polymer, having a DP equal to DP_w (the weight-average DP) of a sample of a polydispersed polymer, then the curves for the dependence of f on E for the two samples will cross; specifically, it is found (by averaging the f's over the distribution at each value of E, for the polydispersed sample) that the slope df/dE is steeper for the polydispersed sample at low values of E, but then decreases at high values of E, requiring higher fields to attain the limiting (saturation) value. This same behavior, in which the longer molecules orient more easily at low fields, with the shorter ones requiring higher fields for orientation, appears in other orientation-type experiments, *e.g.*, in flow birefringence in polydispersed systems.[42] Thus, eq 14 and 15 must be modified to take account of the polydispersity.

If eq 3b is substituted for f_l in eq 27, then the analogs of eq 14 and 15 for a polydispersed system become

$$\mu_2 = 3.24kT\left[\frac{DP_2}{DP_4}\right]^2\left[-\frac{A_1}{A_0}\right]^{1/2};\ \mu_w = \mu_2\left[\frac{DP_w}{DP_2}\right] \tag{28}$$

$$g = 1.515 \times 10^{-4}\left[\frac{A_0}{\mathrm{abs}_{E=0}}\right]\left[\frac{kT}{\mu_2}\right]^2 \tag{29}$$

where $\mu_2 = DP_2(\mu_r)_{\|}$, $\mu_w = DP_w(\mu_r)_{\|}$, and

$$\begin{aligned} DP_2 &= \left(\sum_l h_l DP_l^2\right)^{1/2} \\ DP_4 &= \left(\sum_l h_l DP_l^4\right)^{1/4} \\ DP_n &= \frac{1}{\Sigma h_l/DP_l} \\ DP_w &= \Sigma h_l DP_l \\ DP_z &= \frac{\Sigma h_l DP_l^2}{\Sigma h_l DP_l} \end{aligned} \tag{30}$$

where DP_n, DP_w, and DP_z are the number-, weight-, and z-average DP's, respectively. From eq 30, it can be seen that

$$DP_2 = (DP_z DP_w)^{1/2} \tag{31}$$

and that

$$DP_4 > DP_z > DP_2 > DP_w > DP_n \tag{32}$$

and

$$DP_z/DP_w < DP_w/DP_n \tag{33}$$

For a PBLG sample, Scoffone, *et al.*,[43] found that DP_w/DP_n = 1.25 (which is a fairly narrow distribution). From data on *fractions* from this sample,[43] the polydispersity corrections can be shown to be 0.78 for μ_w and 1.42 for g. This example shows that the polydispersity corrections must be taken into account, even for narrow molecular weight distributions.

II. Experimental Section

(A) Materials. Well-fractionated, characterized PBIC samples (Table II) were the kind gift of Dr. A. J. Bur.[44] The preparation,

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[41] V. N. Tsvetkov, I. N. Shtennikova, Y. I. Ryumtsev, and V. S. Skazka, *Polym. Sci. USSR*, 7, 1231 (1965).

[42] H. A. Scheraga, *J. Chem. Phys.*, 19, 983 (1951).

[43] E. Scoffone, E. Peggion, A. Consani, and M. Terbojevich, *Biopolymers*, 3, 535 (1965).

[44] A. J. Bur, Polymers Division, National Bureau of Standards.

fractionation, and characterization of the samples were described by Bur, *et al.*[16,17,45] The monomer was polymerized in a vacuum system at $-78°$ in toluene solution, using *n*-butyllithium as the initiator and, usually, methanol as a terminator. After purification by reprecipitation, the polymer was fractionated using CCl_4 as the solvent and methanol as the precipitant, thereby reducing DP_w/DP_n from ~4.5 to ~1.2 for the samples used here. The weight- and number-average molecular weights, used in computing this ratio, were determined from light-scattering and osmotic pressure measurements, respectively. The average square of the dipole moment $\langle\mu^2\rangle_B$, the relaxation time τ, and the half-width $W/2$ of the curve of dielectric loss *vs.* log frequency were obtained from dielectric constant measurements on PBIC in CCl_4 solutions. Once calibrated, τ was found to be a sensitive measure of DP_w; $W/2$ was found to be a measure of the polydispersity. Further details of the properties of PBIC may be found in the papers of Bur, *et al.*[16,17,45]

All solvents used in the ED measurements were spectroquality. Solutions were prepared by agitation either at room or elevated (50–75°) temperatures, after which they were sometimes clarified by passage through a 5-μ Teflon Millipore filter. The solutions were subsequently diluted to the appropriate concentration range for ED measurements; sometimes they were then heated above 50° to accelerate dispersal of any aggregates that may have been present in the more concentrated stock solution.

(B) Determination of Concentration. In ED measurements, $\text{abs}_{E=0}$ rather than absolute concentration is required in order to determine A_0 and A_1, and hence μ and g. However, it was desirable to have a rough measure of the concentration in order to be sure that it was low enough to avoid aggregation (which could be assessed by a comparison with Bur's results). The absorbance at various wavelengths λ was obtained with a Cary Model 14 spectrophotometer. The extinction coefficient was determined by carrying out micro-Kjeldahl nitrogen analyses on aliquots of the solution whose absorption spectrum was measured. Because of the small amounts of samples available, it was not possible to obtain a precision of better than 10% in the nitrogen content (and hence in ϵ), the (molar) residue extinction coefficient being ϵ_{249} 4700. The range of optical densities (OD) used in the ED field-dependence measurements was 0.35–1.00, which corresponds to <0.1 mg/ml in CCl_4 and <0.022 mg/ml in cyclohexane (C_6H_{12}) (since measurements were made at different wavelengths in the two solvents). Since Bur and Roberts[17] found that the value of τ at 0.1 mg/ml differed very little from its "infinite dilution" value in CCl_4, and we obtained consistent results in repeated ED measurements (at $\lesssim 0.1$ mg/ml), we may conclude that there is no aggregation below 0.1 mg/ml.

(C) Apparatus and Measurement. The ED apparatus is described in the previous paper.[5] Like those of Bur,[17] all measurements were made at room temperature (which was not controlled). A relatively narrow spectral half-band width of 1.0 mμ was used for all measurements.

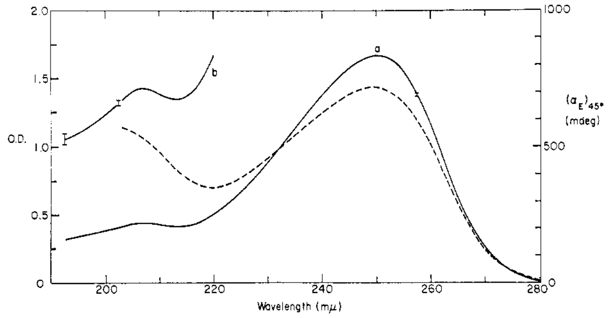

Figure 4. Optical density of sample 4 of Table II (at ~0.030 mg/ml) in the absence of electric field (– – –), and ED spectrum of sample 3 of Table II (at OD_{250} = 0.69, which corresponds to ~0.015 mg/ml) at (a) 45 and (b) 90 statvolts/cm, respectively (——), of PBIC in cyclohexane (θ = 45° and path length = 1 cm); in the ED spectrum, 8 mdeg was subtracted at all wavelengths to correct for the EB artifact discussed in paper I.[5]

Since prolonged exposure to the external electric field might lead to precipitation of the polymer, the absence of such precipitation was assured, not only by visual observation, but also by the absence of any detectable decrease in $(\alpha_E)_{45°}$ during the measurements. However, short-time variations in $(\alpha_E)_{45°}$, due to ohmic heating, were observed (see section III). Further, the absorption spectrum of PBIC remained unchanged after carrying out the ED measurements.

The data for the dependence of $(\alpha°)_{45°}$ on E were fit to the equation

$$(\alpha_E)_{45°} = A_0E^2 + A_1E^4 \tag{34}$$

neglecting the E^6 term of eq 13, by a least-squares program, using a PDP-8/S computer, where $(\alpha_E)_{45°}$ is the change in the rotation of the major axis of the slightly elliptical output polarized light induced by the electric field, **E**, when the direction of propagation of the light is perpendicular to **E** and the direction of polarization is at 45° with respect to **E**.

III. Results

As shown in the preceding paper,[5] a test that α_E arises from ED is its dependence on sin 2θ in an absorbing region, where θ is the angle between the applied electric field and the plane of polarization of the output light beam. The ED data for PBIC followed a sin 2θ dependence very well, both at the wavelengths 207 and 270 mμ in the absorbing (but weakly dichroic) spectral regions and also in the strongly dichroic region of 249 mμ. Further, at 300 mμ, a nonabsorbing region, typical measurement of the dependence of α_E on θ gave an average value of $|\alpha_E|$ which was 2.5% of $(\alpha_E)_{45°}$ at 270 mμ. Thus, the EB artifact, discussed in paper I,[5] is negligible in these measurements (but was nevertheless corrected for—see legend of Figure 4). We conclude from the dependence of the ED on θ that the major contribution to $(\alpha_E)_{45°}$ for PBIC is ED. Also, since the value of $(\alpha_E)_{45°}$ outside the absorption bands was negligibly small, we infer that there is no significant amount of form dichroism[5] in these measurements.

The absorption and ED spectra of PBIC in C_6H_{12} are shown in Figure 4. In the absorption spectrum, λ_{max} is 249 ± 1 mμ for the main band, and is shifted to longer wavelengths by ~2 mμ in CCl_4 and ~5 mμ in $CHCl_3$.[23] In the far-ultraviolet, there is a second band with λ_{max} <203 mμ which could not be observed in absorption measurements, probably because the polymer samples contained slight amounts of absorbing solvent impurities.

However, the ultraviolet-absorbing solvent impurities did not interfere with the ED measurements since their orientation in the electric field is negligible compared to that of the polymer. Thus, the ED spectrum was determined to 192.5 mμ in C_6H_{12}. Even though the ED spectrum may in general contain both positive and negative peaks, the ED for PBIC is positive for λ >192.5 mμ. Of the two ED bands, the dominant one is associated with the skewed absorption band at 249 mμ. The shape of this ED band is similar to the absorption band in the same spectral region, except for the slightly more rapid decrease of $(\alpha_E)_{45°}$ on the low-wavelength side of the maximum.

The second ED band at ~207 mμ is very weak, and much of the rotation $(\alpha_E)_{45°}$ in this spectral region may arise from the short-wavelength tail of the 249-mμ ED band. This 249-mμ tail is also probably the cause of the shift of the low-wavelength ED band (which appears at <203 mμ in the absorption spectrum) to 207 mμ. Nevertheless, Figure 4 shows that the ED from the <203-mμ band is positive. Using an

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(45) A. J. Bur, *J. Chem. Phys.*, 52, 3813 (1970).

TABLE III
WAVELENGTH DEPENDENCE OF g OF PBIC

λ, mμ	$g_{\text{obsd},\lambda}$	% of $g_{\text{obsd},250}$
207	0.096	36
225	0.20	75
250	0.27	100
270	0.25	94

oriented film of PBIC, Rosenheck[46] has observed similar absorption and LD spectra.

The relative values of $g_{\text{obsd},\lambda}$ at several wavelengths are given in the third column of Table III. These were computed from the dependence of OD and $(\alpha_E)_{45°}$ on λ, given in Figure 4, using eq 11 with $f(x)$ being the same at all wavelengths. Since only relative values were computed, the different concentrations for the two curves of Figure 4 did not matter. It can be seen that g passes through a maximum at 250 mμ. From the theory of ED, we would expect g (and ξ) to remain constant in an absorption band in which the magnitude and direction of $\boldsymbol{\mu}_{0i}$ did not vary. We believe that $g_{\text{obsd},207}$ is slightly lower than $g_{\text{obsd},250}$ because of spectral errors on the long-wavelength side of the 249-mμ band, arising from the fact that both the OD and $(\alpha_E)_{45°}$ are very small. The lower value of $g_{\text{obsd},225}$ compared to $g_{\text{obsd},250}$ is probably due to overlap with the weak <203-mμ ED band, and possibly to a slight variation of the magnitude and/or direction of $\boldsymbol{\mu}_{0i}$ within the 249-mμ band. The much lower value of $g_{\text{obsd},207}$ compared to $g_{\text{obsd},250}$, of course, arises from the fact that they both pertain to different transitions.

The dependence of $(\alpha_E)_{45°}$ on E was measured at 250 mμ in C_6H_{12}, at 270 mμ in CCl_4, and at 277.4 mμ in benzene (higher wavelengths were used for the latter two solvents because of their high absorbance at lower wavelengths). Unfortunately, it was not possible to obtain μ from eq 14 because of time-dependent changes in $(\alpha_E)_{45°}$, designated $\Delta\alpha_E(t)$, probably due to ohmic heating. $\Delta\alpha_E(t)$ rapidly reached a steady-state value as an equilibrium temperature gradient was established in the ED cell. At the highest fields, $\Delta\alpha_E(t)$ ranged from -7 to $+10\%$ of $(\alpha_E)_{45°}$ in different experiments, but the relative magnitude of $\Delta\alpha_E(t)$ compared to $(\alpha_E)_{45°}$ was the same at all wavelengths. Since ED $\approx E^2$ (in the range of E used in our experiments) and the power output in ohmic heating is $\propto E^2$, we expect $\Delta\alpha_E(t)$ to be $\approx E^4$. Hence, according to eq 13, the heating should affect the coefficient A_1, and not A_0. Since A_1 is required for the evaluation of μ (see eq 14), an accurate value of μ could not be obtained. Even though the least-squares fit of α_E/E^2 *vs.* E^2 was quite good, with a standard deviation in A_0 of <2%, A_1 (as expected) varied significantly in different experiments with the same sample. It should also be noted that the contribution of the A_1E^4 term to $(\alpha_E)_{45°}$ at low fields is a minor one; hence, the computed value of A_1 normally will be quite sensitive to errors in $(\alpha_E)_{45°}$.

But, from the above discussion, A_0 would be expected to be insensitive to $\Delta\alpha_E(t)$. We can demonstrate this as follows. From eq 29

$$(\mu_2{}^2g)_{\text{obsd},\lambda} = [\text{DP}_2(\mu_r)_{\|}]^2 g_{\text{obsd},\lambda} = 1.515 \times 10^{-4}\left[\frac{A_0}{\text{abs}_{E=0}}\right](kT)^2 \quad (35)$$

Using eq 35, $(\mu_2{}^2g)_{\text{obsd},\lambda}$ was computed from two to four experiments on each sample in CCl_4 and C_6H_{12}. The measurements in C_6H_{12}, made at 250 mμ, were converted to values at 270 mμ (for comparison with the measurements in CCl_4), using the data of column 3 of Table III. The resulting values of $(\mu_2{}^2g)_{\text{obsd},270}$ from these experiments agreed to within an average deviation of ±2% for all samples except sample 5, for which the deviation was ~4%; this demonstrates that $(\mu_2{}^2g)_{\text{obsd},\lambda}$ and consequently A_0 are fairly insensitive to heating effects, and may be used (see below) to compute $g_{\text{obsd},\lambda}$ if an independent value of μ is available. (For sample 4 in benzene, $(\mu_2{}^2g)_{277.4}$ was ~15% lower than for the other samples, probably because of errors arising from the fact that 277.4 mμ is at the tail of the absorption band.) Also, because the value of $(\mu_2{}^2g)_{\text{obsd},\lambda}$ is independent of solvent, we may conclude that the conformation of PBIC is essentially the same in CCl_4, C_6H_{12}, and benzene.

If we use the values of $(\mu_2{}^2g)_{\text{obsd},\lambda}$ computed from ED at room temperature with eq 35, and the values of μ obtained by Bur (see Table II) from dielectric constant measurements in CCl_4 at room temperature, we can compute the value of $g_{\text{obsd},270}$. For this purpose, the values of $\langle\mu^2\rangle_B$ of Table II must first be converted to the root-mean-square values μ_2, since the two differ because of an internal field correction and the polydispersity. Bur and Roberts[17] corrected the apparent solution dipole moment to the vacuum value by using the Lorentz correction for the internal field which, at infinite polymer dilution, is

$$\mathbf{E}_{\text{int}} = [(D_\infty + 2)/3]\mathbf{E} \quad (36)$$

where D_∞ is the high-frequency dielectric constant of the solution. On the other hand, this correction is not required in calculating g since the *apparent* dipole moment appears in the ED equations. Therefore, the internal-field correction term must be removed from the dipole moments of Table II by multiplying $\langle\mu^2\rangle_B{}^{1/2}$ by the factor $(D_\infty + 2)/3$, which is 1.411, using Bur's value of $D_\infty = 2.232$ for CCl_4.

As far as the polydispersity is concerned, $(\mu_2{}^2g)_{\text{obsd},\lambda}$ depends on $\text{DP}_2{}^2$, which is DP_zDP_w according to eq 31. On the other hand, $\langle\mu^2\rangle$ in eq 8 of Bur and Roberts[17] is

$$\langle\mu^2\rangle = \text{DP}_w\text{DP}_n\langle\mu_{r\|}{}^2\rangle \quad (37)$$

However, Bur and Roberts[17] have assumed that $M_w \approx M_n$ and, therefore, have effectively multiplied both sides of eq 37 by DP_w/DP_n to obtain

$$\langle\mu^2\rangle_B = \langle\mu^2\rangle(\text{DP}_w/\text{DP}_n) = \text{DP}_w{}^2\langle\mu_{r\|}{}^2\rangle \quad (38)$$

Thus, $\langle\mu^2\rangle_B{}^{1/2}$ is a weight-average dipole moment, and these values must be multiplied by $(\text{DP}_z/\text{DP}_w)^{1/2}$ to obtain $\mu_{2,\text{obsd}}$, the average dipole moment required in eq 35. Values of DP_w/DP_n for the samples of Table II were obtained from Bur's plot[45] of $W/2$ *vs.* M_w/M_n. Then DP_z/DP_w was estimated from DP_w/DP_n, using the inequality of eq 33 and the observation that $\text{DP}_z/\text{DP}_w = 1.15$ when $\text{DP}_w/\text{DP}_n = 1.25$ for a sample of PBLG.[43] The estimated values of $(\text{DP}_z/\text{DP}_w)^{1/2}$ are given in Table II, with the value for sample 5 being assumed, since no polydispersity data (*i.e.*, no values of $W/2$) were available for it.

While there are errors in this polydispersity correction (arising from errors in the original DP_w/DP_n data and from the estimates in applying the inequality of eq 33 to PBLG data to obtain a correction for PBIC), these are not serious since the fractions of PBIC used here have a low enough degree of polydispersity for $(\text{DP}_z/\text{DP}_w)^{1/2}$ to be close to unity. However, the polydispersity correction itself cannot be ignored since it leads to an increase of ~13% in $\mu_{2,\text{obsd}}{}^2$, the quantity required for the computation of g_{obsd}.

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(46) K. Rosenheck, personal communication.

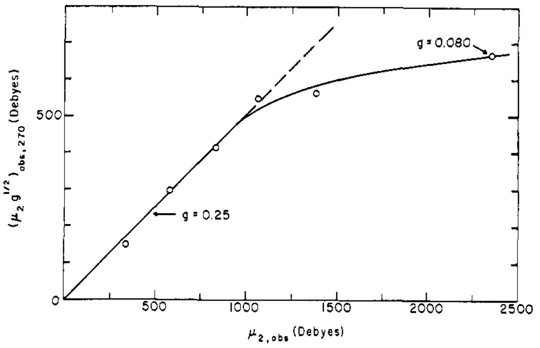

Figure 5. Dependence of ED of PBIC on $\mu_{2,\text{obsd}}$ (*i.e.*, on molecular weight), as reflected in $g_{\text{obsd},270}$.

Overall, taking into account the corrections for the internal field and for polydispersity, the computed values of $\mu_{2,\text{obsd}}$, given in the last column of Table II, are about 50% greater than Bur's values of $\langle\mu^2\rangle_B{}^{1/2}$.

We may use the values of $(\mu_2 g^{1/2})_{\text{obsd},270}$ *vs.* DP (or, equally well, *vs.* $\mu_{2,\text{obsd}}$) as a measure of the dependence of the flexibility of PBIC on DP. In Figure 5, $(\mu_2 g^{1/2})_{\text{obsd},270}$ is plotted against $\mu_{2,\text{obsd}}$. The curve is initially linear, as expected for a rigid-rod-like polymer. From the slope of this linear portion, a value of $g_{\text{obsd},270} = 0.25 \pm 0.02$ is computed for the rigid molecule. According to the data of Table III, this corresponds to a maximum value of g_{obsd} of 0.27 (at 250 mμ) and a value of $\xi_{\max} = 44.3°$. Considering all the errors in the experiment, the uncertainty in $\xi_{\max}$ is $\sim 1.5°$.

Using the relative values of $g_{\text{obsd},250}$ in Table III, $g_{\text{obsd},207}$ is found to be 0.096. Because of the significant overlap with the 249-mμ band, the g value for the isolated <203-mμ transition is probably <0.096, but nevertheless >0. Thus, $51.0° < \epsilon < 54.7°$ for the <203-mμ transition.

The deviation from linearity (in Figure 5) at $\mu_{2,\text{obsd}} \sim 1100$ D (or $\text{DP}_w \sim 600$) reflects the onset of flexibility, at least as it affects the value of g_{obsd}. At $\mu_{2,\text{obsd}} \sim 2360$ D (or $\text{DP}_w \sim 2370$), $g_{\text{obsd},270}$ has fallen to 0.080. Thus, while the samples studied encompass a 12-fold range of molecular weight, $(\mu_2 g^{1/2})_{\text{obsd},270}$ changes only by $\sim$4.5-fold due to the flexibility-induced decreases in μ_r of $\sim$40% (from Bur's data of Table II) and in g of $\sim$65%. As indicated in section ID, g is more sensitive than μ to backbone flexibility.

IV. Discussion

Since $(\mu_2{}^2 g)_{\text{obsd}}$ is obtainable from A_0, and the latter can be measured with good precision ($\pm$<2%), the quantity $(\mu_2{}^2 g)_{\text{obsd}}$ may be used both to determine the DP and to investigate the flexibility of a polymer. Once $(\mu_2{}^2 g)_{\text{obsd}}{}^{1/2}$ is known for one DP for a rigid-rod-like molecule, the DP's of other samples can be determined from the measured values of $(\mu_2{}^2 g)_{\text{obsd}}{}^{1/2}$, since the latter is linear in DP. Even for a flexible molecule, where $(\mu_2{}^2 g)_{\text{obsd}}{}^{1/2}$ is no longer linear in DP, a calibration curve can be obtained from measurements of $(\mu_2{}^2 g)_{\text{obsd}}{}^{1/2}$ at several values of DP, and then used to determine the DP of additional samples from the measured values of $(\mu_2{}^2 g)_{\text{obsd}}{}^{1/2}$, in much the same way that a viscosity–DP relation is used to provide DP's from viscosity measurements.

We have seen that both μ_2 and g are theoretically sensitive to flexibility, g more so than μ. The sensitivity of g to flexibility is demonstrated experimentally in Figure 5. Hence, the product $\mu_2{}^2 g$ is even more sensitive to flexibility. While our primary interest in this paper was the determination of g in the range of DP where the polymer behaves like a rigid rod, nevertheless the availability of several high-DP samples enabled us to conclude that the PBIC molecule exhibits significant flexibility beyond DP $\sim$ 600, a value which is intermediate to those cited by others.[17,21,23]

We have found that the theoretical field dependence of $(\alpha_E)_{45°}$, given by eq 34, describes the experimental behavior of PBIC over the whole range of DP. In agreement with this, Tsvetkov, *et al.*,[18] also obtained the theoretical field dependence of electric birefringence (which is the same as that of ED) of PBIC in the high-DP range where the molecules are flexible. In contrast, Milstein and Charney[23] resorted to a linear term in the field strength in the analysis of their ED data on *high*-DP PBIC, and Charney, *et al.*,[11] similarly used a linear term in the analysis of their ED data on PBLG in ethylene dichloride and in dioxane; in PBLG, the linear term was found[11] to be the major contribution to the ED, even for DP's well within the range in which the molecule behaves like a rigid rod according to dielectric constant studies.[33] We have also studied the ED of PBLG in dioxane,[27] using DP's equal to and higher than some of those of Charney, *et al.*,[11] and find that the data can be fit by the theoretical field dependence of eq 34, without the linear term. Electric birefringence measurements[29,34] also obey eq 34, without the linear term, for samples of PBLG of similar DP. It thus appears that eq 34, without a linear term, describes the ED of these polymers. In further support of our analysis, we note that the coefficient of the E^2 term, in the analysis of Milstein and Charney in the region of rigid behavior of PBIC (which is the region of low molecular weight, where the linear term vanished, according to their data), varies very little with DP, in disagreement with theoretical predictions and our observations.

Concerning the validity of the use of the dipole moments of Bur and Roberts[17] to obtain values of $g_{\text{obsd},\lambda}$ we note that Tsvetkov, *et al.*,[18] have also determined dipole moments on two samples of PBIC (but from EB measurements), and obtained much lower values of $\langle\mu_{r\parallel}{}^2\rangle$ than those found by Bur and Roberts.[17] Since μ appears in eq 35 as a squared term, it is important to have reliable values of μ for computing g. However, we can cite two possible causes for the low values of Tsvetkov, *et al.* First, their samples were unfractionated and, from the results of Yu, *et al.*,[16] polydispersity leads to much lower values of $\langle\mu_{r\parallel}{}^2\rangle$ at a given DP_w because the polydispersed sample probably contains a significant amount of higher DP PBIC, which is flexible. Second, dipole moments determined by dielectric constant measurements[17] are more accurate than those obtained from EB measurements;[18] as in ED experiments, in EB measurements it is difficult to separate the contribution of μ from that of g. Thus, since the dielectric constant measurements[17] were performed on numerous sharply fractionated samples, with precise measurements made throughout essentially the entire frequency range over which the PBIC molecules contributed to the dielectric loss, and since the identical PBIC fractions were used in our ED studies, we feel that the dipole moments of Bur and Roberts[17] are accurate and valid for use as described in section III.

It is important to note that, in contrast to poly(amino acids) (which may have mobile side-chain chromophores), the chromophores of PBIC are located in the rigid backbone (for DP < 600). Hence, we do not encounter the problem of (side chain) mobility in this polymer, and therefore do not need to average over many conformations in order to interpret the observed g values. Thus, the computed values of ξ represent the true directions of the transition dipole moments relative to the helix axis, since no averaging is involved.

From our ED results, the values of ε for PBIC in C_6H_{12} and CCl_4 are ~44 and ~53° for the 249- and <203-mμ absorption bands, respectively, and we must now interpret these ξ values (and also the absence of negative ED in the spectrum) in terms of the structure of PBIC.

For this interpretation, we will consider three special cases for the origin of the electronic transitions. (1) In the first case, it is assumed that there is electronic delocalization over the whole macromolecule because of significant electron orbital overlap from the partial double bond character of the N–(CO) bonds. (2) In the second case, it is assumed that there is no significant overlap of the orbitals of adjacent nitrogen and carbonyl groups [*i.e.*, no partial double bond character in the N–(CO) bonds], but that there is strong exciton coupling between neighboring identical chromophores. (3) In the third case, it is assumed that there is neither significant electronic delocalization nor exciton coupling, *i.e.*, the chain behaves as a collection of essentially independent chromophores. We will show that our ED results on PBIC permit us to eliminate the first two cases as possibilities for describing the electronic character of this polymer.

Since PBIC is helical,[17,20] then, if the electronic system were delocalized over this helix (case 1), the electronic states would have the space group symmetry of a helix. In this case, group theory shows that the only allowed electric dipole transitions are those which are parallel and perpendicular to the helix axis,[47] for which g would be +1.0 and −0.5, respectively. Similarly, strong exciton coupling (case 2) would result in the same transition polarizations. Experimentally, we observe no negative dichroism (ruling out a possible g value of −0.5) and a maximum positive dichroism corresponding to a g value of 0.27 (*i.e.*, much less than 1.0) in an isolated band; these observations enable us to eliminate the special cases 1 and 2 as characterizing the electronic structure of PBIC.

In considering the possibility that PBIC behaves according to case 3 (*i.e.*, the polarization directions would be those of an independent residue of the chain), we examine whether the directions of the transition dipoles correspond to particular directions of groups in the monomeric residue in the helix. For this purpose, we first must know the coordinates of the various atoms of a residue in the helical conformation. To obtain these, we have computed the empirical potential energy surface of poly(methyl isocyanate) to locate the structure of minimum energy, using methods developed in this laboratory for similar computations on polypeptides.[48] In the calculation, two successive dihedral angles, ω_1 and ω_2, were taken as the independent variables, and this pair was assumed to have the same values in every residue; the dihedral angle, χ, for rotation of the side-chain methyl group was maintained fixed at 0° (which is a side-chain conformation in which a CH of the methyl group is cis to the C–N corresponding to ω_1) in every residue. The new convention for polypeptides[49] was used in these calculations, where $\omega_1 = \omega_2 = 180°$ corresponds to the all-trans conformation shown in Figure 6. The lowest energy structures [with $(\omega_1, \omega_2) = (140°, -20°)$ and $(-140°, 20°)$] are near the alternating cis–trans one (the same energy for both the left- and right-handed helical forms) shown in Figure 1 (and referred to as a cis structure in ref 15 and 20), and are similar to that obtained from an analysis of X-ray data on PBIC.[20] Table IV provides a comparison of the computed structure for the methyl derivative with the X-ray structure of the butyl derivative, as well as the angles, θ, between various interatomic directions and the helix axis in the computed minimum-energy structure.

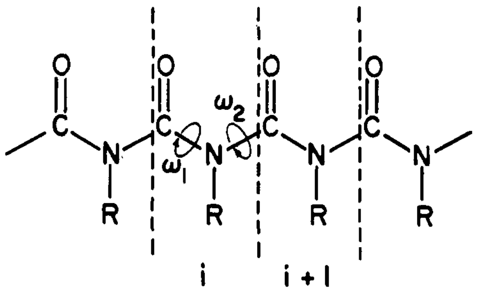

Figure 6. All-trans conformation of poly(methyl isocyanate) which corresponds to $\omega_1 = \omega_2 = 180°$.[49]

From Table IV, we see that the θ values nearest to our observed polarization directions (44 and 53°) are 37 (direction 2) and 58.5° (direction 1); the discrepancy between observed and calculated values (about 6°) may arise from three sources, *viz.;* (1) the use of amide geometry and potential energy (and maintenance of a constant value of χ) in the conformational energy calculations; (2) the computed values for the methyl derivative may vary slightly from those that would have been obtained if the computations had been carried out for the butyl derivative; and (3) even though a transition may lie primarily along a specific interatomic direction, a transition may be polarized in a slightly different direction from the interatomic directions of Table IV because of the complexity of the electronic system of PBIC. Thus, in attempting to identify polarization directions with inter-

Table IV

Computed Values of θ for the Interatomic Directions of the Minimum-Energy Structures of Poly(methyl isocyanate), with $(\omega_1, \omega_2) = (140°, -20°)$ and $(-140°, 20°)$

Interatomic direction	θ, deg
(1) C_i—O_i	58.5
(2) (a) N_i—N_{i+1}	37
(b) C_i—C_{i+1}	
(c) In plane of $\genfrac{}{}{0pt}{}{N\diagdown}{N\diagup}C{=}O$ and perpendicular to C═O	
(3) N_i—O_i	88
(4) N_i—O_{i+1}	32
(5) Perpendicular to plane of $\genfrac{}{}{0pt}{}{N\diagdown}{N\diagup}C{=}O$	72
(6) N_i—C_i	65
(7) N_i—C_{i+1}	18

Helix parameters	Methyl derivative (calcd)	Butyl derivative (X-ray[a])
Translation/residue, Å	1.82	1.94
Rotation/residue, deg	141	135

[a] Reference 20.

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(47) B. Fanconi, B. Tomlinson, L. A. Nafie, W. Small, and W. L. Peticolas, *J. Chem. Phys.*, **51**, 3993 (1969).

(48) H. A. Scheraga, *Advan. Phys. Org. Chem.*, **6**, 103 (1968); *Chem. Rev.*, 71, 195 (1971).

(49) IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970).

atomic directions, we should make allowances for discrepancies of a few degrees. In conclusion, for the model of isolated chromophores, reasonable molecular polarization directions are: primarily parallel to the C=O group for the $<$203-mμ transition, and primarily in the plane of the N—C=O group and perpendicular to the C=O bond for the 249-mμ band.

In attempting to assign these transitions, the unusual structure of this molecule immediately poses a problem, *viz.*, what group should be considered as the isolated chromophore. If the carbonyl group is taken as the isolated chromophore, then the polarization directions for its $\pi \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions compare favorably[50] with those of PBIC (θ = 58.5 and 37°, respectively); however, since these transitions occur below 200 mμ in the isolated carbonyl, they have too high an energy[51] to enable us to identify them with the two transitions observed here in PBIC. The $n \rightarrow \pi^*$ transition of the isolated carbonyl has the correct energy, but it is a forbidden transition.[51] On the other hand, this transition in PBIC could become sufficiently allowed (but with an unpredictable polarization direction) by interaction of the C=O group with the neighboring nitrogens.[52,53] Alternatively, the planar amide-like chromophore might be considered as another model for PBIC. First of all, it is difficult to accept this model since the amide groups would be expected to lead to delocalized electronic structures, as in peptides, whereas there does not appear to be a significant amount of delocalization in PBIC (the amide group in this polymer not being planar). Further, whereas the $\pi_1 \rightarrow \pi^*$ (NV$_1$) transition of a planar amide has an energy and extinction coefficient which are similar to those of PBIC, the intensity decreases as the amide departs from planarity and the partial double bond character of the C–N bond decreases;[53] on the other hand, PBIC has a reasonably high extinction coefficient at 249 mμ. For these reasons, we cannot definitely assign the transitions of PBIC to those of the isolated carbonyl or planar amide groups at this time.

Since the three special cases considered above represent extremes of behavior, it is conceivable that there may be some intermediate degree of electron delocalization. As the orbital overlap increases, there is probably a sharp transition from the localized to the delocalized behavior, with the transitions sharply changing into parallel and perpendicular polarizations over a narrow range of change of the degree of overlap. In such a situation, one might expect to observe large g values with both positive and negative signs, with rapidly varying polarization directions within the complex overlapping transitions. However, this is not observed in the ED spectrum of PBIC, *i.e.*, g is positive and varies very little with wavelength.

The possibility also exists that there might be some intermediate degree of exciton splitting. The spectral effects to be expected in such a case have been considered theoretically by Simpson and Peterson.[54] According to this theory, the $\pi_1 \rightarrow \pi^*$ transition in α-helical polypeptides would be an illustration of intermediate splitting. In this case, a distinct shoulder (appearing about 15 mμ above the absorption peak) arises because of the splitting.[55] The LD varies rapidly, shifting from strongly positive in the high-wavelength predominantly parallel polarization region to strongly negative in the low-wavelength mostly perpendicular polarization region of the band.[56,57] Exciton-induced CD effects and nonidentical oscillator interaction-induced hypochromism accompany these changes. Since the PBIC absorption and ED spectra do not show such behavior, it is unlikely that intermediate exciton splitting is the origin of the ED spectrum.

In addition to overlap with the $<$203-mμ band, there are three possible causes for the slow decrease in g on the low-wavelength side of the 249-mμ band (see Table III). (1) First of all, it could be due to weak exciton coupling,[58] according to the scheme of Simpson and Peterson.[54] (2) Secondly, there could be a weak band of different polarization located at ~230 mμ, beneath the main band. (3) There could be a slight variation of the transition direction within the residue band. Goodman and Chen[26] observed a change in sign of the CD at 234 mμ in optically active polyisocyanates in CHCl$_3$; in poly[(S)-(+)-2-methylbutyl isocyanate], which has no side-chain chromophore, the maximum molar ellipticity (occurring at 253 mμ) is $[\theta] = +13{,}000$. We do not know whether alternative 1 or 3 can account for this CD behavior; however, alternative 2 seems like a reasonable possibility.

For completeness we should mention that there might exist an interaction between nonidentical oscillators which could produce changes in intensity (*i.e.*, hypo- or hyperchromism). These changes in intensity could be accompanied by changes in the direction of the transition moment, *i.e.*, ξ could change. It is not possible to detect the hypo- or hyperchromism from such possible interactions in PBIC, since any attempt to disrupt these interactions would require a change of conformation, *i.e.*, a variation of ω_1 and ω_2 which, in turn, would also alter the intensity, direction of polarization, and wavelength maximum of the transition.[53] Also, in order to bring about a conformational change in PBIC, strong acids are required;[17,21] but these are thought to protonate the backbone,[26] and hence would lead to additional changes in spectrum besides those arising from the desired disruption of the interaction between nonidentical oscillators. It is quite possible that the interaction potential between nonidentical oscillators is too weak to produce significant changes in intensity, since we have already ruled out the possibility of strong or intermediate exciton coupling between identical oscillators.

Since the most consistent interpretation of the electronic structure of PBIC from our ED and absorption data is essentially that of a localized electronic system without exciton effects, it is of interest to consider the spectra of model compounds. Those of *N,N′*-dimethylurea,[59] acetylurea,[60] hydantoin,[60] biuret,[61] barbital,[62] and cyanuric acid[62] do not exhibit peaks in the wavelength region greater than 220 mμ but exhibit only the tails of lower wavelength absorption bands in the region beyond 220 mμ. Although these are

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(50) E. E. Barnes and W. T. Simpson, *J. Chem. Phys.*, **39**, 670 (1963).

(51) H. Suzuki, "Electronic Absorption Spectra and Geometry of Organic Molecules," Academic Press, New York, N. Y., 1967, p 430.

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

(53) J. F. Yan, F. A. Momany, R. Hoffmann, and H. A. Scheraga, *J. Phys. Chem.*, **74**, 420 (1970).

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(57) R. Mandel and G. Holzwarth, *Rev. Sci. Instrum.*, **41**, 755 (1970).

(58) It cannot be due to strong exciton coupling for reasons already cited, and it cannot be due to intermediate coupling for two reasons, *viz.*, (a) because the variation of g with wavelength is small, and (b) because there are no shoulders on the 249-mμ absorption band.

(59) A. Clow and N. L. Helmrich, *Trans. Faraday Soc.*, **36**, 685 (1940).

(60) R. E. Stuckey, *J. Chem. Soc.*, 331 (1947)

(61) Data obtained by us on Fisher Scientific Certified (assay 99.5%) biuret in methanol.

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small molecules, several can have slightly nonplanar amide groups (as deduced from an examination of space-filling models) as in PBIC. Since electron delocalization effects and exciton effects do not seem to be significant in PBIC, we would have expected some of these low-DP model compounds to have similar optical properties as PBIC. The reason seems to be (again, from an examination of space-filling molecular models) that these low-DP model compounds lack one important feature of the PBIC structure, which may account for the lack of similarity of their spectra and that of PBIC. Because of the helical structure of PBIC, the chain is folded in such a manner that the lone-pair electrons of N_{i+1} are close to the C=O group of the *i*th residue shown in Figure 6. Such a conformation might give rise to a new transition on forming the helix, especially since nitrogen–carbonyl transitions (from remote groups in the chain) have been observed previously; for example, Leonard and Oki[63] have observed an apparent charge-transfer transition from the nitrogen lone pair to the antibonding π orbital of the carbonyl group in 6-methyl-6-aza-2-hydroxycyclononanone, with log $\epsilon_{\max} = 3.77$ at 228 mμ.

Certainly, more investigation is required before the <203- and 249-mμ bands can be assigned to specific transitions in the electronically complicated PBIC molecule. Perhaps the molecular orbital calculations on models of PBIC, which Hoffmann[64] is currently carrying out, can show us the origin of the spectral properties, and also whether a charge-transfer transition contributes to the spectrum. Clearly, the quantitative ED polarization data provide important new evidence on the spectral properties of PBIC, which should be a helpful guide in theoretical investigations.

V. Conclusion

A study of the molecular weight dependence of the ED of PBIC has shown that the molecule behaves as a rigid rod up to DP $\sim$ 600. The use of ED to detect the onset of flexibility has been demonstrated.

Three striking features of the ED spectrum are (1) the absence of negative dichroism, (2) the small variation in g with wavelength, and (3) the observation that $g_{\max} = 0.27$. These lead to the following conclusions: (1) the residual double bond character of the N—C bond, which is nonplanar in PBIC, is too weak to cause electron delocalization over the whole polymer; (2) there appears to be at most weak exciton coupling in the spectral region studied; (3) the polarization directions 53 and 44° for the <203- and 249-mμ bands, respectively, seem to correspond to transitions which are primarily polarized along the C=O bond, and in the plane of the N—C=O group but perpendicular to the C=O bond, respectively. Although enough information is not available to be able to make definite assignments for these transitions, it would be worth considering the possibility of a nonneighboring nitrogen–carbonyl transition.

Acknowledgment. We are indebted to Dr. A. J. Bur for the gift of the fractionated PBIC samples, and to Drs. R. F. McGuire and F. A. Momany for carrying out the conformational energy calculations on PBIC.

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Laser Raman Spectroscopy of Polypeptides. I. Water-Soluble Block Copolymers of L-Alanine and D,L-Lysine[1]

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ABSTRACT: Laser Raman spectra of block copolymers of L-alanine and D,L-lysine in the solid state, in salt-free water, and in aqueous salt solutions were obtained. Under all these conditions, the spectra were sensitive to the conformational states of the polymers. In addition, the spectra in aqueous salt solutions indicated the presence of interactions not present in salt-free water and were consistent with an earlier suggestion that, in the presence of salt, the shielding of the charges of the poly(D,L-lysine) end blocks permits the central poly(L-alanine) block to adopt a hairpin-like conformation (with the α helices folded back and forth) which is stabilized by side-chain to side-chain methyl–methyl hydrophobic bonding.

In the continuing search for experimental methods to provide information about protein conformation in solution, attention has turned recently to laser Raman spectroscopy of solutions of polypeptides. This technique has been applied, for example, to monomeric L-alanine and L-lysine[3] in water, to oligomers of alanine[4] in the solid state and in water, and to homopolymers of L-lysine[5,6] and of L-alanine.[7,8] The interpretation of the results from such experimental work was aided materially by theoretical normal-coordinate analyses on poly(L-alanine) by Miyazawa, *et al.*,[9] and by the publication of polarized Raman spectra of poly(L-alanine) by Peticolas, *et al.*[7]

These investigations are of direct applicability to our studies of various water-soluble block copolymers[10–12] to elucidate

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