## (IV) Conclusions

Water-soluble random copolymers containing L-leucine and either $N^5$-(3-hydroxypropyl)-L-glutamine or $N^5$-(4-hydroxybutyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transitions of these copolymers, the Zimm–Bragg parameters $\sigma$ and $s$ for poly(L-leucine) were deduced. The values for $s$ show that L-leucine is a strong helix former, stronger than L-alanine. Comparison with earlier experiments using the block copolymer method indicates similar quantitative results and shows that the state of a residue in a polypeptide or protein, in first approximation, is essentially independent of the chemical nature of its neighbors. The greater stability of the $\alpha$-helical form of poly(L-leucine) compared to those of poly(L-alanine) and polyglycine has been attributed to hydrophobic interactions.

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# Dipole Moments and the Conformations of Polypeptides. I. Some Compounds Related to Poly($\beta$-benzyl L-aspartate)[1]

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ABSTRACT: Dipole moment measurements have been made to obtain information about the orientation of polar side chains with respect to the backbone in several $\alpha$-helical poly(amino acids). The polymers studied were poly($\beta$-*o*-, *m*-, and *p*-chlorobenzyl L-aspartate) as well as poly($\beta$-benzyl L-aspartate) and poly($\gamma$-benzyl L-glutamate) in both ethylene dichloride and methylene chloride. Dipole moments per residue and rotational relaxation times (for the whole molecule) were determined by measurement of the dielectric constant and dielectric loss in the frequency range from 2 MHz to 50 Hz. The polymers behave like rigid $\alpha$ helices in both solvents, with the relaxation times being consistent with those expected from an application of the Perrin equation to a molecule having the known geometry of the $\alpha$ helix. All of the polymers were found to have the same dipole moment, 3.9 ± 0.2 D/residue, in both solvents. These results suggest that the side chains are in random orientation (equivalent to freely rotating side chains) with respect to each other.

For many years, synthetic poly($\alpha$-amino acids) have been used as simple models in theoretical and experimental studies of the interactions which determine the three-dimensional conformations of proteins. From a theoretical point of view, conformational energy calculations have provided insight into the factors responsible for the stability of various polypeptide structures, notably the $\alpha$ helix;[3-5] for example, it has been possible to identify the factors which determine whether a given poly(amino acid) will form a right- or left-handed $\alpha$ helix. A variety of experimental techniques has also been used to examine several aspects of the conformations of such polymers; these include optical rotatory dispersion (ORD),[6] circular dichroism (CD),[7] nuclear magnetic resonance (nmr) spectroscopy,[8-11] infrared[12] and laser Raman[13,14] spectroscopy, linear dichroism,[15] and dipole moment measurements.[16,17]

The conformational energy calculations[3-5] provide additional information about the minimum-energy structure, in particular the orientation of the side chains with respect to the $\alpha$-helical backbone. Thus, it is of interest to determine the conformations of the side chains of an $\alpha$-helical polymer experimentally, and the dipole moment study reported here was undertaken for this purpose. This technique is useful because the dipole moments of the peptide groups of an $\alpha$ helix are almost parallel to the helix axis, leading to an extremely large (backbone) dipole moment of the helical polymer. In fact, the existence of this large dipole moment is additional evidence for the presence of a rigid helical structure. The contribution from the backbone dipole moment will be enhanced or diminished, depending on the orientations of the side chains, if the latter also possess a permanent dipole moment. Thus, the overall dipole moment of the $\alpha$-helical polymer provides information about the orientation of the side chains. However, it should be emphasized that the con-

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formation of the side chain must depend on the nature of the solvent; thus, experimental and computed side-chain conformations can be compared only when they both pertain to the same solvent.

This technique had already been used to study the conformations of poly($\gamma$-benzyl L-glutamate) (PBLG)[18–21] and poly(L-tyrosine).[16] In this paper, we present a dipole moment study of a series of compounds related to (left-handed) $\alpha$-helical poly($\beta$-benzyl L-aspartate) (PBLA), *viz.*, the (left-handed) $\alpha$-helical *o*- and *m*-chloro derivatives[22] and the (right-handed) $\alpha$-helical *p*-chloro derivative[22–25] of PBLA. The location of the chlorine atom in three different positions of the benzene ring provides a convenient way to change the direction of the dipole moment of the chlorobenzyl group and also the conformation of the side chain. Since the backbones are the same (except for the helix sense) for all of these compounds, the *relative* values of the measured dipole moments suffice for providing conformational information about the side chains; thus, errors which affect the absolute but not the relative values of the dipole moments will not alter the conclusions.

## Experimental Section

**Materials.** Matheson Coleman and Bell (MCB) Spectroquality ethylene dichloride (1,2-dichloroethane) (EDC) was purified by shaking with activated alumina (MCB, chromatographic grade) and then filtering through a sintered glass disk. Methylene chloride ($CH_2Cl_2$) (MC) was Fisher Spectranalyzed grade, and was used without further purification. The methanol used in the purification of the polymers was Fisher Spectranalyzed grade, and the dioxane used for lyophilization of the polymers was MCB Spectroquality; both were used without further purification. Fisher Certified *N*,*N*-dimethylformamide (DMF) and Aldrich hexamethylphosphoramide (HMPA) were used without further purification for molecular weight determinations.

Poly($\beta$-*o*-, *m*-, and *p*-chlorobenzyl L-aspartates) were prepared by the *N*-carboxyanhydride method, as described previously.[22] Also, the unsubstituted PBLA and PBLG were synthesized by a similar procedure.

For the polymerization of the *o*-, *m*-, and *p*-Cl PBLA's, an anhydride–initiator (A/I) ratio of 50 was used. For the PBLA and PBLG, the A/I ratio was 25. In addition, the polymerization of the *o*-Cl-PBLA was carried out at 40°, rather than at room temperature, so that the increased solubility of the growing chain at this temperature would result in a larger polymer.

The polymers were purified and fractionated by repeated precipitation from ethylene dichloride solution by the addition of methanol. After the final precipitation, the polymers were lyophilized from dioxane. The solutions used for dipole moment and other measurements were prepared by dissolving the lyophilized material in EDC or MC (occasionally with brief immersion in a water bath at ~80°) and passing through Teflon 5-$\mu$ Millipore filters.

**Methods. (1) Polymer Characterization.** Molecular weights of the *o*-, *m*-, and *p*-chloro derivatives were determined in EDC with a Spinco Model E ultracentrifuge, using a double sector or multichannel Kel-F centerpiece in the cell. Conventional sedimentation equilibrium runs were carried out and the data analyzed to yield $\bar{M}_w$, as described by Chervenka.[26] The density $\rho$ of EDC was taken as 1.245 g/ml[27] at 25°. The value of the partial specific volume $\bar{v}$ for PBLA was that of de Loze, *et al.*,[28] by estimating the contribution of a chlorine atom to $\bar{v}$ from the difference in density of benzene[29] and chlorobenzene,[30] a value of $\bar{v} = 0.708$ ml/g at 25° was estimated for the *o*-, *m*-, and *p*-chloro derivatives of PBLA. Rayleigh interference optics were used for the ortho and meta derivatives, but uv absorption optics were used for the lower concentrations required to avoid nonideality effects in the para derivative.

The molecular weight of PBLG was determined in a similar manner, except that DMF was used as the solvent. Values of 0.945 g/ml[31] for the density of DMF at 25° and $\bar{v} = 0.785$ ml/g for PBLG[28] in this solvent were used in the calculation.

The molecular weight of PBLA was determined from its intrinsic viscosity in HMPA at 25° using the intrinsic viscosity–molecular weight relationship of Hayashi, *et al.*,[32] for PBLA in this solvent. This solvent was used because of the low solubility of PBLA in EDC. Recourse was had to viscosity measurements because of the high optical density of HMPA and because of the difficulty encountered in synthetic boundary runs, making ultracentrifuge measurements impossible.

**(2) Concentration Determinations.** The concentrations of solutions used in all experiments were determined either by a micro-Kjeldahl analysis for nitrogen[33] (after evaporation of the solvent) or by measurement of the optical density in the benzyl absorption region, using extinction coefficients which had been obtained from absorption measurements and nitrogen analyses on a stock solution

**(3) Dielectric Measurements.** All dielectric measurements were carried out in an aluminum concentric cylinder cell of air capacitance of approximately 60 pF at 25°. The inner electrode had an outer diameter of 1.1875 in., while the outer electrode had an inner diameter of 1.3125 in., providing a plate spacing of 0.0625 in. The solution height was 3.6875 in., and the cell required ~20 ml of solution to be filled. The cell had stainless-steel filling tubes on top and bottom so that it could be filled and emptied without disassembly. Teflon spacers were used throughout.

The cell was thermostated in an oil bath (Soltrol 200) at 25.00 ± 0.01°. It was calibrated by measuring its capacitance when filled with air, as well as its capacitance when filled with EDC (dielectric constant $\epsilon = 10.36$)[34] or MC ($\epsilon = 8.90$).[35]

In the 100-kHz to 50-Hz range, the capacitance and conductance were measured with a General Radio 1620-A capacitance-measuring assembly consisting of a Type 1615-A transformer ratio-arm bridge, Type 1310-B oscillator, and Type 1232 tuned amplifier and null detector. The sensitivity of the null point detection, especially at low frequencies, was increased by displaying the output from the amplifier/null detector on an oscilloscope. The lead capacitance was minimized by connecting the cell for three-terminal measurements on a two-terminal capacitor.[36] The conductance range of the bridge was increased by a factor of 100 by replacement of the two 100-kohm (R245 and R246)[36] resistors in the "T" network[36] used for the conductance balance. These were replaced with nominal 10-kohm ± 1% film resistors whose values had been accurately measured before being placed in the bridge. The value of

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(27) J. Timmermans, "Physico-Chemical Constants of Pure Organic Compounds," Vol. II, Elsevier, Amsterdam, 1965, p 189.

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(35) Reference 34, p 5.

(36) Instruction Manual for General Radio Type 1620 capacitance-measuring assembly.

the unknown conductance, $G_x$, can be calculated from the equation[36]

$$G_x = \frac{R_N}{R_A R_B + R_N R_A + R_N R_B} \qquad (1)$$

where (in the modified bridge) $R_A = 10.0647$, $R_B = 10.0392$ kohm and $R_N$ is the resistance of the variable resistor which is read from the bridge dials. The conductance range of the bridge was further extended by the addition of an external resistor ($R = 11.482$ kohm) across the "external standard" terminals of the bridge.[36] The net result of the above modification and external resistor is that conductances from 1 to 100 μmho can be measured with the 1:1 ratio of the bridge arms. This is an important point, since high conductances could thus be measured without the use of the 10, 100, or 1000 to 1 ratio of the bridge arms, which would have involved a consequent loss in the accuracy of the capacitance measurement. We estimate that the *absolute* accuracy of a conductance measurement was better than 0.1%, and the relative accuracy (between measurements at similar frequencies and for similar values of conductance) was about 0.02%. This was usually ~0.002 μmho.

Capacitances could be read over most of the frequency range to 0.01 pF, but were reproducible to only ~0.05 pF, *i.e.*, to about 0.01%.

In the 2-MHz to 100-kHz range, the capacitance and conductance were measured with a General Radio Type 716-CS1 capacitance bridge in conjunction with the Type 1310-B oscillator (and oscilloscope as a null detector). The measurements were made by the the direct method,[37] and appropriate corrections were applied for bridge residuals[38] and for the high-frequency impedance due to the inductance and resistance of the leads.[38] Because of the limitations in reading the bridge itself and the high-frequency range used, measurements in the 2-MHz to 100-kHz region were not as accurate as those in the 100-kHz to 50-Hz region. We estimate that, at the higher frequencies, the capacitance was accurate only to ±~0.5 pF (0.1%), and the conductance to ± ~0.5 μmho (~1%).

In cases where data were obtained with both bridges (*i.e.*, at 100 kHz), the agreement was very good.

**(4) Treatment of Data.** The general procedure for the determination of a molecular dipole moment involves the measurement of the dielectric constant $\epsilon'$ of a dilute solution of the compound. To assure adequate time for the molecule to orient in the external field, $\epsilon'$ should be measured at zero frequency; *i.e.*, in a dc field. However, since the polarization effects of a dc field make this extremely difficult, recourse is had to alternating fields. The primary requirement is that the frequency of the ac field be low enough to allow the molecule to rotate so as to align itself in the field (subject to thermal disorder) before the field reverses direction. Thus, the upper frequency which can be used is determined by the rotational relaxation time of the molecule being considered. In the case of a helical, rodlike polymer, whose dipole moment is parallel to the helix axis, that frequency is low, and can be in the kilohertz region or below. It is at such low frequencies that electrode polarization effects become important and lead to imprecise measurements (see discussion of Cole–Cole plot below).

The long rotational relaxation time, however, is an advantage in that it occurs in a frequency region which is accessible to convenient measurement. Thus, it is possible to measure the dielectric constant of the solution at low frequencies, where the rotating molecule has time to align itself in the alternating field, through the dispersion region where the molecule lags behind the field, and almost (depending on molecular weight) to the high-frequency region where the molecule ceases to rotate because the polarity is changing so rapidly.

As the frequency is varied through this dispersion region, the displacement current due to the charging and discharging of the capacitor develops a component in phase with the applied field, resulting in the absorption of energy.

The frequency behavior of the dielectric constant, $\epsilon'$, and dielectric loss, $\epsilon''$, as the absorption is called, was first described quantitatively by Debye.[39] Assuming an exponential decay of the orientation of the molecules when the orienting field is removed, Debye showed that the frequency response of these two quantities is

$$\epsilon' = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{1 + \omega^2\tau^2} \qquad (2)$$

$$\epsilon'' = \frac{(\epsilon_0 - \epsilon_\infty)\omega\tau}{1 + \omega^2\tau^2} \qquad (3)$$

where $\epsilon_0$ and $\epsilon_\infty$ are the zero-frequency and infinite-frequency limits of $\epsilon'$, $\omega$ is the angular frequency (related to $f$, the frequency in hertz, by $\omega = 2\pi f$), and $\tau$ is a constant, corresponding to the rotational relaxation time of the molecule.[40] A related quantity of interest is $\Delta\epsilon'$, the dielectric increment of the solution over that of the solvent (*i.e.*, $\epsilon'_{\text{solution}} - \epsilon'_{\text{solvent}}$), which is regarded as the contribution to $\epsilon'$ from the solute molecules. These relations are illustrated by the solid curves in Figures 1A and 1B. Thus, by varying the frequency through the dispersion region, we can evaluate not only $\epsilon_0$, the quantity of interest, but we also obtain a direct measure of the rotational relaxation time of the molecule.

The quantities $\epsilon'$ and $\epsilon''$ are not directly observable, but must be calculated from the measured capacitance, $C_{\text{measd}}$, and from the measured conductance, $G_{\text{measd}}$

$$C_{\text{measd}} = \epsilon' C_0 + C_D \qquad (4)$$

where $C_0$ is the air capacitance and $C_D$ is the capacitance of the leads of the cell. The quantities $C_0$ and $C_D$ are determined by calibration of the cell with liquids of known dielectric constant. Also

$$G_{\text{measd}} = G_{\text{ac}} + G_{\text{dc}} \qquad (5)$$

where $G_{\text{ac}}$ is the conductance due to dielectric absorption (the quantity of interest) and $G_{\text{dc}}$ represents a zero-frequency conductance due to ionic impurities in the solution. The value of $G_{\text{dc}}$ must be obtained for each solution by observing the low-frequency value of $G_{\text{measd}}$. Then[41]

$$\epsilon'' = G_{\text{ac}}/\omega C_0 \qquad (6)$$

Debye's derivation is based on the assumption that the solute molecule exhibits a single relaxation time; however, the exact behavior predicted by the above equations is rarely observed in practice. Much of the deviation from Debye-like behavior comes from the existence of a distribution of relaxation times due to variations in the environment of the individual molecules. This distribution of relaxation times is observed even for homogeneous solutes, and is still wider for a polymer with a distribution of molecular weights. The net result of this distribution of relaxation times is to broaden the dispersion and absorption curves.[42,43] We assume, as do previous authors,[42,43] that the measured relaxation time for a polydisperse system corresponds to the species of chain length equal to $\overline{\text{DP}}_w$; since our fractions are fairly sharp, this assumption leads to at most a small error.

In principle, it is possible to extrapolate $\Delta\epsilon'$ to zero frequency by a plot of $\Delta\epsilon'$ *vs.* log $f$ (see Figure 1A). In fact, however, this method is very inaccurate because of the electrode polarization

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[37] Instruction Manual for General Radio Type 716-C capacitance bridge.

[38] Supplement to ref 37 for use with Type 716-CS1 capacitance bridge.

[39] P. Debye, "Polar Molecules," Chemical Catalog Co., Inc., reprinted by Dover Publications, New York, N. Y., 1929.

[40] Although these formulas are quite general, *i.e.*, they can be applied to solution or solvent, our measurements are confined to the low-frequency region, well below the dispersion region of the solvent. Therefore $\epsilon'_{\text{solvent}}$ is a constant throughout the experiments described here; $\epsilon'$ with no subscript refers to the measured, frequency-dependent dielectric constant of the solution. $\epsilon_0$ is then the zero-frequency limit of $\epsilon'$. $\epsilon_\infty$ is the "infinite"-frequency value, *i.e.*, the value of $\epsilon'$ at frequencies higher than the dispersion region of the solute (but not of the solvent); therefore, $\epsilon_\infty = \epsilon'_{\text{solvent}}$. Similarly, since our measurements are made below the absorption region of the solvent, $\epsilon''_{\text{solvent}} = 0$ throughout, and $\epsilon''$ will refer to the dielectric loss of the solution (which arises only from the solute molecules).

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effects which become important at low frequencies. When dielectric loss data are available, in addition to the frequency dependence of $\Delta\epsilon'$, the extrapolation can be carried out more precisely by a plot of $\epsilon''$ *vs.* $\Delta\epsilon'$. Cole and Cole[44] showed that, in the case of a single relaxation time, this plot is a semicircle whose center is on the $\Delta\epsilon'$ axis and which intersects the $\Delta\epsilon'$ axis at $\epsilon_\infty$ and $\epsilon_0$. In the case of a symmetrical distribution of relaxation times, the Cole–Cole plot leads to an arc whose center is below the $\Delta\epsilon'$ axis. In certain cases, it has been observed that the arc is not even symmetrical. This behavior has been attributed to the presence of several relaxation mechanisms.[45] In any of the above cases, however, a plot of $\epsilon''$ *vs.* $\Delta\epsilon'$ is of great help in the extrapolation of $\Delta\epsilon'$ to zero frequency; this is the procedure to be adopted here.

In the particular system under study, a problem arises in calculating the dipole moment of the solute from $\Delta\epsilon_0$, the zero-frequency dielectric increment of the solution. Ideally, one should obtain dipole moment data from measurements in the gas phase. If this is not possible, the second choice is to dissolve the solute in a nonpolar solvent. Because of solubility problems, however, dielectric measurements on synthetic poly($\alpha$-amino acids) must be made in relatively polar solvents. Thus, it becomes more difficult to relate the dielectric constant of the solution to the vacuum dipole moment of the solute. Also, since it is impossible to measure the dipole moment of a polymer in the gas phase, there is no reference value against which the solution value can be checked. An examination of the literature on dielectric measurements of rigid polymers reveals that several treatments of the data have been used. Wada[46] derived an expression for the solvent-dependent dipole moment of an asymmetric rod immersed in a polar solvent; he then provided a shape-dependent correction to convert this dipole moment to that for a molecule in vacuum. It appears that Wada himself never applied this correction term to his own experimentally determined values.[18] Sharp[20] applied the treatments of Onsager,[47] Guggenheim,[48] and Kirkwood[49] to his data, and was able to show that all three gave consistent results only if an empirical correction of Buckingham and LeFevre[50] was applied to the dipole moments calculated from either the Onsager or Guggenheim methods.[51] The most detailed treatment for calculating the "vacuum" dipole moment of a solute dissolved in a polar solvent seems to be that presented by Buckingham.[52] The results of this treatment were presented in a modified form by Applequist and Mahr,[16] and applied to their dielectric data for poly(L-tyrosine) dissolved in quinoline. This equation, in the form as modified by Applequist and Mahr, is

$$\frac{4\pi N C\mu^2 fg}{9kTM} = \frac{(2\epsilon_0 + 1)(\epsilon_0 - n^2)}{3(2\epsilon_0 + n^2)} - \frac{(2\epsilon_\infty + 1)(\epsilon_\infty - n^2)}{3(2\epsilon_\infty + n^2)} \qquad (7)$$

where $N$ = Avogadro's number, $c$ = concentration in grams per milliliter, $\mu$ = dipole moment, $k$ = Boltzmann's constant, $T$ = absolute temperature, $M$ = molecular weight of solute, $n$ = refractive index of the solution,[53] $f$ is a constant relating the actual[52] dipole moment of the molecule to the vapor-phase value, *i.e.*, to the situation in which there would be no reaction field, and $g$ is a constant related to the energy of the molecule in the applied field. $f$ and $g$ are both constants which are defined by the molecular dimensions as well as the dielectric constant and refractive index of the solvent. For the large axial ratios and relatively high solvent polarities of concern to us here, $g \rightarrow 1$ and $f \rightarrow {}^2/_3$ (independent of the dielectric constant and refractive index of the solvent), which are the values used in this paper.

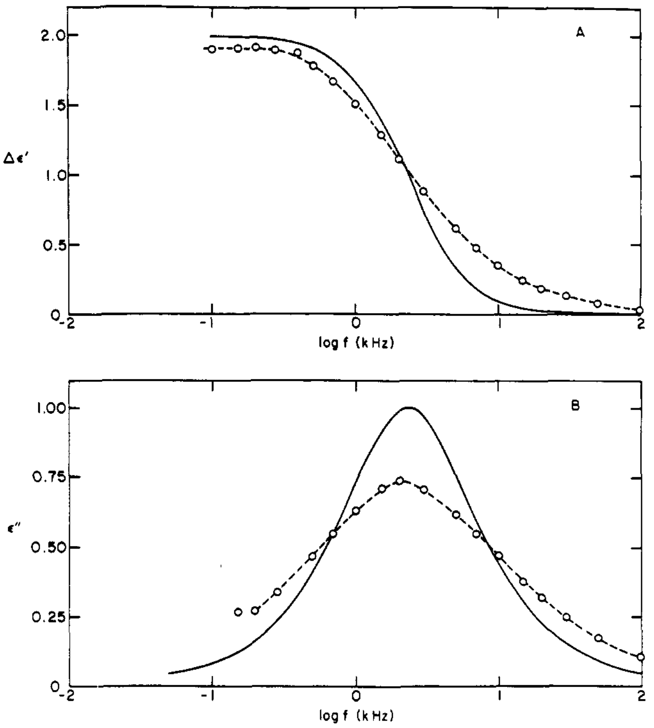

Figure 1. (A) $\Delta\epsilon'$ as a function of $\log f$ for *p*-Cl-PBLA in EDC at 0.70 mg/ml. The dashed line represents the experimental data and the solid line is a theoretical curve calculated from eq 2, with $\epsilon'$ replaced by $\Delta\epsilon'$ in this equation (*i.e.*, taken as the contribution from the solute), and $\epsilon_0 = 2.00$, $\epsilon_\infty = 0$, and $\tau = 6.9 \times 10^{-5}$ sec. (B) $\epsilon''$ as a function of $\log f$ for the same solution as in A. The dashed line represents the experimental data, and the solid line is a theoretical curve calculated from eq 3 with the same parameters as in A.

In addition to obtaining $\mu$ from $\Delta\epsilon_0$, we can gain information about the size, shape, and flexibility of the polymer from the critical frequency, $f_{\text{crit}}$. This is the frequency at which $\epsilon''$ is a maximum. It is related to the rotational relaxation time of the solute by the equation

$$\tau = 1/2\pi f_{\text{crit}} \qquad (8)$$

Strictly speaking, $\tau$, measured in such a manner, is a macroscopic relaxation time, and it is not clear as to whether this corresponds exactly to the molecular relaxation time.[56] To a first approximation, however, we shall assume (as is common practice[16]) that the macroscopic and microscopic relaxation times are the same.

Since the absorption maximum is relatively broad, especially when there is a distribution of relaxation times, it is sometimes difficult to select a precise value for $f_{\text{crit}}$. As an aid in performing this interpolation, we use an empirical distribution function first described by Fuoss and Kirkwood.[57] They found that the absorption data can be fit by the equation

$$\epsilon' = \epsilon_{\text{m}}'' \operatorname{sech} [\beta(\ln \omega/\omega_{\text{m}})] \qquad (9)$$

where $\beta$ is a parameter and $\omega_{\text{m}} = 2\pi f_{\text{crit}}$, *i.e.*, the angular frequency at which the maximum value, $\epsilon_{\text{m}}''$, of $\epsilon''$ occurs. Thus, by rearranging the above to

$$\cosh^{-1}\left(\frac{\epsilon_{\text{m}}''}{\epsilon''}\right) = \beta(\ln \omega - \ln \omega_{\text{m}}) \qquad (10)$$

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(51) This correction is not needed in the Kirkwood treatment.[49]

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(54) Reference 31, p C307.

(55) Reference 31, p C405.

(56) N. E. Hill, W. E. Vaughan, A. H. Price, and M. Davies, "Dielectric Properties and Molecular Behavior," Van Nostrand-Reinhold, London, 1969, p 297.

(57) R. M. Fuoss and J. G. Kirkwood, *J. Amer. Chem. Soc.*, **63**, 385 (1941).

TABLE I
DEGREE OF POLYMERIZATION AND STATIC DIELECTRIC PROPERTIES OF POLYMERS IN EDC

| | $\overline{\mathrm{DP}}_{\mathrm{w}}{}^{a}$ | $\Delta\epsilon_0{}^a/c$, ml/mg | $\mu_{\mathrm{tot}}{}^{b}$ | $\mu$/residue$^{b,c}$ |
|---|---|---|---|---|
| *o*-Cl-PBLA | 320 | 760 | 1190 | 3.73 (3.45) |
| *m*-Cl-PBLA | 145 | 470 | 610 | 4.20 (4.33) |
| *p*-Cl-PBLA | 960 | 2800 | 3710 | 3.86 (4.37) |
| PBLA | 350$^d$ | 1070 | 1330 | 3.80 (3.59) |
| PBLG | 1100 | 3400 | 4180 | 4.11 |

[a] Extrapolated to infinite dilution. [b] Dipole moments are given in Debye units, D. [c] Values in parentheses are for measurements in methylene chloride. [d] This molecular weight was estimated from the intrinsic viscosity in HMPA, using the relation of Hayashi, *et al.*[32]

it is seen that a plot of $\cosh^{-1}(\epsilon_m''/\epsilon'')$ *vs.* $\ln \omega$ yields a straight line whose slope is $\beta$ and which crosses the $\ln \omega$ axis at $\omega = \omega_m$. In using eq 10, even an estimate of $\epsilon_m''$ suffices to provide an accurate value of $\omega_m$.

The relaxation time of the molecule, $\tau$, can be related to the size and shape of the molecule by standard hydrodynamic theories. Thus, Perrin[58] related $\tau$ to molecular parameters for a prolate ellipsoid (a possible model for a rodlike $\alpha$ helix) by the equation

$$\tau = \frac{\pi\eta L^3}{6kT[\ln(L/b) - 0.5]} \qquad (11)$$

where $\eta$ = the viscosity of the solvent,[59] $b$ = the semiminor axis of the equivalent ellipsoid, and $L$ = the length of the particle.

## Results

**Characterization of Materials.** Table I shows the weight-average degree of polymerization, $\overline{\mathrm{DP}}_{\mathrm{w}}$, for each of the polymer fractions used. The shorter polymers, *o*- and *m*-Cl-PBLA, did not exhibit any concentration dependence of $\overline{\mathrm{DP}}_{\mathrm{w}}$ over a range of concentrations from 0.3 to 0.1 mg/ml. The *p*-Cl-PBLA and PBLG exhibited a marked nonideality over the same concentration range, but the values of $\overline{\mathrm{DP}}_{\mathrm{w}}$ (determined with absorption optics) were extrapolated to infinite dilution. There is considerable uncertainty in these values, since the value of $\bar{v}$ for the chloro derivatives was only an estimate and any error is magnified when $1 - \bar{v}\rho$ is calculated; *i.e.*, since $1 - \bar{v}\rho = 0.119$, an error of 0.5% in $\bar{v}$ leads to an error of about 5% in the molecular weight. Nevertheless, EDC was used for the molecular weight determinations, since the dipole moment measurements were made in the same solvent.

**Dielectric Measurements.** Because of considerations of solubility and possible aggregation, ethylene dichloride (EDC) and methylene chloride (MC) were used as solvents for this study. More extensive measurements (to determine the concentration dependence of $\Delta\epsilon_0/c$) were made in EDC than in MC. From the point of view of dielectric work, these are rather poor choices because of the high dc conductance of the solvent, $\sim 10^{-8}$ mho cm, even when pure. Also, because of the relatively high dielectric constant of these solvents, $\epsilon = 10.36$ for EDC and $\epsilon = 8.90$ for MC at 25°, trace impurities which are inevitably bound to the polymer lead to high dc conductances of the solutions. For this reason, it was necessary to extend the conductance range of the bridge as described in the Experimental Section. The number of conducting impurities bound to the polymer was reduced by repeated precipitation as described in the Experimental Section. The solvent system used for the fractionation, EDC–methanol, was chosen particularly for its high dielectric constant; it was felt that a greater percentage of conducting impurities would remain in the solvent during precipitation than if a less polar system had been used. In this manner, it was possible to reduce the dc conductance of solutions of the polymers by about an order of magnitude.

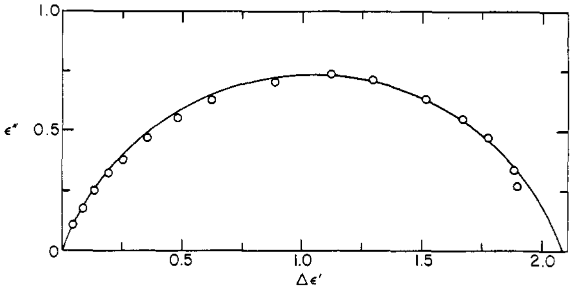

Figure 2. Cole–Cole arc plot of the data of Figure 1.

Even with this purification procedure, a considerable electrode polarization effect was seen at low frequencies. This effect is shown by an increase in apparent capacitance and a decrease in apparent conductance as the frequency is lowered. Although these electrode effects were similar to those observed by Johnson and Cole,[61] our measured capacitance and conductance did not follow the dependence on $G_{\mathrm{dc}}$ and $\omega$ which they had derived empirically. Instead, we were able to obtain some degree of correction for the polarization effect by measurement of the frequency dependence of the apparent capacitance and conductance of EDC and MC of various degrees of purity, *i.e.*, of various amounts of dc conductance. By assuming that the actual capacitance and conductance of the impure solvents were independent of frequency, we ascribed any measured frequency variation to electrode polarization effects. Therefore, the high-frequency values of the measured capacitance and conductance of the impure solvents were assigned as the correct values and were substracted from the measured quantities at each frequency to obtain the polarization effect at that frequency. Of course, this is only a rough correction, since it involves the assumption that the amount of electrode polarization is independent of the nature of the conducting impurity.

We also found it necessary to correct for a continuous drift in the dc conductance of a solution during the course of the measurements over the range of frequencies. This drift may be due to some change in the condition of the surface of the electrode, and we note that the same phenomenon has been reported previously by others.[62] This correction was accomplished by the determination of $G$ immediately before and after the measurement over the entire frequency range. We were thus able to calculate the rate of change of the conductance and extrapolate the values at all frequencies to a common time.

For all of the polymers studied, the measured conductance, $G_{\mathrm{measd}}$, leveled off at low frequencies and was essentially constant at frequencies below $\sim$100 Hz. Thus, we assumed that, at this frequency and below, there was no longer any loss due to the polymer, and the residual conductance was due only to ohmic impurities, *i.e.*, $G_{\mathrm{dc}}$. Hence, at low frequencies, $G_{\mathrm{ac}}$ $(= G_{\mathrm{measd}} - G_{\mathrm{dc}})$ and $G_{\mathrm{measd}}$ were small, so that any small

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(58) F. Perrin, *J. Phys. Radium*, **5**, 497 (1934).

(59) At 25°, $\eta = 0.0078$ P[27] for EDC and 0.00416 P[60] for MC.

(60) Reference 27, p 179.

(61) J. F. Johnson and R. H. Cole, *J. Amer. Chem. Soc.*, **73**, 4536 (1951).

(62) G. Jaffe and J. A. Rider, *J. Chem. Phys.*, **20**, 1077 (1952).

TABLE II
OBSERVED VALUES OF ROTATIONAL RELAXATION TIMES AT 25°

| | EDC | | MC | |
|---|---|---|---|---|
| | $f_{\mathrm{crit}}$[a] kHz | $\tau$, sec | $f_{\mathrm{crit}}$[a] kHz | $\tau$, sec |
| *o*-Cl-PBLA | 80 | $2.0 \times 10^{-6}$ | 130 | $1.2 \times 10^{-6}$ |
| *m*-Cl-PBLA | 350 | $4.5 \times 10^{-7}$ | 1100 | $1.4 \times 10^{-7}$ |
| *p*-Cl-PBLA | 2.8 | $5.7 \times 10^{-5}$ | 4.3 | $3.7 \times 10^{-5}$ |
| PBLA | 70 | $2.3 \times 10^{-6}$ | 90 | $1.8 \times 10^{-6}$ |
| PBLG | 1.6 | $9.9 \times 10^{-5}$ | | |

[a] Extrapolated to infinite dilution. The value shown in Figure 1 pertains to a finite concentration.

error in $G_{dc}$ resulted in a larger error in $G_{ac}$ and therefore in $\epsilon''$.

After applying these corrections and calculating $\epsilon'$ and $\epsilon''$ from eq 4 and 6, respectively, we typically obtained results such as those shown in Figures 1A and 1B. It can be seen that the general shape of the curves is similar to the Debye curves, although both the dispersion and absorption occur over a broader range of frequencies. As pointed out previously, this is to be expected for a polymeric species with a distribution of molecular weights, and hence of relaxation times. The results shown are for *p*-Cl-PBLA in EDC. The curves obtained for the other polymers (and in MC) are similar.

The extrapolation to zero frequency by means of the Cole–Cole arc plot is shown in Figure 2; the data shown are the same as those of Figures 1A and 1B. It can be seen that the data for this particular polymer lead to a depressed arc type of plot, *i.e.*, a semicircle whose center is below the $\Delta\epsilon'$ axis. We find that the shape of this plot depends upon the particular sample of polymer under study. In their study of poly(*n*-butyl isocyanates), Bur and Roberts[43] found the same behavior, and we agree with their conclusion that the differences in shape probably represent differences in molecular weight distributions, and hence differences in the distribution of relaxation times.

This uncertainty as to the expected shape of the curve leads to an uncertainty in the extrapolation to zero frequency (right-hand side of Cole–Cole plot). In the case illustrated, the uncertainty is magnified because of the relatively low value of the critical frequency, $f_{\mathrm{crit}} = 2.8$ kHz, at infinite dilution (see Table II). Thus, the extrapolation to zero frequency must be made to a large extent with low-frequency values of $\Delta\epsilon'$ which are themselves uncertain because of electrode polarization. In this case, it is seen from Figure 2 that the error may be as much as 5%. In the experiments with polymers of lower $\overline{\mathrm{DP}}_{\mathrm{w}}$, this extrapolation is not so doubtful, because the "zero"-frequency limit is reached at higher frequencies where electrode polarization errors are not as large.

Dielectric dispersion measurements were made over a concentration range of 0.2 to 3.0 mg/ml. The lowest usable concentration was dependent on the molecular weight of the polymer, since the longer polymers had a larger dipole moment and gave rise to a larger value of $\Delta\epsilon'$. The concentration dependence of $\Delta\epsilon_0$ for *p*-Cl-PBLA is shown in Figure 3. The good linearity of $\Delta\epsilon_0$ with concentration over the range studied indicates that effects such as aggregation or other strong solute–solute interactions are minimal. Similar linearity of $\Delta\epsilon_0$ with concentration was observed for all of the other polymers studied.

Table I shows the static or zero-frequency dielectric increment per unit concentration extrapolated to infinite dilution, as well as the overall dipole moment and dipole moment per residue as calculated from eq 7. Most of the data shown in Table I are for solutions in EDC; similar results were obtained in methylene chloride, and the dipole moments per residue in this solvent are indicated by the data in parentheses. By dipole moment, we mean the component parallel to the helix axis, because we expect the off-axis components to cancel in polymers of this length. We note that our value of 4.11 D/residue for PBLG is in good agreement with that of Sharp,[20] who measured a value of approximately 4.2 D/residue in *cis*-1,2-dichloroethene, and in fair agreement with that of Wada,[18] who obtained 3.44 D/residue in EDC. In the case of the latter, it is not clear from his paper whether Wada had applied a correction to obtain the vacuum dipole moment or whether his value is uncorrected for solvent.

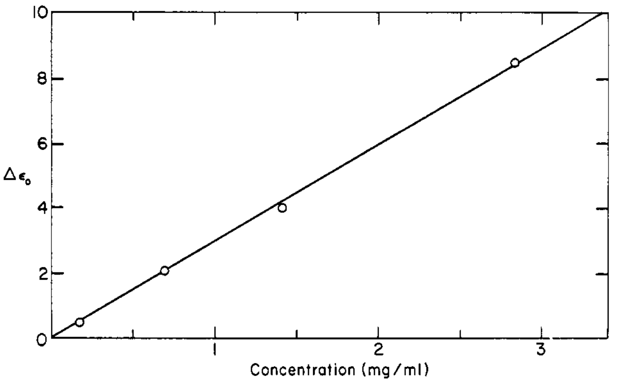

Figure 3. Concentration dependence of $\Delta\epsilon'$ for *p*-Cl-PBLA in EDC.

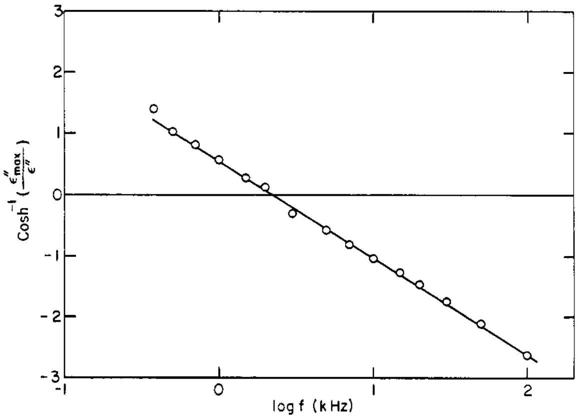

Figure 4. Fuoss–Kirkwood plot for data of Figure 1B.

Aside from the uncertainty involved in using the Buckingham equation to obtain dipole moments from solutions in polar solvents, several other sources of error are present in the final result. These include the above-mentioned errors in molecular weight and uncertainty in $\Delta\epsilon_0$ due to dc conductance, electrode polarization and the exact shape of the Cole–Cole arc plot. The magnitude of each of these errors depends upon the particular polymer and the amount of dc conductance. The effect of each of these errors on the final result, the dipole moment per residue, is reduced somewhat, however, since that quantity varies as the square root of each of the above factors. Thus, we conclude that our calculated values of the dipole moment per residue are probably accurate to about ±5% or ±0.2 D (in addition to the unknown uncertainty arising from the use of the Buckingham equation).

Besides calculating the dipole moment from the static dielectric constant of the solution, we have determined the rota-

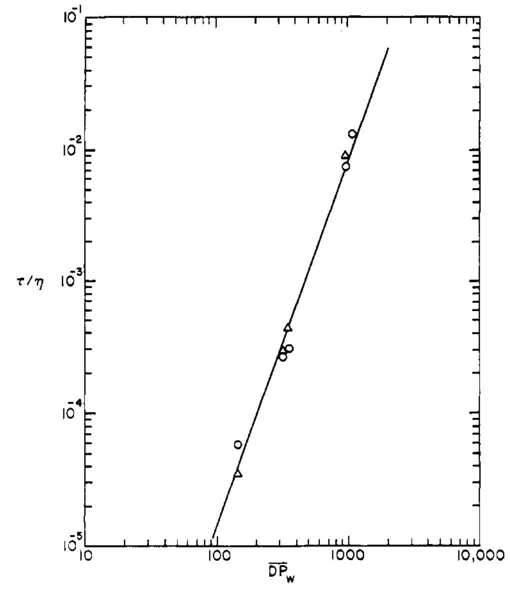

Figure 5. Plot of log $\tau/\eta$ *vs.* log $\overline{\mathrm{DP}}_\mathrm{w}$ for the polymers of Table I in EDC (○) and in MC (△) at 25°. Solid line is the theoretical plot from the Perrin equation (eq 11) using $L = 1.5$ Å × $\overline{\mathrm{DP}}_\mathrm{w}$ and $b = 7.5$ Å.

tional relaxation time for each polymer as shown in Table II. The values of the critical frequency, $f_\mathrm{crit}$, were determined from Fuoss–Kirkwood plots, one of which is shown in Figure 4. There is also some error in this type of procedure, since the Fuoss–Kirkwood plot does not yield a straight line for an asymmetric distribution of relaxation times, as is also implied by the skewness of a Cole–Cole arc plot. The magnitude of this error varies among the various fractions of polymers and probably depends on the asymmetry of the distribution of relaxation times. In addition, we find that the value of $\tau$ is somewhat concentration dependent, particularly in the case of the polymers of higher $\overline{\mathrm{DP}}_\mathrm{w}$ (PBLG and *p*-Cl-PBLA). In general, the rotational relaxation time decreases as the concentration decreases, and the values reported in Table II are those which have been obtained by extrapolation to infinite dilution.

## Discussion

In interpreting the data presented here, we assume that the polymers are $\alpha$ helical in solution, and that specific solvation effects do not affect the computed dipole moments or relaxation times. As for the first assumption, ORD and CD data[22] indicate that PBLA and its ortho and meta derivatives form left-handed $\alpha$ helices, while the para derivative[23] and PBLG[6] form right-handed $\alpha$ helices. The absence of interior breaks in the helix is verified by the fact that the polymers conform to a rigid-rod (more accurately a rigid ellipsoid) model. A plot of log $\tau/\eta$ at 25° *vs.* $\overline{\mathrm{DP}}_\mathrm{w}$ is given in Figure 5 for all of the polymers shown in Table I; the solid line was computed from the Perrin equation (eq 11) using a length per residue of 1.5 Å for an $\alpha$ helix and a value of $b = 7.5$ Å, the latter being obtained from the minimum-energy conformations of these polymers[4] (however, the calculated relaxation time is not very sensitive to the choice of $b$). The fit between the experimental points and the theoretical curve is quite good considering the crude nature of the Perrin equation,[63] the questions raised previously about the relation between macroscopic and microscopic relaxation times, the uncertainty in the determination of $\tau$ in a polydispersed system, and the possible errors in the determination of molecular weights. Despite these problems, the good fit indicates not only that the polymers are $\alpha$ helical in solution but also that specific effects of solvent (other than as a viscosity effect) do not influence the computed values of $\tau$ and, by implication, those of the backbone contribution to $\mu$, thereby establishing the validity of the dielectric technique as used here.

Some measure of the polydispersity can be obtained from the width of the dielectric loss curve.[64] In dielectric measurements on poly(*n*-butyl isocyanate), Bur[42] was able to correlate the width at half peak height ($w/2$) for the absorption peak with $\bar{M}_\mathrm{w}/\bar{M}_\mathrm{n}$. Based on a comparison with the data of Bur, the widths of the absorption peaks for our polymers imply that our fractions have a narrow distribution of molecular weights. For example, for *p*-Cl-PBLA (see Figure 1B), $w/2 = 1.7$; this corresponds to $\bar{M}_\mathrm{w}/\bar{M}_\mathrm{n} \sim 1.2$, which is consistent with our conclusion from the ultracentrifuge data.[64]

The dipole moments presented in this paper can be examined in the context of the conformational energy calculations performed by Yan, *et al.*[4] These authors were very successful in calculating the sense of the helix for many homopoly($\alpha$-amino acids). Their calculations were carried out using the assumption of conformational regularity, *i.e.*, the conformation of the side chain was assumed to be the same for each residue in the polymer. While some degree of side-chain ordering has been inferrred for poly(L-tyrosine) from its optical properties,[65] it is not clear whether this holds for other polymers. The optical spectra of PBLG and PBLA do not provide evidence that the side chains in these polymers are oriented in a regular manner. If they were, one would expect to see different ORD and CD spectra from those observed for homopolymers with nonchromophoric side chains; actually, such differences are not observed, except for the difference in helix sense. The only observed differences have been discussed previously[22] and consist mainly of extremely weak CD bands in the benzyl absorption region for the *p*- and *m*-Cl-PBLA. It is not clear whether the existence of these bands implies side-chain orientation, since optically active bands in this region have been shown to exist in free amino acids with aromatic side chains.[66,67]

The nmr evidence on this point is equally unclear. Bradbury, *et al.*,[9] have examined both the $\beta$-CH$_2$ and the benzyl-CH$_2$ proton spectra for PBLA in chloroform–trifluoroacetic acid (TFA) mixtures. They deduced that, while the conformations of the side chains are not completely random, as evidenced by the splitting of the benzyl-CH$_2$ resonance, there is considerable motion of the side chain. These authors[9] pointed out that it is difficult to interpret this kind of nmr data quantitatively in terms of rotational freedom. PBLG did not even show[9] the splitting of the benzyl protons.

Silverman, *et al.*,[10,11] on the other hand, concluded that there is nmr evidence for ring stacking in PBLG but not in PBLA, in CDCl$_3$–TFA mixtures. From this they concluded that, if there is any orientation of the side groups in PBLA, it

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(63) H. A. Scheraga and L. Mandelkern, *J. Amer. Chem. Soc.*, **75**, 179 (1953).

(64) While the polydispersity could have been determined in terms of $\bar{M}_z/\bar{M}_\mathrm{w}$ from the ultracentrifuge experiments, values for $\bar{M}_z$ are not reported here because of inaccuracies in their determination. These crude values of $\bar{M}_z$ for our *fractionated* polymers led to $\bar{M}_z/\bar{M}_\mathrm{w}$ in the range of 1.1–1.3, indicating that the fractions have a fairly narrow distribution of molecular weights.

(65) A. K. Chen and R. W. Woody, *J. Amer. Chem. Soc.*, **93**, 29 (1971).

(66) A. Moscowitz, A. Rosenberg, and A. E. Hansen, *ibid.*, **87**, 1813 (1965).

(67) E. Iizuka and J. T. Yang, *Biochemistry*, **3**, 1519 (1964).

is not in the conformation predicted by Yan, *et al.*,[4] since that particular arrangement should lead to a detectable ring stacking effect. In all of the above nmr studies, however, the TFA (needed to break up aggregates at the high solute concentrations necessary for these experiments) may have interacted strongly with the polymer, thus altering the conformation of the side chain.

Charney, *et al.*,[68] have used pulsed electric dichroism to study the side-chain orientation of PBLG and PBLA in EDC and MC. On the basis of a nonzero value of the reduced dichroism for PBLG, they concluded that the side chains of this compound are rigidly oriented. However, they found no measurable dichroism for PBLA, and thus concluded that its side chains are either randomly oriented or they are oriented in such a way as to yield a small total dipole moment for the polymer.

Sharp has examined the question of side-chain mobility in his dipole moment study of PBLG.[20] He found that the value of the dipole moment increased with increasing dielectric constant of the solvent, reaching a limiting value of 4.2 D/residue in *cis*-1,2-dichloroethene ($\epsilon$ 9.2). He tentatively interpreted these results to mean that in solvents of low dielectric constant the side chains were restricted in their rotation because of strong dipole–dipole interactions between the side chain and backbone. These forces would become weaker, however, as the dielectric constant of the solvent increased, thus allowing more freedom of rotation; this would account for the fact that, at $\epsilon$ 9.2, the side chains were in random orientation, and thus only the backbone dipole moment was measured (see below).

In order to compare our measured dipole moments with those of the structures predicted by Yan, *et al.*,[4,69] we must calculate the dipole moments to be expected from their conformations. This was done by assigning partial charges to atoms in each of the three polar groups present, *i.e.*, the amide (CONH), ester (COOC), and C–Cl groups. These partial charges were the ones used by Ooi, *et al.*,[3] and Yan, *et al.*[69] They had been selected to reproduce the direction and magnitude of the known dipole moments of small molecules. The groups were assigned partial charges which are consistent with a dipole moment of 3.70 D for the amide, 1.70 D for the ester, and 1.75 D for the C–Cl group. Using these charges, the dipole moment per residue (parallel to the helix axis) was calculated for the polymers in their minimum-energy conformations as predicted by Yan, *et al.*;[4,69] the results are shown in column A of Table III, where the conformation of the backbone and side chains is designated by nomenclature defined in ref 69.

It is also instructive to calculate the dipole moment per residue if we allow freedom of rotation or random orientation around some of the side-chain bonds. Such a calculation can be performed by a "rotational isomeric state" approximation, *i.e.*, the value of the dipole moment is calculated from the average of several rotational positions. Designating the dihedral angles for rotation about the side-chain single bonds by $\chi_1$, $\chi_2$, $\chi_3$,[70] etc., column B of Table III shows the results of such an average for variation of the outermost dihedral angle (*i.e.*, for $\chi_5$ of the Asp series or $\chi_6$ for PBLG) by 360° (calculated in 60° increments). The other dihedral angles, *i.e.*, $\chi_1 \rightarrow \chi_4$ for the Asp series and $\chi_1 \rightarrow \chi_5$ for PBLG, were fixed at the same values as in column A. In a similar manner, averages were computed allowing "free rotation" around the outermost two dihedral angles, *i.e.*, $\chi_4$ and $\chi_5$ for the Asp series and $\chi_5$ and $\chi_6$ for PBLG, while holding the other dihedral angles as in column A. These results are shown in column C.

TABLE III
OBSERVED AND CALCULATED DIPOLE MOMENT PER RESIDUE

| | Conformation[a] | μ/residue (obsd) | μ/residue (calcd) | | | |
|---|---|---|---|---|---|---|
| | | | A[b] | B[c] | C[d] | D[e] |
| *o*-Cl-PBLA | Lt(−) | 3.73 | 2.91 D | 4.34 D | 4.36 D | 3.62 D |
| *m*-Cl-PBLA | Lt(−) | 4.20 | 3.28 | 4.80 | 5.08 | 3.62 |
| *p*-Cl-PBLA | Rt(−) | 3.86 | 1.08 | 1.08 | 1.54 | 3.62 |
| | Rt(−) | | 2.20 | 2.20 | 1.93 | 3.62 |
| PBLA | Lt(−) | 3.80 | 4.76 | 4.76 | 4.76 | 3.62 |
| PBLG | Rl(+) | 4.11 | 3.31 | 3.31 | 3.31 | 3.62 |
| | | Av 3.94 ± 0.17[f] | | | | |

[a] The definition of the nomenclature for describing the conformation is given in ref 69. [b] The first five entries pertain to the low-energy conformations from ref 4, and the last one to the low-energy conformation from ref 69. Two theoretical values are given for *p*-Cl-PBLA because two different Rt(−) conformations had similar energies. [c] For Asp series: $\chi_1 \rightarrow \chi_4$ as in column A, but averaged over $\chi_5$. For PBLG: $\chi_1 \rightarrow \chi_5$ as in column A, but averaged over $\chi_6$. [d] For Asp series: $\chi_1 \rightarrow \chi_3$ as in column A, but averaged over $\chi_4$ and $\chi_5$. For PBLG: $\chi_1 \rightarrow \chi_4$ as in column A, but averaged over $\chi_5$ and $\chi_6$. [e] Assuming random orientation of the side chains; *i.e.*, only the backbone moment contributes to the total dipole moment. [f] This is the average and average deviation of the values in this column. The corresponding quantities in MC are 3.9 ± 0.4.

Wada has pointed out[71] that the contribution of the side chain to the overall dipole moment becomes negligible with respect to that of the backbone when the side chains adopt a random orientation. In accordance with this expectation, we have listed in column D the value of the backbone moment (per residue) parallel to the helix axis for each polymer.[72] These are all the same, since the backbones are identical (except for helix sense). Thus, column D represents the results for completely randomly oriented side chains.

We may compare the theoretical and experimental values of $\mu$/residue by focusing attention primarily on the relative (rather than the absolute) values among polymers, thereby avoiding to some extent the effect of errors in the absolute values, as indicated earlier. The data in columns A, B, and C indicate large variations in $\mu$/residue from polymer to polymer, whereas those in column D are constant and even very close in absolute value to the average of the experimental values (keeping in mind the error of ±0.2 D in the latter). Thus, we conclude that in these poly($\alpha$-amino acids), the side chains have no regular conformation, *i.e.*, they are either "freely" rotating or arranged in some random but immobile orientation. (Of course, we cannot distinguish between these two possibilities.) We say freely rotating with the realization that some conformations will be of sufficiently high energy

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(68) E. Charney, J. B. Milstien, and K. Yamaoka, *J. Amer. Chem. Soc.*, **92**, 2657 (1970).

(69) J. F. Yan, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **49**, 2713 (1968).

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

(71) A. Wada, in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 131.

(72) The value of 3.6 D for the backbone moment differs from that of Applequist and Mahr[16] (4.9 D). However, their value of 4.9 D was the observed one, and (together with assumptions about the side-chain conformation and mobility) was *assumed* to correspond to the backbone dipole moment.

so as to be unlikely; however, at least a large enough region of the conformational space is available so as to effectively randomize the side chains.

It is conceivable that the relative constancy of experimental values might arise from special specific regular orientations of the side chains. For example, the side chains would not contribute to the overall dipole moment if the side chains have a conformation such that their net dipole moment (ester + C–Cl) is zero or (if nonzero) is oriented perpendicular to the helix axis. Alternatively, the side chains of the different polymers could have different conformations, but with the same (small) component of the dipole moment *along* the helix axis. While such possibilities cannot be ruled out, we think it unlikely that such a coincidence could arise in all five polymers. However, such a situation could exist in one or two of the polymers, and might be the reason that stacking in PBLG is observed by nmr[10] but not by dipole moment measurements.

While the constancy of the observed values of $\mu$/residue is thus open to several interpretations, it does at least indicate that these polymers in EDC and MC do not have the compact conformation predicted by Yan, *et al.*,[4,69] since the data in column A of Table III vary considerably from polymer to polymer. As pointed out by Yan, *et al.*,[69] and by Silverman, *et al.*,[10] the calculations[4,69] pertain to a vacuum environment in which the computed conformations would be compact with the side chains close to the backbone (as would also be expected for such nonpolar groups in water). However, in solvents such as EDC and MC, solvation of the side chain would bring the latter away from the backbone where interactions with the solvent would be more favorable (both energetically and entropically[10,69]). If the side-chain conformations of these compounds could be determined in water, one might have the possibility of finding the conformations predicted by Yan, *et al.*[4,69] Such experiments are in progress in our laboratory, using nmr, with nonpolar polymers being solubilized in water by incorporating them between blocks of water-soluble poly(D,L-lysine).[73]

It is of interest to consider two points about the magnitudes of the dipole moments. First, since the computed value of $\mu$/residue for the backbone is 3.62 D (column D of Table III), and the assumed value for the amide dipole moment was 3.70 D, it is seen that the amide dipole is almost (but not completely) parallel to the helix axis. Second, it should be noted that the assumed value of 3.70 D was obtained[3] from the measured dipole moment of small amides, and its applicability to the peptide bond in an $\alpha$ helix is open to some question. As pointed out elsewhere,[71,74] the formation of an intrachain hydrogen bond in the $\alpha$ helix must change the charge distribution and hence the effective dipole moment of the peptide group. Wada[71] estimated that the effective dipole moment in the $\alpha$ helix would be 4–5 D. The data of Momany, *et al.*,[74] also indicate an increase in the dipole moment of an amide when involved in a hydrogen bond. Our experimental results and their interpetation, *viz.*, that $\mu_{\text{obsd}}$/residue = $\mu_{\text{backbone}}$/residue = 3.9 ± 0.2 D (which is greater than 3.6 D), are consistent with this expectation.

We conclude that, in cases such as examined herein, the static and dynamic dielectric properties provide insight into the conformations of these molecules in extremely dilute solutions. The fit of the rotational relaxation time to the Perrin equation corroborates the previous conclusion from optical properties that these poly(amino acids) exist as $\alpha$ helices under the conditions studied.[22] In addition, we have provided evidence for the lack of regularity in the side-chain structure of these polymers.

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