Dielectric Dispersion of Polypeptide Solutions. I. Once-Broken Rod Polypeptide Based on γ-Benzyl L-Glutamate

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ABSTRACT: An improved method was developed for synthesizing once-broken rod molecules. It consists of polymerizing the *N*-carboxy anhydride of γ-benzyl L-glutamate with trimethylenediamine as initiator. This poly(γ-benzyl L-glutamate) (PBLG) molecule, hereafter referred to as PBG-B, consists of two peptide chains jointed by an initiator residue in a head-to-head fashion. It may assume the form of a once-broken rod in helicogenic solvents, because the two peptide chains will assume α-helical conformations, while the intervening initiator residue cannot assume such a rigid conformation. The assumed structure for this polypeptide was confirmed by examinations of the kinetics and mechanisms of the polymerization reaction. Dielectric measurements were carried out on dilute solutions of both PBG-B and ordinary PBLG, PBG-A, in helicogenic solvents. The dipole moments per monomeric residue of PBG-A stayed constant up to a weight-average degree of polymerization, $\bar{N}_w$, of about 300, which proved the rigidity of the helical conformation in this molecular weight range. The dipole moments of PBG-B were also approximately proportional to $\bar{N}_w$ but were smaller by a factor of about 2 than those of the corresponding PBG-A. This was attributed to the fact that the two helical rods of *ca.* 15 Å in diameter may not rotate independently due to steric hindrances, even though the single bonds in the joint portion are free to rotate. The relaxation times of PBG-A and PBG-B showed similar molecular weight dependence characteristic of rodlike molecules. However, the values for PBG-B were smaller than those for PBG-A by a factor of 3 or 4 when compared at the same molecular weights.

In a previous publication,[2] we reported viscosity and sedimentation studies of once-broken rod molecules. The model compound investigated was poly(γ-benzyl L-glutamate) (PBLG) prepared with a bifunctional amine as initiator. This PBLG molecule is expected to be composed of two peptide chains jointed by the initiator residue in a head-to-head fashion. If such a molecule is dissolved in a helicogenic solvent, the two peptide chains will form the α helices, while the intervening initiator residue cannot. Thus the molecule as a whole may be regarded as a once-broken rod consisting of two helical rods connected by the flexible initiator portion. It was found[2] that introduction of a flexible joint into a straight rod had only trivial effects on such hydrodynamic properties as intrinsic viscosity and sedimentation coefficient. Although this finding agreed with theoretical predictions,[2-4] we must accept it with some reservation because the samples used must have been contaminated with straight rod molecules for the reason mentioned below.[2] Very recently, Kihara, et al.,[5] showed that the dipole moment of a once-broken rod PBLG synthesized in this way was smaller by a factor of about $2^{1/2}$ or a little more than that of a straight rod one having the same molecular weight. This factor agrees with the value expected for an ideal flexible joint, but their samples were confined to relatively low molecular weights and were likely to be contaminated with straight rods.

Recently, we have presented an improved method for synthesizing once-broken rod PBLG on the basis of critical examinations of the kinetics and mechanism of the polymerization reaction and proposed possible procedures for characterizing the products to be obtained.[6] The present study describes the synthesis of well-characterized PBLG samples of once-broken rod type and comparison of precise dielectric dispersion data for both straight rod and once-broken rod samples in solution.

The dielectric method will give the dipole moment and relaxation time of the dissolved molecule, both of which are closely related to the molecular conformation. Because polypeptide molecules in the helical conformation have large dipole moments,[5,7-16] it will be particularly powerful at low molecular weights, to which the light-scattering method is not always applicable with accuracy. These are the reasons that we used dielectric measurements in the present study.

Experimental Section

Polypeptide Samples. Two types of poly(γ-benzyl L-glutamate), designated here as PBG-A and PBG-B, were prepared in the following ways.

PBG-A. The PBG-A samples were obtained by polymerizing the *N*-carboxy anhydride of γ-benzyl L-glutamate (BLG-NCA) in dimethylformamide (DMF) with *n*-butylamine as initiator.[6] Sample A-4 was separated into five fractions by precipitation fractionation with the system methanol–dichloromethane. The third fraction A-43 and unfractionated samples A-2, A-3, A-5, and A-6 were used for the present measurements. As expected from the mechanism of polymerization,[6] these samples had relatively narrow distributions of molecular weights; the ratios of weight-average to number-average molecular weights were 1.09 for A-4, and 1.16 for A-6, and that for A-43 was very close to unity. Samples E-4 and A-IV were appropriate fractions extracted from PBG-A samples synthesized by triethylamine initiation.[17] Their molecular-weight distributions were expected to be moderately narrow.

PBG-B. Samples B-1, B-2, and B-3 were obtained by polymerization of BLG-NCA in DMF with trimethylenediamine as initiator.[6] Samples B-1 and B-3 were fractionated by the column elution method with methanol–dichloromethane mixtures as eluent. The middle cuts B-12 and B-3V and B-2 were used for physical measurements. It was difficult to obtain PBG-B samples of high molecular weights by the polymerization in pure DMF. For the reason discussed below, other PBG-B samples were synthesized in the following way. First, BLG-NCA was polymerized in DMF with trimethylenediamine as initiator, fixing the mole ratio of NCA to initiator, *A/I*, at 50. An aliquot of this polymerization mixture was added to a dioxane solution of NCA so that the resultant *A/I* and NCA concentration were adjusted to specified values. The solution was then allowed to stand overnight at room temperature, with stirring, and then poured into a large volume of methanol. The precipitated polymer was freeze dried from a dioxane solution. The amount of DMF in the solvent mixture was kept below 10 vol %. Preparative data are summarized in Table I. Most of the PBG-B samples thus synthesized were fractionated by the column elution method, and appropriate middle fractions were chosen for physical measurements.

Molecular Weight Determinations. Weight-average molecular weights $\bar{M}_w$ were determined by the sedimentation equilibrium method; use was made of a Beckman-Spinco Model E analytical ultracentrifuge equipped with the Rayleigh interference optical system. Number-average molecular weights $\bar{M}_n$ were measured on a Mechrolab Model 502 high-speed membrane osmometer. DMF at 25° was used as the solvent in both determinations. Table II summarizes the numerical data for $\bar{M}_w$, $\bar{M}_n$, and the second virial coefficient A_2. Judging from the values of $\bar{M}_w/\bar{M}_n$ given in this table, samples B-202 and B-212 were almost monodisperse in molecular weight. Intrinsic viscosities in DMF and dichloroacetic acid

Table I
Preparative Data for Trimethylenediamine-Initiated Poly(γ-benzyl L-glutamate)

Sample code	A/I	$[\eta]$,[a] dl/g	$\bar{M}_v \times 10^{-4}$	Yield, %
An-2[b]	20, 140[c]	0.308	3.8	85
B-1	100[d]	0.203	2.2	62
B-2	100	0.166	1.6	65
B-3	300	0.287	3.7	88
B-20	50, 200[c]	0.408	5.5	84
B-21	50, 300	0.546	8.0	92
B-22	50, 750	1.05	19.0	99
B-23	50, 500	0.82	13.5	99
B-24	50, 1000	1.26	24.0	98
B-26	50, 150	0.314	3.9	93
B-27	50, 400	0.665	10.6	94

[a] Intrinsic viscosities in dichloroacetic acid at 25°. [b] PBG-A polymer. [c] Polymerized by initiation with preformed polymer; the preceding number denotes the value of A/I for the preformed polymer and the number which follows represents the total A/I. [d] Polymerized in DMF.

(DCA) at 25° were measured on all samples, which, combined with the above molecular weight data, were used to estimate the molecular weight values given in Table I. The values of A_2 are seen to increase rapidly with decreasing molecular weight, especially at low molecular weights. This may be attributed to end effects, since no such large change in A_2 was observed at higher molecular weights.

Some of the fractionated PBG-B samples exhibited poor solubility in ethylene dichloride (EDC) and in some cases even in DMF. They were soluble in *m*-cresol but showed large conductances. For some samples, extraction with diethyl ether was effective to reduce the conductance to a lower level accessible to dielectric measurements. Samples B-243 and B-223 were finally purified by precipitation of DCA solutions into methanol, followed by freeze drying from dioxane solutions. Unfractionated PBG-B samples behaved similarly. Pure EDC and chloroform, even with the addition of a small amount of DMF, were not suitable for physical measurements, since PBG-B samples displayed appreciable aggregation in these solvents. PBG-A samples behaved much better.

Dielectric Measurements. A transformer bridge (TR1C transformer bridge, Ando Electric Co., Tokyo) was used in conjunction with a HP 3310A Function Generator (Yokogawa-Hewlet-Packard) operating at frequencies between 0.0005 Hz and 5 MHz. Depending on the frequency range investigated, the following three detectors were used for null-point detection: a 1232AP tuned amplifier and null detector (20 Hz–20 kHZ, General Radio), a tuned amplifier and null detector (20–700 kHz, constructed in our laboratory), and a 9R59DS radio receiver (550 kHz–30 MHz, TRIO). A dielectric cell was connected to one arm of the bridge in parallel with a variable capacitor C_B, and a GR1422-CB precision capacitor (General Radio) was connected to the other arm in parallel with another variable capacitor C_A. The precision capacitor was calibrated by the manufacturer between 50 and 1100 pF to 0.1 pF with the accuracy of ±0.3 pF. Balancing of the bridge was achieved by turning the dial of the precision capacitor and the conductance dial of the bridge with C_A and C_B dials affixed at proper positions. The dial readings obtained when the wirings to the cell were disconnected at the cell position were regarded as the lead capacitance and conductance, and they were subtracted from the readings with the cell connected to calculate those of the cell itself. This capacitance-measuring assembly allowed us to cover the frequency range between 20 Hz and 3 MHz, the capacitance range up to 1100 pF, and the conductance range between 0.01 and 3000 μ℧.[18]

Most of the measurements were performed in a brass concentric cylindrical cell, which had a guard circuit and was assembled with Teflon spacers. The cell was calibrated with benzene and monochlorobenzene as reference liquids and had an air capacitance C_0 of approximately 22.5 pF. It was placed in a brass cylinder connected to the earth terminal, which in turn was immersed in a water bath for thermostating. The temperature of the cell, measured by means of a thermocouple attached to the outer cell wall, was regulated to ±0.05°. The cell was sometimes disassembled for cleaning, but the air capacitance was constant to ±1%. It was frequently observed that the capacitance and conductance readings drifted with time, notably soon after the cell had been filled with a test liquid. Similar observations had been reported by previous investigators,[16,19] who attributed them to the polarization near electrode surfaces. Therefore, measurements were usually started after the readings had become constant, which usually took several hours or longer. Readings were taken on both solution and solvent, and the differences between the corresponding readings, *i.e.*, the capacitance increment ΔC and the conductance increment G, were used to evaluate the dielectric increment $\Delta\epsilon'$ and the loss factor $\Delta\epsilon''$ associated with the solute by means of the equations

$$\Delta\epsilon' = \Delta C/C_0 \text{ and } G = G_d + \Delta\epsilon'' C_0 \omega$$

where G_d is the dc conductance and ω ($=2\pi f$) is the angular frequency. The value for G at some low frequency where there was observed no more change in G due to the solute polarization was taken as that for G_d.

Solvents and Solutions for Dielectric Measurements. *m*-Cresol was doubly distilled under reduced pressure (bp 61° (2 mm)). EDC (bp 83.0°) and chloroform (bp 61.3°) were purified according to the standard procedure, dried over calcium hydride, and fractionally distilled. Mixtures of *m*-cresol and EDC or chloroform as well as pure *m*-cresol were used as solvents. Solvent compositions and solute concentrations were determined gravimetrically. The solvent mixtures used showed excellent solubility to both PBG-A and PBG-B and were suitable for dielectric measurements for their relatively low conductivities.

Table II
Numerical Results of Molecular Weights and Second Virial Coefficients for PBG-A and PBG-B Fractions in DMF at 25°

	Sedimentation equilibrium		Osmotic pressure		
Sample code	$\bar{M}_w \times 10^{-4}$	$A_2 \times 10^4$, ml mol g^{-2}	$\bar{M}_n \times 10^{-4}$	$A_2 \times 10^4$, ml mol g^{-2}	$\bar{M}_w/\bar{M}_n$
A-2	0.476	41			
A-3	1.06	13.6			
A-43	1.46	9.7			
A-5	2.30	7.2			
An-22	3.38	4.1			
A-6	3.70	8.4	3.20	5.5	1.16
A-IV	6.35	5.8	5.49	4.0	1.16
E-4	8.08	7.1	7.14	3.25	1.13
B-2	1.57	6.0			
B-12	2.20	6.9			
B-3V	3.47	4.1			
B-26	3.50	3.0			
B-202	5.62	4.7	5.56	2.1	1.01
B-212	8.20	4.4	8.33	2.9	0.98
B-27	10.0	3.1	8.93	2.9	1.12
B-2341	12.3	2.7	9.90	3.1	1.24
B-223	15.6	2.9	14.7	2.6	1.06
B-243	23.8	2.9	17.6	2.7	1.35
	22.5[a]	2.6			

[a] Determined from light-scattering measurements.

Results and Discussion

Polymerization of NCA with a bifunctional amine (trimethylenediamine in the present study) will yield a PBLG molecule having the initiator residue in the middle of the polymer chain.[2,6] If the polymerization reaction is to proceed ideally, the resulting polymer will have a molecular weight distribution of Poisson's type with the average degree of polymerization close to the mole ratio of NCA to initiator, A/I. Furthermore, the two polypeptide chains separated by the initiator residue may be approximately equal in length.

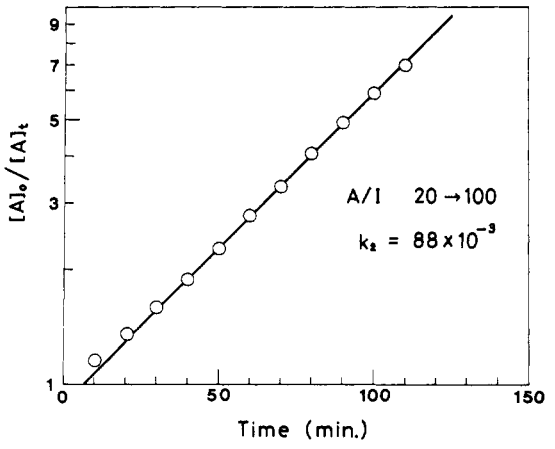

Figure 1. Typical kinetic data for polymerization of BLG-NCA in a dioxane–DMF mixture (67% dioxane): $A/I = 20$, $I = 1.08 \times 10^{-2}$ mol l.$^{-1}$.

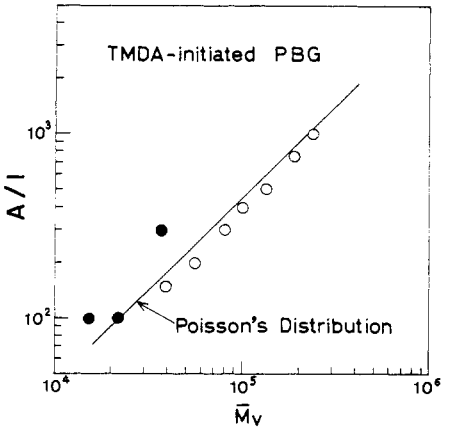

Figure 2. Relation between monomer-initiator mole ratio and molecular weight for PBG-B. The solid line represents the theoretical A/I *vs.* $\bar{M}$ relation expected for the ideal Poisson distribution: (●) polymerized in DMF, and (○) polymerized in dioxane–DMF (90–95% dioxane).

We found that when the polymerization was performed in pure DMF, the degree of polymerization $\bar{N}_v$, determined from intrinsic viscosity in DCA, was proportional to A/I up to $A/I = 100$ but then became increasingly smaller than A/I.[6] This departure from the ideal mechanism was attributed to impurities present in purified DMF, which were capable of initiating polymerization. The PBG-B samples prepared in this way, therefore, must have been contaminated with PBG-A molecules which were produced with such impurities as initiators. The difficulty in purifying DMF might be avoided if it could be replaced by more tractable solvents such as dioxane, tetrahydrofuran, etc. In fact, it was reported that the polymerization in dioxane proceeded in two stages and gave samples of very broad molecular weight distributions.[20,21] Doty and Lundberg[21] proposed for this fact an explanation in which the polymerization rates differ before and after the polymer chain grows long enough to assume the α-helical conformation and found that the critical chain length was about 8. Their kinetic data indicated that the polymerization proceeded ideally even in pure dioxane if initiated by a preformed polymer of sufficient length. We carried out similar experiments to see whether this would hold for polymerization of BLG-NCA in dioxane–DMF mixtures. Figure 1 shows kinetic data from the polymerization carried out in a dioxane (67%)–DMF (33%) mixture with a polymer initiator which had been prepared in DMF at an NCA to *n*-butylamine

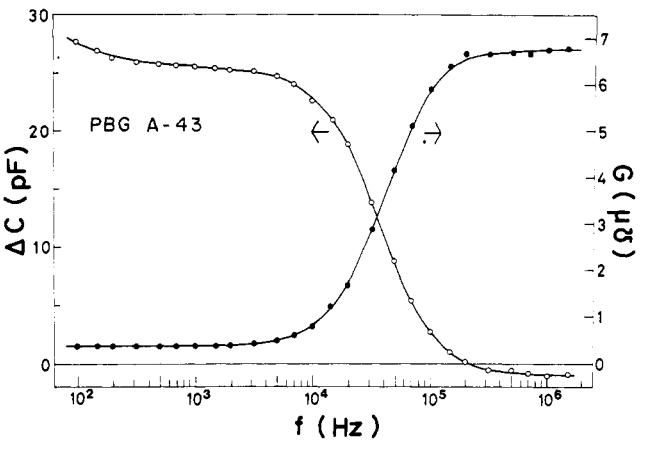

Figure 3. Representative dielectric data for a PBG-A sample A-43 in *m*-cresol at 10°.

ratio of 20. The data follow an ideal first-order reaction scheme, yielding a propagation rate of 0.09 l./mol sec. Essentially the same results were obtained for other solvent mixtures containing 60–75 vol % of dioxane.

On the basis of these findings the following procedure was taken to synthesize PBG-B polymers. First, BLG-NCA was polymerized in DMF with trimethylenediamine as initiator at an A/I of 50. Then an aliquot of the polymerization mixture was used to initiate the polymerization of NCA dissolved in dioxane, with the final value of A/I being adjusted to the desired one. Figure 2 shows the relation between A/I and molecular weight for the PBG-B samples synthesized in this way. The solid line represents the theoretical relation expected for the ideal reaction scheme, and the experimental data are seen to follow it closely up to an N as high as 1000. This, along with the above kinetic evidence, provides an indirect but strong support to our assertion that the PBG-B polymers thus prepared are of the assumed structure: two polypeptide chains of equal length jointed by an initiator residue in a head-to-head fashion.

It had been shown previously[2,6] that helix–coil transition curves of PBG-B samples provided a possible means for confirming their structure. In a thermally induced helix–coil transition, a PBG-B molecule is expected to behave in the same way as does a PBG-A molecule of half its molecular weight, because the two polypeptide chains in the PBG-B molecule separated by the initiator residue will independently participate in helix formation. This expectation was gratified with the present PBG-B samples of higher molecular weights, as had been the case with the lower molecular weight ones examined previously.[6]

The data for polymerization kinetics and mechanism and helix–coil transition presented above all conform to the conditions for the assumed structure of PBG-B. Previous studies[2,5] in which use was made of the samples prepared in dioxane failed to reach such a stage of confirmation, because some observations violated the necessary conditions.

Treatment of Dielectric Data. Dielectric dispersion of a solution characterized by a single relaxation time τ obeys Debye's equations

$$\Delta\epsilon' = \epsilon_\infty + [\Delta\epsilon/(1 + \omega^2\tau^2)]$$

$$\Delta\epsilon'' = \Delta\epsilon\omega\tau/(1 + \omega^2\tau^2)$$

where $\Delta\epsilon = \epsilon_s - \epsilon_\infty$, with ϵ_s being the static dielectric constant of the solution and ϵ_∞ the dielectric constant in the region of high frequency where only the solvent molecules contribute to the orientation polarization. A plot of $\Delta\epsilon''$ *vs.* $\Delta\epsilon'$, called the Cole–Cole plot, forms a semicircle with its

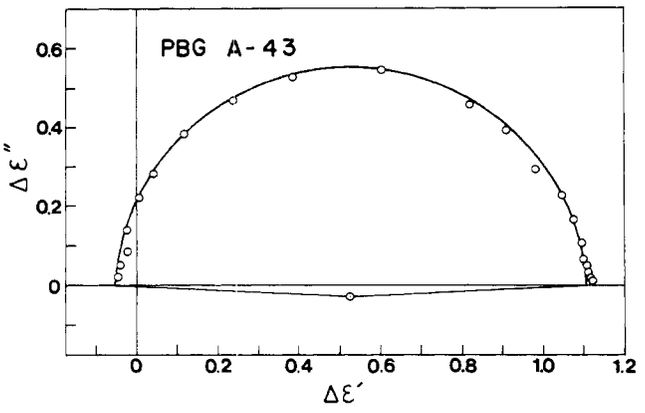

Figure 4. Cole–Cole plot for the data given in Figure 3.

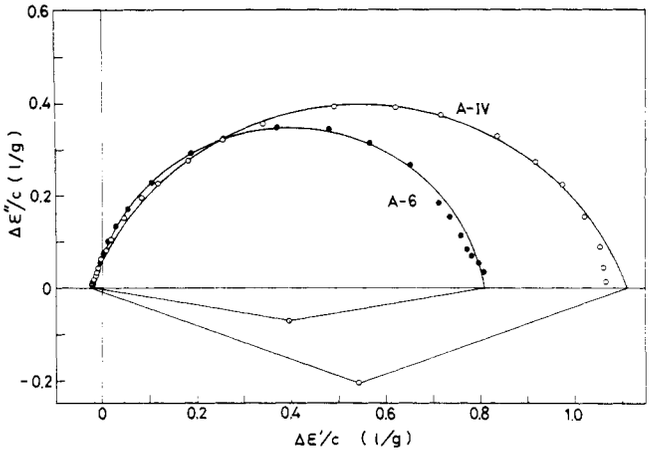

Figure 5. Cole–Cole plots for typical PBG-A samples in *m*-cresol at 25 °.

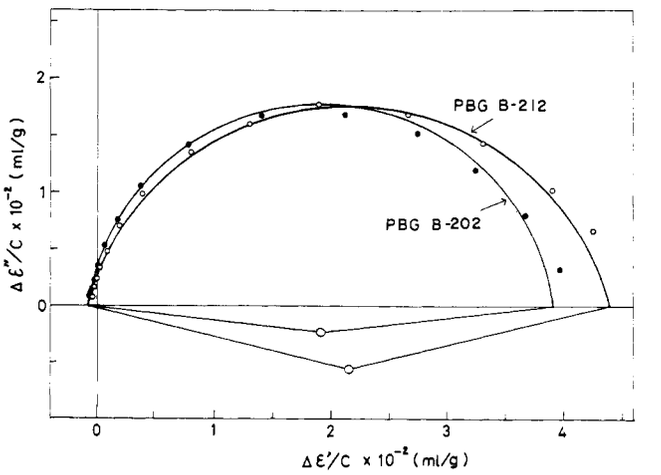

Figure 6. Cole–Cole plots for typical PBG-B samples in *m*-cresol at 25 °.

center on the $\Delta\epsilon'$ axis, and the segment cut out of the horizontal axis by the circle, *i.e.*, the diameter of the circle, gives $\Delta\epsilon$. Such an ideal Cole–Cole plot has never been reported for actual polymer systems, and it has been attributed to probable molecular-weight heterogeneities of the samples investigated.[7-9,14-16] For actual analyses of experimental data, the Cole–Cole plot has such merits that (1) difficulties arising from the electrode polarization at low frequencies can be avoided, (2) $\Delta\epsilon$ may be estimated even from a part of the dispersion curve, and (3) consistency of data between $\Delta\epsilon'$ and $\Delta\epsilon''$ can be checked. Therefore, in the present study, all the dielectric data were treated by Cole–Cole plots.

Data for PBG-A. Typical dielectric data for a PBG-A sample (A-43) in *m*-cresol are illustrated in Figure 3, where the capacitance increment corrected for the lead capacitance, ΔC, and the corrected conductance increment G are plotted against the logarithm of frequency f. It can be seen that the capacitance curve has two flat portions separated by a distinct dispersion region located around 30 kHz. The capacitance displays a gradual change below 1 kHz, which may be due to an electrode polarization originating from conductive impurities. The conductance also shows a dispersion in the same frequency range, and at low frequencies it approaches a constant value which may be taken to be G_d. Figure 4 shows a Cole–Cole plot constructed from the data given in Figure 3. All the data points fall on a semicircle with its center slightly below the $\Delta\epsilon'$ axis, thus almost confirming to a perfect Debye dispersion. A small upward deviation of the data points at low frequencies (on the right-hand side) arises from the electrode polarization. The average relaxation time τ was computed by $\tau = (2\pi f_c)^{-1}$, where f_c is the frequency corresponding to the crest of the arc. Figure 5 shows similar data for samples A-6 and A-IV, both of which have broader molecular-weight distributions than A-43. The center of each arc is displaced appreciably below the $\Delta\epsilon'$ axis. These data confirm the previous authors' inference[7-9,14-16] that polydispersity may give rise to non-Debye type dispersions frequently encountered with polypeptides and suggest that the Cole–Cole plot may be used as a sensitive measure of heterogeneity in molecular weight.

In most of our experiments, values of ΔC were larger than 20 pF and could be determined to within ±0.5 pF. The determining factor for the accuracy of ΔC appeared to be the magnitude of electrode polarization, which could be suppressed considerably by using purified samples or working at higher frequencies but which in no case could be completely neglected. In effect, an accuracy in ΔC of ±5% was achieved in worse cases.

Effects of solute concentration of dielectric dispersion were examined for A-3, A-6, and A-IV in *m*-cresol and for A-6 in *m*-cresol containing EDC or chloroform. Except for A-IV, specific dielectric increments $\Delta\epsilon/c$ and relaxation times τ were independent of c. The values of $\Delta\epsilon/c$ for A-IV increased appreciably with decreasing concentration, and therefore the infinite dilution value $(\Delta\epsilon/c)_0$ was obtained by extrapolation. For other PBG-A samples, data were taken at a single concentration about 5×10^{-3} g/ml.

Data for PBG-B. Measurements on *m*-cresol solutions of PBG-B samples having low molecular weights yielded $\Delta\epsilon$ accurately. On the contrary, *m*-cresol solutions of high-molecular-weight PBG-B samples presented difficulties because their dispersion regions were located at relatively low frequencies where the electrode polarization became pronounced. Since use of mixtures of *m*-cresol and chloroform as solvent displaced the dispersion region to a higher frequency range and largely suppressed the effect of electrode polarization, the specific dielectric increment and relaxation time could be determined with reasonable accuracy. Figures 6 and 7 demonstrate typical Cole–Cole plots for PBG-B samples in *m*-cresol and in a mixture of *m*-cresol and chloroform (80 vol % $CHCl_3$), respectively. Samples B-202 and 212 appeared to be nearly monodisperse in molecular weight when judged from their $\bar{M}_w/\bar{M}_n$ values. However, their Cole–Cole plots exhibit broad distributions of relaxation times. Other PBG-B samples behaved similarly. Thus a PBG-B sample has a broader distribution in relaxation times than a PBG-A sample of similar molecular-weight heterogeneity. This seems to be consistent with the

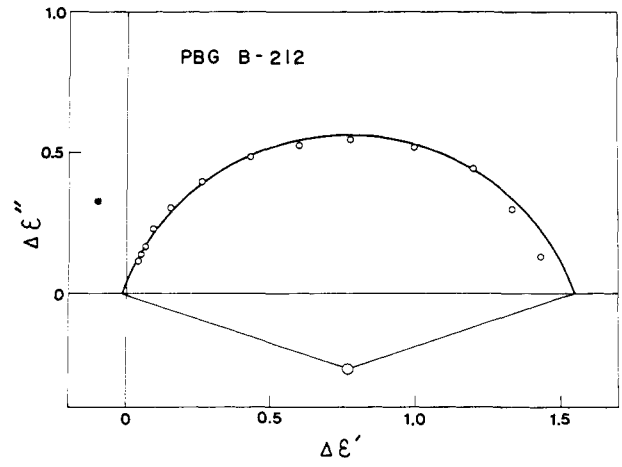

Figure 7. Cole–Cole plot for sample B-212 in a $CHCl_3$–*m*-cresol mixture (80 vol % $CHCl_3$) at 30°.

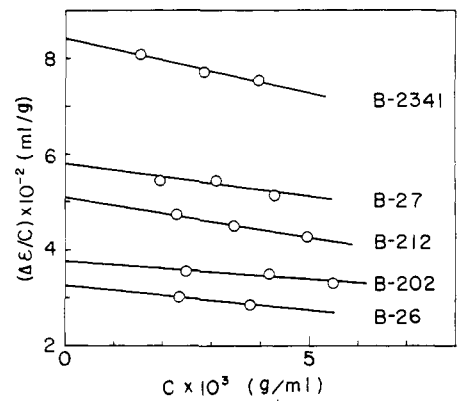

Figure 8. Concentration dependence of specific dielectric increment $\Delta\epsilon/c$ for PBG-B in a $CHCl_3$–*m*-cresol mixture (80 vol % $CHCl_3$).

structure of PBG-B polymer, which has some degree of internal freedom and hence has a multiplicity in relaxation time.

The concentration effect was more remarkable for PBG-B than for PBG-A. Figure 8 shows plots of $\Delta\epsilon/c$ *vs.* c for PBG-B in a *m*-cresol–$CHCl_3$ mixture (80 vol % $CHCl_3$). As was the case with A-IV in *m*-cresol, $\Delta\epsilon/c$ decreased with c in every case studied, while the relaxation time increased with c. Therefore, the infinite-dilution values of $\Delta\epsilon/c$ and τ were obtained by extrapolation. Table III summarizes all the numerical data.

Evaluation of Dipole Moment. The system under study, in which a rodlike macromolecule having a permanent dipole was immersed in a polar liquid, posed the problem of how the "vacuum" dipole moment μ of the molecule can be calculated from the static dielectric increment $\Delta\epsilon$. It was for the first time considered by Wada,[7-9] who derived the following expression similar to that of Oncley[22]

$$\mu^2 = \frac{3kTM(\Delta\epsilon/c)_0}{4\pi N_A} \tag{1}$$

where M is the molecular weight of the solute, k is the Boltzmann constant, T is the absolute temperature, N_A is Avogadro's number, c is the solute concentration in grams per milliliter, and $(\Delta\epsilon/c)_0$ is the specific dielectric increment at infinite dilution. This equation does not take effects of the molecular shape and the surrounding medium on the induced polarization into account. Buckingham[23] took such effects into account in calculating the "vacuum" dipole moment of an ellipsoidal molecule embedded in a polar medium. Recently, Applequist and Mahr[11] recast Buckingham's equation in the following form

$$\frac{4\pi N_A fg\mu^2 c}{3kTM} = \frac{(2\epsilon_s + 1)(\epsilon_s - n^2)}{(2\epsilon_s + n^2)} - \frac{(2\epsilon_\infty + 1)(\epsilon_s - n^2)}{(2\epsilon_s + n^2)} \tag{2}$$

where n is the refractive index of the solution and fg is the parameter given by

$$fg = \frac{\epsilon_s(2\epsilon_s + 1)}{3}\left[\frac{1 + (n_2^2 - 1)A}{\epsilon_s + (n_2^2 - \epsilon_s)A}\right]^2 \tag{3}$$

Here n_2 is the refractive index of the solute and A is the shape factor which depends on the axial ratio p of the molecule as

$$A = \left\{\frac{p}{2(p^2 - 1)^{1/2}} \times \ln\left[\frac{p + (p^2 - 1)^{1/2}}{p - (p^2 - 1)^{1/2}}\right] - 1\right\}(p^2 - 1)^{-1} \tag{4}$$

At the limit of infinite dilution, eq 2 can be rewritten

$$\mu^2 = (3kTM/4\pi N_A)(q/fg)(\Delta\epsilon/c)_0 \tag{5}$$

where

$$q = \frac{4\epsilon_0^2 + 4n_0^2\epsilon_0 - 2n_0^4 + 3n_0^2}{(2\epsilon_0 + n_0^2)^2} \tag{6}$$

with fg in which the dielectric constant ϵ_0 of the solvent is substituted for ϵ_s, and n_0 is the refractive index of the solvent. Equation 5 differs from eq 1 by a factor of q/fg, which tends to 3/2 for large ϵ_0. Erenrich and Scheraga[16] used this limiting value. The value of n_2 needed for this analysis may be equated to that of a particular solvent for which the specific refractive index increment of the solute vanishes. We deduced an n_2 of 1.592 from the data by Strazielle, *et al.*[24] We assumed that the axial ratio p was given by $\bar{N}_w/10$ for PBG-A and $\bar{N}_w/15$ for PBG-B, where $\bar{N}_w$ is the weight-average degree of polymerization of the sample. The assumption for PBG-A was based on the molecular dimensions determined from intrinsic viscosity by Doty, *et al.*[25] The value of p for PBG-B was simply the average of the two values which the molecule would have if it were replaced by a single straight rod and by two individual rods of half its $\bar{N}_w$. In any event, no exact p values are necessary for the present purpose, because the shape factor A appears as a correction in eq 3 and rapidly vanishes with increasing p. It should be noted that the value of μ obtained in this way is actually the root-mean-square dipole moment defined as $\mu = \langle\mu^2\rangle^{1/2}$. The validity of Buckingham's equation has been tested with low-molecular-weight organic liquids and liquid mixtures,[23] but no such test has as yet been reported for polymer systems. Therefore, we used both eq 1 and 5 with $\bar{M}_w$ substituted for M to evaluate μ. All the numerical data are summarized in Table III.

Effects of Solvent Polarity and Temperature on Dipole Moment. Table IV compares the data for sample A-6 in three helicogenic solvents having different dielectric constants. The mean relaxation times corrected for the solvent viscosity η_0 and temperature are constant within experimental errors. The specific dielectric increments show no systematic variation with ϵ_0 in the range between 5.14

Table III
Dielectric Data of PBG-A and PBG-B at Infinite Dilution[a]

Sample	Temp, °C	$(\Delta\epsilon/c)_0$, ml g^{-1}	$\tau T/\eta_0 \times 10^2$, sec deg P^{-1}	μ, D		μ_h, D	
				Wada	A-M	Wada	A-M
			In *m*-Cresol				
A-2	0.4	1.213	0.0613	94.4	78.1	4.35	3.60
A-3	0.3	2.218	0.184	188	196	3.88	4.06
	10.0	2.157	0.174	187	196	3.86	4.06
	25.0	2.203	0.171	195	204	4.03	4.21
A-43	10.0	3.201	0.352	269	296	4.04	4.44
	25.0	3.218	0.345	277	303	4.15	4.55
	40.0	3.231	0.340	284	310	4.26	4.66
A-5	25.0	4.363	1.21	405	463	3.85	4.41
	39.9	4.266	1.05	410	466	3.90	4.44
	60.1	4.290	1.08	424	480	4.04	4.57
An-22	25.0	7.841	3.9	658	769	4.27	4.99
A-6	9.9	8.450	4.07	696	818	4.12	4.84
	25.1	8.348	4.20	710	831	4.20	4.92
	40.0	8.156	3.67	719	838	4.26	4.96
	59.8	8.092	3.59	739	856	4.37	5.07
A-IV	9.9	12.45	16.5	1104	1313	3.81	4.53
	25.1	12.55	15.2	1143	1353	3.94	4.67
	40.0	12.45	13.6	1165	1374	4.02	4.74
	59.8	12.90	13.3	1214	1422	4.19	4.90
B-2	0.3	1.669	0.210	198	211	2.76	2.95
	10.0	1.812	0.210	210	224	2.93	3.12
	25.0	1.866	0.198	219	232	3.05	3.24
B-12	15.0	2.305	0.440	283	315	2.82	3.13
	25.0	2.386	0.449	293	325	2.91	3.23
	40.0	2.339	0.405	297	328	2.96	3.27
B-3V	10.0	2.757	1.10	385	445	2.43	2.81
	25.0	2.757	1.17	395	455	2.49	2.87
	40.0	2.876	1.10	414	474	2.61	2.99
B-26	25.0	3.71	1.17	486	535	3.03	3.34
	40.0	3.84	1.13	507	555	3.16	3.46
B-202	25.0	3.74	2.3	586	682	2.28	2.65
	40.0	4.154	2.1	632	740	2.46	2.88
B-212	25.0	4.45	6.87	766	914	2.05	2.44
	40.0	4.25	6.25	773	912	2.07	2.44
B-27	25.0	7.11	11.0	1080	1280	2.36	2.80
			In *m*-Cresol–$CHCl_3$, 80 vol % $CHCl_3$				
E-4	30.0	13.65	18	1350	1570	3.66	4.25
B-26	30.0	3.28	1.2	440	490	2.75	3.06
B-202	30.0	3.81	3.8	600	680	2.34	2.65
B-212	30.0	5.07	9.1	830	950	2.22	2.54
B-27	30.0	5.80	9.0	990	1130	2.17	2.47
B-2341	30.0	8.40	42	1310	1510	2.33	2.69
B-223	30.0	9.05	53	1530	1760	2.15	2.47
B-243	30.0	14.9	120	2430	2790	2.24	2.57

[a] Terms "Wada" and "A-M" refer to the values calculated by eq 1 and eq 5, respectively.

and 13.16. This is also the case with the dipole moments deduced therefrom. Irrespective of solvent polarity, the values of μ evaluated by eq 1 and 5 differ by a factor of 1.16 ± 0.01. As far as these data are concerned, we find no reason to prefer one of the two equations over the other. On the contrary, there have been reported dielectric data which indicate that the dipole moment of PBLG in the helical conformation depends significantly on solvent.[7-9,13-15] We can give no explanation for the disparity between our results and those of these authors. Wada[7-9] interpreted such a dependence in terms of the effect of reaction field, whereas Block, *et al.*,[14] suggested it to be a reflection of solvation.

Values of $(\Delta\epsilon/c)_0$ increased or decreased slightly as the temperature was raised from 10 to 60°, but the dipole moment showed a slight but definite increase in every case investigated. This observation is in accord with reported results and may be attributed to the side-chain effect as dis-

Table IV
Dielectric Data for A-6 in Solvent Mixtures Having Different Dielectric Constants

Solvent	Temp, °C	ϵ_0	$(\Delta\epsilon/c)_0$, ml g^{-1}	$\tau T/\eta_0 \times 10^2$, sec deg P^{-1}	μ, D[a] Wada	A-M
m-Cresol	25.1	13.09	84.5	4.07	710	835
m-Cresol–EDC[b]	30	9.436	83.3 ± 2	3.8	715	834
m-Cresol–$CHCl_3$[c]	30	5.16	80 ± 5	4.2	700	800

[a] "Wada" refers to values calculated by eq 1 and A-M to those calculated by eq 5. [b] 80% EDC by volume. [c] 80% $CHCl_3$ by volume.

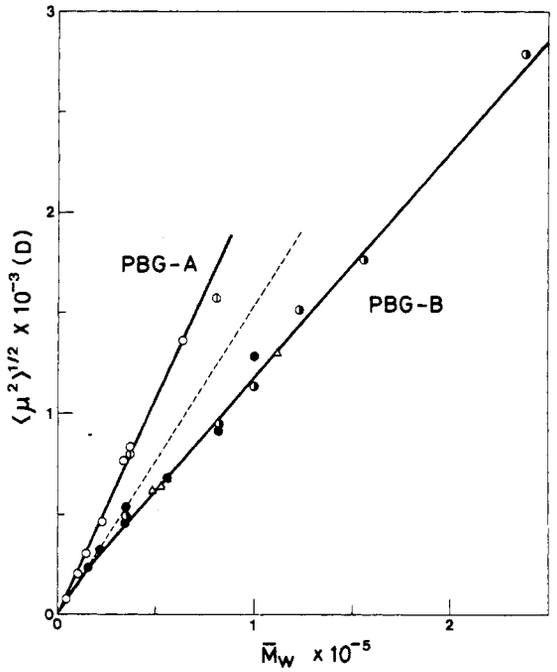

Figure 9. Molecular weight dependence of dipole moment for PBG-A and PBG-B evaluated by eq 5. The solid and dashed lines represent the theoretical relations for the straight and once-broken rods with μ_h of 4.7 D, respectively: ○ and ●, in *m*-cresol; ⊕ and ◑, in a $CHCl_3$–*m*-cresol mixture (80 vol % $CHCl_3$); △, data of Kihara, *et al.*,[5] in *m*-cresol.

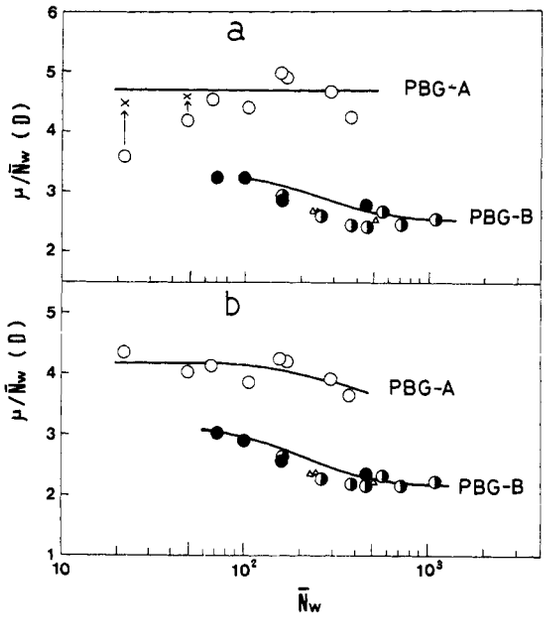

Figure 10. Dipole moments per monomeric residue plotted against $\bar{N}_w$: (a) values computed by eq 5 and (b) values computed by eq 1.

cussed by Wada and Kihara,[10] because PBLG in helicogenic solvents must be essentially helical in this temperature range.[26,27]

Molecular Weight Dependence of Dipole Moment. Figure 9 shows the molecular weight dependence of μ for both PBG-A and PBG-B evaluated by means of eq 5. The data points for PBG-A follow a straight line, yielding an average value of 4.7 ± 0.2 D for the dipole moment per monomeric residue, μ_h. This implies that the helical structure of PBG-A is rigid in the molecular weight range studied. The dashed line represents the relation expected for freely jointed once-broken rods with μ_h = 4.7 D. The data points for PBG-B appear below the dashed line and are in excellent agreement with the recent data by Kihara, *et al.*[5] Since a PBG-B molecule consists of two rigid dipoles jointed by a flexible initiator portion in a head-to-head fashion, the deviation from the dashed line may be attributed to the fact that the average angle between the two dipoles exceeds 90°. Two helical rods of about 15 Å in diameter connected by a short initiator residue may not rotate independently due to steric hindrances, even though the individual single bonds in the joint portion are allowed to rotate freely.[5]

The molecular weight dependence of μ can be more clearly illustrated in Figure 10, where the dipole moment per monomeric residue, $\mu_h = \mu/\bar{N}_w$, is plotted against weight-average degree of polymerization. In the case of PBG-A, the value of μ_h computed by eq 5 exhibits a tendency to decrease at low molecular weights, while that calculated by eq 1 stays almost constant. This latter dependence seems to be in contradiction with ORD data,[27] which indicate that in *m*-cresol the helical conformation of PBLG is destabilized due to unfolding from chain ends if $\bar{N}_w$ is lower than 50; the helical contents for two PBG-A samples of the lowest molecular weights are about 0.71 and 0.91, respectively. The crosses in Figure 10a represent the values corrected for the chain unfolding by means of Nagai's theory[28] on the assumption that only the helical part is responsible for the orientation polarization. They now come to the level of 4.7 ± 0.2 D. Therefore we may conclude that eq 5 is a better representation than eq 1 as far as the shape factor is concerned, and the dipole moment values evaluated by eq 5 will be used in the ensuing discussion. However, their absolute values still leave room for discussion and should be examined by using less polar solvents such as *trans*-1,2-dichloroethylene employed by Block, *et al.*[14]

The data of Wada[7] for relatively high-molecular-weight PBLG in EDC, when reanalyzed by means of eq 5, yielded an average value of 4.0 D for μ_h. Erenrich and Scheraga[16] reported a value of 4.11 D for $\bar{N}_w$ = 1100. These low μ_h for high-molecular-weight samples, together with our data for PBG-A, confirm the previous authors' conclusion[10,13,14] that the helical polypeptide will get a certain degree of flexibility as its chain length becomes longer, which is in harmony with reported light-scattering data for helical polypeptides.[25,29-34]

The apparent μ_h defined by $\mu_h = \mu/\bar{N}_w$ for PBG-B approaches at large $\bar{N}_w$ an asymptotic value of about 2.5 D,

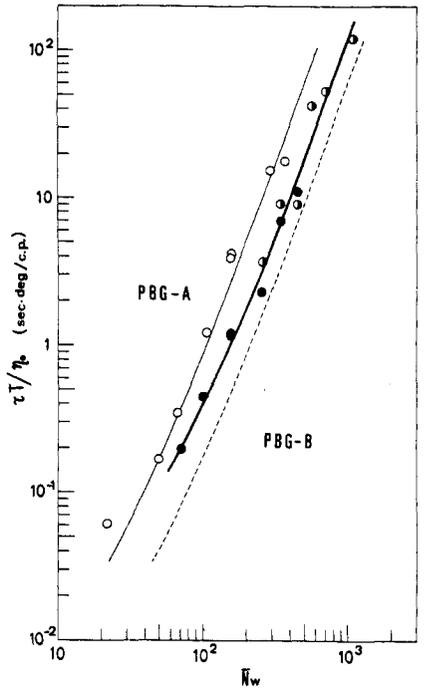

Figure 11. Double logarithmic plots of mean relaxation time *vs.* $\bar{N}_w$. The solid line represents the Perrin's relation for ellipsoids of revolution with the major axis of $1.5\bar{N}_w$ in Å and the minor axis of 19.1 Å, and the dashed line represents the relation for the ellipsoids of half their lengths.[35] The symbols are the same as those in Figure 9.

which is nearly half that for PBG-A. This is a manifestation of the restricted rotation of the constituting helical rods that has been discussed above. As seen in Figures 9 and 10, the recent data of Kihara, *et al.*,[5] come close to the present data. The value of μ_h is seen to go up as $\bar{N}_w$ is decreased, whichever equation is used for the data analysis.

Molecular-Weight Dependence of Mean Relaxation Time. Figure 11 shows double logarithmic plots of mean relaxation time *vs.* $\bar{N}_w$. Here the solid line represents Perrin's relation for prolate ellipsoids of revolution with the major axis = $1.5\bar{N}_w$ Å and the minor axis = 19.1 Å.[35] Except for the lowest-molecular-weight sample, the data points follow this theoretical curve closely, which verifies the rigidity of the helical conformation in the molecular-weight range studied. Wada's data as well as that of Erenrich and Scheraga[16] fall slightly below the theoretical curve. Thus the data for relaxation time are compatible with the conclusion from the dipole moment data that PBLG in helicogenic solvents gains flexibility at higher molecular weights.

The relaxation times of PBG-B display a molecular weight dependence similar to those of PBG-A, but they are smaller by a factor of 3 or 4 than those of the latter when compared at the same molecular weights. For all samples of PBG-B examined, the relaxation times were larger than those for the PBG-A molecules of half their molecular weights (shown by the dashed line in Figure 11), as should be expected. These results do not agree precisely with the theoretical prediction by Yu and Stockmayer[3] that the longest relaxation time of a once-broken rod is half that of a straight rod of equal total length at the limit of infinite length. This disparity may be due to a possible difference between mechanical and dielectric averages of relaxation times.

It is relatively easy to determine from measurements of the molecular weight dependence of μ or τ whether the molecule is *rodlike* or not. However, it is by no means simple to ascertain from such measurements whether the molecular shape is essentially *straight*.

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