Dielectric Study of Polypeptides in Solution. I. Aggregation of Poly(γ -benzyl-L-glutamate) in Ethylene Dichloride

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Poly(γ -benzyl-L-glutamate) (PBLG) takes on a α -helical conformation in ethylene dichloride (EDC),^{1–3} and it was suggested by several authors^{1,4,5} that PBLG molecules formed aggregates in this solvent. Doty, Bradbury and Holtzer¹ reported that the aggregated molecules in EDC were molecularly dispersed by adding formamide to a level of 0.5%. Boeckel, *et al.*,⁵ reported that addition of dimethylformamide (DMF) to 1% was effective. Watanabe⁴ suggested that the aggregation was of the head-to-tail type and consisted of two or three solute molecules. The purpose of the present report is to confirm the existence of the aggregation and to investigate the state of the aggregation in more detail by the method of dielectric dispersion.

PBLG coded 7003 was used⁶ and its degree of polymerization obtained by the relationship of Doty—Bradbury—Holtzer¹ was 340. EDC was distilled with calcium hydride; bp 82.5°C. After distillation, it was dehydrated by shaking with activated alumina. DMF was distilled under reduced pressure and dehydrated in the same way.

Dielectric dispersions of solvents and solutions were measured by a transformer bridge (Ando Denki Co., Tokyo, Japan) in the frequency range from 30 to 3 MHz. A platinum concentric cylinder with an air capacitance of 4.707 pF was used as a liquid cell and calibrated with benzene. During the measurements the cell was kept in a Dewar vessel containing a transformer oil, the temperature of which was maintained at 25°C $\pm$ 0.01°C.

Figure 1. Reduced viscosity of PBLG in EDC with a small fraction of DMF as a function of ϕ (vol % of DMF to EDC): The solute concentration, 6.9×10^{-3} g/ml; $\circ$, data in EDC—DMF mixtures; temp, 25°C; $\bullet$, data in DMF; temp, 25°C.

Figure 1 shows the dependence of the reduced specific viscosity of sample 7003 in EDC—DMF mixtures at 25°C on the vol % ϕ of DMF. According to Boeckel, *et al.*,⁵ this result may be taken to suggest that aggregates present in pure EDC become molecularly dispersed by the addition of DMF up to 1%.

Values for the dipole moment of the solute molecule were computed from the zero- and infinite-frequency limits of the dielectric constants of the solution, ϵ_0 and ϵ_∞ , by the equation due to Applequist and Mahr:⁷

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

Figure 2. Dipole moments per one residue, μ_0 , at 25°C of PBLG in EDC with a small fraction of DMF as a function of ϕ : The solute concentrations, $\circ$, 1.14×10^{-3} g/ml; $\bullet$, 9.7×10^{-4} g/ml. Dipole moments are calculated from Applequist and Mahr equation.⁷

Figure 3. Rotational relaxation times, τ , at 25°C of PBLG in EDC with a small fraction of DMF as a function of ϕ . They are reduced under the condition of 1 poise. The symbols are same as those in Figure 2.

where N =Avogadro's number, C =solute concentration in g/ml, k =Boltzmann's constant, μ =dipole moment of the solute, M =molecular weight of the solute, T =absolute temperature, and n =refractive index of the solution. Here f and g are constants determined by the molecular dimension of the solute and the dielectric constant and refractive index of the solvent. The values $f=2/3$ and $g=1$ ⁸ were adopted in the present analysis.

Figure 2 shows the dipole moment per peptide residue μ_0 plotted vs. ϕ . The values of μ_0 stay constant over the entire range of ϕ examined. This implies that the aggregation of solute molecules, even if present, has little influence on

the average dipole moment.

Figure 3 illustrates the dependence on ϕ of the rotational relaxation time τ of the solute calculated from the critical frequency f_{crit} by $\tau=1/(2\pi f_{crit})$. It can be observed that τ changes appreciably in the region around $\phi=1.0$. This behavior cannot be expected for a molecularly dispersed solute, as is discussed below. The relaxation time of a dispersed solute depends on the rigidity of the backbone chain as well as on the conformation of the side chains. It has been shown in a preceding paper⁶ that the persistent length of PBLG in a helicogenic solvent *m*-cresol is about 1000 Å, which is longer than the total helix length of the PBLG used here. We may, therefore, rule out the possibility that the change in τ is caused by change in the rigidity of the backbone chain. The side chain conformation is not likely to have such a large effect on the change in τ .

These considerations lead us to conclude that the change in τ with ϕ for the system PBLG—EDC—DMF reflects the change in the state of aggregation, with the dipole moment being kept constant at the value for dispersed solutes. Plausible states of aggregation may be: (1) the head-to-tail type with flexible hinges, (2) the linear-rigid type with random direction, (3) the side-by-side type with random direction. The last type of aggregate may be rejected because it should show a faster relaxation than unaggregated molecules. From structural considerations, PBLG is not likely to aggregate in head-to-head fashion. Therefore, the type (1) is most probable, as suggested by Watanabe.⁴

The data in Figure 3 show that τ in the aggregated state is larger by a factor of about two than that in the dispersed state. Yu and Stockmayer¹² calculated τ of a hinged polymer composed of two equal rods connected by a flexible joint to find that τ is four times as large as that of a straight rod having half its total length. This leads to the supposition that the number of polymer molecules per aggregate is two or three and is in equilibrium with molecularly dispersed molecules, in support of Watanabe's conclusion.⁴

The value of μ_0 was estimated to be 4.8 D/residue, which is larger than the 4.11 D in EDC of Erenrich and Scheraga,⁸ the 4.2 D of Scharp

in 1,2-dichloroethene⁹ and the 3.4 *D* of Wada in EDC.^{3,10} Taking into account the difference in the calculation methods the last three values are in rather good agreement with each other. On the other hand, Matsumoto, *et al.*,¹¹ reported that $\mu_0=4.7 D$ in *m*-cresol, which is in good agreement with the data of the present report. The difference between the two values, 4.11 *D* and 4.8 *D*, may be attributed to the difference in molecular weight, because the molecular weight of the sample used by Erenrich and Scheraga is much larger than those used by Matsumoto, *et al.*¹¹ and by the present author.

The dipole moment of the same sample in chloroform was smaller than that in EDC, as found by Marchal and Marchal.¹³ After dissolving in chloroform, the sample was freeze-dried in dioxane. The dipole moment of the so treated sample was smaller than that of the original sample. This suggests that the sample had undergone degradation in chloroform, presumably due to the presence of phosgene.

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