NOTES

Study on the Fluorescence Depolarization of a Chromophore Attached to Polymer Chain End III. Aggregation and Helix-Coil Transition Behavior of Poly(γ -benzyl L-glutamate) in *m*-Cresol and Benzene at Various Temperatures

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Our previous studies on the aggregation and helix-coil transition behavior in the vicinity of the chain end group of poly(γ -benzyl L-glutamate) (PBLG) have shown that the apparent mean rotational relaxation time of a chromophore attached to the chain end of PBLG corresponded to the aggregation behavior of whole PBLG molecules in various helicogenic solvents.^{1,2}

Thermally induced transitions of solvated PBLG molecules from a helix to a random coil conformation have been investigated by well-known methods (heat capacity,³ dielectric relaxation,⁴ optical rotation,⁵⁻⁸ etc). According to Sayama *et al.*,⁶ the helix-coil transition behavior from a random coil to a helix state of PBLG (DP=584) in a dichloroacetic acid (DCA)-ethylene dichloride (EDC) (65:35) mixture begins at about 10°C. The stability of the random coil at lower temperatures is attributed to the selective binding of DCA to PBLG in the mixed solvent which breaks down the peptide hydrogen bonds. Raising temperature decreases the extent of this binding, thus shifting the equilibrium in favor of the helical

form. So the study on the helix-coil transition behavior of PBLG at higher temperatures in mixed solvents presents a special interest in view of the above-mentioned behavior at lower temperatures.

The apparent mean rotational relaxation time of PBLG having a rhodamine B bound at the chain end was measured as a function of temperature in order to study the aggregation and helix-coil transition behavior of PBLG at high temperatures. The present communication describes the results obtained.

Fluorescence spectra and fluorescence depolarization were measured on a JASCO FP-550 spectrofluorometer with polarizing filters. The fluorescent intensity was determined at 585 nm (RhB-NCS in *m*-cresol), 582 nm (PBLG260RhB in *m*-cresol), 576 nm (PBLG480RhB in benzene), and 583 nm (PBLG480RhB in a benzene-DCA (3:2) mixture) using 550 nm as the excitation wavelength, where RhB-NCS represents 5-isothiocyantorhodamine B. In the notation of the samples, the figures between PBLG and RhB *e.g.*, PBLG260RhB mean the approx-

imate degree of polymerization. The temperature was controlled by an Oxford DN704 cryostat.

Fluorescence depolarization of PBLG-RhB was measured at a polymer concentration ranging from 10^{-6} M to 10^{-5} M (0.01 – 0.1 g dl $^{-1}$). Dotite-Luminasol-grade benzene was used without further purification.

The viscosities of *m*-cresol, benzene, and a benzene–DCA (3:2) mixture at various temperatures were measured by an Ubbelohde viscometer in a thermostat.

The emission anisotropy r can be calculated from

$$r = (I_{vv}I_{hh} - I_{vh}I_{hv}) / (I_{vv}I_{hh} + 2I_{vh}I_{hv}) \quad (1)$$

where I is the observed fluorescent intensity of the rhodamine B chromophore, the subscripts v and h denote vertically and horizontally polarized light, and the first letter in the subscript refers to the exciting light and the second to the emitted light. The emission anisotropy r is related to mean rotational relaxation time ρ by

$$r_0/r = 1 + (3\tau/\rho) \quad (2)$$

where τ is the excited singlet lifetime of the chromophore and r_0 is the emission anisotropy (RhB–NCS; $r_0^{-1} = 3.0$) in rigid solution.² If the rate constant k_f of fluorescence emission process is practically independent of temperature, the ratio I_f/I_f^0 is proportional to τ/τ_0 by the relationship of $I_f \propto k_f\tau$, where the subscript and superscript 0 represent the standard, because the rate constant k_f is little influenced by the temperature.^{9,10} Consequently, the ratio ρ/τ_0 at various temperatures can be expressed by

$$\rho/\tau_0 = 3I_f r_0^{-1} / I_f^0 (r^{-1} - r_0^{-1}) \quad (3)$$

The plots for ρ/τ_0 values of RhB–NCS and PBLG260RhB vs. temperature in *m*-cresol are shown in Figure 1. For a globular spherical rotational body, eq 3 can be transformed into eq 4 using the relation of $\rho = 3V\eta/kT$,

$$\rho/\tau_0 = (3V/k\tau_0)(\eta/T) \quad (4)$$

Figure 1. Graph for ρ/τ_0 against temperature of RhB–NCS and PBLG260RhB in *m*-cresol: solid line, RhB–NCS; dashed line, PBLG260RhB.

Figure 2. Dependence of ρ/τ_0 on η/T for RhB–NCS and PBLG260RhB in *m*-cresol: (▼), RhB–NCS; (●), PBLG260RhB.

where V is the globular rotational volume, η is the solvent viscosity, k is the Boltzmann constant, and T is the absolute temperature. The ρ/τ_0 values for the apparent mean rotational relaxation time of PBLG260RhB differ from that of a small molecule model RhB–NCS up to about 110°C . *m*-Cresol is well-known as a helicogenic solvent for PBLG.¹¹ Since the terminal rhodamine B group as a chromophore is bound to the PBLG chain end tightly, the fluorescence depolarization of PBLG–RhB in helicogenic solvents reflects the mean rotational relaxation time of the rod-like polymer as a whole.^{1,2} The plots of ρ/τ_0 vs. η/T for RhB–NCS and PBLG260RhB in *m*-cresol

are shown in Figure 2. According to eq 4, such a plot must be a straight line passing through the origin when the globular rotational volume does not change with the solvent condition. Therefore, the slopes in Figure 2 represent the $3V/k\tau_0$ values. Figure 2 shows that the globular rotational volume of RhB-NCS does not change with rising temperature because the curve of RhB-NCS is linear at temperatures between 40 and 170°C. On the other hand, the slope of the plot for PBLG260RhB changes in the temperature range of 90–110°C in Figure 2. The slope for the plots of ρ/τ_0 vs. η/T for PBLG260RhB in the range of 110–170°C shows the random-coiled state in the vicinity of the chain end group because of the fitness of the lines of RhB-NCS and RBLG260RhB within the range of 110–170°C. It is therefore considered that the globular rotational volume as the vicinity of the chain end group of PBLG260RhB decreases sharply in *m*-cresol at 90–110°C, indicating that the helix-coil transition in peptide groups near the chain end carrying a rhodamine chromophore occurs in this temperature range.

It is well-known that PBLG highly aggregates in benzene, and DCA is a helix-breaking solvent.¹¹ Moreover, the thermally induced helix-coil transition of PBLG in the helicogenic solvent-DCA mixtures proceeds from a random coil to a helix conformation with rising temperature.⁶ The plots of ρ/τ_0 against temperature for PBLG480RhB in benzene and in a benzene-DCA (3:2) mixture are shown in Figure 3. In either solvent, ρ/τ_0 decreases on raising the temperature. However, ρ/τ_0 changes differently in the two solvents studied.

The ρ/τ_0 values of PBLG480RhB in benzene and in a benzene-DCA (3:2) mixture at various temperatures are listed together with the $3V/k\tau_0$ values in Tables I and II, respectively. The plots of ρ/τ_0 vs. η/T for PBLG480RhB in benzene and in the benzene-DCA (3:2) mixture are shown in Figure 4. It seems that the

Figure 3. Graph for ρ/τ_0 for PBLG480RhB against temperature in benzene and in a benzene-DCA (3:2) mixture: solid line, in a benzene-DCA (3:2) mixture; dashed line, in benzene.

Table I. Emission anisotropy and apparent mean rotational relaxation time of PBLG480RhB at various temperature in benzene^a

Temp °C	η cP ⁻¹	I_f/I_f^0	$1/r$	ρ/τ_0	$3V/k\tau_0$ K cP ⁻¹	A
35	0.53	1.1	11	1.2	6.9×10^4	1.8
40	0.50	1.0	13	0.89	5.6×10^4	1.4
45	0.47	0.86	13	0.73	5.0×10^4	1.3
50	0.44	0.84	14	0.68	5.0×10^4	1.3
55	0.41	0.83	14	0.68	5.4×10^4	1.4
60	0.39	0.82	17	0.50	4.3×10^4	1.1
65	0.37	0.77	19	0.44	3.7×10^4	0.94
70	0.35	0.74	19	0.40	3.9×10^4	1.0

^a η is the solvent viscosity, I_f^0 is the fluorescent intensity at 40°C, and A is the relative ratio of globular rotational volume for PBLG480RhB on the basis of that at 70°C.

curve of PBLG480RhB in benzene indicates the dissociation behavior of aggregated PBLG. According to the ρ/τ_0 values in Table I, the apparent mean rotational relaxation time of PBLG480RhB in benzene gradually decreases by a factor of 3 with rising temperature from 35°C to 70°C. On the other hand, the curve of PBLG480RhB in the benzene-DCA (3:2) mixture shows the helix-coil transition behavior. This is because PBLG little aggregates

Table II. Emission anisotropy and apparent mean rotational relaxation time of PBLG480RhB at various temperatures in benzene–DCA (3:2) mixture^a

Temp °C	η cP ⁻¹	I_t/I_t^0	$1/r$	ρ/τ_0	$3V/k\tau_0$ K cP ⁻¹	A
40	0.77	1.0	20	0.51	2.1×10^4	2.3
45	0.72	0.99	21	0.50	2.2×10^4	2.5
50	0.68	0.98	22	0.46	2.2×10^4	2.5
55	0.64	0.96	27	0.36	1.9×10^4	2.1
60	0.60	0.94	30	0.31	1.7×10^4	1.9
65	0.57	0.89	35	0.25	1.5×10^4	1.6
70	0.54	0.87	53	0.15	9.8×10^3	1.1
75	0.51	0.82	53	0.14	9.8×10^3	1.1
80	0.49	0.80	58	0.13	9.4×10^3	1.1
85	0.46	0.76	61	0.12	8.9×10^3	1.0

^a η is the solvent viscosity, I_t^0 is the fluorescent intensity at 40°C, and A is the relative ratio of globular rotational volume for PBLG480RhB on the basis of that at 85°C.

in the benzene–DCA (3:2) mixture as already indicated.² Since the slope of the plots of ρ/τ_0 vs. η/T for PBLG480RhB in the benzene–DCA (3:2) mixture sharply changes in comparison with that in benzene, the leaping phenomenon of the slope is attributable to the helix-coil transition behavior in the vicinity of the chain end group of PBLG. It is understandable that the helix-coil transition in the peptide group near the chain end carrying a rhodamine chromophore occurs readily in a high temperature region.

In conclusion, the measurements of the emission anisotropy r of RhB–NCS and PBLG–RhB in *m*-cresol, benzene, and a benzene–DCA (3:2) mixture at various temperatures show that helix-coil transition in the vicinity of the chain end group of PBLG occurs in a temperature range of 90–110°C in *m*-cresol and of 50–70°C in the benzene–DCA (3:2)

Figure 4. Dependence of ρ/τ_0 on η/T for PBLG480RhB in benzene and in a benzene–DCA (3:2) mixture: (O), in benzene; (●), in a benzene–DCA (3:2) mixture.

mixture, and the dissociation of aggregated PBLG in benzene readily proceeds with rising temperature.

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