Liquid Crystal Formation Coupled with the Coil−Helix Transition in the Ternary System Poly(γ-benzyl L-glutamate)/Dichloroacetic Acid/Dichloroethane

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ABSTRACT: Phase behavior and ordering characteristics were reported for poly(γ-benzyl L-glutamate) (PBLG) in a mixed solvent containing a denaturant acid, dichloroacetic acid (DCA), + dichloroethane (DCE). The solutions of PBLG prepared so as to be liquid crystal at room temperature were observed to undergo a reentrant isotropic transition at low temperature where conformational disordering of PBLG molecules takes place. The deuterium quadrupolar splitting data obtained by the ^{2}H NMR method were analyzed to elucidate the conformation and orientational order parameter of the α-helix in the liquid crystalline phase. The conformation of PBLG molecules in the isotropic state was estimated from the observed ^{13}C NMR chemical shift. The molecular information gained through these experiments was implemented into the theoretical expression set forth by Flory and Matheson. The phase diagrams calculated on this basis have been compared with those observed. The theory has provided a reasonable picture for the high- and low-temperature phase transitions of the PBLG/DCA/DCE system.

Introduction

Poly(γ-benzyl L-glutamate) (PBLG) is an interesting system, which exhibits a reentrant isotropic phase in certain mixed solvents such as dichloroacetic acid (DCA) + dichloroethane (DCE). The reentrant phenomenon has been extensively investigated by Subramanian, Wittebort, and Dupré.[1−3] In the presence of an acid component, the coil form is stable at lower temperatures. Under that condition, the amide hydrogens are free to interact with denaturing solvent. The random-coil configuration prefers the disordered isotropic phase. As temperature is raised, the acid component tends to be excluded from the polymer domain, and the intramolecularly hydrogen-bonded α-helical form becomes more stable. Above a certain concentration, the coil → helix transition leads to the formation of a liquid crystalline phase. The conformational coil → helix transition of the polymer component may be accelerated by the coexisting liquid crystalline phase. These two effects are possibly mutually related. With further increase in temperature, the α-helical backbone once formed becomes more flexible due to the thermal energy, and thus the liquid crystalline phase tends to be less stable. The transition takes place eventually at the point where the free energy of the ordered phase becomes equivalent to that of the conjugated isotropic phase. The advent of the high-temperature isotropic phase is the behavior widely observed for real semirigid macromolecules.[4] In contrast to the low-temperature transition, however, the α-helical architecture of the polymer chain is maintained in the dilute isotropic phase. The biphasic boundaries of the above-mentioned transitions can be determined by optical and NMR measurements, and the phase diagram can be constructed.[1−3]

Theoretical considerations for the possibility of a reentrant isotropic phase of a polypeptide liquid crystal have been described in the literature. Rajan and Woo[5] prescribed a theory based on the de Gennes−Pincus concept,[6] which assumes an attractive orientation-dependent dispersion interaction between α-helical rod molecules. Such dispersion forces are however known to be of secondary importance in the lyotropic system in which polymers are largely surrounded by solvent molecules. On the other hand, Flory and Matheson[7−9] constructed a lattice theory to predict a liquid crystal formation coupled with the helix−coil transformation of polymeric molecules in solution. The free energy change for the helix−coil transition has been incorporated in the lattice scheme by adopting an expression such as[10]

$$-\sum m x_i(\theta \ln s - \varrho \ln \sigma) \qquad (1)$$

where s and σ denote respectively the statistical weight for a unit in the helical state relative to the coil and the weighting factor for initiation of a sequence, m represents the number of repeat units involved in a helical sequence, θ is the fraction of polymer units in the helical conformations, and ρ is the fraction of units that mark the beginning of a helical sequence. The partition functions have been derived in consideration of the equilibrium distribution of rigid and coil segments in the isotropic and anisotropic phases. The marked broadening of the biphasic gap and its shift to higher volume fractions were predicted as a result of the enhanced flexibility of polymer chains.

In this study, the phase behavior of the PBLG/DCA/DCE system which exhibits a reentrant isotropic transition was investigated by using the polarizing microscope and ^{2}H NMR techniques. The deuterium quadrupolar splitting data obtained by the latter method were analyzed to elucidate the conformation and ordering characteristics of the α-helix in the liquid crystalline phase. The conformation of PBLG molecules in the isotropic state was elucidated from the observed ^{13}C NMR

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chemical shift. The molecular information gained through these experiments was implemented into the theoretical expression.[9] The phase diagrams calculated on this basis have been compared with those observed.

Experimental Section

PBLG. PBLG sample was prepared by a standard *N*-carboxy *γ*-benzyl L-glutamate anhydride (NCA) method. The polypeptide formed was purified by repeated precipitation from a chloroform solution in a large volume of methanol. The limiting viscosity number, [*η*], was determined as a function of temperature by using a Ubbelohde suspended-meniscus type capillary viscometer. The molecular weight of the PBLG sample was estimated to be 6.2 × 10^4 from the [*η*] value measured in DCA according to the Doty et al. relation.[11]

PBLG-α-*d*. L-Glutamic acid-α-*d* was prepared by using the method described by Hilton et al.[12] Two PBLG-α-*d* samples were obtained by the NCA method; the molecular weights estimated from intrinsic viscosities were 10.9 × 10^4 and 5.5 × 10^4.

PBLG-*N*,α-*d*$_2$. A desired amount of PBLG-α-*d* was converted to PBLG-*N*,α-*d*$_2$ by dissolving the sample in trifluoroacetic acid-*d* and kept at room temperature for 1 h. The sample was precipitated into methanol and purified as described above.

Measurements. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GSX-500 spectrometer operating at 500 and 125 MHz, respectively. The ^{2}H NMR spectra of anisotropic solutions were recorded with the same spectrometer operating at 76.65 MHz under a complete proton decoupling and nonspinning mode. The spectrometer was equipped with a variable temperature controller.

PBLG solutions were prepared by dissolving the polymer sample in a mixed solvent of DCA/DCE over 1−2 days. The composition of the DCA/DCE mixture employed in most examples was 80/20 in weight ratio or 3.7/1.0 in molar ratio. The polymer volume fraction, v_p, was estimated from the density (1.27 g/cm^3) of PBLG.[13] The phase transitions were observed by using an Olympus polarizing microscope with a Mettler FP82 hot stage. As viewed by microscope, a PBLG solution with $v_p = 0.25$ showed typical cholesteric striations around 45 °C. Cooling to about 20 °C, some isotropic spherulites began to appear and grew in both size and number as the temperature was further reduced. After passing through the biphasic zone, complete extinction of the anisotropic phase took place at −38 °C. On heating the liquid crystalline sample, a cholesteric to isotropic transition was observed over a temperature span of 87−125 °C.

Experimental Results

Phase Diagrams. Following Subramanian and Dupré,[3] experimental phase diagrams for the ternary system PBLG/DCA/DCE were constructed according to the transition temperatures determined by the optical observation and NMR measurement. Figure 1a illustrates the phase diagram obtained as a function of PBLG volume fraction in the mixed solvent DCA/DCE with a given composition (80/20, w/w). The phase diagram is divided into five characteristic zones: The zonings are essentially the same as those adopted by Dupré et al. except the distinction between B$_1$ and B$_2$. Region I$_1$ represents an isotropic phase of orientationally disordered helices: The helix conformation was confirmed by ^{13}C NMR. A biphasic equilibrium between the isotropic solution (I$_1$) and the anisotropic mesophase was observed in region B$_1$. In region LC, microscopic observation gives a homogeneous cholesteric texture. At lower temperatures, the LC phase transforms into the reentrant biphasic zone (B$_2$) in which a helix-rich anisotropic phase is in equilibrium with a dilute isotropic solution (I$_2$) comprising random-coiled PBLG as identified by ^{13}C NMR. In the B$_1$ and B$_2$ zones (v_p = 0.17−0.23), the microscopic examination exhibited only biphasic character at all temperatures.

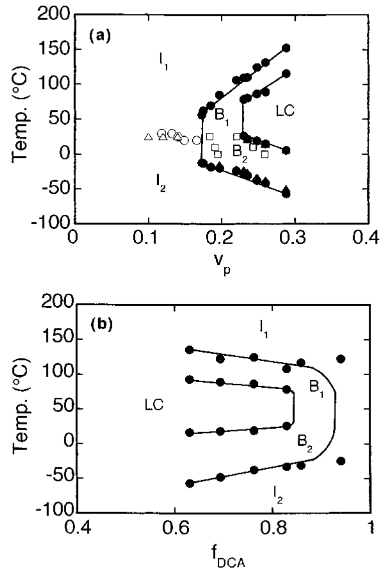

Figure 1. Experimental phase diagrams for a ternary solution comprising PBLG (M_w = 6.2 × 10^4), DCA, and DCE: I$_1$, isotropic phase (orientationally disordered helices); I$_2$, isotropic phase (random coils); LC, liquid crystalline phase; B$_1$, biphasic region where I$_1$ is in equilibrium with LC; and B$_2$, I$_2$ in equilibrium with LC. (a) $T-v_p$ relation for a given solvent composition, DCA/DCE (80/20, w/w): ○, phase transition temperature estimated from ^{13}C NMR (cf. Figure 2); ●, transition temperatures observed at the interphases I$_1$/B$_1$/LC/B$_2$/I$_2$ under microscope; and □, critical volume fractions derived from the centrifugation experiment (cf. Figure 3). The effect of the PBLG/DCA ratio was examined for solutions in which this ratio was kept in invariant: △ for the I$_1$ phase and ▲ for the LC phase. (b) Phase boundary curves as a function of solvent composition at a given polymer concentration (v_p = 0.25), where f_{DCA} (mole fraction) = DCA/(DCA + DCE).

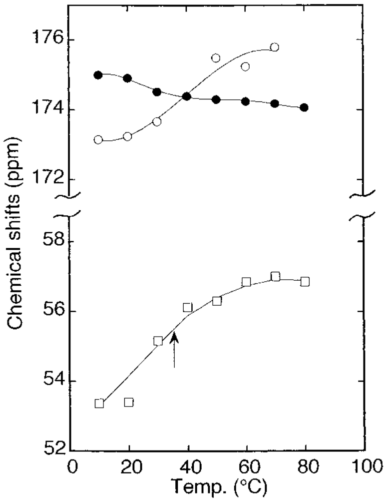

Figure 2. ^{13}C NMR chemical shifts of PBLG observed in dilute solution (v_p = 0.15), PBLG (M_w = 6.2 × 10^4) dissolved in DCA/DCE (80/20, w/w): α-carbon (□), amide carbon (○), and ester carbon (●). The helix−coil transition temperatures (cf. Figure 1a) were defined at the midpoint of the $\delta_{C^\alpha}-T$ curve as indicated by the arrow.

The transformation from the α-helix (I$_1$) to the random-coil form (I$_2$) taking place in the isotropic phase can be easily followed by the ^{1}H or ^{13}C NMR method.[14] Shown in Figure 2 is the temperature dependence of carbon chemical shifts observed for a dilute isotropic solution of PBLG (v_p = 0.15) in the mixed solvent (cf. Figure 1a). On cooling, the resonance peaks due the

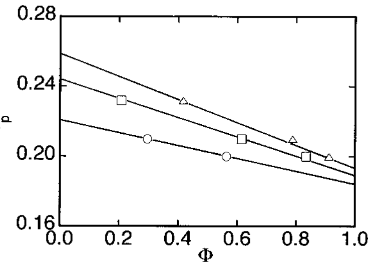

Figure 3. Plots of polymer volume fraction, v_p, versus relative volume of the isotropic phase, Φ, determined for a mixture of PBLG ($M_w = 6.2 \times 10^4$) dissolved in DCA/DCE (80/20, w/w): 25 °C (○), 10 °C (□), and 0 °C (△).

α-carbon and the amide carbonyl carbon exhibit substantial upfield displacements, while the ester carbonyl carbon in the side chain undergoes a small downfield shift: $\delta_{C^\alpha} = 57.0 \rightarrow 53.3$ ppm, $\delta_{CN} = 176.0 \rightarrow 173.2$ ppm, and $\delta_{CO} = 174.1 \rightarrow 175.0$ ppm. The helix–coil transition took place over a considerably wide range of temperatures (20–60 °C). The transition temperatures (I_1/I_2) plotted in Figure 1a (open circles) are those defined at the inflection point of the S-shaped curves observed for the α-carbon (Figure 2). In this transition, the amount of DCA relative to PBLG may play an important role especially in the region where the polymer concentration is dilute. Shown by open triangles are the helix–coil transition temperatures observed when the content of DCA was so adjusted as to maintain the ratio PBLG/DCA invariant. The apparent dependency of the transition temperature on v_p has been suppressed by the adjustment of the DCA concentration. A similar effect arising from the PBLG/DCA ratio becomes less marked in the anisotropic state (filled triangles) as determined by the microscopic examination.

Adopting the method described by Itou and Teramoto,[15] the critical volume fractions, v_p' and v_p'', at the phase boundaries of the biphasic equilibrium were directly estimated by plotting the polymer volume fraction, v_p, against the relative volume of the isotropic phase, ϕ, as shown in Figure 3. A solution adjusted to occur in the B_2 region was found to be easily separated into two layers by applying a centrifugation at a rotor speed of about 2500 rpm. The value of ϕ was obtained from the volumes of the two conjugated phases thus determined. Approximate values of the critical volume fractions can be obtained for given conjugated phases by a linear extrapolation to $\phi = 0$ and 1.0, yielding the results such as $v_p'/v_p'' = 0.18/0.22$ (25 °C), 0.19/0.24 (10 °C), and 0.20/0.26 (0 °C). The critical volume fractions, v_p' and v_p'', are indicated by open squares in Figure 1a. The biphasic gap estimated in this manner is somewhat narrower than that derived from the microscopic observation. After a centrifugation at room temperature, an isotropic solution was separated from the coexisting anisotropic phase. The conformation of PBLG in this solution was studied by measuring the proton chemical shift of α-CH over a certain temperature range. The value of the chemical shift (4.49 ppm) was found to be nearly invariant as long as the solution remained homogeneously isotropic. These observations lead to a conclusion that PBLG molecules exist in a highly coiled state in the conjugated isotropic phase at equilibrium.

The effect of the denaturant acid (DCA) concentration was examined in a separate experiment in which the polymer volume fraction ($v_p = 0.25$) was kept invariant. The results are illustrated in Figure 1b, where variations of the phase boundary curves are illustrated as a function of the DCA mole fraction in the mixed solvent, $f_{DCA} = DCA/(DCA + DCE)$. The experimental condition specified at $v_p = 0.25$ in Figure 1a corresponds to that given at $f_{DCA} = 0.76$ in Figure 1b. With increasing f_{DCA}, the LC phase tends to be destabilized along both high- and low-temperature boundaries. In the range $f_{DCA} > 0.73$, a uniform LC phase could not be detected under the microscope at all temperatures. No attempt was made to determine the phase boundary between I_1 and I_2 in the present study. We have however independent evidence to confirm the distinct difference between these two phases:

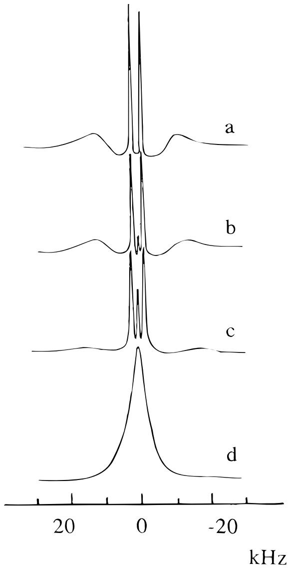

Figure 4. Examples of ^{2}H NMR spectra of PBLG-*N*,α-d_2 ($M_w = 5.5 \times 10^4$) in the mixed solvent DCA/DCE (80/20, w/w) ($v_p = 0.32$) observed at (a) 20, (b) 10, (c) −10, and (d) −35 °C. The outermost splitting is due to α-CD and a sharp doublet at the inner position comes from the deuterium incorporated in DCA. A pair of weak resonance peaks at a remote distance, which is not included here, arises from the amide N–D bond.

"Under a certain condition, PBLG is known to form liquid crystals when dissolved in pure DCA.[16] Similar to the observation in the mixed solvent, the reentrant I_2 phase appears at lower temperatures in addition to the high-temperature I_1 phase. We have carried out ^{1}H NMR measurements for a binary mixture ($v_p = 0.19$) of PBLG and DCA to determine the conformation of the polymer molecule in these two isotropic phases. The observed chemical shifts indicate that PBLG molecules adopt the α-helical arrangement in I_1 (>90 °C) while they become random coils in I_2 (<30 °C)."[17]

Orientational Order and Conformation of PBLG in the LC Phase. As demonstrated in our previous studies,[13,18] the ordering characteristics of PBLG molecules in the liquid crystalline phase can be elucidated by studying the deuterium quadrupolar splitting due to the C^α–D ($\Delta\nu_\alpha$) and/or the amide N–D bond ($\Delta\nu_N$). Examples of the ^{2}H NMR spectra (C^α–D) observed for a PBLG-*N*, α-d_2 sample ($M_w = 5.5 \times 10^4$) are illustrated in Figure 4. A sharp doublet located at the inner position is due to the DCA molecule, indicating that some deuterium exchange takes place between the acid and the amide group. A pair of weak resonance peaks arising from the amide N–D bond appear at a remote distance and are not included in the diagram. In the biphasic region (Figure 4b,c), contribution from the isotropic phase can be noted by the growth of the central peak. Only a broad single peak remains at a low temperature (Figure 4d), where the anisotropic liquid

Table 1. Quadrupolar Splittings $\Delta\nu_\alpha$ and $\Delta\nu_N$ Observed for PBLG-N,α-d_2 ($v_p = 0.32$) in the Mixed Solvent DCA/EDC (80/20, w/w)

temp, °C	$\Delta\nu_\alpha$, kHz[a]	$\Delta\nu_N$, kHz[a]	$\Delta\nu_\alpha/\Delta\nu_N \times 10^2$	S[b]
0 (B_2)	15.26	261.7	5.83	0.979
10 (B_2)	16.60	258.1	6.43	0.965
20 (LC)	17.92	258.1	6.94	0.965
30 (LC)	17.82	255.6	6.97	0.956
40 (LC)	17.57	253.4	6.93	0.947
50 (LC)	17.67	249.5	7.08	0.933
60 (LC)	17.58	247.3	7.11	0.925

[a] The sign of $\Delta\nu_N$ is positive, while $\Delta\nu_\alpha$ is taken to be negative in consideration of the geometry of an α-helix. Only absolute values are available from the experiment. [b] Calculated from the observed values of $\Delta\nu_N$ according to eq 2.[13,18]

crystalline phase is entirely eliminated. The phase boundary LC/B_2 appeared at around 10 °C, being slightly higher than the experimental curve shown in Figure 1a. The orientational order parameter, S, of the PBLG backbone in the liquid crystalline state can be estimated from the observed quadrupolar splittings, $\Delta\nu$ ($=\Delta\nu_\alpha$ or $\Delta\nu_N$), according to the relation such as

$$S = (2/3)\Delta\nu/q_h \tag{2}$$

where q_h is the component of the quadrupolar interaction tensor along the α-helical axis. The q_h values of C^α–D and N–D are invariant for a given α-helical structure. The ratio of the splittings, $\Delta\nu_\alpha/\Delta\nu_N$, derived from a single measurement should bear information regarding the α-helical conformation under a given condition. The results obtained at $v_p = 0.32$ in the mixed solvent are listed in the fourth column of Table 1. The values of $\Delta\nu_\alpha/\Delta\nu_N$ remained nearly invariant with temperature in the anisotropic phase (>20 °C) and were found to fall in a similar range as those previously reported for the α-helical PBLG/$CHCl_3$ liquid crystal system,[13] suggesting that the α-helical conformation remains quite stable in the presence of DCA as long as the molecule exists in the liquid crystalline state. In Table 1, the $\Delta\nu_\alpha$ value tends to decrease somewhat as temperature is lowered beyond the LC/B_2 phase boundary, while $\Delta\nu_N$ remains nearly constant (Table 1). In another independent measurement, however, a slight increase in $\Delta\nu_\alpha$ was observed in the biphasic region. The experimental uncertainty may be larger in the B_2 region (0–10 °C). The total amount of liquid crystalline phase starts diminishing in the biphasic region, and the solution becomes heterogeneous with polydomain structures. The $\Delta\nu$ values observed under such a condition may be affected by the fluctuation of an individual domain axis.

Since the amide N–D bond lies along the α-helical backbone, the magnitude of the observed splitting, $\Delta\nu_N$, is comparatively large, leading to a more accurate estimate of the orientational order parameter, S (see the last column of Table 1). The value of S thus found in the LC region ($v_p = 0.32$) at 30 °C is 0.96, being in the range equivalent to those (0.82–0.97) estimated in conventional helicoidal solvents by the same method.[13,18] In Figure 5, the order parameters, S, listed in Table 1 are compared with those determined in $CHCl_3$ under a similar condition. In both examples, the orientational order tends to decrease gradually with increasing temperature in the liquid crystalline state.[13,18,19] These results are quite consistent with the above-mentioned observation that PBLG molecules remain in a highly rigid α-helical conformation when they are involved in the LC phase.

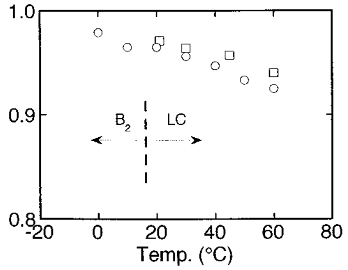

Figure 5. Variation of the order parameter with temperature. Open circles indicate the values obtained for a PBLG sample ($M_w = 10.9 \times 10^4$) dissolved in the mixed solvent DCA/DCE (80/20, w/w) ($v_p = 0.32$). Also shown by open squares are those observed in $CHCl_3$, as reported in the literature.[13]

Theoretical Consideration

Adaptation of Flory–Matheson's Lattice Theory to the Reentrant Phenomenon. Thermodynamic expressions applicable to the present problem have been prescribed by Flory and Matheson within the framework of the lattice model.[7–9] The partition functions derived for a binary system in which the polymeric component is capable of forming either rodlike helical sequences or random coils are solved for the equilibrium condition, i.e.,

$$(\mu_s' - \mu_s^{0\prime})/RT = (\mu_s'' - \mu_s^{0\prime\prime})/RT \tag{3}$$

and

$$(\mu_p' - \mu_p^{0\prime})/RT = (\mu_p'' - \mu_p^{0\prime\prime})/RT \tag{4}$$

where μ_s and μ_p designate respectively chemical potentials of the solvent and the polymeric solute composed of x segments, single-prime and double-prime symbols being appended to distinguish the isotropic and nematic phases. Resulting equations for the fully helical, nematic phase in equilibrium with the isotropic phase are[20]

$$\ln(1 - v_p')/(1 - v_p'') + (1 - 1/x)(v_p' - v_p'') + \chi' v_p'^2 - \chi'' v_p''^2 - 2/y + v_p''(1 - y/x) = 0 \tag{5}$$

and

$$\ln(v_p'/v_p'') + (x - 1)(v_p' - v_p'') + \chi' x(1 - v_p')^2 - \chi'' x(1 - v_p'')^2 - 2x/y + v_p''(x - y) + \ln(y/x)^2 + m \ln[1 - \rho'/(1 - \theta')] + mx \ln s'' + \ln \sigma'' = 0 \tag{6}$$

where y is the disorientation index originally used in Flory's 1956 paper[21] and χ is the solvent–polymer interaction parameter. Since the polymer component is assumed to occur in a highly helical form in the anisotropic phase (see the preceding section), $\theta'' \sim 1.0$ and $\rho'' \sim 1/mx$. Auxiliary relations required for the equilibrium conditions in the individual phases are

$$2/y = -\ln[1 - v_p''(1 - y/x)] \tag{7}$$

$$s' = (1 - \rho'/\theta')/[1 - \rho'/(1 - \theta')] \tag{8}$$

$$\sigma' = \rho'^2/(\theta' - \rho')(1 - \theta' - \rho') \tag{9}$$

For $\theta' = 1.0$, the binodals become identical with those found for systems of rigid rod. Relaxation of rigidity in the isotropic phase renders the transition more gradual from the broad biphasic range to the narrow gap

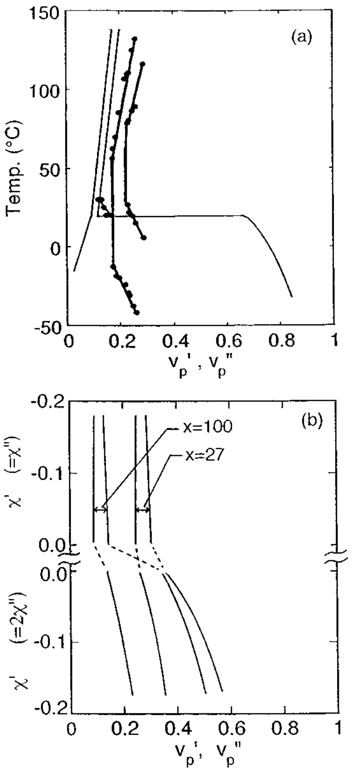

Figure 6. Phase diagrams of PBLG solutions: (a) comparison of experimental binodal curves for PBLG in DCA/DCE (thick lines) with an illustrative sketch of the Miller et al. diagram (thin lines) reported for PBLG/DMF[4b] and (b) phase diagrams calculated according to the Flory–Matheson theory.[9] Two sets of curves are shown with axial ratios $x = 27$ and 100 as indicated for each conjugate pair. Biphasic equilibrium lines in the upper portion are derived by assuming $\chi' = \chi'' \leq 0$, $\theta' = \theta'' = 1.0$, and $\sigma' = \sigma'' = 10^{-4}$ (cf. Figure 1a). Low-temperature reentrant isotropic regions were simulated by using parameters such as ($x = 27$) $\theta' = 0.1$ ($s' = 0.9737$), $\theta'' = 1.0$ ($s'' = 1.039$), $\sigma' = \sigma'' = 10^{-4}$ and ($x = 100$) $\theta' = 0.1$ ($s' = 0.9737$), $\theta'' = 1.0$ ($s'' = 1.009$), on the premise that $\chi' = 2\chi'' \leq 0$.

characteristic of systems of rigid rods as the value of χ parameter becomes more positive. The transition range is shifted simultaneously to higher concentrations. In these calculations,[8] Flory and Matheson assumed $s' = s''$, $\sigma' = \sigma''$, and $\chi' = \chi''$ for simplicity.

In Figure 6a, our phase equilibrium data are compared with an illustrative sketch of the well-known Miller phase diagram.[4b] The latter diagram has been obtained for a binary mixture comprising PBLG ($M_w = 3.1 \times 10^5$) and dimethyl formamide (DMF). Under this condition, PBLG exists in the α-helical form in the isotropic phase at all temperatures. In this work, Miller et al. have demonstrated that the Flory lattice theory for rodlike molecules[21] reproduces at least qualitatively the overall feature of the observed phase diagram. In their calculations, the value of parameter χ was taken to decrease with increasing temperature. Gradual bending of the chimney toward higher concentration range is probably due to the enhanced flexibility caused by the thermal energy at elevated temperatures as normally observed in the real semirigid polymer systems. A high-concentration shift of the biphasic chimney observed in the PBLG/DCA/DCE system (Figure 6a) may be explained by the molecular weight difference between the PBLG samples. As has been demonstrated in various precedents,[22] the original Flory theory has provided a reasonable insight for phase behaviors of solutions containing rodlike molecules.

The transition associated with the reentrant low-temperature isotropic phase is known to be induced by the presence of the acid component, i.e., as temperature is lowered, DCA molecules start attacking the hydrogen bond involved in the α-helical architecture and finally convert the α-helix into a random coil. In the biphasic region, polymer molecules are separated into either the isotropic or the anisotropic phase with higher volume fractions of α-helical molecules in the anisotropic region. These considerations suggest that the value of parameter χ should tend to be more negative with decreasing temperature. In the Flory theory, χ is the parameter defined in units of RT and responsible for the temperature dependence of phase equilibrium conditions. The expression should be valid only for systems in which interacting forces between solvent and solute molecules are sufficiently small, and the random-mixing assumption is effective. Use of parameter χ for the strong interaction between PBLG and DCA may exceed the limit of the original prescription. In this work, we tentatively adopt χ as an arbitrary parameter simply denoting the free energy of interaction. As briefly suggested by Matheson,[7] the parameter χ may adopt different values in the isotropic and nematic phases.

The binodal curves obtained by solving eqs 5–9 are illustrated in the lower part of Figure 6b where the values of parameter χ (χ', χ'') are plotted against polymer volume fraction, v_p (v_p', v_p''). Values of the axial ratio, x, are indicated for each curve; the PBLG sample used in this experiment corresponds to $x = 27$. Calculations were carried out by setting $\sigma' = \sigma'' = 10^{-4}$ and $\theta' = 0.1$ for the isotropic phase. A choice of $\theta'' = 1.0$ for the nematic phase is consistent with what we observed by ^{2}H NMR. Values of s' and s'' estimated correspondingly are given in the legend to Figure 6. The conditions required for the individual states are fulfilled, i.e., $s' < 1.0$ and $s'' > 1.0$. Following the argument given above, interaction parameters are weighted differently: For simplicity, we let $\chi' = 2\chi'' \leq 0$. In the diagram, the dilute isotropic phase (I_2) containing random coils ($\theta' = 0.1$) is in equilibrium with the LC phase ($\theta'' = 1.0$). For an illustrative purpose, we plot the binodals for the high-temperature biphasic gap obtained by choosing $\theta' = \theta'' = 1.0$, thus PBLG remaining in an α-helical rod in the isotropic phase (I_1). Results of numerical calculations for $\chi' = \chi'' \leq 0$ are shown in the upper portion of Figure 6b; the χ values are taken to be increasingly more negative upward.[7–9] The helix ($\theta'' = 1.0$)-to-coil ($\theta' = 0.1$) transition should take place in the intermediate region. To facilitate comparison, a set of high- and low-temperature biphasic gaps are tenatively connected smoothly over this range by the dotted lines in Figure 6b.

Comparison of Experimental Results with Theory. Comparison of parts a and b of FIgure 6 indicates that general features of the experimental phase diagram are reproduced by the lattice theory, with due consideration of the role of denaturant solvent. As we do not know the true χ–T relation, a quantitative comparison is improbable along the ordinate of these figures. With decreasing molecular weight (i.e., $x = 100 \rightarrow 27$), the liquid crystal–isotropic miscibility gap tends to shift toward higher concentrations in both high- and low-temperature transitions. We should point out here that the relative magnitude of χ' and χ'' largely affects the slopes of the phase boundary curve and the width of the biphasic gap for the reentrant isotropic phase. An appreciable bend of the observed phase boundary curves toward higher concentrations at lower temperatures can be explained by an increase in the magnitude of χ parameters due to the enhancement in the solvent power (DCA). Although the curvatures of the high- and low-temperature biphasic gaps are determined by independent causes, they play undoubtedly an important role in the development of the reentrant isotropic phenomenon (Figure 6a).

Experimental observations indicate that PBLG molecules take a highly coiled arrangement in the I_2 phase, while the coexisting LC phase comprises nearly perfect α-helical rods arranged in a highly ordered fashion. An intermediate partially helical configuration is unstable in either of these two phases. In the calculation of theoretical phase diagrams, values of the parameter such as θ and ρ are selected in conformity with these observations. Nearly complete exclusion of heterogeneous species from given phases inevitably enhances cooperativity in the helix−coil transition.

Discussion

Intrinsic viscosities were also measured as a function of temperature. The $[\eta]$ value for PBLG/DCA/DCE shows an increase with temperature from 0 to 40 °C, reaches a plateau, and then is followed by a slight decrease at higher temperature (>50 °C). The initial increases in $[\eta]$ correspond to the coil → helix transition. As temperature increases further, the α-helical backbone tends to be more flexible, and $[\eta]$ decreases gradually with temperature. These observations have been previously reported by Teramoto et al. for a mixture PBLG/DCA/$CHCl_3$.[23]

As shown in Figure 1b, the phase transition temperatures are appreciably affected by the solvent composition. In fact, a slight decrease in the I_1/I_2 boundary (open circles in Figure 1a) for the helix−coil transition in the isotropic phase was found to be improved (open triangles) when the DCA concentration was so adjusted as to keep the PBLG/DCA ratio constant. Possible partitions of the two solvent components between the conjugated isotropic (I_2) and anisotropic (LC) phases were examined for fractions separated by centrifugation. The molar ratios DCA/DCE were estimated for the solutions thus obtained according to the ^{1}H NMR method. The DCA/DCE ratios were found to be slightly higher in the anisotropic phase. Since $v_p' < v_p''$, however, the molar ratio of DCA with respect to PBLG remains indistinguishable between the two phases within the experimental uncertainty. In consideration of these experimental observations, we set $0 > \chi'' > \chi'$ in the Flory−Matheson expression for the low-temperature transition ($I_2 \rightarrow$ LC). As indicated by Matheson,[8,9] the phase boundary curves (Figure 6b) tend to be straight when calculated with $\chi' = \chi''$. It has been suggested that the marked broadening of the biphasic gap at low temperature results from the greater flexibility (random coil) in the isotropic phase. In this experiment, unfractionated PBLG samples were adopted. The molecular weight distributions of PBLG samples have not been determined. To a certain extent, the polydispersity in the degree of polymerization should contribute to the broadening of the biphasic gap.

Finally we wish to emphasize that the Flory−Matheson theory has provided a reasonable picture for the high- and low-temperature phase transition of the PBLG/DCA/DCE system. As put it by Flory,[22] the lattice model is suited, in spite of its artificiality, to treatment of the steric part of the configuration partition function for a system comprising molecules in which volume exclusion plays a dominant role. The subject studied in this work is one of such examples. A correct representation of solvent−solute interactions was found to be of primary importance in predicting the thermal behavior of the system.

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