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Intermolecular Association and Supramolecular Organization in Dilute Solution. 2. Light Scattering and Optical Activity of Poly(*p*-biphenylylmethyl L-glutamate)

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ABSTRACT: Static and dynamic light scattering measurements are reported on dilute solutions of poly(*p*-biphenylylmethyl L-glutamate), PBPMG, in the helicogenic solvent THF, over a temperature range encompassing an observed reversible transition in the chiral optical properties of these solutions. The results confirm the expected helical conformation for this polypeptide, as well as complete dissolution of the polymer at temperatures above the transition temperature. Association is observed at lower temperatures, with the chains in an ordered parallel array within the aggregates. The association occurs with increasingly poor solvent conditions as the temperature is decreased, with the degree of association in the aggregate increasing concurrently with the change in the optical rotation and circular dichroism (CD) spectrum. It is concluded that the intermolecular association of the chains in the parallel array alters the chiral electronic environment of the biphenyl group, without a conformation change in that chromophore, resulting in the observed transition in the optical rotation and the CD spectrum. Unlike the behavior reported for certain other aggregated systems, the optical transition observed with solutions of PBPMG does not involve the initial formation of a disordered aggregate, which orders itself on reduced solvent quality or temperature, but rather a spontaneous association, such as expected on crossing a phase boundary, with a concurrent optical transition.

Introduction

In an earlier study to explore the possible role of intermolecular association as the cause of an unusual thermally reversible change in the optical rotation and the circular dichroism (CD) spectrum of dilute solutions of poly(*p*-biphenylylmethyl L-glutamate), PBPMG,[1−3] in solvents which support the α-helical conformation, viscometric measurements showed a large increase in the intrinsic viscosity over the temperature range associated with the chiral optical changes. Coupled with the fact that several experimental methods showed no evidence of intramolecular conformational change, these observations lead to the hypothesis of a microphase separation similar to that observed with other rodlike polymers exhibiting thermochromic behavior discussed below. Since interpretation of the intrinsic viscosity is complicated because association may have opposite effects on the chain dimensions and molecular weight, static and dynamic light scattering experiments were undertaken to more clearly delineate the association. The results discussed in this contribution demonstrate that the association hypothesis is correct but that the association in PBPMG differs in important ways from that in various other rodlike polymers.

For a number of macromolecules, the intermolecular interactions leading to supramolecular organization in polymers and their concentrated solutions are forecast by thermally reversible intermolecular association in dilute polymer solutions. Although the association may involve equilibrium structures (e.g., as with the well-known tetramer found in normal human hemoglobin[4,5] or end-group association in certain cases[6,7]), nonequilibrium structures are more often observed in polymeric solutions. Intermolecular association is not unusual for solutions of polyglutamates. In particular, association in dilute solutions of poly(γ-benzyl L-glutamate), PBG, has been explored by a variety of methods, to deduce both end-to-end and side-by-side association, depending on the conditions.[7−24] Examples of other systems with potential relevance to this study occur in the intermolecular association frequently observed with solutions of heterocyclic semiflexible or rodlike chains.[25−32] Some of these reveal thermally reversible changes in the electronic absorption as the nature of the intermolecular association changes over a narrow temperature range.[25,29,31,32] With chains dissolved in strong protic acids, a change in the electronic absorption has been attributed in several cases to a decrease in the degree of protonation in the polymer as the aggregated structures collapse and organize to an ordered state,[25,29,31] similar to the process expected for semiflexible or rodlike polymers in concentrated solutions in poor solvents.[33] The domains of associated polymer provide the locally high concentration (in the globally dilute solution) needed to effect this transition as the polymer/solvent thermodynamics are altered by a change in temperature. By contrast, with dilute solutions of poly(3-dodecylthiophene), it appears that the observed reversible thermochromism may result from a thermally reversible change in the organization of aliphatic substituents to improve the order in the chain backbone in preformed supramolecular aggregates, in which the chain backbones are in a roughly nematic order.[32] In a number of

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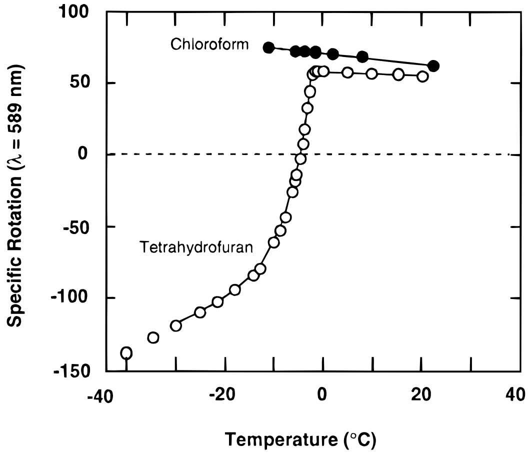

Figure 1. Specific optical rotation as a function of temperature for solutions of poly(p-biphenylylmethyl L-glutamate) in two helicogenic solvents, showing a transition for solution in tetrahydrofuran, but not in chloroform (3 g/L; $\lambda = 589$ nm). After ref 1.

the cited studies, the association process and the structure of the aggregate were elucidated through the use of polarized and depolarized static and dynamic scattering, similar to the methods applied in this study.

Various esters of poly(L-glutamic acid) adopt close to an α-helical conformation in helicogenic solvents, with a characteristic CD spectral signature, while in other solvents, such as dichloroacetic acid, the peptide takes a more random conformation.[15] This is the situation for PBPMG, but as noted above, in addition, in certain of these helicogenic solvents, dilute solutions of PBPMG exhibit a thermally reversible transition in chiral optical properties, as the temperature is lowered below a certain temperature T_{OPT}, even though no helix−coil transition occurs. An example of this behavior is shown in Figure 1 for the D-line optical rotation.[1] No similar transition is observed in solutions in nonhelicogenic solvents or in all helicogenic solvents. The transition occurs within the time required for thermal equilibration after the temperature is changed, and the behavior is nearly independent of concentration over the concentration range 0.7−46 g/L for the polymer studied here.[1,2] For solutions in the helicogenic solvents in which this transition occurs, the CD spectrum at temperatures above the transition is characteristic of an α-helix,[34] with a transition to a very different spectrum at lower temperatures, as seen below.

A strong effect on CD intensity and band shape associated with intermolecular effects has been noted by others.[35] Indeed, optical activity changes are associated with the earliest reports of aggregation of polymers, e.g., the change observed in the formation of a gelatin gel. In this case the chiral optical effects are accompanied by a clear conformational change in the protein constituents.[36] More recently, changes in the chiral optical properties of dilute solutions of poly(n-hexyl isocyanate) have been attributed to conformational changes associated with aggregation, leading to the formation of thermally reversible gels at higher concentrations.[37] In addition chiral optical transitions have been attributed to association or intermolecular order for chiral derivatives of polyacetylene,[38] polysilane,[39] and polythiophene[40] in solution. In general, changes in chiral optical properties may arise with or without conformational changes in the involved chromophores. One of many examples of the latter effect is given by the circular dichroism induced in achiral molecules on dissolution in a chiral mesophase solvent.[41] In the case of solutions of PBPMG, various spectroscopic probes which are known to be sensitive to the conformational properties of polypeptides show virtually no change on moving through the temperatures associated with the chiral optical transition, indicating that the source of the chiral optical transition is in the postulated supramolecular organization of the aggregated chains.[1]

The light scattering results obtained to further understand the properties of PBPMG are obtained in terms of averaged parameters, such as the molecular weight M_{LS}, the root-mean-square radius of gyration $(R_{\mathrm{G}})_{\mathrm{LS}}$, the second virial coefficient $(A_2)_{\mathrm{LS}}$, and possibly the optical anisotropy δ_{LS} if the solute is optically anisotropic, all from static scattering. The correlation length a_{LS} associated with the mutual diffusion coefficient and possibly the rotational diffusion coefficient are obtained from the dynamic scattering. In addition, the dependence of the scattering function $P_{\mathrm{LS}}(q)$ and the photon-count auto-correlation function $g^{(2)}(\tau;q)$ provide insight into the character of the aggregate structure.[7,31,32,42−44] In the following, a description of the light scattering methods is followed by the results. The interpretation of the results in terms of intramolecular parameters and intermolecular association is deferred to the Discussion, where it is concluded that increased intermolecular association is induced below T_{OPT} with decreasing temperature, with the helical PBPMG chains in essentially parallel arrays in the aggregates.

Experimental Section

Methods. The sample of PBPMG used in this study was an aliquot of the sample previously reported;[1] the molecular weight of the sample is discussed below. Reagent grade tetrahydrofuran (THF) was distilled under vacuum over CaH_2 and stored over CaH_2 in the dark until use. Polymers were evacuated (10^{-4}−10^{-3} mmHg) for several days at 60 °C. A stock solution was prepared by weighing the appropriate amount of polymer and about two-thirds of the desired solvent into a centrifuge tube with a screw top cap (Teflon gasket), containing a Teflon-coated stirring bar. After several days, the solution was gently agitated by occasional swirling. After the solution was apparently uniform, the remainder of the solvent was added, and the solution was slowly stirred with the stirring bar for an additional 1−2 weeks. Solutions for scattering prepared by dilution of the stock solution were held at 25 °C until subjected to the thermal histories described below.

Static and dynamic light scattering experiments were carried out using instruments described elsewhere,[28] with incident light with wavelength 514.5 nm from an argon-ion laser (Lexel, Model 85). Solutions in chloroform were filtered into light scattering cells through 0.45 μm Teflon filters, degassed, and sealed on a vacuum line. Cells were centrifuged for 24 h at 7000 rpm in a swinging bucket rotor. Solutions were held at temperature for at least 1 h before measurements were taken.

Data Analysis. The polarized light scattering results were analyzed according to the usual model for isotropic scatterers;[45,46] the depolarized scattering was negligibly small, as seen below. This model provides expressions for the dependence of the vertical component $R(q,c)$ of the Rayleigh ratio with vertically polarized incident light on the concentration c and the modulus $q = (4\pi n_s/\lambda)\sin(\vartheta/2)$ of the scattering angle vector, with n_s the refractive index of the sample, λ the wavelength of light *in vacuo*, and ϑ the angle between the incident and scattered beams. Thus, $R(0,c)$ extrapolated to zero scattering angle provides information on a light scattering averaged molecular weight M_{LS} and the second virial coefficient $(A_2)_{\mathrm{LS}}$, averaged by light scattering:

$$\frac{Kc}{R(0,c)} = M_{\mathrm{LS}}{}^{-1} + 2(A_2)_{\mathrm{LS}}c + 3(A_3)_{\mathrm{LS}}c^2 + ...$$

$$\approx M_{\mathrm{LS}}{}^{-1}\{1 + (A_2)_{\mathrm{LS}}M_{\mathrm{LS}}c + (q_3/2)[(A_2)_{\mathrm{LS}}M_{\mathrm{LS}}c]^2\}^2 \quad (1)$$

where $K = (2\pi^2/N_{\mathrm{A}}\lambda^4)[n_{\mathrm{o}}\partial n/\partial c]^2$, n_{o} is the solvent refractive index, N_{A} is Avogadro's number, and $q_3 = (3A_3/A_2{}^2M - 1)$ is expected to be small.[45,46] When extrapolated to infinite dilution (denoted by a super zero), the angular dependence of the scattering provides a measure of the mean-square radius of gyration $(R_{\mathrm{G}}{}^2)_{\mathrm{LS}}$:[26,45−47]:

$$\left(\frac{Kc}{R(q,c)}\right)^{\mathrm{o}} = \frac{1}{M_{\mathrm{LS}}P_{\mathrm{LS}}(q)} = \frac{1}{M_{\mathrm{LS}}}\left\{1 + \frac{1}{3}(R_{\mathrm{G}}{}^2)_{\mathrm{LS}}q^2 + ...\right\} \quad (2)$$

In these expressions, the subscript LS denotes the appropriate light scattering average, e.g., for homopolymers, with neglect of optical anisotropy (see below), $M_{\mathrm{LS}} = M_{\mathrm{w}}$ and $(R_{\mathrm{G}}{}^2)_{\mathrm{LS}} = \Sigma w_\nu M_\nu (R_{\mathrm{G}}{}^2)_\nu / M_{\mathrm{LS}}{}^{-1}$.[29,46,47] For convenience, the notation $(R_{\mathrm{G}})_{\mathrm{LS}}$ will designate the root-mean-square radius of gyration $(R_{\mathrm{G}}{}^2)_{\mathrm{LS}}{}^{1/2}$ in the following.

Owing to the small amount of sample available for this study, measurements of $\partial n/\partial c$ were not made, but rather $\partial n/\partial c \approx 0.181$ mL/g was estimated for solutions of PBPMG in THF using the Dale−Gladstone relation $\partial n/\partial c \approx (n_{\mathrm{p}} - n_{\mathrm{s}})/\rho_{\mathrm{p}}$, where subscripts p and s denote the polymer (solute) and solvent, respectively, and ρ is the density. In using this relation, the density is calculated for the polymer as the ratio m_{o}/V of the molar mass and volume of the repeat unit, respectively, and n_{p} is estimated from the relation $n \approx 1 + R_{\mathrm{GD}}/V$, where R_{GD} is the molar refraction.[48] Data on $\partial n/\partial c$ as a function of n_{s} for solutions of PBG in several solvents[49] permit the estimates $R_{\mathrm{GD}} \approx 102.9_5$ and $V \approx 177.5$ cm^3/mol with $m_{\mathrm{o}} = 207$ g/mol for PBG. Based on these values, the use of group additivity contributions[50] gives $R_{\mathrm{GD}} \approx 147.8$, $V \approx 138.5$ cm^3/mol, and $m_{\mathrm{o}} = 283$ g/mol for PBPMG. A similar analysis for poly(γ-ethyl L-glutamate) gave estimates of $\partial n/\partial c$ of 0.03 and 0.183 mL/g for solutions in dichloroacetic acid and trifluoroethanol, respectively, in comparison with the reported values of 0.050 and 0.168 mL/g,[49] providing support for the procedure used.

Dynamic light scattering measurements were carried out to give the photon-count autocorrelation function $g^{(2)}(\tau;q,c)$ as a function of scattering angle and correlation time τ.[46,47] With a cumulant analysis:[47]

$$\ln[g^{(2)}(\tau;q,c) - 1]^{1/2} = \ln[g^{(2)}(0;q,c) - 1]^{1/2} - K^{(1)}(q,c)\tau + \frac{1}{2!}K^{(2)}(q,c)\tau^2 + ... \quad (3)$$

where $K^{(1)}(q,c)$ is the first cumulant, etc. For the polarized scattering, the first cumulant is used to compute the hydrodynamic scaling length $a_{\mathrm{LS}}(c)$ by the expression[47]

$$\lim_{q=0} K^{(1)}(q,c) = \frac{kTq^2}{6\pi\eta_{\mathrm{s}}a_{\mathrm{LS}}(c)} \quad (4)$$

with η_{s} the solvent viscosity; $a_{\mathrm{LS}}(c)$ reduces to the hydrodynamic radius $(R_{\mathrm{H}})_{\mathrm{LS}} = M_{\mathrm{LS}}/\Sigma w_\nu M_\nu (R_{\mathrm{H}}{}^{-1})_\nu$ at infinite dilution, with $R_{\mathrm{H}} = kT/6\pi\eta_{\mathrm{s}}D_{\mathrm{T}}$, where D_{T} is the translational diffusion constant.[46,47] For THF, $\partial \ln \eta/\partial T^{-1} \approx 949$ K, and $\eta_{\mathrm{s}} \approx 0.125$ mPa s at 25°C.[29]

Results

Static light scattering was performed on three solutions of PBPMG in THF (concentrations of 0.21, 0.62, and 1.12 g/L), over the temperature range from −25 to +23.7 °C. The depolarized scattering was negligibly small at both +25 and −26 °C, being about 0.5−1% of the corresponding polarized scattering intensity at both temperatures, permitting neglect of the effects of optical anisotropy δ on the light scattering parameters. As illustrated in Figures 2 and 3, curves of $Kc/R(q,c)$ versus $\sin^2(\vartheta/2)$ for different concentrations were parallel at different concentrations and temperatures, despite substantial increase in $R(q,c)$ with decreasing temperature, consistent with minimal change in the structure of the scattering moiety over the range of c studied. In principle, $Kc/R(0,c)$ could change with c if the extent of association depended on c over the range of c studied, as can occur with an equilibrium association. In that case, $(A_2)_{\mathrm{LS}}$ could also depend on c. These effects may be incorporated by putting eq 1 in the form[7,11,43]

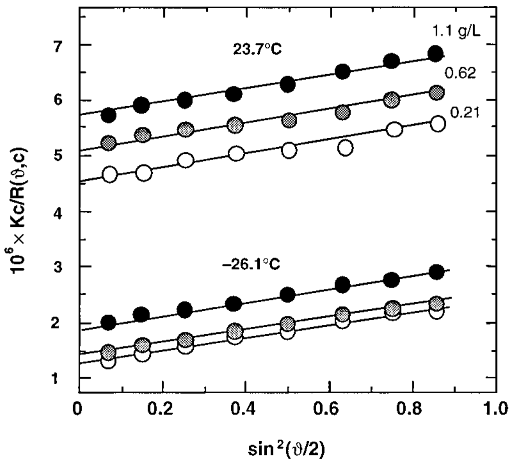

Figure 2. $Kc/R(q,c)$ versus $\sin^2(\vartheta/2)$ for solutions of poly(*p*-biphenylylmethyl L-glutamate) in tetrahydrofuran for the indicated concentrations and temperatures.

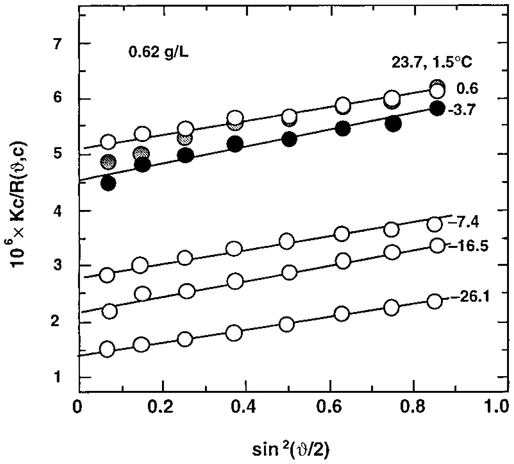

Figure 3. $Kc/R(q,c)$ versus $\sin^2(\vartheta/2)$ for a solution of poly(*p*-biphenylylmethyl L-glutamate) in tetrahydrofuran ($c = 0.62$ g/L) for the indicated temperatures.

$$\frac{Kc}{R(0,c)} = M(c)_{\mathrm{LS}}{}^{-1} + 2[A_2(c)]_{\mathrm{LS}}c + ...$$

$$= M(0)_{\mathrm{LS}}{}^{-1} + \{2[A_2(0)]_{\mathrm{LS}} + [\partial M(c)_{\mathrm{LS}}{}^{-1}/\partial c]_{c=0}\}c + ... \quad (5)$$

Thus, an increase in $M(c)_{\mathrm{LS}}$ with increasing c, as would be expected for an equilibrium association at low c, can give an apparently negative second virial coefficient, even if $[A_2(c)]_{\mathrm{LS}} > 0$ (i.e., $\partial[Kc/R(0,c)]/\partial c < 0$)). In such a case, the molecular weight $M(0)_{\mathrm{LS}}$ obtained by extrapolation to infinite dilution should be that of the fully dissociated solute. No such behavior was observed here in the range of concentrations studied by light scattering, possibly reflecting an equilibrium constant large enough that $M(c)_{\mathrm{LS}}$ does not change appreciably over the range of concentrations studied. Rather, the data extrapolate to the larger values of M_{LS}, taken to be M_{w}

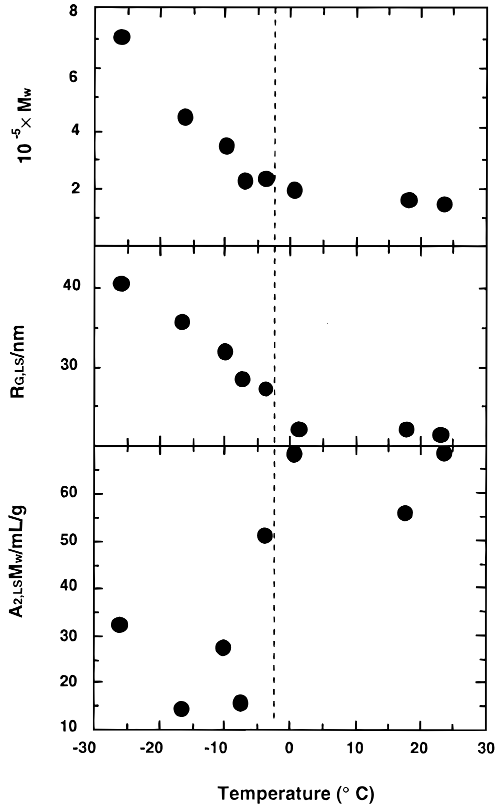

Figure 4. Variation of $M_{LS} \approx M_w$, $(R_G^2)_{LS}$, and $(A_2)_{LS}M_{LS}$ with temperature for poly(*p*-biphenylylmethyl L-glutamate) in tetrahydrofuran as a function of temperature. The vertical dashed line gives the temperature T_{OPT} for transition in the optical rotation from Figure 1.

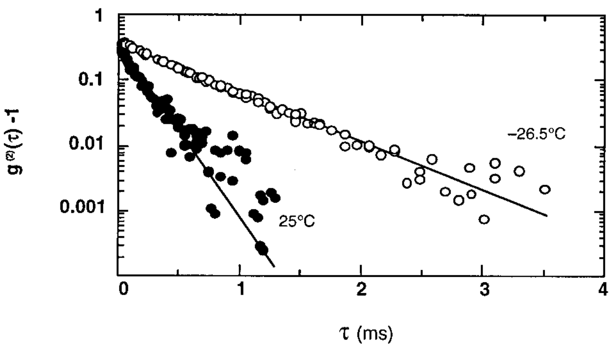

Figure 5. Correlation function observed for a solution of poly(*p*-biphenylylmethyl L-glutamate) in tetrahydrofuran at the indicated temperatures ($c = 1.1$ g/L).

for the associated polymer for the range of c studied. Thus, the data were treated in the usual way[46,47] to obtain estimates of $M_{LS} \approx M_w$, $(R_G^2)_{LS}$, and $(A_2)_{LS}M_{LS}$ given in Figure 4. Both the molecular weight and radius of gyration increased with decreasing temperature, whereas $(A_2)_{LS}M_{LS}$ decreased over the same temperature range.

Dynamic light scattering was performed on two solutions of PBPMG in THF (concentrations of 0.62 and 1.12 g/L), over the temperature range from −25 to +23.7 °C; the intensity was too low to achieve a satisfactory signal-to-noise ratio for the solution with 0.2 g/L concentration. Typical dynamic light scattering experiments are shown in Figure 5 (1.12 g/L at 30° angle for temperature +25.3 and −26.5 °C). As may be seen, the curves are weakly nonexponential (i.e., $K^{(2)}(q,c)/[K^{(1)}(q,c)]^2$ is small: 0.033 at −26.5 °C and 0.067 at 25.3 °C). The hydrodynamic scaling length $a_{LS}(c)$ determined from the first cumulant for the temperature range −25 to +23.7 °C for solutions with 0.62 and 1.12 g/L concentration is shown in Figure 6; $a_{LS}(c)$ is seen to be nearly independent of c within experimental error.

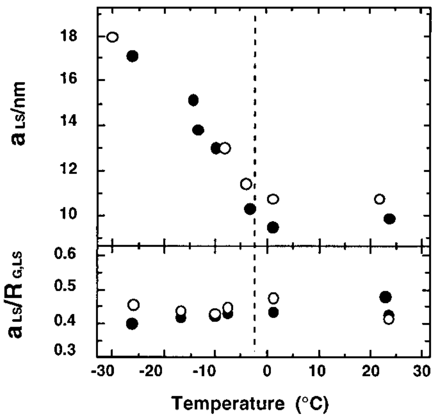

Figure 6. Hydrodynamic scaling parameter $a_{LS}(c)$ and the ratio $a_{LS}(c)/(R_G)_{LS}$ versus temperature for solutions of poly(*p*-biphenylylmethyl L-glutamate) in tetrahydrofuran (c equal to 0.62 and 1.12 g/L for filled and unfilled symbols, respectively). The vertical dashed line gives the temperature T_{OPT} for transition in the optical rotation from Figure 1.

The weakly nonexponential of the correlation function could be due to either or both of two effects: size (or shape) heterodispersity among the scattering moieties and contributions from intramolecular interference effects.[46,51] In the first instance, chain transfer may broaden the molecular weight distribution, even though the polymer was synthesized by an anionic polymerization. Alternatively, for rodlike chains, the rotational diffusion mode to the dynamic scattering could introduce nonexponential behavior, provided $q(R_G)_{LS}$ exceeds about unity. Since $q \le 4\pi n_s/\lambda \approx 0.036$ nm^{-1}, $q(R_G)_{LS}$ is never much greater than unity here, suggesting that intramolecular rotational dynamics are not the source of the observed nonexponential behavior. Since $K^{(1)}(q,c)$ was readily determined, the weak nonexponential behavior was not further investigated.

Discussion

The molecular weight of 1.69×10^5 determined from the scattering data at room temperature is close to the value 1.60×10^5 deduced from the intrinsic viscosity of 56 mL/g reported for solutions of the sample studied in the nonhelicogenic solvent dichloroacetic acid, DCA.[1] For the latter analysis, use is made of the relation

$$[\eta] = (K/m_0)(M_w/m_0)^{\mu} \tag{6}$$

It is assumed that both K and μ are independent of the substituents on the backbone of a linear polymer, though markedly dependent on chain conformation (e.g., helix, random coil, or intermediate structure) or excluded volume effects. Data on solutions of PBG on a number of solvent systems give μ equal to 1.8 in helicogenic solvents, or 0.87 in nonhelicogenic solvents,[15] with $K = 66$ mL/g and $\mu = 0.87$ for solutions of PBG in DCA.[52] Use of the latter gives the estimate $M_w \approx 1.60 \times 10^5$ quoted above. The close agreement of the estimates of M_w from light scattering data in THF solution at room temperature and from analysis of $[\eta]$ in DCA solution suggests that there is essentially no aggregation of PBPMG in THF at room temperature.

For a chain with a persistence length $\hat{a}$ and contour length L, the radius of gyration is given by[45,46,53]

$$R_G{}^2 = \frac{L^2}{12} W(L/\hat{a}) \tag{7a}$$

$$W(L/\hat{a}) \approx 1/\{1 + L/4\hat{a}\} \tag{7b}$$

The contour length is given by $L = M/M_L$, with the mass per unit length given by $M_L = m_o/l$ for repeat units of length l and molar mass m_o. The α-helix has a geometric diameter d_G of 1.6 nm for PBG, a pitch length of 0.54 nm, and 3.6 repeating units per pitch,[15,54] or $l = 0.15$ nm and $M_L \approx 1890$ nm^{-1} for PBPMG. Thus, $L/12^{1/2}$ is calculated to be 26 nm, close to the $(R_G)_{LS} \approx 22$ nm observed at room temperature, suggesting a helical (rodlike) conformation for PBPMG in THF at room temperature, consistent with the circular dichroism reported above (the agreement is closer if L is deduced from $\sqrt{R_G{}^2/W(L/\hat{a})}$ with $\hat{a} \approx 80$ nm). Similarly, for a rodlike chain with hydrodynamic diameter d_h for the repeat unit,[45,46]

$$R_H = \frac{L}{2} \frac{H(L/\hat{a}, d_h/\hat{a})}{\ln(3L/2d_h)} \tag{8}$$

where $H(L/\hat{a}, d_h/\hat{a})$ tends to unity for small $L/\hat{a}$. Accordingly, R_H is calculated to be 10 nm for $M_w = 1.69 \times 10^5$ (using the helicoidal diameter of 1.6 nm for PBG), in reasonable agreement with the observed value of $a_{LS} \approx$ 10 nm. Finally, in general, the intrinsic viscosity may be expressed in the form[55]

$$[\eta] = \pi N_A K_\eta R_G{}^2 R_H / \mathrm{M} \tag{9}$$

where K_η is expected to be unity for a (infinitely) thin, rodlike chain and $\approx {}^{10}/_3$ for a Gaussian chain in the nondraining limit. Data on (fractionated) double-strand helical xanthan[56] give $K_\eta \approx 1.7$. The reason for the deviation of K_η from unity is not certain, but it may be noted that K_η is expected to increase with increasing $d_h/\hat{a}$ for small $L/\hat{a}$.[55,57] It may be noted that the deviation of the observed K_η from unity cannot be due to the effects of polydispersity on the measured parameters, as those would tend to decrease K_η. The empirical value will be accepted here for use in estimates of $[\eta]$ for chains of this type. The calculated value of 85 cm^3/g for $[\eta]_{calc} = 1.7\pi N_A(R_G{}^2)_{LS}a_{LS}/M_{LS}$ using the observed values for M_{LS}, $(R_G{}^2)_{LS}$, $(R_H)_{LS} \approx a_{LS}$ and $K_\eta = 1.7$ is close to the value $[\eta] \approx 95$ cm^3/g reported for measurements of the same polymer in the helicogenic solvent 1,2-dichloroethane.[1] As shown in Figure 7, $[\eta]_{calc}$ increases with decreasing temperature for $T < T_{OPT}$, qualitatively similar to the behavior for $[\eta]$ reported in ref 1 for solutions in 1,2-dichloroethane. The more marked dependence of $[\eta]$ on temperature than observed for $[\eta]_{calc}$ may reflect different states of association in 1,2-dichloroethane and THF solutions.

The negligible depolarized scattering intensity despite the helical conformation is attributed to a small intrinsic optical anisotropy for PBPMG. The α-helical structure of polyglutamates results in a polarizability tensor that is far more isotropic than might be expected for a rodlike conformer. For example, light scattering studies on solutions of PBG in the helicogenic solvent *N*,*N*-dimethylformamide, DMF, were characterized by weak depolarized scattering intensity, with $\delta \approx 0.073$.[58] Since PBPMG has a longer side chain than PBG, the intrinsic optical anisotropy for PBPMG might reasonably be expected to be less than that for PBG, consistent with the very weak depolarized scattering reported here.

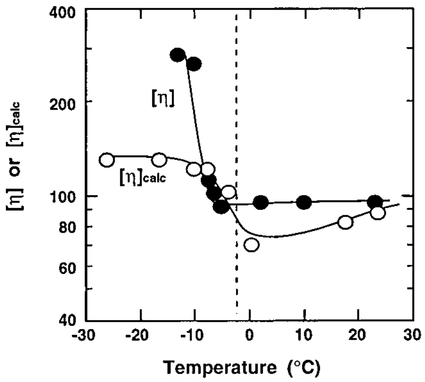

Figure 7. Intrinsic viscosity $[\eta]$ and the quantity $[\eta]_{calc} = \pi N_A(R_G{}^2))_{LS}a_{LS}/M_{LS}$ versus temperature for solutions of poly(*p*-biphenylylmethyl L-glutamate) in 1,2-dichloroethane and tetrahydrofuran, respectively. The vertical dashed line gives the temperature T_{OPT} for transition in the optical rotation from Figure 1. ($[\eta]$ from ref 1.)

The parallelism of the data for $Kc/R(q,c)$ versus $\sin^2(\vartheta/2)$ for different concentrations at fixed T indicates that $(R_G{}^2)_{LS}/M_{LS} = \Sigma w_\nu M_\nu (R_G{}^2)_\nu / M_w{}^{-2}$ is nearly invariant with c at each temperature for the conditions used and is nearly invariant with T as well. Both $(R_G{}^2)_{LS}$ and a_{LS} increase with decreasing T for $T < T_{OPT}$, with the ratio $a_{LS}/(R_G)_{LS}$ essentially independent of temperature over the temperature interval studied, as shown in Figure 6. Taken together, these observations suggest a model in which an ordered supramolecular structure is formed at low temperature, with the rodlike helical PBPMG chains aggregated with their axes parallel, but their ends not in perfect juxtaposition, i.e., similar to a nematic organization in the bulk, with the chains in close contact along their length. End-to-end association would have resulted in a far larger increase in $(R_G{}^2)_{LS}$ and a_{LS} than is observed for the increase in M_{LS}, and parallel association with perfect juxtaposition of chain ends would have resulted in (essentially) no change in these quantities as M_{LS} increased in the range of interest here. The chain packing in the postulated aggregates could tend to make the effective L/m_o for the aggregates relatively independent of the degree of association (neither L nor m_o increase proportionately with M_{LS}). Thus, although the association envisioned would increase d_G, the increase should be much smaller than the degree of association, as is the change in L. If the aggregate is taken to be rodlike, near independence of the effective value of L/m_o for the aggregates of different molecular weight and low degree of association would tend to make $(R_G{}^2)_{LS}/M_{LS}$ independent of c and T, and about proportional to $l(L/m_o)_{eff}M_z/M_w$, even if the molecular weight of the aggregates depended on c.

Since the degree of association is small for $T \approx -8$ °C, which is slightly below T_{OPT}, the decrease in $\partial[Kc/R(0,c)]/\partial c$ at that temperature may safely be attributed to a decrease in $(A_2)_{LS}M_{LS}$ with decreased temperature. For a rodlike solute,[45,46,59]

$$A_2 M = \frac{\pi N_A}{4} \frac{L l d_T}{m_o} \tag{10}$$

where the thermodynamic diameter d_T is expected to be no larger than the geometric diameter d_G (in the absence of electrostatic interactions), and could be zero, as under Flory Θ conditions.[60] Association may alter L, m_o, and d_T, and a change in temperature may alter d_T, even if unaccompanied by association or change in

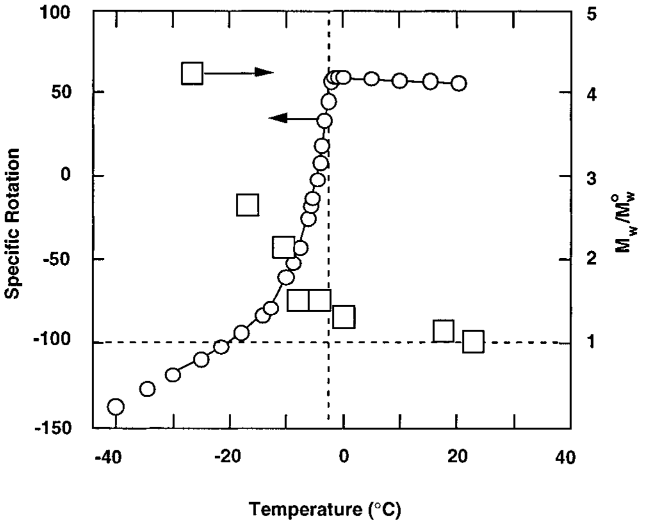

Figure 8. Specific rotation and the reduced molecular weight M_w/M_w^o versus temperature for solutions of poly(p-biphenylylmethyl L-glutamate) in tetrahydrofuran (see Figures 1 and 5). Here, M_w^o is the molecular weight of the fully dissociated polymer.

the chain conformation. For example, a Flory Θ temperature of ≈22 °C (i.e., $A_2 = 0$ at ≈22 °C) has been reported for PBG in the helicogenic solvent DMF, with $A_2 > 0$ for $T > \Theta$.[61] Other studies, however, did not find a Flory Θ temperature in this system,[58,62] leading to the suggestion that the behavior reported originally may reflect contamination of the solution by a small amount of water, with Θ in the experimentally accessible temperature range for the mixed solvent.[58] The decrease in d_T with decreasing T below T_{OPT} for solutions of PBPMG in THF is similar to the behavior reported for PBG in impure DMF, though here A_2 does not decrease to zero. With the solutions of PBPMG in THF, the decrease in solvation power indicated by the decreased d_T at low temperatures may initiate the aggregation. In this regard, it may be noted that the transition is not observed for solutions of PBPMG in all helicogenic solvents (e.g., see Figure 1). These observations suggest that the transition should be induced by adding a nonsolvent to a solution of PBPMG in a helicogenic solvent, provided the nonsolvent does not disrupt the helix conformation, see below.

The data on the temperature dependence of the optical rotation and $M_{LS} \approx M_w$ given in Figures 1 and 4, respectively, are replotted in Figure 8. It is evident there that the aggregates have formed a dimer *on average* by the temperature for which the precipitous drop in optical rotation is complete. The subsequent increase in M_{LS} to form tetramers at the lowest temperature studied is associated with a smaller decrease in the optical rotation. As noted in the Introduction, the effects on the chiral optical properties seem to be nearly independent of concentration over a wide range (0.7−46 g/L).[1,2] This indicates that the optical effects arise even when the state of association is small and that these chiral optical effects are rather insensitive to the size of the aggregated state. This is consistent with the fact that the chiral optical effects are a local probe, insensitive to long range effects. At room temperature the CD spectrum is that expected for a biphenyl group with a moderate excess of left-handed twist conformers.[1,2,63] Steric repulsion between the ortho hydrogen atoms of the benzene rings in the biphenyl group induces a twist angle between the two connected rings. Whereas in the absence of chiral information, the mirror image twist senses produced will have equal energies, the α-helical polypeptide right-handed backbone causes a discrimination between the two twist possibilities, giving rise to the circular dichroism observation above the transition temperature. The change in the chiral optical properties at the transition temperature cannot simply involve a sudden increase in the proportion of one twist sense over the other in the biphenyl group, since that would lead to an increase in magnitude, rather than the observed change in the circular dichroism spectrum. The change in sign of the D-line optical activity at the transition temperature[1,2] does not, therefore, signal a change in the preferred twist sense about the biphenyl group, but rather the creation of an entirely new circular dichroism signal associated with an altered electronic environment of the chromophore, as a consequence of the chirality of the supramolecular structure elucidated here by light scattering, and inferred from the earlier viscosity measurements.[1,3]

The poor solvent condition obtained on decreasing T could promote dimerization through enhancement of axial dipoles in the helical chain or attractive interaction among the biphenyl groups on different chains. In either case, the association could continue beyond the dimer stage, e.g., by aggregation of the dimers. Such a picture could give rise to a thermally reversible gel at a higher concentration, consistent with an unpublished report that such a gel is formed by high molecular weight samples of PBPMG synthesized by ester exchange methods.[64] The well-known gel formation of PBG in certain helicogenic solvents may have a similar origin (e.g., see studies on solutions of PBG in benzyl alcohol[18−22,24]). Indeed, small angle X-ray scattering studies show isolated helical molecules in one PBG system prior to gel formation on decreasing the temperature.[22]

The behavior observed here is different from that for the microphase separation found with dilute solutions of a rodlike heterocyclic macroion[25,29,31] or for dilute solutions of poly(3-dodecylthiophene).[32] In the latter, an associated state forms prior to the optical transition, with an ordered state formed on decreasing the temperature. With the rodlike macroion, this is caused by a decrease in A_2, but with poly(3-dodecylthiophene) the effect appears to be driven by order formation among the alkyl moieties. In the systems studied here, the formation of the ordered aggregate is not preceded by the formation of disordered aggregates but occurs spontaneously as the temperature is lowered, apparently driven by "poor solvent conditions" with decreasing temperature (i.e., smaller A_2). As mentioned above, this suggests that the temperature for the transition might be manipulated by control of the solution thermodynamics. Consistent with this, T_{OPT} for the transition in the specific rotation and the circular dichroism spectrum is increased by the addition of a nonsolvent (DMF) to solutions of PBPMG in dichloroethane.[1,2] This picture of the aggregation of PBPMG suggests that PBG, which is not reported to undergo optical activity transitions in dilute solution parallel to those for PBPMG, could undergo an induced transition by reducing the solvent quality by the addition of nonsolvents. This is seen on reduction of the temperature of solutions of PBG in the mixed solvent 1,2-dichloroethane + ethanol (a nonsolvent); $\eta_{sp}/c \approx 41$ dL/g at $c = 0.34$ g/dL in dichloroethane for the PBG used, uncorrected to low

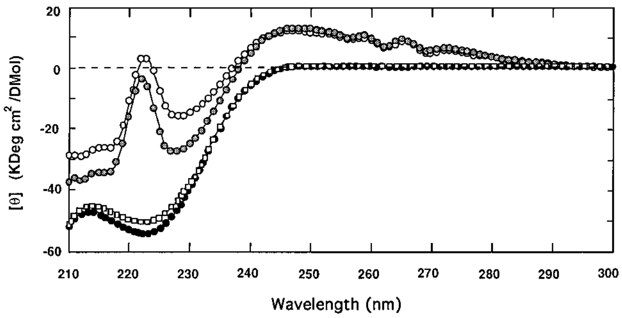

Figure 9. Circular dichroism spectrum as a function of temperature for solutions of poly(γ-benzyl L-glutamate) in dichloroethane/ethanol (63/37 by volume); $c = 1.38$ g/L. The clear and shaded circles are for −13.7 and −10.2 °C, respectively, and the solid circles are for −6.7, −3.2, and 2.0 °C. The squares are for 19.8 °C.

shear rate. While no transition in the CD spectrum is observed for PBG in dichloroethane over a wide temperature, a transition is observed with decreasing temperature range, on addition of 37.1 vol % ethanol; see Figure 9. A precipitate slowly forms on standing at temperatures below the transition shown in Figure 9, providing clear evidence of aggregation. It may be significant that within the wavelength range studied, the CD spectrum below T_{OPT} is identical to that observed for the cholesteric liquid crystal formed in solutions of PBG in good solvents at higher concentration.[65] The similarity of the CD spectrum below T_{OPT} with that of the cholesteric liquid crystal is consistent with the essentially parallel organization determined by the light scattering results for PBPMG. Although cholesteric solutions of PBPMG have been formed in preliminary work, measurements of the CD spectrum have yet to be reported to permit a direct comparison with the CD spectrum obtained here for $T < T_{\mathrm{OPT}}$.

Conclusion

Static and dynamic light scattering measurements on dilute solutions of PBPMG in THF confirm a helical conformation, as well as complete dissolution of the polymer at temperatures above the temperature T_{OPT} for the transition in chiral optical properties. The data also reveal increasingly poor solvent conditions as the temperature is decreased, with the formation of aggregates with the chains in ordered parallel array $T < T_{\mathrm{OPT}}$. The degree of association in the aggregate increases concurrently with the decrease in the optical rotation and change in the CD spectrum. The intermolecular association of the chains in parallel array alters the chiral electronic environment about the biphenyl group, without any evidence for a conformational change in the chromophore, but effecting the spectral characteristics seen in the optical rotation and the CD spectrum. Unlike the behavior reported for certain other systems discussed above, the optical transition observed with solutions of PBPMG does not involve the initial formation of a disordered aggregate, which orders itself on reduced solvent quality or temperature, but rather a spontaneous association, such as expected on crossing a phase boundary, with a concurrent optical transition.

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