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Mechanism of the screw-sense reversal of tightly hydrogen-bonded α-helical network triggered by the side-chain conformation

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ABSTRACT

The paper presents theoretical treatment of a screw-sense reversal of tightly hydrogen-bonded α-helical polypeptide chains. The transition is triggered by the conformational free-energy difference (s) of the side chain flanking the chiral (right(r)- or left(ℓ)-handed) backbone. The two-parameter scheme (s, σ) corresponding to a zipper-type transition model starting from one terminal has been constructed and applied to the thermally-induced helix–helix transition characteristic of polyaspartic acid esters observed in the helicoidal solution. The weighting parameter σ takes care of the instability associated with the ℓ/r junction, i.e., the unfolded site where at least three successive hydrogen-bonds should become free to change their partner. The enthalpy of transition (ca. 2 kJ/mol) of poly(β-phenethyl L-aspartate) (PPLA) ($Mv = 6 \times 10^4$) observed in the lyotropic liquid crystalline state in 1,1,2,2-tetrachloroethane has been reproduced with the instability parameter $\sigma = 0.001–0.0001$. Attempts were further extended to interpret the thermally-induced helix–helix transition behaviors of the PPLA homopolymers and random copolymers involving benzyl L-aspartate residues observed under various conditions.

1. Introduction – a short description of the background

Under certain conditions, polypeptides that are capable of adopting two α-helical forms, i.e., right(r)- and left(ℓ)-handed screw, may undergo a sharp conformational transition. (In the following, symbols r and ℓ will serve in two capacities: i.e., representing either the screw-sense of an α-helix or the handedness of a given unit constituting the α-helical array.) Blout et al. [1,2] have first found that poly(β-benzyl L-aspartate) (PBLA) has a helical sense opposite to that of more popular poly(γ-benzyl L-glutamate) (PBLG) in chloroform solution. PBLA α-helix normally takes the ℓ-form, the r-form being reported only under certain limited conditions [3–8]. Goodman et al. [9] have demonstrated that random copolymers of β-benzyl and β-nitrobenzyl L-aspartate exhibit a sharp transition of the Moffit b_0 value in chloroform solution from positive for PBLA to negative for poly(β-nitrobenzyl L-aspartate) (PNBLA) when plotted against the β-nitrobenzyl content. While PBLA prefers the ℓ-helix, the most stable form of PNBLA is r at room temperature. Since then, the so-called helix–helix transition has been widely recognized as a characteristic of polyaspartic acid esters [10–15].

These polymers are known to exhibit solvent-induced as well as thermally-induced helix–helix transitions depending on the polarity of the media and the chemical structure of the pendant side groups [9–18]. Scheme 1 is constructed to demonstrate briefly the phase transition behaviors of poly(β-phenethyl L-aspartate) (PPLA). PPLA takes the α-helical r-form in 1,1,2,2-tetrachloroethane (TCE) at moderate temperatures. The transition to the ℓ-helix occurs at 85–90 °C in the isotropic solution as well as in the lyotropic liquid crystalline (LC) state [19]. In the presence of a denaturant acid, PPLA also exhibits a transformation to the ℓ-form by lowering temperature. In Scheme 1, the two coil states which appear in the higher- and lower-temperature regions are also included [16,20–23]. Accordingly, a ternary mixture such as PPLA/TCE/trifluoroacetic acid (TFA) could possibly exhibit as many as five phases under a proper condition at a polarizing microscopic examination (the outermost framework of the scheme) [18]. PPLA is also known to undergo an irreversible helix–helix transition around 135 °C in the solid state [24]. On the contrary, PBLG is known to exist only in the r-α-helix in conventional helix-forming solvents as well as in bulk. As shown above, PBLG and PPLA are mutually isomeric in their chemical structures: i.e., PBLG can be converted into PPLA by interchanging a γ-CH_2 with the ester group in the side chain. The major difference

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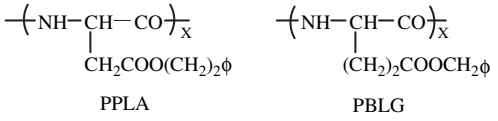
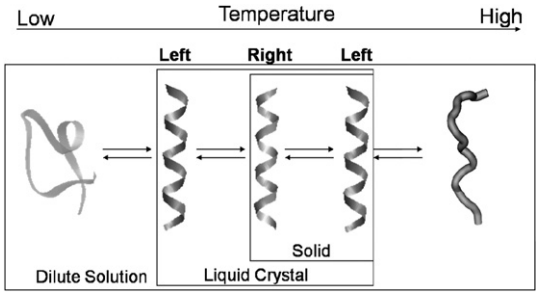
arises in the rotational characteristics around the first articulated χ_1 bond, which controls the interaction between the side chain and the helical backbone [25].



In general, cooperative formation of a hydrogen-bond (H-bond) array along the backbone is an essential feature involved in the stabilization of α-helical forms of polypeptides and proteins [26]. The formation and collapse of such an H-bonded array are customarily interpreted as a pseudo-first-order transition of linear chains. The most well-known example is the transition between the helix and coil of polypeptides observed in solution. Zimm and Bragg [27] have demonstrated that the characteristics of such transitions can be well described by the one-dimensional Ising lattice on the assumption that helical and coil units are free to occur anywhere along the chain. More sophisticated Ising expressions have been later proposed by Nagai [28], and Lifson and Roig [29]. In the r↔ℓ-helix transition, however, the conformational free-energy difference between the two oppositely α-helical networks approaches zero at certain point where the transformation takes place. As manifestly shown in a series of ^{2}H NMR studies on PPLA in the lyotropic LC state [30–34], the orientation of the α-helical backbone remains unaffected during the helix–helix transition, suggesting that the screw-sense reversal does not include any coil configuration in the intermediate state. At the transition site, some minimum number of monomer units must be liberated from the regular α-helical H-bond framework in order to achieve the reversal of the α-helical arrangement [34,35].

Studies on the transition behaviors of random copolymers comprising the two opposite helical preferences, i.e., β-benzyl (BLA) and β-phenethyl L-aspartate (PLA) residues, revealed that the transition temperature tends to be lowered by inclusion of the former component (BLA content of 0–50%), but the sharpness of the transition curve remains almost unaffected. The experimental results on the copolymer systems were summarized in the preceding paper [19]. An example derived from ^{2}H NMR studies on partially deuterated copoly(22BLA-ran-78PLA) is shown in Fig. 1, where the two components, BLA (22%) and PLA (78%), are separately labeled [31]. The two transition curves, ℓ-content f_{left} ($=1-f_{right}$) vs. temperature, obtained from copoly(22BLA-$\beta_R d$-ran-78PLA) (○) and copoly(22BLA-ran-78PLA-$\beta_R d$) (●) respectively



Scheme 1. Phase transition diagram of PPLA observed in the isotropic solution (five phases), in the lyotropic LC state (three phases), and in the crystalline solid (two phases). Each of the three square frameworks indicates the experimental condition and the transitions observed therein.

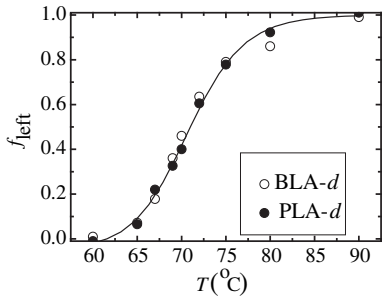


Fig. 1. Comparison of the transition curves from the two copoly(22BLA-ran-78PLA) samples where the two components are separately deuterated as indicated in the diagram. ^{2}H NMR observations were carried out in the lyotropic LC state (20 wt% in TCE).

by probing BLA and PLA residues almost exactly overlap with each other, indicating that the screw-sense inversion proceeds in a highly cooperative manner along the chain. Theoretical models assuming the intermediate state comprising multi-block arrangements of r- and ℓ-sequences may be safely excluded [36,37]. In his pioneering work on PBLA monolayers, Malcolm [4,5] offered a model in which the unfolded regions (the ℓ/r junction) are propagated along the backbone from one or both terminals.

In view of the above-mentioned experimental evidences, we have concluded that the transition follows a single-front-moving zipper-type scheme, propagating from one terminal to the other. An independent experiment by using properly designed block copolymers with γ-benzyl L-glutamate (BLG) suggests that the rewinding of α-helical network predominantly starts from the N-terminal of the chain with raising temperature [38]. In this paper, a theoretical description is presented on the basis of the molecular mechanism described above. Attempts are further extended to interpret the thermally-induced helix–helix transition data of copolymer systems reported in the preceding paper [19].

2. Theoretical prescription

Scheme 2 represents an ideal zipper-type process (a single-front-moving model) consisting of three representative steps: i.e., the initial, propagation and final stages.

Reversal of the helix-sense may be assumed to start from one terminal [38]. The second row indicates an intermediate state of the propagation. Following the conventional Zimm–Bragg formalism [27], the transition may be treated by Ising's method using the two parameters s and σ. Here as usual, s designates the stability of ℓ-relative to r-unit.

$$\begin{aligned} \ln s &= \Delta S_{\ell-r}/R - \Delta H_{\ell-r}/RT \\ &= \Delta H_c/RT_c(1 - T_c/T) \end{aligned} \tag{1}$$

where $\Delta S_{\ell-r}$ and $\Delta H_{\ell-r}$ designate the entropy and enthalpy difference between ℓ- and r-units respectively. Adopting the conventional thermodynamic notations, ΔH_c and T_c are used in the latter expression.

Initial	rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr
Intermediate	ℓℓℓℓℓℓℓℓℓℓℓℓσrrrrrrrrrrrrrrrrrrrrr
Final	ℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓℓ

Scheme 2. Schematic diagram of the zipper-type model of the helix-sense reversal. Parameter σ represents the instability inherent to the H-bond rearrangement at the transition site.

Since the reversal of the screw-sense takes place only at the moving front, parameter σ takes care of the instability associated with the ℓ/r junction, i.e., the transition state where at least three successive H-bonds should become free to change their partner. In Scheme 2, σ is symbolically included to represent such an irregular event. The configurational partition function Z for an α-helical rod of n units may be expressed by the matrix-multiplication scheme:

$$Z = J_0 M^n J_n \tag{2}$$

where

$$M = \begin{array}{c} \\ R \\ L \end{array} \begin{array}{c} \begin{array}{cc} R & L \end{array} \\ \begin{bmatrix} 1 & \sigma s \\ 0 & s \end{bmatrix} \end{array} \tag{3}$$

and J_0 and J_n are respectively the row and column vectors to collect the desired elements. For simplicity, n may be taken to be the degree of polymerization (DP). In this particular example, however, Z can be alternatively replaced by a simple algebraic expression such as

$$\begin{aligned} Z &= s^n + \sigma(s^{n-1} + s^{n-2} + \cdots + s^2 + s) + 1 \\ &= s^n + \sigma \sum_{k=1}^{n-1} s^k + 1 \\ &= 1 + s^n + \sigma s(s^n - 1)/(s - 1) \end{aligned} \tag{4}$$

The fraction of ℓ-unit in a given α-helical array can be calculated by

$$\begin{aligned} f_{\text{left}} &= (1/n)\partial \ln Z/\partial \ln s \\ &= \mathrm{A}/n\mathrm{B} \end{aligned} \tag{5}$$

where

$$\mathrm{A} = ns^n + \sigma s\left[\left(n-1\right)s^n - ns^{n-1} + 1\right]\Big/(s-1)^2 \tag{6}$$

and

$$\mathrm{B} = s^n + \sigma s(s^{n-1} - 1)\Big/(s-1) + 1 \tag{7}$$

In Fig. 2a, the effect of DP on the transition curves was examined at a fixed σ value (0.001) by plotting f_{left} against ln s for various values of n. The slope becomes sharper as n increases. In the range $n > 200$, the variation of f_{left} becomes less sensitive with n. Alternatively, the f_{left} vs. ln s plots calculated for various values of σ at $n = 300$ are put together in Fig. 2b. The slope of the curve becomes sharper as σ decreases, approaching the limiting value in the range $\sigma < 0.001$. As manifestly shown by Eq. (4), σ is closely related to the fraction of the molecular species existing in the intermediate state. The average number of species containing the ℓ/r junction is given by.

$$\begin{aligned} \nu &= \partial \ln Z/\partial \ln \sigma \\ &= \sigma \Sigma s^k/Z \\ &= 1 - (s^n + 1)/Z \end{aligned} \tag{8}$$

When $\sigma = 0$, the equilibrium should take place between the partitioned all r- and all ℓ-form without any intermediate state. The value of ΔH_c obtained under such an ultimate condition corresponds to the enthalpy change defined by the two-state equilibrium of the van't Hoff theory [39].

3. Analysis of the observed helix–helix transition data – elucidation of σ and ΔH_c

Shown in Fig. 3 is an example of the theoretical treatment of the experimental data of the screw-sense inversion of PPLA observed by ^{2}H NMR in the LC state. The deuterium quadrupolar splittings due to the β_R-CD bond in the side chain varies sensitively with the helical sense of the backbone: $\Delta\nu \approx 20$ kHz and 40 kHz, respectively, for the r- and ℓ-regime. The relative intensity of these peaks can be used as a probe to follow the transition. The transition behaviors studied in this manner provides a basis to examine the validity of the theoretical framework (Eq. (1)–(7)). The computational procedure is as follows. (1) The values of s can be estimated by using Eq. (5)–(7) for given set of fraction-temperature data, σ being kept constant. In all calculations, the value of n may be set equal to 300 in consideration of the molecular weight (Mv $= 6 \times 10^6$) of the sample. (2) The transition enthalpy, ΔH_c is then obtained from the standard relation given in Eq. (1). (3) The process may be repeated for another possible choice of σ. In the example shown in Fig. 3, the experimental curve (filled circles) is well reproduced by the calculation (open circles) using a set of parameters such as $\Delta H_c = 2.42$ kJ/mol and $\sigma = 0.001$.

The DSC measurements on PPLA solutions gave an estimate of the transition enthalpy in the range 1.5–1.8 kJ/mol for the LC state (~25 wt% in TCE). Although the DSC peaks are much broader, and thus less accurate for dilute isotropic solutions (~5 wt%), the

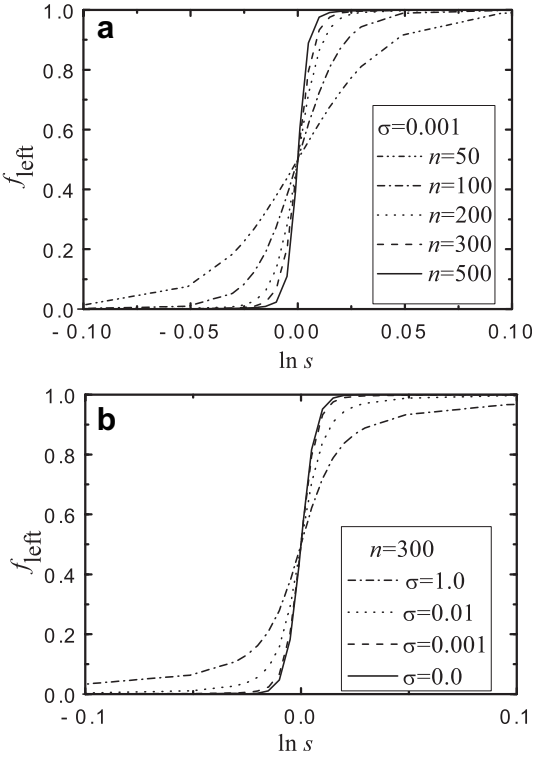


Fig. 2. (a) n-Dependence of transition curves calculated with $\sigma = 0.001$, and (b) σ-dependence of the transition curves for $n = 300$.

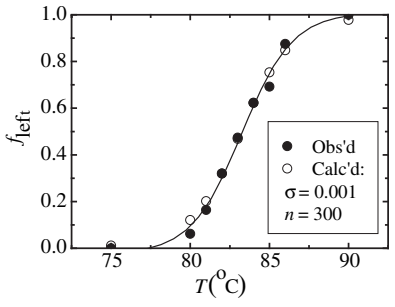


Fig. 3. Reproduction of experimental observations on PPLA (sample designated as E1) by theory. The transition enthalpy ΔH_c thus estimated is 2.42 kJ/mol for the LC phase.

magnitude of the transition enthalpies estimated from the peak area was nearly identical with those for the more concentrated LC phase [40]. The conformational analysis of the ^{2}H NMR data collected from PPLA carrying fully deuterated side chains gave an estimate of 1.1 kJ/mol for the energy difference between the two helical forms [32,35]. The side-chain conformation and the energy difference derived from ^{2}H NMR measurements have been well reproduced by calculations such as MD simulation and rotational isomeric state (RIS) energy minimization [35].

Theoretical treatments were extended to include all other helix–helix transition data obtained by using the ^{2}H NMR technique in the LC state. The results are accommodated in Table 1. In addition to the two independent measurements on the same PPLA homopolymer samples (E1, E2), copolymers comprising BLA and PLA [19] are also studied. In all cases, the deuterium atom introduced at the C-β_R position of the side chain was used as a probe to detect the r↔ℓ equilibration. The data adopted in the 4th row of the table were obtained from a 1:1 mixture of PPLA-$\beta_R d$ and PBLG-N*d* in a lyotropic LC state in TCE (25 wt%) [31]. Since the α-helical PBLG remains in the rigid r-form over the entire temperature range, the r↔ℓ helix-sense inversion of the guest (PPLA-$\beta_R d$) should take place in a highly ordered LC environment of the host. The two experiments, with and without the host, yielded a comparable fraction vs. temperature plot characterized by the parameters such as (with) $T_c = 86.6$ °C and the van't Hoff energy $\Delta H_v = 503.0$ kJ/mol (the 4th row), and (without) $T_c = 83.2$ °C and $\Delta H_v = 656.2$ kJ/mol (the 2nd row) respectively. Essentially the same result was obtained from the experiment in which the host PBLG was replaced by poly(γ-benzyl D-glutamate) (PBDG-N*d*) having the opposite chirality.

The transition enthalpies ΔH_c listed in the 4th column of Table 1 were calculated according to the zipper model for various combinations of σ and s. In most cases, the observed transition curves were found to be reproduced equally well by using σ values in the range 0–1. In consideration of the experimental DSC data of PPLA [40], the results of calculations are shown for two values of σ (0.0001 and 0.001) in the 4th column of Table 1. Also listed in the table are the transition temperature T_c and van't Hoff energy (expressed in kJ/mol of polymers) obtained directly from the experimental transition curve. In the range $\sigma < 0.0001$, the value of ΔH_c remains nearly invariant. For copolymers involving BLA residues, the ΔH_c values tend to be somewhat smaller than those from homopolymer PPLA.

Shown in Table 2 are the summary of the analysis of the experimental data collected in the dilute, isotropic TCE solution. Three different techniques such as ^{1}H NMR, FTIR, and ORD (b_0) were employed to follow the transition behaviors of PPLA. As noted in the preceding paper [19], the ^{1}H NMR measurements were hampered by the low solubility of PPLA at lower temperatures: the transition curve thus derived was found to be less steep than those from the other measurements. For given polymers, the slope of the thermal transition curve becomes moderate in the isotropic state [35]. These observations are reflected in the lower values of the van't Hoff energy ΔH_v in Table 2 relative to those in Table 1. Nevertheless the transition temperatures T_c remain nearly unaffected regardless of whether the solution is isotropic (Table 2) or liquid crystalline (Table 1), suggesting that the transition mechanism is more or less identical.

As indicated in Table 2, larger σ values (0.01 and 0.1) are adopted for the isotropic dilute solution. Use of the same σ values (0.0001–0.001) as in Table 1 leads to much lower estimates of ΔH_c than those of the experimental enthalpy change (1.6 kJ/mol) of PPLA [40]. The difference in σ values may be attributable to a higher chain flexibility in the dilute solution. Copolymerization with BLA up to 24% does not affect the value of ΔH_c for a given σ value (4th–6th rows of Table 2). The ΔH_c value obtained for Copoly (49BLA-$\beta_R d$-ran-51PLA) tends to be much lower however (the bottom row). The physical meaning of the instability factor σ will be further examined in the following sections.

4. The effect of the instability factor σ

In the present theoretical framework, we cannot offer any reasonable way to determine the magnitude of the instability factor σ. Under such circumstances, the most probable σ value may be selected in consideration of the experimental ΔH_c data. The ΔH_c values of PPLA shown in Table 1 fall in the range of the enthalpy change ΔH associated with the thermal transition of PPLA in the LC state in TCE. For copolymers with BLA, as one may expect, the values of ΔH_c derived for the LC state tend to be lower than those of the homopolymer. The reliable estimate of ΔH becomes more difficult in the isotropic dilute solution (Table 2). When chains are isolated in a dilute solution, the α-helical backbone may gain higher flexibility: a larger disorientation at the ℓ/r junction may facilitate the transition, and lead to a larger σ value than those assumed in the LC state. Adoption of a higher value of σ enhances the magnitude of ΔH_c, but never to the range of those listed in Table 1 for the LC state.

To examine the effect of σ at given DPs, calculations were tentatively carried out for two representative sets of experimental data: PPLA (E1) and copoly(49BLA-ran-51PLA) obtained in the LC state. For given sets of σ and n, ΔH_c values were so adjusted as to reproduce the experimental observations. Fig. 4 indicates variation of ΔH_c with σ and n. Two σ values are examined: i.e., 0.0001 (cf. Table 1) and 0.01 (cf. Table 2). The ΔH_c value decreases rapidly in the range $n < 200$, but tends to be moderate around $n = 300$ for given values of σ. The DP of the polymers and copolymers used in the experiments [19,31] remain mostly in this range. The effect of σ is appreciable for all n. At $n = 300$, the values of ΔH_c are depressed by about 1.5 and 1.0 kJ/mol respectively for PPLA (E1) and copoly (49BLA-ran-51PLA) with reduction in σ from 0.01 to 0.0001. Taking the observed heat of transition into account, the former σ value

Table 1
Transition enthalpies elucidated from the ^{2}H NMR data in TCE in the nematic LC state: ΔH_v from the van't Hoff relation and ΔH_c for the zipper model with $n = 300$.

Sample	T_c (°C)	van't Hoff ΔH_v (kJ/mol)	Zipper model ΔH_c (kJ/mol)		Ref.
			$\sigma = 0.0001$	(0.001)	
PPLA-$\beta_R d$ (E1)	83.2	656.2	2.21	(2.42)	[31]
PPLA-$\beta_R d$ (E2)	82.8	767.2	2.59	(2.82)	[31]
PPLA-$\beta_R d$ in PBLG	86.6	503.0	1.69	(1.85)	[31]
Copoly(22BLA-ran-78PLA-$\beta_R d$)	71.0	310.7	1.05	(1.14)	[31]
Copoly(22BLA-$\beta_R d$-ran-78PLA)	70.4	343.7	1.16	(1.27)	[31]
Copoly(24BLA-$\beta_R d$-ran-76PLA)	76.1	224.6	0.76	(0.83)	[19]
Copoly(49BLA-$\beta_R d$-ran-51PLA)	37.2	418.7	1.41	(1.54)	[19]

Table 2
Transition enthalpies elucidated from the data observed in TCE in a dilute isotropic state: ΔH_v from the van't Hoff relation and ΔH_c for the zipper model with $n = 300$.

Sample	T_c (°C)	van't Hoff ΔH_v (kJ/mol)	Zipper model ΔH_c (kJ/mol)		Ref.
			$\sigma = 0.01$	(0.1)	
PPLA (^{1}H NMR)[a]	87.6	159.0	0.93	(1.57)	[19]
PPLA (FTIR)	82.1	234.0	1.46	(2.70)	[41]
PPLA (ORD, b_0)	90.7	188.4	1.09	(1.86)	[19]
Copoly(09BLA-ran-91PLA) (ORD, b_0)	83.9	174.8	1.02	(1.73)	[19]
Copoly(24BLA-ran-76PLA) (ORD, b_0)	66.7	192.4	1.12	(1.90)	[19]
Copoly(49BLA-ran-51PLA) (ORD, b_0)	41.4	107.9	0.63	(1.06)	[19]

[a] As noted in Ref. [19], the ^{1}H NMR measurements are hampered by the low solubility of PPLA in TCE at lower temperatures.

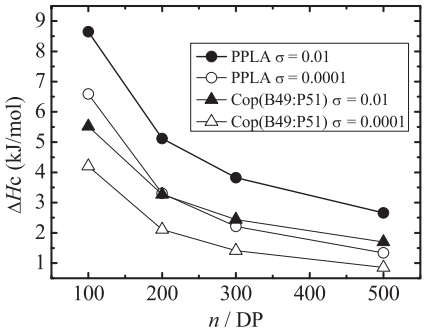


Fig. 4. n-dependence of ΔH_c for PPLA-$\beta_R d$ (circles) and Copoly(49BLA-$\beta_R d$-ran-51PLA) (triangles) derived from the analysis of ^{2}H NMR data in the LC state, estimated for two σ values as indicated in the diagram.

exceeds the upper limit for these polymers in the LC state. In the range $\sigma < 0.0001$, the effect on the ΔH_c value becomes practically nil at $n = 300$. These considerations underlie the choice of σ values in Tables 1 and 2.

5. Discussion

As stated earlier (cf. Eq. (4)), the assumption of $\sigma = 0.0$ entirely eliminates the intermediate fraction, leading to the equilibrium between the two chemical species, i.e., all-r and all-ℓ form. This is the concept of the van't Hoff model. The values of ΔH_c given in the 3rd column of Tables 1 and 2 correspond to this limit. The results of the analysis indicate that despite the thermodynamically unfavorable aspects of the ℓ/r junction, there is a finite probability that a polymer molecule contains the transient configurations intervening between the two helical portions. The observed transitions have been successfully described by the two-parameter scheme set forth in this work. In the present treatment, σ is introduced as the instability parameter at the ℓ/r junction which corresponds to the moving front of the zipper transition. In the conventional Zimm–Bragg model [27], the transition is assumed to take place through a multi-block intermediate state. The factor σ is then defined as the nucleation parameter associated with the extra free energy to create a junction at a given site along the chain. Experiments on the random copolymers of PLA and BLA strongly suggest that the transition proceeds through a single-front-moving mechanism [19]. The characteristics of the σ parameter appreciably differ from those customarily adopted for the helix–coil transition.

Nevertheless the role of σ in the mathematic operation is similar to that originally introduced in the treatment of the helix–coil transition [27]. Following the argument presented by Applequist [42], the instability parameter may be understood as an equilibrium constant for the formation of an interruption

$$\sigma = \exp(-\Delta G_0/RT) \tag{9}$$

where ΔG_0 is the extra free energy of the ℓ/r junction formation. Since the number of H-bonds remains constant during the transition process, the energy contribution (ΔH_0) may be insignificant. Thus

$$\sigma \approx \exp[(\Delta S_0/R)] \tag{10}$$

where ΔS_0 accounts for the entropy change to create an interruption for the rearrangement of H-bonds. The intermediate state involves at least 3 monomeric residues or four hydrogen bonds to complete the reversal of the α-helical screw-sense. Let $\sigma = 0.001$, Eq. (9) leads to $\Delta S_0 \approx -57$ J/mol K, or $\Delta S_0/3 \approx -19$ J/mol K for a residue. Adoption of a larger σ value (0.01) from the isotropic solution yields $\Delta S_0 \approx -38$ J/mol K, or $\Delta S_0/3 \approx -13$ J/mol K for a residue. The entropy penalty to create an unfolded site is slightly higher in the LC state. Our interest is now to elucidate the chain configuration at the transition site. A RIS simulation has been attempted and results were reported in the literature [34,35]. In the range of σ estimated above, however, the fraction of the junction sites seems to be so low that they may easily elude experimental observations. In spite of such experimental difficulties, it may be worth investigating the ΔH_c–σ relation for copolymers of higher BLA content (>50%).

Eq. (1) is a standard thermodynamic expression for the temperature-dependence of the free-energy difference between the two states. While the front factor $A = \Delta H_c/RT_c$ is responsible for the slope of the ln s vs. T relation, the succeeding tern $(1 - T_c/T)$ takes care of the thermal variation of ln s in terms of reduced temperature. It is important to note that the characteristics of the individual polymer systems are included in term A. Variation of T_c as a function of PLA content of copolymers is previously shown in Fig. 11 of the preceding paper. In contrast to T_c, the accurate estimate of the steepness of the transition curves is difficult, and thus ΔH_c values listed in Tables 1 and 2 inevitably involve larger uncertainty. Nevertheless, it should be interesting to examine how A varies with the copolymer composition. In Fig. 5, the A values elucidated from the IR and b_0 measurements in the dilute isotropic solution (Table 2) are plotted against the PLA content of copolymers. With the reason stated earlier (cf. Section 3), the A value derived from ^{1}H NMR measurements is omitted from the plot. The general trend of the plot is followed by a linear fitting. A long extrapolation to PLA content = 0 yields $A = 0.09$, suggesting that the magnitude of $\Delta H_c/RT_c = \Delta S_c/R$ declines toward the PBLA limit. The values of A obtained from Table 1 for the LC state are somewhat larger, and when plotted, the points are more scattered. The decrease in A illustrated in Fig. 5 is consistent with the observation that PBLA takes the ℓ-form in conventional helicoidal solvents at room temperature [9–12], and easily transforms into the coil state by addition of a small amount of organic acids [43,44]. Such vulnerability of the PBLA system is strongly related to the smaller free-energy difference between the two chiral states. In our model, the average values of the side-chain conformation energy should be approximately proportional to the copolymer composition. The screw-sense of copolymers remains in the ℓ-regime below PLA content = ca. 0.4 at room temperature (cf. Fig. 3 of the preceding paper).

As shown in Scheme 1, PPLA also exhibits an inverse-type helix–helix transition in a mixed solvent such as TCE/TFA. The rotational characteristics of bond χ_1 are sensitively affected by the presence of an organic acid (TFA). According to the ^{1}H NMR observations [16,18], the fraction of the gauche conformation (χ_1) tends to be higher with increasing acid concentration or alternatively with decreasing temperature. The free-energy change in the side-chain conformation triggers the screw-sense inversion of α-helix from the r- to ℓ-form with descending temperature.

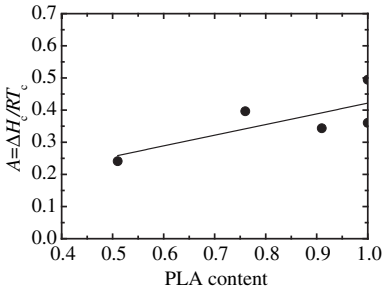


Fig. 5. Variation of $A = (\Delta H_c/RT_c)$ with PLA content.

Essentially the same mechanism may be assumed for the inverse-type transition as long as the H-bond network along the α-helical backbone is effectively maintained. As temperature is further lowered, the solution becomes biphasic where the α-helical ℓ-form is in equilibrium with the coil state [18] (cf. Scheme 1).

6. Concluding remarks

It would be interesting to examine the effect of residues other than aspartates. Inclusion of a few mol% of BLG residue as a comonomer is enough to prevent the completion of the screw-sense inversion of PPLA [31]. According to the previous analysis of the side-chain conformation, the χ_1 bond of PLA is very susceptible to temperature or polarity of solvent. The origin of such flexibility may be traced back to the chemical constitution of the χ_1 bond. When a C–C bond carries polarizable groups on both sides, the energy difference between the *t* and *g* states tends to be lowered, and often a pronounced gauche preference (a gauche effect) is detected [45,46]. Since BLG includes another methylene unit between C_α and the ester carbonyl group, no such gauche-stabilization effect can be expected. While the PLA side chain gains more freedom in the high-temperature ℓ-form by taking a $\chi_1 = g^-$ arrangement, BLG stubbornly prefers an extended $\chi_1 = t$ conformation [47]. Our observation is consistent with the results of Bradbury et al. [48] who examined the compatibility of BLA with the two isomers of γ-benzyl glutamate by preparing the random copolymer. While copolymers with the D-isomer (ℓ-preference) remain in the ℓ-form over the entire composition, the helical sense of the copolymer with the L-isomer (r-preference) is largely determined by the glutamate residue and the b_0 value remained below −400 over the range of BLA content = 0–85%.

PPLA is known to exhibit an irreversible screw-sense inversion from the r-α-helix to ℓ-π-helix in the solid state at about 130°C [24]. Luijten et al. [49] have reported a reversible transition in PPLA films end-grafted on silicon or quartz substrates: the original r-α-helix has been recovered from the high-temperature π-helix by immersion in chloroform. It is of interest to note that a racemic mixture of PPLA with its optical antipode PPDA undergoes a reversible transition to the high-temperature α-form at about 200 °C [31]. In polyaspartates having somewhat longer side chains, a similar transition takes place reversibly in the solid state [50–54]. These observations are quite consistent with the zipper-type mechanism of the screw-sense inversion described above. Detailed studies on the mechanism of the screw-sense inversion in the solid state are in progress in our laboratory.

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