Dimensional Changes of Polypeptides in the Helix-Sense Inversion Region

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ABSTRACT: Dimensional changes of polypeptides in the helix-sense inversion region are analyzed by a theory based on the three-state, nearest-neighbor interaction model. Each peptide residue in a chain is assumed to take on right- and left-handed α -helical states as well as a random coil state, and the helix stabilization effect due to hydrogen-bond interactions is taken into account. Such interactions between four consecutive residues have explicitly been discussed in a 9×9 statistical-weight matrix in a previous theory [*Macromolecules*, **17**, 1599 (1984)], while in the present model we simplify this matrix into the form of a 3×3 matrix by considering the interactions only between two consecutive residues. Calculations of the average helix fraction and the number of helix sequences are correspondingly simplified with maintaining quantitative agreement in numerical results. The new theory is also applied to the analyses of the length distribution and the higher moments of helix. Theoretical transition curves illustrating the general characteristics of the sense inversion phenomena suggest a method for evaluating the transition parameters.

KEY WORDS Polypeptide / Helix-Sense Inversion / Chain Dimension / Nearest-Neighbor Interaction Model /

In a previous study¹ we have developed a theory of the helix-sense inversion in polypeptides for interpreting the unusual conformational transitions exhibited by poly(aspartate ester)s. For example, poly(β -phenethyl L-aspartate) (PPELA), being in a right-handed α -helical form (RH α) in chloroform, undergoes a sense inversion to a left-handed α form (LH α) when a small amount of denaturing solvent such as dichloroacetic acid or trifluoroacetic acid is added.² The LH α -helix thus formed can then be transformed into a random coil form by further increasing the acid concentration. Moreover, PPELA in an appropriate chloroform-acid mixture undergoes a thermally induced sense inversion from RH to LH α -helix upon heating. An indication of this latter type of inversion has also been reported for poly(β -*n*-propyl L-aspartate) in chloroform.^{3,4} Generally, the stabilities of the

two α -helical forms of poly(aspartate ester)s are known to be low and close together.³⁻⁸ The sense inversion can therefore be induced relatively easily in this class of polypeptides when the solution conditions are appropriately changed.

In the theory in ref 1 (hereafter referred to as Part I) we have assumed that each peptide residue in a chain can take on three conformational states (RH α , LH α and coil states), and that the conformational stability of a given residue depends on the states of its neighboring three residues. This latter requirement arises since a hydrogen-bond bridge stabilizing the helix sequence forms only when three consecutive residues are cooperatively distorted to the α -helical state of the same sense. A statistical-weight matrix representing this long-range hydrogen-bond interaction is given by a 9×9 matrix with five non-zero

eigenvalues. The partition function of the entire chain is derived in terms of these five eigenvalues and used to obtain explicit expressions for average fractions of the two α -helical conformers. The theory in Part I is successful in reproducing the fundamental aspects of the sense inversion phenomena; nevertheless, its scope and applicability are expected to be further expanded before we proceed to the analyses of experimental observations.

Particularly, the following problems are to be noted. In most current experiments, conformational properties of polypeptides are examined by polarimetric measurements such as optical rotatory dispersion (ORD) and circular dichroism (CD). In the case of a simple helix-coil transition both ORD and CD can readily determine an average helix fraction: However, when the chain undergoes the sense inversion, individual values of fractions of RH and LH α conformers can no longer be separated. The quantity measurable in these experiments is a fractional difference between the two α -helical conformers ($f_r - f_l$); while the sum of their fractions ($f_r + f_l$), *i.e.*, a total helicity, is left undetermined. Estimation of the latter quantity should require independent experimental informations (*e.g.*, the mean-square radius of gyration, the dipole moment, or the Kerr constant from electric birefringence measurement) that reflect the average dimension of α -helices irrespective of their right- or left-handednesses. It is for this reason that we have carried out in a recent study⁹ the measurement of electric birefringence of poly(ϵ -carboxy L-lysine) (PCBL) in the helix-coil transition region. This particular polypeptide is chosen since it undergoes a sharp transition and detailed conformational properties have already been established through several experimental studies.¹⁰⁻¹³ The observed changes in birefringence can well be reproduced from ORD data by using the Zimm-Bragg theory¹⁴ where only the nearest-neighbor interactions between two consecutive residues

are considered. This reference experiment suggests that the informations needed to analyze the sense inversion mechanism can similarly be obtained from electric birefringence measurements (and probably from other two experiments as well), and that the nearest-neighbor interaction model should work in these analyses. This second conclusion is important since all aforementioned studies require theoretical estimation of the higher order moments of helix for analyzing the observed informations (*e.g.*, the electric birefringence, or the Kerr constant, is in proportion to an average of the cubed helix lengths).⁹ To facilitate these calculations and hence provide a means of quantitative analyses of experimental observations, we simplify below the theory in Part I by eliminating mathematical complexity associated with the long-range hydrogen-bond interactions.

The nearest-neighbor interaction model including three conformational states was once discussed by Scott.¹⁵ His primary interest was to see how the α -helical conformation of one sense can be stabilized in a long polypeptide chain in spite that the energy difference between RH and LH α states of each residue has been predicted to be only marginal.^{16,17} Although he was aware of possibility of coexistence of RH and LH α -helices in the intermediate stages of the helix-coil transition, no further attention was paid to the sense inversion phenomena. In the following we analyze the dimensional changes of a polypeptide chain in the sense inversion region based on this nearest-neighbor interaction model, and propose a method for estimating the thermodynamic parameters of the transition.

THEORY

Nearest-Neighbor Interaction Model

Some basic assumptions introduced in Part I to describe the sense inversion phenomena are as follows. A polypeptide chain under consideration is regarded to consist of a linear

array of monomeric residues ($-\text{CO}-\text{C}^\alpha\text{HR}-\text{NH}-$), with R being a side chain attached to the α -carbon atom. All monomeric residues are indexed as $1, 2, \dots, N$ from the free carboxyl end, so that an intramolecular hydrogen-bond bridge forms between the amido hydrogen atom of a given residue (say, $i-2$) and the amido oxygen atom of its fourth succeeding residue ($i+2$) when intervening three residues ($i-1$, i , and $i+1$) are all distorted to the α -helical state. Such a residue, distorted to the α -helical state and stabilized by at least one hydrogen-bond bridge, is defined as a helix (h) unit. Since each individual residue is assumed to interconvert its conformational state between RH and LH α -helical states, the h units are distinguished into two types. One which has the rotational angles about the skeletal single bonds $\text{C}-\text{C}^\alpha$ and $\text{C}^\alpha-\text{N}$ equal to those characteristic of the RH α conformation is defined as a r unit, and the other one having the rotational angles equal to those of the LH α conformation is defined as a l unit. A residue assuming any arbitrary sets of angles other than the above two is then called a random coil unit and denoted by c.

According to the requirement that the helix sequence becomes stabilized when it involves three consecutive helix units, occurrences of chc and chhc ($h=r$ or l) sequences with only one or two helix units are disregarded.^{18,19} This latter tetrad chhc is the longest sequence whose interaction is to be considered, hence a statistical-weight matrix (or a transition matrix) assumed in Part I has originally had a dimension of $3^3 \times 3^3$. Fortunately, some of these 27 triads have been excluded by considering the steric hindrance effects between r and l units and the hydrogen-bond interactions. The dimension of this matrix has finally been reduced to 9×9 after eliminating redundant matrix elements.

In the nearest-neighbor interaction model adopted here, we disregard some detailed structural characteristics of the hydrogen-bond bridge and instead introduce a hypo-

thetical bridge. That is, when a given residue (i) turns into an α -helical state, a hypothetical hydrogen-bond bridge forms with its neighboring residue ($i-1$) and thus stabilizes the helical conformation of the i th residue. A statistical-weight matrix W sufficient to describe this situation may have the following structure¹⁵

$$W = \begin{array}{c|ccc} & i-1 & & & \\ & \hline & r & l & c \\ \hline r & S_r & 0 & 1 \\ l & 0 & S_l & 1 \\ c & \sigma_r S_r & \sigma_l S_l & 1 \end{array} \quad (1)$$

where S_h and σ_h ($h=r, l$) represent an equilibrium constant and a cooperative parameter of the helix formation, respectively. Fundamentals of our three-state, nearest-neighbor interaction model are self-explanatory in eq 1. The (1, 1) and (2, 2) elements of W having the statistical weight S_h characterize the helix propagation process maintaining the same sense, while the (3, 1) and (3, 2) elements with $\sigma_h S_h$ characterize the helix initiation process from coil residues. In the third column the same statistical weight of unity is assigned to all coil residues irrespective of the conformational state of their preceding residues. The (1, 2) and (2, 1) elements of W , whose statistical weights are taken to be zero, prohibit the successive occurrence of r and l units (*cf.* Part I). Hence, in the matrix W in eq 1, the sense inversion is expressed in terms of the coexistence of two equilibria; $\text{RH } \alpha \rightleftharpoons \text{coil}$ and $\text{coil} \rightleftharpoons \text{LH } \alpha$. In other words, the inversion is assumed to proceed with retaining at least one coil residue between RH and LH α -helices.

The matrix contraction by the nearest-neighbor interaction model may require certain adjustments of the elements. In a real polypeptide chain, the growth of helix sequence may continue from either end of the sequence with an equal probability. The 9×9 transition matrix in Part I was large enough to

distinguish the first and last residues in the sequence, hence we were able to represent this two-directional growth of helix by assigning the same statistical weight $\sqrt{\sigma_h}$ to these two terminal residues. However, in the contracted 3×3 matrix W in eq 1 we cannot designate the last helix residue in the sequence since every h unit, except the first one in the sequence, is given the same statistical weight S_h no matter whether it is followed by h or c. Consequently, the $\sqrt{\sigma_h}$ value assumed in Part I for the last helix residue is not counted in the present matrix W . To compensate this we assign a statistical weight of the order of σ_h to the (3, 1) and (3, 2) elements of W which are responsible for the helix initiation process. Then, for the purpose of simplicity, we replace this σ_h value by $\sigma_h S_h$ and let all matrix elements in the first and second columns include S_h in common. It should be clear that our S_h and σ_h parameters have the same physical meanings as those discussed in the Zimm-Bragg theory of the helix-coil transition.^{14,20} As will be seen below, mathematical calculations of various molecular averages can be simplified by this modification without causing any significant influences to the final numerical results (note that $S_h \simeq 1$, hence $\sigma_h S_h \simeq \sigma_h$, in the region of our interest).

Partition Function

The partition function of a polypeptide chain consisting of N monomeric residues is given as

$$Z_N = e_1 W^{N-2} e_N \quad (2)$$

The end vectors associated with the first and N th residues, which are assumed never to be involved in the helix section, should have the form

$$e_1 = (0, 0, 1) \quad (3)$$

and

$$e_N = (1, 1, 1)^T \quad (4)$$

where the superscript T denotes the transpose

of a vector. To evaluate Z_N , the matrix W may be expanded by its eigenvalues and eigenvectors. The secular equation of W is given as

$$(\lambda - S_r)(\lambda - S_l)(\lambda - 1) - \sigma_r S_r (\lambda - S_l) - \sigma_l S_l (\lambda - S_r) = 0 \quad (5)$$

and its three roots, i.e., the eigenvalues of W , are denoted by λ_i ($i=1-3$) in the order of magnitude. The right-hand side and left-hand side eigenvectors of W , denoted respectively by $u(\lambda)$ and $v(\lambda)$ for $\lambda = \lambda_1, \lambda_2$, and λ_3 , can then be calculated as follows in the normalized form

$$u(\lambda) = \left(1, \frac{\lambda - S_r}{\lambda - S_l}, \lambda - S_r \right)^T \quad (6)$$

and

$$v(\lambda) = C(\lambda) \left(1, \frac{\sigma_l S_l}{\sigma_r S_r} \frac{\lambda - S_r}{\lambda - S_l}, \frac{\lambda - S_r}{\sigma_r S_r} \right) \quad (7)$$

where

$$C(\lambda) = \sigma_r S_r \frac{\lambda - S_l}{\lambda - S_r} F(\lambda) \quad (8)$$

$$F(\lambda) = \{(\lambda - S_r)(\lambda - S_l) + A_r(\lambda) + A_l(\lambda)\}^{-1} \quad (9)$$

$$A_r(\lambda) = (\lambda - S_r)(\lambda - 1) - \sigma_r S_r \quad (10)$$

$$A_l(\lambda) = (\lambda - S_l)(\lambda - 1) - \sigma_l S_l \quad (11)$$

By combining eq 6—11, we expand W as

$$W = \sum_{\lambda} \lambda u(\lambda) v(\lambda) \quad (12)$$

and hence

$$W^{N-2} = \sum_{\lambda} \lambda^{N-2} u(\lambda) v(\lambda) \quad (13)$$

Introduction of eq 13 to 2 gives the final expression of the partition function Z_N :

$$\begin{aligned} Z_N &= \sum_{\lambda} \lambda^{N-2} e_1 u(\lambda) v(\lambda) e_N \\ &= \sum_{\lambda} \lambda^{N-1} (\lambda - S_r)(\lambda - S_l) F(\lambda) \end{aligned} \quad (14)$$

Average Dimension of RH and LH α -Helices

In the matrix W in eq 1, occurrence of the RH α state at the i th residue is represented by the two different joint conformations, cr and

rr. The former characterizes the r residue appearing at the beginning of the RH α sequence, and the latter characterizes that located either within or at the end of the sequence. Since both joint conformers have S_r in their statistical weights in common, the average number of RH α residues per chain is given by the exponent of S_r in Z_N :

$$\begin{aligned} N_{S_r} &= \frac{\partial \ln Z_N}{\partial \ln S_r} \\ &= \frac{1}{Z_N} \sum_{\lambda} \lambda^{N-1} \{N(\lambda - S_r)(\lambda - S_l) \\ &\quad \times A_l(\lambda) F^2(\lambda) - I_r(\lambda)\} \end{aligned} \quad (15)$$

where

$$\begin{aligned} I_r(\lambda) &= 2\lambda(\lambda - S_r)\{(\lambda - S_l)B(\lambda)F(\lambda) - 1\} \\ &\quad \times A_l(\lambda)F^2(\lambda) \end{aligned} \quad (16)$$

and

$$B(\lambda) = (\lambda - S_r) + (\lambda - S_l) + (\lambda - 1) \quad (17)$$

On the other hand, the exponent of σ_r gives the average number of RH α -helices in the chain, since σ_r characterizes the initiation of the RH α sequence

$$\begin{aligned} N_{\sigma_r} &= \frac{\partial \ln Z_N}{\partial \ln \sigma_r} \\ &= \frac{1}{Z_N} \sum_{\lambda} \lambda^{N-2} \{N(\lambda - S_r)^2(\lambda - S_l) \\ &\quad \times A_l(\lambda)F^2(\lambda) - J_r(\lambda)\} \end{aligned} \quad (18)$$

where

$$\begin{aligned} J_r(\lambda) &= (\lambda - S_r)[2\lambda(\lambda - S_r)(\lambda - S_l)B(\lambda)F(\lambda) \\ &\quad - \{2\lambda^2 - S_r(\lambda + S_l)\}A_l(\lambda)F^2(\lambda)] \end{aligned} \quad (19)$$

Corresponding averages for the LH α -helix are

$$\begin{aligned} N_{S_l} &= \frac{1}{Z_N} \sum_{\lambda} \lambda^{N-1} \{N(\lambda - S_r)(\lambda - S_l) \\ &\quad \times A_r(\lambda)F^2(\lambda) - I_l(\lambda)\} \end{aligned} \quad (20)$$

and

$$\begin{aligned} N_{\sigma_l} &= \frac{1}{Z_N} \sum_{\lambda} \lambda^{N-2} \{N(\lambda - S_r)(\lambda - S_l)^2 \\ &\quad \times A_r(\lambda)F^2(\lambda) - J_l(\lambda)\} \end{aligned} \quad (21)$$

with

$$\begin{aligned} I_l(\lambda) &= 2\lambda(\lambda - S_l)\{(\lambda - S_r)B(\lambda)F(\lambda) - 1\} \\ &\quad \times A_r(\lambda)F^2(\lambda) \end{aligned} \quad (22)$$

and

$$\begin{aligned} J_l(\lambda) &= (\lambda - S_l)[2\lambda(\lambda - S_r)(\lambda - S_l)B(\lambda)F(\lambda) \\ &\quad - \{2\lambda^2 - S_l(\lambda + S_r)\}A_r(\lambda)F^2(\lambda)] \end{aligned} \quad (23)$$

Average fractions of helix units, f_h ($h=r, l$), are then calculated as follows by considering again that the two chain-end residues do not adopt the α -helical conformation

$$f_h = N_{S_h}/(N-2) \quad (24)$$

As the length N increases, contribution of the leading eigenvalue λ_1 to N_{S_h} will become predominant over those of other two eigenvalues ($\lambda_1^N \gg \lambda_2^N, \lambda_3^N$). Thus, by introducing eq 15 (or 20) into 24 we have an asymptotic form of f_h at infinitely large N as

$$f_h = A_g(\lambda_1)F(\lambda_1) \quad (g \neq h) \quad (25)$$

Length Distribution Function and Moments of Helix

The simplified 3×3 matrix model enables us to deal with several more complex averages associated with the dimensional changes of a polypeptide chain in the sense inversion region. This advantage is taken below to calculate the length distribution and the higher order moments of helix.

The probability of occurrence of a RH α sequence consisting of v successive r residues, $P_r(v)$, may be given as¹⁹

$$\begin{aligned} P_r(v) &= \frac{1}{Z_N} \{S_r^v (\partial Z_{N-v+1} / \partial S_r) \\ &\quad - 2S_r^{v+1} (\partial Z_{N-v} / \partial S_r) \\ &\quad + S_r^{v+2} (\partial Z_{N-v-1} / \partial S_r) \} \end{aligned} \quad (26)$$

Here the first term derived from

$$\frac{S_r^v}{Z_N} \frac{\partial Z_{N-v+1}}{\partial S_r} = \frac{S_r^v}{Z_N} \sum_{j=2}^{N-v} \{e_1 W^{j-2} (\partial W / \partial S_r) W^{N-j-v} e_N\} \quad (27)$$

calculates the probability of finding of RH α sequences of length v and longer, while the second and third terms offset the over-measured contributions from longer sequences. On introducing eq 14 into 26 we have

$$P_r(v) = \frac{\sigma_r S_r^v}{Z_N} \sum_{\lambda} \lambda^{N-v-2} (\lambda - S_r)(\lambda - S_l) \times [(N-v-1)(\lambda - S_r)(\lambda - S_l) + 2\lambda\{(\lambda - S_r)A_r(\lambda) + (\lambda - S_l)A_l(\lambda)\}F(\lambda)]F^2(\lambda) \quad (28)$$

A corresponding equation for a LH α sequence, $P_l(v)$, is given by replacing $\sigma_r S_r^v$ in eq 28 with $\sigma_l S_l^v$. With these $P_h(v)$ values, we can subsequently calculate the n th order moment of helix $M_h^{(n)}$ as

$$M_h^{(n)} = \sum_{v=1}^{N-2} v^n P_h(v) \quad (29)$$

Note that the first moment $M_h^{(1)}$ satisfies the relation

$$M_h^{(1)} = \sum_{v=1}^{N-2} v P_h(v) = N_{S_h} \quad (30)$$

hence reproduces N_{S_h} in eq 15 and 20, *i.e.*, the average number of α -helical residues per chain. In fact, introduction of eq 28 into 30 results in the same analytical expression for N_{S_r} given in eq 15.

RESULTS AND DISCUSSION

Numerical Results

To analyze the theory numerically, for a case of thermally induced transition, we define the equilibrium constant of the helix formation

S_h as a function of the absolute temperature T :

$$S_h = \exp\{-(\Delta H_h/RT)(1 - T/T_h)\} \quad (31)$$

where ΔH_h is the enthalpy change associated with the helix-coil transition occurring at T_h and R is the gas constant. Transition parameters are taken from Part I so that the results of the two calculations can be compared under the same condition: $\Delta H_r = -400$ cal mol⁻¹, $T_r = 310$ K, $\Delta H_l = -300$ cal mol⁻¹, $T_l = 318$ K, and $\sigma_r = \sigma_l = 5 \times 10^{-5}$. The predominant chain conformation determined by this set of parameters shifts from RH α to coil *via* an intermediate LH α state as T is increased. Figure 1 illustrates the temperature dependence of the average number of α -helical residues N_{S_h} calculated at $N=400$. An initial increase in N_{S_l} and an associated decrease in N_{S_r} represent the formation of LH α -helices proceeding at the expense of RH α -helices. A subsequent decrease in N_{S_l} corresponds to the melting of LH α -helices into the random coil conformation. The total number of α -helical residue $N_{S_r} + N_{S_l}$ decreases only monotonically in the entire range of temperature, indicating that the sense inversion occurs without forming a respectably long coil sequence between RH and LH α -helices. In other words, the propagation of the center of inversion along the chain should dominate the RH $\alpha \rightarrow$ LH α transition.

The temperature dependence of the average number of α -helical sequences N_{σ_h} is shown in Figure 2. N_{σ_r} in the RH α region decreases asymptotically toward unity as T is decreased. A value of $N_{\sigma_r} = 1.11$ at $T = 250$ K suggests, when compared with $N_{S_r} = 385$, that the chain at low temperatures includes a fairly long RH α sequence. As T is increased to shift the conformer equilibrium to favor the LH α conformation, N_{σ_l} begins to increase while N_{σ_r} decreases. Comparison of the N_{σ_l} curve with the N_{S_l} curve in Figure 1 indicates that the LH α -helix grows in this region in both its average length and number. The N_{σ_l} curve reaches its maximum at $T = 310$ K. Correspondingly the total number of helices $N_{\sigma_r} + N_{\sigma_l}$ runs up to

Figure 1. Temperature dependence of the average number of α -helical residues N_h in a polypeptide chain of $N=400$: Transition parameters assumed are $\Delta H_r = -400 \text{ cal mol}^{-1}$, $T_r = 310 \text{ K}$, $\Delta H_l = -300 \text{ cal mol}^{-1}$, $T_l = 318 \text{ K}$, and $\sigma_r = \sigma_l = 5 \times 10^{-5}$.

1.95 at the same temperature. This value is indicative of the coexistence of two α -helices. Since $N_{\sigma_l} > N_{\sigma_r}$, these coexisting helices are expected to consist mainly of LH α -LH α and LH α -RH α pairs.

Further details of the sense inversion are seen in the helix-length distribution curves $P_h(v)$ in Figure 3. For example, the RH α -helix at 250 K has the average length of $N_{S_r} = 385$ (cf. Figure 1), the maximum $P_r(v)$ value of 0.050 at $v = 393$, and as much as 90% of RH α residues are involved in sequences with $v = 350$ –398. The peak height of the $P_r(v)$ curve decreases continuously with T , while the $P_l(v)$ curve, though much broader in width than $P_r(v)$, begins to rise in the LH α region above 280 K. At 310 K where $N_{\sigma_r} + N_{\sigma_l}$ is at its maximum in Figure 2, both $P_r(v)$ and $P_l(v)$ curves exhibit almost linear decrease with v . The higher probabilities for the shorter helix sequences and $P_l(v) > P_r(v)$ are congruous with the coexistence of LH α -LH α and LH α -RH α

Figure 2. Temperature dependence of the average number of helix sequences N_{σ_h} at $N=400$.

Figure 3. Helix-length distribution curves $P_h(v)$ at (a) $T = 250 \text{ K}$, (b) 260 K, (c) 270 K, (d) 280 K, (e) 290 K, (f) 300 K, (g) 310 K, (h) 320 K, and (i) 350 K.

pairs suggested above.

Figure 4 shows the plots of $f_r - f_l$ vs. T at several different values of N . The $f_r - f_l$ curves should correspond to the experimental transition curves determined by polarimetric meth-

Figure 4. Curves of $f_r - f_l$ vs. T at various values of N .

Figure 5. Curves of $f_r + f_l$ vs. T at various values of N .

ods such as ORD and CD. The absolute $f_r - f_l$ value at any given temperature increases as N is increased, *i.e.*, the transition becomes sharper for a longer chain. However, the midpoint of the RH $\alpha \rightarrow$ LH α transition remains at the same temperature independently of N . This critical temperature of the sense inversion, T_{inv} , is uniquely determined from eq 31 by equating $S_r = S_l$ for a given set of ΔH_h and T_h values (T_{inv} in the present example is 288.2 K). Then, it should be straightforward to show, if $\sigma_r = \sigma_l$, that $N_{S_r} = N_{S_l}$ and $N_{\sigma_r} = N_{\sigma_l}$ in eq 15–23, hence the chain at T_{inv} includes the RH and LH α -helical sequences having the same average conformation. The assumption that $\sigma_r = \sigma_l$ may naturally be admitted for α -helices coexisting in homopolypeptides. Even if $\sigma_l \neq \sigma_r$, since the $f_r - f_l$ value at T_{inv} is less influenced by the difference in σ_h (note that $\sigma_h \ll S_h$ or unity), a shift of the crossover point will be confined within a very narrow range. Consequently, the convergence of the $f_r - f_l$ vs. T curves at T_{inv} appears as a general characteristic of the sense inversion phenomena occurring in homopolypeptides. This finding leads

to the following suggestion concerning a method of estimation of the transition parameters: ΔH_h and T_h .

The N dependence of the total helix fraction $f_r + f_l$ is depicted in Figure 5. The $f_r + f_l$ curve undergoes a sigmoidal decrease with T , and shifts continuously to the higher temperature region as N is increased. Knowing in Figure 4 that $f_r - f_l = 0$ at the inversion midpoint, we obtain the $f_r = f_l$ value from Figure 5 as a function of N . Under a condition that $S_r = S_l$ ($= S$) and $\sigma_r = \sigma_l$ ($= \sigma/2$) all our equations are reduced to those given by the Zimm–Bragg theory¹⁴ for a simple helix–coil transition: Mathematics necessary for evaluating S and σ from the f vs. N curve can be found in the theories subsequently developed.^{21–23} Having had the S and σ values established at T_{inv} , we can then optimize the ΔH_h and T_h parameters by analyzing either the $f_r - f_l$ curves in Figure 4 or the $f_r + f_l$ curves in Figure 5 as a function of temperature (*via* eq 31). It may be noted that the number of independent parameters to be optimized decreases to two (for the simplest case where $\sigma_r = \sigma_l$) because of the aforemen-

Figure 6. Curves of $m^{(n)} = (M_r^{(n)} + M_l^{(n)}) / (N-2)^n$ ($n=1-3$) at $N=400$.

tioned peculiarity of the RH $\alpha \rightarrow$ LH α sense inversion.

The above analyses may alternatively be carried out by using the higher moments of helix. Figure 6 illustrates how the first three moments, $m^{(n)} = (M_r^{(n)} + M_l^{(n)}) / (N-2)^n$ ($n=1-3$), vary with temperature in the sense inversion region. The higher moments reflect the mode of helix-length distribution more sensitively, hence can reveal subtle changes in the average chain dimension. For example, the $m^{(2)}$ and $m^{(3)}$ curves show a shallow dent in the sense inversion region, whilst no such a change is seen when the first moment $m^{(1)} (\equiv f_r + f_l)$ alone is examined. Further refinement of the theory to include the effects of spatial distribution and flexibility of α -helices coexisting in a chain is certainly possible, and will be discussed in detail for the case of the electric birefringence of PCBL in the helix-coil transition region.⁹

Comparison with Previous Calculations

To compare the numerical results obtained

in the present study with those in Part I, we evaluate the root-mean-square (rms) errors between the two sets of results:

$$\sqrt{(1/n) \sum_{i=1}^n (x_1 - x_2)^2}$$

where x_1 and x_2 are the values from previous and present calculations, respectively, and $n=101$ is the number of data points between $T=250$ K and 300 K. The rms error for N_{S_r} at $N=400$ is calculated to be 0.864 and that for N_{S_l} to be 0.955. Both values are satisfactorily small compared with the total number of residues $N=400$, hence differences between the two sets of results cannot be distinguished in the curves in Figure 1. Similar agreement is also observed for N_{S_h} at different values of N and for other averages.

The primary difference between the previous and present theories lies in the degree of approximation concerning the helix stabilization effects due to hydrogen-bond interactions. In Part I, such interactions between four consecutive residues are explicitly expressed in the 9×9 statistical-weight matrix. Among five non-zero eigenvalues of this matrix, only three have the values of the order of S_h or unity, while the remaining two are in the order of $\sqrt{\sigma_h} \approx 10^{-2}$. Obviously, contributions of the latter two eigenvalues to the partition function are smaller than those of the three leading eigenvalues. The present theory, based on the 3×3 statistical-weight matrix, reproduces these three major eigenvalues. Although short, unfavorable helix sequences (chc and chhc) are not excluded in this nearest-neighbor interaction model, their influences to the final numerical results are found to be only trivial.

Implication with Experiments

As we have pointed out above, analyses of the sense inversion mechanism should require not only the $f_r - f_l$ value available from ORD and CD experiments, but also the $f_r + f_l$ value or its equivalent that reflects the average dimension of helix. The most straightforward

method for estimating $f_r + f_i$ should be a ultraviolet absorption measurement. There are, however, several other feasible approaches that can discern the details of the average chain dimension. They include the analyses of the higher order moments of helix rather than the first moment. Especially, the mean-square dipole moment and the Kerr constant, which respectively reflect the second and third moments of helix (see Figure 6), are of our interest. Omura *et al.*¹² have analyzed the dipole moment of PCBL in the helix-coil transition region, resulting in a σ value coincident with that obtained from ORD measurements. More recently, we have shown for the same polypeptide that the changes in its electric birefringence can similarly be reproduced from ORD data.⁹ These studies suggest that the average dimension of helix is experimentally measurable, hence can be collaborated with ORD and CD data in the analyses of the sense inversion mechanism. With the new theory it is even possible to calculate the mean-square radius of gyration, which may be compared with the informations from light scattering measurements. The Nagai theory of the helix-coil transition,¹⁹ in which helix sequences (rigid rods) and coil sequences (random flight chains) are assumed to be alternately connected by flexible joints, can be expanded to apply to the sense inversion case. Determination and analyses of these quantities are beyond the scope of this study; nevertheless, it should just be noted that the $f_r - f_i$ curves in Figure 4 can reproduce the ORD data reported for PPELA,² thus confirming the validity of our theory.

In summary, the theory of the helix-sense inversion in polypeptides, simplified within a framework of the three-state, nearest-neighbor interaction model, improves its applicability and provides a mathematical basis for analyzing the experimental observations. The new theory is shown to be particularly advantageous in calculating several complex aver-

ages associated with dimensional changes of polypeptides. Analyses of the calculated transition curves reveal general characteristics of the sense inversion phenomena and suggest a procedure for evaluating the transition parameters. The proposed method will be subjected to a quantitative comparison with experiments in subsequent studies.

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