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Thermodynamic Study on Charge-Induced Helix–Coil Transition of Ionizable Polypeptide. 1. Free Energy of Helix–Coil Transition

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ABSTRACT: Isothermal charge-induced helix–coil transitions of ionizable polypeptides are discussed by means of ΔF°, which is defined as the nonelectrostatic part of the free energy of the conformational transition, obtained from potentiometric titration. ΔF° for homopolypeptides and copolypeptides is formulated on the basis of the conformational partition function by introducing statistical theories of the helix–coil transition. It is found that $\Delta F^\circ/N$ is not $-RT \ln s$ but a function of the Zimm–Bragg parameters s and σ, the degree of polymerization N, and the composition and the sequence distribution of the copolymer and does not directly reflect the conformational stability of an amino acid residue. Thus, the conventional method of charge-induced transition in which $\Delta F^\circ/N$ is considered to depend only on the parameter s may lead to incorrect estimations. From the theoretical results obtained in this study, a method to analyze experimental data on the potentiometric titration is proposed.

There have been many investigations of the conformational stability of ionizable polypeptides, both homopolymers and copolymers, in aqueous solution.[1-10] These studies have been performed mainly with the theories of Zimm–Rice[1] and Nagasawa–Holtzer,[2] in which ΔF°, the nonelectrostatic part of the free energy of the conformational transition, has been evaluated from potentiometric titration.

However, these investigations are confined to the evaluation of ΔF°, and seem to be insufficient to analyze the characteristic parameters of the helix–coil transition, i.e., the Zimm–Bragg parameters s and σ,[11] the degree of polymerization N, and so on. Accordingly, the conformational stability of monomer unit associated with the parameter s has not been estimated properly from the titration method, especially in the case of copolymers.

In this paper, we formulate ΔF° with a conformational partition function on the basis of statistical theories of the helix–coil transition for homopolymers and copolymers[11-14] and calculate the value of ΔF° for given values of these parameters. The aim of this study is to elucidate the character of ΔF° and point out problems included in the conventional methods of charge-induced transition. In the following paper,[15] we will analyze our experimental data by means of the method described here.

Theoretical Section

Homopolypeptide. The partition function for a system composed of a homopolypeptide with ionizable side chains and a solvent as a dielectric continuum is written[1]

$$\Xi = \sum_{\mathbf{c}}\sum_{\tilde{\eta}} Q(\mathbf{c},\tilde{\eta}) \exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta} \qquad (1)$$

where $\mathbf{c}$ specifies the secondary structure of the polymer chain, $\mathbf{c} = \{c_i\}$ $(1 \le i \le N)$, and thus c_i implies, for example, the dihedral angles of the ith residue. Similarly $\tilde{\eta}$ specifies the charge state, $\tilde{\eta} = \{\eta_i\}$ and $\eta = \sum_i \eta_i$, where η_i can be either 0 or 1. The electrostatic potential W is a function of $\mathbf{c}$ and $\tilde{\eta}$, and the internal partition function Q is also a function of $\mathbf{c}$ and $\tilde{\eta}$, including the interaction between a charge on the side chain and the main chain. Finally, the variable λ plays the role of absolute activity and is related to the intrinsic dissociation constant $\mathrm{p}K_0$ by

$$\log \lambda = \mathrm{pH} - \mathrm{p}K_0$$

Further, the partition function for the standard state is defined by

$$\Xi_{\mathrm{c}} = \sum_{\mathbf{c}'}\sum_{\tilde{\eta}} Q_{\mathrm{c}}(\mathbf{c}',\tilde{\eta}) \exp[-W(\mathbf{c}',\tilde{\eta})/RT]\lambda^{\eta} \qquad (2)$$

where Q_{c} is the same as Q except that the interaction which leads to the formation of a hydrogen bond in the α-helical conformation is excluded, and $\mathbf{c}'$ is concerned with the variables which specify only the random coil conformation. From eq 1 and 2, the degrees of ionization α and α_{c} for the respective systems are obtained by

$$\alpha = \frac{1}{N}\left(\frac{\partial \ln \Xi}{\partial \ln \lambda}\right) \qquad \alpha_{\mathrm{c}} = \frac{1}{N}\left(\frac{\partial \ln \Xi_{\mathrm{c}}}{\partial \ln \lambda}\right) \qquad (3)$$

ΔF° can be expressed by relating the partition function given above to the potentiometric titration curves by

$$\begin{aligned} \Delta F^\circ &= -RT \ln Z \\ &= NRT \int_{\lambda(0)}^{\lambda(1)} (\alpha - \alpha_{\mathrm{c}})\, \mathrm{d}(\ln \lambda) \end{aligned} \qquad (4)$$

with

$$Z = \frac{\Xi[\lambda(0)]}{\Xi_{\mathrm{c}}[\lambda(0)]} = \frac{\sum_{\mathbf{c}} Q(\mathbf{c},\mathbf{0})}{\sum_{\mathbf{c}'} Q_{\mathrm{c}}(\mathbf{c}',\mathbf{0})} \qquad (5)$$

where $\lambda(0)$ and $\lambda(1)$ denote the λ values at $\alpha = 0$ and $\alpha = 1$, respectively, $\mathbf{0}$ means the zero vector whose elements are all zero, and the fully charged state is assumed to be in purely random coil conformation; thus

$$\Xi[\lambda(1)] = \Xi_{\mathrm{c}}[\lambda(1)] \qquad (6)$$

From these equations we obtain the following conclusions. First, ΔF° obtained by the titration method indicates directly the free energy change of the polymer chain as a whole, from an uncharged fully random coil state to a conformation at $\lambda(0)$, which is not necessarily in a completely α-helical state and, moreover, independent of the specific nature of the chain conformations. Second, the standard state for ΔF° is not the so-called extended coil but a random coil which does not include any effect of electrostatic interaction.

To investigate the dependence of ΔF° on the parameters of the helix–coil transition, eq 5, which includes only short-range interactions, is written in the form of a nearest-neighbor Ising model as[11]

$$Z = \mathbf{e}\mathbf{W}^{N}\mathbf{e}^{+} \qquad (7)$$

with

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$$\mathbf{e} = (0 \quad 1) \qquad \mathbf{W} = \begin{pmatrix} s & 1 \\ s\sigma & 1 \end{pmatrix} \qquad \mathbf{e}^{+} = \begin{pmatrix} 1 \\ 1 \end{pmatrix}$$

where s and σ are the Zimm–Bragg helix–coil transition parameters, on the assumption that the polymer chain cannot form any second-order structure other than the α helix. Then, from the parameter s defined in eq 7, we finally obtain $\Delta F^{\circ}_{\text{res}}$, the free energy for converting an end residue of a helical sequence from the random coil to the α-helical structure, which directly reflects the conformational stability of an amino acid residue

$$\Delta F_{\text{res}} = -RT \ln s \tag{8}$$

It should be noted that the method of temperature-induced transition is directly concerned with $\Delta F^{\circ}_{\text{res}}$, but not ΔF°.

Obviously, at $\lambda = \lambda(0)$ the polymer chain can exist in any conformation described by eq 7. In the conventional method of charge-induced transition, however, the conformation at $\lambda(0)$ was assumed to take a completely α-helical form, and eq 9 was used instead of eq 7. Equation

$$Z = s^{N} \tag{9}$$

9 should be understood as an approximation which leads to $\Delta F^{\circ}/N = \Delta F^{\circ}_{\text{res}}$. In other words, eq 9 means that ΔF° is simply given by the summation of the values for the monomer units.

Copolypeptide. We consider copolypeptides composed of two kinds of amino acids, one of which has an ionizable side chain. Instead of eq 1, the partition function for such copolypeptides is written as

$$\Xi = \sum_{\tilde{\delta}} \sum_{\mathbf{c}} \sum_{\tilde{\eta}} Q(\tilde{\delta},\mathbf{c},\tilde{\eta}) \exp[-W(\tilde{\delta},\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta} \tag{10}$$

where $\tilde{\delta}$ specifies the sequence distribution of the components, $\tilde{\delta} = \{\delta_i\}$. Defining Ξ_{c}, α, α_{c}, and Z for the copolymer in a manner similar to that in eq 2, 3, and 4, we obtain the relation

$$\Delta F^{\circ} = -RT \ln Z = -RT\{\ln [\sum_{\tilde{\delta}} \sum_{\mathbf{c}} Q(\tilde{\delta},\mathbf{c},\mathbf{0})] - \ln [\sum_{\tilde{\delta}} \sum_{\mathbf{c}'} Q_{\text{c}}(\tilde{\delta},\mathbf{c}',\mathbf{0})]\} = NRT \int_{\lambda(0)}^{\lambda(1)} (\alpha' - \alpha_{\text{c}}') \, \text{d}(\ln \lambda) \tag{11}$$

with $\alpha' = \alpha f_{\text{A}}$ and $\alpha_{\text{c}}' = \alpha_{\text{c}} f_{\text{A}}$, where f_{A} is the mole fraction of the A residue carrying the ionizable side chain. The partition function corresponding to eq 11 can be estimated with the aid of statistical theories for copolymers.[12–14]

The statistical theories for copolymers have been developed with respect to the temperature-induced transition for the purpose of evaluating the differential of the partition function, i.e., the helical content θ_{h}. The approximate theory, which is sufficiently valid for the temperature-induced transition,[16] may not necessarily give the same value of ΔF° as that calculated by the exact theory. By comparing both values of ΔF° obtained by approximate and exact theories, one can examine the validity of approximate theories for the charge-induced transition. Here we employ the linear approximation as the conventional method,[7–10] the approximate theories of Lifson[12] and of Allegra[13] for random copolymers, and the exact theory of Lehman–McTague.[14]

A. Linear Approximation

$$Z = Z_{\text{A}}^{f_{\text{A}}} Z_{\text{B}}^{f_{\text{B}}} \tag{12}$$

where $f_{\text{B}} = 1 - f_{\text{A}}$ and Z_{A} and Z_{B} are, respectively, the partition functions at $\lambda(0)$ for homopolymer A and homopolymer B having the same value of N. It is seen from eq 12 that ΔF° is a linear function of f_{A}

$$\Delta F^{\circ} = f_{\text{A}} \Delta F_{\text{A}}{}^{\circ} + f_{\text{B}} \Delta F_{\text{B}}{}^{\circ} \tag{13}$$

with

$$\Delta F_{\text{A}}{}^{\circ} = -RT \ln Z_{\text{A}} \qquad \Delta F_{\text{B}}{}^{\circ} = -RT \ln Z_{\text{B}}$$

With the approximation shown in eq 9, eq 13 leads to the relation which has been used conventionally for copolymers.[7–10] Equation 12 can also be regarded as the partition function for an A–B-type block copolymer, neglecting the effect of the junction between the two blocks.

B. Lifson's Approximation[12]

$$Z = \mathbf{e}\mathbf{W}^{N}\mathbf{e}^{+} \tag{14}$$

where

$$\mathbf{W} = \begin{pmatrix} f_{\text{A}} s_{\text{A}} + f_{\text{B}} s_{\text{B}} & 1 \\ f_{\text{A}} s_{\text{A}} \sigma_{\text{A}} + f_{\text{B}} s_{\text{B}} \sigma_{\text{B}} & 1 \end{pmatrix}$$

in which s_{A} and s_{B} are the equilibrium constants of A and B and σ_{A} and σ_{B} are the cooperativity constants of A and B, respectively.

C. Allegra's Approximation[13]

$$Z = \mathbf{e}\mathbf{W}^{N}\mathbf{e}^{+}/(\gamma_{\text{A}} + \gamma_{\text{B}})^{N} \tag{15}$$

where

$$\mathbf{W} = \begin{pmatrix} \gamma_{\text{A}} s_{\text{A}} + \gamma_{\text{B}} s_{\text{B}} & \gamma_{\text{A}} + \gamma_{\text{B}} \\ \gamma_{\text{A}} s_{\text{A}} \sigma_{\text{A}} + \gamma_{\text{B}} s_{\text{B}} \sigma_{\text{B}} & \gamma_{\text{A}} + \gamma_{\text{B}} \end{pmatrix}$$

and γ_{A} and γ_{B} satisfy the equations

$$f_{\text{A}} = \frac{1}{N}\left(\frac{\partial \ln Z}{\partial \ln \gamma_{\text{A}}}\right) \qquad f_{\text{B}} = \frac{1}{N}\left(\frac{\partial \ln Z}{\partial \ln \gamma_{\text{B}}}\right) \tag{16}$$

D. Lehman–McTague's Exact Theory[14]

$$Z = \exp[C_{N}(1)] \tag{17}$$

where $C_N(1)$ is the value of $C_N(x)$ at $x = 1$ and $C_N(x)$ is obtained from the functional equation

$$\frac{N}{N-1} C_N(x) = f_{\text{A}} \ln (x + s_{\text{A}}) + C_{N-1}\left(\frac{x + s_{\text{A}}\sigma_{\text{A}}}{x + s_{\text{A}}}\right) + f_{\text{B}} \ln (x + s_{\text{B}}) + C_{N-1}\left(\frac{x + s_{\text{B}}\sigma_{\text{B}}}{x + s_{\text{B}}}\right) - C_{N-1}(1) + C_1(x)/(N - 1) \tag{18}$$

with

$$C_1(x) = f_{\text{A}} \ln (x + s_{\text{A}}\sigma_{\text{A}}) + f_{\text{B}} \ln (x + s_{\text{B}}\sigma_{\text{B}})$$

Numerical Results

The dependence of ΔF° on the parameters will be evaluated by means of the theoretical equations described above. Comparing $\Delta F^{\circ}/N$ with $\Delta F^{\circ}_{\text{res}}$, we will elucidate the meaning of ΔF° and point out the problem involved in the conventional method.

Homopolypeptide. As is obvious from Figures 1–4, differences are observed between $\Delta F^{\circ}/N$ and $\Delta F^{\circ}_{\text{res}}$ as functions of the parameters s, σ, N, and T. The conventional method, in which $\Delta F^{\circ}/N = \Delta F^{\circ}_{\text{res}}$ was assumed, may be applicable only under limited conditions for these parameters.

In Figure 1, the dependence of $\Delta F^{\circ}/N$ on s is compared with that of $\Delta F^{\circ}_{\text{res}}$. Obviously, a difference is clearly recognized in the region $s \lesssim 1$; i.e., as s approaches 0, $\Delta F^{\circ}/N$ approaches 0, while $\Delta F^{\circ}_{\text{res}}$ approaches infinity. Inherently, $\Delta F^{\circ}/N$ can never take a positive value. Such behavior is more remarkable in Figure 2, where both quantities are shown as functions of σ. Namely, $\Delta F^{\circ}/N$ is a monotonically decreasing function of σ starting from

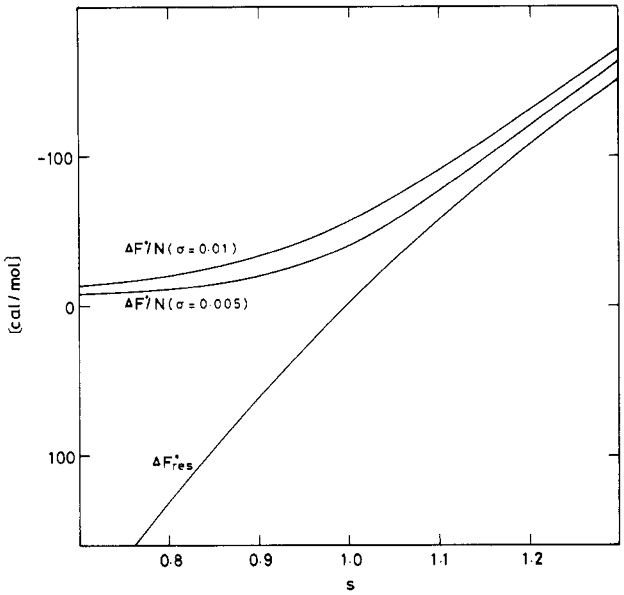

Figure 1. $\Delta F^\circ/N$ and $\Delta F^\circ_{\text{res}}$ plotted against s at T = 25.0 °C for N = 1000.

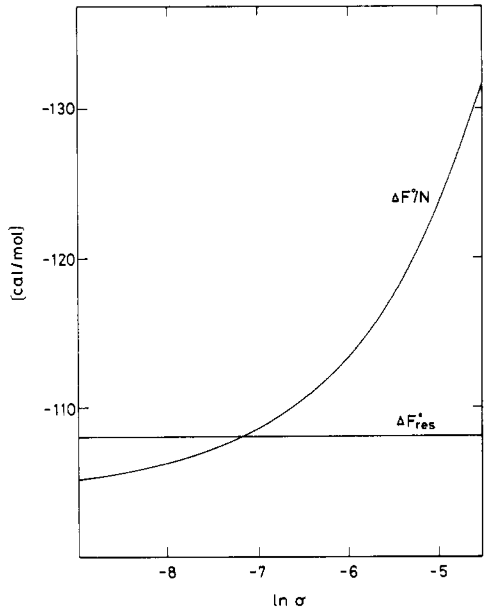

Figure 2. $\Delta F^\circ/N$ and $\Delta F^\circ_{\text{res}}$ plotted against ln σ at T = 25.0 °C for N = 1000 and s = 1.2.

zero at σ = 0. Figure 3 shows the N dependence of $(\Delta F^\circ/N)/(\Delta F^\circ/N)_{N\to\infty}$, $(\Delta F^\circ_{\text{res}})/(\Delta F^\circ_{\text{res}})_{N\to\infty}$, and $\theta_{\text{h}}/(\theta_{\text{h}})_{N\to\infty}$, values relative to those for infinite chain length. It is found that the behavior of $(\Delta F^\circ/N)/(\Delta F^\circ/N)_{N\to\infty}$ and $\theta_{\text{h}}/(\theta_{\text{h}})_{N\to\infty}$ is very similar. Thus, most experimental data on $\Delta F^\circ/N$ cannot be analyzed without considering the N dependence, as for θ_{h}.[17] Finally, Figure 4 shows that $\Delta F^\circ/N$ is not linear against T, when $\Delta F^\circ_{\text{res}}$ is a linear function of T and σ is a constant. In other words, ΔH° (enthalpy part of ΔF°) and ΔS° (entropy part of ΔF°) depend on T.

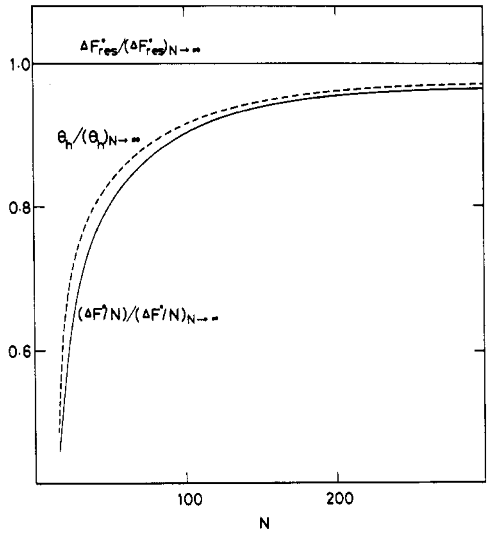

Figure 3. Dependence on N of $\Delta F^\circ/N$, $\Delta F^\circ_{\text{res}}$, and θ_{h}, for values relative to those for the infinite chain at T = 25.0 °C for s = 1.2 and $\sigma = 5.0 \times 10^{-3}$.

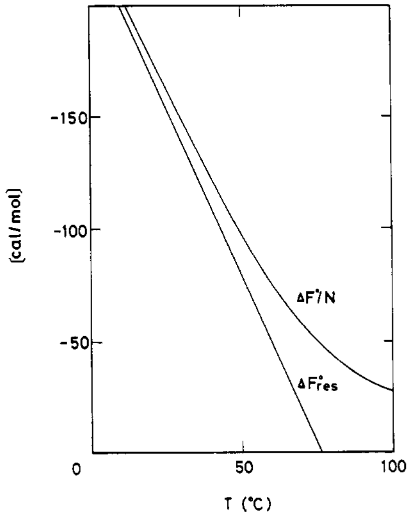

Figure 4. Temperature dependence of $\Delta F^\circ/N$ and $\Delta F^\circ_{\text{res}}$ for $\Delta H^\circ_{\text{res}}$ = −1050 cal/mol, $\Delta S^\circ_{\text{res}}$ = −3.0 eu/mol, N = 1000, and $\sigma = 5.0 \times 10^{-3}$.

Copolypeptide. First, we deal with the dependence of $\Delta F^\circ/N$ on the copolymer composition f_{A}. As shown in Figure 5, the approximate theories, especially the linear approximation, result in large discrepancies from the value obtained with the exact theory when the differences in the values of s_{A} and s_{B} are large. Thus, the experimental results are not correctly analyzed with these approximate theories. Concerning the validity of these theories, Allegra's method is a higher order approximation than that of Lifson in the case of the temperature-induced transition.[18] However, for charge-induced transition, their validity is almost of the same order, and the values of ΔF° are in the following order for all the values of the parameters:

$$\Delta F^\circ(\text{Lifson}) < \Delta F^\circ(\text{exact}) < \Delta F^\circ(\text{Allegra})$$

In any case, the validity of approximate theories should be discussed by comparing the value with that calculated by the exact theory whenever the experimental data are analyzed by means of an approximate theory.

Second, the effect of sequence distribution should be discussed. In Figure 6, a comparison is made of copolymers with a random sequence distribution and with a Markovian sequence distribution by using the exact theory. These calculations are performed by introducing the nearest-neighbor correlated sequence in the equation of Lehman–McTague.[14] Model chains used for calculation are the following:

1. A two-component block copolymer composed of 10 A-block sequences and 10 B-block sequences, as an example of a higher order of blockiness (curve 2 in Figure 6).
2. A two-component copolymer in which the residues of the minor component are not adjacent to the same kind

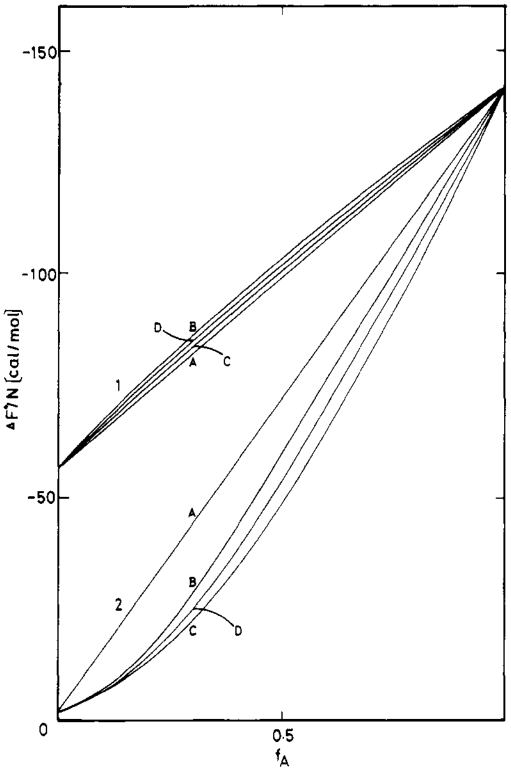

Figure 5. Effect of composition on $\Delta F°/N$ for a random copolymer of $N = 1000$ at $T = 25.0$ °C with numerical values $s_A = 1.25$, $\sigma_A = 5.0 \times 10^{-3}$, $s_B = 1.1$, $\sigma_B = 3.0 \times 10^{-4}$ (group 1) and $s_A = 1.25$, $\sigma_A = 5.0 \times 10^{-3}$, $s_B = 0.9$, $\sigma_B = 3.0 \times 10^{-4}$ (group 2): (A) linear approximation (eq 12); (B) Lifson's approximation (eq 14); (C) Allegra's approximation (eq 15); (D) Lehman-McTague's exact theory (eq 17).

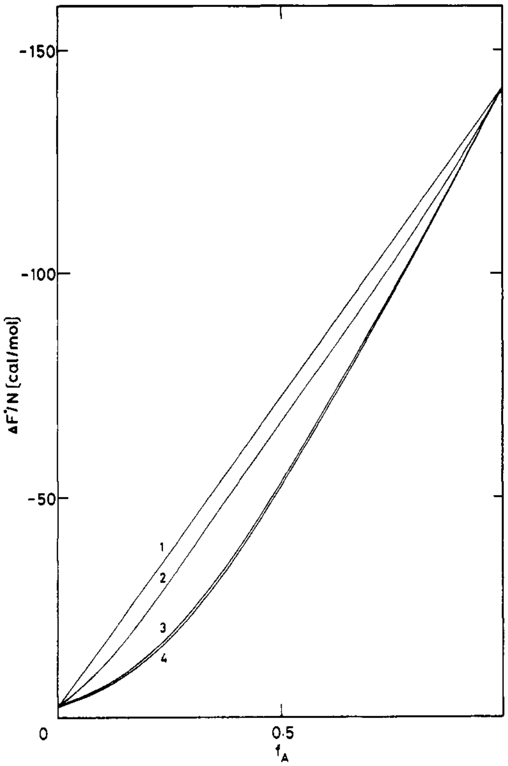

Figure 6. Effect of sequence distribution. $\Delta F°/N$ plotted against f_A with numerical values $T = 25.0$ °C, $N = 1000$, $s_A = 1.25$, $\sigma_A = 5.0 \times 10^{-3}$, $s_B = 0.9$, and $\sigma_B = 3.0 \times 10^{-4}$. Curve 1, linear approximation (eq 12); curve 3, exact theory for random sequence distribution (eq 17); curves 2 and 4; Markovian sequence distribution (see text).

of residue, as an example of the lowest order of blockiness (curve 4 in Figure 6).

As is obvious from Figure 6, $\Delta F°/N$ is also dependent on the sequence distribution. The $\Delta F°/N$ curve approaches that of the linear approximation with increasing blockiness. Such a finding agrees with the comment made above that the linear approximation is applicable to A-B-type diblock copolymers.

In conclusion, the conventional method, in which the approximations of eq 9 and 12 are used, may lead to erroneous results. In other words, $\Delta F°/N$ obtained by the titration method is a function of various parameters and does not directly reflect the conformational stability of an amino acid residue.

Discussion

In this section, $\Delta F°/N$ is calculated in an analytical form, and the dependence of $\Delta F°/N$ on the parameters is explained more explicitly.

Homopolypeptide. The approximation for infinite chain length is employed to circumvent the difficulty in resolving $\Delta F°/N$ into its factors. When $N \rightarrow \infty$, $\Delta F°/N$ of a homopolypeptide can be written in the form

$$\Delta F°/N = -RT \ln w_1 = -RT \ln [\tfrac{1}{2}(s + 1 + \{(s-1)^2 + 4s\sigma\}^{1/2})] = \theta_h \Delta F°_{res} + \theta_s \Delta F°_{int} - RT \ln g_1 \quad (19)$$

where w_1 is the largest eigenvalue of $\mathbf{W}$ in eq 7 and $\Delta F°_{int}$, the free energy of initiation of a helical region, θ_h, θ_s, and g_1 are defined by

$$\Delta F°_{int} = -RT \ln \sigma$$

$$\theta_h = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln s}\right) \qquad \theta_s = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln \sigma}\right)$$

$$g_1 = \left[\frac{1-\theta_h}{1-\theta_h-\theta_s}\right] \times \left[\frac{\theta_h(1-\theta_h-\theta_s)}{(\theta_h-\theta_s)(1-\theta_h)}\right]^{\theta_h}\left[\frac{(\theta_h-\theta_s)(1-\theta_h-\theta_s)}{\theta_s^{\,2}}\right]^{\theta_s}$$

Assuming that σ is independent of T, in other words, that the entropy contribution prevails, $\Delta H°$ and $\Delta S°$ can be written as

$$\Delta H° \sim \Delta U° = RT\left(\frac{\partial \ln w_1}{\partial \ln T}\right) \sim \theta_h \Delta H°_{res} \quad (20)$$

$$\Delta S° = \theta_h \Delta S°_{res} + \theta_s R \ln \sigma + R \ln g_1 \quad (21)$$

where $\Delta H°_{res}$ and $\Delta S°_{res}$ are the enthalpy and entropy parts in $\Delta F°_{res}$, respectively. $\Delta F°/N$ in eq 19 consists of three terms, i.e., (1) $\theta_h \Delta F°_{res}$, (2) $\theta_s \Delta F°_{int}$, and (3) $RT \ln g_1$. It is noted that the second and third terms are completely entropic.

These three terms, together with the sum of them, are plotted against s in Figure 7. Equations 19, 20, and 21, as well as Figure 7, indicate the importance of the entropy terms in $\Delta F°/N$, particularly in the region $s \lesssim 1$, and explain the features of Figures 1–4. The σ dependence shown in Figure 2 may be attributed mainly to the second term of eq 19. It should be noted that $\Delta F°/N$ of eq 19 approaches $\Delta F°_{res}$ as $\sigma \rightarrow 0$, while $\Delta F°/N$ of eq 7 approaches 0. The discrepancy in the behavior of $\Delta F°/N$ at $\sigma = 0$ between finite and infinite chain length can be explained by the end effect in the following way. As σ becomes smaller, i.e., as the cooperativity becomes larger, the end effect may affect remote residues and makes the coil state more favorable. At $\sigma = 0$, the polymer chain

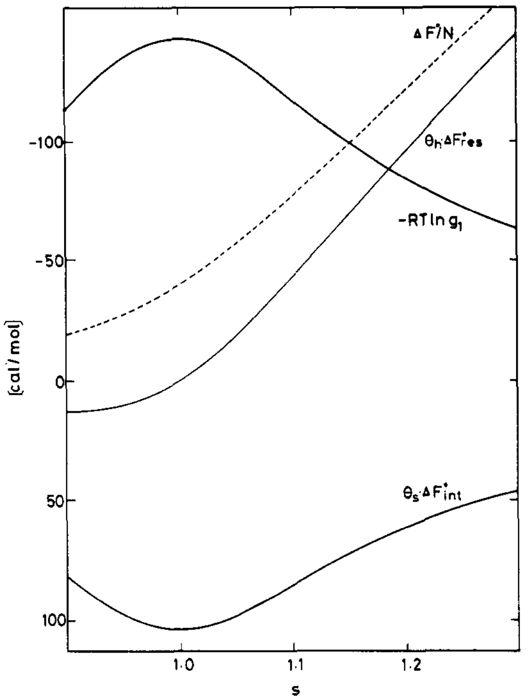

Figure 7. $\theta_h \Delta F°_{res}$, $\theta_s \Delta F°_{int}$, $-RT \ln g_1$, and $\Delta F°/N$ (eq 19) plotted against s at $T = 25.0$ °C for $\sigma = 5.0 \times 10^{-3}$.

finally achieves the fully random coil state independent of other parameters, so that $\Delta F°/N$ approaches 0. On the other hand, the end effect is neglected in eq 19. The N dependence of $(\Delta F°/N)/(\Delta F°/N)_{N\to\infty}$ shown in Figure 3, which is almost identical with that of $\theta_h/(\theta_h)_{N\to\infty}$, may arise mainly from the N dependence of θ_h in the first term of eq 19. Finally, the nonlinear dependence of $\Delta F°/N$ on T indicated in Figure 4 results from the fact that $\Delta H°$ and $\Delta S°$ are functions of θ_h and θ_s, both of which are also dependent on T, as clearly indicated in eq 20 and 21.

Copolypeptide. We now define $\langle s \rangle$ and $\langle \sigma \rangle$ as

$$\langle s \rangle = x_A s_A + x_B s_B \tag{22a}$$

$$\langle \sigma \rangle = \frac{x_A s_A \sigma_A + x_B s_B \sigma_B}{x_A s_A + x_B s_B} \tag{22b}$$

where x_A and x_B are equivalent to f_A and f_B in Lifson's approximation and to $\gamma_A/(\gamma_A + \gamma_B)$ and $\gamma_B/(\gamma_A + \gamma_B)$ in Allegra's approximation, respectively. Then we have

$$\theta_h = \frac{1}{N}\left[\left(\frac{\partial \ln z}{\partial \ln s_A}\right) + \left(\frac{\partial \ln z}{\partial \ln s_B}\right)\right] = \frac{1}{N}\left(\frac{\partial \ln z}{\partial \ln \langle s \rangle}\right) \tag{23a}$$

$$\theta_s = \frac{1}{N}\left[\left(\frac{\partial \ln z}{\partial \ln \sigma_A}\right) + \left(\frac{\partial \ln z}{\partial \ln \sigma_B}\right)\right] = \frac{1}{N}\left(\frac{\partial \ln z}{\partial \ln \langle \sigma \rangle}\right) \tag{23b}$$

If $\langle s \rangle$ and $\langle \sigma \rangle$ are replaced by s and σ, respectively, then eq 14 and 15 coincide with eq 7, and the above discussion for homopolymers is applicable to random copolymers. It can thus be concluded that the partition function for a random copolymer can be described with $\langle s \rangle$ and $\langle \sigma \rangle$, i.e., approximate mean values of the s and σ values for the homopolymer. Since $\Delta F°$ obtained with the exact theory is between that of Lifson and that of Allegra, as shown in Figure 5, the exact values of $\langle s \rangle$ and $\langle \sigma \rangle$ are obtained by substituting a value between f_A and $\gamma_A/(\gamma_A + \gamma_B)$ for x_A in eq 23, and similarly for x_B. However, it should be noted that the behavior of $\langle s \rangle$ and $\langle \sigma \rangle$ in the transition region is considerably different from those of homopolymers.

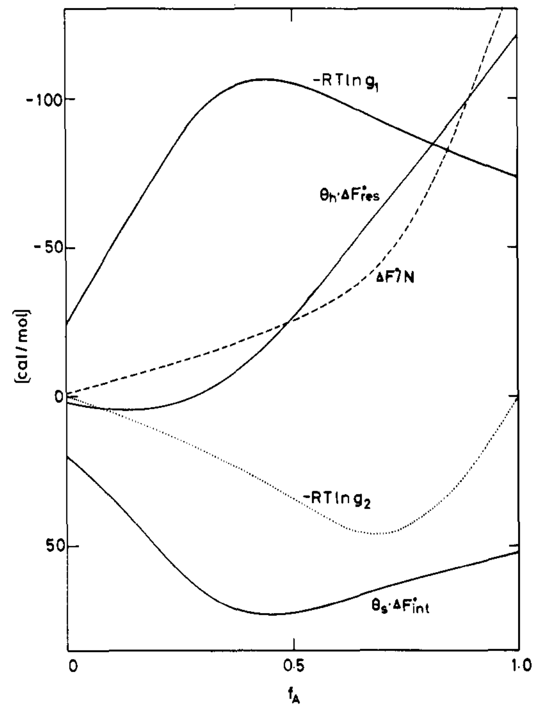

Figure 8. $\theta_h \Delta F°_{res}$, $\theta_s \Delta F°_{int}$, $-RT \ln g_1$, $-RT \ln g_2$, and $\Delta F°/N$ (eq 24) plotted against f_A, according to Allegra's method with numerical values $T = 25.0$ °C, $s_A = 1.25$, $\sigma_A = 5.0 \times 10^{-3}$, $s_B = 0.9$, and $\sigma_B = 3.0 \times 10^{-4}$.

For the purpose of arriving at $\Delta F°/N$ of the random copolymer, the fluctuation of the composition is neglected in addition to the infinite chain length approximation in a manner similar to that described above. Thus, $\Delta F°/N$ can be written in the form

$$\Delta F°/N = \theta_h \Delta F°_{res} + \theta_s \Delta F°_{int} - RT \ln g_1 - RT \ln g_2 \tag{24}$$

where

$$\theta_h = \theta_{hA} + \theta_{hB}$$

$$\theta_{hA} = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln s_A}\right) \qquad \theta_{hB} = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln s_B}\right)$$

$$\theta_s = \theta_{sA} + \theta_{sB}$$

$$\theta_{sA} = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln \sigma_A}\right) \qquad \theta_{sB} = \frac{1}{N}\left(\frac{\partial \ln w_1}{\partial \ln \sigma_B}\right)$$

w_1 is the largest eigenvalue of $\mathbf{W}$ in eq 14 or 15, and

$$\Delta F°_{res} = -RT(\ln s_A + \ln s_B)$$

$$\Delta F°_{int} = -RT(\ln \sigma_A + \ln \sigma_B)$$

$$g_2 = \left[\frac{(1-\theta_h)(1-f_A)}{(1-\theta_h-f_A+\theta_{hA})}\right] \times \left[\frac{(\theta_h-\theta_s)(1-\theta_h-f_A+\theta_{hA})}{(1-\theta_h)(\theta_h-\theta_s-\theta_{hA}+\theta_{sA})}\right]^{\theta_h} \times \left[\frac{(\theta_h-\theta_s-\theta_{hA}+\theta_{sA})\theta_s}{(\theta_s-\theta_{sA})(\theta_h-\theta_s)}\right]^{\theta_s} \times \left[\frac{(\theta_h-\theta_s-\theta_{hA}+\theta_{sA})(f_A-\theta_{hA})}{(\theta_{hA}-\theta_{sA})(1-\theta_h-f_A+\theta_{hA})}\right]^{\theta_{hA}} \times \left[\frac{(\theta_{hA}-\theta_{sA})(\theta_s-\theta_{sA})}{(\theta_h-\theta_s-\theta_{hA}+\theta_{sA})\theta_{sA}}\right]^{\theta_{sA}} \left[\frac{(1-\theta_h-f_A+\theta_{hA})f_A}{(f_A-\theta_{hA})(1-f_A)}\right]^{f_A}$$

From eq 24, $\Delta F^\circ/N$ of a random copolymer is interpreted to include not only the terms of eq 19 but also the combinatorial entropy term which arises from random arrangement of A and B residues. Figure 8 shows the f_A dependence of each term of eq 24 calculated by means of Allegra's approximation. It is found from Figures 7 and 8 that the plots against s and against f_A have almost the same meaning; i.e., the curves of $\theta_h\Delta F^\circ_{res}$, $\theta_s\Delta F^\circ_{int}$, and $-RT \ln g_1$ in the two figures are almost identical, but the term $-RT \ln g_2$ is added in Figure 8. This term can also explain the deviation from the numerical result of the linear approximation which does not include any effect of sequence distribution.

References and Notes

(1) Zimm, B. H.; Rice, S. A. *Mol. Phys.* **1960**, *3*, 391.
(2) Nagasawa, M.; Holtzer, A. *J. Am. Chem. Soc.* **1964**, *86*, 538.
(3) Hermans, J., Jr. *J. Phys. Chem.* **1966**, *70*, 510.
(4) Olander, D. S.; Holtzer, A. *J. Am. Chem. Soc.* **1968**, *90*, 4549.
(5) Bychkova, V. E.; Ptitsyn, O. B.; Barskaya, T. V. *Biopolymers* **1971**, *10*, 2161. Barskaya, T. V.; Ptitsyn, O. B. *Ibid.* **1971**, *10*, 2181.
(6) Kubota, S.; Gaskin, F.; Yang, J. T. *J. Am. Chem. Soc.* **1972**, *94*, 4328.
(7) Miller, W. G.; Nylund, R. E. *J. Am. Chem. Soc.* **1965**, *87*, 3542. Snipp, R. L.; Miller, W. G.; Nylund, R. E. *Ibid.* **1965**, *87*, 3547.
(8) Nitta, K.; Sugai, S. *Bull. Chem. Soc. Jpn.* **1967**, *88*, 46.
(9) Sugiyama, H.; Noda, H. *Biopolymers* **1970**, *9*, 459.
(10) Snell, C. R.; Fasman, G. D. *Biopolymers* **1972**, *11*, 1723.
(11) Zimm, B. H.; Bragg, J. K. *J. Chem. Phys.* **1959**, *31*, 526.
(12) Lifson, S. *Biopolymers* **1963**, *1*, 25.
(13) Allegra, G. *J. Polym. Sci., Part C* **1967**, *16*, 2815.
(14) Lehman, G. W.; McTague, J. P. *J. Chem. Phys.* **1968**, *49*, 3170.
(15) Kidera, A.; Nakajima, A. *Macromolecules*, following paper in this issue.
(16) von Dreele, P. H.; Lotan, N.; Ananthanarayanan, V. S.; Andreatta, R. H.; Poland, D.; Scheraga, H. A. *Macromolecules* **1971**, *4*, 408.
(17) Teramoto, A.; Fujita, H. *J. Macromol. Sci., Rev. Macromol. Chem.* **1976**, *C15*, 165.
(18) von Dreele, P. H.; Poland, D.; Scheraga, H. A. *Macromolecules* **1971**, *4*, 396.

Thermodynamic Study on Charge-Induced Helix–Coil Transition of Ionizable Polypeptide. 2. Potentiometric Titration of Poly(L-glutamic acid) and Copolypeptides of L-Glutamic Acid with L-Alanine or L-Valine

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ABSTRACT: The charge-induced helix–coil transition of poly(L-glutamic acid) and two series of random copolypeptides composed of L-glutamic acid and L-alanine or L-valine in 0.1 N NaCl is investigated. The Zimm–Bragg parameter s for L-glutamic acid, L-alanine, and L-valine is obtained by using the method described in the preceding paper together with the numerical solution of the Poisson–Boltzmann equation and the Zimm–Rice parameter s'. The values of s for L-glutamic acid and L-alanine agree well with those obtained from the temperature-induced transition; however, that for L-valine does not. These results are discussed in connection with the method employed in this study.

In the preceding paper,[1] the charge-induced helix–coil transition of ionizable polypeptides was investigated by means of ΔF°, which is defined as the nonelectrostatic part of the free energy of the conformational transition, obtained from potentiometric titration. By introducing the statistical theories[2–5] of the helix–coil transition, we proposed a theory in which ΔF° was formulated with the conformational partition functions. It was found that $\Delta F^\circ/N$, N being the degree of polymerization, was a function of not only the Zimm–Bragg equilibrium parameter s but also the cooperativity parameter σ, the degree of polymerization, and the composition and the sequence distribution of copolymers. Thus, $\Delta F^\circ/N$ does not necessarily reflect the conformational stability of an amino acid residue associated with the parameter s.

In this paper, we apply the theory proposed in paper I to the analysis of the experimental data of potentiometric titration and then estimate the value of s. Experiments are performed with poly(L-glutamic acid) (PGA) as a homopolymer and two series of random copolymers of varying composition containing L-glutamic acid as the major component and L-alanine or L-valine as the minor component, i.e., random poly(L-glutamic acid-*co*-L-alanine) (GA) and random poly(L-glutamic acid-*co*-L-valine) (GV). From the potentiometric titration data for these polymers, the values of s for L-glutamic acid, L-alanine, and L-valine are obtained. These results are discussed in connection with the sequence distribution of the sample polymers and compared with those obtained from the temperature-induced transition.[6–8]

Estimation of $\Delta F^\circ/N$

The experimental data on potentiometric titration are related to the titration equation for weak polyacids

$$\mathrm{p}K = \mathrm{pH} - \log\frac{\alpha}{1-\alpha} = \mathrm{p}K_0 + \frac{1}{\ln 10}\frac{e\psi}{RT} \qquad (1)$$

where pK is the apparent ionization constant, α the degree of ionization defined in eq I-3, pK_0 the intrinsic ionization constant, e the protonic charge, and ψ the electrostatic potential at the position from which the hydrogen ion is dissociated. Then, eq I-4 can be converted to a form corresponding to eq 1

$$\begin{aligned}\Delta F^\circ/N &= RT\int_0^1(\mathrm{p}K_c - \mathrm{p}K)\,\mathrm{d}\alpha \\ &= \frac{e}{\ln 10}\int_0^1(\psi_c - \psi)\,\mathrm{d}\alpha \quad \text{(area OSQ in Figure 1)}\end{aligned} \qquad (2)$$

where pK_c and ψ_c are the apparent ionization constant and

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