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.

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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

$$pK = pH - \log \frac{\alpha}{1-\alpha} = pK_0 + \frac{1}{\ln 10}\frac{e\psi}{RT} \qquad (1)$$

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

$$\Delta F^\circ/N = RT\int_0^1 (pK_c - pK)\, d\alpha = \frac{e}{\ln 10}\int_0^1 (\psi_c - \psi)\, d\alpha \quad \text{(area OSQ in Figure 1)} \qquad (2)$$

where pK_c and ψ_c are the apparent ionization constant and

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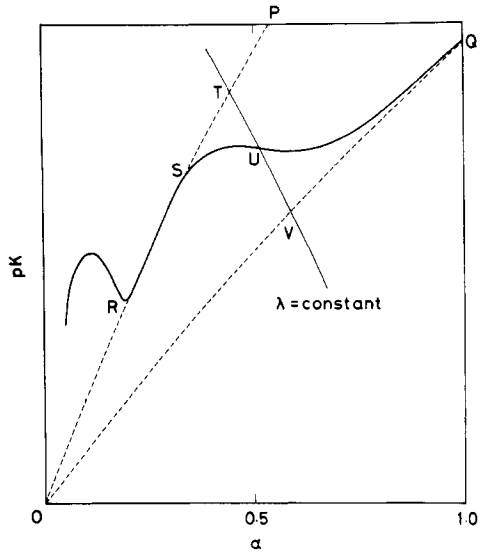

Figure 1. Schematic representation of potentiometric titration curves. The solid curve denotes the experimental data and the broken curves denote the theoretical data.

the electrostatic potential for the standard state, respectively, and both pK and pK_c are assumed to have the same pK_0.

As schematically shown in Figure 1, the values of ψ in the region of $\alpha \lesssim 0.2$, which is the so-called aggregation region[9] (curve OR in Figure 1), and ψ_c (curve OQ in Figure 1) are required in addition to the experimental data in order to estimate $\Delta F^\circ/N$ by means of eq 2.

In this study, these values are obtained from the numerical solutions of the cylindrically symmetric Poisson-Boltzmann equation.[10] In a large excess of uni-univalent added salt, the cylindrically symmetric Poisson-Boltzmann equation can be written in the form

$$\frac{1}{r}\frac{d}{dr}\left(r\frac{d\psi}{dr}\right) = \kappa^2(RT/N_A e)\sinh(e\psi/RT) \quad (3)$$

where r is the radial distance from the axis of the polyion rod, κ the Debye-Hückel parameter, and N_A Avogadro's number. For given values of the characteristic parameters of a polyion, the number of charges ν per unit length, and the radius r_s of the rod, eq 2 is solved by means of the Hamming method under the boundary conditions

$$\phi'(x_s) = -\kappa^2\nu/4\pi n x_s$$
$$\phi(\infty) = 0 \quad (4)$$

and the initial conditions

$$\phi(x_i) = -\phi'(x_s)\frac{K_0(x_i)}{K_1(x_s)}$$
$$\phi'(x_i) = \phi'(x_s)\frac{K_1(x_i)}{K_1(x_s)} \quad (5)$$

where $x = \kappa r$, $x_s = \kappa r_s$, $\phi = e\psi/RT$, and $\phi' = d\phi/dx$. $K_n(x)$ is the modified Bessel function of the second kind, and x_i is the smallest value which satisfies

$$|\phi(x_i) - \sinh[\phi(x_i)]| < 10^{-7}$$

The values of the characteristic parameters ν and r_s for the curves OP and OQ in Figure 1 are chosen in the following manner. First, for ν values of the curves OP and OQ, the axial translations of the α helix and of a fully extended coil form are chosen, respectively. Next, the values of r_s are chosen as adjustable parameters to give such curves as shown in Figure 1; that is, r_s of the curve OP gives an extrapolation of the experimental curve in the region $\alpha \sim 0.3$, and r_s of the curve OQ gives the standard curve for zero-helical content ($\theta_h = 0$) which approaches asymptotically the experimental curve in the region $\alpha \gtrsim 0.75$ and finally overlaps at $\alpha = 1$ in accordance with eq I-6; i.e.

$$\Xi[\lambda(1)] = \Xi_c[\lambda(1)]$$

It should be noted that the curve OP does not mean the standard curve for $\theta_h = 1$, and the curve OQ does not mean a curve which is given by extrapolating the experimental curve in the region of $\alpha \gtrsim 0.75$.

Estimation of σ (Definition of s')

Since σ is one of characteristic parameters which describe the helix-coil transition of nonionic polymers, the value of σ should be estimated from the nonelectrostatic part in the free energy $\Delta F^\circ/N$. However, $\Delta F^\circ/N$ is a function of s, σ, and N (in the case of random copolymers, $\langle s\rangle$ and $\langle\sigma\rangle$ defined by eq I-22 in place of s and σ), and both s and σ cannot be estimated at the same time, although N can be evaluated from other experimental procedures.

Hence, we will estimate the value of σ by using the Zimm-Rice parameter s'[11] and further assume that σ is constant independent of the charge state.

Then, s' can be defined by the following two methods.

A. Definition I. By extending eq I-5 to $\alpha > 0$, one can obtain the following quantity from the titration curves

$$Z_1[\lambda(\alpha)] = \frac{\Xi[\lambda(\alpha)]}{\Xi_c[\lambda(\alpha)]} = \exp\left[\int_{\lambda(\alpha)}^{\lambda(1)}(\alpha_c - \alpha)\, d(\ln\lambda)\right] \quad (6)$$

(area UQV in Figure 1)

where Ξ, Ξ_c, and $\alpha_c - \alpha$ are defined by eq I-1, eq I-2, and eq I-3, respectively, and $\log\lambda = pH - pK_0$. To a good approximation, $\mathbf{c}'$ in Ξ_c can be extended to $\mathbf{c}$, and the internal partition function Q is factorized into two parts, Q_h, a part of Q resulting from the formation of hydrogen bonds in the α-helical conformation, and Q_c, the remaining part of Q and defined in eq I-2. Then, Z_1 can be written in the form

$$Z_1[\lambda(\alpha)] = \frac{\sum_{\mathbf{c}}\sum_{\tilde{\eta}} Q_h(\mathbf{c},\tilde{\eta})Q_c(\mathbf{c},\tilde{\eta})\exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta}}{\sum_{\mathbf{c}}\sum_{\tilde{\eta}} Q_c(\mathbf{c},\tilde{\eta})\exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta}} \quad (7)$$

The right side of eq 7 proves to be a value of Q_h averaged with $Q_c\exp[-W/RT]\lambda^{\eta}$. Since Z in eq I-7 is also understood to be a value of Q_h averaged with Q_c, we can express Z_1 also in a charged state as follows:

$$Z_1 = \mathbf{e}\mathbf{W}^N\mathbf{e}^+ \quad (8)$$

where

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

Equation 8 is the first definition of s'.

B. Definition II. Similarly, the following quantity can be obtained from the titration curves:

$$Z_2[\lambda(\alpha)] = \frac{\Xi_h[\lambda(\alpha)]}{\Xi_c[\lambda(\alpha)]} =$$
$$\exp\left[\int_{\lambda(0)}^{\lambda(1)}(\alpha_c - \alpha)\, d(\ln\lambda) - \int_{\lambda(0)}^{\lambda(\alpha)}(\alpha_c - \alpha_h)\, d(\ln\lambda)\right] \quad (9)$$

(area OSQ - OTV in Figure 1)

where Ξ_h is a partition function which represents the curve

OP in Figure 1 and $\alpha_h = (1/N)(\partial \ln \Xi_h/\partial \ln \lambda)$. Following the description of the preceding section, we can define Ξ_h as

$$\Xi_h = \Xi \quad \text{for } \lambda \leq \lambda_S$$

$$\Xi_h = \Xi_S \quad \text{for } \lambda \geq \lambda_S$$

$$\Xi_S = \sum_c \sum_{\tilde{\eta}} Q_h^S(\mathbf{c},\tilde{\eta}) Q_c(\mathbf{c},\tilde{\eta}) \exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta} \quad (10)$$

λ_S is λ at the point S in Figure 1 and Q_h^S is an internal partition function which makes Ξ_S represent the conformation at the point S for $\lambda \geq \lambda_S$. Then Z_2 can be written in the form

$$Z_2 = Z_1 \quad \text{for } \lambda \leq \lambda_S$$

$$Z_2 = \frac{\sum_c \sum_{\tilde{\eta}} Q_h^S(\mathbf{c},\tilde{\eta}) Q_c(\mathbf{c},\tilde{\eta}) \exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta}}{\sum_c \sum_{\tilde{\eta}} Q_c(\mathbf{c},\tilde{\eta}) \exp[-W(\mathbf{c},\tilde{\eta})/RT]\lambda^{\eta}} \quad \text{for } \lambda \geq \lambda_S \quad (11)$$

The right side of eq 11 proves to be a value of Q_h^S averaged with $Q_c \exp[-W/RT]\lambda^{\eta}$. Thus, in a manner similar to that of eq 8, Z_2 for $\lambda \geq \lambda_S$ can be expressed as

$$Z_2 = \sum_{N_h} \sum_{N_s} s'^{N_h} \sigma^{N_s} g(N_h,N_s) P_S(N_h,N_s) \quad (12)$$

where

$$g(N_h,N_s) = \frac{N_h!}{(N_h - N_s)!N_s!} \frac{(N - N_h)!}{(N - N_h - N_s)!N_s!}$$

and $P_S(N_h,N_s)$ is the probability of occurrence of N_h helical units and N_s helical sequences at $\lambda = \lambda_S$; that is

$$P_S(N_h,N_s) = s_S'^{N_h} \sigma^{N_s} g(N_h,N_s)/Z_1(\lambda_S)$$

where s_S' is the value of s' at $\lambda = \lambda_S$. Equation 12 is the second definition of s'.

Zimm and Rice[11] assumed the curve OP in Figure 1 simply as the standard curve of $\theta_h = 1$ and defined s' by eq 13 instead of eq 12. This equation is equivalent to the

$$Z_2 = s'^N \quad (13)$$

approximation of eq I-9 and thus includes the problems pointed out in paper I.

Now using the parameter s' defined by the above two definitions, we will estimate σ in the following manner. By employing the fact that both Z_1 and Z_2 should give the same value of s' for a reasonable value of σ, we define the following quantities for a given value of σ:

$$\tau_a(\sigma) = \int_{\lambda(0)}^{\lambda(1)} |\theta_h^1(\lambda) - \theta_h^2(\lambda)| \, d(\ln \lambda)$$

$$\tau_b(\sigma) = \int_{\lambda(0)}^{\lambda(1)} |Z_1(\lambda) - Z_1'(\lambda)| \, d(\ln \lambda) \quad (14)$$

where θ_h^1 and θ_h^2 are helical contents which are calculated by differentiating Z_1 and Z_2 with respect to s', respectively, and Z_1' is a value of Z_1 calculated with s' defined by Z_2. Then we take the best value of σ as the one which minimizes τ_a and τ_b.

Similarly, Z_1 and Z_2 for random copolypeptides can be defined by replacing s' and σ with $\langle s' \rangle$ and $\langle \sigma' \rangle$, defined in analogy with eq I-22; that is

$$\langle s' \rangle = x_A s_A' + x_B s_B'$$

$$\langle \sigma' \rangle = \frac{x_A s_A' \sigma_A + x_B s_B' \sigma_B}{x_A s_A' + x_B s_B'} \quad (15)$$

where x_A and x_B are the same parameters as those defined in eq I-22, s_A' and s_B' are the Zimm–Rice parameters for A and B residues, respectively, and both σ_A and σ_B are assumed to be constant independent of the charge state. By denoting the values of s' and σ for L-glutamic acid as s_A' and σ_A, respectively, we define τ_a and τ_b for random copolypeptides for a given value of σ_B in a manner similar to that of eq 14 and then obtain the value of σ_B by choosing a value which minimizes τ_a and τ_b.

Table I
Characterization of Polymers

sample	f_A[a]	$[\eta]$[b]	N[c]
PGA	1.00	1.17	400
GA1	0.93	0.97	330
GA2	0.85	1.47	550
GA3	0.78	1.80	720
GV1	0.96	1.91	680
GV2	0.94	1.31	450
GV3	0.87	1.23	440

[a] From amino acid analyses. [b] In 0.1 N NaCl aqueous solution at 25.5 °C. [c] Estimated from limiting viscosity number.

Experimental Section

Materials. Poly(γ-benzyl L-glutamate) (PBLG), random poly(γ-benzyl L-glutamate-*co*-L-alanine) (PBGA), and random poly(γ-benzyl L-glutamate-*co*-L-valine) (PBGV) were synthesized by polymerization of *N*-carboxy amino acid anhydrides (NCA) in a 1:1 (v/v) mixture of dioxane and methylene chloride with triethylamine as an initiator ([A]/[I] ~ 50). In order to avoid heterogeneity of the copolymer composition with increasing conversion,[12] we stopped the polymerization at about 70% conversion for PBGA and at about 40% for PBGV. After the dimethylformamide-insoluble portion of the product was removed, debenzylation (dry HBr in benzene solution) gave poly(L-glutamic acid) (PGA), random poly(L-glutamic acid-*co*-L-alanine) (GA), and random poly(L-glutamic acid-*co*-L-valine) (GV). Finally, after dialysis the samples were freeze-dried.

Sample Characterization. The absence of the γ-benzyl group in the polymers was confirmed by measuring the absorption at 259 nm, the benzyl ester chromophore, using a Hitachi EPS-3T recording spectrophotometer. The amino acid composition was determined by amino acid analysis, performed at the Protein Research Foundation, Osaka. The degree of polymerization was estimated from the viscosity–molecular weight relationship proposed by Hawkins and Holtzer.[13] Viscosity measurements were carried out with an improved Ostwald viscometer at 25.5 °C in 0.1 N NaCl solution. Their concentrations were determined by conductometric titration, using a Yanagimoto Conductivity Outfit MY-8. Table I summarizes the composition, the intrinsic viscosity, and the degree of polymerization of the polymers. The circular dichroism (CD) spectra were measured at 25 ± 1 °C at wavelengths ranging from 200 to 250 nm, using a Jasco J-20 CD/ORD spectropolarimeter. The CD spectra shown in Figure 2 clearly indicate that the sample polymers do not form any second-order structure other than α helix in the acidic region (pH ~4.8); the values of ΔF° estimated here are confirmed to entirely result from the α helix–coil transition.

Measurement of pH. A Hitachi-Horiba F-7_{ss} pH meter was used with a 1026-05T glass electrode and a 2535-05T double-junction type reference electrode. The instrument was calibrated with Horiba standard buffer solutions (pH 6.86 and 4.01) at 25.0 °C. The leakage of Cl^- from the reference electrode was less than 5×10^{-5} N/h, and the drift of the instrument was less than 5×10^{-3} pH unit during the titration.

Potentiometric Titration. The freeze-dried sample was dissolved in twice-distilled water to give a ca. 3×10^{-3} (residue mol)/L solution and deionized by using a mixed-bed ion exchanger (Bio-Rad AG501X8). The deionized solution was mixed with 1 N NaCl aqueous solution to give a 0.1 N NaCl solution. The solution was titrated in nitrogen gas saturated with water vapor at 25 ± 0.05 °C with a 0.1 N NaOH solution by means of a Terumo MS-N50 microsyringe. The titration end point was indicated by the volume of added base at which the change in pH per unit

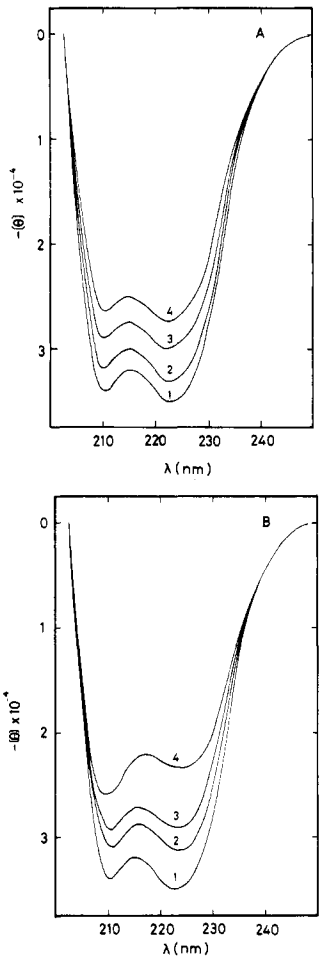

Figure 2. CD spectra of the polymers at 25 °C and pH 4.8 in 0.1 N NaCl: (A) (1) PGA; (2) GA1; (3) GA2; (4) GA3. (B) (1) PGA; (2) GV1; (3) GV2; (4) GV3.

Table II
Values of $\Delta F^\circ/N$

sample	$\Delta F^\circ/N$, cal/mol
PGA	−177.1 ± 0.8
GA1	−167.6 ± 0.1
GA2	−160.4 ± 0.5
GA3	−150.9 ± 0.4
GV1	−163.8 ± 1.4
GV2	−156.6 ± 1.6
GV3	−128.1 ± 1.5

volume of added base is greatest. It was assumed that the activity of H^+ of the solution was not influenced by the presence of the polyion in determining the degree of ionization.

Results

The experimental data for the potentiometric titration are shown in Figure 3, where the abscissa is scaled with $\alpha' = \alpha f_A$, f_A being the mole fraction of L-glutamic acid in the polymer. The experimental points are reproducible within $\pm 5 \times 10^{-3}$ pK unit. The parameters of the calculated curves for both GA's and GV's are $1/\nu = 1.5$ Å, $r_s = 14$ Å for curve OP, $1/\nu = 3.6$ Å, $r_s = 11$ Å for curve OQ, and $pK_0 = 4.375$. As described in the previous section, curve OP is chosen as the extrapolation of the experimental curve at $\alpha \sim 0.3$ to pK_0, and curve OQ as the standard curve for $\theta_h = 0$. It is noted from these titration curves that difference in the conformational stability of each polymer appears only in the middle of the transition region.

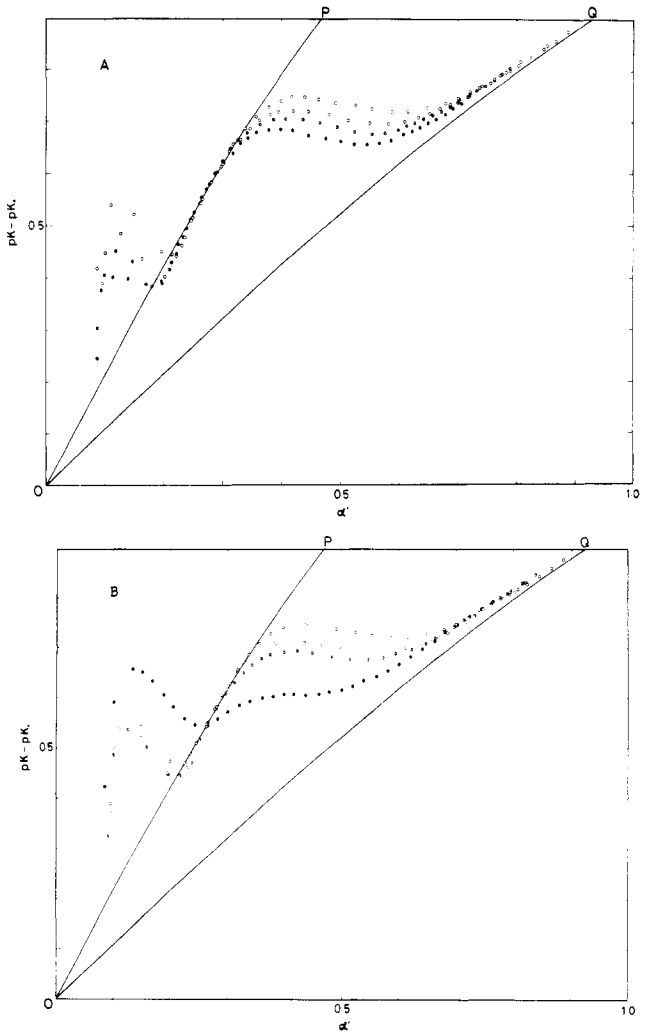

Figure 3. Potentiometric titration curves at 25.0 °C in 0.1 N NaCl: (A) (○) PGA; (◎) GA1; (Φ) GA2; (●) GA3. (B) (○) PGA; (◎) GV1; (Φ) GV2; (●) GV3.

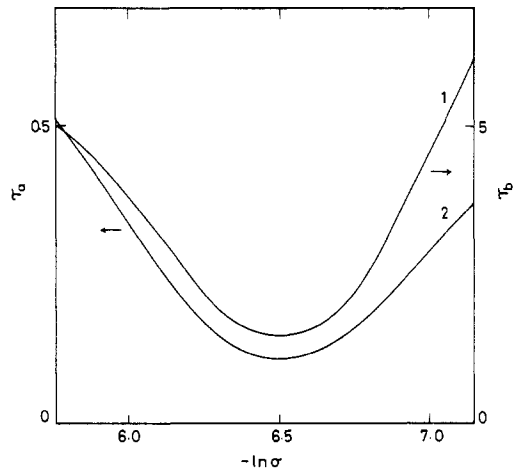

Figure 4. τ_a and τ_b plotted against $-\ln \sigma$ for PGA.

Now let us estimate the values of s for L-glutamic acid, L-alanine, and L-valine. First, the titration curves are integrated numerically in accordance with eq 2 to give $\Delta F^\circ/N$. Table II shows the results for $\Delta F^\circ/N$ together with their ranges of reproducibility. Second, the value of σ is estimated. In Figure 4, τ_a and τ_b calculated from the titration curve of PGA are plotted against σ. Thus we obtained $\sigma = 1.5 \times 10^{-3} \pm 0.3 \times 10^{-3}$ for L-glutamic acid. Finally, substituting the results of N, $\Delta F^\circ/N$ and σ into

Table III
Comparison of Values of s Calculated with Approximate Theories and Exact Theory

sample	linear	Lifson	Allegra	exact
		L-Alanine		
GA1	1.08 ± 0.01	1.07 ± 0.01	1.11 ± 0.01	1.09 ± 0.01
GA2	1.12 ± 0.01	1.07 ± 0.01	1.10 ± 0.01	1.09 ± 0.01
GA3	1.10 ± 0.01	1.06 ± 0.01	1.10 ± 0.01	1.08 ± 0.01
		L-Valine		
GV1		0.43 ± 0.08	0.79 ± 0.03	0.68 ± 0.04
GV2		0.45 ± 0.07	0.80 ± 0.03	0.68 ± 0.03
GV3		0.49 ± 0.03	0.80 ± 0.01	0.69 ± 0.01

Table IV
Values of s at 25 °C

amino acid	s
L-glutamic acid	1.36 ± 0.01
L-alanine	1.09 ± 0.02
L-valine	0.68 ± 0.04

eq I-7, we obtained the value of s for L-glutamic acid as 1.36 ± 0.01.

The analyses of the data on the random copolypeptides are carried out in accordance with three approximate theories and an exact theory, i.e., linear approximation (eq I-12), Lifson's approximation[3] (eq I-14), Allegra's approximation[4] (eq I-15), and the exact theory of Lehman–McTague[5] (eq I-17). τ_a and τ_b were calculated from the titration curves of the random copolymers to estimate σ_B for L-alanine and L-valine. However, τ_a and τ_b did not depend on σ_B within experimental error. These results may arise from the fact that f_A is close to unity and the values of σ for L-alanine and L-valine are much smaller than that for L-glutamic acid. Actually, if σ_B is only of the order of 10^{-4}, a change in σ_B gives a negligible effect on $\Delta F^\circ/N$. Hence, the analysis of the random copolymers is performed by assuming only $\sigma_B = 10^{-4}$. Then using the results, N and f_A in Table I, $\Delta F^\circ/N$ in Table II, and s_A and σ_A for L-glutamic acid obtained above, we obtained the values of s for L-alanine and L-valine. They are shown in Table III. The data for the GV samples could not be analyzed by linear approximation because the values of $\Delta F_B^\circ/N$ obtained from eq I-13, which must be inherently negative, give positive values: 155.4 cal/mol for GV1, 164.6 cal/mol for GV2, and 199.8 cal/mol for GV3. The data shown in Table III indicate that the approximate theories are applicable for the present system only to a more limited region than for the temperature-induced transition. The estimated values of s for L-glutamic acid, L-alanine, and L-valine are summarized in Table IV.

Discussion

Theoretical Titration Curve Calculated by the Poisson–Boltzmann Equation. Nagasawa[14,15] and Sugai[16] suggested that a theoretical titration curve calculated by eq 3 shows a disagreement with the observed one at low degrees of ionization and that such a disagreement is attributable to the flexibility of the polymer chain and the nonuniform charge distribution on the polyion surface.

In the present study, the effect of nonuniform charge distribution in the calculated curves shown in Figure 3 may be compensated by regarding the radius of the polyion rod, r_s, as an adjustable parameter, for which we actually chose a larger value than the realistic one. On the other hand, the effect of chain flexibility should be inherently included in Ξ_c, defined by curve OQ, as is obvious in its definition (eq I-2), though curve OP concerns a polymer chain having scarcely any flexibility because of its high helical content. Hence, curve OQ, in accordance with the condition of eq I-6, should be understood as the one which concerns a polymer chain maintaining the conformation at $\lambda(1)$ throughout the whole range of α. In other words, $\Delta F^\circ/N$ obtained here represents the change in free energy from the uncharged conformation at $\lambda(1)$ to the conformation at $\lambda(0)$. Such an effect on $\Delta F^\circ/N$, however, is expected to be sufficiently reduced in the solution of 0.1 N added salt used in this experiment.

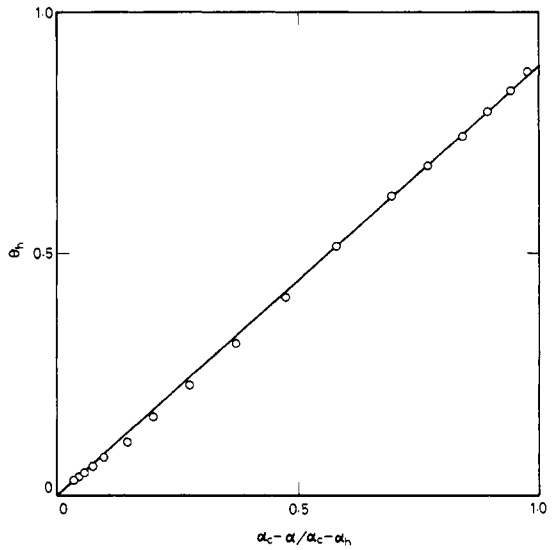

Figure 5. θ_h plotted against $(\alpha_c - \alpha)/(\alpha_c - \alpha_h)$ for PGA.

On the Assumption of Charge Independency of σ. If a rodlike polyion is assumed for the behavior of titration curves, then the helical content θ_h can be related directly to the titration curves by

$$\theta_h = \frac{\alpha_c - \alpha}{\alpha_c - \alpha_h}\theta_h^S \qquad (16)$$

where θ_h^S $(=N_h^S/N)$ is the helical content at the point S in Figure 1. It is noted that eq 14 in the limit of $\theta_h^S = 1$ is the equation which was employed by Peller[17] and also by Nagasawa and Holtzer.[18] Since eq 14 is entirely independent of the results of the statistical theories based on eq 8 and 13, the validity of the assumption of charge independence of σ can be examined by plotting the value of θ_h obtained from eq 8 or 13 against $(\alpha_c - \alpha)/(\alpha_c - \alpha_h)$. The result is shown in Figure 5, in which the experimental points fall almost on a straight line given by $\theta_h^S(\alpha_c - \alpha)/(\alpha_c - \alpha_h)$. Hence, it is concluded that the assumption of charge independence of σ is valid at least within the range of validity of the rodlike polyion model in the transition region.

Effect of Sequence Distribution. In general, the temperature-induced transition of random copolymers has been investigated by assuming randomness in their sequence distribution, which implies that the constituent monomers are arranged randomly enough to neglect the effect of specific sequence distribution on θ_h. Though the assumption of randomness is adopted also in this study, it should be confirmed whether the sequence distribution of the sample polymers used here has a negligible effect on $\Delta F^\circ/N$.

Concerning GV samples, we employ the numerical results on sequence distribution of random poly(γ-benzyl L-glutamate-*co*-L-valine) reported by Ishiwari.[19] The sequence distribution was estimated as a function of conversion by using the equation of Skeist[20] and the method of Frensdorff[21] with monomer reactivity ratios for γ-benzyl L-glutamate NCA (1) and L-valine NCA (2) of $r_1 = 2.96$ and $r_2 = 0.17$.

Table V
Effect of Sequence Distribution for Poly(L-glutamic acid-co-L-valine)

f_A	$\Delta F^\circ/N$, cal/mol	
	random dist	Markovian dist
0.9	−139.1696	−139.1696
0.8	−100.5881	−100.5881
0.7	−64.4655	−64.7979
0.6	−34.5286	−35.0314
0.5	−15.4446	−16.4181
0.4	−6.5736	−7.1612
0.3	−2.9981	−3.2999
0.2	−1.4272	−1.5930
0.1	−0.6120	−0.6590

By applying the results of dyad distribution at 90% conversion to Lehman–McTague's method,[5] which considers the nearest-neighbor correlated sequence, we also estimated the values of $\Delta F^\circ/N$ for the Markovian sequence distribution. The values of $\Delta F^\circ/N$ for random poly(L-glutamic acid-*co*-L-valine) are shown in Table V. Comparing these values, one can see that the blockiness at 90% conversion produces little effect on $\Delta F^\circ/N$. Actually, GV samples whose conversions are about 40% may have a lower order of blockiness than those calculated above because the order of blockiness increases in proportion to conversion. Further, the GA samples may have a sequence distribution closer to the random one than the GV samples because the reported values[22] of monomer reactivity ratios for γ-benzyl L-glutamate NCA (1) and L-alanine NCA (2) are $r_1 = 2.36$ and $r_2 = 0.50$. In conclusion, the assumption of randomness for the present copolypeptides is sufficiently valid.

Comparison with Results of Temperature-Induced Transition. Using water-soluble random copolymers containing (hydroxypropyl)-L-glutamine (HPG) or (hydroxybutyl)-L-glutamine (HBG) as the major component, i.e., the so-called host component, the values of s for L-glutamic acid,[6] L-alanine,[7] and L-valine[8] were obtained from the temperature-induced helix–coil transition on the basis of statistical theories for copolymers. The values of s at 25 °C were reported as 1.32 for L-glutamic acid (pH 2.3), 1.06 for L-alanine, and 0.95 for L-valine. The values for L-glutamic acid and L-alanine agree well with those of the present study. However, the value for L-valine is much larger than ours.

We can identify two differences between the methods in this study and in the temperature-induced transition which may cause such a discrepancy. One is the difference in the standard state as mentioned in the preceding section; that is, the standard state in this study is not the random coil of nonionic polypeptides but a conformation defined at λ(1), which may be relatively extended by the electrostatic potential of the dissociated side chains. Hence, it is expected that an L-valine residue, which has a bulky side chain, is more sensitive to such an extension. The other is the difference in host component. L-Valine was reported to perform an inverse transition in water unlike L-glutamic acid and L-alanine; that is, L-valine increases α-helix stability with increasing temperature. Such a feature implies that the conformation of the L-valine residue depends predominantly on the interaction with H_2O molecules. Therefore, it is expected that the difference in host components has a significant effect on the conformational stability of the L-valine residue by varying the state of solvation.

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