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POLYMER CONCENTRATION DEPENDENCE OF THE HELIX TO RANDOM COIL TRANSITION OF A CHARGED POLYPEPTIDE IN AQUEOUS SALT SOLUTION

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The helix to coil transition of poly(L-glutamic acid) was investigated in 0.05 and 0.005 M aqueous potassium chloride solutions by use of potentiometric titration and circular dichroism measurement. Polymer concentration dependence of the transition was observed in the range from 0.006 to 0.04 monomol/ℓ in 0.005 M KCl solution. The polymer concentration dependence can be interpreted by current theories of the transition of charged polypeptides and of titration curves of linear weak polyelectrolytes taking the effect of polymer concentration into consideration.

1. Introduction

Because the helix to random coil transition of polypeptides is an intramolecular transconformation, it should be regarded as independent on polymer concentration at least within the first order approximation. However, in case of charged polypeptides, it is a little complicated because of the long-range electric monopole–monopole interaction. One of the factors to induce the transition is the difference of electrostatic free energy between helical and coiled polypeptides. If the electrostatic free energy per residue is dependent on polymer concentration, the transition should be considered to be dependent on polymer concentration. Several groups of researchers have recently published theories of the polymer concentration dependence of the electrostatic free energy of polyelectrolyte in aqueous solutions [1–3]. They have all indicated the dependence especially in the case of lower added salt concentration than the polymer concentration, and have well interpreted the experimental potentiometric titration curves of weak polyacids [3]. So the dependence of the helix–coil transition is expected in such cases.

The aim of the present investigation is to observe the above concentration dependence and to examine whether the dependence can be interpreted or not by current theories of the helix–coil transition of charged polypeptides [4,5] and of the polymer concentration dependence of the electrostatic free energy of linear polyelectrolytes in aqueous salt solutions.

2. Materials and methods

2.1. Samples

Poly(γ-benzyl L-glutamate) was prepared by the ordinary method [6] from γ-benzyl L-glutamate purchased from Protein Research Foundation, Minoh, Osaka. It was converted to poly(L-glutamic acid) [poly(Glu)] [6], and dialyzed against distilled water for a week and then freeze-dried. The degree of debenzylation was found to be more than 99.5% by use of ultraviolet absorption at 260 nm. The molecular weight of the poly(Glu) was determined from limiting viscosity number in 0.4 M aqueous NaCl and sodium phosphate solution (pH 6.8) [7] as 56000. Aqueous poly(Glu) solutions were deionized by use of a mixed-bed ion-exchange column (Amberlite IR-120 and IRA-410). The concentrations of the poly(Glu) solutions were determined by potentiometric titration. Other chemicals were guaranteed reagent grade purchased from Nakarai Chemicals, Ltd., Osaka.

Aqueous potassium hydroxide of 1 N was decarbonated according to essentially the same method as literature [8] but to remove barium hydroxide, Chelex-100, Bio-Rad Laboratories, was used instead of Amberlite IR-120. The salt concentration of the titrant and the poly(Glu) solutions was brought to the desired value by addition of a measured volume of a concentrated KCl solution.

2.2. Potentiometric titration

A Radiometer pH M4c instrument was used with a radiometer G202C glass electrode and a Horiba #2630-05T calomel electrode, of which inner solution is 3.3 M KCl solution. The calomel electrode was kept immersed in 3.3 M KCl in a thermostated glass vessel, and a KCl liquid-junction tube from the vessel was joined to a glass tube having a pin hole at its end which contacted with the measured solution. The leakage of KCl was estimated by conductivity measurement as to be less than 0.00005 M during a few hours. The instrument was calibrated with Radiometer standard buffer S1001 (6.48 ± 0.02) at 25°C, and checked also with Beckmann standard buffers 3005 (pH 4.008) and 3007 (pH 7.00) to be accurate to 0.02 pH units.

The poly(Glu) solutions were titrated in a nitrogen atmosphere at 25 ± 0.1°C using 1 N KOH with a micrometer syringe. Drift of the instrument is less than 0.005 pH units during the titration.

2.3. Circular dichroism measurement

Circular dichroism was measured at 222 nm using a Jasco J-20 instrument with 1 mm or 0.1 mm fused quartz cell. The cell was thermostated to 25°C by use of a water-jacketed cell holder. The instrument and the cells were calibrated with D-10-camphor-sulphonic acid [9].

2.4. Computation

Surface electric potential of cylindrical polyelectrolytes was calculated with the method described previously [3] by use of FACOM 230-60 or 230-75 in Hokkaido University Computing Center.

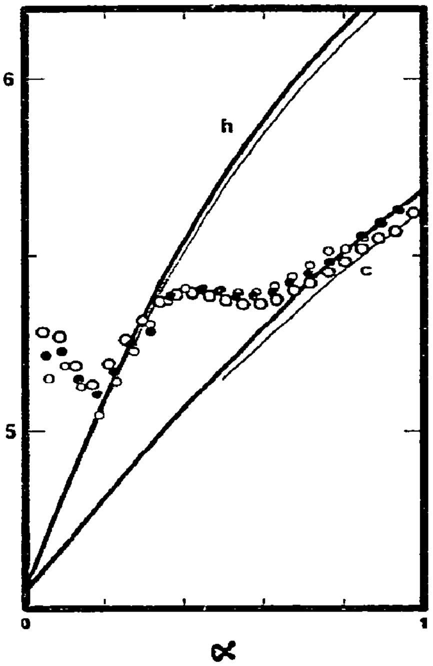

Fig. 1. Titration curves for poly(Glu) at 25°C in 0.05 M KCl. Polymer concentrations are 0.0065 (small open circles), 0.0132 (small filled circles) and 0.0277 monomol/ℓ (large open circles). The heavy and light lines are calculated for helical (h) and coiled (c) polymer at infinite dilution and C_p = 0.0277 monomol/ℓ, respectively [3]. The curves for the other C_p's essentially coincide with that for infinite dilution. The parameters used are: a = 14.0 Å (the closest approach of added salt ions to the polymer) and L = 1.50 Å (length for monomeric unit) for helical, and a = 11.5 Å and $2L$ = 7.23 Å for coiled polypeptides.

3. Results

Potentiometric titration curves of poly(Glu) were obtained in aqueous solutions with added salt concentrations (C_s) of 0.05 and 0.005 M and in the range of polymer concentration (C_p) from 0.006 to 0.04 monomol/ℓ and shown in figs. 1 and 2. Theoretical titration curves [3] were computed with lengths of monomeric unit of 1.500 and 3.615 Å for helical and coiled poly-

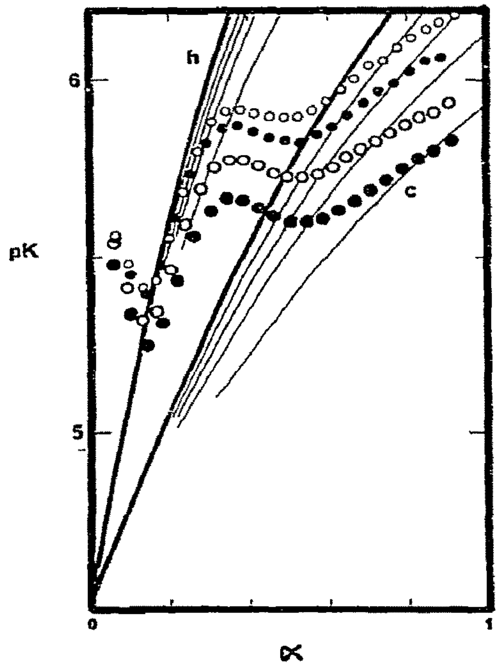

Fig. 2. Titration curves for poly(Glu) at 25°C in 0.005 M KCl. The polymer concentrations are 0.0065 (small open circles), 0.0119 (small filled circles), 0.0191 (large open circles) and 0.0363 monomol/ℓ (large filled circles). The heavy and light lines are calculated for helical (h) and coiled (c) polymer at infinite dilution and each C_p, respectively [3], with the same parameters as cited in fig. 1.

peptides, respectively, and the best fits to the experimental curves were obtained at the closest approaches of added salt ions to the polymer of 14.0 and 11.5 Å, respectively. These values agree with the published ones for poly(Glu) obtained by the pH titration method [10,11]. The value of pK_0, the intrinsic dissociation constant, were obtained as 4.55 in 0.05 M KCl or 4.53 in 0.005 M KCl, by use of the above nonlinear extrapolation. This value agrees essentially well with that of oligomers of glutamic acid [11] or poly(DL-glutamic acid) [12]. Expectedly, the titration curve is little dependent on C_p when it is lower than C_s but is dependent when C_p is higher than C_s, and the pH value at the helix–coil transition also seems to be dependent on C_p in the same manner. Helical fraction of residues f_h in 0.005 M KCl obtained from the titration curves [13] is shown in fig. 3 as a function of pH of the solution, and is really dependent on C_p.

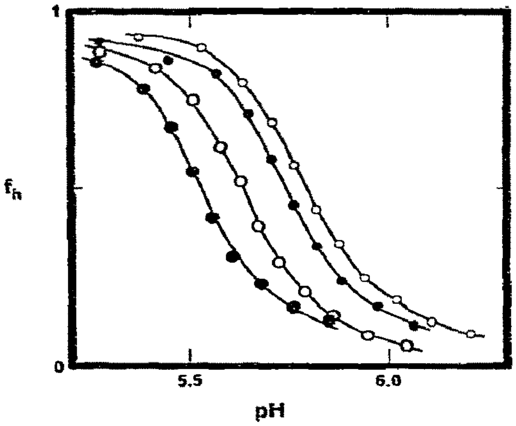

Fig. 3. Helical fraction of residues as a function of pH for poly(Glu), determined by pH titration in 0.005 M KCl. Polymer concentrations are the same as cited in fig. 2.

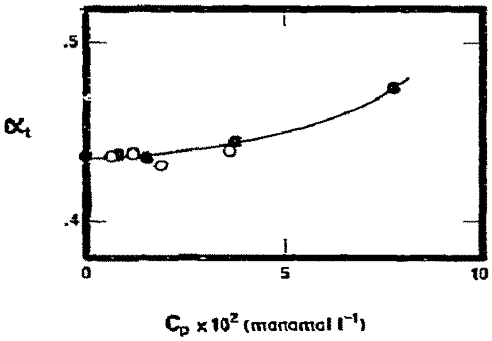

Fig. 4. Polymer concentration dependence of the degree of ionization at the midpoint of the helix–coil transition in 0.005 M KCl. Open circles: determined from pH titration, filled circles: calculated with $\Delta G_0^\circ/N$ of 297 cal/mol.

However, the degree of ionization of γ-carboxyl groups at the midpoint of the transition α_t is scarcely dependent on C_p within the range of C_p employed, as shown in fig. 4. The standard Gibbs free energy change per residue, of the transition un-ionized helix to un-ionized coil, is obtainable from the following integral [13]:

$$\Delta G_0^\circ/N = (RT\ln 10)\log s_0 = (RT\ln 10)\oint \mathrm{pK}\,\mathrm{d}\alpha\,, \quad (1)$$

where s_0 is the equilibrium constant for the transfer of a monomeric unit to a section of the helix from

Table 1
Parameters for the helix–coil transition of poly(Glu) in 0.005 M KCl

		C_p (monomol/ℓ)				
		0.0065	0.0119	0.0191	0.0363	Mean
$\Delta G_0^\circ/N$ (cal/mol)	a	304	300	291	301	299 ± 5
	b	300	301	297	292	297 ± 5
	c	265	270	285	288	277 ± 12
$\sigma \times 10^3$	d	2.7	2.5	3.1	2.8	2.8 ± 0.3
	e	2.9	2.4	2.0	3.0	2.6 ± 0.6

a) From titration with eq. (1).
b) From titration with eq. (2).
c) From CD with eq. (2).
d) From titration with eq. (11) of ref. [16].
e) From CD with eq. (11) of ref. [16].

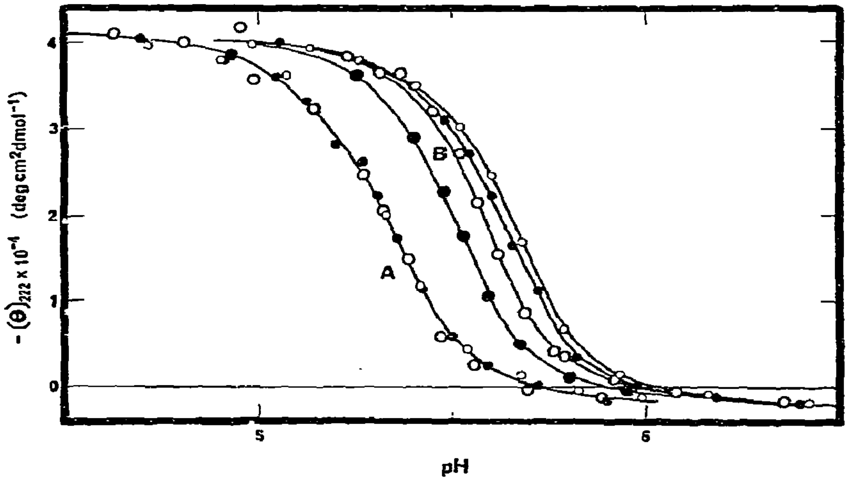

Fig. 5. Circular dichroism of poly(Glu) as a function of pH. A: $C_s = 0.05$ M and C_p's are the same as cited in fig. 1. B: $C_s = 0.005$ M and C_p's are the same as cited in fig. 2.

the adjacent section of the random coil at uncharged state. The integration is taken over the charge cycle, helix at $\alpha = 0$ through coil at $\alpha = 1$ to coil at $\alpha = 0$. The free energy change was obtained by graphical integration and listed in table 1 in the case that $C_s = 0.005$ M. This quantity coincides with that obtained by Nagasawa and Holtzer in 0.005 M NaCl [13], and the dependence on C_p is not observed. It is reasonable that the transition is independent on C_p at the uncharged state because in this state the long-range electrostatic monopole–monopole interaction disappears.

Circular dichroism at 222 nm, which is characteristic of helical polypeptides [14], was measured on poly(Glu) in the same range of C_s and C_p as in the pH titration and shown in fig. 5. Circular dichroism is scarcely dependent on C_p in 0.05 M KCl but dependent in 0.005 M KCl, in the same manner as that in pH titration. The standard free energy change $\Delta G_0^\circ/N$, which is defined in eq. (1), is also determined from the following integration [13]:

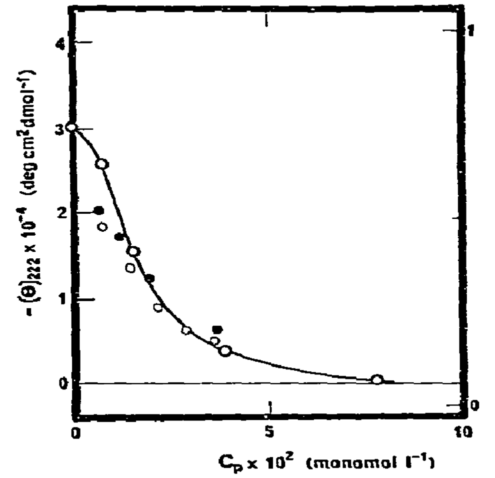

Fig. 6. Circular dichroism of poly(Glu) in 0.005 M KCl at pH of 5.65. Small open circles are directly measured and small filled circles are interpolated from the data in fig. 5. Large open circles are helical contents calculated with $\Delta G_0^\circ/N$ of 277 cal/mol and σ of 2.6 × 10^{-3}.

$$\Delta G_0^\circ/N=(RT\ln 10)\int_{-\infty}^{\mathrm{pH_m}}(\alpha_c-\alpha_h)\mathrm{dpH}\,, \qquad (2)$$

where $\mathrm{pH_m}$ is pH at the midpoint of the transition, and α_h and α_c are the degrees of ionization of the γ-carboxyls in the helix and the random coil section, respectively. The free energy change was again obtained with $\mathrm{pH_m}$ determined from the circular dichroic measurement and summarized in table 1. To demonstrate clearly the C_p dependence of the transition, the circular dichroism was measured at constant pH and is shown in fig. 6.

According to the theory of Zimm and Rice [5], the equilibrium constant s, which has the same meaning as s_0 at any pH, can be evaluated from titration data as follows:

$$\log s=\log s_0+\log s'$$

$$=\log s_0+\int_{-\infty}^{\mathrm{pH}}(\alpha_h-\alpha_c)\mathrm{dpH}\,. \qquad (3)$$

The parameter s' indicates the contribution to s only from the electrostatic interactions. The fraction f_h of high molecular weight polypeptide is given by the following equation [15]:

$$f_h=\frac{1}{2}\left(1+\frac{s-1}{\sqrt{(s-1)^2+4\sigma s}}\right), \qquad (4)$$

where σ is the initiation parameter characterizing the initiation of a helical section. The circular dichroic data and f_h's from the titration were replotted against $\log s'$ and compared with f_h calculated theoretically from eq. (4) and are shown in figs. 7 and 8. They seem to be independent on C_p as far as they are plotted against $\log s'$. Reproducible C_p dependence of the parameter σ [17] was not observed within the range of C_p employed in the present study, and observed values of σ coincided with those obtained at higher C_s [16,18], as shown in table 1.

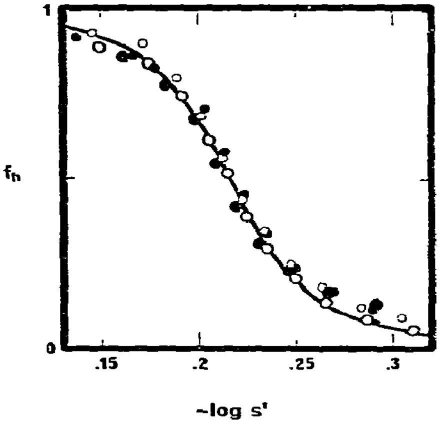

Fig. 7. Comparison of theoretical (solid line, calculated from eq. (4); σ = 2.6 × 10^{-3}, $\Delta G_0^\circ/N$ = 297 cal/mol) and experimental f_h of poly(Glu) in 0.005 M KCl determined from pH titration. Polymer concentrations are the same as cited in fig. 2.

4. Discussion

The pH value where the helix–coil transition occurs decreases as C_p increases, and the C_p dependence of the helix–coil transition is considered to be a result only from the electrostatic interactions, as shown in figs. 3–8. This phenomenon can be interpreted with fig. 9 as follows. A section of a charged polypeptide

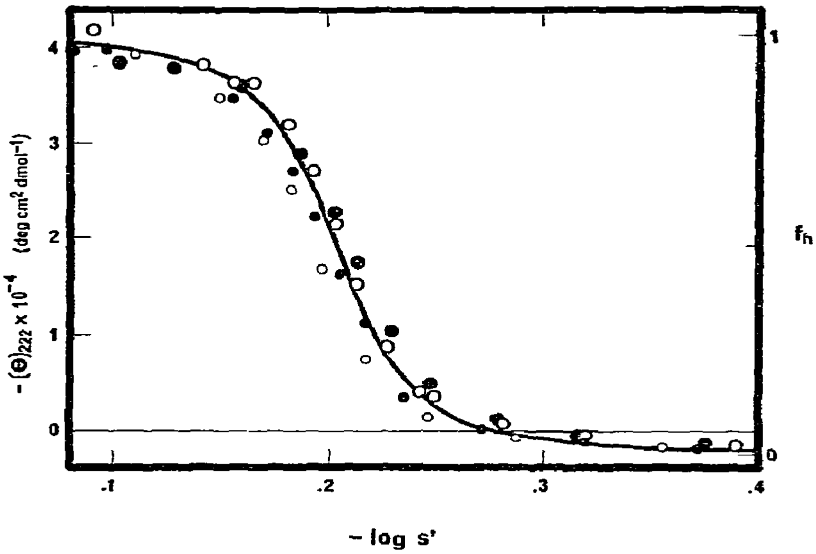

Fig. 8. Comparison of theoretical f_h (solid line, calculated from eq. (4); $\sigma = 2.6 \times 10^{-3}$, $\Delta G_0^\circ/N = 277$ cal/mol) and circular dichroism of poly(Glu) at 222 nm in 0.005 M KCl. Polymer concentrations are the same as cited in fig. 2.

at an arbitrary pH is either in helical conformation with the degree of ionization of side chain groups of α_h or in coiled conformation with α_c [4,5], and the relation between α_h and α_c is:

$$\alpha_h < \alpha_c \,, \tag{5}$$

due to *intramolecular* electrostatic interaction. As C_p increases especially to higher value than C_s, these degrees of ionization increase because of the *intermolecular* electrostatic interaction. As shown in fig. 9, the relation of the increase of α is:

$$\alpha_h^h - \alpha_h^l \leqslant \alpha_c^h - \alpha_c^l \,, \tag{6}$$

where superscript l denotes low C_p and h denotes high C_p. The inequality (6) can be accepted from inequality (5) because in "pure" coiled conformation the *ionizable* groups are surrounded by more *ionized* groups of the other polymer molecules than those in "pure" helical conformation. In fact the inequality (6) holds up to such a high pH value that α_c can scarcely increase ($\alpha_c \geqslant 0.9$). Consequently,

$$\int_{-\infty}^{\text{pH}} (\alpha_h^h - \alpha_c^h)\text{dpH} \leqslant \int_{-\infty}^{\text{pH}} (\alpha_h^l - \alpha_c^l)\text{dpH} \,, \tag{7}$$

and from eq. (3),

$$\log s^h \leqslant \log s^l \,. \tag{8}$$

Namely, at the higher C_p helical conformation is less stable at the condition that $C_p \leqslant C_s$ as far as the pH and C_s are common.

The values of $\Delta G_0^\circ/N$ near 300 cal/mol in 0.005 M KCl obtained in the present study are considerably higher than those of 130–200 cal/mol obtained at higher C_s [12,18–21]. However, Nagasawa and Holtzer have obtained a similar value to that in the present study in 0.005 M NaCl [13], and more recently Rinaudo and Domardo have reported a higher value of 390 cal/mol in salt-free aqueous solution at 25°C [11]. This dependence of $\Delta G_0^\circ/N$ can hardly be interpreted with ill chosen pK_0. To obtain the similar value of $\Delta G_0^\circ/N$ to those at higher C_s, one would assume abnormal pK which would be higher than 5.5 for γ-carboxyl groups of glutamyl residues in salt-free solution. One may accept the C_s dependence as an experimental result, as far as one accepts the Katchalsky–Gillis equation (eq. (13) of ref. [22]) and eq. (1). A part of the integration process of eq. (1) involves discharging from coil at $\alpha = 1$ to coil at $\alpha = 0$. This hypothetical process is only the discharging pro-

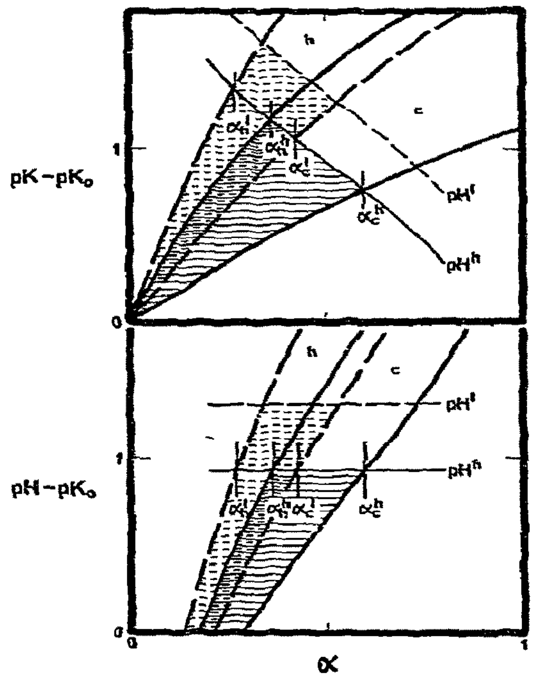

Fig. 9. Representative titration curves for "pure" helix (h) and "pure" coil (c), calculated with parameters cited in fig. 1 at the condition that C_s = 0.005 M. Heavy dashed lines: $C_p < C_s$, and heavy solid lines: C_p = 0.0775 monomol/ℓ. Superscript h denotes high C_p and l denotes low C_p. The values of pH^l and pH^h give the same value of log s'. See discussion.

cess and the final product should be considered to maintain expanded conformation of highly charged polymer, and the configuration is considerably different from those at different C_s. That is to say, the coil at $\alpha = 0$ is different from each other at least in configurational entropy. One of the causes of the C_s dependence may be due to the above mentioned configurational entropy, in addition to that discussed by Olander and Holtzer [12].

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