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DEPENDENCE ON IONIC STRENGTH OF CHARGE-INDUCED COIL-HELIX TRANSITION OF SODIUM POLY(L-GLUTAMATE) IN AQUEOUS SOLUTION

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Helix contents of sodium poly(L-glutamate) in aqueous NaCl solutions were estimated as functions of the degree of ionization, α, and the salt concentration by CD measurement. The helix content increases with increasing salt concentration but this helix stabilization decreases with decreasing α and at $\alpha < 0.25$ the helix conformation is destabilized by salt addition. The α dependence of the helix stabilization was qualitatively interpreted by Manning's theory in which electrostatic interactions between charges on alternatively arrayed coil and helix segments in a simplified polymer model were incorporated.

1. Introduction

While the helix-coil transition of charged polypeptides has been investigated for the charge-, temperature-, or solvent-induced transition [1–8], less attention has been paid to the dependence on ionic strength. Recently, however, the counterion condensation theory developed by Manning [9] has been applied to synthetic polyelectrolytes to elucidate the colligative properties [10–13] as well as to biological polyelectrolytes to analyze the ionic strength dependence of the conformational transition [14,15]. For example, Record et al. [16–19] have successfully analyzed the salt concentration dependence of the melting temperature of DNA. The Poisson-Boltzmann school [20–23], however, is dubious about Manning's model of the counterion distribution around a linear polyelectrolyte. On the other hand, efforts have been made to bridge the gap between Manning's idealized polymer model, i.e., an infinitely long linear and regular array of univalent point charges, and real polyelectrolytes [24,25]. These theoretical studies have revealed that in the limit of $\kappa \to 0$ (κ, Debye parameter), a helical array of fixed charges reduces to Manning's model but real short chain polymers cannot be approximated by the infinitely long charge array.

The helix-coil transition of poly(L-glutamic acid) (PLG) in aqueous salt solution should also be treated by taking into account the above limitation. Because of the relatively short cooperative unit ($\simeq 30$ residues) in the helix-coil transition of PLG, a special consideration of the electrostatic interaction between charges on adjacent helix and coil segments is required in the analysis of the ionic strength dependence of the helix content.

This paper describes the helix stability of sodium poly(L-glutamate) (PLGNa) in aqueous NaCl in a relatively low salt concentration region (10^{-3}–10^{-1} M) and gives a qualitative explanation for the experimental results with a polymer model which takes account of the electrostatic interactions between charges on relatively short coil and helical segments.

2. Theoretical background

Here we outline Manning's theory and its application to the helix-coil transition of DNA [26].

Suppose a linear polyion is dissolved in a solvent containing a simple salt under the condition $I \gg C_p$,

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where I is the ionic strength and C_p the equivalent concentration of the polyion. A parameter proportional to the axial charge density of the linear polyion, ξ, is defined as

$$\xi = e^2/DkTb \tag{1}$$

where e is the protonic charge, D the bulk dielectric constant of solvent, k the Boltzmann constant, T the Kelvin temperature and b the average axial charge spacing. When the counterion is monovalent, the degree of condensation, λ, is expressed as

$$\lambda = 1 - \xi^{-1} \tag{2}$$

Since b_c in the coil conformation is larger than b_h in the helix, ξ_c is smaller than ξ_h, and hence the coil-to-helix transition involves a further counterion condensation onto the chain. Taking this into account, Record et al. [19] have treated the helix-coil transition of DNA as follows;

$$\mathrm{COIL} + \Delta n \mathrm{M}^{+} \overset{K}{\rightleftharpoons} \mathrm{HELIX} \tag{3}$$

where COIL and HELIX represent cooperative units in coil and helix conformations, respectively, Δn is the difference in the stoichiometric amounts of counterion condensed on cooperative units in coil and helix conformations. and M^+ is a monovalent counterion.

From eq. (2),

$$\Delta n = N_u(\xi_c^{-1} - \xi_h^{-1}) \tag{4}$$

where N_u is the number of monomer residues contained in a cooperative unit. The equilibrium constant of the coil-helix transition observed, K_{obs}, and K in the equilibrium (3) can be related as

$$\ln K_{obs} = \ln K + \ln \gamma_c/\gamma_h + N_u(\xi_c^{-1} - \xi_h^{-}) \ln a_{+} \tag{5}$$

Manning expressed the activity coefficient γ of a cooperative unit as

$$\ln \gamma = -N_u \xi^{-1} \ln(1 - e^{-\kappa b}) \tag{6}$$

From eqs. (5) and (6), the following equation can be derived.

$$\mathrm{d} \ln K_{obs}/\mathrm{d} \ln C_s = N_u(\xi_c^{-1} - \xi_h^{-})/2 \tag{7}$$

where it is assumed that in the limit a_+ (activity of the counterion)→0. $a_+ \simeq C_s$ (concentration of added salt) and $\ln(1-e^{-\kappa b}) \simeq \ln \kappa b$. It is to be noted that eq. (7) is applicable only to fully neutralized DNA or PLG. In the case of half-neutralization, eq. (7) is modified as eq. (8) by considering the difference in the degrees of ionization, α_c and α_h in coil and helix conformations, respectively [17].

$$\begin{aligned} \mathrm{d} \ln(\theta/1-\theta)/\ln C_s &= N_u\{(\xi_c^{-1} - \xi_h^{-1})/2 + \alpha_h - \alpha_c\} \\ &\quad \text{for } \alpha_c\xi_c > 1,\ \alpha_h\xi_h > 1 \\ &= N_u(\alpha_h - \xi_h^{-1}/2 - \alpha_c^2\xi_c/2) \\ &\quad \text{for } \alpha_c\xi_c \leqslant 1,\ \alpha_h\xi_h > 1 \\ &= N_u(\alpha_h^2\xi_h - \alpha_c^2\xi_c)/2 \\ &\quad \text{for } \alpha_c\xi_c \leqslant 1,\ \alpha_h\xi_h \leqslant 1 \end{aligned} \tag{8}$$

where θ is helix content. The results obtained in this study are discussed in terms of the plot of $\ln(\theta/1-\theta)$ versus $\ln C_s$. Eq. (8) means that the slope estimated at certain θ gives the free energy change of a cooperative unit having the length at $\theta = 0.5$.

3. Experimental

3.1. Materials

PLGNa used was kindly supplied by Kyowa Hakko Co. The molecular weight was determined as 8.4×10^4 by viscometry. The polymer was ultrafiltrated through an Ultra Filter UP 20 (Toyo Roshi) to eliminate low molecular weight substances, and then collected by freeze-drying.

A stock solution for CD and pH measurements was prepared by dissolving the polymer in water at a concentration determined by the weight, 1.776×10^{-2} basemol/l. The degree of neutralization of PLGNa was adjusted by adding appropriate aliquots of 0.1 N HCl. Deionized and afterward distilled water was used throughout the experiments.

3.2. Apparatus and methods

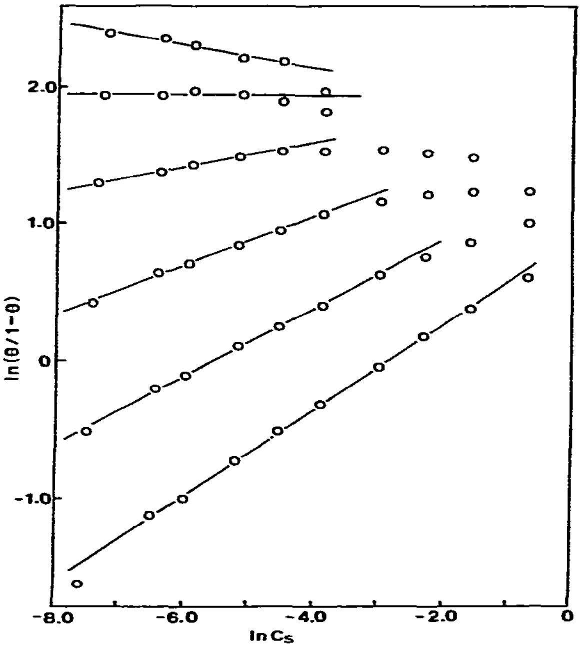
CD measurements were performed at 25.0±0.05°C with a Jasco J 20 automatic recording spectropolarimeter. The helix content, θ, was determined according to the relationship, $\theta = -[\theta]_{222}/40000$, where $[\theta]_{222}$ is the molar ellipticity at 222 nm.

Polymer solution for CD measurement was prepared as follows: 5.00 ml of a solution of PLGNa having the desired degree of neutralization and 5.00 ml of aqueous salt solution were mixed. In the preparation, careful pipetting of the polymer solution under stirring was essential for obtaining data with high reproducibility. The polymer concentration of the sample solution was 8.88×10^{-4} basemol/l.

The measurement of the helix content was performed for the PLG solutions under a constant degree of neutralization. The variation of the salt concentration gives rise to a deviation of the degree of ionization of PLG in the relevant solutions. Therefore, the helix content under the constant degree of ionization was estimated by reverting the degree of neutralization to the degree of ionization from the pH measurements of the relevant solutions. The plots of $\ln(\theta/1-\theta)$ versus $\ln C_s$ were made for PLG under constant degrees of ionization.

4. Experimental results

Fig. 1 shows the plots of $\ln(\theta/1-\theta)$ versus $\ln C_s$ for PLGNa in aqueous NaCl at 25°C. $\ln(\theta/1-\theta)$ changes linearly with $\ln C_s$, although deviations from the linear relations are seen in the higher C_s region, especially for PLGNa having small α. Under such high salt concentration, so called 'specific' interactions between the polymer and the salt-constituting ions may come into work. We exclude, therefore, the deviation from the present analysis, which should be applied to the relevant observations under sufficiently low salt concentration. Similar plots have been obtained for other systems, i.e., alkali and alkaline earth metal chlorides, results for which are to be reported in a subsequent paper [26]. The slopes estimated from the linear parts, given in the second column of table 1, decrease to a negative value with decreasing α. The third column of table 1 shows the slopes calculated by eq. (8) with parameters obtained by other experiments; α_c and α_h from the potentiometric titration curve shown in fig. 2 and N_u by the relation $N_u = \sigma^{-1/2}$, where σ, the cooperativity parameter, was estimated from viscometric data [27]. It is found in table 1 that eq. (8) does not explain the decreasing trend of the slope with decreasing α, and seems to be extrapolated to a positive slope at $\alpha = 0.2$. Such a failure of eq. (8) in explaining the α dependence of the slope seems to stem from the short cooperativity unit ($N_u \approx 30$), since the formalism in section 2 has assumed an infinitely long linear polyion and hence has been successfully applied to DNA which has much longer cooperative units (N_u of the order of 10^2). For a cooperativity unit which is shorter than the Debye length, κ^{-1}, the polymer model used by Manning for estimation of the electrostatic interaction energy is



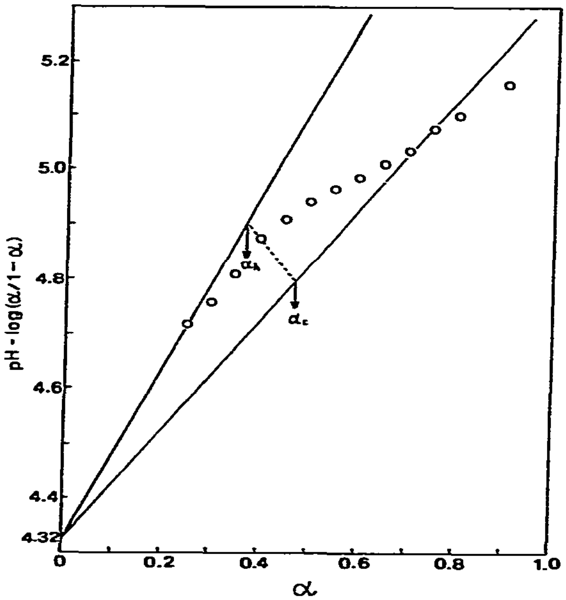


Fig. 1. Plot of $\ln(\theta/1-\theta)$ versus $\ln C_s$ for PLGNa in aqueous NaCl at 25°C: $\alpha=0.20, 0.25, 0.30, 0.35, 0.40, 0.45$ from top to bottom.

Fig. 2. Titration curve of PLGNa in aqueous NaCl: $C_s=0.1$ M, $C_p=3.49\times10^{-2}$ basemol/l.

Table 1

Values of $d\ln(\theta/1-\theta)/d\ln C_s$ experimentally obtained and calculated by eq. (8), and the data used for the calculation

α	$d\ln(\theta/1-\theta)/d\ln C_s$		θ[a]	α_c[b]	α_h[c]	$\sigma(\times10^3)$
	Expt.	Calc. by eq. (8)				
0.20	−0.09		0.91	0.25	0.195	
0.25	0.0	0.72	0.875	0.31	0.24	3.1[c]
0.30	0.09	0.94	0.785	0.36	0.28	2.4
0.35	0.17	1.4	0.625	0.40	0.32	1.9[c]
0.40	0.25	1.1	0.41	0.44	0.34	1.4
0.45	0.31	1.5	0.205	0.47	0.37	1.0
0.50	0.35	1.2	0.075	0.51	0.395	0.78

[a] Values at $C_s=10^{-3}$ M.

[b] Obtained from the pH titration curve of PLGNa using Nagasawa's method [3]: $\theta=(\alpha_c-)/(\alpha_c-\alpha_h)$.

[c] Obtained by assuming a smooth change of σ with α.

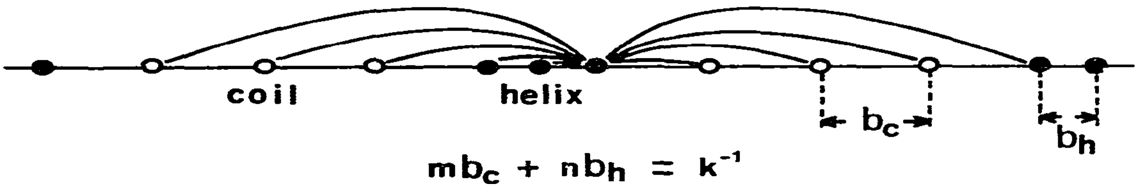


Fig. 3. Polymer and electrostatic interaction model for PLG in the transition region.

inadequate. Manning calculated the electrostatic interaction energy per charged group, G_p/RT, by the following equation [28,29],

$$G_p/RT = -\xi \sum_{j=1}^{\nu-1} (1-j/\nu) \exp(-\kappa jb)/j \simeq -\xi \sum_{j=1}^{\infty} \exp(-\kappa jb)/j = \xi \ln(1-e^{-\kappa b}) \tag{9}$$

where ν is the number of fixed charged groups on a polyion or on a cooperative unit. The use of infinity as ν means that the polyion is infinitely long. For 'a short cooperative unit', interactions between charges on different conformational segments must be considered. Thus, we adopt the following polymer model for PLG.

5. Polymer model

We replace a real PLG chain in the transition region by a linear wire composed of two alternately arrayed components, h and c, which are provided with discrete charges with different spacings, as shown in fig. 3. This linear polymer model may be applied for PLG even at low charge density because the chain becomes less flexible with decreasing α through the helix formation. We have reported that the Mark-Sakurada-Houwink parameter became higher than 1.0 for PLGNa in 0.1 M NaCl at $\alpha = 0.3$ [30].

For simplicity's sake, components c and h are supposed to have average numbers of charges, n, with constant spacings, respectively, although n has a distribution in a real PLG chain [31].

$$n_h = \alpha_h \sqrt{\theta/(1-\theta)\sigma} \qquad n_c = \alpha_c \sqrt{(1-\theta)/\theta\sigma} \tag{10}$$

b is calculated on the usually accepted poly(amino acid) dimension,

$$b_h = 1.5/\alpha_h \ \text{Å} \qquad b_c = 3.6/\alpha_c \ \text{Å} \tag{11}$$

6. Calculation of electrostatic free energy

We calculate the electrostatic free energy according to Manning's method; pair-wise summation of the Debye Hückel's screened coulomb potential. However, we cut off the summation at a conditioned distance, because a real PLG chain is not infinitely long and the linear model given in fig. 3 should be applied to relatively short segments in a polymer chain. We introduce a cut-off distance given by $l_{c.o.} \simeq \kappa^{-1}$ by assuming that electrostatic interactions beyond this distance are sufficiently screened and negligible in comparison with those within $l_{c.o.}$. In fact, the underestimation due to the employment of the cut-off is only 10% even in comparison with the estimation by eq. (9).

For example, in the case of PLGNa in the coil conformation for $\alpha = 1.0$ and $C_s = 10^{-3}$ M, G_p/RT with the cut-off is given by

$$G_p/RT = -\xi \sum_{j=1}^{n} \exp(-\kappa jb)/j, \tag{12}$$

where $n=27 \simeq 1/\kappa b$ ($\kappa = 1.04 \times 10^6$ cm^{-1}, $b = 3.6 \times 10^{-8}$ cm), as 6.13, while eq. (9) estimates it as 6.54. Similarly, for $C_s = 10^{-1}$ M, eqs. (12) and (9) give values of 2.05 and 2.30, respectively.

Pair-wise summation of electrostatic interaction based on the polymer model was carried out by dividing the calculation into the following items. $\Sigma_{cc}(\Sigma_{hh})$, electrostatic energy between charges on a coil (helix) segment; $\Sigma_{cc'}(\Sigma_{hh'})$, electrostatic energy between charges on different coil (helix) segments, and $\Sigma_{ch}(\Sigma_{hc})$, electrostatic energy between a charge on a coil segment and one on an adjacent helix segment. Details of these terms are given in the Appendix.

7. Discussion

The helix-coil transition in charged polypeptides was theoretically treated by Zimm and Rice [1], who, utilizing the grand partition function in the matrix form, considered only electrostatic interactions within a unit composed of four contiguous residues. As we intend to take account of the counterion condensation, electrostatic interaction within a longer distance must be considered, especially at a low salt concentration.

If cutting-off of electrostatic interaction at $l_{c.o.} = \kappa^{-1}$ were applied, a matrix of thousands of orders would be required to treat statistically the helix-coil transition of PLGNa, e.g., in 10^{-3} M NaCl. This is the reason we use the simple polymer model described above. We evaluate the dependence of the helix content on the salt concentration based on the polymer model constructed with data on PLGNa at an equilibrium state.

7.1. *Contribution of electrostatic interaction to* d ln(θ/1 − θ)/d ln C_s

As the electrostatic part in d ln($\theta/1-\theta$)/d ln C_s can be obtained as the difference of electrostatic energy of charges in helix and coil segments, it is expressed with Σ terms as

$$\{\mathrm{d}\ln(\theta/1-\theta)/\mathrm{d}\ln C_s\}_{el} = \{\alpha_c^2\xi_c(\Delta\Sigma_{cc} + b_c n_c^{-1}\Delta\Sigma_{cc'} + b_c n_c^{-1}\Delta\Sigma) \\ - \alpha_h\xi_h(\Delta\Sigma_{hh} + b_h n_h^{-1}\Delta\Sigma_{hh'} + b_h n_h^{-1}\Delta\Sigma_{hc})\} N_u/\Delta\ln C_s \tag{13}$$

where $\Delta\Sigma/\Delta \ln C_s$ is the average change of Σ value per unit ln C_s change at C_s. It is to be noted that eq. (13) corresponds to d ln($\theta/1-\theta$)/d ln C_s for α with which counterion condensation does not take place. The calculated values of $\{\mathrm{d}\ln(\theta/1-\theta)/\mathrm{d}\ln C_s\}_{el}$ with eq. (13) are shown for $C_s = 10^{-3}$ M in table 2. It is seen that eq. (13) gives negative values having an increasing trend with decreasing α, while the third equation of eq. (8) for the condition of an uncondensed counterion predicts positive values having a decreasing trend with decreasing α. This contrast comes from the difference in the polymer models on which the two equations are based. Namely, eq. (9) predicts that the electrostatic energy of charges on an isolated helix chain decreases more rapidly than that on a coil with increasing ionic strength, which results in the positive values for the slope given by eq. (8), i.e., stabilization of helix. However, in the case of the polymer model shown in fig. 3, the electrostatic energy of charges on a helix segment decreases less rapidly than that on an isolated helix because of the interaction with charges on coil segments. Similarly, the electrostatic energy of charges on a coil segment decreases more rapidly than that on an isolated coil because of the interaction with charges on helix segments. Such electrostatic interactions between charges on helix and coil give negative values for the electrostatic part in the slope through eq. (13).

Eq. (13) is compared with the experimental value of d ln($\theta/1-\theta$)/d ln C_s at α where counterion

Table 2

Values of $\{d \ln(\theta/1-\theta)/d \ln C_s\}_{el}$ calculated by eqs. (13) and (8), and $\Delta\Sigma/\Delta \ln C_s$ terms [a]

α	$\{d \ln(\theta/1-\theta)/d \ln C_s\}_{el}$		$-\frac{\Delta\Sigma_{cc}}{\Delta \ln C_s}$	$-\frac{b_c\Delta\Sigma_{cc'}}{n_c\Delta \ln C_s}$	$-\frac{\Delta\Sigma_{hh}}{\Delta \ln C_s}$	$-\frac{b_h\Delta\Sigma_{hh'}}{n_h\Delta \ln C_s}$	$-\frac{\alpha_h\xi_h b_h\Delta\Sigma_{hc}}{\Delta \ln C_s}$
	eq. (13)	eq. (8)					
0.25	−0.30	0.76	0.027	0.161	0.137	0.252	0.648
0.30	−0.36	1.2	0.066	0.205	0.113	0.230	1.188
0.35	−0.79	2.0	0.111	0.183	0.093	0.128	1.940
0.40	−1.4	2.3	0.210	0.142	0.062	0.009	2.457
0.45	−1.3	3.4	0.333	0.087	0.048	0	2.436

[a] The other data used for the calculation are shown in table 1.

condensation does not take place. Below such a very low α, if we use 0.2 for the α as expected from eq. (2), both the experimental and calculated slopes seem to be negative: the latter appears to give a negative value at $\alpha = 0.2$ judged from the changing trend with decreasing α (the missing value at $\alpha = 0.2$ in table 2 is due to the lack of data of σ and increasing uncertainty of the polymer model in this study with decreasing the charge density because the error coming from the employment of an integer for charge number n becomes significant at such a small n as 1 or 2).

7.2. *Estimation of the degree of counterion condensation*

In the above discussion, we estimated the contribution of electrostatic interaction to the slope, $d \ln(\theta/1-\theta)/d \ln C_s$, for PLGNa with uncondensed counterion. The degrees of counterion condensation, which explain the results within the framework of this study, can be estimated by solving the following equation,

$$\{d \ln(\theta/1-\theta)/d \ln C_s\}_{exp} = N_u\Big[\alpha_h\xi_h + \alpha_c^2\xi_c\{\Delta\Sigma_{cc} + b_c n_c^{-1}\,\Delta\Sigma_{cc'} + b_c n_c^{-1}(1-\lambda_h)\Delta\Sigma_{hc}\}/\Delta \ln C_s$$
$$-\alpha_h^2\xi_h\{(1-\lambda_h)^2\,\Delta\Sigma_{hh} + b_h n_h^{-1}(1-\lambda_h)^2\,\Delta\Sigma_{hh'} + b_h n_h^{-1}(1-\lambda_h)\Delta\Sigma_{hc}\}/\Delta \ln C_s\Big] \qquad (14)$$

where λ_h is the degree of counterion condensation on helical PLGNa and it is assumed that that on coil, $\lambda_c = 0$ below $\alpha = 0.45$. The values of λ_h thus calculated are shown together with those by Manning theory, $\lambda_h = 1 - \alpha^{-1}\xi^{-1}$, in table 3. The former show a reasonable trend that changes with α, being similar to the latter, because the degree of counterion condensation will decrease with decreasing the charge density of the polyion. The smaller value, especially at $\alpha = 0.45$, than the latter may be caused by the fact that the mean length of helix segments decreases with increasing α, deviating from the infinitely long polymer model on which the latter is based.

Table 3

Values of λ_h estimated by eq. (14) and Manning s theory

α	λ_h from eq. (14)	$\lambda_h = 1 - \alpha_h^{-1}\xi_h^{-1}$
0.25	0.13	0.13
0.30	0.18	0.24
0.35	0.24	0.34
0.40	0.28	0.38
0.45	0.25	0.43

In summary, a qualitative interpretation of the salt concentration dependence of the helix content of PLGNa in aqueous NaCl was made by taking account of electrostatic interaction between charges on conformationally different segments. Manning's theory, if the limited length of helical and random coil segments of PLGNa are taken into consideration, predicts the decreasing slope of $\ln(\theta/1-\theta)$ versus $\ln C_s$ plot with decreasing α.

Appendix

The Σ terms were reckoned by the following expressions.

$$(1)\ \Sigma_{cc}(\Sigma_{hh}) = \sum_{j=1}^{n-1} (1-j/n)\exp(-\kappa jb)/j \quad \text{when } (n-1)b \leqslant \kappa^{-1}$$

$$= \left\{(n-n')\sum_{j=1}^{n'} \exp(-\kappa jb)/j + \sum_{n''=1}^{n'-1}\sum_{j=1}^{n''} \exp(-\kappa jb)/j\right\}/n \quad \text{when } (n-1)b > \kappa^{-1}$$

where n is the number of charged groups on a coil or a helix segment and n' is an integer which satisfies $n'b \simeq \kappa^{-1}$.

$$(2)\ \Sigma_{cc'} = \sum_{p=2}^{w} (p-1)\exp[-\kappa\{(n_h-1)b_h+pb_c\}]/\{(n_h-1)b_h+pb_c\}$$

$$\text{when } (n_h-1)b_h+2b_c \leqslant \kappa^{-1},\ \text{where } w \simeq \{\kappa^{-1}-(n_h-1)b_h\}/b_c$$

$$(2')\ \Sigma_{hh'} = \sum_{q=0}^{z} (q+1)\exp[-\kappa\{(n_c+1)b_c+qb_h\}]/\{(n_c+1)b_c+qb_h\}$$

$$\text{when } (n_c+1)b_c \leqslant \kappa^{-1},\ \text{where } z \simeq \{\kappa^{-1}-(n_c+1)b_c\}/b_h$$

$$(3)\ \Sigma_{hc} = \sum_{m=0}^{x}\sum_{m'=1}^{y} \exp\{-\kappa(mb_h+m'b_c)\}/(mb_h+m'b_c)$$

$$\text{where } x = n_h-1,\ \text{when } (n_h-1)b_h+b_c \leqslant \kappa^{-1}$$

$$\simeq (\kappa^{-1}-b_c)/b_h,\ \text{when } (n_h-1)b_h+b_c > \kappa^{-1}$$

$$y = n_c,\ \text{when } mb_h+n_cb_c \leqslant \kappa^{-1}$$

$$\simeq (\kappa^{-1}-mb_h)/b_c,\ \text{when } mb_h+n_cb_c > \kappa^{-1}$$

The values of $\Delta\Sigma/\Delta \ln C_s$ terms are summarized in table 2.

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