Molecular Theory of the Helix–Coil Transition in Polyamino Acids. III. Evaluation and Analysis of s and σ for Polyglycine and Poly-l-alanine in Water

3 AUTHORS, INCLUDING:

Mitiko Go
Cornell University
112 PUBLICATIONS **2,827** CITATIONS

Nobuhiro Go
Japan Atomic Energy Agency
238 PUBLICATIONS **9,966** CITATIONS

Molecular Theory of the Helix–Coil Transition in Polyamino Acids. III. Evaluation and Analysis of s and for Polyglycine and Polylalanine in Water

Mitiko G, Nobuhiro G, and Harold A. Scheraga
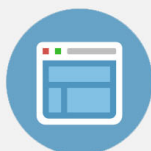
[8] J. T. Miller and D. E. Irish, Can. J. Chem. **45**, 147 (1967).

[9] D. L. Nelson, Ph.D. thesis, University of Waterloo, Waterloo, Ontario, 1969.

[10] S. A. Al-Baldawi, M. H. Brooker, T. E. Gough, and D. E. Irish, Can. J. Chem. **48**, 1202 (1970).

[11] F. J. C. Rossotti and H. Rossotti, *The Determination of Stability Constants* (McGraw-Hill, New York, 1961).

[12] M. M. Jones, *Elementary Coordination Chemistry* (Prentice-Hall, Englewood Cliffs, N.J., 1964).

[13] R. Bane and P. Peterson (private communication).

[14] A. I. Vogel, *Textbook of Quantitative Inorganic Analysis* (Longmans Green, London, 1951), 2nd ed.

[15] D. E. Irish and H. Chen, J. Phys. Chem. **74**, 3796 (1970).

[16] G. J. Janz, K. Balasubrahmanyam, and B. G. Oliver, J. Chem. Phys. **51**, 5723 (1969).

[17] S. S. Krishnamurthy and S. Soundararajan, J. Inorg. Nucl. Chem. **28**, 1689 (1966).

[18] S. K. Ramalingam and S. Soundararajan, J. Inorg. Nucl. Chem. **29**, 1763 (1967).

[19] N. Bjerrum, *Selected Papers* (Einar Munksgaard, Copenhagen, 1949), p. 279.

[20] M. T. Beck, *Chemistry of Complex Equilibria*, translated by R. A. Chalmers (Van Nostrand, London, 1970), p. 32.

[21] H. Irving and H. S. Rossotti, J. Chem. Soc. **1953**, 3397.

[22] R. Garnsey and D. W. Ebdon, J. Am. Chem. Soc. **91**, 50 (1969).

[23] J. R. Ferraro, C. Cristallini, and I. Fox, J. Inorg. Nucl. Chem. **29**, 139 (1967).

[24] D. E. Irish, B. McCarroll, and T. F. Young, J. Chem. Phys. **39**, 3436 (1963).

[25] R. B. Ellis, J. Electrochem. Soc. **113**, 485 (1966).

[26] J. H. R. Clarke and C. Solomons, J. Chem. Phys. **47**, 1823 (1967).

[27] H. Brintzinger and R. E. Hester, Inorg. Chem. **5**, 980 (1966).

[28] R. E. Hester, and W. E. L. Grossman, Inorg. Chem. **5**, 1308 (1966).

Molecular Theory of the Helix–Coil Transition in Polyamino Acids. III. Evaluation and Analysis of s and σ for Polyglycine and Poly-L-alanine in Water[*],[†]

Mitiko Gō, Nobuhiro Gō,[‡] and Harold A. Scheraga[§]

Department of Chemistry, Cornell University, Ithaca, New York 14850

(Received 27 October 1970)

Previously, the Zimm–Bragg parameters s and σ for the helix–coil transition in polyglycine and poly-L-alanine were calculated in terms of molecular quantities. These calculations are extended here to take into account the effects of water as the solvent and are analyzed to deduce the relative importance of the various interaction terms for the helix–coil transition. The screening effect of the electrostatic interactions by the water molecules which lie between or near two charged atoms is taken into account by using a dielectric constant $D = 4.0$ for short-range interactions, and by cutting off all interactions of longer range than about 6.0 Å. The calculated values of the parameter σ, which are very sensitive to the value of D, agree well with experimental ones. The origin of the greater stability of the α-helical conformation of poly-L-alanine compared to that of polyglycine is analyzed. The effect on the value of the parameter s, arising from the binding of water molecules to free CO and NH groups of residues in the coil state, is considered. Parameters which express the strength of the binding of water molecules are adjusted so as to make the calculated values of s for poly-L-alanine in water as a function of temperature fit best with experimental values. When the same parameters are used in the calculation of s for polyglycine in water, the theory predicts that polyglycine should be in the random coil form in water in the temperature range of 0–100°C, which agrees with experiment. The values obtained for these parameters are compared with experimental data for binding water molecules to a small-model peptide molecule. Finally, a simple but realistic model of the helix–coil transition, deduced from the calculation of s and σ in terms of molecular quantities, is proposed as a first approximation not only for homopolymers but also for co-polymers.

I. INTRODUCTION

In the first paper[1] of this series, we presented the formulation of a method to calculate (from molecular quantities) the parameters s and σ of the Zimm–Bragg[2] theory of the helix–coil transition in polyamino acids. In the second paper,[3] the formulation was amplified and improved, and was applied to the evaluation of s and σ for polyglycine and poly-L-alanine in vacuum (except for the use of a dielectric constant of $D = 4.0$ in some cases, and $D = 1.0$ in others, to account partially for the solvent effect). It was also shown[3] that the values of σ for the polyamino acids studied would not change much when solvent effects were taken into account explicitly in the calculation. Thus, the calculated values of σ in vacuum (but with $D = 1.0$ and

4.0, respectively) were compared with experimental data obtained for polyamino acids in solution, with fair agreement.

In the present paper, new definitions of the enthalpy and entropy of a residue in a helical and in a coil state, respectively, are introduced (in Sec. II) for the convenience of the later discussion, and then primarily four things are accomplished. First, in Sec. III, the long-range electrostatic interactions are cut off in order to take into account the screening effect by the water molecules which lie between or near two charged atoms. Better agreement between the calculated values of σ and the experimental ones is obtained by this cutting-off procedure. Second, in Secs. IV and VII, various contributions to the value of s are analyzed, and the origin of the greater stability of the α-helical conforma-
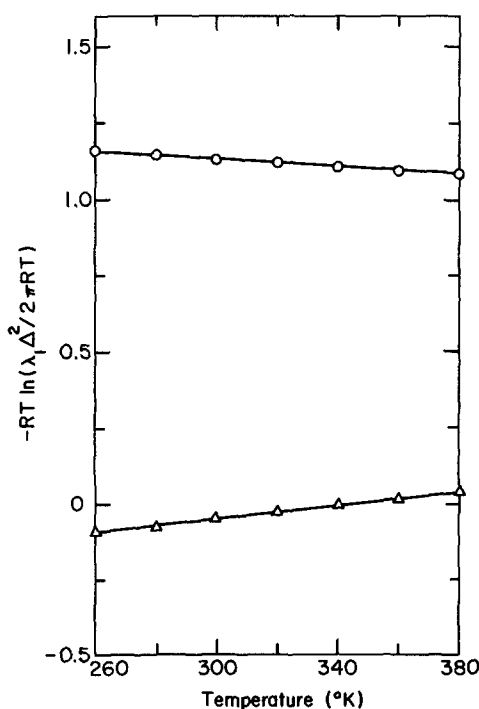
FIG. 1. Free energy of a residue in a coil state in vacuum, $-RT \ln(\lambda_1\Delta^2/2\pi RT)$ (with RT given in kilocalorie/mole) vs temperature for polyglycine (O) and poly-L-alanine (△) for parameter set A.

tion of poly-L-alanine compared to that of polyglycine is identified. Third, in Sec. V, the effect on the value of the parameter s, arising from the binding of water molecules to free CO and NH groups of residues in the coil state, is considered. The binding constants are treated as adjustable parameters to make the calculated values of s fit best with experimental ones. The values obtained for these parameters are compared with experimental data for binding water molecules to a small-model peptide molecule. Fourth, in Sec. VI, the results of the calculation of s and σ in terms of molecular quantities are summarized in the form of a simple model, which possesses the most essential features of the helix–coil transition, and is a realistic one not only for homopolymers but also for copolymers.

II. BREAKDOWN OF FREE ENERGY INTO ENTHALPY AND ENTROPY TERMS

The free energy, enthalpy, and entropy associated with the parameter s of Zimm and Bragg[2] are given by Eqs. (II.10), (II.13), and (II.14), respectively. In this section, new definitions of the free energies of a residue in a helical or coil state, respectively, will be introduced by rearranging terms in these equations. In later sections, these new definitions will be found to be convenient for the analysis of various contributions to the values of s and σ.

The curves of $-RT \ln s$ vs temperature T, shown in

Figs. 11–13 of Paper II for the range from 200 to 1000°K, deviate slightly from exact straight lines. The values of the enthalpy and entropy in Table VIII of Paper II were obtained from the tangential lines at the points where $s=1$. In this paper, however, we will restrict our discussion to the temperature range between 0 and 100°C because we are considering water as the solvent. Therefore, the values of $-RT \ln s$ were replotted against T on an expanded scale in this range and good straight lines were obtained. In order to understand this linearity, Eq. (II.10) [i.e., Eq. (10) of Paper II] is written in a slightly different way, viz.,

$$-RT \ln s^{(0)} = f + RT \ln g + RT \ln(\lambda_1\Delta^2/2\pi RT). \quad (1)$$

The superscript (0) on $s^{(0)}$ (and on $\Delta H_s^{(0)}$ and $\Delta S_s^{(0)}$ appearing later) indicates that the quantity is one for a polyamino acid in vacuum; the corresponding quantities for a polyamino acid in water will be expressed by symbols without the superscript (0). Equation (1) is obtained by transferring $2\pi RT$ from the second to the third term on the right-hand side of Eq. (II.10). While the resulting equation may now appear as if logarithms are taken of quantities which have physical dimensions, this difficulty is avoided by a proper choice of units for the energy scale. Since f and g, as defined in Eqs. (II.11) and (II.12), respectively, are independent of T, the linearity of Eq. (1) means that the last term is linear in T. This linearity is shown in Fig. 1 for parameter[4] set A; the same linearity, though not shown here, was obtained for parameter[4] sets B and C. Therefore, in the temperature range between 0 and 100°C, the free energy of a residue in a coil state in vacuum, can be expressed formally as

$$-RT \ln(\lambda_1\Delta^2/2\pi RT) = H_c^{(0)} - TS_c^{(0)}, \quad (2)$$

where the two constants $H_c^{(0)}$ and $S_c^{(0)}$ will be referred to as the enthalpy and entropy, respectively, of a residue in the coil state in vacuum. The values of these quantities, obtained from the slopes and intercepts of plots such as Fig. 1, are given in Table I; the following considerations provide insight into their physical significance. It can be seen that the entropy of a residue in the random coil state of polyglycine is larger than that of poly-L-alanine. A similar difference was reported by Brant et al.[5] This difference arises because the side-chain methyl group of poly-L-alanine restricts the chain to a much smaller region of conformational space than that available to polyglycine. When the approximation of Eq. (II.18) [i.e., the neglect of the term $B(\psi, \phi)$ defined by Eq. (I.17)] is used for $\lambda_1\Delta^2$, $H_c^{(0)}$ can be expressed as

$$H_c^{(0)} = \langle A \rangle - RT, \quad (3)$$

where $\langle A \rangle$ is the average value given by Eq. (II.20) of $A(\phi, \psi)$ defined by Eq. (I.16). If the function $A(\phi, \psi)$ could be approximated by a two-dimensional parabola around the global minimum point in the (ϕ, ψ) plane, then Eq. (3) would imply that $H_c^{(0)}$ is the value of

TABLE I. The enthalpy and entropy associated with s in vacuum between 0 and 100°C.

Parameter set	Polyamino acid	$H_c^{(0)}$ [a]	$S_c^{(0)}$ [a]	$H_h^{(0)}$	$S_h^{(0)}$	$\Delta H_s^{(0)}$	$\Delta S_s^{(0)}$
A	Gly[b]	1.30	0.55	−2.75	−7.80	−4.05	−8.35
	Ala(R)[b]	−0.38	−1.11	−5.76	−9.40	−5.38	−8.29
	Ala(L)[b]	−0.38	−1.11	−5.20	−7.90	−4.82	−6.79
B	Gly	1.88	0.59	−2.29	−8.08	−4.17	−8.67
	Ala(R)	0.51	−1.23	−4.65	−9.62	−5.16	−8.39
	Ala(L)	0.51	−1.23	−4.39	−8.34	−4.90	−7.11
C	Gly	12.73	−1.67	9.58	−7.64	−3.15	−5.97
	Ala(R)	11.62	−2.60	7.39	−9.46	−4.23	−6.86
	Ala(L)	11.62	−2.60	7.48	−7.81	−4.14	−5.21

[a] The units are kilocalorie/mole·residue and entropy units/residue for the enthalpy (H or ΔH) and entropy (S or ΔS), respectively.

[b] Here, and hereinafter, Gly refers to polyglycine, and Ala(R) and Ala(L) refer to the right- and left-handed α helices, respectively, of poly-L-alanine.

$A(\phi, \psi)$ at the global minimum and RT is the mean thermal excitation. However, the correct values of $H_c^{(0)}$ shown in Table I [i.e., those including the contribution from the term $B(\psi, \phi)$] are larger than the global minimum value of $A(\phi, \psi)$, not shown here, by an amount which is larger than the average value of $B(\psi, \phi)$; this means that $A(\phi, \psi)$ cannot be approximated by a two-dimensional parabola. Instead, in order to define and obtain $H_c^{(0)}$ and $S_c^{(0)}$, we have used the interesting *empirical* fact [arising because $2\pi RT$ was transferred to the last term in Eq. (1)] that $-RT \ln(\lambda_1 \Delta^2 / 2\pi RT)$ is linear in T in the temperature range between 0 and 100°C. Despite the nonapplicability of the parabolic approximation for $A(\phi, \psi)$, $H_c^{(0)}$ can be given the approximate physical interpretation implied by Eq. (3) and the above discussion.[6,7]

From Eq. (1), the enthalpy and entropy of a residue in a helical state in vacuum are defined by

$$H_h^{(0)} = f \tag{4}$$

and

$$S_h^{(0)} = -R \ln g, \tag{5}$$

respectively, so that the enthalpy and entropy associated with the parameter s are given by

$$\Delta H_s^{(0)} = H_h^{(0)} - H_c^{(0)} \tag{6}$$

and

$$\Delta S_s^{(0)} = S_h^{(0)} - S_c^{(0)}. \tag{7}$$

Though we defined the enthalpy and entropy of the coil and helical states by Eqs. (2), (4), and (5), their absolute values have no significance. They acquire meaning only when the differences in these parameters [as in Eqs. (6) and (7)] are considered. The values of $H_h^{(0)}$, $S_h^{(0)}$, $\Delta H_s^{(0)}$, and $\Delta S_s^{(0)}$ are given in Table I.

The free energy, enthalpy, and entropy associated with the parameter σ of Zimm and Bragg[2] are given by Eqs. (II.21), (II.24), (II.25), (II.27), and (II.28). Since the curve of $\ln C$ vs $1/T$ is fairly straight and passes through the origin, as shown in Fig. 6 of II,[8] the

TABLE II. The contributions[a,b] from the short- and long-range interactions to ΔH_σ and $\Delta H_{\sigma, \text{cut}}$.

Parameter set	Polyamino acid	Short-range interactions (1)	Short-range interactions (2)	Long-range interactions	ΔH_σ	$\Delta H_{\sigma, \text{cut}}$
A	Gly	1.52	−0.17	2.36	3.70	1.34
	Ala(R)	0.89	1.20	2.98	5.07	2.09
	Ala(L)	1.48	0.41	2.83	4.73	1.89
B	Gly	1.17	0.27	2.93	4.37	1.44
	Ala(R)	0.96	1.26	3.18	5.39	2.22
	Ala(L)	1.20	0.68	2.97	4.85	1.88
C	Gly	8.44	0.10	11.34	19.88	8.54
	Ala(R)	7.90	1.71	12.58	22.19	9.61
	Ala(L)	7.96	1.13	11.83	20.92	9.09

[a] Since these values were obtained for nonregular helices, the energy gain (E_{diff}) from the diffuseness at the ends of a helical sequence was classified into the three categories of interactions shown in this table, and these interactions were subtracted from the corresponding interactions for regular helices.

[b] Given in kilocalorie/mole of long helical sequence.

TABLE III. The contributions to ΔS_σ.

Parameter set	Polyamino acid	$R \ln g_0$ (eu)	$2(S_h^{(0)} - S_c^{(0)})$ (eu)	ΔS_σ (eu)
A	Gly	1.1	−16.7	−15.6
	Ala(R)	5.4	−16.6	−11.2
	Ala L)	0.9	−13.6	−12.7
B	Gly	3.4	−17.4	−14.0
	Ala(R)	6.5	−16.8	−10.3
	Ala(L)	3.3	−14.2	−10.9
C	Gly	−2.8	−12.0	−14.8
	Ala(R)	5.9	−13.7	−7.8
	Ala(L)	1.8	−10.4	−8.6

expressions cited above for the enthalpy and entropy associated with σ can be simplified as follows:

$$\Delta H_\sigma = f_0 - RT \ln C + 2(H_h^{(0)} - H_c^{(0)}), \qquad (8)$$

$$\Delta S_\sigma = R \ln g_0 + 2(S_h^{(0)} - S_c^{(0)}). \qquad (9)$$

The values of ΔH_σ and ΔS_σ for nonregular helices,[9a] which are obtained by Eqs. (8) and (9), respectively, are given in Tables II and III, respectively. The values of $\Delta H_s^{(0)}$, $\Delta S_s^{(0)}$ (in Table I), ΔH_σ, and ΔS_σ differ somewhat from those given in Tables VIII and IX of Paper II, because the values in the present paper were obtained in the temperature range between 0 and 100°C, while the values given in the tables in Paper II were obtained at the helix–coil transition temperatures in vacuum, which were much higher than room temperature, ranging from 212 to 528°C.

In the present paper, we are concerned with the calculation of s and σ for a polyamino acid in water. Various types of effects of solvent on the values of s and σ were discussed, and classified into three groups in Sec. II.F of Paper II, viz.: (1) specific interactions of one or more solvent molecules with the backbone NH and CO groups of the residues in *coil* sequences, (2) effect of the solvent molecules in which they are regarded as a bulk medium with a dielectric constant other than unity, and (3) additional interaction energies between atoms, or groups of atoms, in a polyamino acid, arising from the presence of nearby solvent molecules (e.g., hydrophobic bonding). Effects of type (1) are considered in Sec. V in the present paper. Effects of type (2) were already studied in Paper II by calculating s and σ for a dielectric constant of $D = 4.0$ as well as for $D = 1.0$. In the present paper, these effects are reconsidered by introducing a cutoff of the long-range electrostatic interactions. In Sec. VII, effects of type (3) in the systems under consideration (poly-glycine or poly-L-alanine in water) are shown to be small, compared to other effects. The major effects of water as a solvent on the values of s and σ for poly-glycine and poly-L-alanine come from the binding of water molecules or clusters of water molecules to

backbone NH and CO groups of the residues in *coil* sequences. Therefore, the value of s for a polyamino acid in water can be obtained from the value of $s^{(0)}$ for a polyamino acid in vacuum (except for the treatment of the dielectric constant) by considering the binding of water molecules, or, as was shown in Sec. II.F of Paper II, from the expression

$$s = s^{(0)} \exp(b/RT), \qquad (10)$$

where b is the free energy of binding.

We shall make the simplifying assumption that the binding constants K of water to NH and CO groups are the same. Hence, the free energy of binding is given by

$$b = -2RT \ln(1 + Ka), \qquad (11)$$

where a is the activity of water. Then Eq. (10) becomes

$$s = s^{(0)} (1 + Ka)^{-2}. \qquad (12)$$

For a dilute solution of a polymer, a is a constant, which is independent of the polymer concentration and has a value characteristic of pure water. Expressing the dimensionless quantity Ka as

$$Ka = \exp[-(\Delta H_B - T\Delta S_B)/RT], \qquad (13)$$

we shall refer to ΔH_B and ΔS_B as the enthalpy and entropy changes, respectively, due to water binding. If we assumed (as we do in Sec. V) that ΔH_B and ΔS_B are independent of temperature in the range between 0 and 100°C, then the enthalpy and entropy associated with the parameter s for a polyamino acid in water are given by

$$\Delta H_s = \Delta H_s^{(0)} - 2\Delta H_B Ka(1 + Ka)^{-1} \qquad (14)$$

and

$$\Delta S_s = \Delta S_s^{(0)} - 2R \ln(1 + Ka) - [2\Delta H_B T^{-1} Ka/(1 + Ka)].$$
$$\qquad (15)$$

It should be noted that, unlike $\Delta H_s^{(0)}$ and $\Delta S_s^{(0)}$, ΔH_s and ΔS_s are temperature dependent through the temperature dependence of Ka.

As was already shown in Sec. II.F of Paper II, the value of σ is not affected by a solvent effect of type (1), i.e., by the binding of water molecules to CO and NH groups. (This point will be rediscussed in Sec. VI from a slightly different point of view.) Therefore, Eqs. (8) and (9) hold both in the absence and presence of water binding. This is the reason why the superscript (0) was not introduced in discussing the enthalpy and entropy parameters associated with σ.

III. ANALYSIS OF VARIOUS CONTRIBUTIONS TO σ FOR POLYAMINO ACIDS IN VACUUM AND IN WATER

In this section, the various terms contributing to ΔH_σ and ΔS_σ are compared and analyzed. In Paper II, the value of σ was calculated for both regular and nonregular helices, the former treatment being an approxi-

mation to the latter, and the σ's were distinguished by subscripts, viz., as σ_{reg} and σ_{nonreg}. In this paper, however, values of σ for only nonregular helices are discussed, and hence no subscript is used.

We consider first the entropy. In Table III, the two terms on the right-hand side of Eq. (9) are compared. The second term is the same as twice the value of $\Delta S_s^{(0)}$, the latter being the entropy change when a residue is transferred from the coil state to the helical state at the end of a long helical section in vacuum. The first term, $R \ln g_0$, according to Eq. (II.23), accounts for the fact that a few residues at the ends of a long helical section are in different molecular environments and, therefore, have different ranges of fluctuations compared to residues in the middle of a helical section. From Table III, it appears that the second term is the dominant one, and therefore, that $\Delta S_\sigma \cong 2 \Delta S_s^{(0)}$, viz., the entropy associated with σ is roughly equal to twice the entropy change arising from the restriction of the dihedral angles ϕ and ψ when a residue is incorporated into a helical section from the coil state. This is only an approximate conclusion because the relative contribution of the first term, $R \ln g_0$, to ΔS_σ ranges from -76% to $+18\%$, which is not small. Nevertheless, the above statement provides a simple, rough picture about the value of the entropy associated with σ.

It should be mentioned here that the simple relation described above between ΔS_σ and $\Delta S_s^{(0)}$ does not hold between ΔS_σ and ΔS_s, because ΔS_s is affected by the solvent binding as shown by Eq. (15), while ΔS_σ is not.

In order to see which interactions are most important for determining the value of ΔS_σ, the terms of Eq. (9) are analyzed. The first term, $R \ln g_0$, is relatively small and of short range, as can be seen from the range of diffuseness at the ends of nonregular helices shown in Fig. 8 of II. In the second term, $S_c^{(0)}$ is, of course, determined by the short-range interactions which are accounted for in the calculation of the energy of the coil section (see Sec. III.C of Paper II). The entropy of the helix, $S_h^{(0)}$ is calculated from $\det \mathbf{A}_j$ by Eqs. (5) and (II.12), where the matrix elements of $\mathbf{A}_j$ are the second derivatives of the total energy of an α helix of j residues with respect to $\phi_1, \psi_1, \cdots, \phi_j, \psi_j$ [see Eq. (I.25)]. As discussed in Paper I, the matrix $\mathbf{A}_j$ exhibits a periodicity and "diagonal" character because of the helical structure. Since the elements of $\mathbf{A}_j$ are the second derivatives of the energy, it would be expected and indeed was borne out by the calculations that the hydrogen bond potential energy, which shows the sharpest change among the various energy terms for a small deviation of the conformation from the minimum point, contributes dominantly to the matrix elements of $\mathbf{A}_j$. Actually, from 80% to 90% of the value of the diagonal elements of $\mathbf{A}_j$ arises from the hydrogen bond energy, and the other contribution comes from the nonbonded interactions between pairs of atoms which belong to residues separated by zero to three inter-

mediate residues, and are very near each other in space because of the α-helical structure. In summary, ΔS_σ is determined mainly by short-range interactions in both the random coil and helical states; among those in the helical states, the hydrogen bond energy is the most important.

We turn next to a consideration of ΔH_σ. In Paper II, the influence of a type (2) solvent effect (see Sec. II.F of Paper II and Sec. II of this paper) on the value of σ was assessed by calculating σ for $D = 4.0$ as well as for $D = 1.0$. The values of ΔH_σ were found to be very dependent on the value of D, being very large for $D = 1.0$ and rather large for $D = 4.0$ but smaller than for $D = 1$. In this section, we examine the way in which ΔH_σ depends on the value of D, to see if a more realistic treatment, using a distance-dependent D, will give better values of σ.

For this purpose, we consider Eq. (8). The quantity f_0 is equal to $f_{0, \text{nonreg}}$ of Paper II, which is equal to $f_{0, \text{reg}} - E_{\text{diff}}$, where the values of $f_{0, \text{reg}}$ and E_{diff} (the energy decrease due to diffuseness at the ends of a helical sequence) are given in Tables V and VI of Paper II, respectively. By using Eqs. (4), (II.17), and (II.26), Eq. (8) can be written as

$$\Delta H_\sigma = \sum_{i=0}^{\infty} \left[E_i' - (i-2) E_i \right] - E_{\text{diff}} - RT \ln C - 2 H_c^{(0)}.$$

$$(16)$$

For $i \geq 1$, i.e., when E_i and E_i' do not refer to the self-energy, E_i' is essentially the same as E_i as was shown in Fig. 7 of II. Therefore, the contributions from the energies E_i and E_i' to ΔH_σ reduce essentially to the form of $-(i-3) E_i$ for $i \geq 1$. The values of E_i and its breakdown into its electrostatic, nonbonded, and other contributions are given in Table IV for the case of parameter set A. As stated in Sec. III.A of Paper II, the electrostatic and nonbonded interactions between the N and O, and H and O atoms were omitted from the energy function and, instead, the Lippincott–Schroeder function was used when these atoms form a hydrogen bond; when the Lippincott–Schroeder function is used, the sum of the interaction energies between the N, H, and O atoms is designated as the hydrogen bond energy in Table IV. The values of the other two terms, $RT \ln C$ and $2 H_c^{(0)}$, in Eq. (16) are given in Table IV[8] of Paper II and Table I of this paper (see also Fig. 6 of II). Because of the factor $(i-3)$, the energy term E_3 does not make any contribution to ΔH_σ. In particular, the energy of hydrogen bond formation, which is included in E_3, does not influence the value of ΔH_σ explicitly. Though E_i itself converges rapidly to zero as shown in Fig. 7 in II and Table IV, the contribution from long-range interactions to ΔH_σ is appreciable because of the factor $(i-3)$. The large positive values of $E_{1, \text{el}}$ arise from the electrostatic repulsion between the electric dipoles of the neighboring amide groups, which are located almost side by side to each other.

TABLE IV. The interaction energies between peptide groups.[a]

i	Gly				Ala(R)				Ala(L)			
	E_i [b]	$E_{i,el}$	$E_{i,nb}$	$E_{i,others}$	E_i [b]	$E_{i,el}$	$E_{i,nb}$	$E_{i,others}$	E_i [b]	$E_{i,el}$	$E_{i,nb}$	$E_{i,others}$
0	−0.61	0.0	−0.61		−1.74	0.0	−1.75	0.01	−1.14	0.0	−1.15	0.01
1	3.52	5.00	−2.04	0.56	2.88	5.00	−2.70	0.57	2.55	5.00	−3.01	0.56
2	−1.52	−0.04	−1.48		−1.66	−0.00	−1.66		−1.98	−0.04	−1.94	
3	−2.90	−0.13	−0.93	−1.84	−3.21	−0.09	−1.43	−1.69	−3.26	−0.12	−1.30	−1.84
4	−0.63	−0.31	−0.32		−1.35	−0.33	−1.02		−0.75	−0.31	−0.44	
5	−0.17	−0.13	−0.04		−0.23	−0.13	−0.10		−0.19	−0.13	−0.07	
6	−0.13	−0.12	−0.02		−0.15	−0.12	−0.03		−0.14	−0.12	−0.03	
7	−0.09	−0.08	−0.01		−0.11	−0.09	−0.02		−0.10	−0.08	−0.01	
8	−0.05	−0.05	−0.00		−0.06	−0.05	−0.01		−0.05	−0.05	−0.01	
9	−0.04	−0.03	−0.00		−0.04	−0.04	−0.00		−0.04	−0.03	−0.00	
10	−0.03	−0.03	−0.00		−0.03	−0.03	−0.00		−0.03	−0.03	−0.00	
15	−0.01	−0.01	−0.00		−0.01	−0.01	−0.00		−0.01	−0.01	−0.00	
20	−0.00	−0.00	−0.00		−0.00	−0.00	−0.00		−0.00	−0.00	−0.00	

[a] These energies (in kilocalorie/mole·residue) were obtained for parameter set A.
[b] E_i is the interaction energy between the peptide groups which are separated by $i-1$ intermediate groups (for $i=0$, it corresponds to the self-energy); it is the sum of the electrostatic interaction energy ($E_{i,el}$), the nonbonded interaction energy ($E_{i,nb}$) and the other interaction energies ($E_{i,others}$). The other interaction energies $E_{i,others}$ are the torsional energy of the side chain for $i=0$, the torsional energy around ϕ and ψ for $i=1$, and the hydrogen bond energy for $i=3$.

The important role of electrostatic interactions between amide groups in the α helix has been recognized by Arridge and Cannon.[9b] The small values of $E_{2,el}$ arise from the special geometrical arrangement that the next-nearest-neighbor amide groups have to each other. As the amide groups become more separated along the helix, their dipoles assume a head-to-tail geometrical arrangement, so that $E_{i,el}$ becomes negative for $i \geq 3$. When we take the factor $-(i-3)$ into account, we see from this behavior of $E_{i,el}$ that the contribution to ΔH_σ from the electrostatic interactions is always positive except for a very small contribution from $E_{2,el}$. The inverse proportionality of this positive contribution to D explains why ΔH_σ was large for $D=1.0$ (parameter set C) compared with the value for $D=4.0$ (parameter sets A and B).

From Table IV, one can also see that the interactions between peptide groups which are separated by more than four intermediate groups are primarily electrostatic. Hence, the contribution to ΔH_σ from long-range interactions are mainly electrostatic. In order to see the relative importance of the contributions to ΔH_σ from short- and long-range interactions, a breakdown of ΔH_σ according to the range of interactions is given in Table II. The interactions are classified into three types: (i) *the short-range interactions (1)*, including the self-energies, the nearest-neighbor interactions, the next-nearest-neighbor interactions in helices, and $RT \ln C$ from junctions and $H_c^{(0)}$ from coils; (ii) *the short-range interactions (2)*, including the intergroup interactions between groups separated by two or three intermediate groups; and (iii) *the long-range interactions*, including the intergroup interactions between groups separated by more than three intermediate groups. The enthalpy

of the coil state, $H_c^{(0)}$, defined by Eq. (2) includes interaction energies between atoms whose interatomic interaction energies (in the α-helical form) are included in E_0, E_1, or E_2, and therefore classified as consisting of *short-range interactions (1)*. *The short-range interactions (2)*, which include E_3, E_3', E_4, and E_4' in the case of regular helices, are of longer range *sequencewise* than *the short-range interactions (1)*. However, they both include interactions of more or less the same spacewise range, since the groups separated by two or three intermediate groups are located near each other in space because of the α-helical structure. Striking differences are observed between the values for sets A and B on the one hand and those for set C on the other for *the short-range interactions (1)* and *the long-range interactions*, respectively. This is because contributions from the nearest-neighbor dipole–dipole repulsion and long-range dipole–dipole attractions are included in *the short-range interactions (1)* and *the long-range interactions*, respectively.

So far, the solvent effect of type (2) has been accounted for by calculating s and σ for $D=4.0$ as well as for $D=1.0$. However, the use of a simple Coulomb law with a certain value of the dielectric constant D should be regarded as a purely empirical procedure, as was discussed in Sec. II.F of Paper II. In fact, as was also discussed there, the use of a distance-dependent value of D may be more realistic when the solvent is water. For example, even though the value of $D=4.0$ is not expected to be very far from reality for short-range electrostatic interactions, the electrostatic interaction between two (partial) charges separated by more than several angstrom units in water would be weakened appreciably by the presence of water molecules between

TABLE V. The values of σ and its entropic contribution in water.

Parameter set	Polyamino acid	σ		$(-T\Delta S_\sigma)/(-RT\ln\sigma)$	
		300°K	360°K	300°K	360°K
A	Gly	4.0×10^{-5}	5.9×10^{-5}	0.78	0.81
	Ala(R)	1.1×10^{-4}	1.9×10^{-4}	0.62	0.66
	Ala(L)	6.9×10^{-5}	1.2×10^{-4}	0.67	0.71
B	Gly	7.7×10^{-5}	1.1×10^{-4}	0.75	0.78
	Ala(R)	1.3×10^{-4}	2.4×10^{-4}	0.58	0.63
	Ala(L)	1.7×10^{-4}	2.9×10^{-4}	0.64	0.68
C	Gly	3.5×10^{-10}	3.9×10^{-9}	0.34	0.38
	Ala(R)	1.9×10^{-9}	2.7×10^{-8}	0.20	0.23
	Ala(L)	2.5×10^{-9}	3.3×10^{-8}	0.22	0.25

and near those charges in such a way that the effective value of D would approach the value for bulk water, viz., ~80. In order to take into account this (partial) screening effect due to water, a cutoff of the long-range interactions in the helix is employed as one of the simplest procedures. A complete cutoff would correspond to the approximation in which the dielectric constant of the bulk water (~80) is regarded as infinite. While the definition of a long-range interaction is somewhat arbitrary, the one used in Table II is employed here, viz., an interaction between pairs of atoms separated by about 6 Å or more. Even though such atoms may not be separated by a layer of water molecules *between* them, the effective value of D for the electrostatic interaction between them would be expected to be quite large, because most of the lines of force between those charges pass through the nearby water medium which has a large dielectric constant. Even though *the long-range interactions* as defined in Table II are mainly electrostatic, as discussed above, we applied the cutoff also for the *weak* nonbonded interactions of long range, since the long-range attractive part of the nonbonded interaction (the London force) arises basically from electric interactions (in fact the London force can be derived by a second-order perturbation of the electrostatic interactions). No cutoff was applied explicitly to the interactions in the coil since long-range interactions had already been neglected in the calculation of the statistical weight of a coil section.

In principle, all the calculations described in Paper II would have had to be redone for the interaction energies with the cutoff mentioned above. But, in practice, it was not necessary, because minimum-energy conformations and their conformational entropies are not sensitive to *the long-range interactions*. The insensitivity of the conformational entropies to *the long-range interactions* was shown earlier in this section. The insensitivity of the minimum-energy conformation to *the long-range interactions* was mentioned in Sec. III.D of Paper II; this is also seen from the fact that the diffuseness at the ends of nonregular helices is confined to about five residues (see Sec. III.E of paper II). This means that the interactions responsible for the minimum-energy conformation range up to about five residues, because otherwise diffuseness would be observed in longer terminal sections. The only quantities which are affected appreciably by the cutoff are the calculated energies. Hence, in order to introduce the cutoff beyond about 6 Å, we can simply neglect the contribution to ΔH_σ from *the long-range interactions* given in Table II. The enthalpies obtained in this way are given in Table II as $\Delta H_{\sigma,\,\text{cut}}$. By introducing the cutoff in this manner, the values of ΔH_σ were reduced (from ΔH_σ to $\Delta H_{\sigma,\,\text{cut}}$) by 40%–80% depending on the parameter sets and polyamino acids.

In Table V, the value of $\sigma = \exp[-(\Delta H_{\sigma,\,\text{cut}} - T\Delta S_\sigma)/RT]$ and the fraction of the contribution from $-T\Delta S_\sigma$ to $-RT\ln\sigma$ at two temperatures are given; the values of $\Delta H_{\sigma,\,\text{cut}}$ and ΔS_σ are those of Tables II and III, respectively. The agreement of the calculated value of σ with experimental data[10] is quite good for parameter sets A and B, but is very poor for parameter set C. From this we can conclude that the very rough picture, in which $D = 4.0$ is used for the short-range interactions and $D = \infty$ for interactions of a range longer than about 6 Å, is consistent with the experimental result, while the use of $D = 1.0$ for the short-range interactions is not. It should be noted that the temperature dependence of the values of σ is still appreciable, even though the values of ΔH_σ were reduced considerably by the cutoff of the long-range interactions. The importance of the entropic contribution to the value of σ can be seen from the last two columns of Table V. The fraction of the entropic contribution to the free energy associated with σ is seen to be about 60% to 80% for parameter sets A and B. Parameter set C is no longer regarded as being realistic.

The way in which $\Delta H_{\sigma,\,\text{cut}}$ is broken down into *the short-range interactions* (1) and (2) depends on the polyamino acid, as seen from Table II. For polyglycine,

the short-range interactions (1) are dominant over the other, while for poly-L-alanine both contribute more or less equally to $\Delta H_{\sigma,\,\text{cut}}$.

IV. ANALYSIS OF VARIOUS CONTRIBUTIONS TO s FOR POLYAMINO ACIDS IN VACUUM AND IN WATER

In this section, the influence of a type (2) solvent effect on the value of s will be reexamined by applying the cutoff procedure for *the long-range interactions* discussed in Sec. III. The influence of a type (1) solvent effect, i.e., the effect of binding of water molecules to free CO and NH groups, will be discussed in Sec. V. Because the amount by which a coil state is stabilized by binding water molecules to its free CO and/or NH group(s) may be regarded as the same both for poly-glycine and for poly-L-alanine, the relative stabilities of the α-helical conformations of these polyamino acids can be discussed without taking into account the binding effect explicitly. Hence, in this section, in addition to an analysis of the contributions to s, an examination will also be made of the source of the greater stability of the α-helical conformation of poly-L-alanine compared to that of polyglycine.

The enthalpy $\Delta H_s^{(0)}$ in vacuum can be expressed by Eqs. (4), (6), and (II.17) as

$$\Delta H_s^{(0)} = \sum_{i=0}^{\infty} E_i - H_c^{(0)}. \qquad (17)$$

The terms on the right-hand side of Eq. (17) are classified into three groups, *the short-range interactions (1)* which is $E_0 + E_1 + E_2 - H_c^{(0)}$, *the short-range interactions (2)* which is $E_3 + E_4$, and *the long-range interactions* which is

$$\sum_{i=5}^{\infty} E_i.$$

The contributions from these three groups are given in Table VI. The contributions from the *long-range interactions*, being mainly electrostatic, are almost independent of the polyamino acid and the helical sense, and are also small in numerical values as compared with the same contributions to ΔH_σ given in Table II. Nevertheless, these small contributions must also be cut off, as discussed in Sec. III, in order to take into account the (partial) screening effect of the long-range interactions by water. The values thus obtained for the enthalpy associated with s, denoted as $\Delta H_{s,\,\text{cut}}^{(0)}$, are given in Table VI. It is seen from Table VI that, after the small contributions from the *long-range interactions* are cut off, the stabilization of the α-helical conformation against the random coil state arises mainly because of *the short-range interactions (2)*, which are the interaction energies between groups of atoms located near each other because of the spiraling of the backbone in the α-helical structure. This amount of stabilization depends on the polyamino acids (poly-glycine or poly-L-alanine) and also on the helical sense

(the right or left handedness). The small contributions from the *short-range interactions (1)* do not have an appreciable dependence on the polyamino acid or on the helical sense.

Experimentally, the α-helical conformation of poly-glycine in water has not been observed,[11–13] while the right-handed α-helical conformation of poly-L-alanine has been observed.[10,14] A theoretical explanation of these findings will be given in Sec. V after the effect of water binding is taken into account explicitly. Meanwhile, in this section, we consider only the difference between the stabilities of the α-helical conformations of poly-glycine and poly-L-alanine since this difference is not affected by binding water to the random coil. Since there is almost no difference between $\Delta S_s^{(0)}$ for poly-glycine and the right-handed α-helix of poly-L-alanine, as shown in Table I, the greater stability of the right-handed α-helical conformation of poly-L-alanine compared to that of polyglycine must be attributed to the difference in $\Delta H_{s,\,\text{cut}}^{(0)}$. As was just noted, this difference arises mainly from the differences in the interaction energies E_3 and E_4. The breakdown of these terms for parameter set A is given in Table IV, where it is seen that the stabilization of the right-handed α-helix of poly-L-alanine over that of polyglycine arises mainly from the nonbonded interactions, the difference being 0.5 and 0.7 kcal in E_3 and E_4, respectively. The same qualitative conclusion was obtained for the other parameter sets examined. This difference in the nonbonded interaction energies arises mainly from the side-chain–backbone interactions in poly-L-alanine which are absent in polyglycine.

In order to discuss the relative stability of the right- and left-handed α-helices of poly-L-alanine, we have to consider the difference in $\Delta S_s^{(0)}$ as well as in $\Delta H_{s,\,\text{cut}}^{(0)}$ since both differences are appreciable, as shown in Tables I and VI, respectively. The right-handed α helix is more favored enthalpywise than the left-handed one, while the latter is more favored entropywise than the former for parameter sets A and B. The balance between these two competing factors leads to a conformational transition from the right- to the left-handed α helix of poly-L-alanine in vacuum, as the temperature is raised. But the balance is so subtle that we cannot draw any quantitative conclusion about the transition temperature in vacuum from the present calculation because of uncertainties in the values of the parameters in the conformational energy function. The difference in $\Delta H_{s,\,\text{cut}}^{(0)}$ for the right- and left-handed α helix of poly-L-alanine also arises from the nonbonded interaction energies included in E_3 and E_4, the differences being 0.13 and 0.58 kcal, respectively.

V. BINDING OF WATER MOLECULES TO FREE NH AND CO GROUPS

In this section, the values of the enthalpy and entropy changes, ΔH_B and ΔS_B, respectively, arising from water binding to free NH and CO groups, and defined

TABLE VI. The contributions[a] from the short- and long-range interactions to $\Delta H_{s,\text{cut}}^{(0)}$.[b]

Parameter set	Polyamino acid	Short-range interactions (1) $\sum_{i=0}^{2} E_i - H_c$	Short-range interactions (2) $(E_3 + E_4)$	Long-range interactions $\sum_{i=5}^{20} E_i$[c]	$\Delta H_{s,\text{cut}}^{(0)}$
A	Gly	0.09	−3.53	−0.61	−3.44
	Ala(R)	−0.14	−4.57	−0.67	−4.71
	Ala(L)	−0.19	−4.02	−0.61	−4.21
B	Gly	−0.01	−3.54	−0.62	−3.55
	Ala(R)	−0.13	−4.36	−0.67	−4.49
	Ala(L)	−0.27	−4.02	−0.62	−4.28
C	Gly	3.84	−4.78	−2.21	−0.94
	Ala(R)	3.73	−5.59	−2.37	−1.86
	Ala(L)	3.32	−5.24	−2.22	−1.92

[a] Given in kilocalorie/mole·residue.
[b] $\Delta H_s^{(0)}$ given in Table I and $\Delta H_{s,\text{cut}}^{(0)}$ are the sums of Columns 3–5 and 3–4, respectively.

[c] The sum of E_i for i larger than 20 is negligible. Hence, this quantity represents essentially

$$\sum_{i=5}^{\infty} E_i.$$

by Eq. (13), are determined from the requirement that the calculated curve for the fraction of helical content, θ, of poly-L-alanine vs temperature for a given set of values of ΔH_B and ΔS_B fit best with experimental data.[10] The 2×2 matrix formulation of Zimm and Bragg[2] was used to calculate θ as a function of s and σ. The values of s and σ were calculated from $\exp[-(\Delta H_{s,\text{cut}} - T\Delta S_s)/RT]$ and $\exp[-(\Delta H_{\sigma,\text{cut}} - T\Delta S_\sigma)/RT]$, respectively, where $\Delta H_{s,\text{cut}}$ and ΔS_s are given by Eqs. (14) (with ΔH_s and $\Delta H_s^{(0)}$ being replaced by $\Delta H_{s,\text{cut}}$ and $\Delta H_{s,\text{cut}}^{(0)}$, respectively) and 15, respectively, and the values of $\Delta H_{\sigma,\text{cut}}$ and ΔS_σ are given in Tables II and III, respectively. The calculated data for parameter set A were used, because this is the only one among the three parameter sets examined for which the right-handed α helix of poly-L-alanine was found to be more stable than the left-handed one at room temperature (which agrees with the experiments[10,14]). By using a least-squares method to fit $s(T)$ and $\sigma(T)$ to experimental data,[10] the values $\Delta H_B = -2.74 \pm 0.02$ kcal and $\Delta S_B = -5.78 \pm 0.06$ eu were obtained. The reasonableness of these numbers will

TABLE VII. Constant Ka for water binding and the fraction $Ka/(1+Ka)$ of free CO and NH groups to which water molecules are bound.

Temperature (°K)	Ka	$Ka/(1+Ka)$
280	7.43	0.881
300	5.35	0.843
320	4.02	0.801
340	3.12	0.757
360	2.49	0.713

be discussed later in this section. The values of Ka of Eq. (13) and the fraction of free CO and NH groups to which water molecules are bound, viz., $Ka/(1+Ka)$, calculated from these values, are given in Table VII.

The values of ΔH_B and ΔS_B, determined above by comparing the experimental and calculated values of θ for the right-handed α helix of poly-L-alanine, can also be used for the calculation of s, ΔH_s, and ΔS_s for the right- or left-handed α helix of polyglycine and the left-handed α helix of poly-L-alanine. The calculated values of ΔH_s and ΔS_s for these polyamino acids in water are given in Table VIII. An interesting result is that both ΔH_s and ΔS_s are quite temperature dependent, as was already pointed out after Eq. (15). This finding implies that the assumption, that ΔH_s is independent of temperature (made in most, but not all, analyses of experimental data), may not be a good one. The values in Table VIII may be compared with $\Delta H_w = -188$ cal/mole and $\Delta S_w = -0.55$ eu, obtained experimentally[10] by assuming that ΔH_w and ΔS_w are independent of temperature, where ΔH_w and ΔS_w are the enthalpy and entropy associated with the Lifson–Roig[15] parameter w; the differences between ΔH_s and ΔH_w, and ΔS_s and ΔS_w, respectively, are usually very small. The values of ΔH_w and ΔS_w determined experimentally with the assumption that they are independent of temperature, lie in the range of values of $\Delta H_s(T)$ and $\Delta S_s(T)$ in Table VIII. However, because of the small differences between ΔH_s and ΔH_w, and ΔS_s and ΔS_w, respectively, the transition temperature defined as $T = \Delta H_w/\Delta S_w$ does not agree with the one defined as that for which $s(T) = 1$ (especially when both ΔH_w and ΔS_w are small). This is the reason why we obtained $s = 1$ at 323°K, while $\Delta H_w/\Delta S_w$ was found to be 342°K.[10] The calculated values of s are shown in Fig. 2, and lead to

TABLE VIII. ΔH_s (kilocalorie/mole) and ΔS_s (entropy units) in water.[a]

Temperature (°K)	Gly		Ala(R)		Ala(L)	
	ΔH_s	ΔS_s	ΔH_s	ΔS_s	ΔH_s	ΔS_s
280	1.38	0.39	0.11	0.45	0.61	1.95
300	1.17	−0.34	−0.10	−0.28	0.40	1.22
320	0.94	−1.08	−0.33	−1.02	0.17	0.48
340	0.70	−1.80	−0.57	−1.74	−0.07	−0.24
360	0.47	−2.48	−0.80	−2.42	−0.30	−0.92

[a] $\Delta H_B = -2.74$ kcal/mole and $\Delta S_B = -5.78$ eu were used for water binding. The values of $\Delta H_{s,\text{cut}}^{(0)}$ and $\Delta S_s^{(0)}$ for parameter set A were used.

three important observations. First, polyglycine in water always remains in the random coil state (in the temperature range of Fig. 2, and undoubtedly over a wider range), and a helix–coil transition never takes place. The reason for this is that the value of $\Delta H_{s,\text{cut}}^{(0)}$ is not large enough (compared with the enthalpy gain of the coil state when water molecules are bound to NH and CO groups) to stabilize the α-helical conformation of polyglycine in water. This fact is seen in the numerical values of ΔH_s for polyglycine which are always positive, as shown in Table VIII. Second, even though a conformational transition from the right- to the left-handed α helix was predicted for poly-L-alanine in vacuum (sec. IV.C of Paper II), the left-handed α helix does not exist *in water*; hence, poly-L-alanine in water exhibits a conformational transition from the right-handed α helix to the random coil when the temperature is raised.[16] This is because the random coil form becomes, and remains, more stable than the α-helical conformations before the left-handed α helix becomes more stable than the right-handed one, as the temperature is raised.[16] Third, when the temperature is lowered (in the lower temperature range of Fig. 2), the values of s become smaller for all three curves, in contrast to the opposite tendency in the values of s for these polyamino acids in vacuum (see Fig. 11 of

II). This is because the binding of water molecules to free CO and NH groups becomes stronger when the temperature is lowered, as can be seen from Table VII. Essentially this same effect of solvent binding accounts for the so-called inverse helix–coil transition of poly-γ-benzyl-L-glutamate in a mixed organic solvent.[17] The calculated values of θ are shown in Fig. 3 for three different degrees of polymerization, 160, 450, and 1000, together with the experimental data.[10] The decrease of θ below 290°K arises because of the decrease of s in the same range. The poor fit of the calculated values of θ with the experimental data at high temperatures, especially for high DP's, may arise because of possible errors in the experimental data at high temperature, as discussed by the original authors.[10] These high-temperature points were given less weight in the determination of ΔH_B and ΔS_B by the least-squares procedure. Another possible reason for the poor fit will be considered in Sec. VII in connection with the discussion of possible effects of hydrophobic bonding on the values of s.

In order to see if the value of ΔH_B determined in this section is a reasonable one, an analysis is made of the heat of dimerization of N-methylacetamide molecules in carbon tetrachloride, ΔH_{CCl_4}, and in water,

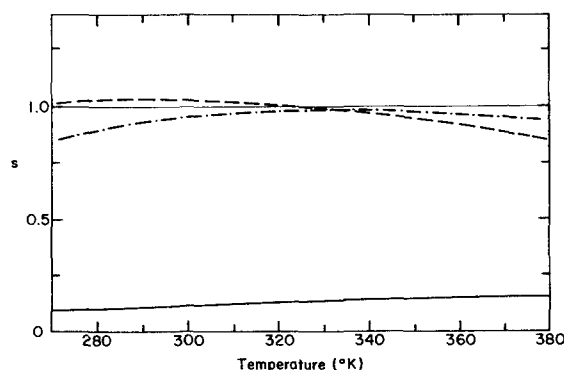
FIG. 2. The statistical weights of helix in water for the right- or left-handed α helix of polyglycine (—), for the right-handed α helix of poly-L-alanine (- - -) and for the left-handed α helix of poly-L-alanine (-·-·-). $\Delta H_B = -2.74$ kcal and $\Delta S_B = -5.78$ eu were used for water binding.
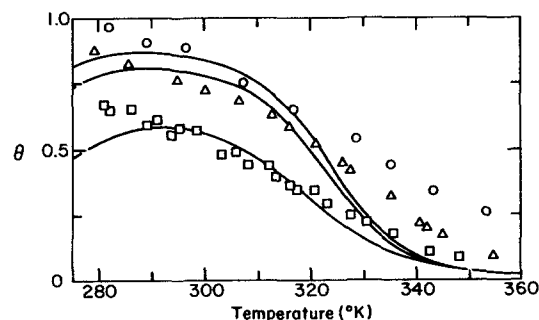

FIG. 3. Curves for the fraction of helical content (θ) vs temperature (T°K) for the right-handed α helix of poly-L-alanine in water which were calculated to give the best fit to the experimental points for the degrees of polymerization 160 (□), 450 (△), and 1000 (○). The experimental data are from Ref. 10. The calculated curves were obtained for parameter set A, with $\Delta H_B = -2.74$ kcal and $\Delta S_B = -5.78$ eu being used for water binding.

$\Delta H_{\mathrm{H_2O}}$, obtained by Kresheck and Klotz.[18] They reported the following values: $\Delta H_{\mathrm{CCl_4}} = -5.1$ kcal, $\Delta H_{\mathrm{H_2O}} = 0.0$ kcal. Here, we assume that the dimerization in carbon tetrachloride involves only hydrogen bond formation, and that the following processes are involved in the dimerization in water: (a) release of water molecules from the NH and CO groups of the monomer molecules, (b) formation of the hydrogen bond between the freed NH and CO groups, and (c) formation of hydrophobic bond(s) between the methyl groups.[19] With these assumptions, $\Delta H_{\mathrm{CCl_4}} - \Delta H_{\mathrm{H_2O}} = -5.1$ kcal should be interpreted as (i) the heat of binding of water molecules to the NH and CO groups of a monomer minus (ii) the heat of formation of hydrophobic bond(s) between the methyl groups. If we make the further assumptions that the fraction $Ka/(1+Ka)$ of NH and CO groups in N-methylacetamide to which water molecules are bound is the same (viz., 0.85 at 25°C) as found in Table VII, that the binding constants for water to NH and CO groups are the same (as we have done in Sec. II), and that the formation of hydrophobic bond(s) contributes a heat of formation of about $+0.4$ kcal,[19] we come to the conclusion that $\Delta H_B = -2.76$ kcal/mole, in excellent agreement with the value of -2.74 kcal/mole obtained from the poly-L-alanine data.

It is a bit more difficult to rationalize the large negative value of $\Delta S_B = -5.78$ eu. Perhaps the binding of a water molecule to a free NH or CO group promotes the formation of additional icelike regions around it, thereby reducing the entropy of the system. This possibility is suggested by the cooperative nature of hydrogen bond formation.

VI. A SIMPLE MODEL OF THE HELIX–COIL TRANSITION

In this section, we introduce a simple model of the helix–coil transition, and will develop it further in a subsequent paper.[20] We are led to this model by the present calculations of s and σ from molecular quantities. The purpose of introducing it is twofold. First, we wish to summarize the results obtained in this series of papers by expressing the most essential factors responsible for the values of s and σ in the form of a simple model; at the same time, the approximate nature of such a model will be emphasized by pointing out the terms which are neglected in order to obtain it. Second, it is desirable to have a model of the helix–coil transition that is a realistic first approximation not so much for homopolymers but primarily for co-polymers.[20]

The basic assumptions underlying the phenomenological theories of the helix–coil transition in homopolyamino acids, e.g., that of Zimm and Bragg,[2] are that (a) the statistical weight of a certain conformation of the chain is given by Eq. (I.1), i.e., by a product of the statistical weights of the alternating helical and coil sequences, and (b) the statistical weights of the helical and coil sequences are such that the convergence

of Eq. (I.3) is rapid. The success of the phenomenological theories justifies these assumptions. In the calculations reported in this series of papers for the Zimm–Bragg parameters s and σ from molecular quantities, assumption (a) was also made (there being several reasons for the validity of this assumption), and assumption (b) was justified by the calculations, viz., by the fact that E_i given in Table IV or Fig. 7 of II has an appreciable value only for $0 \leq i \leq 4$, together with the fact that $\ln[(2\pi RT)^j (\det A_j)^{-1/2}]$ plotted against j in Figs. 9 and 10 of II rapidly approaches a straight line, implying that the convergence limit in Eq. (I.3) is attained at about $j=4$, and also by the fact that the important intramolecular interactions responsible for the helix–coil transition extend only over a range of up to several residues. Even though the interactions extend over several residues, the nearest-neighbor interaction model (or the 2×2 matrix formulation) of Zimm and Bragg[2] is nevertheless a very good one for the helix–coil transition in a *homopolyamino acid* (but the empirical parameters s and σ in the theory must be interpreted as containing contributions from beyond the nearest neighbors.[21,22]

For homopolymers, the contribution from each residue to the statistical weight of a helical sequence can be varied as long as the statistical weight of a helical sequence consisting of j hydrogen bonds is still given by σs^j. In the formulation of Zimm and Bragg,[2] the following rule (rule A) is used as one of the possible choices: the first residue whose CO group forms a hydrogen bond with the NH group of the residue four places ahead carries the statistical weight σs, each of the subsequent residues whose CO group forms a hydrogen bond carries the statistical weight s, and each of the four last residues of the helical sequence whose NH group forms a hydrogen bond with a former residue carries the statistical weight 1, the same as for a coil residue. Another possible rule (rule B, the physical incentive for which will be given below) is to assign $\sigma^{-1/4} s^{1/2}$ to each of the first and last residues, $\sigma^{1/4} s^{1/2}$ to each of the six residues located at the second through fourth places from either end of the helix, and s to each of the residues in the middle. The two rules mentioned above give the same statistical weight σs^j for a helical sequence in a homopolymer. This arbitrariness in the way statistical weights are divided up for each residue in a homopolymer is not present in the case of a co-polymer, because the value of s for each residue is different depending on the species of the amino acid. [For example,

$$\prod_{k=1}^{j} s_k$$

for rule A, with s_k being the value of s of the kth residue, is not the same as

$$(s_1 s_2 s_3 s_4)^{1/2} (\prod_{k=5}^{j} s_k)(s_{j+1} s_{j+2} s_{j+3} s_{j+4})^{1/2}$$

TABLE IX. The definition and the statistical weights of eight states of a residue.

| State | (ϕ,ψ)[a] | Hydrogen bond on[b] | | Statistical weight | | |
		CO	NH	rule A[c]	rule B[d]	
1	c	No	No	1	1	1
2	c	No	Yes	1	$\beta^{1/2}$	$\sigma^{-1/4}s^{1/2}$
3	c	Yes	No	σs	$\beta^{1/2}$	$\sigma^{-1/4}s^{1/2}$
4	c	Yes	Yes	σs	β	$\sigma^{-1/2}s$
5	h	No	No	1	α	$\sigma^{1/2}$
6	h	No	Yes	1	$\alpha\beta^{1/2}$	$\sigma^{1/4}s^{1/2}$
7	h	Yes	No	s	$\alpha\beta^{1/2}$	$\sigma^{1/4}s^{1/2}$
8	h	Yes	Yes	s	$\alpha\beta$	s

[a] The symbols c and h correspond to the states of the dihedral angles ϕ and ψ in which they are not, and they are, confined to the small range in the (ϕ,ψ) space characteristic of the α helix, respectively.

[b] The existence and nonexistence of a hydrogen bond on the CO and NH groups are designated by yes and no, respectively.

[c] The statistical weights according to the rule of Zimm and Bragg.[2]

[d] Proposed statistical weights, expressed in two different ways.

for rule B when the s_k's are not equal to each other.] In other words, the statistical weight for each residue in a homopolymer does not necessarily have a direct relation to the intramolecular interactions in the chain (because of the arbitrariness mentioned above), while that for each residue in a co-polymer must be directly related to the intramolecular interactions (because this arbitrariness does not exist). Therefore, in order to establish a reasonable model of the helix–coil transition in co-polymers, (at least) the main factors determining the values of s and σ must be known or reasonably assumed.

The value of s is determined by three factors, (a) the enthalpy change $\Delta H_{s,\text{cut}}^{(0)}$ and (b) the entropy change $\Delta S_s^{(0)}$, when a residue is incorporated into the end of a long helical sequence from the random coil state, and (c) the binding of a water molecule or a cluster of water molecules to free NH and CO groups. Short-range intramolecular interactions between pairs of atoms belonging to amino acid residues separated by up to three intermediate residues are responsible for the value of $\Delta H_{s,\text{cut}}^{(0)}$. For polymers in water, interactions of longer range do not contribute much to $\Delta H_{s,\text{cut}}^{(0)}$ because of the (partial) screening of the long-range electrostatic interactions by water molecules. Among the short-range interactions, the contributions from those included in E_0, E_1, and E_2 (defined in Sec. II.E of Paper II) for the helical conformation almost cancel with those from interactions included in $H_c^{(0)}$ for the random coil state (see Table VI). The interactions included in E_3 (defined in Sec. II.E of Paper II) account for from 67% to 84% of the total contributions to $\Delta H_{s,\text{cut}}^{(0)}$, as can be seen from Tables IV and VI. It should be mentioned here that the energy of hydration in the β_1–α_4 hydrophobic bond is not included in the numbers in Table IV. When included, it changes E_4 by about -0.2 kcal at 300°K, which does not change appreciably the numbers quoted here to indicate the

primary importance of E_3. Hence, in order to simplify the matter and to obtain a model which can serve as a first-order approximation, we can neglect all contributions to $\Delta H_{s,\text{cut}}^{(0)}$ other than E_3. It should be noted from Table IV that, even though the hydrogen bond energy (the one expressed by the Lippincott–Schroeder function) accounts for more than 50% of E_3 [63% in Gly, 53% in Ala(R), and 56% in Ala(L) for set A], the nonbonded interactions also account for a substantial part of E_3 [32% in Gly, 45% in Ala(R), and 40% in Ala(L) for set A]. Nonetheless, we shall refer to E_3 as "the hydrogen bond energy" because there is no experimental means to distinguish between the two main contributions to E_3 mentioned above. Thus, if the dihedral angles of three consecutive amino acid residues assume values characteristic of the α helix, one "hydrogen bond" is formed. The quantity $\Delta S_s^{(0)}$ expresses the loss of entropy accompanying the confinement of the values of the dihedral angles ϕ and ψ to the smaller range in the (ϕ,ψ) space characteristic of the α helix when a residue is transferred from the random coil state to the α-helical conformation. This factor, together with the effect of water binding to free NH and CO groups, favors the random coil state.

From the results summarized in the above paragraph, we are naturally led to the idea of classifying the conformational states of a residue in a polypeptide chain into eight types according to three independent factors: (a) whether or not the values of the two dihedral angles ϕ and ψ around the $C^{\alpha}N$ and $C^{\alpha}C'$ bonds, respectively, are confined to the small region in the (ϕ,ψ) space characteristic of the right-handed α helix, (b) whether or not a hydrogen bond is formed between the CO group of the residue in question and the NH group of the residue four places ahead, and (c) whether or not a hydrogen bond is formed between the NH group of the residue in question and the CO group of the residue four places back. The definition of the eight states is given in Table IX together with the statistical weights of each state of a residue according to rules A and B. An example of a conformational state of a peptide chain is given in Fig. 4, in which each residue is designated as being in one of the eight possible states. Rule B is obtained by the following considerations. State 1 is chosen as the reference state, or, in other words, the statistical weight of state 1 is taken to be unity. In states 5–8 the dihedral angles ϕ and ψ are confined to the small range in the (ϕ,ψ) space characteristic of the α helix, and the corresponding entropy loss decreases the statistical weights of these states by a factor designated as α in the sixth column of Table IX. Whenever a hydrogen bond is formed, the corresponding enthalpy gain stabilizes, or increases the statistical weight of, the state by a factor designated as $\beta^{1/2}$ in the sixth column of Table IX. Thus, if a state has one hydrogen bond on its CO or NH group (as in states 2, 3, 6, and 7), the state carries the factor $\beta^{1/2}$. If a state has two hydrogen bonds on its CO and NH groups (as in states 4 and 8), the state carries the factor of β. The

isolated helical section with j hydrogen bonds is expressed by the following sequence of states:

$$\cdots 1\,1\,1\,3\,7\,7\,7\,8\,8\cdots 8\,8\,6\,6\,6\,2\,1\,1\,1\,\cdots.$$
$$\underbrace{}_{(j-4)8\text{'s}}$$

According to the above rule the statistical weight of this sequence is $\alpha^2(\alpha\beta)^j$. This has the form of σs^j if $\sigma = \alpha^2$ and $s = \alpha\beta$. The parameters α and β defined above have the same physical meaning as the ones introduced by Nagai.[23] But Nagai's rule for the statistical weights is different from both rule A and rule B of Table IX. The seventh column of Table IX is obtained by expressing α and β in terms of s and σ.

As a consequence of the above considerations, based on the simplifying assumption that all contributions to $\Delta H_{s,\,\text{cut}}^{(0)}$ but that from E_3 can be neglected (and, hence, on the concept that the statistical weight of a residue depends on the state of "hydrogen bonding" of its NH and CO groups and on near-neighbor side-chain–backbone interactions), the Zimm–Bragg parameter σ is given by α^2; it is the small positive number accounting for the entropy loss when two sets of (ϕ,ψ)'s are confined to the small "α-helical" region. This is indeed what we have observed in Sec. III, viz., about 60%–80% of the free energy associated with σ is an entropic term (Table V, sets A and B); of the entropic term, the major part is $2\Delta S_s^{(0)} [\Delta S_s^{(0)} = S_h^{(0)} - S_c^{(0)}$ being related to α by $\alpha = \exp(\Delta S_s^{(0)}/R)]$, and the other (minor) part is $R \ln g_0$, which accounts for the extra amount of entropy which a few residues at each end of a helical section have (compared to residues in the middle of a helical sequence) because of the looseness of the structure at the ends $[2\Delta S_s^{(0)}$ and $R \ln g_0$ have opposite signs, and the ratio $R \ln(g_0)/2\Delta S_s^{(0)}$ ranges between -0.06 and -0.39 (Table III, sets A and B; set C not being regarded as realistic)]. One particular aspect of the simplifying assumption of the exclusive importance of E_3 is that the parameter σ is purely entropic and therefore independent of temperature. However, this is not a very good picture because, if the enthalpy term ΔH_σ is completely neglected, then the values of σ become larger than those given in Table V by a factor of 9.3 to 40.4 at 300°K. For polymers in water, the enthalpy term ΔH_σ comes from the short-range interactions up to E_4. Since the enthalpy term in σ, or the temperature dependence of σ, is important, its neglect places a limitation on the applicability of the proposed simple model. Nevertheless, this model is a good first-order approximation, having the essential features of the helix–coil transition, and is necessary for the study of the complex problem of co-polymers.

TABLE X. Number of binding sites in the conformational states I and II.

State of a residue	Number of binding sites per residue	Number of residues		Number of binding sites	
		I	II	I	II
1	2	2	5	4	10
2	1	1	1	1	1
3	1	1	1	1	1
4	0	1	0	0	0
5	2	3	0	6	0
6	1	3	3	3	3
7	1	3	3	3	3
8	0	2	3	0	0
Total		16	16	18	18

The simplified model introduced above retains one of the most essential aspects of the helix–coil transition derived in Sec. II.F of Paper II, viz., that the value of the parameter σ is not affected by the binding of solvent to the free CO and NH groups. This will be shown here, since the proof based on the simplified model gives us more insight into the physical reason for the fact that σ is independent of solvent binding. We consider two conformational states, I and II, of a chain consisting of 16 residues, where the number of hydrogen bonds is the same but the number of helical sequences is two and one, respectively, viz.,

state I   1 3 5 5 5 4 7 7 7 8 8 6 6 6 2 1

state II   1 1 1 1 3 7 7 7 8 8 8 6 6 6 2 1.

State I is the same as that shown in Fig. 4. The statistical weights are $\sigma^2 s^7$ and σs^7 for states I and II, respectively, according to either rule A or rule B. The ratio of these two statistical weights is σ. By making an apparent generalization to more general states, we see that σ may be described as the equilibrium constant for the formation of an interruption in a sequence of hydrogen bonds by a process which maintains a constant number of hydrogen bonds.[24] The independence of σ of solvent binding to free CO and NH groups can be demonstrated by showing that the ratio of the statistical weights of states I and II is not affected by the binding. This is done in Table X by counting the number of binding sites in states I and II. Because the numbers of binding sites in states I and II are the same, as shown in Table X, the ratio of the statistical weights of states I and II does not depend on solvent binding.

So far the simplified model has been discussed only in connection with homopolymers. For homopolymers, however, both rules A and B give the same statistical weight σs^j for a helical section consisting of j hydrogen bonds. In other words, our simple model (rule B) is identical with the Zimm–Bragg model (rule A) when applied to homopolymers. But, when applied to co-

NCCNCCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCCNCC
  1   3   5   5   5   4   7   7   7   8   8   6   6   6   2   1

FIG. 4. An example of a conformational state of a peptide chain consisting of 16 amino acid residues.

TABLE XI. Comparison of the free energy of a minimum-strength hydrophobic bond with other quantities responsible for the helix–coil transition in poly-L-alanine in water.

Temperature (°K)	$\Delta F_{H\phi}$[a] (kcal)	$\Delta H_{s,\mathrm{cut}}^{(0)} - T\Delta S_s^{(0)}$ (kcal)	$2RT \ln(1+Ka)$ (kcal)	$-RT \ln s$ (kcal)
280	−0.292	−2.389	2.374	−0.015
300	−0.345	−2.223	2.206	−0.017
320	−0.384	−2.057	2.053	−0.004
340	−0.411	−1.891	1.915	0.024
360	−0.424	−1.726	1.791	0.065

[a] These values include a term $Z_R E_R$ [Eq. (11) of Ref. 19] which is −0.15 kcal/mole. This term should be subtracted, thereby making the values of $\Delta F_{H\phi}$ less negative by 0.15, because it corresponds to a non-bonded interaction between the β_1-methyl CH$_3$ group and α_4-CH group, which has already been taken into account in the nonbonded interactions in the helix.

polymers, they are different, rule B being closer to reality because it is directly related to the structure of the molecule, whereas rule A is not. The simplest, yet realistic, model of the helix–coil transition in co-polyamino acids would be one in which (a) the state of each residue is classified into one of the eight states defined in Table IX, and (b) each residue carries a statistical weight according to rule B, where s is dependent on the type of the residue while σ is not. Even though we know that both s and σ depend on the type of residues, only the dependence of s is retained in the simplest model of the helix–coil transition in copolymers, because the conformational state of a polyamino acid chain depends very sharply on the value of s but not as much on the value of σ. The use of the parameter s, which depends only on the type of the one residue in question but not on the types of neighboring residues, is valid as long as side-chain–side-chain interactions do not play a major role in the helix–coil transition. In other words, the validity of assigning the values of the parameter s to each type of amino acid residue is based on the assumption that the side-chain–side-chain interaction energy in E_3 is negligible, meaning that the dependence of E_3 on the type of amino acid residue is mainly due to side-chain–backbone interactions; this is an assumption which has been made (with some justification) in considering the conformations of individual residues in proteins.[25–27] At least the difference in the values of s for polyglycine and poly-L-alanine arises from the side-chain–backbone interactions in poly-L-alanine which are absent in polyglycine. The method of calculating physical quantities for copolymers, based on rule B, will be presented elsewhere.[20]

VII. DISCUSSION

In this section, we will confine the discussion to two points, first, the effects of hydrophobic bonding on the helix–coil transition in poly-L-alanine in water and, second, the implication of the calculations in this series of papers for estimates of the change in the conformational entropy of a polypeptide chain when a protein molecule denatures.

In Paper II, we showed that β_1–α_4 hydrophobic bonds in the α helix[19] do not affect the value of σ (because, as developed further here in Sec. III, the values of g and g_0 are determined mainly by the energies of interaction between atoms participating in the formation of intra-chain hydrogen bonds), and we consider here their effect on s. As shown earlier,[19,28] hydrophobic bonds of this type stabilize the α helix. Hence, the free energy of formation of a hydrophobic bond, $\Delta F_{H\phi}$, must be added to $-RT \ln s$, and correspondingly for the enthalpy ($\Delta H_{H\phi}$) and entropy ($\Delta S_{H\phi}$) terms. Since the β_1–α_4 hydrophobic bond in poly-L-alanine is considered to be one of minimum strength,[19,28] $\Delta F_{H\phi}$ is given by

$$\Delta F_{H\phi} = 1830 - 12.2T + 0.165T^2 \qquad (18)$$

in calorie per residue.[19] In Table XI, this quantity is compared with $\Delta H_{s,\mathrm{cut}}^{(0)} - T\Delta S_s^{(0)}$ (the free energy associated with $s_{\mathrm{cut}}^{(0)}$) and with $2RT \ln(1+Ka)$ (the contribution to the free energy from the binding of water to the CO and NH groups). Considering the comment in Footnote a of Table XI, it is apparent that $\Delta F_{H\phi}$ is negligibly small compared to the other two contributions to the free energy, i.e., as far as the effect of water is concerned, the binding to CO and NH groups is much more important than the hydrophobic bond. Thus, the values of ΔH_B and ΔS_B, obtained by the procedure in Sec. V, are not very sensitive to the existence of the hydrophobic bond.

At this point, a comment should be made about the accuracy of the calculated values of s. Several approximations (involving neglecting terms of the order of 0.1 kcal in $-RT \ln s$, such as the β_1–α_4 hydrophobic bond) were made in the calculation of s. Therefore, the accuracy of the calculated values of $-RT \ln s$ is not better than 0.1 kcal. However, the helical fraction θ is a very sensitive function of s in the region near $s=1$. In fact, θ changes from 0.75 to 0.25 for a change of s from $1+1.15\sigma^{1/2}$ to $1-1.15\sigma^{1/2}$. The value of $-RT \ln s$ changes only by $1.4\sigma^{1/2}$ at $T=300°K$ (which is 0.02 kcal for $\sigma = 2\times10^{-4}$) for the same change in s. This means that, in order to calculate θ reasonably accurately in the region where it is not very close to either zero or

unity, $-RT \ln s$ must be calculated with an accuracy of better than 0.01 kcal, which is impossible at the present time. Therefore, the present molecular theory of s and σ cannot be used to predict the absolute value of θ, unless s is very much different from unity, for which θ is very close to either zero or unity. Thus, the inclusion of the very small contribution from $\Delta F_{H\phi}$ could easily alter the behavior of $s(T)$ or $\theta(T)$ for poly-L-alanine, e.g., the decreases at low temperature shown in Figs. 2 and 3, respectively. Since the values of s for the right- and left-handed α helices of poly-L-alanine are so close to unity, and to each other, in the transition range, this uncertainty prevents us from drawing any final conclusions here about the relative stabilities of the right- and left-handed α helices of poly-L-alanine in water. These uncertainties do not affect the conclusions drawn for polyglycine in water (or for the relative stabilities of polyglycine and poly-L-alanine in water) since the calculated values of s for polyglycine are so much smaller than unity. The present molecular theory of s and σ can be used instead to deduce the values of adjustable parameters in the energy function by comparing the calculated values of s and σ with experimental data, as we have done to obtain ΔH_B and ΔS_B. The utility of the simple model of the helix–coil transition in copolymers proposed in Sec. VI is the same. There, the model was derived by neglecting a number of terms of second-order importance. However, we can obtain information about the magnitude of terms of first-order importance from the values of s and σ obtained by comparing the calculated helical fraction with experimental data.

Turning to the second point, viz., the entropy of protein denaturation, we recall that $\Delta S^{(0)}$ for protein denaturation has been estimated[29],[30] as 3 to 4 eu per residue, with the solvation and other contributions being added in separately. If we regard the helix–coil transition in homopolyamino acids as a model for the denaturation of proteins, then the calculations reported here (based on intermolecular interactions) lead to a value of $\Delta S^{(0)}$ closer to about 7–8 eu per residue (see the data for sets A and B in Table I), i.e., it appears that there is a greater change in the conformational entropy than heretofore realized.[29],[30]

[1] N. Gō, M. Gō, and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S. 59, 1030 (1968), hereafter referred to as I, with equation numbers cited as (I.).

[2] B. H. Zimm and J. K. Bragg, J. Chem. Phys. 31, 526 (1959).

[3] M. Gō, N. Gō, and H. A. Scheraga, J. Chem. Phys. 52, 2060 (1970), hereafter referred to as II, with equation numbers cited as (II.).

[4] See Sec. IIIA of Paper II for the numerical values of the various sets of parameters used in the conformational energy calculations.

[5] D. A. Brant, W. G. Miller, and P. J. Flory, J. Mol. Bio. 23, 47 (1967).

[6] It is of interest to note here that Brant[7] obtained $H_c^{(0)}$ as $\langle A \rangle$, i.e., without subtracting the term RT. However, he did subtract RT from the corresponding term for a residue in a helical state.

[7] D. A. Brant, Macromolecules 1, 291 (1968).

[8] In Table IV of Paper II, a minus sign should be inserted so that the heading of the last three columns becomes $-RT^2 \partial \ln C / \partial T$.

[9] (a) See Sec. II.C of Paper II for the definitions of regular and nonregular helices. In the following discussion, the numerical analysis will be carried out only for nonregular helices, since these helices are more realistic conformations than regular ones, because of the diffuseness at the ends of helices. (b) R. G. C. Arridge and C. G. Cannon, Proc. Roy. Soc. (London) 278A, 91 (1964).

[10] R. T. Ingwall, H. A. Scheraga, N. Lotan, A. Berger, and E. Katchalski, Biopolymers 6, 331 (1968).

[11] It is known that, in the solid state, polyglycine forms extended multiple-stranded structures[12] whereas, in nonaqueous[13a,b] and aqueous[13c] solution, the structure is disordered. In this series of papers, we have confined our attention to ordered structures of the α-helical type, and have not considered the extended structures observed for polyglycine in the solid state.

[12] (a) C. H. Bamford, L. Brown, E. M. Cant, A. Elliott, W. E. Hanby and B. R. Malcolm, Nature 176, 396 (1955); (b) F. H. C. Crick and A. Rich, ibid. 176, 780 (1955).

[13] (a) H. Block and J. A. Kay, Biopolymers 5, 243 (1967); (b) R. D. B. Fraser, B. S. Harrap, T. P. Macrae, F. H. C. Stewart, and E. Suzuki, ibid. 5, 251 (1967); (c) V. S. Ananthanarayanan, R. H. Andreatta, D. Poland, and H. A. Scheraga Macromol. (to be published).

[14] W. B. Gratzer and P. Doty, J. Am. Chem. Soc. 85, 1193 (1963).

[15] S. Lifson and A. Roig, J. Chem. Phys. 34, 1963 (1961).

[16] For parameter set A, the transition temperature for the conversion of the right- to the left-handed form of poly-L-alanine in vacuum was reported as 363°K in Paper II. Because of the use of $\Delta H_{s,\text{cut}}^{(0)}$ here, this value is lowered to 335°K in vacuum. However, since the transition from the right-handed helix to the coil occurs at a lower temperature (323°K), the transition from the right- to the left-handed helix in water is not observed.

[17] P. Doty and J. T. Yang, J. Am. Chem. Soc. 78, 498 (1956).

[18] G. C. Kresheck and I. M. Klotz, Biochemistry 8, 8 (1969).

[19] G. Némethy and H. A. Scheraga, J. Phys. Chem. 66, 1773 (1962).

[20] N. Gō, P. N. Lewis, M. Gō, and H. A. Scheraga, Macromolecules (to be published).

[21] D. Poland and H. A. Scheraga, Physiol. Chem. Phys. 1, 389 (1969).

[22] D. Poland and H. A. Scheraga, Theory of Helix–Coil Transitions in Biopolymers (Academic, New York, 1970), p. 135.

[23] K. Nagai, J. Phys. Soc. Japan 15, 407 (1960).

[24] J. Applequist, J. Chem. Phys. 38, 934 (1963).

[25] D. Kotelchuck and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S. 61, 1163 (1968); 62, 14 (1969).

[26] D. Kotelchuck, M. Dygert, and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S. 63, 615 (1969).

[27] P. Lewis, N. Gō, M. Gō, D. Kotelchuck, and H. A. Scheraga, Proc. Natl. Acad. Sci. U.S. 65, 810 (1970).

[28] M. Bixon, H. A. Scheraga, and S. Lifson, Biopolymers 1, 419 (1963).

[29] J. A. Schellman, Compt. Rend. Trav. Lab. Carlsberg 29, 230 (1955).

[30] H. A. Scheraga, in The Proteins, edited by H. Neurath (Academic, New York, 1963), 2nd ed., Vol. 1, p. 477.