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## Molecular Theory of the Helix–Coil Transition in Poly(amino acids). IV. Evaluation and Analysis of $s$ for Poly(L-valine) in the Absence and Presence of Water<sup>1</sup>

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**ABSTRACT:** The Zimm–Bragg parameter  $s$  for the helix–coil transition in poly(L-valine) in water was evaluated from the intramolecular interaction energies taking into account two kinds of solvent effects, *viz.*, the change in hydration around the side-chain methyl groups (including hydrophobic bonding) which accompanies the conformational change and the binding of water molecules to the free NH and CO groups of residues in the coil state. The entropy loss from the restricted rotational freedom of the side chain in the  $\alpha$  helix (in the presence and absence of water) and the binding of water molecules to the free NH and CO groups destabilize the  $\alpha$  helix of poly(L-valine); however, the change in hydration around the side-chain methyl groups stabilizes the  $\alpha$  helix over the random coil. The  $s$  vs. temperature curve of poly(L-valine) in water shows a maximum around 50° which originates from the change in hydration around the side-chain methyl groups. Also, a method for including further interactions beyond those in the random-coil form of the dipeptide is proposed, and the effective range of these additional interactions under experimental conditions is estimated.

In the previous papers<sup>3–5</sup> of this series, we reported the general formulation of the molecular theory of the helix–coil transition in poly(amino acids)<sup>3</sup> and the evaluation of the Zimm–Bragg parameters<sup>6</sup>  $s$  and  $\sigma$  for polyglycine and poly(L-alanine) in the absence and presence of water.<sup>4,5</sup> In this paper, we extend the computations to obtain the values of  $s$  for poly(L-valine) in the absence and presence of water. The evaluation of  $\sigma$  for poly(L-valine) was not carried out because of the large amount of computer time required. These computations provide a theoretical basis for understanding the behavior of  $s$  and  $\sigma$  (which are also being determined experimentally for the naturally occurring amino acids using random copolymers<sup>7</sup>) and for computing the tendency of each amino acid to form or disrupt the  $\alpha$ -helical conformation in proteins.<sup>8</sup> Since poly(L-valine) has bulkier side chains than poly(L-alanine), the nonbonded and hydrophobic interactions between the side chains would be expected to play an important role. In poly(L-al-

anine), these interactions between the side chains contribute negligibly.<sup>5</sup>

The formulation and the notation of the molecular quantities follow the previous papers<sup>3–5</sup> except that the recently adopted standard nomenclature convention<sup>9</sup> is used for the description of the conformations in the present paper. Only the parameter set  $A^4$  (with  $D = 4$ ) is used for the calculations in the present paper, since this parameter set is more reasonable than the others.<sup>4</sup> Also, only the right-handed  $\alpha$  helix is considered here, and not the left-handed one.

### I. Energy Minimization

The conformational energy was minimized with respect to the dihedral angles  $\phi_i$ ,  $\psi_i$ , and  $\chi_i^1$ , with  $\chi_i^{2,1}$  and  $\chi_i^{2,2}$  held fixed at 180° in both the helical and coil states. (Hereafter,  $\chi_i^1$  will be denoted simply as  $\chi_i$ .)

**A. Coil State.** In paper I,<sup>3</sup> the energy of the coil state was taken as a sum of interaction energies  $F^{(1)}(\phi_i)$ ,

	i - 1				i				i + 1				i + 2					
	H <sup>a</sup>	R	C'	O	H <sup>a</sup>	N	C <sup>a</sup>	H <sup>a</sup>	R	C'	O	H <sup>a</sup>	N	C <sup>a</sup>	H <sup>a</sup>	R	C'	O
N <sup>a</sup>																		
C <sup>a</sup>																		
H <sup>a</sup>																		
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C'																		
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**Figure 1.** Representation of pairs of atoms whose interaction energies are included in  $F^{(0)}$ ,  $F^{(1)}$ ,  $F^{(2)}$ , ...,  $F^{(8)}$ . All points in region A appear in region C or D. All points in region B are independent of  $\phi$ ,  $\psi$ , and  $\chi$ . Region C, which is either blank or numbered, contains those pairs of atoms included in  $F^{(0)}$  up to  $F^{(8)}$ . The pairs of atoms numbered 0, 1, 2, ..., 8 are those whose interatomic interactions are included in  $F^{(0)}(\chi_i)$ ,  $F^{(1)}(\phi_i, \chi_i)$ ,  $F^{(2)}(\chi_i, \psi_i)$ ,  $F^{(3)}(\phi_i, \psi_i)$ ,  $F^{(4)}(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$ ,  $F^{(5)}(\phi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$ ,  $F^{(6)}(\chi_i, \psi_i, \phi_{i+1}, \psi_{i+1})$ ,  $F^{(7)}(\phi_i, \psi_i, \phi_{i+1}, \psi_{i+1})$ , and  $F^{(8)}(\chi_i, \psi_i, \phi_{i+1}, \psi_{i+1}, \phi_{i+2}, \chi_{i+2})$ , respectively. The blanks in region C refer to pairs which are identical with those already listed. All pair interactions in region D are of longer range than  $F^{(8)}$ , and are neglected in the calculation of the statistical weight of the coil sequence.

$F^{(2)}(\psi_i)$ ,  $F^{(3)}(\phi_i, \psi_i)$ ,  $F^{(4)}(\psi_i, \phi_{i+1})$ , etc., for polyglycine and poly(L-alanine) with  $\chi$  fixed. For poly(L-valine), where  $\chi$  is allowed to vary, this sum of interaction energies must be modified to take into account the dependence of interactions with the side chains on  $\chi$ ; thus, we require  $F^{(0)}(\chi_i)$ ,  $F^{(1)}(\phi_i, \chi_i)$ ,  $F^{(2)}(\chi_i, \psi_i)$ ,  $F^{(4)}(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$ ,  $F^{(5)}(\phi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$ ,  $F^{(6)}(\chi_i, \psi_i, \phi_{i+1}, \psi_{i+1})$ , etc.;  $F^{(3)}(\phi_i, \psi_i)$  is not modified since it does not depend on  $\chi$ . The pairs of atoms which contribute to the terms  $F^{(0)}$ ,  $F^{(1)}$ ,  $F^{(2)}$ , ...,  $F^{(8)}$  are listed in Figure 1. In polyglycine and poly(L-alanine), the contribution to the free energy of the coil from interactions beyond  $F^{(4)}$  was negligible;<sup>5</sup> however, since the side chain of poly(L-valine) is larger and bulkier, we consider the possibility of longer range interactions, up to  $F^{(8)}$ . In this section, we evaluate the free energy of the coil by including interactions only up to  $F^{(4)}$ . In section IV, a method to include the longer range interactions will be considered, together with the evaluation of the free energy of the coil with the inclusion of terms up to  $F^{(6)}$ .

In analogy with eq I-16 and I-17, we define quantities  $A$  and  $B$  which now include a dependence on  $\chi_i$ .

$$A(\phi_i, \chi_i, \psi_i) = F^{(0)}(\chi_i) + F^{(1)}(\phi_i, \chi_i) + F^{(2)}(\chi_i, \psi_i) + F^{(3)}(\phi_i, \psi_i) \quad (1)$$

and

$$B(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1}) = F^{(4)}(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1}) \quad (2)$$

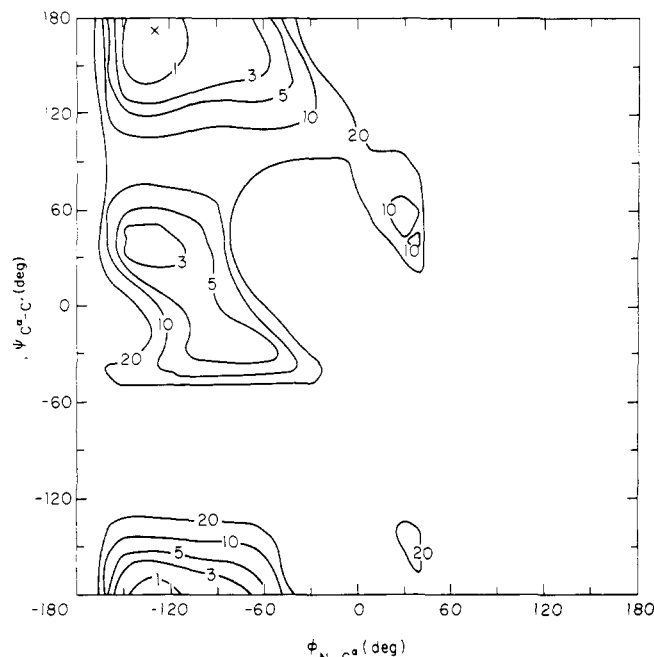
In eq 1,  $F^{(0)}$  ( $\chi_i$ ) is the sum of pair interactions which depend only on  $\chi_i$ .  $A(\phi_i, \chi_i, \psi_i)$  and  $B(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$  are each divided into two terms, viz.

$$A(\phi_i, \chi_i, \psi_i) = A_b(\phi_i, \psi_i) + A_s(\phi_i, \chi_i, \psi_i) \quad (3)$$

and

$$B(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1}) = B_b(\psi_i, \phi_{i+1}) + B_s(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1}) \quad (4)$$

In eq 3 and 4,  $A_b$  and  $B_b$  are the sums of the interaction energies between backbone atoms,  $A_s$  is the sum of the backbone-side-chain interactions and intra-side-chain interactions, and  $B_s$  is the sum of the backbone-side chain and inter-side-chain interactions.  $A_s$  was minimized with respect to  $\chi_i$  at fixed  $\phi_i$  and  $\psi_i$ , starting at the three minima ( $\pm 60$  and  $180^\circ$ ) of the torsional energy around the  $C^\alpha-C^\beta$  bond. The minimum-energy values of  $\chi_i$  (at fixed  $\phi_i$  and  $\psi_i$ ) lie near  $60$ ,  $180$ , and  $-60^\circ$ , and are designated  $\chi_I$ ,  $\chi_{II}$ , and



**Figure 2.** Energy contour map  $A(\phi_i, \chi_I, \psi_i)$  of the dipeptide of valine in vacuum.  $\chi_I$  is the value of  $\chi_i$  obtained by minimizing  $A_s(\phi_i, \chi_i, \psi_i)$  with respect to  $\chi_i$  at each fixed value of  $\phi_i$  and  $\psi_i$ , starting at  $\chi_i = 60^\circ$ .  $\times$  is the minimum point of  $A(\phi_i, \chi_I, \psi_i)$  in the  $\phi$ - $\psi$  plane.

$\chi_{III}$ , respectively. However, some of the corresponding values of  $A_s(\phi_i, \chi_\rho, \psi_i)$  (with  $\rho = I, II$ , or  $III$ ), which depend on  $\phi_i$  and  $\psi_i$ , are very high; i.e., the side-chain conformations for these particular backbone conformations are not allowed. Therefore, the number of allowed values of  $\chi_i$  depends on the backbone conformation  $\phi_i$  and  $\psi_i$ .  $B_s$  was minimized with respect to  $\chi_i$  and  $\chi_{i+1}$  at various values of  $\psi_i$  and  $\phi_{i+1}$  where the dipeptide energy  $A(\phi_i, \chi_\rho, \psi_i)$  (with  $\rho = I, II$ , or  $III$ ) is low (in the  $\phi_i$ - $\psi_i$  plane), with the value of  $\phi_{i+1}$  in  $B_s(\chi_i, \psi_i, \phi_{i+1}, \chi_{i+1})$  taken equal to that of  $\phi_i$  in  $A(\phi_i, \chi_\rho, \psi_i)$ . The minimum-energy values of  $\chi_i$  and  $\chi_{i+1}$  lie within a few degrees of  $\pm 60$  and  $180^\circ$ . Thus, even when side-chain-side-chain interactions are allowed (as in  $B_s$ ), the torsional energy dominates to determine the minimum-energy values of  $\chi_i$  and  $\chi_{i+1}$ , as it does when  $A_s$  is minimized.

The energy maps of the dipeptide,  $A(\phi_i, \chi_I, \psi_i)$ ,  $A(\phi_i, \chi_{II}, \psi_i)$  and  $A(\phi_i, \chi_{III}, \psi_i)$  are shown in Figures 2, 3, and 4, respectively. The number of values of  $\chi_\rho$  (with  $\rho = I, II$ , or  $III$ ) where  $A(\phi_i, \chi_\rho, \psi_i)$  is low (i.e., within 3.9 kcal above the global minimum) when  $A_s$  is minimized with respect to  $\chi_i$  at fixed  $\phi_i$  and  $\psi_i$  is shown in Figure 5 to illustrate the number of allowed positions of the side chain at each backbone conformation. It can be seen that, near the  $\beta$  conformation [which is the minimum of  $A(\phi_i, \chi_I, \psi_i)$ ], all three values of  $\chi_\rho$  are allowed but, near the right- ( $\alpha_R$ ) and left-handed ( $\alpha_L$ ) helices, only one position (viz.,  $\chi_{III}$ ) is allowed. From an examination of molecular models, it can be seen easily that, in the  $\alpha_R$  and  $\alpha_L$  conformations, the  $H_i^\gamma$  atoms are very close to the  $H_i^N$  atom and the  $C_i^\gamma$  atoms are very close to the  $O_{i-1}$  atom, when  $\chi_i \approx 60$  and  $180^\circ$ ; hence, repulsive interactions between these pairs of atoms exclude the  $\chi_I$  and  $\chi_{II}$  conformations. On the other hand, all three positions  $\chi_I$ ,  $\chi_{II}$ , and  $\chi_{III}$  have almost equal energies for a  $\beta$  backbone conformation.

**B. Helical State.** In the regular helical conformation, the energy was minimized with respect to  $\phi_i$ ,  $\psi_i$ , and  $\chi_i^1$  for an infinite chain, starting with the values<sup>10</sup>  $\phi = -48.4^\circ$  and  $\psi = -57.3^\circ$  for the right-handed  $\alpha$  helix, and  $\chi^1 = \pm 60$  and  $180^\circ$ . Besides the steric interactions in  $A(\phi_i, \psi_i, \chi_I$  or  $\chi_{II})$  at  $\alpha_R$ , cited above, there are additional repulsions between the  $C=O$  of the  $i$ th residue and the  $C^\gamma H_3$  of the  $(i+3)$ th

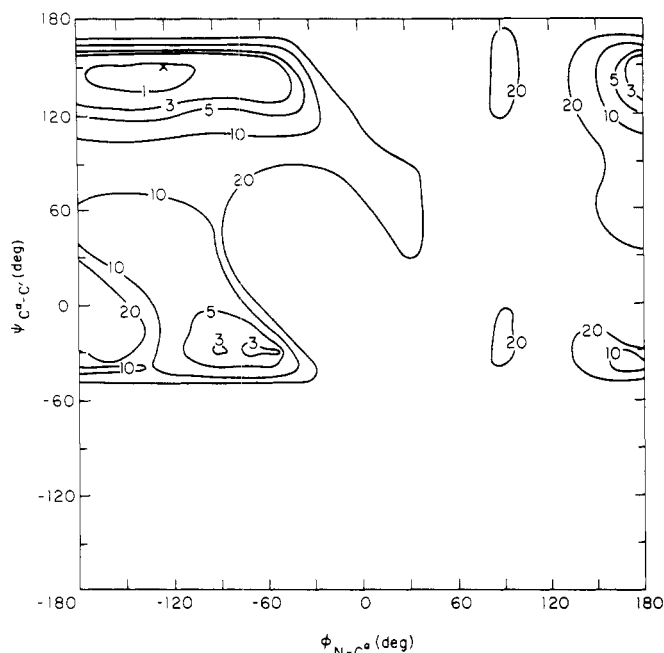


Figure 3. Same as Figure 2, but for  $\chi_{II}$ , starting at  $\chi_i = 180^\circ$ .

residue when  $\rho = I$  or  $II$ ; thus,  $\chi_{III}$  is the only allowed position of the side chain in the  $\alpha_R$  conformation. The minimum-energy conformation of the regular  $\alpha_R$  helix of poly(L-valine) is  $\phi = -48.6^\circ$ ,  $\psi = -57.8^\circ$ , and  $\chi^1 = -73.7^\circ$ .

## II. Free Energy (Including Entropy Contribution from Side-Chain Rotation)

In the case of poly(L-alanine), the entropy contributions from the rotational motion of the side chains to the residues in the helical and coil states are almost the same, and therefore cancel in the calculation<sup>4</sup> of  $s$ . However, in the case of poly(L-valine), this cancellation does not occur because the interactions between the backbone and side chain differ in the helical and coil states, as seen in section I. The entropy contribution from the side-chain rotation was computed from a parabolic approximation of  $A_s(\phi_i, \chi_\rho, \psi_i)$  at the minimum points  $\chi_I$ ,  $\chi_{II}$ , and  $\chi_{III}$ . Thus,  $A(\phi_i, \chi_i, \psi_i)$  was approximated as

$$A(\phi_i, \chi_i, \psi_i) = A(\phi_i, \chi_\rho, \psi_i) + \frac{1}{2} \frac{\partial^2 A_s(\phi_i, \chi_i, \psi_i)}{\partial \chi_i^2} \bigg|_{\chi_\rho} (\chi_i - \chi_\rho)^2 \quad (5)$$

where  $\rho = I, II$ , or  $III$  correspond to the possible minima of  $A_s$  at fixed  $\phi_i$  and  $\psi_i$  since  $A_b$  does not depend on  $\chi_i$ .

**A. Coil State.** The free energy of the coil was calculated for three different degrees of approximation, viz., the case where interactions up to  $F^{(3)}$  are included (approximation 1), the case where interactions up to  $F^{(4)}$  are included (approximation 2), and the case where interactions up to  $F^{(6)}$  are included (approximation 3). Since the sum of  $F^{(0)}$ ,  $F^{(1)}$ ,  $F^{(2)}$ , and  $F^{(3)}$  is the sum of all pair interactions in a dipeptide whose conformation is described by a set of values of  $\phi_i$ ,  $\psi_i$ , and  $\chi_i$ , approximation 1 is the dipeptide approximation. Approximations 1 and 2 will be described in this section, but approximation 3 will be deferred until section IV.

For approximation 1, only  $A(\phi_i, \chi_i, \psi_i)$  is taken into account. As shown in paper III,<sup>5</sup> the free energy of the coil state under vacuum is given by

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

where

$$\lambda_1 \Delta^2 = \int_0^{2\pi} \int_0^{2\pi} \int_0^{2\pi} \exp[-\beta A(\phi, \chi, \psi)] d\phi d\chi d\psi \quad (II-30)$$

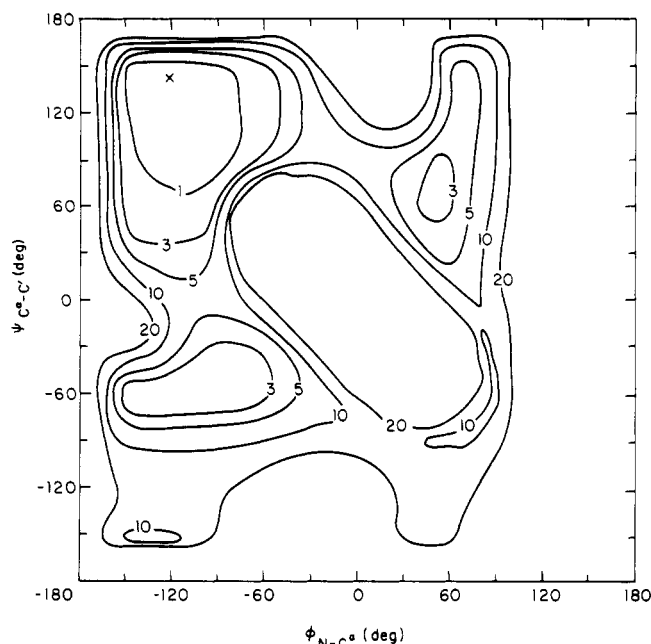


Figure 4. Same as Figure 2, but for  $\chi_{III}$ , starting at  $\chi_i = -60^\circ$ .

and  $\beta \equiv 1/RT$ . From eq 5 and II-30,  $\lambda_1 \Delta^2$  is given by

$$\lambda_1 \Delta^2 = (2\pi RT)^{1/2} \sum_{\rho=1}^{III} \int_0^{2\pi} \int_0^{2\pi} \left[ \frac{\partial^2 A_s(\phi, \chi, \psi)}{\partial \chi^2} \bigg|_{\chi_\rho} \right]^{-1/2} \times \exp[-\beta A(\phi, \chi_\rho, \psi)] d\phi d\psi \quad (II-31)$$

$H_c^{(0)}$  is given by

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

where  $\langle A \rangle$  is defined by

$$\langle A \rangle = \frac{\int_0^{2\pi} \int_0^{2\pi} \int_0^{2\pi} A(\phi, \chi, \psi) \exp[-\beta A(\phi, \chi, \psi)] d\phi d\chi d\psi}{\int_0^{2\pi} \int_0^{2\pi} \int_0^{2\pi} \exp[-\beta A(\phi, \chi, \psi)] d\phi d\chi d\psi} = \frac{\sum_{\rho=1}^{III} \int_0^{2\pi} \int_0^{2\pi} A(\phi, \chi_\rho, \psi) \left[ \frac{\partial^2 A_s}{\partial \chi^2} \bigg|_{\chi_\rho} \right]^{-1/2} \exp[-\beta A(\phi, \chi_\rho, \psi)] d\phi d\psi}{\sum_{\rho=1}^{III} \int_0^{2\pi} \int_0^{2\pi} \left[ \frac{\partial^2 A_s}{\partial \chi^2} \bigg|_{\chi_\rho} \right]^{-1/2} \exp[-\beta A(\phi, \chi_\rho, \psi)] d\phi d\psi} \quad (6)$$

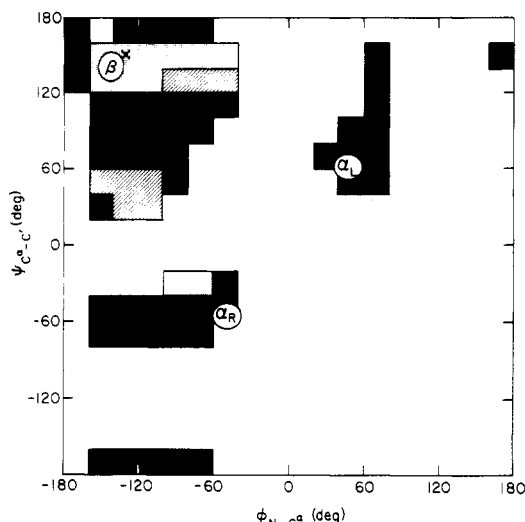
The entropy  $S_c^{(0)}$  is calculated from eq III-2 and III-3 as

$$S_c^{(0)} = \langle A \rangle / T - R + R \ln \left\{ \frac{\sum_{\rho=1}^{III} \int_0^{2\pi} \int_0^{2\pi} \left[ \frac{\partial^2 A_s}{\partial \chi^2} \bigg|_{\chi_\rho} \right]^{-1/2} \exp[-\beta A(\phi, \chi_\rho, \psi)] d\phi d\psi}{(2\pi RT)^{1/2}} \right\} \quad (7)$$

For the evaluation of the integrals with respect to  $\phi$  and  $\psi$  in eq II-31 and 6, the matrix  $\mathbf{R}_\rho$  (with  $\rho = I, II$ , or  $III$ ), whose  $(j, k)$  element is defined by eq 8, was used, where  $N$  was taken as 18; i.e.,  $A(\phi, \chi_\rho, \psi)$  was computed at every  $20^\circ$  in  $\phi$  and  $\psi$ .

$$\mathbf{R}_\rho(j, k) = (2\pi RT)^{1/2} \left[ \frac{\partial^2 A_s(2\pi j/N, \chi_\rho, 2\pi k/N)}{\partial \chi^2} \bigg|_{\chi_\rho} \right]^{-1/2} \times \exp[-\beta A(2\pi j/N, \chi_\rho, 2\pi k/N)] \quad (j, k = 1, 2, \dots, N) \quad (8)$$

The contribution from the freedom of rotation of the side chain to the statistical weight at a certain backbone conformation,  $[\partial^2 A_s / \partial \chi^2]_{\chi_\rho}^{-1/2}$ , is shown in Figures 6, 7, and 8



**Figure 5.** The number of values of  $\chi_\rho$  (with  $\rho = \text{I, II or III}$ ) where  $A(\phi_i, \chi_\rho, \psi_i)$  is low (i.e., within 3.9 kcal above the global minimum) when  $A_s(\phi_i, \chi_i, \psi_i)$  is minimized with respect to  $\chi_i$  at fixed  $\phi_i$  and  $\psi_i$ .  $A > 3.9$  kcal/mol in the blank region. [Blank], [Diagonal lines], and [Solid black] are regions where three, two, and one values of  $\rho$ , respectively, are available for low  $A(\phi_i, \chi_\rho, \psi_i)$ .

for  $\rho = \text{I, II, and III}$ , respectively. By comparing Figures 6, 7, and 8 with Figures 2, 3, and 4, respectively, it can be seen that  $[\partial^2 A_s / \partial \chi^2]_{\chi_1}^{-1/2}$  has a high value when the value of  $A(\phi, \chi_\rho, \psi)$  is low; thus, when the Boltzmann factor  $\exp[-\beta A(\phi, \chi_\rho, \psi)]$  is high, the freedom of rotation of the side chain is high. Therefore, the probabilities for the occurrence of low-energy conformations of the valine dipeptide are enhanced by this entropy contribution from the rotation of the side chain.

For approximation 2, where the interactions from  $F^{(0)}$  to  $F^{(4)}$  are included, the free energy is still given by eq III-2; however,  $\lambda_1 \Delta^2$  is not obtained from eq II-30 but rather from the largest eigenvalue<sup>3</sup> of the matrix product  $\mathcal{R}\mathcal{S}$ , where  $\mathcal{R}$  and  $\mathcal{S}$  are defined by

$$\mathcal{R} = \begin{bmatrix} \mathbf{R}_\text{I} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_\text{II} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R}_\text{III} \end{bmatrix} \quad (9)$$

and

$$\mathcal{S} = \begin{bmatrix} \mathbf{S}_{\text{I,I}} & \mathbf{S}_{\text{I,II}} & \mathbf{S}_{\text{I,III}} \\ \mathbf{S}_{\text{II,I}} & \mathbf{S}_{\text{II,II}} & \mathbf{S}_{\text{II,III}} \\ \mathbf{S}_{\text{III,I}} & \mathbf{S}_{\text{III,II}} & \mathbf{S}_{\text{III,III}} \end{bmatrix} \quad (10)$$

where  $\mathbf{0}$  is the zero matrix,  $\mathbf{R}_\rho$  (with  $\rho = \text{I, II, or III}$ ) is the matrix defined by eq 8, and  $\mathbf{S}_{\rho,\eta}$  (with  $\rho, \eta = \text{I, II, or III}$ ) is the matrix whose  $(j,k)$  element is given by

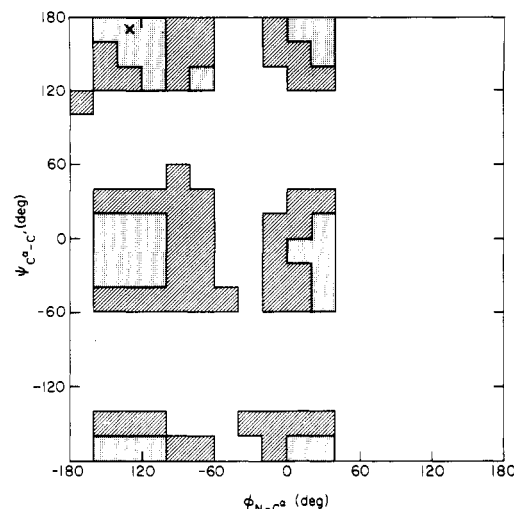
$$\mathbf{S}_{\rho,\eta}(j,k) = \exp[-\beta B(\chi_\rho 2\pi j/N, 2\pi k/N, \chi_\eta)] \quad (11)$$

Since  $N$  was taken equal to 18 (i.e.,  $\Delta = 2\pi/18$ ),  $\mathcal{R}$  and  $\mathcal{S}$  are  $54 \times 54$  dimensional matrices. When  $\lambda_1 \Delta^2$  is calculated as a function of temperature from the largest eigenvalue of  $\mathcal{R}\mathcal{S}$ ,  $H_c^{(0)}$  and  $S_c^{(0)}$  are obtained from the intercept and slope, respectively, of a plot of  $-RT \ln(\lambda_1 \Delta^2 / 2\pi RT)$  vs.  $T$  (see eq III-2).

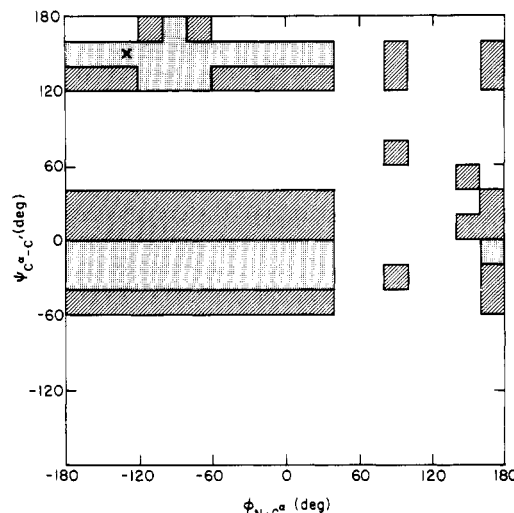
**B. Helical State.** From eq II-17 and III-4, the enthalpy  $H_h^{(0)}$  of a residue in a helical state under vacuum is expressed by

$$H_h^{(0)} = \sum_{i=0}^{\infty} E_i \quad (12)$$

where  $E_i$  represents the interaction energies between the



**Figure 6.** The contribution from the freedom of rotation of the side chain,  $[\partial^2 A_s(\phi, \chi, \psi) / \partial \chi^2]_{\chi_1}^{-1/2}$  in  $\text{kcal}^{-1/2}$  to the statistical weights of various conformations of valine under vacuum.  $x$  is the minimum of  $A(\phi, \chi_1, \psi)$  in the  $\phi$ - $\psi$  space. [Blank], [Diagonal lines], and [Solid black] are regions where  $[\partial^2 A_s / \partial \chi^2]_{\chi_1}^{-1/2}$  is more than 0.1, less than 0.1 but more than 0.05, and less than 0.05, respectively.



**Figure 7.** Same as Figure 6, but for  $\rho = \text{II}$ .

$j$ th and  $(j+i)$ th groups in a helical sequence (see Figure 2 of paper II for the grouping). Considering the screening effect of the long-range electrostatic interactions, as discussed in the previous papers,<sup>4,5</sup> the enthalpy contribution is expressed by

$$H_{h,\text{cut}}^{(0)} = \sum_{i=0}^4 E_i \quad (13)$$

The entropy,  $S_h^{(0)}$ , of a residue in a helical state is given by eq III-5, where  $g$  is defined by eq II-12. The  $(k,l)$  element of the matrix  $\mathbf{A}_j$  in eq II-12 is given by

$$a_{kl} = \left[ \frac{\partial^2 F_j(q)}{\partial q_k \partial q_l} \right]_{q_0} \quad (\text{I-25})$$

where  $q$  is an independent variable (dihedral angle),  $q_0$  is the minimum-energy conformation and  $F_j(q)$  is the total energy of a helical sequence of  $j+1$  peptide units. For polyglycine and poly(L-alanine), where  $\chi$  was fixed,  $q_k$  and  $q_l$  (with  $k, l = 1, 2, \dots, 2j$ ) in eq I-25 are the dihedral angles of the backbone,  $\phi_1, \psi_1, \phi_2, \dots, \phi_j, \psi_j$ . However, for poly(L-valine), where the side-chain dihedral angles  $\chi_i$ 's must be considered,  $q_k$  and  $q_l$  (with  $k, l = 1, 2, \dots, 2j$ ) are the dihe-

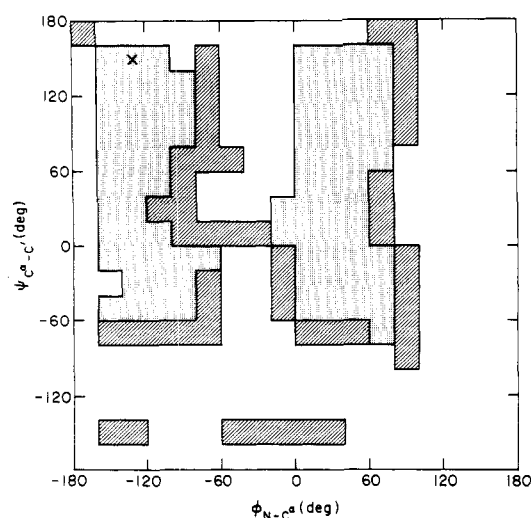


Figure 8. Same as Figure 6, but for  $\rho = \text{III}$ .

dihedral angles of the backbone,  $\phi_1, \psi_1, \phi_2, \dots, \phi_j, \psi_j$ , and (with  $k, l = 2j + 1, \dots, 3j$ ) are the dihedral angles of the side chains,  $\chi_1, \chi_2, \dots, \chi_{j-1}, \chi_j$ . As discussed in paper I, the matrix  $\mathbf{A}_j$  exhibits a periodicity and "diagonal" character because of the helical structure. Also, from the calculation of the matrix  $\mathbf{A}_j$  for polyglycine and poly(L-alanine),<sup>5</sup> it was concluded that from 80 to 90% of the values of the diagonal elements of  $\mathbf{A}_j$  arise from the hydrogen bond energy, and that the "nondiagonal" elements of  $\mathbf{A}_j$  (*viz.*,  $a_{k,k+8}, a_{k,k+9}, \dots$ ) were negligibly small compared to the "diagonal" elements (*viz.*,  $a_{k,k}, a_{k,k+1}, \dots$  and  $a_{k,k+7}$ ); the width of the band of the "diagonal" elements is a reflection of the correlation between the backbone dihedral angles which are restricted by the hydrogen bond in the  $\alpha$  helix. In contrast to polyglycine and poly(L-alanine), the elements  $a_{kl}$  for poly(L-valine) are the sums of the second derivatives of the interaction energies between backbone and backbone, between side chain and backbone, between side chain and side chain, and between the atoms in a given side chain in a helical sequence of  $j + 1$  units. These interaction energies (all of the above except that between backbone and backbone) are the nonbonded energies and the torsional energies for rotating around the single bonds in the side chains, but no hydrogen-bond energies are included. Thus, the longer range interactions contribute a lesser amount to the second derivative (with respect to the dihedral angles) of the sum of the interaction energies between backbone and side chain, between side chain and side chain, and between the atoms in a given side chain. Therefore, one would expect that the additional elements  $a_{kl}$  for poly(L-valine), beyond those that already appeared in  $\mathbf{A}_j$  for polyglycine and poly(L-alanine), would have a more "diagonal" character than those for the backbone (with  $q_k$  and  $q_l$  being  $\phi$ 's and  $\psi$ 's). Thus, among the new elements  $a_{kl}$  for poly(L-valine), beyond those for the backbone, the off-diagonal elements (*i.e.*, those for which  $k \neq l$ ) were approximated as zero and only the diagonal elements  $a_{kk}$  (with  $k = 2j + 1, \dots, 3j$ ), *i.e.*,  $\partial^2 F_j / \partial \chi_k^2$  (with  $k = 1, 2, \dots, j$ ) were taken into account. Hence,  $g$  in eq II-12 for poly(L-valine) is divided into two factors, one ( $g_b$ ) being the contribution from the second derivatives with respect to the backbone dihedral angles only (*e.g.*,  $\partial^2 F_j / \partial \phi_2 \partial \phi_4$ ) and the other ( $g_s$ ) being the contribution from the second derivatives with respect to the side-chain dihedral angles only (*e.g.*,  $\partial^2 F_j / \partial \chi_2^2$ ); thus

$$g = g_b g_s \quad (14)$$

In eq 14,  $g_b$  is defined by eq II-12 with  $\mathbf{A}$  and  $g$  being replaced by  $\mathbf{A}_b$  and  $g_b$ , respectively; thus,  $g_b$  is given by

Table I  
Thermodynamic Parameters<sup>a</sup> for a Helical Residue of Poly(L-valine)

$H_{h,\text{cut}}^{(0)}$	-9.76 kcal/mol
$S_h^{(0)}$	-15.37 eu
$-R \ln g_b$	-10.60 eu
$-R \ln g_s$	-4.77 eu

<sup>a</sup> These are independent of temperature.

$$g_b = \lim_{j \rightarrow \infty} (\det \mathbf{A}_{b,j+1} / \det \mathbf{A}_{b,j})^{1/2} \quad (15)$$

where the matrix  $\mathbf{A}_{b,j}$  is a submatrix of the matrix  $\mathbf{A}_j$ , and the elements of  $\mathbf{A}_{b,j}$  are the second derivatives of  $F_j$  with respect to the backbone dihedral angles, *i.e.*

$$a_{b,kl} = \left[ \frac{\partial^2 F_j}{\partial q_k \partial q_l} \right]_{q_0} \quad (16)$$

with  $k, l = 1, 2, \dots, 2j$  or  $q_k, q_l = \phi_1, \psi_1, \dots, \phi_j, \psi_j$ . The factor  $g_s$  in eq 14 is defined by

$$g_s = \lim_{j \rightarrow \infty} \left[ \frac{(\partial^2 F_{j+1} / \partial \chi_1^2)(\partial^2 F_{j+1} / \partial \chi_2^2) \cdots (\partial^2 F_{j+1} / \partial \chi_{j+1}^2)}{(\partial^2 F_j / \partial \chi_1^2)(\partial^2 F_j / \partial \chi_2^2) \cdots (\partial^2 F_j / \partial \chi_j^2)} \right]_{\chi_{\text{III}}}^{1/2} \quad (17)$$

For a sufficiently large value of  $m$  (taken as  $m = 7$  in the calculations performed here), eq 17 may be replaced by

$$g_s = (\partial^2 F_{2m} / \partial \chi_m^2)_{\chi_{\text{III}}}^{1/2} \quad (18)$$

Thus, the entropy of a residue in a helical sequence under vacuum is given by

$$S_h^{(0)} = -R \ln g_b - R \ln g_s \quad (19)$$

Recently, we proposed a simple method<sup>11</sup> to calculate  $g_b$  directly from the matrix elements of  $\mathbf{A}_{b,j}$ , rather than from eq 15, and this method was used in the present paper for the evaluation of  $g_b$ . The values of  $H_{b,\text{cut}}^{(0)}$ ,  $-R \ln g_b$ ,  $-R \ln g_s$ , and  $S_h^{(0)}$  for poly(L-valine) are given in Table I.

### III. Effect of Solvent (Water)

When the helix-coil transition of poly(L-valine) in water is treated, there are considered to be two additional sources of free energy which affect the parameter  $s$ , *viz.*, (1) the free-energy change accompanying the binding of water molecules to the free NH and CO groups in the coil state, and (2) the free-energy change accompanying the removal of water molecules from the first layer of hydration around the side-chain methyl groups when the atoms of the poly(amino acid) approach the methyl groups within a certain distance; the latter includes the hydrophobic interactions<sup>12</sup> between the side chains and between side chains and backbone of poly(L-valine) in both the helical and coil states. Solvent effect (1) favors the coil state over the helix, whereas solvent effect (2) favors the helix over the coil because the  $C\gamma$  atoms of the  $i$ th residue approach the backbone and side-chain atoms of the  $i$ , ( $i \pm 1$ ), ( $i \pm 3$ ), and ( $i \pm 4$ )th residues more closely in the  $\alpha$  helix than in the random coil.

**A. Water Binding to NH and CO.** The free-energy change accompanying the binding of water molecules to the free NH and CO groups in the coil state (*i.e.*, in the conformation in which the NH and CO do not form an intramolecular hydrogen bond) was determined semiempirically in the case of poly(L-alanine) in water.<sup>5</sup> It is reasonable to use the same free-energy change in the case of poly(L-valine) in water if we make the assumption that the binding of water to the free NH and CO groups is not influenced by the side-chain atoms. In this approximation, the NH and CO groups were not allowed to lose their bound water even if they approach the  $C\gamma H_3$  groups. Thus, the change in hydration around the N,  $H^N$ ,  $C'$ , and O atoms was taken into account

only in terms of water binding, using the same binding constant determined for poly(L-alanine). Hence, the enthalpy and entropy contributions to  $s$  from water binding to the free NH and CO groups in the coil state<sup>13</sup> are given by

$$\Delta H_b = 2\Delta H_B K\alpha(1 + K\alpha)^{-1} \quad (20)$$

and

$$\Delta S_b = 2R \ln(1 + K\alpha) + [2\Delta H_B T^{-1} K\alpha / (1 + K\alpha)] \quad (21)$$

respectively, where

$$K\alpha = \exp[-(\Delta H_B - T\Delta S_B)/RT] \quad (\text{III-13})$$

and the values of  $\Delta H_B$  and  $\Delta S_B$  are the same as those determined empirically for poly(L-alanine) in water.

**B. Change of Hydration around the Methyl Groups.** When  $\Delta H_B$  and  $\Delta S_B$  were determined<sup>5</sup> from the difference between the calculated value of  $s$  for poly(L-alanine) under vacuum and the experimental result for this poly(amino acid) in water, the effect of hydrophobic bonds was neglected since their contribution to  $s$  is much smaller than that from water binding to the free NH and CO groups. Since we use the same values of  $\Delta H_B$  and  $\Delta S_B$  for poly(L-valine), we will assume that there is no difference in the first hydration layers of the  $C^\alpha$  and  $C^\beta$  groups between the helical and coil forms, even when the  $C^\alpha$  and  $C^\beta$  interact with the  $C^\gamma$ 's; the correctness of this assumption will be discussed in section VI. Thus, we consider only the change in hydration around the  $C^\gamma$  methyl groups for poly(L-valine). The effect of this change in hydration of the methyl groups in the helix-coil transition can be manifested in two ways, *viz.*, (i) by a change in the value of the conformational free energy and (ii) by a change in the second derivative of the free energy, *i.e.*, in the curvature of the free-energy surface, giving rise to an additional change in entropy.

If  $Y_h$  and  $Y_c$  are the number of water molecules in the first hydration layers of the methyl groups in the helix and coil forms, respectively, then the difference,  $Y_h - Y_c = \Delta Y$ , is the change in hydration accompanying the coil-to-helix transition. The value of  $\Delta Y$  was computed to determine the change in hydration, accompanying the change in conformation (*i.e.*, change in  $\phi$ 's,  $\psi$ 's, and  $\chi$ 's), as recommended by Gibson and Scheraga.<sup>14</sup>

The change in hydration of the NH and CO groups was taken into account in terms of water binding, as described by eq 20 and 21. However, the value of  $\Delta Y$  was computed for the interaction of the hydrated  $C^\gamma H_3$  groups with hydrated  $C^\alpha H$ ,  $C^\beta H$ , and  $C^\gamma H_3$  groups and N,  $H^N$ , C, and O atoms. When the number of waters of hydration was computed, the  $C^\gamma H_3$ ,  $C^\beta H$ , and  $C^\alpha H$  groups were taken as unit-ed atoms; however, separate C and H atoms were used for the nonbonded interactions under vacuum. The maximum value of  $Y_h$  and  $Y_c$  for one methyl group of valine was taken as 6; thus, a maximum of 12 water molecules could be hydrated around the  $C^\gamma H_3$  groups of a residue.

For the coil state, the value of  $Y_c$  for the methyl groups in the dipeptide was calculated as a function of  $\phi$  and  $\psi$ , with  $\chi$  fixed at  $\chi_I$ ,  $\chi_{II}$ , and  $\chi_{III}$ , respectively. The results obtained show that  $Y_c$  is insensitive to changes in the values of  $\phi$  and  $\psi$  in the sterically allowed regions. In other words, the free energy of hydration of the methyl groups is essentially constant over the  $(\phi, \chi, \psi)$  plane, compared to  $A(\phi, \chi, \psi)$  which varies with  $\phi$  and  $\psi$  at fixed  $\chi_\rho$ . Therefore, hydration effect (ii) is negligible compared with the contribution from the curvature of the  $A(\phi, \chi, \psi)$  surface under vacuum. Since only hydration effect (i) contributes to the free energy of the coil, and since this effect is essentially independent of  $\phi$  and  $\psi$  at fixed  $\chi_\rho$ , the number of water molecules in the first layer around the  $C^\gamma$ 's was calculated for the  $\beta$  conformation ( $\phi = -130^\circ$ ,  $\psi = 150^\circ$ ) for which  $A(\phi, \chi, \psi)$  is a min-

imum (making the  $\beta$  conformation the predominant one for the dipeptide). For the helical state, whose conformational entropy originates (roughly speaking) from a narrower volume of conformational space than that allowed for the random coil, hydration effect (ii) is negligible. Hence, only hydration effect (i) pertains to the helix; it was calculated for the values of  $\phi$ ,  $\psi$ ,  $\chi_{III}$  which were obtained by energy minimization under vacuum.

Once  $\Delta Y$  is known for a given interaction, then the corresponding change in free energy is given<sup>12</sup> by

$$\Delta F_w = (957 - 6.08T + 0.00824T^2)\Delta Y \quad (22)$$

in cal/mol of residues for  $T$  in  $^\circ K$ . The quantity  $\Delta F_w$  is to be added to that already computed for the coil-to-helix transition (*i.e.*, for  $s$ ) in vacuum. Since there are three  $\beta$  conformations, corresponding to the three minimum-energy values of  $\chi_\rho$ , the value of  $Y_c$  used to compute  $\Delta Y$  was taken as the average of the three values for the three  $\beta$  conformations. The numerical values of  $Y_c$  depend on whether the longer range interactions in the random coil are taken into account; this higher approximation will be discussed in section IV. The corresponding enthalpy and entropy changes are

$$\Delta H_w = (957 - 0.00824T^2)\Delta Y \quad (23)$$

in cal/mol of residues and

$$\Delta S_w = (6.08 - 0.01648T)\Delta Y \quad (24)$$

in eu, respectively. Since the hydration effect (ii) has been neglected, the only remaining contribution to the entropy of hydration is that ( $\Delta S_w$ ) due to  $\Delta Y$  in the coil-to-helix transition;  $\Delta S_w$  dominates<sup>12</sup> over  $\Delta H_w$  in leading to a favorable value of  $\Delta F_w$ .

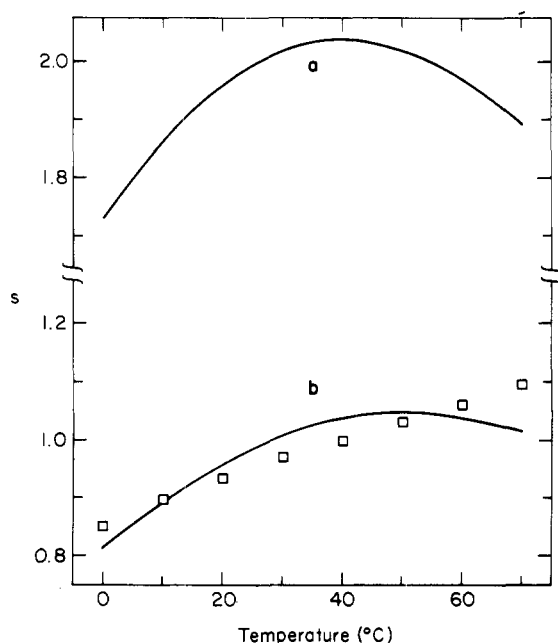
#### IV. Further Interactions Beyond the Dipeptide Approximation in the Random Coil

Under the  $\theta$  condition,<sup>15</sup> the dimensions of the random coil depend only on short-range interactions. However, we have no theoretical basis to determine how far these "short-range" interactions extend. Therefore, it would be desirable to determine this range empirically for polyamino acids by comparing the experimental value of  $s$  at the  $\theta$  condition with the theoretical value, computed for several different approximations of the range of interaction in the coil. Unfortunately, it is fairly difficult to attain the  $\theta$  condition in experimental systems involving polypeptides.<sup>16</sup> Thus, we are forced to assume that the experimental conditions do not differ very much from the (ideal)  $\theta$  condition and, therefore, that the experimental data can be compared with the results computed under the assumption that we can neglect the longer range interactions along the polymer chain (beyond a range that is to be determined). Specifically, we will determine the range of the interatomic interactions empirically for poly(L-valine) in water by comparing the experimental value of  $s$  with the theoretical value, computed for several different approximations of the range of interaction in the coil. Thus far, we have considered approximations 1 and 2, by taking interactions up to  $F^{(3)}$  and  $F^{(4)}$ , respectively. In this section, we will treat approximation 3. To see the difference between approximations 1 and 2, *i.e.*, the effect of  $F^{(4)}$ , the thermodynamic parameters for the coil form of polyglycine,<sup>5</sup> poly(L-alanine),<sup>5</sup> and poly(L-valine), respectively, in vacuum are compared in Table II. The contribution of  $F^{(4)}$  to the entropy of the coil is almost negligible for the three poly(amino acids), but that to the enthalpy is significant (becoming more negative with increasing size of side chain). Further, the contribution to the enthalpy term is comparable to the value of  $F^{(4)}$  itself at the minimum in  $A(\phi, \chi, \psi)$ , *i.e.*, at the  $\beta$  conformation, as

**Table II**  
Contribution of  $F^{(4)}$  to the Entropy and Enthalpy of the Coil under Vacuum

Poly- (amino acid)	Approximation (1) <sup>a</sup>		Approximation (2) <sup>b</sup>		Difference		$F^{(4)}(\beta)$ (kcal)
	$H_{c,1}^{(0)}$ (kcal)	$S_{c,1}^{(0)}$ (eu)	$H_{c,2}^{(0)}$ (kcal)	$S_{c,2}^{(0)}$ (eu)	$H_{c,2}^{(0)} - H_{c,1}^{(0)}$ (kcal)	$S_{c,2}^{(0)} - S_{c,1}^{(0)}$ (eu)	
Polyglycine	1.39	0.59	1.30	0.55	-0.09	-0.04	-0.096
Poly(L-alanine)	-0.20	-1.15	-0.38	-1.11	-0.18	0.04	-0.302
Poly(L-valine)	-4.02	-5.14	-4.62	-5.18	-0.61	-0.04	-0.61 <sup>c</sup>

<sup>a</sup> Including interactions in the random coil from  $F^{(0)}$  up to  $F^{(3)}$ . <sup>b</sup> Including interactions in the random coil from  $F^{(0)}$  up to  $F^{(4)}$ . <sup>c</sup> Obtained by averaging over nine pairs of values of  $\chi_i$  and  $\chi_{i+1}$ .



**Figure 9.**  $s$  vs.  $T$  curves for poly(L-valine) in water. (a) and (b) were calculated with approximations 2 and 3, respectively. The squares are the experimental results.<sup>7</sup>

shown in Table II. The negligible contribution of  $F^{(4)}$  to the entropy term originates from the flatness of  $F^{(4)}$  compared to that of  $A(\phi, \chi, \psi)$ , which is equal to the sum of  $F^{(0)}$ ,  $F^{(1)}$ ,  $F^{(2)}$ ,  $F^{(3)}$ . This behavior is easily understood from the fact that the  $\beta$  conformation is the most probable one in the dipeptide approximation, and the  $F^{(4)}$  interaction contributes only a small correction; i.e.,  $F^{(4)}$  is small compared to  $A(\phi, \chi, \psi)$  and does not depend on the variable dihedral angles as much as  $A(\phi, \chi, \psi)$  does. We assume that this behavior applies to the higher interactions  $F^{(5)}$ ,  $F^{(6)}$ , etc., and, hence, calculate only the enthalpy contribution, neglecting the entropy contribution.<sup>17</sup> Thus, it seems like a good approximation to calculate the entropy of the coil from  $A(\phi, \chi, \psi)$ , and use the longer range interactions to calculate the additional enthalpy (beyond the dipeptide), with the additional portions of the chain maintained in the  $\beta$  conformation. A similar treatment was required for poly(L-lysine).<sup>19</sup>

The enthalpy and entropy of the coil in the dipeptide approximation are given by eq III-3 and 7, respectively. When the further interactions are included, the enthalpy of the coil becomes

$$H_c^{(0)} = \langle A \rangle - RT + \sum_{j=4}^{k(\theta)} F^{(j)}(\phi_\beta' s, \chi_\beta' s, \psi_\beta' s) \quad (25)$$

where  $k(\theta)$  is to be determined experimentally. When more than one variable dihedral angle appears in  $F^{(j)}$  in eq 25,  $F^{(j)}(\phi_\beta' s, \chi_\beta' s, \psi_\beta' s)$  should be understood as an average value over pairs of  $\chi$  values ( $\chi_I$ ,  $\chi_{II}$ , and  $\chi_{III}$ ) since the ener-

**Table III**  
Values of Hydration Numbers and  $H_c^{(0)}$  for Poly(L-valine)

Approximation	$Y_h$	$Y_c$	$\Delta Y$	$H_c^{(0)}$ (kcal/mol)
	mol/Residue			
2 <sup>a</sup>	3.6	8.0	-4.4	-4.62
3 <sup>b</sup>	3.6	7.8	-4.2	-5.01

<sup>a</sup> Including interactions in the coil from  $F^{(0)}$  up to  $F^{(4)}$ .

<sup>b</sup> Including interactions in the coil from  $F^{(0)}$  up to  $F^{(6)}$ .

gies for the three values of  $\chi_\rho$  are almost equal in the  $\beta$  conformation. The terms  $F^{(5)}$ ,  $F^{(6)}$ , etc., are negligible for polyglycine and poly(L-alanine) but not so for poly(L-valine). Because of the presence of the branched side chain in the valine polymer, some of the backbone and side-chain atoms (whose interactions are included in  $F^{(5)}$ ,  $F^{(6)}$ , etc.) are near each other in the  $\beta$  conformation. Thus, the nonbonded side-chain–backbone and side-chain–side-chain interactions give rise to non-negligible values of  $F^{(5)}$ ,  $F^{(6)}$ , etc., in the  $\beta$  conformation, and are included in eq 25. In addition, the values of  $Y_c$  decrease in the presence of additional groups beyond those of the dipeptide, because of increased interactions with the atoms in the additional portions, and thus contribute to  $\Delta H_w$  and  $\Delta S_w$ .

### V. Dependence of $s$ on $T$ in Water

The enthalpy and entropy contributions to  $s$  in water are expressed as

$$\Delta H_s = H_{h, \text{cut}}^{(0)} - H_c^{(0)} - \Delta H_b - \Delta H_w \quad (26)$$

and

$$\Delta S_s = S_h^{(0)} - S_c^{(0)} - \Delta S_b - \Delta S_w \quad (27)$$

respectively. Since  $H_c^{(0)}$  in eq 26 is defined by eq 25, it contains the parameter  $k(\theta)$  which must be determined by comparing the calculated and experimental values of  $s$ . The parameter  $k(\theta)$  is also included in  $\Delta H_w$  of eq 26 and  $\Delta S_w$  of eq 27, since  $\Delta Y$  in eq 23 and 24 depends on longer range interactions than those in the dipeptide. We have evaluated  $\Delta H_s$  and  $\Delta S_s$  for approximations 2 and 3, and the numbers of hydrated water molecules around the methyl groups,  $Y_h$  for the  $\alpha$ -helix and  $Y_c$  for the  $\beta$  conformation, and their difference  $\Delta Y$ , together with the values of  $H_c^{(0)}$  for these two approximations are given in Table III.

The  $s$  vs.  $T$  curves are calculated with

$$S = \exp[-(\Delta H_s - T\Delta S_s)/RT] \quad (28)$$

for approximations 2 and 3, and are shown in Figure 9 along with experimental data obtained by the host-guest technique.<sup>7</sup> In approximation 2, the  $s$  vs.  $T$  curve is higher than the experimental one, and the  $\alpha$ -helix is more stable than the random coil between 0 and 70°. When  $F^{(5)}$  and  $F^{(6)}$  are included (approximation 3), curve b is obtained, which agrees fairly well with the experimental results, indi-

**Table IV**  
Thermodynamic Parameters for the Coil-to-Helix Transition in Poly(L-valine) in Water

$T$ (°K)	$\Delta F_s$ (kcal/mol)	$\Delta H_s$ (kcal/mol)	$\Delta S_s$ (eu)
280	0.077	1.401	4.73
293	0.024	1.006	3.35
300	0.003	0.785	2.61
320	-0.028	0.125	0.48
340	-0.016	-0.573	-1.64

cating that the helix is stable ( $s > 1$ ) only at higher temperature. When the further interactions beyond  $F^{(6)}$  are included [e.g.,  $F^{(7)}$  and  $F^{(8)}$ ], the calculated values of  $s$  become smaller than those for approximation 3; i.e., the  $\alpha$  helix becomes less stable. Thus,  $k(\theta)$  in eq 25 is taken as 6. The computed curves show maxima at around 40 and 50° for approximations 2 and 3, respectively.

The existence of the maximum in the  $s$  vs.  $T$  curve originates from the change in hydration in the coil-to-helix transition. If  $\Delta H_w$  and  $\Delta S_w$  were not included in eq 26 and 27, respectively,  $s$  would decrease monotonically between 0 and 70°, being 0.4 at 50°.

Since approximation 3 gives better agreement with experiment than does approximation 2, we will consider only approximation 3 in the remainder of this paper. The thermodynamic parameters for the coil-to-helix transition in poly(L-valine) in water are shown in Table IV. The experimental values<sup>7</sup> at 293°K are  $\Delta F_s = 0.040$  kcal/mol,  $\Delta H_s = 0.64 \pm 0.14$  kcal/mol,  $\Delta S_s = 2.05 \pm 0.47$  eu.

## VI. Discussion

By taking interactions up to  $F^{(6)}$  into account in the random coil, good agreement is attained between the calculated and experimental values of  $s$  for poly(L-valine) in water. The maximum in the  $s$  vs.  $T$  curve, and the range of values of  $s$ , have been shown to arise in large part from the changes in hydration around the methyl groups. This suggests that the same explanation should hold for poly(L-leucine) and poly(L-isoleucine) in water, and indeed experimental results for poly(L-leucine) in water obtained by two different techniques<sup>20,21</sup> show the existence of a maximum in the  $s$  vs.  $T$  curve.

Since the change in hydration plays an important role in determining  $s$  for poly(L-valine) in water, it is of interest to examine in detail the change in hydration around the nonpolar groups in the coil-to-helix transition. Actually, the hydration around  $C^\alpha H$  and  $C^\beta H$ , as well as that around  $C^\gamma H_3$  groups, was calculated for the  $\alpha$  helix and  $\beta$  conformation of poly(L-valine) in approximation 3. However, no water molecules remained around  $C^\alpha H$  and  $C^\beta H$  in both conformations; thus, there was no change in hydration of the  $C^\alpha H$  and  $C^\beta H$  groups in the coil-to-helix transition. This result supports the assumption made in section III that there is no difference in the hydration of the  $C^\alpha$  and  $C^\beta$  groups in the helix and coil forms of poly(L-valine). A value of 12 is taken as the number of water molecules in the first layer around two methyl groups of valine. In the  $\beta$  conformation, the side chains of consecutive residues are far apart, on opposite sides of the backbone chain; hence, side-chain-side-chain interaction would not reduce the hydration number below 12. However, 4.2 of these 12 water molecules are removed by the proximity of backbone atoms (including  $C^\beta$ ) near the methyl groups in the  $\beta$  conformation. Thus, the hydration of a residue in the random coil was taken as 7.8 molecules, since it was shown that the  $\beta$  conformation is the dominant one for a dipeptide under vacuum, and that the hydration is essentially independent of  $\phi$  and  $\psi$  in the sterically allowed region. In the right-handed

**Table V**  
Contributions to  $\Delta H_s^{(0)}$

Poly- (amino acid)	Short-Range Inter- actions (1) <sup>a,b</sup>	Short-Range Inter- actions (2) <sup>a,b</sup>	$[\Delta H_s^{(0)}]^{a,c}$
	$\sum_{i=0}^2 E_i - H_c^{(0)}$	$E_3 + E_4$	
Poly(L-valine)	1.33	-6.07	-4.75
Poly(L-alanine)	-0.14	-4.57	-4.71

<sup>a</sup> In kcal/mol. <sup>b</sup> See text for definitions of short-range interactions (1) and (2). <sup>c</sup>  $\Delta H_s^{(0)}$  is the sum of columns 2 and 3.

$\alpha$ -helical conformation, 8.4 of the 12 water molecules are excluded; of these 8.4, 1.8 are excluded by the proximity of  $C_1^{\gamma 2}$  and  $C_2^{\gamma 1}$  (i.e., by  $C^\gamma$ 's on neighboring side chains) and of  $C_1^{\gamma 2}$  and  $C_5^{\gamma 1}$  (i.e., by the  $C^\gamma$ 's of residues 1 and 5 in the helical conformation), and 6.6 are excluded by the proximity of the  $C^\gamma$ 's to various backbone atoms (including  $C^\beta$ ). Therefore, the change in hydration in the transition from coil to helix is  $8.4 - 4.2 = 4.2$  water molecules.

Further interactions, beyond those in the dipeptide, are taken into account for the random coil under vacuum. While such interactions were negligible for polyglycine and poly(L-alanine), they are appreciable for poly(L-valine), and also for poly(L-lysine).<sup>19</sup> From a comparison of the calculated and experimental<sup>7</sup> values of  $s$ , it was found that interactions up to  $F^{(6)}$  in the coil are required in order to obtain the correct enthalpy of the coil state. The most dominant contribution to  $s$  from  $F^{(5)}$  and  $F^{(6)}$  is the enthalpy decrease (-0.39 kcal/mol) in the coil, arising from nonbonded interactions between the backbone and side-chain atoms. The change in hydration,  $\Delta Y$ , when  $F^{(5)}$  and  $F^{(6)}$  are included, is only -0.2 (i.e., an additional 0.2 water molecule is removed); this corresponds to contributions of -0.04 kcal/mol and -0.2 eu in  $\Delta H_s$  and  $\Delta S_s$ , respectively, at 300°K.

Since several approximations were made in the calculation of  $s$ , the present molecular theory of  $s$  cannot be used to predict the absolute value of the helix content, which is very sensitive to small changes in  $s$  when  $s$  is very close to unity.<sup>5</sup> Nevertheless, the experimental inverse transition curve (whereby random coil is converted to  $\alpha$  helix as the temperature is raised) is obtained theoretically; this provides strong support for inclusion of the terms  $\Delta H_w$  and  $\Delta S_w$  (i.e., the effect of a change in hydration), even though (within the present degree of precision of the theory) the calculated curve cannot reproduce exactly the experimental almost-linear dependence of  $s$  on  $T$  in the experimental range of temperature (i.e., the calculated curve shows a maximum).

In the present theory, we have discussed the helix-coil transition within a single chain, and therefore only intrachain interactions have been considered. The results obtained indicate that  $s$  is near unity and that the  $\beta$  conformation of a residue in a single chain is the dominant one for the random coil in water. These two results suggest that, if intermolecular interactions were allowed for, the interchain  $\beta$  structure of poly(L-valine) might be more stable than the  $\alpha$  helix in water. Comparing a multichain  $\beta$  structure with a single-chain one, the former would be more favored by hydrogen bonds, additional nonbonded interactions, and hydrophobic bonds. On the other hand, the multichain structure would be less favored because of an entropy loss arising from restriction of dihedral angles, and a loss of free energy from the release of water molecules from the NH and CO groups. A quantitative treatment of these compensating free energy gains and losses would be



Table VI  
Contributions to  $\Delta S_s^{(0)}$

Poly-(amino acid)	$[S_h^{(0)}]^a$	$[S_c^{(0)}]^a$	$[\Delta S_s^{(0)}]^a$
Poly(L-valine)	-15.37	-5.14	-10.23
Poly(L-alanine)	-9.40	-1.11	-8.29

<sup>a</sup> In eu.

required for an assessment of the stability of the multichain  $\beta$  structure. In fact, there are experimental data which show that poly(L-valine) forms an intermolecular  $\beta$  structure in water.<sup>22</sup>

Despite the contribution from hydrophobic bonding in poly(L-valine), the stability of the  $\alpha$  helix of this poly(amino acid) is comparable to that of poly(L-alanine).<sup>5</sup> We show below that the effect of hydrophobic bonding [which favors the poly(L-valine) helix over that of poly(L-alanine)] is compensated by the conformational entropy [which favors the helical form of poly(L-alanine)]. In the absence of solvent, the enthalpy and entropy changes are defined<sup>5</sup> by

$$\Delta H_s^{(0)} = H_{h,\text{cut}}^{(0)} - H_c^{(0)} \quad (29)$$

or

$$\Delta H_s^{(0)} = (E_0 + E_1 + E_2 - H_c^{(0)}) + (E_3 + E_4) \quad (30)$$

and by

$$\Delta S_s^{(0)} = S_h^{(0)} - S_c^{(0)} \quad (31)$$

respectively. The terms within the first and second parentheses on the right-hand side of eq 30 are referred to<sup>5</sup> as *short-range interactions (1)* and *short-range interactions (2)*, respectively. The terms in eq 30 and 31 are shown in Tables V and VI for both polymers. It can be seen that  $\Delta H_s^{(0)}$  is essentially the same for both poly(amino acids), and that  $\Delta S_s^{(0)}$  is more positive for poly(L-alanine) than for poly(L-valine).<sup>23</sup> As far as the enthalpy term is concerned, the short-range interactions (1) favor the helix over the coil for poly(L-alanine) but *vice versa* for poly(L-valine); however, the short-range interactions (2) favor the helix over the coil in both poly(amino acids)—more so for poly(L-valine) than for poly(L-alanine). The sum, *i.e.*,  $\Delta H_s^{(0)}$ , indicates that the helical form of both poly(amino acids) is favored to the same extent. The difference between the values of  $S_h^{(0)}$  for the two poly(amino acids), -5.97 eu, is more negative than the difference between their values of  $S_c^{(0)}$ , -4.03 eu. This behavior, which results mainly from the difference between the number of allowed positions for the dihedral angle  $\chi$  in the helical forms, can be seen as follows. In poly(L-alanine), three ranges of  $\chi$  are allowed in both the helical and coil forms, yielding no entropy contribution to  $s$  from freedom of rotation of the side chain. However, in poly(L-valine), one and three ranges of  $\chi$  are allowed in the helical and coil forms, respectively, yielding an entropy gain of 2.2 eu in going from helix to coil.

## References and Notes

- (1) This work was supported by research grants from the National Science Foundation (GB-28469X3) and from the National Institute of General Medical Sciences of the National Institutes of Health, U. S. Public Health Service (GM-14312).
- (2) (a) On leave of absence from the Department of Applied Physics, Waseda University, Tokyo, Japan, Summer, 1971, and from the Department of Physics, Faculty of Science, Kyushu University, Fukuoka, Japan, Summer, 1972; Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan. (b) On leave from the Van't Hoff Laboratorium der Rijksuniversiteit, Utrecht, Netherlands, 1971-1972.
- (3) (c) On leave of absence from the Department of Physics, Faculty of Science, Tokyo University, Tokyo, Japan, Summer, 1971, and from the Department of Physics, Faculty of Science, Kyushu University, Fukuoka, Japan, Summer, 1972.
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- (14) Equation II-29 should be corrected as follows
 
$$b = -RT \ln \left( 1 + \sum_k K_{NH,k} a_k \right) - RT \ln \left( 1 + \sum_l K_{CO,l} a_l \right)$$
 This equation is a general one for a mixed solvent, *i.e.*, one for which  $k$  and  $l$  are solvent molecules. However, since we have dealt only with water as the solvent, heretofore, this correction does not influence any of the results which we have obtained previously in this series of papers.
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- (18) In the calculation of the unperturbed dimensions of the random coil, good agreement was obtained<sup>18</sup> between the calculated value (including terms only up to  $F^{(3)}$ ) and the experimental value. This agreement was achieved by demonstrating<sup>18</sup> the necessity of including dipole-dipole interactions, in addition to the nonbonded and torsional energies; *i.e.*, dipole-dipole interactions play a dominant role in determining the unperturbed dimensions. However, while this situation pertains to the unperturbed dimensions, it does not pertain to the calculation of  $s$ . Because  $F^{(4)}$  is very flat compared to  $A(\phi, \chi, \psi)$ , and because a similar behavior is expected for  $F^{(5)}$ ,  $F^{(6)}$ , etc., the unperturbed dimensions of the coil would not be expected to be very sensitive to  $F^{(4)}$ ,  $F^{(5)}$ , etc.; this is presumably the reason why these terms could be neglected in the calculation of the unperturbed dimensions. However, in the calculation of the value of  $s$ , the higher terms beyond  $F^{(3)}$  cannot be neglected because they contribute to  $\Delta H_s$ , even though they do not contribute to  $\Delta S_s$  because of their flatness.
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- (24) It is of interest to relate the interaction energies  $F^{(0)}$ ,  $F^{(1)}$ , ...,  $F^{(6)}$ , etc., which are defined according to their dependence on specific dihedral angles, to the inter-unit interaction energies  $E_0$ ,  $E_1$ ,  $E_1'$ ,  $E_2$ ,  $E_2'$ , ..., etc. From the definition of  $E_0$ ,  $E_1$ ,  $E_1'$ , etc., which is given in paper II, and the definition of  $F^{(0)}$ ,  $F^{(1)}$ , ..., etc., which is given in section IA of this paper, it follows that
 
$$\sum_{i=0}^3 F^{(i)} = E_0 + E_1'$$
 and
 
$$\sum_{i=0}^6 F^{(i)} = E_0 + E_1 + (\text{a part of } E_2) + (\text{a part of } E_3)$$
 $E_0$ ,  $E_1$ ,  $E_1'$ ,  $E_2$ , and  $E_2'$  in the helix and coil constitute the short-range interactions (1) and  $E_3$ ,  $E_3'$ ,  $E_4$ , and  $E_4'$  the short-range interactions (2). Since interactions from  $F^{(0)}$  up to  $F^{(6)}$  are included in  $H_c^{(0)}$  in the present calculation of  $s$ ,  $H_c^{(0)}$  may be compared with the short-range interactions (1) when discussing the difference between the enthalpies of the helix and coil.