Theory of the Cooperative Transition between Two Ordered Conformations of Poly(L-proline). I. Phenomenological Theory[1]

Seiji Tanaka[2a] and Harold A. Scheraga*[2b]

Department of Chemistry, Cornell University, Ithaca, New York 14853.
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ABSTRACT: The states of three residues are correlated in a nearest-neighbor Ising model matrix treatment of a one-dimensional phase transition, in which nucleation is assumed to differ at each end of a regular sequence (asymmetric nucleation). The correlation of the states of three residues requires a 4 × 4 matrix, which cannot be reduced in size because of the asymmetric nature of the nucleation. Also, because of the asymmetry, at least four independent parameters for a homopolymer (rather than the two usually encountered in the helix-coil transition), and at least five for a specific-sequence copolymer, are required to describe the transition behavior. The most important current interest in such a treatment (for a homopolymer) is its applicability to the poly(L-proline) form I ⇄ form II interconversion. The earlier treatment of Schwarz, using the nearest-neighbor Ising model (with a correlation of only two residues), is identical with the above treatment, and requires only a 2 × 2 matrix which greatly simplifies numerical computations, which are presented in the next two papers of this series. However, the 4 × 4 matrix treatment is required in order to make the asymmetric nature of the nucleation explicit and physically understandable, for a homopolymer; for a specific-sequence copolymer, such as a protein, it is essential in order to show how the asymmetric nature of helix nucleation differs from one amino acid to another.

Extensive theoretical and experimental investigations have been carried out to obtain quantitative information about the nature and magnitude of the interactions which determine the stable conformations of polypeptides and proteins in solution.[3] In this connection, it is necessary to study cooperative conformational transitions in simple linear systems in order to understand the interactions that determine the conformations of more complex biological macromolecules. Such knowledge would be useful for predicting protein conformations, and for determining the mechanism for the folding of proteins.

Among the naturally occurring amino acids, the L-proline residue is unique in its chemical structure and physical properties. A number of investigations[4] have shown that poly(L-proline) and oligomeric derivatives of L-proline can exist in the trans and/or cis isomeric forms in solution. Furthermore, experimental equilibrium studies[4] have demonstrated that poly(L-proline) undergoes a conformational transition between two helical forms, conventionally designated as form I and form II.[5,6] In the form I conformation, the peptide groups are in the cis conformation and the helical sense is right handed. In the form II conformation, the peptide groups are in the trans conformation and the helical sense is left handed.

A number of theoretical treatments have been proposed to explain the α-helix ⇄ coil transition in polyamino acids.[7] The phenomenological theories of order ⇄ disorder transitions have been successful in interpreting experimental data in terms of a few parameters;[7] e.g., Zimm and Bragg[8] used only two parameters, s and σ, to describe the α-helix ⇄ coil transition. These phenomenological parameters have also been accounted for on a molecular basis by Gō et al.[9-12] In contrast to the α-helix ⇄ coil transition, that for poly(L-proline), form I ⇄ form II, is a cooperative order ⇄ order transition,[13-15] a phenomenological theory for which has been proposed by Schwarz[16] and Applequist.[17] In section IB of this paper, we will compare our theory with that of Schwarz, Applequist, and Zimm and Bragg.

Schwarz's treatment correlates the states of only two residues (nearest-neighbor Ising model), and thus requires a 2 × 2 matrix for formulating the partition function. As a result, the asymmetric character of the nucleation, while present, does not appear explicitly in the elements (statistical weights) of the matrix (in section IB, we will explain why the nucleation is asymmetric). We have, therefore, formulated a treatment, involving a correlation of three residues (and hence a 4 × 4 matrix) in the formulation of the partition function; in this matrix, the elements clearly reflect the asymmetric character of the nucleation. However, since the present formulation in terms of a 4 × 4 matrix is also based on a nearest-neighbor model, with no interactions between the $(i-1)$th and $(i+1)$th residues, the present theory and Schwarz's theory yield the same results for the conformational transition *in a homopolymer*; this is different from the case of the helix–coil transition, in which the 2 × 2 matrix formulation[18] differs slightly from, but is a good approximation to, the Lifson–Roig treatment[19] in terms of a 4 × 4 matrix. However, by initially using the 4 × 4 matrix treatment, the meaning of asymmetric nucleation becomes clear and explicit. Furthermore, the present formulation can be applied easily to the conformational transition in a specific-sequence copolymer, where asymmetric nucleation must be taken into account, whereas it is impossible to apply Schwarz's theory to a specific-sequence copolymer, in practice, because the 2 × 2 matrix contains the nucleation parameters at either the N or C terminus, but not both. It should be noted that, in contrast to the Lifson–Roig theory[19] of the helix–coil transition, the 4 × 4 matrix for conformational transitions, such as that in the poly(L-proline) form I ⇄ form II interconversion, cannot be reduced to a 3 × 3 matrix because of the asymmetric character of the nucleation in the poly(L-proline) transition.

In this paper, we will present a phenomenological 4 × 4 matrix treatment of the poly(L-proline) transition as the most interesting example of a conformational transition in a homopolymer. With the meaning of the statistical weights thus made clear, we will summarize the 2 × 2 matrix treatment of Schwarz which is more easily adaptable to numerical computation. In paper II,[6] we will present a *molecular* theory of this transition, in the absence of solvent. Finally, in paper III,[20] we will introduce the role of solvent in the molecular theory.

I. 4 × 4 Matrix Formulation

A. Theoretical Formulation. In order to formulate the partition function for a one-dimensional polymeric system consisting of two states per residue, we assume that any conformation of the chain consists of alternating sequences of *regular* conformations, and that the free energy of a residue depends on its state and on the states of its immediate

neighbors (the basic assumption of the one-dimensional nearest-neighbor Ising model).

To be more general at the outset, we designate the two possible conformational states of a residue as α and β. The free energy G of a particular conformation of a chain of N residues[21] may be expressed in terms of the free energy of the ith residue, G_i, and of the interactions between the ith residue and its neighbors on either side, $G_{i-1,i}$ and $G_{i,i+1}$, viz.,

$$G = |G_1 + (G_{1,2}/2)| + \sum_{i=2}^{N-1} |(G_{i-1,i}/2) + G_i + (G_{i,i+1}/2)| + |(G_{N-1,N}/2) + G_N| \quad (1)$$

It should be noted that the term $G_{i-1,i,i+1}$ does not appear in eq 1, even though the states of three residues are correlated in the present matrix treatment. This differs from the 4×4 (or, equivalently, 3×3) matrix formulation of the Lifson–Roig model.[19] In the latter model, the term $G_{i-1,i,i+1}$ results from the hydrogen-bond interaction in the α-helix. Equation 1 may be expected to be a good approximation to the free energy, when treating the form I $\rightleftarrows$ form II interconversion in poly(L-proline), where no backbone hydrogen bond is involved. This may be stated alternatively by regarding eq 1 as a good approximation, for treating the poly(L-proline) transition, even though $G_{i-1,i,i+1}$ may not be negligible, in the sense that the nearest-neighbor Ising model treatment[18] of the helix–coil transition is a good approximation to the Lifson–Roig treatment.[19] Thus, the treatment presented here is that for a nearest-neighbor Ising model, although the states of three successive residues are correlated in the matrix to be described shortly.

We assign a statistical weight w_α to an ith residue in the α state, when both of its neighbors are in the α state, i.e.,

$$w_\alpha = \exp|-\{(G^{\alpha\alpha}{}_{i-1,i}/2) + G^{\alpha}{}_i + (G^{\alpha\alpha}{}_{i,i+1}/2)\}/RT| \quad (2)$$

where R and T are the gas constant and absolute temperature, respectively. Similarly, for the middle (ith) residue in a $\beta\beta\beta$ conformation.

$$w_\beta = \exp|-\{(G^{\beta\beta}{}_{i-1,i}/2) + G^{\beta}{}_i + (G^{\beta\beta}{}_{i,i+1}/2)\}/RT| \quad (3)$$

By considering other possible triad conformations, we can introduce parameters which pertain to nucleation or termination; the notation for these parameters differs according to the location of a residue, its conformational state, and the direction of the conformational transition (i.e., from N to C terminus, or vice versa). Thus, consider the triad conformation $\alpha\alpha\beta$ for residues $i-1$, i, and $i+1$, as an example. The free energy of $\alpha\alpha\beta$, assigned to the ith residue, is

$$(G^{\alpha\alpha}{}_{i-1,i}/2) + G^{\alpha}{}_i + (G^{\alpha\beta}{}_{i,i+1}/2) \quad (4)$$

If we use the same statistical weight, w_α, for the ith residue of $\alpha\alpha\beta$ as was used for the ith residue of $\alpha\alpha\alpha$, then (from eq 2) we would have

$$-RT \ln w_\alpha = (G^{\alpha\alpha}{}_{i-1,i}/2) + G^{\alpha}{}_i + (G^{\alpha\alpha}{}_{i,i+1}/2) \quad (5)$$

However, since the net free energy for the ith residue in $\alpha\alpha\beta$ is given by eq 4, we can obtain the correct free energy by subtracting $G^{\alpha\alpha}{}_{i,i+1}/2$ and adding $G^{\alpha\beta}{}_{i,i+1}/2$ in eq 5; this results from the change of the conformational state of the $(i+1)$th residue from α to β, for which we define a free energy change

$$-RT \ln \sigma_C{}^{\alpha} = (G^{\alpha\beta}{}_{i,i+1}/2) - (G^{\alpha\alpha}{}_{i,i+1}/2) \quad (6a)$$

or

$$\sigma_C{}^{\alpha} = \exp|-\{(G^{\alpha\beta}{}_{i,i+1}/2) - (G^{\alpha\alpha}{}_{i,i+1}/2)\}/RT| \quad (6b)$$

The parameter $\sigma_C{}^{\alpha}$ may have either of two meanings for a conformational transition from α to β in the direction from the N to the C terminus,[21] viz., (1) the termination of the α sequence at the ith residue (at the C terminus of the α sequence), or (2) the nucleation of the β sequence at the $(i+1)$th residue (at the N terminus of the β sequence). Thus, the statistical weight of the ith residue in the triad $\alpha\alpha\beta$ is $\sigma_C{}^{\alpha} w_\alpha$. The physical meaning of $\sigma_C{}^{\alpha}$, deduced from eq 6a or 6b, is as follows: the nucleation of a β state at the $(i+1)$th residue is difficult if $G^{\alpha\beta}{}_{i,i+1} > G^{\alpha\alpha}{}_{i,i+1}$. In a similar manner, the statistical weight for the ith residue of the triad $\beta\beta\alpha$ is $\sigma_C{}^{\beta} w_\beta$. For the triad $\alpha\beta\beta$, for which the free energy is $(G^{\alpha\beta}{}_{i-1,i}/2) + G^{\beta}{}_i + (G^{\beta\beta}{}_{i,i+1}/2)$, the excess free energy accompanying the nucleation of α at residue $i-1$ is $(G^{\alpha\beta}{}_{i-1,i}/2) - (G^{\beta\beta}{}_{i-1,i}/2)$, analogous to eq 6a, when the parameter w_β is obtained from eq 3. Thus, we may define a parameter $\sigma_N{}^{\beta}$ as

$$\sigma_N{}^{\beta} = \exp|-\{(G^{\alpha\beta}{}_{i-1,i}/2) - (G^{\beta\beta}{}_{i-1,i}/2)\}/RT| \quad (7)$$

Hence, the statistical weight for the ith residue is $\sigma_N{}^{\beta} w_\beta$. It should be noted that the subscripts $i-1,i$ in eq 7 differ from those $(i,i+1)$ that appear in eq 6b. Thus, the parameter $\sigma_N{}^{\beta}$ may have either of two meanings for a conformational transition from β to α in the direction from the C to the N terminus,[21] viz., (1) the termination of the β sequence at the ith residue (at the N terminus of the β sequence), or (2) the nucleation of the α sequence at the $(i-1)$th residue (at the C terminus of the α sequence). In a similar manner, the statistical weight for the ith residue of the triad $\beta\alpha\alpha$ is $\sigma_N{}^{\alpha} w_\alpha$.

By referring to the triad conformations in column 1 of Table I, the free energy of the middle (ith) residue can be written as in column 2. Then, by analogy to eq 4–7, we obtain the statistical weights of the ith residue in column 3, for the conformational states in column 1, where

$$\sigma_N{}^{\alpha} = \exp|-\{(G^{\beta\alpha}{}_{i-1,i}/2) - (G^{\alpha\alpha}{}_{i-1,i}/2)\}/RT| \quad (8)$$

$$\sigma_C{}^{\beta} = \exp|-\{(G^{\beta\alpha}{}_{i,i+1}/2) - (G^{\beta\beta}{}_{i,i+1}/2)\}/RT| \quad (9)$$

The physical meaning of α in $\sigma_N{}^{\alpha}$ (and β in $\sigma_C{}^{\beta}$) can be understood by substituting β for α in case (2) of the illustration for $\sigma_N{}^{\beta}$ described above [or by substituting α for β in case (2) of the illustration for $\sigma_C{}^{\alpha}$].

It should be noted that all w and σ parameters introduced here depend on the states of *three* residues, $i-1$, i, and $i+1$, and thus differ from corresponding quantities in Schwarz's treatment, in which the states of only two residues determine the magnitudes of these parameters.

The number of statistical weights may be reduced by defining four other statistical weights as follows:

$$s = w_\alpha / w_\beta \quad (10)$$

$$\sigma_N = (\sigma_N{}^{\alpha} \sigma_N{}^{\beta})^{1/2} \quad (11a)$$

$$\sigma_C = (\sigma_C{}^{\alpha} \sigma_C{}^{\beta})^{1/2} \quad (11b)$$

$$\beta_N = (\sigma_N{}^{\alpha} / \sigma_N{}^{\beta})^{1/2} \quad (12)$$

$$\beta_C = (\sigma_C{}^{\alpha} / \sigma_C{}^{\beta})^{1/2} \quad (13)$$

For the conformational transition in a *homopolymer*, the parameters σ_N and σ_C have the same values because $G^{\alpha\beta}{}_{i,i+1} = G^{\alpha\beta}{}_{i-1,i}$, $G^{\beta\alpha}{}_{i,i+1} = G^{\beta\alpha}{}_{i-1,i}$, $G^{\alpha\alpha}{}_{i,i+1} = G^{\alpha\alpha}{}_{i-1,i}$, and $G^{\beta\beta}{}_{i,i+1} = G^{\beta\beta}{}_{i-1,i}$ in eq 6–9, which yields

Table I
Conformational States and Statistical Weights

Conformational state of $i-1$	i	$i+1$	Free energy of ith residue[a]	Statistical wt of ith residue	Relative statistical wt of ith residue: For homopolymer	Relative statistical wt of ith residue: For specific-sequence copolymer	Dummy statistical wt
α	α	α	$(G^{\alpha\alpha}/2) + G^{\alpha} + (G^{\alpha\alpha}/2)$	w_α	s	s	q_1
α	α	β	$(G^{\alpha\alpha}/2) + G^{\alpha} + (G^{\alpha\beta}/2)$	$\sigma_C{}^{\alpha} w_\alpha$	$\sigma^{1/4}\beta_C{}^{1/4}s$	$\sigma_C{}^{1/4}\beta_C{}^{1/4}s$	q_2
α	β	α	$(G^{\alpha\beta}/2) + G^{\beta} + (G^{\beta\alpha}/2)$	$\sigma_N{}^{\beta}\sigma_C{}^{\beta} w_\beta$	$\sigma^{1/2}\beta_N{}^{-1/4}\beta_C{}^{-1/4}$	$\sigma_N{}^{1/4}\sigma_C{}^{1/4}\beta_N{}^{-1/4}\beta_C{}^{-1/4}$	q_3
β	α	α	$(G^{\beta\alpha}/2) + G^{\alpha} + (G^{\alpha\alpha}/2)$	$\sigma_N{}^{\alpha} w_\alpha$	$\sigma^{1/4}\beta_N{}^{1/4}s$	$\sigma_N{}^{1/4}\beta_N{}^{1/4}s$	q_4
α	β	β	$(G^{\alpha\beta}/2) + G^{\beta} + (G^{\beta\beta}/2)$	$\sigma_N{}^{\beta} w_\beta$	$\sigma^{1/4}\beta_N{}^{-1/4}$	$\sigma_N{}^{1/4}\beta_N{}^{-1/4}$	q_5
β	α	β	$(G^{\beta\alpha}/2) + G^{\alpha} + (G^{\alpha\beta}/2)$	$\sigma_N{}^{\alpha}\sigma_C{}^{\alpha} w_\alpha$	$\sigma^{1/2}\beta_N{}^{1/4}\beta_C{}^{1/4}s$	$\sigma_N{}^{1/4}\sigma_C{}^{1/4}\beta_N{}^{1/4}\beta_C{}^{1/4}s$	q_6
β	β	α	$(G^{\beta\beta}/2) + G^{\beta} + (G^{\beta\alpha}/2)$	$\sigma_C{}^{\beta} w_\beta$	$\sigma^{1/4}\beta_C{}^{-1/4}$	$\sigma_C{}^{1/4}\beta_C{}^{-1/4}$	q_7
β	β	β	$(G^{\beta\beta}/2) + G^{\beta} + (G^{\beta\beta}/2)$	w_β	1	1	q_8

[a] For convenience, the subscripts have been omitted from the G's. However, it should be understood that the subscripts $i-1, i$ apply to the first term, i to the second term, and $i, i+1$ to the third term.

$$\sigma = \sigma_N = \sigma_C \tag{14}$$

However, this is not true for the conformational transition in a *specific-sequence copolymer*, since the equivalence of the free energy terms (depending on i) no longer holds, in general, because of the different chemical species at residues $(i-1)$, i, and $(i+1)$; thus, in this case $\sigma_N \neq \sigma_C$. A discussion of the reduction in the number of variables (eq 10–13) and of the *physical* meaning of s, σ, β_N, and β_C will be deferred until section IIA. The right-hand sides of eq 11–13 are squared because the corresponding free energy terms are divided by 2 in the definition of $\sigma_C{}^{\alpha}$, $\sigma_C{}^{\beta}$, $\sigma_N{}^{\alpha}$, and $\sigma_N{}^{\beta}$. For the case of a specific-sequence copolymer, we may rewrite these quantities as

$$\sigma_N{}^{\alpha} = \sigma_N{}^{1/4}\beta_N{}^{1/4} \tag{15}$$

$$\sigma_N{}^{\beta} = \sigma_N{}^{1/4}\beta_N{}^{-1/4} \tag{16}$$

$$\sigma_C{}^{\alpha} = \sigma_C{}^{1/4}\beta_C{}^{1/4} \tag{17}$$

$$\sigma_C{}^{\beta} = \sigma_C{}^{1/4}\beta_C{}^{-1/4} \tag{18}$$

and, for the case of a homopolymer, the use of eq 14 in eq 15–18 gives

$$\sigma_N{}^{\alpha} = \sigma^{1/4}\beta_N{}^{1/4} \tag{19}$$

$$\sigma_N{}^{\beta} = \sigma^{1/4}\beta_N{}^{-1/4} \tag{20}$$

$$\sigma_C{}^{\alpha} = \sigma^{1/4}\beta_C{}^{1/4} \tag{21}$$

$$\sigma_C{}^{\beta} = \sigma^{1/4}\beta^{-1/4} \tag{22}$$

Using eq 15–22, and dividing by w_β (to obtain *relative* statistical weights), and defining s as w_α/w_β, we obtain the quantities in column 4 (for a homopolymer) and in column 5 (for a specific-sequence copolymer) of Table I. For the familiar helix–coil transition in a homopolyamino acid (where $\alpha = h$ and $\beta = c$), the usually made assumption of symmetric nucleation leads to

$$\sigma_N{}^{c} = \sigma_C{}^{c} = 1 \tag{23}$$

and

$$\beta_N = \beta_C = (\sigma_N{}^{\beta})^2 = (\sigma_C{}^{\beta})^2 = \sigma \tag{24}$$

The exponent, 2, in eq 24 arises from the fact that the free energies were divided by 2 in the definition of $\sigma_N{}^{\alpha}$ (or $\sigma_N{}^{\beta}$) and $\sigma_C{}^{\alpha}$ (or $\sigma_C{}^{\beta}$) in eq 8 and 6b, respectively. Further discussion of σ will be presented in section IB.

So far, we have described the conformational states and their statistical weights for the residues in the interior of the chain. In section VI of paper III,[20] we suggest that end effects have to be considered in order to account for the experimental results, especially for polymers of low molecular weight. For this purpose, we now examine the statistical weights for the residues at the N and C termini of the chain. Consider first the conformational states and statistical weights for the first residue at the N terminus.[21] In order to take the end effects into account, we now define the excess free energy, $G^{\alpha}{}_N$, for the first N terminal residue of the chain (when it is in an α state) as that arising from the specific properties of the end residues. The contributions to $G^{\alpha}{}_N$ may be regarded as the decrease in free energy because of the absence of interactions between the first residue and its (missing) neighbor on the left, $-G^{\alpha}{}_{01}/2$, and the free energy contribution from specific interactions between the solvent and the N terminal group, $G^{\alpha}{}_{\mathrm{solv},N}$. Thus

$$G^{\alpha}{}_N = (-G^{\alpha}{}_{01}/2) + G^{\alpha}{}_{\mathrm{solv},N} \tag{25}$$

If, say, residues 1 and 2 are both in α states, then the free energy for this $\alpha\alpha$ dyad is given by

$$G^{\alpha}{}_N + G^{\alpha}{}_1 + (G^{\alpha}{}_{1,2}/2) \tag{26}$$

We then define a parameter $\gamma_N{}^{\alpha}$, related to the free energy of eq 25, as

$$\gamma_N{}^{\alpha} = \exp[-G^{\alpha}{}_N/RT] \tag{27}$$

Thus, using eq 25 and 27, we can assign the statistical weight $\gamma_N{}^{\alpha} w^{\alpha}$ to the first residue at the N terminus when the first two residues are in the $\alpha\alpha$ state. From similar considerations of all other possible conformational states at the N and C termini (see the second column of Table II), we obtain the statistical weights that are given in the third column of Table II, where

$$\gamma_N{}^{\beta} = \exp[-G^{\beta}{}_N/RT] \tag{28}$$

$$\gamma_C{}^{\alpha} = \exp[-G^{\alpha}{}_C/RT] \tag{29}$$

and

$$\gamma_C{}^{\beta} = \exp[-G^{\beta}{}_C/RT] \tag{30}$$

and $\sigma_C{}^{\alpha}$, $\sigma_N{}^{\beta}$, $\sigma_N{}^{\alpha}$ and $\sigma_C{}^{\beta}$ are defined in eq 6b, 7, 8, and 9, respectively. By analogy to eq 12 and 13, the parameters ϵ_N

Table II
The Statistical Weights of the End Residues of the Chain

Chain end	Conformational states of the first or last two residues at the ends of the chain	Statistical wt for the nearest-neighbor Ising model[a]			Statistical wt[c] for the helix–coil transition in polyamino acids	Dummy statistical wt
		Statistical wt[b] of the end residue	Relative statistical wt[b] of the end residue			
			For homopolymer	For specific-sequence copolymer		
N terminus	α α	$\gamma_N^{\alpha} w_{\alpha}$	$\epsilon_N^{1/2} s$	$\epsilon_N^{1/2} s$	q_4	$q_N^{(1)}$
	α β	$\gamma_N^{\alpha} \sigma_C^{\alpha} w_{\alpha}$	$\epsilon_N^{1/2} \sigma^{1/4} \beta_C^{1/4} s$	$\epsilon_N^{1/2} \sigma_C^{1/4} \beta_C^{1/4} s$	q_6	$q_N^{(2)}$
	β α	$\gamma_N^{\beta} \sigma_C^{\beta} w_{\beta}$	$\sigma^{1/4} \beta_C^{-1/4}$	$\sigma_C^{1/4} \beta_C^{-1/4}$	q_7	$q_N^{(3)}$
	β β	$\gamma_N^{\beta} w_{\beta}$	1	1	q_8	$q_N^{(4)}$
C terminus	α α	$\gamma_C^{\alpha} w_{\alpha}$	$\epsilon_C^{1/2} s$	$\epsilon_C^{1/2} s$	q_2	$q_C^{(1)}$
	α β	$\gamma_C^{\beta} \sigma_N^{\beta} w_{\beta}$	$\sigma^{1/4} \beta_N^{-1/4}$	$\sigma_N^{1/4} \beta_N^{-1/4}$	q_5	$q_C^{(2)}$
	β α	$\gamma_C^{\alpha} \sigma_N^{\alpha} w_{\alpha}$	$\epsilon_C^{1/2} \sigma^{1/4} \beta_N^{1/4} s$	$\epsilon_C^{1/2} \sigma_N^{1/4} \beta_N^{1/4} s$	q_6	$q_C^{(3)}$
	β β	$\gamma_C^{\beta} w_{\beta}$	1	1	q_8	$q_C^{(4)}$

[a] These statistical weights are applicable to the form I $\rightleftarrows$ form II interconversion of poly(L-proline), in which the α and β states are taken as form I and form II, respectively. [b] If circular boundary conditions (i.e., no end effects) are applied to the chain, the statistical weights are given by: $\gamma_N^{\alpha} = \gamma_N^{\beta} = \gamma_C^{\alpha} = \gamma_C^{\beta} = 1$ or $\epsilon_N = \epsilon_C = 1$. [c] This column pertains to the helix–coil transition in the polyamino acid model, where α and β refer to the α-helical and coil states, respectively, and the 0th and $(N + 1)$th residues are taken as c states. The parameters q_i are those given in the last column of Table I.

and ϵ_C are defined by taking one of the two possible states (i.e., α and β) at the first residue as a standard state, since we are interested only in the relative stabilities of two states when thermodynamic quantities are computed; thus

$$\epsilon_N = (\gamma_N^{\alpha}/\gamma_N^{\beta})^2 \tag{31}$$

$$\epsilon_C = (\gamma_C^{\alpha}/\gamma_C^{\beta})^2 \tag{32}$$

Using eq 31 and 32, the relative statistical weights for residues 1 and N (at the N and C terminus, respectively) are given in columns 4 and 5 of Table II; these are analogs of those for residues in the interior of the chain (given in columns 4 and 5 of Table I).

With the statistical weights of Table I (expressed as the dummy statistical weights of the last column, for convenience), we may correlate the states of three residues, requiring a 4 × 4 matrix, $\mathbf{W}_i$ for the ith residue, viz.

$$\mathbf{W}_i = \begin{array}{cc|cccc} & i+1 & \alpha & \beta & \alpha & \beta \\ i-1 & i & \alpha & \alpha & \beta & \beta \\ \hline \alpha & \alpha & q_1 & q_2 & 0 & 0 \\ \alpha & \beta & 0 & 0 & q_3 & q_5 \\ \beta & \alpha & q_4 & q_6 & 0 & 0 \\ \beta & \beta & 0 & 0 & q_7 & q_8 \end{array} \tag{33}$$

In contrast to the Lifson–Roig matrix, the matrix of eq 33 cannot be reduced to a 3 × 3 one because of the asymmetric nature of the nucleation. The partition function, Z, may then be written as

$$Z = \mathbf{u}\left(\prod_{i=2}^{N-1} \mathbf{W}_i\right)\mathbf{u}^{+} \tag{34}$$

where

$$\mathbf{u} = (q_N^{(1)}, q_N^{(2)}, q_N^{(3)}, q_N^{(4)}) \tag{35}$$

and

$$\mathbf{u}^{+} = (q_C^{(1)}, q_C^{(2)}, q_C^{(3)}, q_C^{(4)})^{+} \tag{36}$$

and the superscript plus sign designates the transpose of the vector. The statistical weights for the end residues (in eq 35 and 36) are given as dummy statistical weights in the last column of Table II; the actual statistical weights are those of columns 3, 4, and 5 of Table II. These statistical weights are used for the end residues in the same way that the statistical weights of columns 3, 4, and 5 of Table I are used in eq 33 for the interior residues.

If the present formulation is applied to the helix–coil transition in polyamino acids (using the statistical weights q_i, for i = 1–8, of Table I), then the statistical weights $q_N^{(j)}$ and $q_C^{(j)}$ (for j = 1–4) of eq 35 and 36 are replaced by the statistical weights for the coil state (β, in the present formulation), i.e., (q_4, q_6, q_7, q_8) for the first residue and (q_2, q_5, q_6, q_8) for the last residue (see column 6 of Table II). This is the way the end residues have been treated in earlier formulations[8,18,19] of the helix–coil transition. Hence, for the helix–coil transition in polyamino acids, eq 34 can be simplified to

$$Z = \mathbf{e}\left(\prod_{i=1}^{N} \mathbf{W}_i\right)\mathbf{e}^{+} \tag{37}$$

since

$$\mathbf{u} = (q_4, q_6, q_7, q_8) = \mathbf{e}\mathbf{W}_1 \tag{38}$$

and

$$\mathbf{u}^{+} = (q_2, q_5, q_6, q_8)^{+} = \mathbf{W}_N\mathbf{e}^{+} \tag{39}$$

where

$$\mathbf{e} = (0, 0, 1, 1) \tag{40}$$

and

$$\mathbf{e}^{+} = (0, 1, 0, 1)^{+} \tag{41}$$

In general, the average fraction, θ_i, of state i in the system (where i = 1 to 8) is obtained directly from the partition function as

$$\theta_i = \frac{1}{N}\frac{\partial \ln Z}{\partial \ln q_i} \tag{42}$$

For the case of interest here, we seek θ_{α}, which is defined as

$$\theta_{\alpha} = \frac{1}{N}\frac{\partial \ln Z}{\partial \ln q_{\alpha}} \tag{43}$$

Table III
Comparison of the Nucleation Parameters of Several Theories

Theory	An example of a conformational sequence of α's and β's and the statistical weights for nucleation
	Residue 1 (N terminus) ... $\beta, \beta, \beta, \beta, \alpha, \alpha, \alpha, \alpha, \beta, \beta, \beta, \beta, \ldots$ Residue N (C terminus)
Zimm–Bragg[a]	σs
Applequist[b]	$\beta t_{\beta,\alpha}$ $\alpha t_{\alpha,\beta}$ αt_α for α or βt_β for β
Tanaka–Scheraga[c]	$q_N^{(i)}$ $(i = 1\text{–}4)$ q_7 q_4 q_2 q_5 $q_C^{(i)}$ $(i = 1\text{–}4)$

[a] The α and β states should be regarded as α-helical and coil states, respectively.[8] [b] From ref 17. [c] Present study. The dummy statistical weights listed here are given explicitly in Tables I and II.

where

$$\frac{\partial}{\partial \ln q_\alpha} = \frac{\partial}{\partial \ln q_1} + \frac{\partial}{\partial \ln q_2} + \frac{\partial}{\partial \ln q_4} + \frac{\partial}{\partial \ln q_6} \quad (44)$$

B. Discussion. In section IA, we assumed that the free energy of a residue depends only on its state and on the states of its immediate neighbors. This is an extremely simplified (one-dimensional Ising) model of a real polyamino acid chain that undergoes a helix–coil (or helix–helix) transition. Indeed, in all theories thus far proposed for these transitions, the free energy of any *regular* sequence depends only on the nature of the conformation and on the number of residues in the sequence; it is further assumed that the sequences are independent of one another, i.e., there is no energy of interaction between neighboring sequences. However, it is not necessary to make the latter assumption in order to treat the conformational properties in terms of the one-dimensional Ising model. In a one-dimensional nearest-neighbor Ising model, we may include longer-range interactions by taking larger units than a single residue. Thus, for example, if we define new units as $\alpha\alpha\alpha\alpha$, $\alpha\alpha\beta\beta$, $\beta\beta\alpha\alpha$, $\beta\beta\beta\beta$, $\alpha\beta\alpha\beta$, etc., we can calculate *all* interactions within a tetrad, and then build up a nearest neighbor Ising model matrix from these tetrads. Each tetrad that is a combination of α and β states would contain various nucleation parameters, and the number of such nucleation parameters would depend on the length of these new units. The lengths of these new units can be taken as large as necessary to account for the long-range interactions. Although such a new formulation would involve additional computational effort, the free energy of a sequence in a real polyamino acid chain would then depend not only on the length of the sequence but also, more or less, on the length of its neighboring sequences. Thus, the initiation parameter, assigned to a residue at a junction between two different sequences, would depend on the junction free energy arising from the interaction between neighboring sequences. In this respect, the Zimm–Bragg[8] nucleation parameter σ (which they assigned to the first residue of a helical sequence) would depend not only on the length of the helical sequence to which it is assigned but also on the length of the preceding coil sequence. However, the contributions of most of the residues in the preceding coil sequence to the junction free energy seem to be negligible, to a first approximation. Thus, one of the assumptions in the Zimm–Bragg theory could be that σ depends on the length of only the helical sequence. However, Zimm and Bragg[8] made the more restricted assumption that σ is independent of the length of the helical sequence; i.e., they used a single value of σ, and assumed it to be constant for the first residue of any helical sequence when the helical sequence was longer than two residues. In the molecular theory of Gō et al.,[9–12] it was also assumed that the value of σ is independent of the length of the preceding coil sequence (the junction residue being involved in the helical sequence), and was defined for a succeeding helical sequence of infinite length. However, in the molecular theory of the poly(L-proline) transition developed here and in the accompanying papers,[6,20] the nucleation parameter σ is treated as being dependent on the lengths of *both* sequences that meet at a junction; the contributions of the long-range interactions between the form I and form II sequences thus appear in the junction free energy. Even though the parameter σ involves sugh long-range interactions, it can be evaluated effectively from a phenomenological nearest-neighbor model in which long-range interactions do not appear explicitly in the simple low-order matrix used in the theory, because (after matrix multiplication) the parameter σ appears only at the first residue of a helical sequence of various lengths.

A similar argument can be presented for the parameter s. A constant value of s for an infinite chain (instead of values which, in principle, depend on the lengths of the helical sequences) may be derived from a nearest-neighbor model of the transitions in polyamino acids[9–12] and in poly(L-proline).[6,20] Thus, many successful interpretations of experimental results[6–12,18,20] have demonstrated that the nearest-neighbor interaction models (even those using a 2×2 matrix) can simulate the conformational transition of a real polyamino acid chain (without the more tedious computations required when higher-order matrices are used to take long-range interactions into account in the one-dimensional Ising model, as described above).

We next compare various theories proposed for conformational transitions in polyamino acids and poly(L-proline), viz., those of Zimm and Bragg,[8] Schwarz,[16] Applequist,[17] and the present authors. The main differences among these theories are in the treatment of the nucleation process, whereas the propagation step is treated in the same manner in all theories. For this comparison, the nucleation parameters of these theories are summarized in Table III, using a particular set of sequences of α and β as an example. Schwarz's treatment is not included in Table

III because his theory requires the specification of both directions of the conformational transition (i.e., from N to C terminus, and from C to N terminus) in order to assign the statistical weights (for nucleation) to conformational sequences such as that given in Table III. In contrast, in the other theories, only one direction (which may be taken from the N to the C terminus, as usual,[21] in accordance with the direction of the matrix multiplication of eq 34 or 37) needs to be specified to assign the statistical weights to conformational sequences such as that of Table III. Therefore, the nucleation parameters of Schwarz's theory will be discussed separately in section IIB.

As seen in Table III, the nucleation parameter of the Zimm–Bragg theory is assigned only to the initial residue of the α (i.e., α-helical) sequence. In Applequist's treatment, two different parameters, $t_{\alpha,\beta}$ and $t_{\beta,\alpha}$, are used to describe the nucleation of the β and α sequences, respectively, in the interior of the chain. In both the theories of Zimm and Bragg, and Applequist, it was assumed that the conformational transition can occur only in the direction from the N to the C terminus in accordance with the direction of matrix multiplication. On the other hand, in our theory, the conformational transition can occur in both directions, although the matrix multiplication is carried out from the N to the C terminus. The nucleation statistical weights q_7 and q_2 are assigned to the nucleation of α at the N terminus of an α sequence and of β at the N terminus of a β sequence, respectively (or to the termination of a β and an α sequence, respectively), in a transition occurring from the N to the C terminus of the chain; q_4 and q_5 are assigned to the nucleation of β at the C terminus of a β sequence and of α at the C terminus of an α sequence, respectively, in a transition occurring from the C to the N terminus of the chain. As for the end effects, these are not taken into account in the Zimm–Bragg theory, whereas it is taken into consideration only at the C terminus of the chain in the Applequist theory. However, in our theory, the end effects, which can play an important role in the transition when the polymer chains are short (see discussion in paper III[20]), are taken into account explicitly (see also section IA).

In the theory presented in this paper, as well as in the theories of Schwarz and Applequist, the asymmetric properties of nucleation were introduced. In general, this asymmetry may be caused by the asymmetric chemical structure of amino acid residues even in a homopolymer (i.e., one end of the chain has an amino group, and the other end a carboxyl group), and by the effect of the amino acid sequence on the energies of interaction in a specific-sequence copolymer. Indeed, for the form I $\rightleftarrows$ form II interconversion in poly(L-proline), the junction free energies for the sequences I–II and II–I differ considerably, as shown in paper II;[6] this difference leads to the asymmetric nucleation of the helical structures.

According to the formulation of section IA, the possible end effects are incorporated in the end-effect parameters ϵ_N and ϵ_C. These parameters influence the statistical weights at the ends of the chain; i.e., $\epsilon_N{}^{1/2}s$ or $\epsilon_C{}^{1/2}s$ are used at the ends (see Table II) rather than s which is assigned to residues in the interior of the chain (see Table I). On the other hand, the nucleation parameters σ_N, σ_C, β_N, and β_C can be used at the end residues as well as in the interior of the chain (see Tables I and II). Hence, hereafter we will designate $\gamma_N{}^{\alpha}$, $\gamma_N{}^{\beta}$, $\gamma_C{}^{\alpha}$, and $\gamma_C{}^{\beta}$ (or ϵ_N and ϵ_C) as "end effect" parameters.

If circular boundary conditions are assumed, as is done in treating an infinite chain in the one-dimensional Ising model, then the additional interaction between residues 1 and N (e.g., $G^{\alpha\alpha}{}_{N,1}$) is treated as an interior interaction (e.g., $G^{\alpha\alpha}{}_{i,i+1}$), neglecting the contribution of specific interactions between the solvent and the end groups. Thus, the "end effect" parameters $\gamma_N{}^{\alpha}$, $\gamma_N{}^{\beta}$, $\gamma_C{}^{\alpha}$, and $\gamma_C{}^{\beta}$ (or ϵ_N and ϵ_C) vanish, and the four parameters s, σ, β_N, and β_C are sufficient to describe the transition.

In summary, in order to describe a transition in a polymer chain in which asymmetric nucleation must be taken into account, four interesting cases can be distinguished, viz., (i) an infinite homopolymer chain, in which the four parameters s, σ, β_N, and β_C are necessary, i.e., if circular boundary conditions are assumed; (ii) a real (open) homopolymer chain ($\epsilon_N \neq \epsilon_C$), in which the various end effects have to be taken into account, and the six parameters s, σ, β_N, β_C, ϵ_N, and ϵ_C are necessary; (iii) an infinite specific-sequence copolymer chain, in which it is necessary to differentiate the nucleation parameters at the N and C termini of a *sequence*, and σ_N and σ_C ($\sigma_N \neq \sigma_C$) must then be used instead of the single parameter σ (i.e., the five parameters s, σ_N, σ_C, β_N, β_C are required); (iv) a real (open) specific-sequence copolymer chain, in which the seven parameters s, σ_N, σ_C, β_N, β_C, ϵ_N, and ϵ_C are required. In order to decide which approximation to use to describe a conformational transition in a particular polymer chain, we must consider the chain length, the species of end groups, and the interactions between the end groups and the solvent.

II. Schwarz's 2 × 2 Matrix Formulation

A. Summary. Having shown the structure and meaning of the statistical weights, we now summarize the results of Schwarz's 2 × 2 matrix theory since this leads to a quadratic secular equation, with which it is relatively easy to compute θ_α.

It is also possible to formulate the theory of the conformational transition by defining the free energies $G^{\eta}{}_i$ and $G^{\eta\xi}{}_{i,i+1}$ corresponding to the internal properties of the ith residue and to the nearest-neighbor interaction between the ith and $(i + 1)$th residues which are in the conformational states η and ξ, respectively (where η and ξ may be replaced by α and β) (alternatively $G^{\eta\xi}{}_{i-1,i}$ may be used instead of $G^{\eta\xi}{}_{i,i+1}$). In the treatment of section I, we assigned half of $G^{\eta\xi}{}_{i,i+1}$ to the ith residue and half to the $(i + 1)$th residue to obtain eq 1 for the total free energy, G, of a chain of N residues. Using the present assignment of free energies, the corresponding equation to eq 1 is

$$G = (G_1 + G_{1,2}) + \sum_{i=2}^{N-1}(G_i + G_{i,i+1}) + G_N \quad (45)$$

Equation 45 implies that a possible conformational state of the chain can be represented by a sequence of statistical weights whose general form is

$$u_{\eta\xi} = \exp[-(G^{\eta}{}_i + G^{\eta\xi}{}_{i,i+1})/RT] \quad (46)$$

We also define the quantity

$$u_\eta = \exp(-G^{\eta}{}_i/RT) \quad (47)$$

Since we are interested here in the poly(L-proline) I $\rightleftarrows$ II interconversion, we shall replace η and ξ (or α and β) by I and II, respectively.[22] The appropriate 2 × 2 statistical weight matrix for the ith residue is

$$\mathbf{W}_i = \begin{array}{c|cc} & i+1 & \\ & \text{II} & \text{I} \\ \hline \text{II} & u_{\text{II,II}} & u_{\text{II,I}} \\ \text{I} & u_{\text{I,II}} & u_{\text{I,I}} \end{array} \quad (48)$$

Table IV
Schematic Description of Elementary Processes and Equilibrium Constants, Based on Schwarz's Theory

Elementary process[a]		Equilibrium constant	Illustrative description of the process for the theoretical formulation of the statistical wt	
Growth process	...II, II, I... ⇌ ...II, I, I...	s	$\underbrace{\text{II, II, II...II, II}}_{j+1}, \underbrace{\text{I, I, I...I, I}}_{j} \rightleftharpoons \underbrace{\text{II, II, II...II, II, II}}_{j}, \underbrace{\text{I, I...I, I, I}}_{j+1}$	(IV-1)
Nucleation of an isolated I at an internal residue	...II, II, II... ⇌ ...II, I, II...	σs		*b*
Nucleation of an isolated II at an internal residue	...I, I, I... ⇌ ...I, II, I...	σ/s		*b*
Nucleation of I at the N terminus of a II sequence	0, II, II... ⇌ 0, I, II...	$\sigma'_{\text{I}}s$	$\underbrace{\text{II, II...II, II, II}}_{2j} \rightleftharpoons \underbrace{\text{I, I...I, I,}}_{j} \underbrace{\text{II, II...II, II}}_{j}$	(IV-2)
Nucleation of I at the C terminus of a II sequence	...II, II, 0 ⇌ ...II, I, 0	$\sigma''_{\text{I}}s$	$\underbrace{\text{II, II...II, II, II}}_{2j} \rightleftharpoons \underbrace{\text{II, II...II, II,}}_{j} \underbrace{\text{I, I, I...I, I}}_{j}$	(IV-3)
Nucleation of II at the N terminus of a I sequence	0, I, I... ⇌ 0, II, I...	σ'_{II}/s	$\underbrace{\text{I, I, I...I, I, I}}_{2j} \rightleftharpoons \underbrace{\text{II, II...II, II,}}_{j} \underbrace{\text{I, I...I, I}}_{j}$	(IV-4)
Nucleation of II at the C terminus of a I sequence	...I, I, 0 ⇌ ...I, II, 0	σ''_{II}/s	$\underbrace{\text{I, I...I, I, I}}_{2j} \rightleftharpoons \underbrace{\text{I, I...I, I,}}_{j} \underbrace{\text{II, II...II, II}}_{j}$	(IV-5)

[a] The symbol 0 denotes the absence of an interresidue interaction (see text), and *not* the end of the chain. When 0 appears at the left end of a sequence, it means that the ith residue (as the first one) interacts with residue $(i + 1)$ and *not* with residue $(i - 1)$, and it does not matter what the conformation of residue $(i - 1)$ is. An analogous meaning pertains to a 0 at the right end of a sequence. [b] It is not necessary to introduce σ in the original formulation since the value of σ may be calculated on the basis of IV-2 to IV-5, using eq 63.

Any possible conformational state of a chain of N residues can be represented by a sequence of the symbols I and II. The statistical weight of the given sequence may be written as a product of statistical weights assigned to each state (which, in turn, depend on the conformation of each residue and that of its nearest neighbor), i.e., as a product of the elements of the matrix of eq 48.

The statistical weights introduced by Schwarz[16] (and which differ from those of section I) were defined as follows, with the aid of the elementary processes described in Table IV: s_{I} is assigned to a I which is followed by a I, i.e.

$$s_{\text{I}} = w_{\text{I,I}} \tag{49}$$

and s_{II} to a II followed by a II, i.e.

$$s_{\text{II}} = w_{\text{II,II}} \tag{50}$$

The growth process (IV-1 in Table IV) then has an equilibrium constant

$$w_{\text{II,I}}w_{\text{I,I}}/w_{\text{II,II}}w_{\text{II,I}} = s_{\text{I}}/s_{\text{II}} \tag{51}$$

which is defined as s. The nucleation of a I at the N terminus of a II sequence (IV-2 in Table IV) has an equilibrium constant $w_{\text{I,II}}/w_{\text{II,II}}$, which may be written as

$$w_{\text{I,II}}w_{\text{I,I}}/w_{\text{I,I}}w_{\text{II,II}} = \sigma'_{\text{I}}s \tag{52}$$

where σ'_{I} is defined as

$$\sigma'_{\text{I}} = w_{\text{I,II}}/w_{\text{I,I}} \tag{53}$$

Likewise, the nucleation of a II at the N terminus of a I sequence (IV-4 in Table IV) has an equilibrium constant σ'_{II}/s, where σ'_{II} is defined as

$$\sigma'_{\text{II}} = w_{\text{II,I}}/w_{\text{II,II}} \tag{54}$$

Similarly, the nucleation of a I at the C terminus of a II sequence (IV-3 of Table IV) has an equilibrium constant

$$w_{\text{II,I}}u_{\text{I}}/w_{\text{II,II}}u_{\text{II}} = \sigma''_{\text{I}}s \tag{55}$$

where

$$\sigma''_{\text{I}} = w_{\text{II,I}}u_{\text{I}}/w_{\text{I,I}}u_{\text{II}} \tag{56}$$

Likewise, the nucleation of a II at the C terminus of a I sequence (IV-5 in Table IV) has an equilibrium constant σ''_{II}/s, where

$$\sigma''_{\text{II}} = w_{\text{I,II}}u_{\text{II}}/w_{\text{II,II}}u_{\text{I}} \tag{57}$$

Finally, the nucleation of an isolated I at an internal residue (line 2 in Table IV) has an equilibrium constant

$$w_{\text{II,I}}w_{\text{I,II}}/w_{\text{II,II}}w_{\text{II,I}} = \sigma'_{\text{I}}\sigma'_{\text{II}}s = \sigma s \tag{58}$$

where $\sigma = \sigma'_{\text{I}}\sigma'_{\text{II}}$, and the nucleation of an isolated II at an internal residue (line 3 in Table IV) has an equilibrium constant

$$w_{\text{I,II}}w_{\text{II,I}}/w_{\text{I,I}}w_{\text{I,I}} = \sigma''_{\text{I}}\sigma''_{\text{II}}/s = \sigma/s \tag{59}$$

where $\sigma = \sigma''_{\text{I}}\sigma''_{\text{II}}$.

Thus, eq 48 can be rewritten as

$$\mathbf{W} = \begin{array}{c|cc} & i+1 & \\ & \overline{\text{II}} & \overline{\text{I}} \\ \hline \text{II} & s_{\text{II}} & \sigma'_{\text{II}}s_{\text{II}} \\ \text{I} & \sigma'_{\text{I}}s_{\text{I}} & s_{\text{I}} \end{array} \quad (i) \tag{60}$$

It should be noted that, in contrast to eq 33, eq 60 [which correlates the states of the ith and $(i + 1)$th residues] treats

a transition as if it occurred only in the direction from the N to the C terminus; i.e., only σ'_{I} and σ'_{II} appear, but not σ''_{I} and σ''_{II}. Nevertheless, restricting our consideration to the conformational transition in a homopolymer, the *numerical* values (such as the partition function and thermodynamic quantities computed from the partition function) calculated by using eq 60 are the same as those obtained from theories (such as that developed in section IA) in which the two directionalities of the conformational transition (i.e., from N to C terminus, and vice versa) are taken into account (as will be proven in the next paragraph). The reason for this is that conformational nucleations in the interior of the chain may be expressed in terms of a single nucleation parameter, σ, regardless of whether, say, a I is nucleated from the N to the C terminus ($\sigma'_{I}\sigma'_{II}$ or σ_C in the formulation of section IA) or from the C to the N terminus ($\sigma''_{I}\sigma''_{II}$ or σ_N), because $\sigma = \sigma'_{I}\sigma'_{II} = \sigma''_{I}\sigma''_{II}$ or $\sigma = \sigma_C = \sigma_N$ for a homopolymer. (See also the description of the physical meaning of σ, β', and β'' in the next paragraph and in section IIB.)

We stated in section IA that our 4 × 4 matrix theory and Schwarz's 2 × 2 matrix theory led to the same result. In order to demonstrate this, consider the following sequence as an example

$$\overset{1}{\text{II}},\overset{2}{\text{II}},\overset{3}{\text{I}},\overset{4}{\text{II}},\overset{5}{\text{II}} \text{ or } \overset{1}{\beta},\overset{2}{\beta},\overset{3}{\alpha},\overset{4}{\beta},\overset{5}{\beta} \tag{61}$$

Then, let us consider the statistical weights for this sequence by using our formulation and that of Schwarz, *tentatively* neglecting the end effects at residues 1 and 5. On the basis of section IA (column 3 of Table I), the statistical weights are $\sigma_C{}^{\beta}w_{\beta}$, $\sigma_N{}^{\alpha}\sigma_C{}^{\alpha}w_{\alpha}$, and $\sigma_N{}^{\beta}w_{\beta}$ for residue 2 of triad $\beta^1\beta^2\alpha^3$, for residue 3 of triad $\beta^2\alpha^3\beta^4$, and for residue 4 of triad $\alpha^3\beta^4\beta^5$, respectively. The product of these statistical weights is $\sigma w_{\beta}{}^2w_{\alpha}$ (using eq 11a, 11b, and 14) for a homopolymer. On the other hand, on the basis of Schwarz's formulation (see eq 60), regarding the sequence of expression 61 as resulting from a conformational transition from the N to the C terminus of the chain, the statistical weights are $\sigma'_{II}s_{II}$, $\sigma'_{I}s_{I}$, and s_{II} for residue 2 of the dyad II^2I^3, for residue 3 of the dyad I^3II^4, and for residue 4 of dyad II^4II^5, respectively. The product of these statistical weights is $\sigma s_{II}{}^2s_{I}$ (using eq 58, or the later eq 63), which is exactly the same as $\sigma w_{\beta}{}^2w_{\alpha}$ if α and β are replaced by I and II and w is replaced by s. Applying the above reasoning to any sequence of I's and II's in the whole chain, the equivalence of our formulation (section IA) and that of Schwarz is demonstrated. Thus, if the statistical weights for the end residues of the chain, which have been omitted from the above discussion, are treated in the same manner in both theories, then both will lead to the same result; case (i), in the discussion of the treatment of end groups at the end of section IB, corresponds to the treatment of end groups in Schwarz's theory.

The six parameters $w_{I,I}$, $w_{I,II}$, $w_{II,I}$, $w_{II,II}$, u_I, and u_{II} of eq 46 and 47 are the independent variables. However, since the values for one of $w_{I,I}$, $w_{I,II}$, $w_{II,I}$, and $w_{II,II}$, and for one of u_I and u_{II}, may be chosen arbitrarily (as the choice of standard state), eq 49–60 may be expressed in terms of any four of the independent variables. Thus, the equilibrium behavior of the system may then be described by the degree of polymerization, N, and the four new independent parameters (defined in terms of those of eq. 49–59) as

$$s = s_I/s_{II} \tag{62}$$

$$\sigma = \sigma'_{I}\sigma'_{II} = \sigma''_{I}\sigma''_{II} \tag{63}$$

$$\beta' = \sigma'_{I}/\sigma'_{II} \tag{64}$$

$$\beta'' = \sigma''_{I}/\sigma''_{II} \tag{65}$$

The relationships between the elementary processes and these equilibrium constants are summarized in Table IV. The quantity s of eq 62 may be regarded as the equilibrium constant for the growth process, II,II,I $\rightleftarrows$ II,I,I. The physical meaning of σ, β', and β'' of eq 63–65 may be understood by comparison with σ, β_C, and β_N of the formulation presented in section IA (this may be seen by substituting I and II for α and β in eq 12′, and comparing it with eq 64″, likewise, by comparing eq 13′ and 64′; all of these equations appear later). For this purpose, it is instructive to express the parameter σ in terms of free energy by using eq 46, 53, 54, and 63 which are related to the quantities σ'_{I} and σ'_{II} (or eq 46, 47, 56, 57, and 63 which are related to the quantities σ''_{I} and σ''_{II}) based on Schwarz's formulation, viz.

$$-RT \ln \sigma = (G^{I,II} + G^{II,I}) - (G^{I,I} + G^{II,II}) \tag{63′}$$

In terms of the formulation of section IA, the corresponding expression is

$$-RT \ln \sigma = (G^{\alpha\beta}{}_{i,i+1} + G^{\beta\alpha}{}_{i,i+1}) - (G^{\alpha\alpha}{}_{i,i+1} + G^{\beta\beta}{}_{i,i+1}) \tag{14′}$$

by using eq 6b, 9, 11b, and 14 or

$$-RT \ln \sigma = (G^{\alpha\beta}{}_{i-1,i} + G^{\beta\alpha}{}_{i-1,i}) - (G^{\alpha\alpha}{}_{i-1,i} + G^{\beta\beta}{}_{i-1,i}) \tag{14″}$$

by using eq 7, 8, 11a, and 14. As seen in eq 63′, 14′, and 14″, the nucleation parameter σ is determined by the difference in free energy between the junction free energies (I,II and II,I) and the free energies of the homogeneous pairs (I,I and II,II), which means that the value of σ measures the difficulty of nucleation of the junctions I,II and II,I relative to I,I and II,II. Also, as seen in eq 63′, 14′, and 14″, the parameter σ is common to both directions of the conformational transition in a *homopolymer* (see the last paragraph of this section for an analogous discussion for a specific-sequence copolymer).

In order to understand the physical meaning of the parameters β' and β'' of Schwarz, or β_C and β_N of our formulation, it is also helpful to express these parameters in terms of free energies. Thus, we obtain

$$-RT \ln \beta' = -RT \ln (\sigma'_{I}/\sigma'_{II}) = (G^{I,II} - G^{I,I}) - (G^{II,I} - G^{II,II}) \tag{64′}$$

by using eq 46, 53, 54, and 64, and

$$-RT \ln \beta'' = -RT \ln (\sigma''_{I}/\sigma''_{I}) = (G^{II,I} - G^{I,I}) - (G^{I,II} - G^{II,II}) \tag{64″}$$

by using eq 46, 47, 56, 57, and 65. In terms of the formulation of section IA, we obtain

$$-RT \ln \beta_N = -RT \ln (\sigma^{\alpha}{}_{N}/\sigma^{\beta}{}_{N})^2 = (G^{\beta\alpha}{}_{i-1,i} - G^{\alpha\alpha}{}_{i-1,i}) - (G^{\alpha\beta}{}_{i-1,i} - G^{\beta\beta}{}_{i-1,i}) \tag{12′}$$

by using eq 8, 7, and 12 and

$$-RT \ln \beta_C = -RT \ln (\sigma^{\alpha}{}_{C}/\sigma^{\beta}{}_{C})^2 = (G^{\alpha\beta}{}_{i,i+1} - G^{\alpha\alpha}{}_{i,i+1}) - (G^{\beta\alpha}{}_{i,i+1} - G^{\beta\beta}{}_{i,i+1}) \tag{13′}$$

by using eq 6b, 9, and 13. Substituting I and II for α and β in eq 12′ and 13′, we obtain the equations expressed in a notation directly applicable to the form I $\rightleftarrows$ form II interconversion in poly(L-proline). As seen in eq 64′ (or eq 13′), the parameter β' (or β_C in our formulation) measures the difficulty of nucleation of a II (or β) state at the N terminus of a II sequence relative to the nucleation of a I (or α) state at the N terminus of a I sequence for a conformation-

al transition occurring from the N to the C terminus of the chain; hence, β' (or β_C) depends on the directionality of the transition. Expressed in another way, we may say that, for a conformational transition occurring from the C to the N terminus of the chain, β' (or β_C) measures the difficulty of termination of a I (or α) state at the C terminus of a I sequence relative to the termination of a II state at the C terminus of a II sequence. On the other hand, β'' (or β_N) measures the difficulty of nucleation of a II state at the C terminus of a II sequence relative to the nucleation of a I state at the C terminus of a I sequence for a conformational transition occurring from the C to the N terminus of the chain. Expressed in another way, we may say that, for a conformational transition occurring from the C to the N terminus of the chain, β'' (or β_N) measures the difficulty of termination of a I state at the N terminus of a I sequence relative to the termination of a II state at the N terminus of a II sequence.

As stated in section IA, the free energy difference on the right-hand side of eq 14′ is equal to $-RT \ln \sigma_N$ (see eq 11a), and that of eq 14″ is equal to $-RT \ln \sigma_C$ (see eq 11b). For a specific-sequence copolymer, $-RT \ln \sigma_N$ and $-RT \ln \sigma_C$ are not always the same because different types of amino acid residues, in general, are present at positions $i - 1$, i, and $i + 1$; thus σ_N and σ_C [and also β' (or β_C) and β'' (or β_N)] can depend on the direction of the conformational transition.

B. Discussion. The parameters σ'_I and σ'_{II} (hence β') are the nucleation parameters assigned to the N termini of sequences of II's and I's, respectively, even in the middle of a chain, whereas σ''_I and σ''_{II} (hence β'') are assigned to the C termini of such sequences. If the conformational transition occurred only in one direction, only two such σ parameters, instead of four, would be required, but another set of two σ parameters would then be required for a transition in the opposite direction. For the special case, where nucleation in either direction is equivalent (symmetric nucleation), only two nucleation parameters are required.

In Schwarz's treatment, it was necessary to specify the direction of the conformational transition in order to assign the nucleation parameters σ'_I and σ'_{II} (hence β') and σ''_I and σ''_{II} (hence β'') to their appropriate conformational states.[23]

As an illustration, consider the sequence in expression 66

$$\overset{1\ 2\ 3\ 4\ 5\ 6\ 7\ 8}{\ldots \text{II, II, II, I, I, I, II, II, } \ldots} \qquad (66)$$

where we have numbered the sequence 1 to 8 for convenience. Let us suppose that the sequence of expression 66 was achieved by a transition in a certain direction (i.e., from the N to the C terminus of the chain or vice versa) and that, for the transition from the N to the C terminus, the direction of interaction is taken from i to $i + 1$. Correlating the state of the ith residue with that of the $(i + 1)$th, we may write the free energy for the conformational state of expression 66 as

$$\begin{aligned} G = \ldots &+ (G^{\mathrm{II}} \overset{1}{+} G^{\mathrm{II,II}}) + (G^{\mathrm{II}} \overset{2}{+} G^{\mathrm{II,II}}) + \\ &(G^{\mathrm{II}} \overset{3}{+} G^{\mathrm{II,I}}) + (G^{\mathrm{I}} \overset{4}{+} G^{\mathrm{I,I}}) + (G^{\mathrm{I}} \overset{5}{+} G^{\mathrm{I,I}}) + \\ &(G^{\mathrm{I}} \overset{6}{+} G^{\mathrm{I,II}}) + (G^{\mathrm{II}} \overset{7}{+} G^{\mathrm{II,II}}) + \\ &(G^{\mathrm{II}} \overset{8}{+} \ldots) + \ldots \end{aligned} \qquad (67)$$

where the parentheses group the free energies assigned to each residue according to eq 46. From the above argument, the sequence of expression 66 should be regarded as arising in a transition from the N to the C terminus because of the direction of interaction from i to $i + 1$. According to eq 46 and 47, the statistical weight of the conformation in expression 66 may be written as

$$\overset{1\quad 2\quad 3\quad 4\quad 5\quad 6\quad 7\quad 8}{\ldots w_{\mathrm{II,II}} w_{\mathrm{II,II}} w_{\mathrm{II,I}} w_{\mathrm{I,I}} w_{\mathrm{I,I}} w_{\mathrm{I,II}} w_{\mathrm{II,II}} \ldots} \qquad (68)$$

With the aid of eq 49, 50, 53, and 54, this may be written as

$$\overset{1\ 2\quad 3\quad 4\ 5\quad 6\quad 7}{\ldots s_{\mathrm{II}} s_{\mathrm{II}} (\sigma'_{\mathrm{II}} s_{\mathrm{II}}) s_{\mathrm{I}} s_{\mathrm{I}} (\sigma'_{\mathrm{I}} s_{\mathrm{I}}) s_{\mathrm{II}} \ldots} \qquad (69)$$

Thus, for a transition from the N to the C terminus σ'_{II} and σ'_I are assigned to the C termini of sequences of II's and I's (i.e., to residues 3 and 6 in expression 66). It should be noted that the parameter σ, which is $\sigma'_I\sigma'_{II}$, according to eq 63, is a measure of the nucleation of a sequence of like states, e.g., $I^4I^5I^6$ in expression 66. In the Zimm–Bragg theory of the helix $\rightleftarrows$ coil transition,[8] the parameter σ was assigned to the first residue of a sequence of like states, and no σ was assigned to the last residue of such a sequence (see Table III).

Now consider the reverse direction for the conformational transition, i.e., the sequence of expression 66 is regarded as arising in a transition from the C to the N terminus and the direction of interaction is taken from i to $i - 1$. Then

$$\begin{aligned} G = \ldots &\overset{1}{+} G^{\mathrm{II}}) + (G^{\mathrm{II,II}} \overset{2}{+} G^{\mathrm{II}}) + (G^{\mathrm{II,II}} \overset{3}{+} G^{\mathrm{II}}) + \\ &(G^{\mathrm{II,I}} \overset{4}{+} G^{\mathrm{I}}) + (G^{\mathrm{I,I}} \overset{5}{+} G^{\mathrm{I}}) + (G^{\mathrm{I,I}} \overset{6}{+} G^{\mathrm{I}}) + \\ &(G^{\mathrm{I,II}} \overset{7}{+} G^{\mathrm{II}}) + (G^{\mathrm{II,II}} \overset{8}{+} G^{\mathrm{II}}) + \ldots \end{aligned} \qquad (70)$$

Then, the statistical weight is

$$\overset{1\ 2\quad 3\quad\quad 4\quad\quad 5\ 6}{\ldots w_{\mathrm{II,II}} w_{\mathrm{II,II}} (w_{\mathrm{II,I}} w_{\mathrm{I}} / u_{\mathrm{II}}) w_{\mathrm{I,I}} w_{\mathrm{I,I}} \times} \overset{7\quad 8}{(w_{\mathrm{I,II}} u_{\mathrm{II}} / u_{\mathrm{I}}) w_{\mathrm{II,II}} \ldots} \qquad (71)$$

or

$$\overset{1\ 2\ 3\quad 4\quad 5\ 6\quad 7\quad 8}{\ldots s_{\mathrm{II}} s_{\mathrm{II}} (\sigma''_{\mathrm{I}} s_{\mathrm{I}}) s_{\mathrm{I}} s_{\mathrm{I}} (\sigma''_{\mathrm{II}} s_{\mathrm{II}}) s_{\mathrm{II}} \ldots} \qquad (72)$$

Thus, for the transition, from the C to the N terminus, σ''_I and σ''_{II} are assigned to the N termini of sequences of I's and II's (i.e., to residues 4 and 7 in expression 66). Thus, if (and only if) the *direction* of the transition is specified, the parameters σ'_I and σ'_{II} (hence β') and σ''_I and σ''_{II} (hence β'') can be assigned explicitly to any residue of the chain. This was the reason why Schwarz's nucleation parameters could not be compared to those of other theories in Table III, and why, in Schwarz's formulation,[16] it is impossible to express the elements of the matrix (such as eq 60) in terms of the six parameters, s_I, s_{II}, σ'_I, σ'_{II}, σ''_I, and σ''_{II}, and hence impossible to express these elements in terms of s, σ, β', and β'', because the matrix "looks only in one direction" (from i to $i + 1$ in the case of eq 60), corresponding to the direction of matrix multiplication in eq 34 (or eq 73). However, this difficulty does not arise in the matrix of eq 33. Nevertheless, both Schwarz's and our theories yield the same result, as discussed in section IIA.

C. Evaluation of Partition Function and Transition Curve. The partition function Z and thermodynamic quantities such as θ_I, the average fraction of residues in form I, may be evaluated by the matrix method, using the matrix of eq 60

$$Z = \mathbf{u}\mathbf{W}^{N-1}\mathbf{v} \qquad (73)$$

where

$$\mathbf{u} = (1, 1) \qquad (74)$$

because the first residue at the N terminus may be in ei-

ther a I or a II state, which differs from the usual treatment of the end groups in the helix–coil transition,[18] and[24]

$$\mathbf{v} = \begin{bmatrix} (\sigma'_{\mathrm{I}}\sigma''_{\mathrm{I}})^{1/2}s_{\mathrm{I}} \\ (\sigma'_{\mathrm{II}}\sigma''_{\mathrm{II}})^{1/2}s_{\mathrm{II}} \end{bmatrix} = \begin{bmatrix} u_{\mathrm{I}} \\ u_{\mathrm{II}} \end{bmatrix} \quad (75)$$

The last equality makes use of the definition of u_η of eq 47.

The eigenvalues $\lambda_{1,2}$ of the matrix of eq 60 are

$$\lambda_{1,2} = \tfrac{1}{2}\{s_{\mathrm{I}} + s_{\mathrm{II}} \pm [(s_{\mathrm{I}} - s_{\mathrm{II}})^2 + 4\sigma s_{\mathrm{I}} s_{\mathrm{II}}]^{1/2}\} \quad (76)$$

The left- and right-hand eigenvector matrices are given by

$$\mathbf{M} = \begin{bmatrix} \sigma'_{\mathrm{II}}s_{\mathrm{II}} & \sigma'_{\mathrm{II}}s_{\mathrm{II}} \\ \lambda_1 - s_{\mathrm{II}} & \lambda_2 - s_{\mathrm{II}} \end{bmatrix} \quad (77)$$

and

$$\mathbf{M}^{-1} = \frac{1}{\sigma'_{\mathrm{II}}s_{\mathrm{II}}(\lambda_1 - \lambda_2)}\begin{bmatrix} s_{\mathrm{II}} - \lambda_2 & \sigma'_{\mathrm{II}}s_{\mathrm{II}} \\ \lambda_1 - s_{\mathrm{II}} & -\sigma'_{\mathrm{II}}s_{\mathrm{II}} \end{bmatrix} \quad (78)$$

where

$$\mathbf{W}^n = \mathbf{M}\begin{bmatrix} \lambda_1^n & 0 \\ 0 & \lambda_2^n \end{bmatrix}\mathbf{M}^{-1} \quad (79)$$

Using eq 47–50 of Schwarz,[16] we may obtain $\mathbf{W}^n\mathbf{v}$ and $\mathbf{u}\mathbf{W}^n$. The partition function is given by Schwarz's eq 51. The probability $F_{\mathrm{I}(i)}$ that the ith residue is in conformation I is given[16] by

$$F_{\mathrm{I}(i)} = \left(\frac{1}{Z}\right)(\mathbf{u}\mathbf{W}^{i-1})\left(\frac{\partial \mathbf{W}_i}{\partial \ln s_{\mathrm{I}}}\right)(\mathbf{W}^{N-i-1}\mathbf{v}) \quad (80)$$

and θ_{I} is then given by

$$\theta_{\mathrm{I}} = (1/N)\sum_{i=1}^{N} F_{\mathrm{I}(i)} \quad (81)$$

(see Schwarz's eq 66). This value of θ_{I} depends on the values of N, s, σ, β', and β''. The evaluation of s, σ, β', and β'', in terms of interatomic potentials, in the absence and presence of solvent, is given in papers II[6] and III,[20] respectively.

References and Notes

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(2) (a) From Kyoto University, 1972–1975; (b) address correspondence to this author.

(3) For a recent review, see H. A. Scheraga, *Chem. Rev.*, **71**, 195 (1971).

(4) For reviews on the conformational properties of proline, see: (a) E. Katchalski, A. Berger, and J. Kurz, "Aspects of Protein Structure", G. N. Ramachandran, Ed., Academic Press, New York, N.Y., 1963, p 205; (b) L. Mandelkern, "Biological Macromolecules", Vol. I, G. D. Fasman, Ed., Marcel Dekker, New York, N.Y., 1967, p 675.

(5) According to the recommendations of an IUPAC Commission on nomenclature [*Biochemistry*, **9**, 3471 (1970)], the terms "form I" and "form II" helices for poly(L-proline) refer to the conformation about the peptide bond, viz., $\omega = 0°$ and 180°, respectively. In this paper, we will follow this convention to designate *regular* helices of poly(L-proline), even though the minimum-energy values of ω indicate slight deviations from planarity of the peptide group, i.e., ω is close to, but not exactly equal to, 0 and 180°, respectively (see Table II of paper II[6]).

(6) S. Tanaka and H. A. Scheraga, *Macromolecules*, part II following in this issue.

(7) For example, see D. Poland and H. A. Scheraga, "Theory of Helix-Coil Transitions in Biopolymers", Academic Press, New York, N.Y., 1970.

(8) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

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(10) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **52**, 2060 (1970).

(11) M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, **54**, 4489 (1971).

(12) M. Gō, F. T. Hesselink, N. Gō, and H. A. Scheraga, *Macromolecules*, **7**, 459 (1974).

(13) Mattice and Mandelkern[14] reported that poly(L-proline) can exist as a nonregular chain of form II in solution, rather than as a regular rigid structure of form II. As we will discuss in a later paper,[15] this is not yet a completely settled question; hence, in the present series of three papers, we will assume that the poly(L-proline) chain consists of alternating sequences of regular conformations (form I and form II) except at the junctions between these regular conformations.

(14) W. L. Mattice and L. Mandelkern, *J. Am. Chem. Soc.*, **93**, 1769 (1971).

(15) S. Tanaka and H. A. Scheraga, *Macromolecules*, submitted for publication.

(16) G. Schwarz, *Biopolymers*, **6**, 873 (1968).

(17) J. Applequist, *Biopolymers*, **6**, 117 (1968).

(18) D. Poland and H. A. Scheraga, *J. Chem. Phys.*, **43**, 2071 (1965).

(19) S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).

(20) S. Tanaka and H. A. Scheraga, *Macromolecules*, part III following in this issue.

(21) In this paper, we are interested primarily in the conformational transitions in polyamino acids, including poly(L-proline). Therefore, we employ the conventions and nomenclature proposed by an IUPAC-IUB Commission [*Biochemistry*, **9**, 3471 (1970)]. Thus, a chain of N residues is numbered from residue 1 at the N terminus to residue N at the C terminus.

(22) In general, the symbols I and II may designate the conformational states of either a residue or a segment of several residues. In these papers, we use these symbols to represent the conformational states of the residue.

(23) The argument in this section is presented in terms of s_{I}, s_{II}, σ'_{I}, σ'_{II}, σ''_{I}, and σ''_{II}. However, it can also be presented in terms of s, σ, β', and β'' by using eq 62 and the following altered forms of eq 63–65: $\sigma'_{\mathrm{I}} = (\sigma\beta')^{1/2}$; $\sigma'_{\mathrm{II}} = (\sigma/\beta')^{1/2}$; $\sigma''_{\mathrm{I}} = (\sigma\beta'')^{1/2}$; $\sigma''_{\mathrm{II}} = (\sigma/\beta'')^{1/2}$.

(24) The expression for $\mathbf{v}$ is obtained by considering the following sequence at the C terminus

$$\ldots \mathrm{II,II,0} \rightleftharpoons \ldots \mathrm{II,I,0} \quad \text{(a)}$$

(the choice of other sequences, such as ... I,I,0 ⇌ ... I,II,0, would yield the same result for $u_{\mathrm{I}}/u_{\mathrm{II}}$ as in eq f). The equilibrium constant may be written as

$$\frac{w_{\mathrm{II,I}}u_{\mathrm{I}}}{w_{\mathrm{II,II}}u_{\mathrm{II}}} = \sigma''_{\mathrm{I}}\left(\frac{s_{\mathrm{I}}}{s_{\mathrm{II}}}\right) \quad \text{(b)}$$

or

$$\frac{u_{\mathrm{I}}}{u_{\mathrm{II}}} = \sigma''_{\mathrm{I}}\left(\frac{s_{\mathrm{I}}}{s_{\mathrm{II}}}\right)\left(\frac{w_{\mathrm{II,II}}}{w_{\mathrm{II,I}}}\right) \quad \text{(c)}$$

Using eq 54, eq c may be rewritten as

$$\left(\frac{u_{\mathrm{I}}}{u_{\mathrm{II}}}\right)^2 = \left(\frac{\sigma''_{\mathrm{I}}}{\sigma'_{\mathrm{II}}}\right)^2\left(\frac{s_{\mathrm{I}}}{s_{\mathrm{II}}}\right)^2 \quad \text{(d)}$$

From eq 63

$$\sigma''_{\mathrm{II}} = \frac{\sigma'_{\mathrm{I}}\sigma'_{\mathrm{II}}}{\sigma''_{\mathrm{I}}} \quad \text{(e)}$$

Substituting eq e in eq d, we obtain

$$\frac{u_{\mathrm{I}}}{u_{\mathrm{II}}} = \left(\frac{\sigma'_{\mathrm{I}}\sigma''_{\mathrm{I}}}{\sigma'_{\mathrm{II}}\sigma''_{\mathrm{II}}}\right)^{1/2}\frac{s_{\mathrm{I}}}{s_{\mathrm{II}}} \quad \text{(f)}$$

which leads to eq 75.