tial energy functions may be small in the range in which they are usually used. Thus, the assumption that the potential energy is independent of temperature may not lead to significant errors since we are not interested in the temperature-induced transition, but mainly in the solvent-induced transition. (However, see ref 36.)

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(18) F. A. Momany, L. M. Carruthers, R. F. McGuire, and H. A. Scheraga, *J. Phys. Chem.*, **78**, 1595, 1621 (1974).

(19) See ref 4 of paper I.[3]

(20) F. Gornick, L. Mandelkern, A. F. Diorio, and D. E. Roberts, *J. Am. Chem. Soc.*, **86**, 2549 (1964).

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(25) S. Knof, H. Strassmair, J. Engel, M. Rothe, and K. D. Steffen, *Biopolymers*, **11**, 731 (1972).

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

(27) While there is no NH group in poly(L-proline), there is one in other polyamino acids. In such cases, the statistical weight may be written as the product of terms corresponding to the CO and NH groups. In this connection, we point out a typographical error in eq 29 of ref 7, which should have been written as

$$b = -RT \ln (1 + \sum_k K_{\mathrm{NH},k} a_k) - RT \ln (1 + \sum_l K_{\mathrm{CO},l} a_l)$$

This typographical error appeared only in the final paper, and does not affect any of the computations reported in ref 7.

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(29) M. Bixon, H. A. Scheraga, and S. Lifson, *Biopolymers*, **1**, 419 (1963).

(30) D. C. Poland and H. A. Scheraga, *Biopolymers*, **3**, 283 (1965).

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

(32) In the up and down geometries used here, the bond lengths and bond angles have been maintained close to those observed by X-ray diffraction, as described in ref 17; thus the ϕ and χ^1 values must differ for the up and down gometries to maintain the X-ray geometry for the two types of puckering. Although we mentioned only C^γ puckering in describing the proline geometry in our previous paper,[15] the position of the C^β atom also changes slightly as ϕ is altered by −7.4° from −67.6 to −75.0°. However, the dominant puckering of the pyrrolidine ring may be described as "C^γ puckering" because χ^1 is altered by 24.8° from the up (−6.1°) to the down (18.7°) ring conformation, which is larger than the variation (−7.4°) in ϕ. This discussion will not change any other part of our previous paper.[15]

(33) Uniform pseudo-random numbers were generated by using RANDU of the IBM Scientific Subroutine Package.

(34) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).

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(36) The relative statistical weights for the four types of ring puckering in form I and form II chains were computed as a function of temperature to investigate the thermally induced transition under vacuum. In ref 16, we pointed out that we were not concerned with the *thermal* transition in the text of this paper, and thus were not concerned about the temperature dependence of the potential energy. However, we consider the thermal transition only in this reference, and retain the assumption that the potential energy is independent of temperature even though we are considering a large temperature range here. From an energy (i.e., enthalpy) point of view, the transition takes place in the following order as the temperature is raised: form I (regular D) → form I (random A or random B) → form I (regular U) → form II (regular D) → form II (regular U) → form II (random A or random B) (see Table II). However, from a free energy point of view, form I (D puckering) is overwhelmingly the most stable conformation between 0 and 700°K [e.g., see the statistical weights (at 303°K) of section IIIA]. At 700°K, the relative statistical weights become 1, 10^{-7}, 10^{-7}, 10^{-7}, 10^{-1}, 10^{-4}, 10^{-6}, and 10^{-5} for regular D, regular U, random A, and random B of form I and regular D, regular U, random A, and random B of form II, respectively. At 885°K, a transition from regular D (form I) to regular D (form II) takes place; this may be regarded as an all-or-none transition for a chain length of $n = 30$. Above 885°K, and up to ~5000°K, form II (regular D puckering) is the most stable form. Above 5000°K form II is the most stable, but with a mixture of ring puckerings (regular D, regular U, random A, and random B); however, between 885 and 5000°K, the regular U, random A, and random B puckerings do not exist. Thus, the only thermally induced transition in the range of 0 to 5000°K is that of form I to form II, with regular D puckering in both forms.

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Theory of the Cooperative Transition between Two Ordered Conformations of Poly(L-Proline). III. Molecular Theory in the Presence of Solvent[1]

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ABSTRACT: Phenomenological theories of the form I ⇄ form II interconversion in poly(L-proline) have been presented by Schwarz (using the parameters s, σ, β', and β'' in a 2 × 2 matrix formulation) and by the present authors (using the parameters s, σ, β_C, and β_N in a 4 × 4 matrix formulation). In addition, a molecular theory was developed to compute s, σ, β', and β'' under vacuum. In this paper, we take into account the effect of solvent on the parameters s, σ, β', and β'' of the isothermal poly(L-proline) form I ⇄ form II interconversion. The growth parameter s is sensitive to the binding of solvent molecules to the peptide CO groups, but the nucleation parameters σ, β', and β'' are not affected by this type of solvent effect. The calculated values of s and σ under vacuum are in good agreement with the corresponding values derived from experimental data. By combining the theoretical values of s, σ, β', and β'' under vacuum with experimentally determined equilibrium constants for the binding of alcohols to the peptide CO groups (which differ in magnitude for form I and form II), it was possible to reproduce the experimental transition curves satisfactorily. Alternatively, the binding constants for alcohols, obtained by combining our theoretically computed parameters under vacuum with experimental equilibrium transition curves, are in satisfactory agreement with those evaluated independently by infrared spectral measurements of the binding of alcohols to the peptide CO groups. It is pointed out that significant errors may arise in analyzing experimental data if short chains are included with long chains in the determination of s, σ, β', and β'' from the equilibrium transition curves. The transition of poly(L-proline) from form II to form I when n-butyl alcohol is added to a solution of the polymer in benzyl alcohol is brought about by the slight difference in the binding free energies of both alcohols to the carbonyl groups of form II. The different binding affinities of the two alcohols, ROH, to form II may arise from (a) the different hydrogen-bond strength between the alcohol and the proline carbonyl group, and (b) possible differences in nonbonded and electrostatic interactions between the R group and the binding-site environment of the proline carbonyl group. The greater binding affinity of form II (compared to form I) for a *given* alcohol is attributed to the more open and extended conformation of form II.

In accompanying papers, we considered the phenomenological theory (paper I[3]) and a molecular theory in the absence of solvent (paper II[4]) for the form I ⇄ form II interconversion of poly(L-proline). In this paper, we consider the effects of solvent on this transition. Reference to equations in papers I and II will be made as I-1, II-1, etc.

More specifically, in paper I, we presented Schwarz's phenomenological theory[5] (using the parameters s, σ, β', and β'' in a 2×2 matrix formulation) and ours (using the parameters s, σ, β_C, and β_N in a 4×4 matrix formulation), and showed that both treatments are equivalent. In paper II, we formulated a molecular theory for s, σ, β', and β'', and computed these quantities under vacuum. We now consider the influence of solvent on s, σ, β', and β'' at constant temperature.

In paper II, we discussed the three types of solvent effect on the poly(L-proline) transition, viz., (1) direct binding of solvent molecules to the functional groups of the polymer, i.e., to the CO groups of poly(L-proline); (2) effect of the solvent on the intramolecular interactions of the polymer chain, e.g., the effect of the dielectric constant on electrostatic interactions; and (3) mutual effect of the solvent and polymer on each other, e.g., hydrophobic bonding in aqueous solution. It was concluded in paper II that a type 2 solvent effect has little influence on the parameters s, σ, β', and β'' since the form I $\rightleftarrows$ form II interconversion takes place over a very narrow range of solvent composition in which the dielectric constant varies very little, and that a type 3 solvent effect cannot play a significant role if this transition occurs in organic solvents. However, a type 1 solvent effect can influence the value of s (see eq II-41), but not those of β', β'', or σ (see eq II-42 to II-44). In fact, the values of σ calculated for chains of regular down[6] and regular up[6] ring puckering (under vacuum) are in good agreement with those observed experimentally in organic solvents (see section IIIC of paper II). Furthermore, the form I $\rightleftarrows$ form II interconversion takes place with a preservation[4] of D ring puckering,[6] without any contribution from U,[6] random A,[6] or random B[6] puckering. Therefore, our primary interest in this paper is on the effect of solvent on s, β', and β'' for chains with regular D puckering; i.e., D puckering will be assumed in all computations in this paper, except where we compare the calculated value of s for a chain (under vacuum) with regular U puckering with the "observed" value under vacuum.

In section I, we compute the effect of solvent on s directly from observed transition curves. In section II, we compute the same quantity, making use of equilibrium binding constants from infrared data. In section III, we compare the results of sections I and II, and show the reasonableness of the computed values of s, β', and β''. In section IV, we compare the values of s computed theoretically with those determined from experiment. In section V, we compare the computed and experimental transition curves and, in section VI, we provide some additional comparisons with experiment.

I. Evaluation of $s^{(s)}$ from Experimental Transition Curves

Ganser et al.[7] studied the solvent-induced transition between forms I and II of poly(L-proline) in n-butyl alcohol (BU):benzyl alcohol (BA) and n-butyl alcohol (BU):trifluoroethanol (TFE) mixtures. The transition behavior of this polymer can be described by five parameters, viz., s [or $s^{(s)}$ in solution; see the definition of $s^{(s)}$ in eq II-41], σ, β', and β'', and the degree of polymerization, N. The value of N was determined independently; ideally, the molecular weight distribution should also be determined since it influences the transition curve significantly, especially in samples of low molecular weight (see the next paragraph). The four other parameters were determined by fitting[7] the experimental transition data with the theory of Schwarz[5] in terms of s [or $s^{(s)}$], σ, β', β'', and N, using eq I-42 or I-81.

We assume that the values of σ, β', and β'', computed under vacuum,[4] also apply in solution[8] (see eq II-42 to II-44). In this section, we compute $s^{(s)}$ by using the theoretical values[4] of σ, β', and β'' and adjust $s^{(s)}$ as a function of f_{BU} (the volume fraction of n-butyl alcohol, BU, in a mixture of this alcohol with benzyl alcohol, BA) until we obtain a best fit of the computed (eq I-81) and experimental[7] transition curves at 70°. In this computation, it must be realized that experimental data for short chain lengths are unreliable for two reasons: (1) heterogeneity of molecular weight has its largest effect for shorter chains, and (2) the end groups of the chain may contribute nonnegligible effects in short chains, even if there were no molecular weight heterogeneity. Thus, in the curve fitting procedure, we gave greater weight to longer chains, and omitted the data from the lowest molecular weight sample ($N = 14$) of Ganser et al.[7] The resulting best-fit curves are shown by solid lines in Figure 1, where the fraction of form I is plotted against f_{BU}. The computed relationship between $s^{(s)}$ and f_{BU}, which yielded the best-fitting curves of Figure 1, is shown as a solid curve in Figure 2.

II. Evaluation of $s^{(s)}$, Using Binding Data

A. Binding Data from Infrared Measurements. We can obtain $s^{(s)}$ in an alternative manner by combining the theoretical values of s under vacuum with equilibrium constants for binding A and B molecules (i.e., BU and BA) to the peptide CO groups; these equilibrium constants were obtained independently by infrared spectral measurements.[9,10]

From eq II-37, II-38, and II-41 (with $i = 2$), $s^{(s)}$ in a binary solvent mixture is

$$s^{(s)} = s\,\frac{1 + K^{\mathrm{I}}{}_{\mathrm{A}}a_{\mathrm{A}} + K^{\mathrm{I}}{}_{\mathrm{B}}a_{\mathrm{B}}}{1 + K^{\mathrm{II}}{}_{\mathrm{A}}a_{\mathrm{A}} + K^{\mathrm{II}}{}_{\mathrm{B}}a_{\mathrm{B}}} \tag{1}$$

where a_{A} and a_{B} are the activities of BU and BA, respectively. The activities of n-butyl alcohol in benzyl alcohol were measured[7] as a function of the mole fraction of n-butyl alcohol and a relation was obtained[7] to convert mole fraction to volume fraction, f. Thus, a_{A} and a_{B} may be expressed in terms of f_{BU} and f_{BA}, respectively. The equilibrium constants for binding BU and BA to the peptide CO group in forms I and II, $K^{\mathrm{I}}{}_{\mathrm{BU}}$, $K^{\mathrm{II}}{}_{\mathrm{BU}}$, $K^{\mathrm{I}}{}_{\mathrm{BA}}$, and $K^{\mathrm{II}}{}_{\mathrm{BA}}$, were estimated from infrared spectral measurements.[9,10] Actually, the measurements were made on forms I and II of poly(o-acetylhydroxyproline)[9] (models for cis and trans proline, respectively) and cyclo(tri-L-proline)[10] (a model for cis proline), but we may assume that they pertain to poly(L-proline). According to Strassmair et al.[9] and Knof et al.,[10] the binding constants at 25° are

$$K^{\mathrm{I}}{}_{\mathrm{BU}} = 6 \tag{2}$$

$$K^{\mathrm{I}}{}_{\mathrm{BA}} = 6 \tag{3}$$

$$K^{\mathrm{II}}{}_{\mathrm{BU}} = 15 \tag{4}$$

$$K^{\mathrm{II}}{}_{\mathrm{BA}} = 20\text{–}30 \tag{5}$$

in units of (mole fraction)$^{-1}$. As in the analysis of the experimental data,[9,10] we also assume that the binding constants are independent of temperature, in order to compare the results obtained from infrared measurements at 25°[9,10] with those obtained from the transition equilibrium studies at 70°.[7] Substituting the values in eq 2–4, and $K^{\mathrm{II}}{}_{\mathrm{BA}} = 30$, in eq 1, together with a value of $s = 3.26$ (from Table VIII of paper II[4]), we calculate $s^{(s)}$ as a function of f_{BU}. The results are given by the dashed curve of Figure 2. Another curve for $s^{(s)}$ vs. f_{BU} (the short and long dashed curve in Figure 2) was computed by using the values in eq 2–4, but with $K^{\mathrm{II}}{}_{\mathrm{BA}} = 25$ and $s = 3.26$.

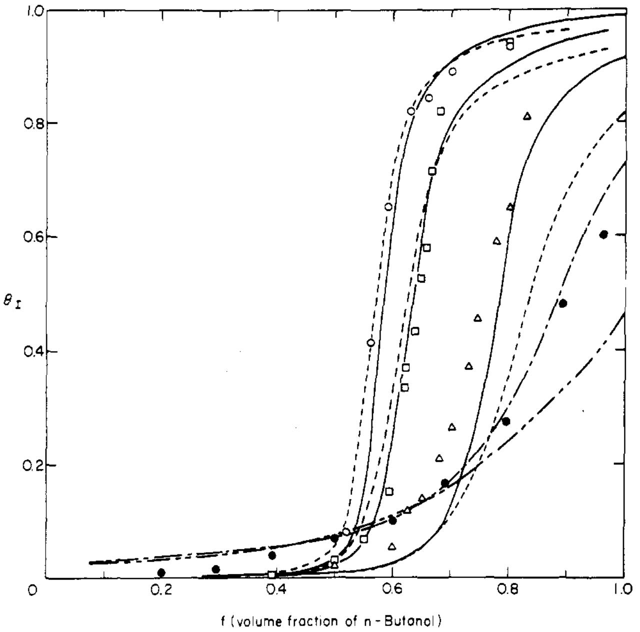

Figure 1. Transition curves of poly(L-proline) in *n*-butyl alcohol: benzyl alcohol at 70°. The fraction of form I helix, θ_I, is plotted against the volume fraction of *n*-butyl alcohol, f_{BU}. The experimental points are shown for the degree of polymerization $N = 217$ (○), $N = 90$ (□), $N = 33$ (△), and $N = 14$ (●), quoted from Figure 5 of ref 7. The solid curves were obtained by the fitting method for treating experimental data, described in section I. The dashed curves were calculated by combining the theoretical values of s, σ, β', and β'' (for D puckering) with the binding constants of eq 2–4 and $K^{II}{}_{BA} = 30$ from infrared spectral measurements.[9,10] Curves – - – and – - - - for $N = 14$ were obtained by methods similar to those which gave the solid and dashed curves, respectively, except that $\sigma = 4.57 \times 10^{-3}$ was used for $N = 14$ (given in Table I) instead of $\sigma = 9.12 \times 10^{-4}$ for infinite chain length (given in Table IX of paper II[4]). See section V of the text for more details.

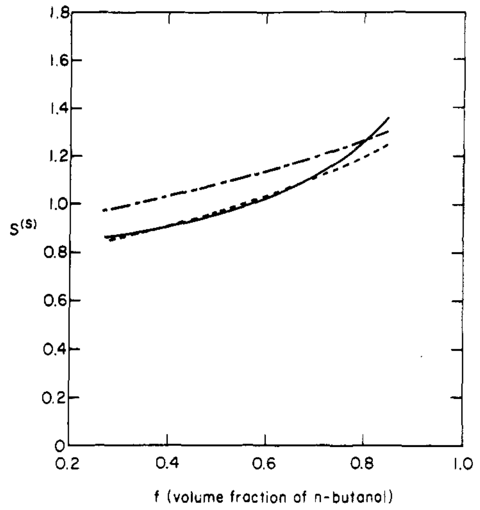

Figure 2. Dependence of the growth parameter $s^{(s)}$ in *n*-butyl alcohol:benzyl alcohol mixtures on the volume fraction of *n*-butyl alcohol, f_{BU}. The solid curve was obtained by the best-fit method for treating experimental data, described in section I, in which the theoretical values of σ, β', and β'' are used. The dashed curve was obtained by combining the theoretical value of s with the binding constants $K^{I}{}_{BU} = 6$, $K^{I}{}_{BA} = 6$, $K^{II}{}_{BU} = 15$, and $K^{II}{}_{BA} = 30$, which were determined by infrared spectral measurements.[9,10] The curve of - - - was obtained by using $K^{I}{}_{BU} = 6$, $K^{I}{}_{BA} = 6$, $K^{II}{}_{BU} = 15$, and $K^{I}{}_{BA} = 25$.

Equations 2–5 mean that the binding of BU and BA molecules to the peptide CO group is about 2–4 times larger when the polymer is in form II than when it is in form I. This appears reasonable in terms of the minimum-energy structures of these two helices in Figures 3 and 4 of paper II.[4] The carbonyl groups are almost perpendicular to the helix axis in form II, while they are almost parallel to the helix axis in form I. It is obvious that the carbonyl groups in form II are much more exposed to the solvent than in form I, where a carbonyl group is shielded by three pyrrolidine rings and two neighboring carbonyl groups (Figure 3 of paper II). This steric effect is probably the most important factor affecting the binding of small molecules to the peptide carbonyl groups in poly(L-proline); the polarizability of the carbonyl groups *may* also depend on the conformation of the chain. It is more difficult to account for the fact that $K^{II}{}_{BA} > K^{II}{}_{BU}$. However, it is this inequality, i.e., the better binding of BA than BU to form II, that favors form II at high concentrations of BA. It can be shown that this (small) difference in binding free energy (leading to only a small change in solvent binding at a sufficiently high value of f_{BA}, viz., that in the transition region) is sufficient (because of the cooperativity of the transition) to lead to form II. When f_{BU} increases, the solvation term in eq 1 changes in such a way that $s^{(s)}$ increases (see Figure 2) and form II is converted to form I. Perhaps the reasons that $K^{II}{}_{BA} > K^{II}{}_{BU}$ are: (a) the hydrogen-bond strengths between the proline carbonyl group and the two alcohols (ROH) may differ, and/or (b) there may be differences in nonbonded and electrostatic interactions between the R group and the binding-site environment of the proline carbonyl group.

B. Determination of Relative Binding Constants from Transition Data. Instead of using the infrared data to obtain the binding constants, we can obtain their relative values, using a procedure of Ganser et al.,[7] by combining our theoretical values of σ, β', and β'' and the solid curve of Figure 2.

Ganser et al.[7] found a linear relationship between activity, *a*, and mole fraction, *x*, in the range of composition of the transition, i.e.

$$a_A = \alpha_A + \delta_A x_A \qquad (6)$$

$$a_B = \alpha_B + \delta_B x_B \qquad (7)$$

where α and δ are the intercept and slope, respectively, of the approximately straight line. The relation between x_A and f_A (volume fraction of A) is given[7] by

$$x_A = tf_A/[1 - f_A(1 - t)] \qquad (8)$$

where t denotes the ratio of the molar volumes of solvent B and solvent A.

Substituting eq 6–8 for the corresponding quantities of eq 1, we obtain

$$s^{(s)} = P(1 + f_A Q)/(1 + f_A R) \qquad (9)$$

where

$$P \doteq s\frac{1 + K^{I}{}_{A}\alpha_A + K^{I}{}_{B}(\alpha_B + \delta_B)}{1 + K^{II}{}_{A}\alpha_A + K^{II}{}_{B}(\alpha_B + \delta_B)} \qquad (10)$$

$$Q = \frac{(t - 1) + K^{I}{}_{A}[t(\alpha_A + \delta_A) - \alpha_A] + K^{I}{}_{B}[t\alpha_B - \alpha_B - \delta_B]}{1 + K^{I}{}_{A}\alpha_A + K^{I}{}_{B}(\alpha_B + \delta_B)} \qquad (11)$$

$$R = \frac{(t-1) + K^{\mathrm{II}}{}_{\mathrm{A}}[t(\alpha_{\mathrm{A}} + \delta_{\mathrm{A}}) - \alpha_{\mathrm{A}}] + K^{\mathrm{II}}{}_{\mathrm{B}}[t\alpha_{\mathrm{B}} - \alpha_{\mathrm{B}} - \delta_{\mathrm{B}}]}{1 + K^{\mathrm{II}}{}_{\mathrm{A}}\alpha_{\mathrm{A}} + K^{\mathrm{II}}{}_{\mathrm{B}}(\alpha_{\mathrm{B}} + \delta_{\mathrm{B}})} \quad (12)$$

By varying P, Q, and R to obtain a best fit of eq 9 to the solid curve of Figure 2, we obtain the values: $P = 0.79$, $Q = -0.61$, and $R = -0.85$. The values of the α's and δ's can be obtained from Figure 3 of ref 7. Substituting these values of P, Q, R, α_{A}, α_{B}, δ_{A}, and δ_{B} in eq 10–12, the following relations among the binding constants are obtained:

$$K^{\mathrm{I}}{}_{\mathrm{BA}} = 0.23K^{\mathrm{II}}{}_{\mathrm{BA}} - 0.76 \quad (13)$$

$$K^{\mathrm{I}}{}_{\mathrm{BU}} = 0.63K^{\mathrm{II}}{}_{\mathrm{BU}} - 0.37 \quad (14)$$

and

$$K^{\mathrm{II}}{}_{\mathrm{BA}} = 6.9K^{\mathrm{II}}{}_{\mathrm{BU}} + 5.9 \quad (15)$$

Equations 13–15 agree with the conclusions from eq 2–5 that the equilibrium constants for the binding of BU and BA molecules to the peptide CO groups of form II are about 2–4 times greater than those of form I.

III. Comparison of Values of $s^{(\mathrm{s})}$

We now compare the $s^{(\mathrm{s})}$ vs. f_{BU} curves obtained by the procedures of sections I and II. It can be seen in Figure 2 that these curves are sensitive to the experimental value of $K^{\mathrm{II}}{}_{\mathrm{BA}}$. Also, both the solid curve (obtained by the procedure of section I) and the dashed curve (obtained by the procedure of section II, with $K^{\mathrm{II}}{}_{\mathrm{BA}} = 30$) coincide reasonably well, not only in their intercepts at $s^{(\mathrm{s})} = 1$, but also in their shapes in the important region of the transition ($0.9 < s^{(\mathrm{s})} < 1.15$). The latter coincidence implies that the theoretical values[8] of β' and β'' are reasonable. Also, the theoretical values of s, which led to these values of $s^{(\mathrm{s})}$, are also reasonable.

IV. Comparison of Theoretical and Experimental Values of s

The coincidence of the two curves at $s^{(\mathrm{s})} = 1$ implies that our theoretical value of $s = 3.26$ at 70° is reasonable. This result may be considered in another way. In section II, we used the theoretical value of s, together with theoretical values of σ, β', and β'' and experimental binding constants, to obtain $s^{(\mathrm{s})}$. However, an "experimental" value of s may be estimated using only experimental quantities. An experimental curve for $s^{(\mathrm{s})}$ vs. f_{BU} was derived from the equilibrium transition curves.[7] Applying eq 1 to this curve (using the experimental binding constants), s was varied to obtain a best fit to this $s^{(\mathrm{s})}$ vs. f_{BU} curve. The resulting value of s is an "experimental" one. By this procedure, Strassmair et al.[9] obtained $s = 2.7$ and 2.9 at 70° in BU:BA and in BU:TFE mixtures, respectively. If we take into account the experimental errors and also the assumptions that the activity is equal to the mole fraction, that the equilibrium constants for the binding of these alcohol molecules to poly(*o*-acetylhydroxyproline), cyclo(tri-L-proline), and poly(L-proline) are the same, and that the binding constants are independent of temperature, then our theoretical value of $s = 3.26$ at 70° (for regular D puckering) is in reasonably good agreement with the experimental one.

Since our theoretical value for a chain with regular U puckering is $s = 0.65$ at 70° (Table VIII of paper II[4]), it is suggested that the puckering in poly(L-proline) is regular D rather than regular U. As can be seen from eq 1, if $s = 0.65$, the binding of the alcohol molecules to form I would have to be greater than that to form II to make $s^{(\mathrm{s})} = 1$ at $f_{\mathrm{BU}} = 0.55$ at 70°, which is contrary to the results of the infrared data (eq 2–5); this also suggests that the ring puckering cannot be regular U.

Finally, the reproduction of the curvature of the θ_{I} vs. f_{BU} curve in the transition region (see section V), using the experimental values of the binding constants (i.e., eq 2–4 and $K^{\mathrm{II}}{}_{\mathrm{BA}} = 30$), attests to the reasonableness of the theoretical values of s, σ, β', and β'' (with regular D ring puckering).

V. Computation of Transition Curves

We may now compute the transition curves for $N = 217$, 90, and 33 (using eq I-42 or I-81) by combining the theoretical values of s, σ, β', and β'' at 70° with the binding constants of eq 2–4 and $K^{\mathrm{II}}{}_{\mathrm{BA}} = 30$. As seen in Figure 1, the agreement between the calculated curves and the experimental points is satisfactory for $N = 217$ and 90. The disagreement for $N = 33$ may be due to experimental errors, molecular weight heterogeneity, and relatively large end effects at this low molecular weight.

There is another possible reason for the discrepancy at low molecular weight, especially for the very short chain, $N = 14$, but also for $N = 33$. It can be seen in Figure 6 of paper II[4] that $f_1 + f_2$, which gives the enthalpic contribution to σ (see eq II-25), converges slowly as the number of residues, n, increases, while $-R \ln g_1 g_2$, which gives the entropic contribution to σ (see eq II-26), converges rapidly. This is also shown by comparing Table I of this paper and the first row of Table IX of paper II.[4] Thus, σ should depend considerably on chain length in the range of short chains, because of the dependence of ΔH_σ on n. The values of ΔH_σ and ΔS_σ for $n = 12$, 14, and 30 are given in Table I, the data for which were computed in exactly the same way as the data of Table IX of paper II for infinite chains. It can be seen that the values of ΔH_σ for short chains differ considerably from those for infinite chain length. [Parenthetically, it should be noted that β' and β'' do not depend on chain length because $f_1 - f_2 - 2jf_0$ (which gives $\Delta H_{\beta'}$ at sufficiently large chain length; see eq II-28), $f_3 - f_4 - 2jf_0$ ($\Delta H_{\beta''}$; see eq II-35), $\{-R \ln (g_1/g_2) + 2jR \ln g_0\}$ ($\Delta S_{\beta'}$; see eq II-29), and $\{-R \ln (g_3/g_4) + 2jR \ln g_0\}$ ($\Delta S_{\beta''}$; see eq II-36) are constants or converge rapidly at small values of n; furthermore, s also converges rapidly at small values of n because f_0 of Figure 5 of paper II (which gives ΔH_s; see eq II-18) and g_0 of Figure 11 of paper II (which gives ΔS_s; see eq II-19) converge rapidly.] The behavior of ΔH_σ suggests that σ values for infinite chain length should not be used in analyzing data for short chains. Indeed, if we use $\sigma = 4.57 \times 10^{-3}$ for $n = 14$ at 70° (see Table I) instead of the infinite-chain value of 9.12×10^{-4} (see Table IX of paper II[4]) in computing θ_{I} (using the infinite-chain values of s, β', and β'') we obtain better agreement with the experimental data for $N = 14$, as seen in Figure 1. The use of the infinite-chain value of $\sigma = 9.12 \times 10^{-4}$ for $N = 14$ leads to a curve in which the values of θ_{I} are much lower in the range of $f_{\mathrm{BU}} > 0.7$, compared with the curve drawn in Figure 1.

In the helix–coil transition in homopolyamino acids, a nucleation parameter, σ, assigned to a hydrogen-bonded state followed by $(m - 1)$ helical states may depend on the number m. However, in the Zimm–Bragg theory,[11] a unique value of σ (independent of m for $m \geq 3$) was assumed for the sake of simplicity. If σ, determined for long chains, is used to compute a transition curve of a very short chain, that latter would exhibit a transition which may be described as of the *all-or-none* type (see the discussion of chapter 2 of ref 12). Now, by observing only the equilibrium phenomenological properties of a polyamino acid system, it is impossible to know the exact distribution of conformational states of the system. In other words, one can-

Table I
σ for Finite Chains of Poly(L-proline)

No. of proline[a] residues in a sequence, n	ΔH_σ, kcal/mol sequence	ΔS_σ, eu/mol sequence	σ	
			at 25°	at 70°
12	4.03	1.60	2.50×10^{-3}	6.09×10^{-3}
14	4.22	1.60	1.80×10^{-3}	4.57×10^{-3}
30	4.77	1.60	7.06×10^{-4}	2.03×10^{-3}

[a] For poly(L-proline) sequences with regular D ring puckering conformations.

not determine, for example, whether an observed average phenomenological property arises from a large number of states or simply from the two states of the all-or-none type, because both distributions might lead to similar average (equilibrium) properties. For oligomers of L-proline, mixed conformations of trans and cis in a molecule have been observed by NMR experiments,[13] and also computed theoretically.[14] This observation implies that the form I $\rightleftarrows$ form II interconversion may not be of the all-or-none type for very short chains. It thus suggests that the parameter σ has to depend on chain length for short chains; i.e., the infinite-chain value of σ should not be used in describing the transition in short chains of poly(L-proline).

VI. Additional Comparisons with Experiment

In principle, similar data in BU:TFE mixtures[7] could be analyzed in the same manner. However, we have not done so because they are not very extensive and pertain to short chains, thus making them less reliable than the data in BU:BA mixtures. Alternatively, in this section, we provide some additional discussion of the following three aspects of the experimental results of Ganser et al.[7] in light of the present theoretical results. (1) According to their analysis, the values of β' are 5×10^{-2}, $<10^{-6}$, and 9×10^{-4}, and the values of β'' are 5.6×10^{-2}, 5×10^{3} to 5×10^{4}, and 1.5×10^{4} in BU:BA at 70°, in BU:TFE at 70°, and in BU:TFE at 25°, respectively, from which they concluded that β' and β'' are affected significantly by solvent effects. On the other hand, σ is 10^{-5} in BU:BA at 70°, 10^{-6} to 10^{-5} in BU:TFE at 70°, and 5×10^{-6} in BU:TFE at 25°, from which they concluded that σ is independent of solvent. In obtaining these results, Ganser et al.[7] gave the same weight to data for short chains (N = 12 and 14) as well as for longer ones. (2) Whereas the experimental curves for $s^{(s)}$ vs. f_{BU} in BU:BA and BU:TFE, respectively, must intersect at 100% BU (since $s^{(s)}$ must be the same in pure n-butyl alcohol no matter what solvent mixtures are used), this was not the case in the data of Ganser et al.[7] (see Figure 8 of ref 7). (3) Strassmair et al.[9] obtained two curves for $\ln s^{(s)}$ vs. f_{BU} for the BU:TFE system, one by varying $s^{(s)}$, σ, β', and β'' independently to obtain a best fit to the transition curves of Ganser et al.,[7] and the other by combining binding constants[9,10] with the value of s obtained by suitable adjustment, using eq 1. These two curves for the BU:TFE system did not agree with each other, even though the two curves for the BU:BA system (obtained by the same methods) did (see Figure 5 of ref 9). It appears to us that the data in the BU:BA system are more reliable because they were obtained with longer chain lengths. The behavior in point 3 is related to the discrepancy indicated in point 2, and probably arises from the nonnegligible end effects of the low molecular weight samples studied in the BU:TFE system. Point 1 is discussed in the next paragraph.

Consider point 1, i.e., the conclusion[7] that β' and β'' depend on the nature of the solvent, but σ does not. In paper II,[4] we concluded that β', β'', and σ are unaffected by a type 1 solvent effect (see eq II-42 to II-44). While we anticipated that β', β'', and σ would be unaffected by type 2 and type 3 solvent effects, nevertheless the possibility does exist that type 2 and type 3 solvent effects might affect these quantities. However, if they do, then they should affect *all three* quantities which are related through σ'_{I}, σ'_{II}, σ''_{I}, and σ''_{II} (see eq I-63–I-65). Thus, it was inconsistent to conclude[7] that σ is unaffected by solvent, and then to say[7] that β' and β'' *are* influenced by type 2 and type 3 solvent effects; i.e., one cannot attribute different solvent effects on the nucleation at the ends of a sequence compared with the nucleation of an isolated residue in the interior of a sequence (see eq I-63 to I-65). As to the end effects, which cannot be neglected, especially in short chains, one might define new parameters ϵ_N and ϵ_C for the N and C termini, respectively, differing from β' and β'' in that they would not be related to σ by eq I-63 to I-65. These new parameters could then include explicitly special solvation effects at the ends of the chain, in addition to the absence of interactions from the missing neighboring residues (see section IA of paper I[3]). Then one could account for special solvation end effects, especially in short chains. In fact, ϵ_{N} and ϵ_{C} could then include even a type 1 solvent effect (special binding by end groups) which does not appear in β' and β''. We believe that the apparent solvent effects on β' and β'', obtained by Ganser et al,[7] who included short chains in their analysis, may be the result of identifying β' and β'' with ϵ_{N} and ϵ_{C}, as already pointed out above in the discussion of points 2 and 3. For this reason, we cannot compare our theoretical values of β' and β'' with the experimental values that Ganser et al.[7] observed in the solvent system BU:TFE at 70° (i.e., $\beta' < 10^{-6}$ and $5 \times 10^{3} < \beta'' < 5 \times 10^{4}$) and BU:TFE at 25° [$\beta' = 9 \times 10^{-4}$ and $\beta'' = 1.5 \times 10^{4}$]. As we had already concluded in the first paragraph of section III, our theoretical values of β' and β'' are reasonable; these values (given in Tables X and XI of paper II) agree qualitatively with those observed in the BA:BU system ($\beta' = 5 \times 10^{-2}$ and $\beta'' = 5.6 \times 10^{-2}$) in that $\beta' < 1$ and $\beta'' < 1$, because samples of longer chain length (with negligible end effects) were used in the study in the BA:BU system. The remaining discrepancies may arise from the method of treating the data; i.e., we omitted the data from the lowest molecular weight sample in our analysis, whereas Ganser et al.[7] gave equal weight to all chain lengths in their analysis. Thus, short chains should not be used in obtaining the phenomenological parameters $s^{(s)}$, σ, β', and β'' from experimental transition curves. A statistical mechanical theory of the form I $\rightleftarrows$ form II interconversion including ϵ_N and ϵ_C in addition to s, σ, β', and β'', where β' and β'' pertain to both ends of a sequence, and ϵ_N and ϵ_C to both ends of the chain, was formulated in section IA of paper I.[3] These parameters could be evaluated if the structure of the terminal groups and the nature of their interactions with solvents were known. However, in the analyses presented thus far,[7] β' and β'', and ϵ_N and ϵ_C, are not distinguished.

Finally, we consider an additional point, which is related to the above discussion. As can be seen in Figures 5 and 7 of ref 7, the transition curves for the BU:TFE system cross, but those for the BU:BA system do not. This crossing phenomenon cannot be accounted for by the Zimm–Bragg theory,[11] but can be by the theory presented in paper I.[3] In principle, it is possible to produce transition curves of various shapes by varying β' and β'', and the chain length N, as shown by Schwarz;[5] this cannot be done with the single initiation parameter of the Zimm–Bragg theory. However, we cannot conclude from the different behavior in Figures 5 and 7 of ref 7 that β' and β'' differ in the BA:BU and BU:TFE systems. Alternatively, different end-effect parameters, ϵ_C and ϵ_N, for the short chain length samples in the BA:BU and BU:TFE systems can produce transition curves of different shapes, in the same way that Schwarz obtained transition curves of various shapes by varying β', β'', and N. Thus, ϵ_N and ϵ_C would differ for the two systems, as discussed under point 1 above, and we thus attribute the different behavior in Figures 5 and 7 of ref 7 to ϵ_N and ϵ_C; β' and β'' are probably independent of solvent in these systems.

VII. Concluding Discussion

In previous sections, we compared the theoretical results with experimental ones. In this section, we discuss other theoretical studies related to the present one. As far as we know, the only related studies are those of Ganser et al.,[7] Holzwarth and Backman,[15] and Holzwarth and Chandrasekaran,[16] all of whom tried to calculate the conformational energy of sequences with and without a junction, and related this to the parameter σ. It appears that no other calculations of s, β', and β'' have been reported. In the previous calculations of σ,[7,15,16] only the conformational energy, and not the entropic contribution, was evaluated. Since the entropic contribution to σ amounts to about -1 to $+1$ kcal/mol (in the form $T\Delta S_\sigma$) under the usual experimental conditions, this contribution cannot be neglected in determining values of σ in the range of $10^{-3} < \sigma < 10^{-6}$, which corresponds to $\Delta G_\sigma = 4$ to 7 kcal/mol. Further, the previous authors[7,15,16] did not allow for ring puckering, and did not minimize the conformational energy to determine the most stable form I and form II helical structures. Holzwarth et al.[15,16] attributed the source of cooperativity in the poly(L-proline) transition to the electrostatic interaction energy. However, we find that the electrostatic energies are almost the same for the *minimum-energy* form I and form II helices (see Table II of paper II[4]). It is the nonbonded interaction energies, especially the attractive interactions in the compact conformation of form I, that play the important role in determining the relative stabilities of the helical conformations of poly(L-proline) under vacuum (and possibly in nonactive solvents).

Finally, keeping in mind the caveats of ref 16 and 36 of paper II,[4] it should be possible to calculate the effect of temperature on the transition, and the dependence of the transition temperature on solvent composition. For this purpose, it would be necessary to know the temperature dependence of the empirical energy functions, of the binding constants, and of the activities of the various solvents in the given mixtures. With this information, the treatment presented here could be extended to account for data on the thermally induced transition of poly(L-proline) in mixed solvents.

References and Notes

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