to-end distance. It has been suggested[31] that in isotactic chains in solution the 3_1 helix extends undisturbed for a considerable number of units. The only allowed helix reversal if of the type $(gt)(tg)$, the junction $(tg)(gt)$ being sterically forbidden. Since we have shown that in PVC, the *r* sequences are almost as conformationally regular as *m* sequences, then the majority of "kinks" in a PVC chain would occur at *m–r* junctions of the form $(tg)(tt)$ and $(gt)(tt)$ or their mirror images.

(31) P. J. Flory, J. E. Mark, and A. Abe, *J. Amer. Chem. Soc.*, **88**, 639 (1966).

Cooperativity in Poly-L-proline I–II Transitions

G. Holzwarth and R. Chandrasekaran

Departments of Biophysics and Chemistry, The University of Chicago, Chicago, Illinois.
Received January 20, 1969

ABSTRACT: The poly-L-proline I–II transition is known to exhibit positive cooperativity. This effect has hitherto been only qualitatively explained. In this study, the contributions of intramolecular steric and electrostatic interactions to this phenomenon are computed. The calculations suggest that the total end effects are +11.5 kcal/mol for form I but only +3.3 kcal/mol for form II. Similar calculations for a polyproline molecule with a junction between form I and form II, and between form II and form I, yield intramolecular junction free energies of −0.8 and +2.3 kcal/mol for these two structures. The minimum intramolecular junction free energy *J* for simultaneous formation of a I–II and a II–I junction, +1.5 kcal/mol, arises from a +2.1-kcal/mol electrostatic term and a −0.6-kcal/mol van der Waals term. Since the observed value of *J* is +4 to +7 kcal/mol, these calculations suggest that, in contrast to the α-helix–random coil transition, the poly-L-proline I–II transition derives much of its cooperativity from polymer–solvent interactions.

Poly-L-proline is known to undergo a reversible conformational transition from a right-handed helix with *cis* peptide groups, termed form I, to a left-handed helix of *trans* peptide units, termed form II.[1,2] This conformational transition occurs over a narrow range of solvent composition,[3] suggesting that it is characterized by positive cooperativity. That is, under solvent conditions in which form I and form II are, on the average, equally probable, individual polymer chains possess long sequences of peptide groups in identical conformation rather than containing a truly random distribution of *cis* and *trans* units. This observation can be introduced into a thermodynamic description of the phaselike I–II transformation by assigning a "junction energy" to each intramolecular junction between form I units and adjacent form II units. A positive junction energy then yields positive cooperativity. Indeed, this junction energy has been measured by two different techniques and found to lie between +4 and +7 kcal/mol.[4–6] The molecular origin of this junction energy is the primary subject of the present paper.

The positive cooperativity of polyproline has been ascribed to three distinguishable phenomena. The first, suggested by Schimmel and Flory,[7] is best viewed as a van der Waals end effect in form I. These authors point out that form I, which occurs in poor solvents, must possess more favorable intramolecular van der Waals interactions than form II. Since residues at the ends of form I sections are deprived of their full share of these intramolecular interactions, these end residues will be energetically unfavorable in comparison to residues within a long form I chain. Thus, under solvent conditions in which forms I and II both have significant average probability, *cis* units will usually be found only in long, all-*cis* sequences. Long all-*trans* sequences must then also occur, lending positive cooperativity to the I–II transition.

The second proposal, suggested by Rifkind and Applequist,[5] can best be described as intramolecular steric hindrance at the junction between form II and form I. According to them, molecular model studies of junctions give evidence for extensive overlap of atoms near the junction. Thus the first and second proposals are essentially of a steric nature, but neither has been placed on a quantitative basis.

Recently, a third source for the cooperativity, the electrostatic interactions of peptide dipoles with one another, was suggested.[8] Calculations of the intramolecular peptide interaction, in dipole–dipole approximation, show that a positive junction energy of 1–2 kcal/mol arises from this source. However, since the observed junction energy is 4–7 kcal/mol, electrostatic interactions are unable to account fully for the observed effects.

In order to establish quantitatively whether intramolecular steric interactions make a significant contribution to the cooperativity, we have calculated the van der Waals energy and have examined for steric hindrance junctions between the two forms. In addition, the electrostatic energy is calculated in monopole ap-

(1) E. Katchalski, A. Berger, and J. Kurtz in "Aspects of Protein Structure," G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1963, p 205.
(2) L. Mandelkern in "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Decker, New York, N. Y., 1967, p 675.
(3) F. Gornick, L. Mandelkern, A. F. Diorio, and D. E. Roberts, *J. Amer. Chem. Soc.*, **86**, 2549 (1964).
(4) J. Engel, *Biopolymers*, **4**, 945 (1966).
(5) J. M. Rifkind and J. Applequist, *J. Amer. Chem. Soc.*, **90**, 3651 (1968).
(6) G. Schwarz, *Biopolymers*, **6**, 873 (1968).
(7) P. R. Schimmel and P. J. Flory, *Proc. Nat. Acad. Sci. U. S.*, **58**, 52 (1967).
(8) G. Holzwarth and K. Backman, *Biochemistry*, **8**, 883 (1969).

TABLE I
VALUES OF THE SHORT CONTACT LIMITS AND THE POTENTIAL CONSTANTS FOR THE NONBONDED INTERACTIONS

Atom pair	Short contact limit, Å		Potential constants	
	Normal	Extreme	$A \times 10^4$	B
H···H	2.00	1.90	0.83	46.7
H···N	2.40	2.20	5.33	156.1
H···O	2.40	2.20	3.83	124.1
H···C^α	2.40	2.20	5.97	127.0
H···C′	2.40	2.20	7.79	165.8
N···N	2.70	2.60	40.44	547.1
N···O	2.70	2.60	29.36	446.2
N···C^α	2.90	2.80	47.19	445.7
N···C′	2.90	2.80	60.49	571.4
O···O	2.70	2.60	21.66	368.9
O···C^α	2.80	2.70	34.12	363.8
O···C′	2.80	2.70	43.31	461.9
C^α···C^α	3.00	2.90	55.90	363.2
C^α···C′	3.00	2.90	71.61	465.3
C′···C′	3.00	2.90	92.39	600.2

proximation. The calculations show that junctions between form I and form II may actually be stabilized by a van der Waals energy of −1.6 kcal/mol, whereas junctions between form II and form I are destabilized by +1.0 kcal/mol or more. The monopole electrostatic contributions are about 1 kcal/mol positive in both cases, in agreement with the results for dipole–dipole calculations. However, the minimum sum of intramolecular electrostatic and van der Waals interactions is only −0.8 for form I–II and +2.3 for form II–I. These two figures yield a total junction energy of +1.5 kcal/mol, which is inadequate to account fully for the observed cooperativity. This difference of 2.5–5.5 kcal/mol from the observed suggests that the positive cooperativity in poly-L-proline arises partly from energetically unfavorable solvent–solute interactions at the junction, in addition to the intramolecular processes here examined.

It is noteworthy that similar positive cooperativity is observed in the α-helix–random-coil transition.[9,10] Although electrostatic interactions contribute to the cooperativity in both cases,[8,11,12] the molecular mechanism must otherwise be different because polyproline lacks the intramolecular hydrogen bonds and entropy changes which are believed to make large positive contributions to cooperativity in the α-helix.[10,13]

Method of Calculation

van der Waals Energy. The van der Waals energy was calculated for a poly-L-proline chain possessing eight peptide units. The peptide units and their numbering scheme are shown in Figure 1. Because of obvious complications introduced by the slightly different dimensions of forms I and II, our definition of the monomer differs from the usual one,[14] *i.e.*, we associate

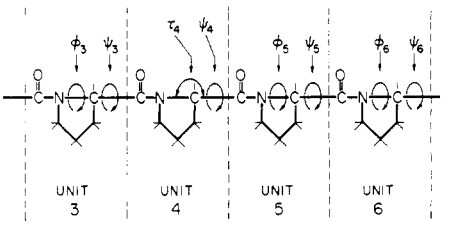

Figure 1. Structure and angle nomenclature of the middle four proline subunits, no. 3–6, for the 8-mer whose interactions are examined in detail. Junctions between form I and form II occur at the dotted line between units 4 and 5. The special junction bond angle τ_4 and junction dihedral angle ψ_4 shown in the figure are the two angles which are varied in the calculations.

a given side chain with the peptide group toward the amino end, rather than toward the carboxyl end of the chain. The basic geometry of the proline unit is taken from published data of X-ray analysis. Thus, the dimensions of the form I proline unit are those found by Traub and Shmueli[15] and the dimensions of the form II proline unit are those of Sasisekharan.[16] The peptide groups themselves are set planar in spite of the small deviations from planarity (+0.8 and −0.6°) in the published coordinates. Similarly, the dihedral angles ϕ about the N–C^α bond are locked at the X-ray values, 97 and 102°, which are fixed by the pyrollidine ring closure, and the bond angle N–C^α–C′ is also set at the X-ray values, 115 and 110°, in these two forms. Finally, seven hydrogen atoms, whose coordinates are not given by the X-ray analysis, are affixed in their appropriate places on each proline unit by assuming a C–H bond length of 1.1 Å, an H–C–H angle of 110° and an otherwise symmetric disposition with respect to the atom to which they are bound.

The chain of eight peptide units is constructed by placing the first unit in a suitable Cartesian coordinate system and then generating the succeeding units by appropriate translations and rotations of the coordinate frame. Such operations are conveniently handled by matrix methods which are fully described elsewhere.[17]

Once the atoms of the chain of eight units are fixed in a common coordinate system, the van der Waals energy $V_{a,b}$ of each pair of atoms (a,b), which are separated by a distance r, is calculated using a Buckingham potential of the form

$$V_{a,b} = Ae^{-\mu r} - Br^{-6} \quad (1)$$

The semiempirical constants A and B, as well as the value 4.6 assigned to μ, are those found useful in earlier studies on polypeptides and proteins[17]; they yield $V_{a,b}$ in kilocalories per mole if r is expressed in ångströms. The values of A and B are listed in Table I.

The exact calculations are limited to the octamer. Within the octamer interactions are considered for all pairs of atoms separated by more than two atoms. In addition, all interatomic distances in the 8-mer are examined for short contacts which could lead to steric hindrance. The two sets of contact limits, termed the

(9) B. H. Zimm, P. Doty, and K. Iso, *Proc. Nat. Acad. Sci. U. S.*, **45**, 1601 (1959).
(10) O. B. Ptitsyn in "Conformation of Biopolymers," G. N. Ramachandran, Ed., Academic Press, London, 1967, p 381.
(11) R. G. C. Arridge and G. C. Cannon, *Proc. Roy. Soc.* (London), **A278**, 91 (1964).
(12) D. A. Brant, *Macromolecules*, **1**, 291 (1968).
(13) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(14) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966).
(15) W. Traub and U. Shmueli, *Nature*, **198**, 1165 (1963).
(16) V. Sasisekharan, *Acta Cryst.*, **12**, 897 (1959).
(17) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 283 (1968); C. M. Venkatachalam and G. N. Ramachandran, *Ann. Rev. Biochem.*, in press.

"normal limit" and the "extreme limit," are also shown in Table I. The values conform to general usage.[17]

The calculations were carried out for octamers of four basic conformations as follows.

a. Form I. All peptide groups are *cis:* this is essentially an 8-mer of form I. For this basic conformation, the dihedral angles ψ_1 to ψ_7 are varied in concert between 280 and 370° at 10° intervals.

b. Form II. All peptide groups are *trans:* this is essentially an 8-mer of form II. For this basic conformation, the dihedral angles ψ_1 to ψ_7 are varied in concert as above.

c. Form I–II. The first four peptide groups are *cis*, with a form I type of L-proline units; the second four peptide groups are *trans*, with a form II type of proline units. The bond angles τ_1, τ_2, and τ_3 and the dihedral angles ψ_1, ψ_2, ψ_3, are 115 and 337°, respectively, as in form I. Similarly, τ_5, τ_6, τ_7 and ψ_5, ψ_6, ψ_7 are assigned the crystallographic form II values, 110 and 327°. The values of τ_4 and ψ_4 at the junction between *cis* and *trans* sections of the chain are varied (at suitable intervals) over the range 102–120° and 0–360°, respectively. The τ_4 range studied includes distortions of ±8° from the tetrahedral angle; distortions greater than 8° are rarely observed except in small ring systems.

d. Form II–I. The first four peptide groups are *trans*, with a form II type of L-proline unit; the second four peptide groups are *cis* with a form I type of proline unit. The bond angles and dihedral angles are similar to those in form I–II, with appropriate alterations corresponding to the reversed positions of *cis* and *trans* units. As in form I–II, the junction bond angle τ_4 and junction dihedral angle ψ_4 are varied from 102 to 120° and 0 to 360°, respectively.

The nonbonded energies for the 8-mers are established in the form of 8 × 8 matrices. Each matrix element $M(i,j)$ represents the nonbonded interaction of groups i and j. Thus, the total energy of the 8-mer is given by

$$\sum_{i=1}^{8}\sum_{i=j}^{8} M(i,j)$$

The energy of an N-mer can be readily calculated from appropriate elements of the 8 × 8 matrices and is conveniently described by an $N \times N$ matrix with each element $M(i,j)$ having the same meaning as above. It was found useful to determine the 100 × 100 matrix describing the interaction energies within a 100-mer of form I, form II, form I–II, and form II–I. The energies of shorter chains are then readily found by using only a portion of each large matrix. For pure form I and pure form II, the 100 × 100 matrices are easily established, since the interaction of any two residues i and j is determined solely by the absolute value of $(i - j)$ and not by the positions of the residues within the chain. For $|i - j| \leq 7$, values from the matrix for the 8-mer can be used directly; for $|i - j| \geqq 8$, zero interaction is assumed. Inspection of the energy matrices shows that the neglected nonbonded terms for $|i - j| \geqq 8$ are smaller than 0.01 and 0.00 kcal/mol for forms I and II.

Long molecules containing a I–II or II–I junction are similarly approached. The junction is placed at the center of the chain, and a "graft" of the appropriate *cis* or *trans* peptide units is added to each end of the 8-mer. Thus, for a 100-mer with a I–II junction, 46 *cis* proline units are added to the amino end of the 8-mer of I–II geometry, and 46 *trans* proline units are added to the carboxyl end of the 8-mer. The 100 × 100 energy matrices are then readily assembled. Interactions among the first 50 units are identical with those in polyproline I, whereas the interactions among units 51–100 are those of polyproline II. The interactions among units 47–54 are given by the special 8 × 8 matrix for the I–II geometry. The interactions between units i and j are neglected if $|i - j| \geq 8$. In addition, the interactions of prolines 44–46 with 51–53, as well as the interactions of units 48–50 with 55–57, are set equal to zero. These involve $4 \leq |i - j| \leq 7$, but take place across the junction, so that the matrix elements of form I and form II are inappropriate. The sum of these 12 neglected elements in the 100 × 100 matrix is unlikely to exceed the value −0.25 which they attain in form I, a very compact structure. To preserve internal consistency when comparing the energies of forms I, II, I–II, and II–I, these same terms are set equal to zero in the matrices for the pure form I and form II helices.

Once the energies for polymers of arbitrary length are at hand, it becomes possible to analyze the data for an "effective end energy" E and an "effective junction energy" J, corresponding to the nearest-neighbor model adopted in the statistical-thermodynamic theories of chain conformation.[6,13,18] This model suggests that the energy $W(N)$ of an N-mer with M *cis* proline units and $(N - M)$ *trans* proline units should be of the form

$$W(N) = MW_c + (N - M)W_t + E_a + E_b - J \quad (2)$$

where W_c is the energy of a *cis* unit, W_t is the energy of a *trans* unit, E_a and E_b are the end energies of the amino and carboxyl terminals, and J is the energy of the junction. The energies E_a and E_b are evaluated from the energies of form I and form II helices, which lack a junction term J, by determining the intercept in a plot of $W(N)$ *vs.* N. The energy of a form I end, E_I, and the energy of a form II end, E_{II}, having been established, the junction energies J_{I-II} and J_{II-I} are found from eq 2.

Electrostatic Energy. The electrostatic energy corresponding to the interaction of proline units is evaluated in monopole approximation. Clearly, knowledge of the location, direction, and magnitude of the dipole moment of the proline unit is a prerequisite for this calculation. Now, the dipole moments of a number of substituted alkylamides ranging from N-methylformamide and acetamide to N,N-dimethylacetamide are found to lie between 3.6 and 3.8 D.[19] This remarkable invariance to substitution suggests that the NC′O framework common to these molecules is the predominant source of the observed moment; the presence or absence of hydrogens at the nitrogen nucleus, as well as saturated hydrocarbon groups attached to N and C′, are apparently not significant. In contrast to other workers,[20,21] therefore, we assume that significant charges

(18) J. Applequist, *Biopolymers*, **6**, 117 (1968).
(19) R. M. Meighan and R. H. Cole, *J. Phys. Chem.*, **68**, 503 (1964).
(20) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).
(21) D. Poland and H. A. Scheraga, *Biochemistry*, **6**, 3791 (1967).

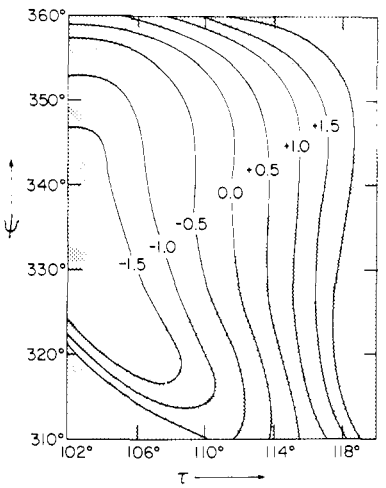

Figure 2. Steric interaction map for a junction between forms I and II in poly-L-proline. The coordinates τ and ψ correspond to the junction angles τ_4 and ψ_4 in Figure 1. The shaded area engenders contacts shorter than the "extreme limits" of Table I. The van der Waals junction energy, in kilocalories per mole, is shown on the contours.

reside only on the N, C′, and O nuclei. The direction of the dipole moment is taken to be identical with that established in formamide,[22] *i.e.*, parallel to a line which passes through the O nucleus, is in the NC′O plane, and makes an angle of 39.6° with the C′–N bond. The effective charges used, +0.22 electronic charge at N, +0.26 at C′, and −0.48 at O, simulate a dipole moment of 3.8 D at long distances. In evaluating the electrostatic energy according to Coulomb's law, the effective dielectric constant of the medium between charges is assumed to be 3.5 in accordance with current usage.[17,20,23] These energies for the 8-mer are found exactly, similar to the van der Waals energies. For longer chains, interactions among all proline units less than eight units apart are considered. The absolute magnitude of terms for prolines separated by more than seven units does not exceed 0.03 kcal/mol.

Results

Poly-L-proline I and Poly-L-proline II. In both forms the variation of van der Waals energy with dihedral angle ψ follows closely the earlier work of de Santis, *et al.*[24] For form I, a sharp minimum in energy is observed at $\psi = 330°$, and energies about 1.5 kcal/mol greater than the minimum are found at ψ equal to 325 and 350°. The van der Waals stabilization per proline unit is −14.5 kcal/mol. In contrast to form I, form II shows a much broader potential well with a minimum at 345° and 1.5 kcal/mol destabilization for ψ equal to 295 and 360°. The intramolecular van der Waals energy of form II is only −8.7 kcal/mol. These results confirm earlier conclusions of de Santis, *et al.*,[24] and Schimmel and Flory[7] that polyproline I is stabilized largely by intramolecular van der Waals interactions. The calculated van der Waals intramolecular end effect for form I at $\psi = 337°$ is found to be +10.5 kcal/mol for each end, confirming earlier suggestions of Rifkind and Applequist[5] and Schimmel and Flory[7] that form I possesses large positive van der Waals end effects. The van der Waals end effect for form II is, by contrast, only +3.0 kcal/mol. The electrostatic end effects, +1.0 kcal/mol for form I and +0.3 for form II, agree roughly with the results found for dipole–dipole interaction, +2.2 and +0.6 kcal/mol, respectively.[8]

TABLE II
CALCULATED ENERGIES OF POLY-L-PROLINE

	Energy, kcal/mol			
	Form I	Form II	Form I–II	Form II–I
Energy of an interior unit				
van der Waals	−14.5	−8.7		
Electrostatic, monopole	−0.4	−0.6		
Electrostatic, dipole	−1.2	−1.1		
Total	−14.9	−9.3		
End effects				
van der Waals	+10.5	+3.0		
Electrostatic, monopole	+1.0	+0.3		
Electrostatic, dipole	+2.2	+0.6		
Total	+11.5	+3.3		
Minimum total junction energy[a]				
van der Waals			−1.6	+1.0
Electrostatic, monopole			+0.8	+1.3
Total			−0.8	+2.3
Electrostatic junction energy for $\tau_4 = 112°$ and $\psi_4 = 332°$				
Monopole			+1.2	+1.4
Dipole			+1.5	+2.0

[a] Actual (τ, ψ) pairs are as follows: I–II junction (104, 335); II–I junction (112, 350).

Polyproline with a I–II Junction. The intramolecular van der Waals junction energy of poly-L-proline with a I–II junction reveals a striking potential minimum in the region defined by values of τ_4 between 102 and 120° and values of ψ_4 between 310 and 360°, as shown in Figure 2. Noteworthy is the region of *negative* junction energy near $\tau = 102°$, $\psi = 330°$. This negative region spans a significant portion of the $\tau - \psi$ map and provides quantitative support of conclusions drawn by Rifkind and Applequist[5] from model studies. Also shown in Figure 2 are the regions which exhibit short contacts by the extreme limit criteria of Table I; these are indicated by shading. Nonshaded regions of Figure 2 are therefore of greater interest, since the molecule presumably will shun configurations causing excessively short contacts even though the total calculated van der Waals energy may be lower in such a configuration than in any others. It can be seen that values of τ_4 between 106 and 110°, and ψ_4 between 330 and 355°, lead to junction energies between −1.6 and 0 kcal/mol. This means that intramolecular steric hindrance is not present at the I–II junction. The contacts which restrict permitted values of τ_4 and ψ_4 in the region of the potential minimum are $H^{\beta}(2)\cdots H^{\alpha}(5)$ and N(4)···O(5), which have values as small as 1.72 and 2.56 Å, respectively.

Within the sterically allowed region, the electrostatic junction energy varies between +0.5 and +1.2 kcal/mol.

(22) R. J. Kurland and E. B. Wilson, *J. Chem. Phys.*, **27**, 585 (1957).
(23) K. D. Gibson and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, **58**, 420 (1967).
(24) P. De Santis, E. Giglio, A. M. Liquori, and A. Ripamonti, *Nature*, **206**, 456 (1965).

It is noteworthy that the electrostatic junction energy, though not a large number, is invariably positive. Hence, the total intramolecular junction energy for the I–II junction varies between −0.8 and +2.6 kcal/mol over the sterically allowed region, for τ_4 between 104 and 118°. These totals are obtained from an examination of the total junction energy at various τ and ψ.

Polyproline with a II–I Junction. The intramolecular van der Waals junction energy of poly-L-proline with a II–I junction, for values of the junction bond angle τ_4 between 102 and 120°, and dihedral angle ψ_4 between 295 and 360°, reveals a broad sterically allowed region of positive energy, as shown in Figure 3. There is also a large region which is free of short contacts, as shown by the unshaded area of this figure. Thus, the II–I junction has positive intramolecular van der Waals energy of +0.8 to +1.8 kcal/mol. These findings are consistent with qualitative conclusions drawn from molecular model studies by Rifkind and Applequist.[5]

The electrostatic junction energy in monopole approximation varies between +1.2 and +1.7 kcal/mol over the sterically permitted region. Consequently, the total intramolecular junction energy varies from +2.3 to +3.4 kcal/mol. The findings for forms I, II, I–II, and II–I are summarized in Table II.

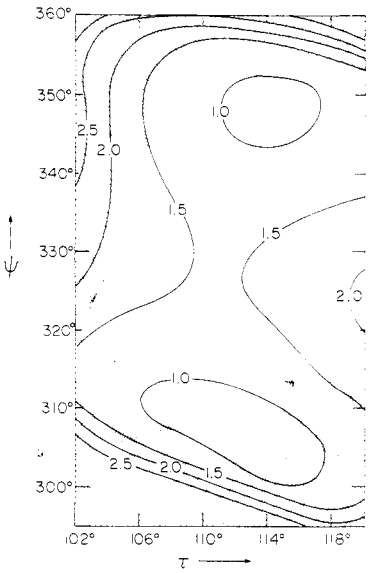

Figure 3. Steric interaction map for a junction between forms II and I in poly-L-proline. The coordinates τ and ψ correspond to the junction angles τ_4 and ψ_4 in Figure 1. The shaded area engenders contacts shorter than the "extreme limits" of Table I The van der Waals junction energy, in kilocalories per mole, is shown on the contours.

Discussion

Critique of the Calculations. The determinations of sterically permitted regions of τ_4 and ψ_4, and the calculations of nonbonded energy, are supported by the success of similar calculations in analyzing the conformations of a variety of polypeptides.[17, 25, 26] In effect, they represent quantitative model building with space-filling models. Figures 2 and 3 show clearly that I–II and II–I junctions are sterically allowed by the "extreme limit" criteria. Indeed, both junctions are also permitted over a range of τ_4 and ψ_4 by the more restrictive "normal limit" criteria as well. However, form II itself does not fully satisfy these criteria, so these data are not shown. Moreover, abundant empirical evidence suggests that the extreme limit is the more realistic one.[17]

It is important to note that suitable conformations are found upon variation of only two parameters. It is likely that additional variations in the structure, for example in the proline rings, could lead to allowed conformations of yet more favorable van der Waals junction energies.

Two additional factors which could influence the intramolecular steric junction energies shown in Figures 2 and 3 are the distortion energy for τ_4 and the hindrance potential characterizing ψ_4. The potential well for τ_4 is in fact crudely approximated by restricting τ_4 to ±8° from the tetrahedral angle. The range of ψ_4 permitted by other factors is too small to engender significant changes in the hindrance energy.[27]

The calculated electrostatic energies in monopole approximation are in reasonable agreement with the earlier results for the dipole–dipole interaction,[8] as indicated in Table II, for the particular values of τ_4 and ψ_4 used previously. The observed differences arise from the approximations to the interaction energy; the monopole energy is presumably more accurate. The energies obtained by either method of calculation are subject to significant alteration from at least two sources. First, the assumed dielectric constant, 3.5, may not accurately represent the effects of solvent and solute polarizability. Second, effective charges on the remainder of the proline side chain[21] may contribute to the system energy. In spite of these uncertainties, it is clear that the electrostatic interactions cause the ends of form I to be less stable than those of form II. In addition, both junctions gain positive cooperativity from electrostatic interactions.

Comparison between Theory and Experiment. The calculated end effects and junction energies may be compared with analogous quantities derived from experimental observations, as follows.

1. End Effects. The studies of Engel[4] show that in the solvent system benzyl alcohol–*n*-butyl alcohol (40:60) polymer with degree of polymerization (DP) equal to 34 is largely in form II, whereas polymer with DP 220 is primarily in form I. These experiments suggest that form II has more favorable end effects than does form I. This observation is in accord with the results of our calculations, which show that the intramolecular van der Waals end effects for both ends in form II are 15 kcal/mol more favorable than those of form I. The electrostatic end effects also favor form II by 1.4 kcal/mol. Thus Engel's observations that short form I helices are less stable than form II can be explained largely by the unfavorable van der Waals end effects of form I, as suggested earlier by Schimmel and Flory.[7] Additional agreement between theory and experiment is found in conformational studies on oligomers with DP between 2 and 8 in

(25) P. J. Flory in "Conformation of Biopolymers," G. N. Ramachandran, Ed., Academic Press, London, 1967, p 339.
(26) H. A. Scheraga, *Advan. Phys. Org. Chem.*, **6**, 103 (1968).
(27) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966).

water, acetonitrile, and methanol.[28] The conformation of the oligomers with DP 6 and 8 is found to be form II, yet high molecular weight polyproline appears to be in form I in methanol.[29] The end effects in this solvent thus also favor form II. Some counterevidence to this attractive agreement between theory and experiment exists. For example, oligomers with DP 6 and 12 were shown by Yaron and Berger[30] to be capable of adopting form I in a certain solvent (unspecified); presumably the solvent was so poor that end effects were overpowered. However, studies in the solvent system trifluoroethanol–*n*-propyl alcohol for polyprolines with DP 17 and 550 (Backman and Holzwarth, unpublished) show an absence of end effects, *i.e.*, the I–II transition curves are symmetrical about the same solvent composition for both DP's. These experiments suggest that the magnitude of the end effect may depend upon the nature of the particular solvent system employed.

2. Junction Energy. The experimental evaluations of junction energy are commonly expressed in terms of a parameter σ, where σ is the weight factor used in statistical-thermodynamic theories of conformational transitions.[6,13,18] Since σ equals $\exp[-J/RT]$, where J is an average junction energy, the experimental junction energies, +4 and +7 kcal/mol, correspond to $\sigma = 10^{-3}$ and 10^{-5}. The two experimental evaluations of junction energy are based upon the pressure dependence of the proline I–II transition[5] and upon the kinetics of the I–II transformation.[4,6] The former measurement, carried out by Rifkind and Applequist,[5] actually yields the quantity $\Delta\bar{V}^2/\sigma$, where $\Delta\bar{V}$ is the change in partial residue-molal volume for the *trans–cis* conversion at infinite dilution. Unfortunately, $\Delta\bar{V}$ is not known, so that the value of σ obtained is uncertain. Similarly, the kinetic measurements of Engel[4] yield σ directly only under certain simplifying assumptions.[6] Thus neither value of σ is untainted.

Now, the experimental evaluations of σ correspond to an equilibrium constant for the process in which a molecule with q *cis* sequences is converted into a molecule with $q + 1$ *cis* sequences while the total number of *cis* residues remains invariant. Such a process must, on the average, be compared to $J_{I-II} + J_{II-I}$ since both a I–II and a II–I junction are created as q increases by 1. The quantity $J_{I-II} + J_{II-I}$ is conveniently designated as J. The calculated intramolecular value of J then falls between +1.5 and +6.0 kcal/mol, depending upon the particular value of τ_4 and ψ_4 chosen. The lower calculated value of J consists of a +2.1-kcal/mol unfavorable electrostatic term and a favorable −0.6 kcal/mol net van der Waals term as indicated in Table II.

These results suggest that the cooperativity of the poly-L-proline does not originate primarily in intramolecular steric effects. Rather intramolecular electrostatic and intermolecular polymer–solvent interactions appear to be the source of the observed positive cooperativity. Polymer–solvent interactions are implicated here because the smaller calculated value of J is less than half the lower of the two experimental values of J. Convincing experimental evidence for the importance of these interactions in polyproline must however await measurements of σ by the same method in a variety of solvents.

Acknowledgments. The authors are grateful to the U. S. Public Health Service for financial support of this work through Grants NB 07286 and AM 11493. We thank, with great pleasure, Professor G. N. Ramachandran for his interest in this work and financial help to R. C. Finally we wish to acknowledge helpful comments on J by a reviewer.

(28) T. Isemura, H. Okabayashi, and S. Sakakibara, *Biopolymers*, **6**, 307 (1968).
(29) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Amer. Chem. Soc.*, **82**, 5263 (1960).
(30) A. Yaron and A. Berger, *Bull. Res. Council Israel*, **10A**, 46 (1961).

The Configurational Entropy of Polyethylene at the Melting Point

Richard E. Robertson

General Electric Research and Development Center, Schenectady, New York 12301.
Received January 8, 1969

ABSTRACT: The configurational entropy of polyethylene at the melting point is obtained from the measured entropy of fusion, the excess nonconfigurational entropy of the glass calculated from heat capacity data, and an estimated nonconfigurational entropy for expanding the liquid from the volume of the glass to the volume of the melt at atmospheric pressure. The value obtained is 0.536 ± 0.045 J/°K g (1.79 ± 0.15 eu/mol of CH_2). Other, more simply made estimates compare as follows. The entropy of fusion is 33% larger; Mandelkern's approximate entropy of fusion at constant volume is within our estimated error; and our estimate of the entropy of fusion at constant volume is 25% smaller.

The configurational entropy is the entropy of the variety of shapes assumed by the polymer molecules. It describes the variability in the internal rotation about each single bond and, to a minor extent, the variability in bond lengths and bond angles. Unfortunately, the configurational entropy cannot be measured directly but must be derived from other quantities.

The simplest estimate for the configurational entropy of a liquid at the melting point is the entropy of fusion. Since the configurational entropy is all developed as the molecules are released from their sameness of