Conformational Properties of Poly(L-proline) Containing a Flexible Pyrrolidine Ring[1]

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ABSTRACT: Two conformational maps for the dipeptide unit in the interior of poly(L-proline) containing peptide bonds in the planar trans conformation are found to correctly predict the characteristic ratios experimentally observed for this polypeptide at 5 and 30°. These two conformational maps differ from previous maps in allowing for flexibility in the pyrrolidine ring. They suggest that there are two rotational isomeric states, separated by about 180° rotation about ψ, for poly(L-proline) containing peptide bonds in the planar trans conformation. While one of these rotational states is populated to only a minor extent at 30°, it nevertheless exerts a significant effect upon the unperturbed dimensions of poly(L-proline). The conformational maps are also consistent with Torchia's analysis of the ring vicinal couplings in the 220-MHz nmr spectrum of poly(L-proline) in aqueous solution.

Several theoretical analyses have been reported which attempt to predict the conformational properties of poly(L-proline) containing peptide bonds in the planar trans conformation.[4-12] Conformational maps, usually presented as energy *vs.* ψ[13] at constant ϕ, have been obtained for helical poly(L-proline),[4,6,7,11,12] for the L-prolyl-L-prolyl dipeptide unit in the interior of a poly(L-proline) chain,[5,8-10,12] and for the dipeptide L-prolyl-L-proline.[7] The various studies differ significantly in the manner in which the conformational energy depends upon the dihedral angles. However, they all find a low conformational energy for ψ = 145–146°, corresponding to the conformation adopted in the solid state by helical poly(L-proline) when the peptide bonds are in the planar trans conformation.[14,15]

A more sensitive test of the validity of a conformational map is the successful prediction of an experimentally measurable quantity which depends upon the statistical weights for all portions of the map. The most frequently used such experimental quantity is the characteristic ratio,[16] defined[17] for polypeptides as $\langle r^2\rangle_0/n_\mathrm{p}l_\mathrm{p}^2$, where $\langle r^2\rangle_0$ is the unperturbed mean-square end-to-end distance for a polypeptide containing n_p virtual bonds of length l_p. The characteristic ratios obtained from the various conformational maps for poly(L-proline) vary by more than an order of magnitude.[18,19] None of the conformational maps published through 1971[4-11] could satisfactorily account for both the measured characteristic ratio and its temperature dependence.[18,19] We wish to report here that a recently published[12] conformational map for the L-prolyl-L-prolyl dipeptide unit in the interior of a poly(L-proline) chain not only contains a low energy for the geometry observed in the solid state[14,15] but also is consistent with the characteristic ratios observed for poly(L-proline) at 5 and 30°.[18] The reasons for the success of this recent theoretical study[12] are examined.

Calculational Procedure

Conformational Map. The procedure utilized by Nishikawa and Ooi[12] to compute the conformational map for the internal dipeptide unit in poly(L-proline) is summarized here. For additional details, see ref 12.

The structure used in the computation is presented in Figure 1. The dihedral angles ϕ_i and ψ_i were allowed to vary while ϕ_{i+1} was held constant at −60° and ω_i and ω_{i+1} were held constant at 180°.[20] Two different positions were considered for C_i^γ: in the plane of C_i^α–N_i–C_i^δ (γ^1 position) and in the plane of C_i^β–C_i^α–N_i (γ^2 position). The unspecified bond angles in the pyrrolidine ring of residue *i* are functions of both ϕ_i and the location of C_i^γ.

Bending energies for the three variable bond angles in residue *i* were taken to be proportional to the square of the degree of distortion from 109.5°.[21] Lennard-Jones 6–12 potentials and torsional energies for the bonds in the main chain were those used previously.[22] A hydrogen atom radius of 1.275 Å was employed. Threefold torsional potentials with barrier heights of 3 kcal/mol and maxima at the cis conformation were assumed for bonds χ_1, χ_2, χ_3, and χ_4 in residue *i*.[20] The electrostatic energy was evaluated in the monopole approximation with a dielectric constant of 4 and partial changes on the carbonyl group which produced a dipole moment of 2.4 D.

Characteristic Ratio. The averaged transformation matrix was obtained as described by Flory.[23] Intervals of 10° were used for ϕ_i and ψ_i. Regions of the conformational maps with energies higher than 10 kcal/mol above the minimum were excluded. It was assumed that the change in the conformational properties upon cooling from 30 to 5° was due solely to the change in the temperature of the Boltzmann factors. The unperturbed mean-square end-to-end distance for polypeptides of various degrees of polymerization was computed from eq 29, chapter IV, ref 16. In computing the characteristic ratio, l_p was taken as

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Table I
Characteristic Ratios Predicted by Figure 2 for a Degree of Polymerization of 1000

Section of Conformational Map Used	C_i^γ Position	Characteristic Ratio	
		5°	30°
Entire map	γ^1	13.0	12.4
	γ^2	20.0	17.0
Restrict ψ_i to 150 ± 50°	γ^1	29.7	29.1
	γ^2	31.0	30.2
Restrict ϕ_i to −50°	γ^1	42.1	41.5
	γ^2	10.5	9.7
Restrict ϕ_i to −60°	$\gamma^1 = \gamma^2$	19.3	17.2
Restrict ϕ_i to −70°	γ^1	10.2	9.5
	γ^2	49.3	47.8
Restrict ϕ_i to −80°	γ^1	21.7	19.7
	γ^2	56.1	54.5
Observed, organic solvent[a]		22.0 ± 2.0[b]	19.4 ± 1.6[c]
Observed, water[a]		14.8 ± 1.0	13.7 ± 0.9

[a] Observed characteristic ratios are from W. L. Mattice and L. Mandelkern, *J. Amer. Chem. Soc.*, **93**, 1769 (1971). [b] Measured in trifluoroethanol and in propionic acid. [c] Measured in trifluoroethanol, in propionic acid, and in acetic acid.

3.825 Å, corresponding to the separation of adjacent α-carbon atoms in Figure 1.

Results and Discussion

The conformational energy maps computed for the structure in Figure 1 with two different conformations of the pyrrolidine ring of residue *i*, the γ^1 and γ^2 positions, are shown in Figure 2. The energy contours are at 1, 2, 5, and 10 kcal per mol above the energy minimum, which is at ϕ_i, ψ_i = −70°, 160–170° for the γ^1 position and at −70°, 170° for the γ^2 position. In each case two regions of low energy are found, one including the energy minimum and the other near ϕ_i, ψ_i = −60°, −50°. The γ^1 and γ^2 positions for C_i^γ lead to nearly identical conformational maps. The only significant difference is the lower energy of the small region near ψ_i = −50° for the γ^1 position.

Because of the presence of the pyrrolidine ring, ϕ_i is limited to the range of *ca.* ±20° from the planar structure of the ring (ϕ_i = −60°).[21] In the region ψ_i = 140 ± 20°, the energy decreases when ϕ_i rotates slightly in either direction from ϕ_i = −60°. This situation arises because the dihedral angles χ_1, χ_2, χ_3, and χ_4 in residue *i* are in the cis conformation when the pyrrolidine ring is planar, which is the most unfavorable state so far as torsional energy is concerned.

The averaged transformation matrices calculated from the conformational maps in Figure 2, using a temperature of 30°, are given in eq 1 and 2. The characteristic ratios

$$\langle \mathbf{T} \rangle_{\gamma 1} = \begin{bmatrix} 0.423 & 0.474 & 0.548 \\ -0.589 & -0.221 & 0.582 \\ 0.640 & 0.667 & 0.186 \end{bmatrix} \qquad (1)$$

$$\langle \mathbf{T} \rangle_{\gamma 2} = \begin{bmatrix} 0.444 & 0.523 & 0.589 \\ -0.589 & -0.272 & 0.644 \\ 0.641 & -0.686 & 0.175 \end{bmatrix} \qquad (2)$$

calculated from the conformational maps in Figure 2 are presented in Table I along with the experimental results.[18] Characteristic ratios of 13.0 at 5° and 12.4 at 30° are predicted by the conformational map for the γ^1 position, and the γ^2 position leads to the slightly higher characteristic ratios of 20.0 at 5° and 17.0 at 30°. These predictions are in excellent agreement with the experimentally observed values, both with regard to the result at a given temperature and to the increase of roughly 10% in the characteristic ratio upon cooling from 30 to 5°. The γ^1 position leads to results similar to those obtained in water, while the γ^2 position agrees well with the results obtained in organic solvents, suggesting the possibility that the observed solvent effect on the characteristic ratio might be due to solvent-induced alterations in the energetics of the puckering of the pyrrolidine ring. Other possible origins of the solvent effect on the characteristic ratio may also exist, as will be brought out below.

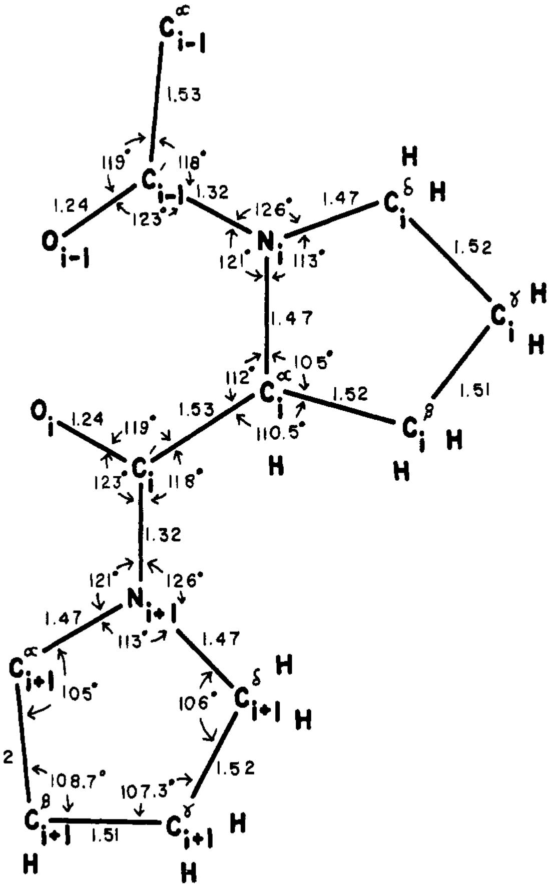

Figure 1. The structure considered in computing the conformational energy map.[12] The angles ϕ, ψ, ω, and χ are not designated in this figure. They are defined in ref 13.

None of the other conformational maps reported for poly(L-proline)[4-11] can satisfactorily account for the observed conformational properties.[18,19] It is of interest to ascertain to what extent the agreement noted in Table I is due to the presence of a second region of low energy near ψ_i = −50°, to the allowance of some freedom of rotation about ϕ_i, and to the two locations for C_i^γ.

The effect of the second region of low energy near ψ_i = −50° can be assessed by ignoring this region of the conformational map in the computation, *i.e.*, by restricting ψ_i to 150 ± 50°. The resulting characteristic ratios are presented in the second set of entries in Table I. Elimination of the second region of low energy near ψ_i = −50° leads to a substantial increase in the characteristic ratio so that the agreement with experiment is decreased. The predicted characteristic ratios are nearly identical for the two locations of C_i^γ when ψ_i is limited to 150 ± 50°. The effect of the location of C_i^γ on the characteristic ratio, noted in the first set of entries in Table I, apparently is due primarily to the differences in the depth of the minimum near ψ_i = −50° and in the values of ϕ_i for this minimum. The observed solvent effect upon the characteristic ratio of poly(L-proline) could be rationalized if the solvent can, by any

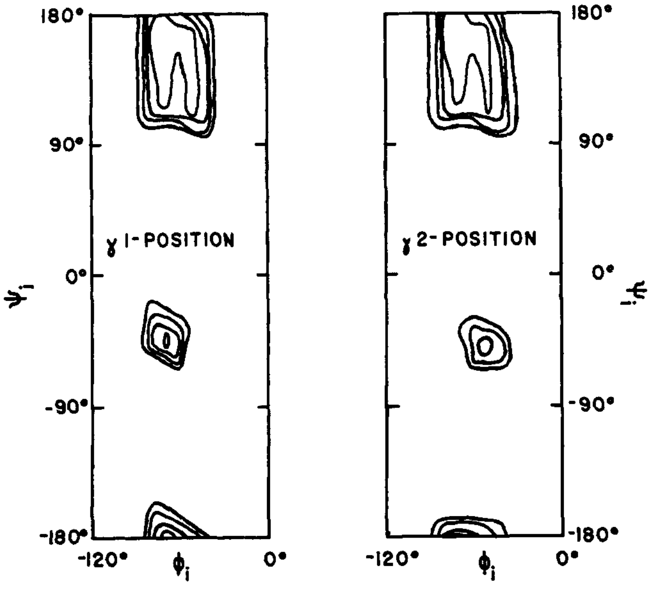

Figure 2. Conformational energy maps for the γ^1 and γ^2 positions.[12] The contours are drawn at 1, 2, 5, and 10 kcal per mol above the energy minimum.

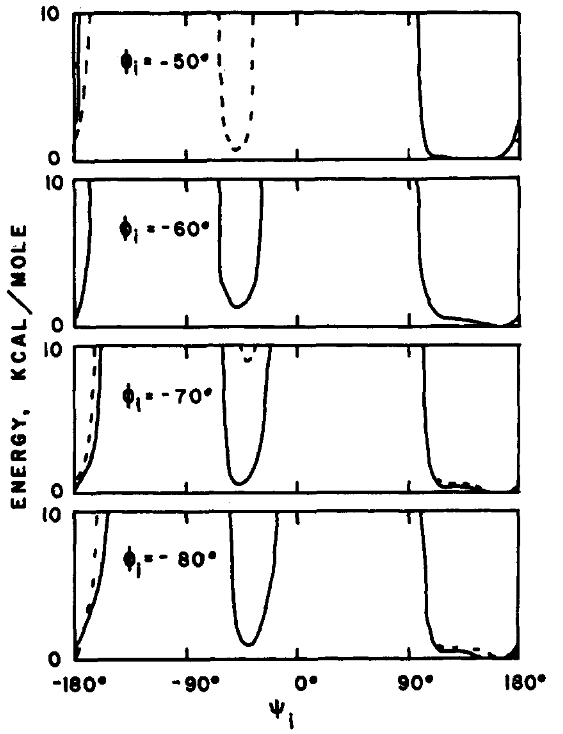

Figure 3. Conformational maps obtained from Figure 2 at constant ϕ. The vertical position for each curve has been shifted to yield a minimum energy of 0 kcal/mol in each case. The solid line is for the γ^1 position and the broken line is for the γ^2 position. When $\phi_i = -60°$ the γ^1 and γ^2 positions are identical.

mechanism, induce slight alterations in the precise location and depth of this second region of low energy. The electrostatic interaction of the adjacent peptide bonds makes only a minor contribution to the conformational energy. This small contribution, however, is attractive near $\psi_i = 150°$ and repulsive near $\psi_i = -50°$. If the local dielectric constant is slightly higher in water than in the organic solvents, the region near $\psi_i = -50°$ will be of slightly lower energy, relative to $\psi_i = 150°$, in water than in the organic solvents. The resulting solvent effect on the characteristic ratio would be in the direction which is observed.

Previous calculations of the conformational map for poly(L-proline) have been conducted by assigning a constant value, in the range of $-78°$ to $-58°$, to ϕ_i. In order to ascertain the effect of ignoring rotation about ϕ_i, characteristic ratios were computed from Figure 2 with ϕ_i held constant at -50, -60, -70, and $-80°$. The corresponding slices through the conformational map, adjusted on the vertical scale so that the minimum for each curve is at 0 kcal/mol, are shown in Figure 3. The definitions of the γ^1 and the γ^2 positions lead to precisely the same ring structure, a planar pyrrolidine ring, when $\phi_i = -60°$. The relative energies of the region at $\psi = 150°$ and $\psi = -50°$ are extremely sensitive to the choice of ϕ_i and the position of C_i^{γ}. When $\phi_i = -50°$ with the γ^1 position and when $\phi_i = -80°$ with the γ^2 position the region near $\psi = -50°$ has an energy more than 10 kcal/mol higher than that at $\psi = 150°$.

The characteristic ratios computed from Figure 3 are shown in the third through sixth sets of entries in Table I. They are found to be extremely sensitive to which value of ϕ_i is chosen. If C_i^{γ} is in the γ^1 position, the low characteristic ratio of 9.5 at 30° is obtained when $\phi_i = -70°$, while a value over four times larger is computed when $\phi_i = -50°$. With C_i^{γ} in the γ^2 position, the characteristic ratio of 9.7 at 30° is obtained when $\phi_i = -50°$. A result more than five times larger is calculated when $\phi_i = -80°$ with C_i^{γ} in the γ^2 position. The results summarized in Table I also show that the characteristic ratio is extremely sensitive to the conformation assumed for the pyrrolidine ring if ϕ_i is fixed at -50, -70, or $-80°$. This situation arises because the relative energies of the regions near $\psi_i = -50°$ and $\psi_i = 170°$, at constant ϕ_i, are highly dependent upon the choice of ϕ_i and the geometry of the pyrrolidine ring.

Essentially identical characteristic ratios are predicted when ϕ_i is allowed to vary while C_i^{γ} is in the γ^2 position, when ϕ_i is held constant at $-60°$, and when ϕ_i is held constant at $-80°$ with C_i^{γ} in the γ^1 position. Therefore no distinction, based solely upon the ability to correctly predict the characteristic ratio of poly(L-proline), can be made between limited rotation about ϕ_i, fixing ϕ_i at $-60°$ with a planar pyrrolidine ring, or fixing ϕ_i at $-80°$ with C_i^{γ} in the γ^1 position. A recent examination[24] of the ring vicinal couplings in the 220-MHz nmr spectrum of poly(L-proline) provides the basis for the elimination of two of these possible structures for poly(L-proline). It was shown that the pyrrolidine ring in poly(L-proline) in aqueous solution is not planar, that the only rigid pyrrolidine ring structures which are consistent with experiment have C^{α} slightly exo[25] or C^{β} slightly endo and that agreement with experiment can also be obtained if it is assumed that the pyrrolidine ring rapidly interconverts between at least two different puckered conformations.[24] Therefore the rigid planar pyrrolidine ring is not a reasonable structure for poly(L-proline), even though the characteristic ratios obtained with $\phi_i = -60°$ agree with experiment. The rigid ring with $\phi_i = -80°$ and the γ^1 position can also be eliminated because C_i^{β} is exo under these circumstances, and this is not consistent with the nmr results obtained by Torchia.[24]

In contrast, the results calculated from the entire conformational maps in Figure 2, and which predict the characteristic ratios in the first set of entries in Table I, presume the existence of a range of puckerings at one of the carbon atoms in the pyrrolidine ring. When C_i^{γ} is in the γ^1 position, C_i^{β} will be exo if ϕ_i is less than $-60°$, the pyr-

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(25) According to Torchia,[24] "An out-of-plane ring atom is endo if it lies on the same side of the ring plane as the carbonyl carbon. An exo ring atom lies on the opposite side of the ring."

rolidine ring is planar when $\phi_i = -60°$, and C_i^{β} will be endo if ϕ_i is greater than $-60°$. On the other hand, when C_i^{γ} is in the γ^2 position, C_i^{δ} will be endo if ϕ_i is less than $-60°$, the pyrrolidine ring is planar when $\phi_i = -60°$, and C_i^{δ} will be exo when ϕ_i is greater than $-60°$. Since the γ^1 position and the γ^2 position each correspond to the assumption of the existence of a range of puckered geometries for the pyrrolidine ring when there is limited rotation about ϕ_i, it seems probable that they may also be consistent with the observed ring vicinal couplings.[24]

The conformational maps in Figure 2 suggest that two rotational isomers, seperated by nearly 180° rotation about ψ_i, may exist in poly(L-proline) when the peptide bonds are in the planar trans conformation. Evaluation of the relative populations of these two rotational isomers, using Figure 2 with 10° intervals in ϕ_i and ψ_i and a temperature of 30°, indicates that the fraction of the amino acid residues which have ψ_i located at $-50 \pm 20°$ would be about 0.06 and 0.02, respectively, for the γ^1 and γ^2 positions. Even though the conformational maps in Figure 2 predict that only a small fraction of the L-prolyl residues populate this second minimum, this small number of residues can lead to a marked reduction in the unperturbed dimension of a long polypeptide chain because each such residue causes a comparatively sharp bend in the chain. The ability to detect such a small fraction of residues populating the region of $\psi_i = -50 \pm 20°$ by a technique in which all L-prolyl residues contribute equally to the signal would depend upon the sensitivity of the technique and whether or not the signal from the L-prolyl residues with $\psi = -50 \pm 20°$ can be adequately separated from the much larger signal due to the more numerous L-prolyl residues with $\psi = 150 \pm 50°$.

A second minimum located near $\psi = -50°$ also occurs in three other conformational maps for poly(L-proline).[7,8,10] In a conformational map reported for L-prolyl-L-proline the two minima are of essentially equal energy,[7] leading to the extremely low characteristic ratio of 5.1.[19] For *N*-acetyl-*N'*-prolylprolylamide the second minimum at $\psi = -50°$ was found to be about 10 kcal/mol higher than the absolute minimum at $\psi = 140°$.[10] The characteristic ratio evaluated from this map is 118.[19] Gō and Scheraga report four different conformational maps for the internal L-prolyl-L-prolyl dimer, each based upon a rigid pyrrolidine ring, but with different puckering at C^{γ}.[8] They found that the energy at $\psi = -45 \pm 40°$ was extremely sensitive to the geometry chosen for the pyrrolidine ring.[8] In one case the energy in this region was found to be only ~8 kcal/mol above the energy at the absolute minimum at $\psi = 140 \pm 40°$.[8] The four conformational maps calculated by Gō and Scheraga[8] predict characteristic ratios of 29, 39, 68, and 70,[19] illustrating that different rigid pyrrolidine ring geometries substantially alter the conformational properties of poly(L-proline).

The energy of the area near $\psi_i = -50°$ is raised substantially relative to the energy at $\psi_i = 150°$ when the computation is performed for a polypeptide chain containing several L-proline residues, each with the same location of the C^{γ} atom, in which ϕ and ψ are varied simultaneously at each residue.[12] For this reason it is not anticipated that poly(L-proline) will be observed to form an ordered structure with all ψ near $-50°$, even though a small fraction of the L-prolyl residues in the statistical coil may have ψ near $-50°$.

The importance of considering the flexibility of amino acid side chains may apply to other polypeptides in which there is a substituent attached to the peptide nitrogen atom. Dipeptide conformational maps computed for poly(*N*-methyl-L-alanine)[26] and poly(*N*-methylglycine)[27] do not find a low energy near $\phi,\psi = -60°, -50°$. In both of these calculations the *N*-methyl was situated so that one of its hydrogen atoms eclipsed the peptide bond. This position corresponds to a rotation of about 60° from the orientation of the C^{δ} atom in the proline residue. Inspection of CPK models reveals that the repulsions generated at $\phi,\psi = -60°, -50°$ by the substituent attached to the nitrogen atom are much more severe if it has the orientation selected in the studies of poly(*N*-methyl-L-alanine)[26] and poly(*N*-methylglycine)[27] than is the case if the orientation is that found in the proline residue. Therefore the conformational maps computed for these two polypeptides may be altered significantly if rotation of the methyl substituent is allowed.

The conformational maps in Figure 2 are the only published maps which have allowed for flexibility in the pyrrolidine ring. C_i^{β} and C_i^{δ} move with respect to the remainder of the pyrrolidine ring in the γ^1 and γ^2 positions, respectively. Many other types of flexibility in the pyrrolidine ring can be imagined.[28] Evidence for puckering at C^{γ} is supplied from X-ray diffraction studies on crystals of small linear peptides containing L-proline.[29-31] Puckering at C^{β}, corresponding to the γ^1 position, has recently been documented in a study of crystalline *cyclo*-L-prolyl-L-leucyl.[32] We cannot exclude the possibility that consideration of types of flexibility in the pyrrolidine ring other than those assumed here might also lead to a conformational map which would correctly predict the conformational properties observed for poly(L-proline).

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