Conformational Properties of Poly(γ-hydroxy-L-proline) Based on Rigid and Flexible Pyrrolidine Rings[1a]

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ABSTRACT: Conformational energy maps have been computed for the internal dipeptide unit in poly(γ-hydroxy-L-proline) containing planar trans peptide bonds. The conformational energy maps based on rigid pyrrolidine rings which have the conformation observed in the solid state exhibit one low-energy region at $\psi = 145° \pm 40°$ (using the convention in which $\phi,\psi = 180°,180°$ for the fully extended chain). The characteristic ratios for this geometry are much higher than the result obtained experimentally for poly(L-proline). Conformational energy maps based on flexible pyrrolidine rings contain a region of low energy near $\psi = -50°$ in addition to the low-energy region at $\psi = 150° \pm 70°$. The characteristic ratio based on the opportunity for flexibility in the pyrrolidine rings of residues i and $i + 1$ is close to the result obtained experimentally for poly(L-proline) in water. The configurational entropies per residue for poly(L-proline) and poly(γ-hydroxy-L-proline) are ~3-5 cal/(mol deg) smaller than the results obtained by Brant, Miller, and Flory for polyglycine and poly(L-alanine). The hydroxyl group in the γ-hydroxy-L-proline residue decreases the flexibility of the pyrrolidine ring, leading to a smaller configurational entropy for the γ-hydroxy-L-proline residue than for the L-proline residue. This effect would lead to an increased thermal stability of the collagen triple helix when γ-hydroxy-L-proline is substituted for L-proline.

The conformational properties of poly(L-proline) and poly(γ-hydroxy-L-proline) are similar in many respects. The ϕ,ψ[2] of poly(L-proline) form II[3,4] and poly(γ-hydroxy-L-proline) A[5] are virtually identical in the solid state, dilute aqueous solutions exhibit quantitatively similar circular dichroism, and the conformation is altered by the isothermal addition of calcium chloride.[6] However, a variety of techniques indicate that some change in the conformational properties of polypeptides occurs when γ-hydroxy-L-proline is substituted for L-proline. Intrinsic viscosity and circular dichroism measurements show that poly(γ-hydroxy-L-proline) is more resistant than poly(L-proline) to the conformational change induced by calcium chloride.[6,7] The pyrrolidine ring in poly(L-proline) exists in two equally populated conformations, and interconversion between these conformations is rapid.[8] The pyrrolidine ring in poly(γ-hydroxy-L-proline) is found to preferentially populate one conformation,[9] with the C^γ atom exo.[10] Reduced flexibility of the pyrrolidine ring in the poly(γ-hydroxy-L-proline) as compared to poly(L-proline) is evident in the ring atom correlation times obtained from ^{13}C nmr spectra.[11] Differences in the conformational properties in dilute solution are also observed with sequential copolypeptides in which γ-hydroxy-L-proline is substituted for L-proline.[9,11-13]

The unperturbed dimensions, and their temperature dependence, of poly(L-proline)[14] can be explained by conformational energy maps based on a flexible pyrrolidine ring.[15] This communication presents conformational energy maps for the internal dipeptide unit in poly(γ-hydroxy-L-proline) based on a rigid pyrrolidine ring with puckering at the C^γ atom, as observed in the solid state,[5] and also based on flexible pyrrolidine rings of the type used in poly(L-proline).[15] The conformational properties predicted for poly(γ-hydroxy-L-proline) by these two approaches are found to be different, and they also differ from the results obtained previously for poly(L-proline).[15]

Calculational Procedure

Structures. The atoms considered in the computation of the conformational energy maps for the internal dipeptide unit in poly(γ-hydroxy-L-proline) are shown in Figures 1 and 2. The bond lengths and bond angles in Figure 1 were used to obtain the conformational energy maps based on rigid pyrrolidine rings.[5] The O–H and C–H bond lengths were 1.00 Å and the C^γ–O–H angle was 109.5°. The angle between the C –N–C^α–C^β and C^β–C^γ–C^δ planes was 17°, with the C^γ atom exo.[16] The conformational energy was computed at 5° increments of ψ for $\chi_5 = 60°$, 180°, and 300°.

The structure of the internal dimer containing flexible pyrrolidine rings, shown in Figure 2, was obtained by placing two hydroxyl groups on the structure previously used for the internal dimer of poly(L-proline).[15] The C^γ atom can adopt either of two positions as a consequence of rotation about ϕ: in the plane of C^α–N–C^δ (γ^1 position) or in the plane of C^β–C^α–N (γ^2 position).[15,17,18] The unspecified bond angles in the pyrrolidine rings are functions of ϕ and the position of the C^γ atoms for that ring.[17] The hydrogen and oxygen atoms attached to the C^γ atom were located perpendicular to the C^β–C^γ–C^δ plane, the C^γ–O and C^γ–H bonds each made an angle of 54.75° with this plane, and χ_5 was held constant at 60°. The conformational energy was computed at 10° intervals for ϕ_i, ψ_i, and ϕ_{i+1}, for both positions of the C_i^γ and C_{i+1}^γ atoms. The peptide bonds were maintained in the planar trans conformation.

Energy Functions. The energy functions used in the previous work on poly(L-proline) were adopted for poly(γ-hydroxy-L-proline).[15,17-19] The torsional potential about χ_5 was assumed to be threefold. Electrostatic interactions involving the hydroxyl groups were ignored except when specifically stated to the contrary. In those cases where they were considered, the charges on the O–H atoms were those used by Poland and Scheraga,[20] and the charge on the C^γ atom was chosen to yield a net charge of zero for the C^γ–O–H unit.

Conformational Properties Ignoring Interdependence of Rotations in Neighboring Residues. The averaged transformation matrix was obtained from eq 1[21] using 10°

$$\langle \mathbf{T}_i \rangle = \mathbf{R}(\xi,0)\langle \mathbf{R}(\theta^\alpha, \pi - \phi_i)\mathbf{R}(-\eta, -\psi_i)\rangle \tag{1}$$

intervals for ψ_i, or ϕ_i and ψ_i, for a particular conformation of residue $i + 1$. The N–C^α–C^β angle is θ^α, η is the angle between the C′–C^α bond and the virtual bond, and ξ is the angle between the C^α–N bond and the virtual bond. Regions with energies above the 10 kcal/mol contour were ignored in the evaluation of $\langle \mathbf{T}_i \rangle$. The temperature dependence was assumed to arise entirely from the change in the temperature term in the Boltzmann factors. The unperturbed mean-square end-to-end distance, $\langle r^2 \rangle_0$, for a chain containing n_p virtual bonds was computed from eq 2.[22] The matrices $\mathbf{J}^*$, $\mathbf{G}_1$, $\mathbf{G}$, and $\mathbf{J}$ are defined by Flory.[23]

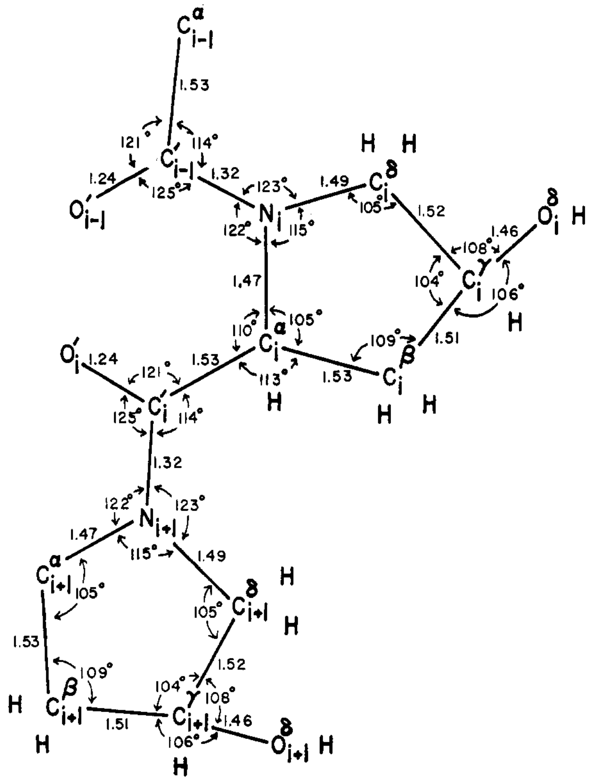

Figure 1. Internal dipeptide geometry used for rigid pyrrolidine rings.

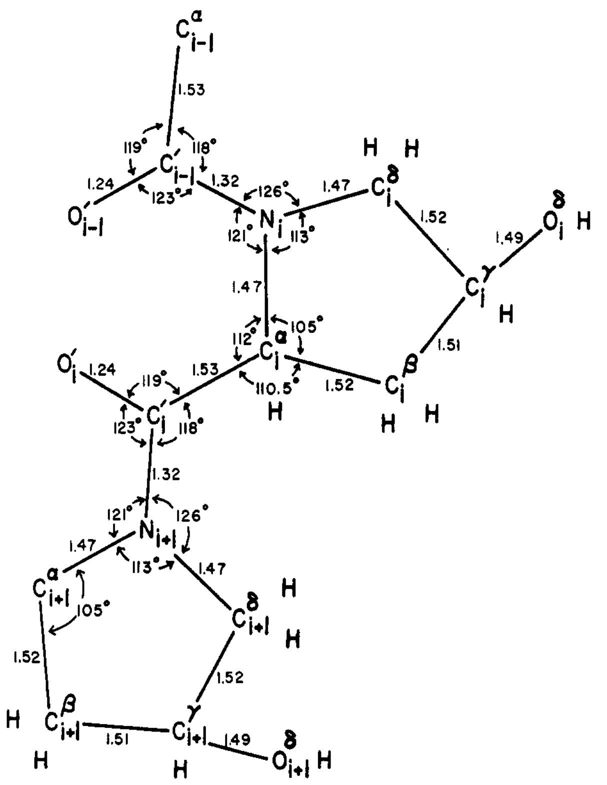

Figure 2. Internal dipeptide geometry used for flexible pyrrolidine rings.

$$\langle r^2\rangle_0 = 2Z^{-1}\mathbf{J}^*\mathbf{G}_1\mathbf{G}^{n_p-1}\mathbf{J} \qquad (2)$$

The partition function, Z, is unity in this case. The configurational entropy per residue, S, was computed from eq 3[24] using 10° intervals for ϕ_i and ψ_i.

$$S = R\ \ln \sum_{\phi_i}\sum_{\psi_i} \exp(-E(\phi_i,\psi_i)/RT) + \langle E\rangle/T \qquad (3)$$

Conformational Properties Including Interdependence of Rotations in Neighboring Residues. The conformational energy map for residue i depends upon the state of residue $i - 1$. Flory has described methods for determining the conformational properties of a polymer in which rotation about bond i depends upon the rotation at bond $i - 1$.[25] This procedure can be adapted to the conformational energy maps presented here by viewing the carboxyl terminus as the beginning of the chain, thereby making residue $i + 1$ (in the customary notation[2]) precede residue i. The average transformation matrix is now given by eq 4 instead of eq 1.

$$\langle \mathbf{T}_i\rangle = \mathbf{R}(\eta, 0)\langle \mathbf{R}(\theta^\alpha, \pi - \psi_i)\mathbf{R}(-\xi, -\phi_i)\rangle \qquad (4)$$

The various states for γ-hydroxy-L-proline residue i were assigned as follows. Residue i can have $\phi_i = -70$, -60, or $-50°$, and atom C_i^γ may assume either the γ1 or γ2 position when $\phi_i = -70$ or $-50°$. The γ^1 and γ^2 positions are identical when $\phi_i = -60°$. Each of these five situations is divided into two states, representing the low-energy regions near $\psi_i = 170$ and $-50°$. The statistical weight assigned to each of these ten states was evaluated by summing the Boltzmann factors at 10° intervals for ψ_i in the appropriate low-energy region (near $\psi_i = -50$ or 170°) and the appropriate ϕ_i in the proper conformational energy map in Figures 3 or 4. The statistical weight was set equal to zero if all of the energies were greater than 6 kcal/mol above the minimum. The nonzero statistical weights and average value of ψ_i, both evaluated at 30°, are shown in Table I. The statistical weight matrix at 30° for each residue, except that situated at the carboxyl terminus of the chain, is formed from these statistical weights, with the rows describing the state of residue $i + 1$ and the columns the state of residue i in Figure 2.

A different statistical weight matrix is required for the carboxyl terminal residue in the chain because it cannot interact with another residue further toward the carboxyl terminus. The statistical weights for this unit reflect the changes in the energy of an individual γ-hydroxy-L-proline ring as it interconverts between $\phi = -70$, -60, and $-50°$ for the γ^1 and γ^2 position. The total energy for this residue was a minimum for the γ^1 position and $\phi = -70$ or $-50°$, was 0.69 kcal/mol higher for the γ^2 position and $\phi = -70$ or $-50°$, and was 1.26 kcal/mol above the minimum for $\phi = -60°$. The statistical weight matrix for the carboxyl terminus contains elements in the first row made up of the Boltzmann factor obtained from the appropriate ring energy.

The poly(γ-hydroxyl-L-proline) configuration partition fuction taking account of interdependent rotations in neighboring residues, Z', is different from the configuration partition function, Z, described by Flory,[26] because Z' must take account of the pyrrolidine ring conformations of every amino acid residue in the chain, including the terminal residues. It can be evaluated from eq 5, which has a different range for the subscript i than the

$$Z' = \mathbf{J}^*\left[\prod_{i=1}^{n_p+1}\mathbf{U}_i\right]\mathbf{J} \qquad (5)$$

equation used to evaluate Z.[26] The matrices $\mathbf{J}^*$ and $\mathbf{J}$ are defined by Flory,[26] and the $\mathbf{U}_i$ are the statistical weight matrices. The *a priori* probability, $p_{\rho,i}$, that pyrrolidine ring i is in state ρ can be obtained from eq 6, which differs

$$p_{\rho,i} = Z'^{-1}\mathbf{J}^*\left[\prod_{h=1}^{i-1}\mathbf{U}_h\right]\mathbf{U}'_{\rho,i}\left[\prod_{j=i+1}^{n_p+1}\mathbf{U}_j\right]\mathbf{J} \qquad (6)$$

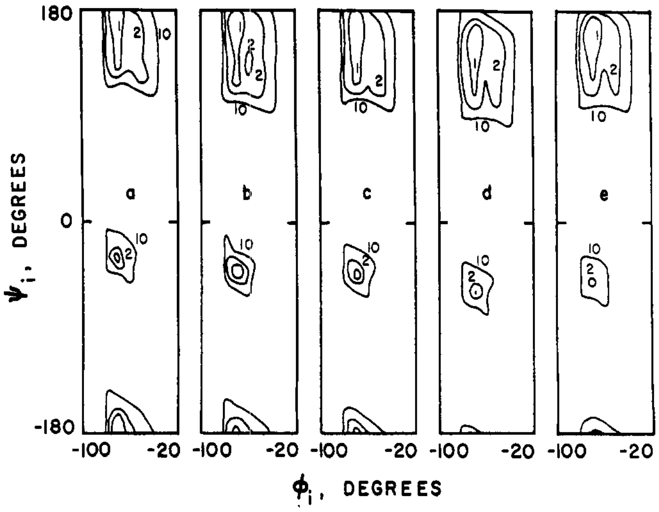

Figure 3. Conformational energy maps for residue i in the γ^1 conformation, with residue $i + 1$ in the following conformations: (a) γ^1, $\phi = -70°$; (b) γ^2, $\phi = -70°$; (c) $\gamma^1 = \gamma^2$, $\phi = -60°$; (d) γ^1, $\phi = -50°$; (e) γ^2, $\phi = -50°$. Contours are drawn at 1, 2, and 10 kcal per mol relative to the lowest energy, which occurs in (b), (c), and (e).

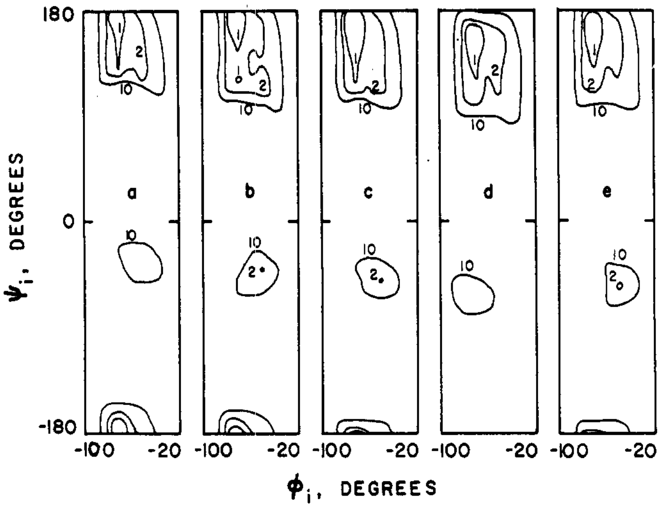

Figure 4. Conformational energy maps for residue i in the γ^2 conformation, with residue $i + 1$ in the following conformations: (a) γ^1, $\phi = -70°$; (b) γ^2, $\phi = -70°$; (c) $\gamma^1 = \gamma^2$, $\phi = -60°$; (d) γ^1, $\phi = -50°$; (e) γ^2, $\phi = -50°$. Contours are drawn as in Figure 3.

from the equation described by Flory[27] in the range of h and j due to the differences in Z and Z'. The only nonzero column in $\mathbf{U}'_{p,i}$ is the column corresponding to state p.

The unperturbed mean-square end-to-end distance for a chain with n_p virtual bonds is obtained from eq 2 with Z being replaced by Z' for a chain with n_p residues. Numbering begins at the carboxyl terminus in the application of eq 2, 5, and 6 described in this section. The transformation matrices are given by eq 4, with $\langle \mathbf{R}(\theta^\alpha, \pi - \psi_i)\mathbf{R} \times (-\xi, -\phi_i)\rangle$ for each state being evaluated using the ϕ_i for the state and 10° intervals for ψ_i through the appropriate low energy region.

Results and Discussion

Conformational Energy Maps Based on a Rigid Pyrrolidine Ring. The conformational energy maps based on rigid pyrrolidine rings with the structure shown in Figure 1, and the C^γ atom exo by 17°,[16,28] are shown in Figure 5. The energy scale for each curve was adjusted to yield a minimum energy of zero. Slightly lower energies at the minimum were obtained with $\chi_5 = 60°$ than with $\chi_5 =$ 180 or 300°, but the manner in which the conformational energy varies with ψ_i was found to be nearly the same for each orientation of the hydroxyl group. The low-energy region occurs at $\psi_i = 145 \pm 40°$, with the minimum at $\psi_i = 170°$. These general features are similar to several conformational energy maps obtained at constant ϕ for poly(L-proline)[29-36] and poly(γ-hydroxy-L-proline)[37] containing planar trans peptide bonds. Another low-energy region, nearly 20 kcal/mol higher than the minimum at $\psi_i = 170°$, occurs near $\psi_i = -50°$.

Table I
Nonzero Statistical Weights for γ-Hydroxy-L-proline Residue *i* in Poly(γ-hydroxy-L-proline) at 30°

C_i^γ Position	$-\phi_i$[a]	C_{i+1}^γ Position	$-\phi_{i+1}$[a]	$\langle\psi_i\rangle$[a]	Statistical Wt
γ^1	70	γ^1	70	167	3.803
γ^1	70	γ^1	70	−30	0.452
$\gamma^1{=}\gamma^2$	60	γ^1	70	−34	0.024
$\gamma^1{=}\gamma^2$	60	γ^1	70	160	0.350
γ^1	50	γ^1	70	144	0.428
γ^2	70	γ^1	70	166	2.458
γ^2	50	γ^1	70	−35	0.061
γ^2	50	γ^1	70	144	0.322
γ^1	70	$\gamma^1{=}\gamma^2$	60	−45	0.582
γ^1	70	$\gamma^1{=}\gamma^2$	60	155	4.803
$\gamma^1{=}\gamma^2$	60	$\gamma^1{=}\gamma^2$	60	−47	0.025
$\gamma^1{=}\gamma^2$	60	$\gamma^1{=}\gamma^2$	60	150	0.508
γ^1	50	$\gamma^1{=}\gamma^2$	60	140	0.772
γ^2	70	$\gamma^1{=}\gamma^2$	60	154	3.045
γ^2	50	$\gamma^1{=}\gamma^2$	60	−49	0.052
γ^2	50	$\gamma^1{=}\gamma^2$	60	141	0.547
γ^1	70	γ^1	50	−59	0.255
γ^1	70	γ^1	50	143	3.377
$\gamma^1{=}\gamma^2$	60	γ^1	50	−62	0.013
$\gamma^1{=}\gamma^2$	60	γ^1	50	141	0.399
γ^1	50	γ^1	50	135	0.679
γ^2	70	γ^1	50	−62	0.020
γ^2	70	γ^1	50	143	2.281
γ^2	50	γ^1	50	138	0.490
γ^1	70	γ^2	70	−42	1.261
γ^1	70	γ^2	70	162	4.264
$\gamma^1{=}\gamma^2$	60	γ^2	70	−44	0.032
$\gamma^1{=}\gamma^2$	60	γ^2	70	153	0.412
γ^1	50	γ^2	70	136	0.604
γ^2	70	γ^2	70	159	2.584
γ^2	50	γ^2	70	−41	0.043
γ^2	50	γ^2	70	158	0.409
γ^1	70	γ^2	50	−50	0.112
γ^1	70	γ^2	50	153	3.486
$\gamma^1{=}\gamma^2$	60	γ^2	50	−48	0.002
$\gamma^1{=}\gamma^2$	60	γ^2	50	153	0.420
γ^1	50	γ^2	50	144	0.614
γ^2	70	γ^2	50	156	2.569
γ^2	50	γ^2	50	−55	0.068
γ^2	50	γ^2	50	146	0.425

[a] Degrees.

Inclusion of the electrostatic interaction of the atoms in the hydroxyl group does not alter the conformational energy map if $\chi_5 = 180$ or 300°. The hydroxyl proton of residue $i + 1$ participates in a weak electrostatic interaction with the carbonyl oxygen atom of residue $i - 1$ if ψ_i is near 110° and $\chi_5 = 60°$, resulting in a slight decrease in the conformational energy. Since the electrostatic energy is more negative when $\psi_i = 110°$ than when $\psi_i = 170°$, the difference in the conformational energy at these two points is sensitive to the choice of dielectric constant. The difference in energy is only slightly greater than 1 kcal/mol if the dielectric constant is two, $\chi_5 = 60°$, and the electrostatic effects due to the hydroxyl group are included.

The predicted characteristic ratios are shown in Table II. Deletion of the electrostatic interaction due to the hy-

Table II
Dimensional Properties at 30° Based on Rigid Pyrolidine Rings with the Geometry Observed in the Solid State

χ_5 (deg)	Dielectric Constant	Hydroxyl Electrostatic Interaction Included	Characteristic Ratio[a]	d ln $\langle r^2\rangle_0$/dT × 10^3 [a]
60	4	No	139	−6.5
180	4	No	138	−6.5
300	4	No	140	−6.5
60	4	Yes	121	−6.4
60	2	Yes	54	−4.1

[a] At a degree of polymerization of 1000.

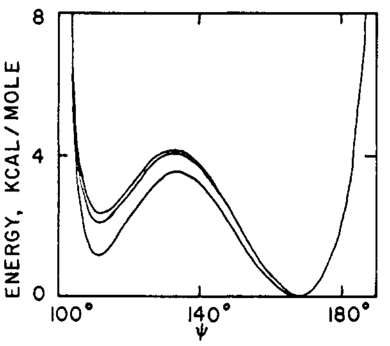

Figure 5. Conformational energy maps based on rigid pyrrolidine rings with the geometry observed in the solid state and with χ_5 = 60°. The energy scale for each curve is adjusted to yield a minimum of zero kcal/mol. Curves (a) to (c), with (a) being the curve of highest energy at 110° and (c) the lowest, are as follows: (a) dielectric constant = 4, hydroxyl electrostatic interactions deleted; (b) dielectric constant = 4, hydroxyl electrostatic interactions included; (c) dielectric constant = 2, hydroxyl electrostatic interactions included.

droxyl group, and the use of a dielectric constant of four, yields a characteristic ratio of about 140 at 30°. The characteristic ratio is diminished by interactions which decrease the difference in energy between the conformations with ψ_i = 110 and 170°. In all cases d ln $\langle r^2\rangle_0$/dT is strongly negative. The characteristic ratio of poly(γ-hydroxy-L-proline) at 30° would be higher, by a factor of at least 2.5, and perhaps by as much as an order of magnitude, than the characteristic ratio observed[14] for poly(L-proline) *if* poly(γ-hydroxy-L-proline) exists in solution with precisely the same pyrrolidine ring geometry which is observed in the solid state.[5]

Conformational Energy Maps Based on Flexible Pyrrolidine Rings. The conformational energy maps when the C_i^γ atom is in the γ^1 and γ^2 position are shown in Figures 3 and 4, respectively. The χ_5 are 60° in each case, corresponding to the lowest energy value found in the computations based on rigid pyrrolidine rings. The 1-kcal/mol contour in the minimum near ψ_i = 170° is always restricted to the vicinity of ϕ_i = −70°. A different result was obtained with the internal dimer of poly(L-proline),[15] in which the 1-kcal/mol contour included ϕ_i = −50° as well as ϕ_i = −70°. The preference for ϕ_i = −70° in poly(γ-hydroxy-L-proline) is due to slight atomic overlaps between the O^δ atom and hydrogen atoms attached to the C^β and C^δ atoms. Since these interactions depend upon the dihedral angles in the pyrrolidine ring, there is a greater tendency for the pyrrolidine ring in poly(γ-hydroxy-L-proline) to maintain a specific conformation than is the case of the pyrrolidine ring in poly(L-proline).

The locations and relative energies of the minima are shown in Table III. Slightly higher energies are obtained when the C_i^γ atom is in the γ^2 position. The energy of the minimum near ψ_i = −50° is always found to be closer to the energy at ψ_i = 170° than was the case in the calculations based upon rigid pyrrolidine rings. The fractional population of the minimum near ψ_i = −50°, based on the evaluation of the Boltzmann factors at 30°C and 10° intervals for ϕ_i and ψ_i, is shown in Table III. The result varies from 0.8 to 19.3% as a consequence of the differences in the relative energies of the two minima in the conformational energy maps.

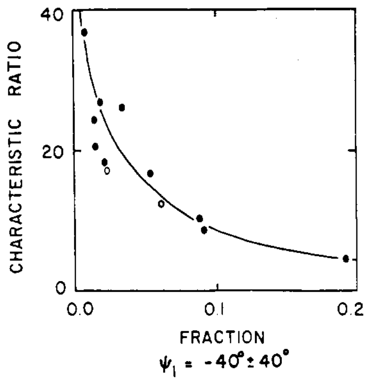

Figure 6. Characteristic ratio at 30° and a degree of polymerization of 1000 based on a flexible pyrrolidine ring at residue i with various fixed conformations at residue i + 1 for poly(γ-hydroxy-L-proline) (●) and poly(L-proline)[15] (○).

The characteristic ratios at 30° predicted by each of the conformational energy maps in Figures 3 and 4, evaluated neglecting the interdependence of events at residue i and i + 1, are shown in Table III. Figure 6 shows these characteristic ratios as a function of the population of the minimum near ψ_i = −50°. The relative population of the two minima is more important than the precise location of the minima in the determination of the dimensional properties of poly(γ-hydroxy-L-proline). The two open symbols in Figure 6 are from the analogous treatment of poly(L-proline),[15] and follow the same trend observed for poly(γ-hydroxy-L-proline).

Calcium chloride produces a pronounced reduction in the intrinsic viscosity of aqueous poly(L-proline)[7,14] and poly(γ-hydroxy-L-proline).[6] This process has been suggested to reflect an increased freedom of rotation about ψ[38-40] or cis–trans isomerization about the peptide bond.[14,41-43] Computations have shown that a substantial decrease in intrinsic viscosity would accompany the random isomerization of peptide bonds in poly(L-proline) from the trans to the cis conformation.[14] It is apparent from Figure 6 that a large reduction in the intrinsic viscosity of poly(L-proline) and poly(γ-hydroxy-L-proline) would also be expected if the population of the region near ψ_i = −50° increases in the presence of the salt.

The predicted temperature coefficients of the unperturbed dimensions are shown in the final column of Table III. They are negative in every case, and tend to be more negative for those conformational energy maps which yield a high characteristic ratio at 30°.

Conformational Properties Based on Interdependent Rotations at Neighboring Residues. It is apparent from Table III that the conformational properties computed from the conformational energy map for residue i depend upon the conformation selected for residue i + 1. A similar situation exists in polysarcosine and poly(N-methyl-L-alanine),[44] which also bear substituents on the nitrogen atom. An improved estimate of the conformational properties of poly(γ-hydroxy-L-proline), based on flexible pyrrolidine rings, should come from taking account of the interdependence of the events in adjacent residues.

Table III
Results from the Conformational Energy Maps Based on a Flexible Pyrrolidine Ring in Residue *i*

			Lowest Minimum			Second Minimum					
C_i^{γ}	C_{i+1}^{γ}	$-\phi_{i+1}{}^a$	$-\phi_i{}^a$	$\psi_i{}^a$	Energy[b]	$-\phi_i{}^a$	$-\psi_i{}^a$	Energy[b]	f[c]	CR[d]	T Coef[e]
γ^1	γ^1	70	70	180	0.1	70	30	0.6	0.091	8.7	−1.1
γ^1	γ^2	70	70	170	0.0	70	40	0.1	0.193	4.4	−1.0
γ^1	$\gamma^1{=}\gamma^2$	60	70	160–170	0.0	70	50	0.7	0.088	10.2	−1.7
γ^1	γ^1	50	70	150	0.1	70	60	0.9	0.054	16.8	−2.1
γ^1	γ^2	50	70	160	0.0	70	50	1.4	0.034	26.4	−3.7
γ^2	γ^1	70	70	180	0.3	50	40	2.1	0.022	18.5	−3.6
γ^2	γ^2	70	70	170	0.2	50	40	2.0	0.014	20.5	−3.1
γ^2	$\gamma^1{=}\gamma^2$	60	70	170	0.2	50	50	1.9	0.014	24.5	−3.5
γ^2	γ^1	50	70	160	0.4	70	60	2.5	0.008	36.7	−3.5
γ^2	γ^2	50	70	160	0.3	50	50	2.0	0.017	26.8	−4.2

[a] Degrees. [b] In kcal/mol relative to the lowest energy (10.0 kcal/mol) computed. [c] Fraction of residue i with $\psi_i = -40 \pm 40°$ at 30°. [d] Characteristic ratio at a degree of polymerization of 1000 and 30°. [e] $d \ln \langle r^2\rangle_0/dT \times 10^3$ at a degree of polymerization of 1000 and 30°.

Table IV
Conformational Properties of a Poly(γ-hydroxy-L-proline) Chain Containing Flexible Pyrrolidine Rings and Taking Account of the Interdependence of Events at Neighboring Residues[a]

	Fraction of Residues with Indicated Conformation						
Temp (°C)	$\phi = -70°$	$\phi = -60°$	$\phi = -50°$	γ^1 [b]	γ^2 [b]	$\psi = -40 \pm 40°$	$\psi = 150 \pm 70°$
5	0.865	0.049	0.085	0.574	0.377	0.083	0.917
30	0.837	0.057	0.105	0.561	0.381	0.088	0.912
70	0.804	0.075	0.120	0.543	0.382	0.084	0.916

[a] Computed for a degree of polymerization of 1000. [b] The total of the γ^1 and γ^2 position is less than unity because it does not include those residues for which $\phi = -60°$, where the γ^1 and γ^2 positions are identical.

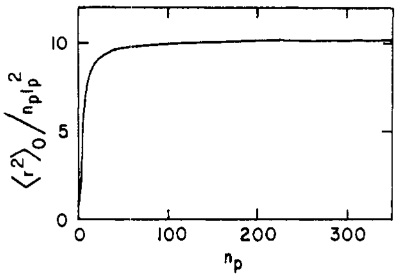

Figure 7. Characteristic ratio for poly(γ-hydroxy-L-proline) at 30° based on flexible pyrrolidine rings at residues i and $i + 1$.

Table IV presents the composition of a poly(γ-hydroxy-L-proline) chain with a degree of polymerization of 1000, evaluated as described in the section entitled **Calculational Procedure.** These results actually reflect the situation for residue 514 in a chain containing 1026 residues, but they can be equated to the average of all residues in a chain of 1000 residues because end effects are negligible. There is a marked preference for $\phi = -70°$, which is expected from the location of the 1-kcal/mol contour line in Figures 3 and 4. The γ^1 position is favored somewhat over the γ^2 position. The fraction of residues with $\psi = -40 \pm 40°$ is 0.088 at 30°, which is higher than the average (0.054) of the entries in column 10 of Table III due to the pronounced preference of ϕ for −70°. Four combinations with ϕ_i, $\phi_{i+1} = -70°, -70°$ are present in Table III, and the two of lowest energy contribute fractional populations of 0.091 and 0.193.

The characteristic ratio at 30°, based on flexible pyrrolidine rings and interdependent events at neighboring residues, is shown as a function of n_p in Figure 7. The computations were not extended beyond $n_p = 350$ due to the time required to form the product $G_1G^{n_p-1}$. The characteristic ratio of about 10 at 30° is consistent with the population of the region $\psi = -40 \pm 40°$, shown in Table IV, and the pattern presented in Figure 6. The temperature coefficient, $d \ln \langle r^2\rangle_0/dT$, is close to zero. An appreciable uncertainty must be attached to the computed characteristic ratio of ~10. The population at $\psi = -40 \pm 40°$, and hence the characteristic ratio, could be substantially altered by small changes in the conformational energies at $\psi = -40$ and 150° in a particular conformational energy map, or by similar small alterations in the relative energies at the minima in the various conformational energy maps. More states for residue i in poly(γ-hydroxy-L-proline) could be included in the computation, with a possible effect on the computed characteristic ratio, by using an interval smaller than 10° for ϕ, and also assigning more states to the minimum near $\psi = 170°$. These same considerations suggest that there is an appreciable uncertainty in the computed $d \ln \langle r^2\rangle_0/dT$.

A reasonable appraisal of the results in this section is that they predict a characteristic ratio for poly(γ-hydroxy-L-proline) at 30° which is close to the result obtained for poly(L-proline).[14] In contrast, the results predicted for poly(γ-hydroxy-L-proline) based on rigid pyrrolidine rings with the conformation observed in the solid state[5] are substantially higher than the characteristic ratio of poly(L-proline). An experimental characteristic ratio has not been obtained for poly(γ-hydroxy-L-proline) due to the unavailability of samples of poly(γ-hydroxy-L-proline) of high molecular weight. It may be significant, however, to point out that samples of poly(L-proline) with $M_w = 9200 \pm 500$ and poly(γ-hydroxy-L-proline) with $M_w = 9000$ have nearly identical intrinsic viscosities in water at 30°.[6] This observation would be consistent with similar unperturbed dimensions for the two polypeptides. A definite conclusion, however, must await the experimental determination of the characteristic ratio of poly(γ-hydroxy-L-proline).

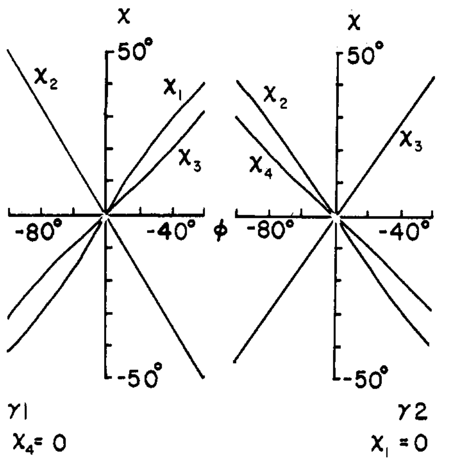

Figure 8. χ_1, χ_2, χ_3, and χ_4 as a function of ϕ for the γ^1 and γ^2 positions.

The values of χ_1, χ_2, χ_3, and χ_4 are shown as a function of ϕ for the γ^1 and γ^2 positions in Figure 8. The data in Table IV shows that the great majority of the pyrrolidine rings in poly(γ-hydroxy-L-proline) would have $\phi = -70°$ and either the γ^1 or γ^2 position for the C^γ atom. An average of the results for these conformations yields negative values for χ_1 and χ_3 and positive values for χ_2 and χ_4. There is agreement as to sign, but not magnitude, with the results deduced for these angles from the ring vicinal couplings in the 220-MHz nmr spectrum of poly(γ-hydroxy-L-proline).[9]

Configurational Entropy per Residue. The configurational entropy per residue at 30° was evaluated for each of the ten conformational energy maps in Figures 3 and 4 and also for the two analogous conformational energy maps for poly(L-proline)[15] using 10° intervals for ϕ_i and ψ_i. The results are shown in Table V along with the results obtained by Brant *et al.*[45] for polyglycine and poly(L-alanine) using the same intervals for ϕ_i and ψ_i. The configurational entropy per residue computed by this procedure includes an additive constant, the size of which depends upon the interval chosen for the summation over ϕ_i and ψ_i.[25] Therefore the only point of interest in Table V is the difference between the configurational entropy per residue for the various polypeptides.

The configurational entropies per residue in Table V are ~3-5 cal/(mol deg) larger for polyglycine and poly(L-alanine) than for poly(L-proline) and poly(γ-hydroxy-L-proline). The low configurational entropy of the L-proline and γ-hydroxy-L-proline residues is recognized as an important factor in the stabilization of the ordered structure of collagen.[46-52] Harrington and Rao[51] used entropy changes upon melting of zero for the L-proline and γ-hydroxy-L-proline residues and 4.1 cal/(mol deg) for the other amino acid residues in their analysis of the melting temperature of various collagens. The difference in configurational entropy per residue of 4.1 cal/(mol deg) is within the range of ~3-5 cal/(mol deg) suggested by the results in Table V.

The configurational entropy per residue is found to be slightly lower for poly(γ-hydroxy-L-proline) than for poly(L-proline) because of the restriction of the 1-kcal/mol contour line to the vicinity of $\phi_i = -70°$ in the former case, while for poly(L-proline) it extends from $\phi_i = -70$ to $-50°$. A reduction in configurational entropy for the statistical coil when γ-hydroxy-L-proline is substituted for L-proline would increase the stability of the ordered structures formed by that polypeptide. This effect may account in part for the recent observations that the thermal transition of monodisperse poly(L-prolyl-γ-hydroxy-L-prolylglycine) is about 35° higher than that of monodisperse poly(L-propyl-L-prolylglycine),[13] and that the thermal transition of an unhydroxylated enzyme-modified collagen precursor is about 15° lower than that of the collagen itself.[53,54]

Table V
Configurational Entropy per Residue Based on 10° Intervals for ϕ_i and ψ_i

Polypeptide	Config Entropy (cal/(mol deg))
Polyglycine[a]	11.5
Poly(L-alanine)[a]	10.0
Poly(L-proline)[b,c]	6.6-6.7
Poly(γ-hydroxy-L-proline)[b]	5.8-6.3

[a] Ref 45. The electrostatic interaction of the peptide units was evaluated in the monopole approximation. [b] Range of values obtained from the conformational energy maps based on a flexible pyrrolidine ring for residue i and a fixed pyrrolidine ring geometry for residue $i + 1$. [c] Evaluated from the conformational energy maps reported in ref 15.

Acknowledgment. We thank Dr. Dennis Torchia for making his results available to us prior to publication.

References and Notes

(1) (a) This work was supported in part by Grant GB-36055 from the National Science Foundation to W. L. M. (b) Address correspondence to this author at Louisiana State University.

(2) The convention used is described by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, 9, 3471 (1970), and *J. Mol. Biol.*, 52, 1 (1970). Torsion angles about the side chain bonds are χ_1, χ_2, χ_3, and χ_4 for the pyrrolidine ring, starting at the C^α-C^β bond, and χ_5 for the C^γ-O bond.

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Application of the Lanthanide-Induced Shifts to the Nuclear Magnetic Resonance Determination of the Helical Conformation of Poly(β-hydroxybutyrate) in Solution

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Received November 1, 1973

ABSTRACT: The method of shift reagents is applied quantitatively to the determination of the internal rotation angles in the helical structure of poly(β-hydroxybutyrate) in solution and results in fair agreement with those deduced from conformational energy and X-ray structure factors calculations.

The minimum energy crystalline conformation of a stereoregular polymer is helical. X-Ray fiber diagrams or electron diffraction patterns of ultra thin (100–200 Å) and often imperfect crystals give very easily the number of residues per turn and the pitch of the molecular helix.[1] They are however not rich enough for a direct determination of the atomic positions. When the repeat unit has several internal rotation angles, there is a large number of conformations compatible with the two helical parameters. Generally a few conformations specified by a set of internal angles are selected on the basis of steric hindrance (space-filling model building) or more refined conformational energy calculations and the intensities of the diffraction patterns are recalculated with the additional parameters coming from the relative positions of the helices in the lattice. Such a process can generally be refined to values of the reliability factor of the order of 0.15. Such a work has recently been performed on poly(β-hydroxybutyrate) (PHB) a natural polyester forming a 2_1 helix with a pitch of 5.96 Å (Figure 1).[2-4]

Since it has been recently shown[5,6] that PHB in solution in $CDCl_3$ and TFE retains essentially its local helical structure, but with enough flexibility to give a well-resolved high-resolution nmr spectrum[7] (Figure 2), we have attempted a more direct and eventually more precise determination of the four internal angles defining the local conformation. Our method rests on the relative pseudocontact shifts experienced by the four types of protons upon binding of a lanthanide chelate to the carbonyl oxygen. This method of conformational analysis has recently been applied quantitatively to small molecules or protein with a single chelating site[8] but has never been applied quantitatively to stereoregular homopolymers where, on one hand, the rapid jump of the lanthanide between equivalent sites must be considered (precluding the simultaneous use of relative broadening) but where, on the other hand, the helical condition reduces considerably the number of conformations.

Nmr Spectrum of PHB and the Determination of Possible Values of the Angle ψ. A first piece of structural information can be derived from the coupling constants J_{AC} and J_{BC} obtained from analysis of the nmr spectrum.

The results are given in Table I where the index *A* has arbitrarily been given to the one of the nonequivalent CH_2 protons with the higher chemical shift and higher coupling constant. Recalculated intensities of the CH_2 signal are shown in Figures 3 and 4. Despite the uncertainties in the use of Karplus relations,[9] one can use the difference between J_{AC} and J_{BC} to derive from the dihedral angle an interval of possible ψ values. Owing to the arbitrariness in the choice of H_A there are two such intervals $0 < \psi < 20°$ and $120° < \psi < 140°$ (Figure 5). It must be remarked that these values are incompatible with the model proposed by Okamura[2] ($\psi = 218°$) and this disagreement has given some impetus to the reexamination of the data.[3]

Principle of Lanthanide-Induced Pseudo-Contact Shift and Its Application to Polymers. It is now well established[10,11] that the shift Δδ experienced by a proton close to lanthanide complexed to a lone pair bearing atom is almost entirely due to pseudo contact and therefore proportional to the product of a magnetic factor *M* related to the anisotropy of the magnetic susceptibility by a geometrical factor

$$F_g = 3\cos^2\theta_i - 1/r_i^3$$

where θ_i and r_i specify the position of the proton relative to the ion (Figure 6). The exact origin of the magnetic factor *M* and the conditions for a proper application of the method for conformational analysis have been recently stressed.[10,11] The actual shift observed at a given lanthanide molar ratio depends upon the equilibrium be-