The Conformational Characteristics in Solution of the Cyclic Hexapeptide ┌Gly-Gly-D-Ala-D-Ala-Gly-Gly┐

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Abstract: Elucidation of the conformation of the synthetic cyclic hexapeptide ┌Gly-Gly-D-Ala-D-Ala-Gly-Gly┐ in solution is described. Nuclear magnetic resonance (nmr) measurements are coupled with an approximate theoretical treatment to determine the conformational characteristics of this cyclic hexapeptide. All of the cyclic conformations generated in the theoretical portion of this study, each of which is considerably different from the recently deduced crystalline conformation, are consistent with the nmr data and may be distinguished from each other on the basis of whether or not they possess a single intramolecular hydrogen bond. Since all of the cyclic conformations generated have nearly the same intramolecular conformational energy, and because there are no severe steric barriers involved in their interconversion, it is concluded that conformations with and without a single intramolecular hydrogen bond are in rapid equilibrium. Thus, the cyclic hexapeptide ┌4Gly-2D-Ala┐ appears to possess considerable conformational freedom, or flexibility, in solution.

Unlike the large proteins, whose native conformations (crystalline or solution) are determined primarily by space-filling and packing requirements,[1] the crystalline and solution conformations of small polypeptides cannot, in general, be expected to be the same. Since several of the hormones, toxins, kinins, and antibiotics are small polypeptides[2,3] (linear and cyclic), it is important to determine their solution conformations in order to begin to establish the relationship between their molecular structure and their biological function. Even though ┌4Gly-2D-Ala┐ has no known biological activity, elucidation of its solution conformation[4] serves to illustrate the possible nonequivalence of the crystalline and solution conformations of small polypeptides, since its crystalline conformation has recently been determined.[5]

The crystalline conformation[5] of ┌4Gly-2D-Ala┐ is presented in Table I in terms of the residue backbone rotation angles[6] ω, φ, and ψ illustrated in Figure 1. The residue numbering scheme is defined in Figure 2. Since each residue in the crystalline conformation has trans peptide bonds, the intramolecular conformational energy of this crystalline structure can be estimated by summing the independent[8] residue energies[9] and adding the stabilizing effect of the two weak intramolecular hydrogen bonds[10] between residues Gly_1 and Gly_4. When this procedure is followed, the total intramolecular conformational energy is estimated to be *ca.* 22 kcal/mol of hexapeptide relative to the minimum energy, nonhydrogen bonded, acyclic conformation.[9]

Table I. Crystalline Conformation[a] of ┌—4-Gly-2D-Ala—┐

Residue	φ, deg	ψ, deg	ω, deg
Gly_1	74.7	11.6	−3.7
Gly_2	109.9	164.9	−8.3
Gly_3	73.5	196.2	2.3
Gly_4	281.1	354.0	1.9
D-Ala_1	245.7	195.2	2.1
D-Ala_2	310.9	148.9	4.1

[a] Reference 5.

This high intramolecular conformational energy is more than compensated for in the crystal[5] by the fact that of the 12 possible hydrogen bonding groups (C=O and N–H) in each hexapeptide, nine are hydrogen bonded to the three H_2O molecules of crystallization (two of the hydrogen bonds to H_2O are of the type NH---OH-H---O=C), one N-H---O=C intermolecular hydrgoen bond is found, and two Gly_1–Gly_4 intramolecular hydrogen bonds are present. However, when this cyclic hexapeptide is dissolved in a hydrogen bonding solvent, many conformations different from the crystalline structure and having a much lower intramolecular conformational energy can be envisaged which would still permit retention of extensive intermolecular hydrogen bonding with the solvent.[11]

On the other hand, in a nonhydrogen bonding solvent, where stabilization originating from intermolecular hydrogen bonds is not possible, the high intramolecular energy of the crystalline conformation precludes its

(1) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience, New York, N. Y., 1969, pp 296–304.
(2) M. O. Dayhoff, Ed., "Atlas of Protein Sequence and Structure," Vol. 4, National Biomedical Research Foundation, Silver Springs, Md., 1969.
(3) W. S. Spector, Ed., "Handbook of Toxicology," Vol. II, W. B. Sanders, Philadelphia, Pa., 1957.
(4) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *Biopolymers*, 4, 121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).
(5) I. L. Karle, J. W. Gibson, and J. Karle, *J. Amer. Chem. Soc.*, **92**, 3755 (1970).
(6) Each of the angles, ω, φ, and ψ is taken[4] as 0° in the all-trans or planar zigzag conformation (see Figure 1) and adopts positive values for right-handed rotations. A more recently proposed convention,[7] which assigns $\omega = \varphi = \psi = 180°$ to the all-trans conformation, is not adopted here to avoid confusion.
(7) J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 6489 (1970); *J. Mol. Biol.*, **52**, 1 (1970).
(8) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).
(9) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).
(10) D. A. Brant, *Macromolecules*, **1**, 297 (1968).
(11) This contention is further supported by the fact that in those solvents, which unlike H_2O may not act simultaneously as both hydrogen bond acceptors and donors, fewer intermolecular hydrogen bonds to the cyclic hexapeptide are possible. Furthermore, since most solvent molecules, including H_2O, are much smaller than the cyclic hexapeptide, formation of intermolecular hydrogen bonds is much less dependent upon the cyclic hexapeptide conformation in solution than in the crystal.

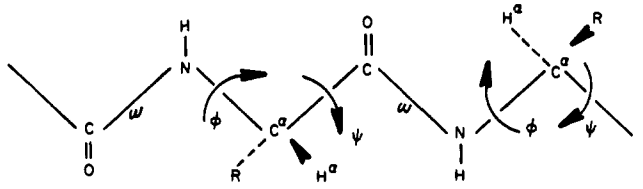

Figure 1. A schematic representation of a portion of a poly-L-peptide in the planar zigzag or all-trans conformation.

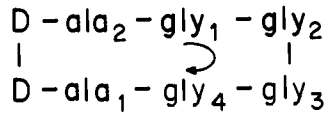

Figure 2. A schematic representation of [4Gly-2D-Ala] where the sense of the arrow indicates movement from N to C^{α} in each residue.

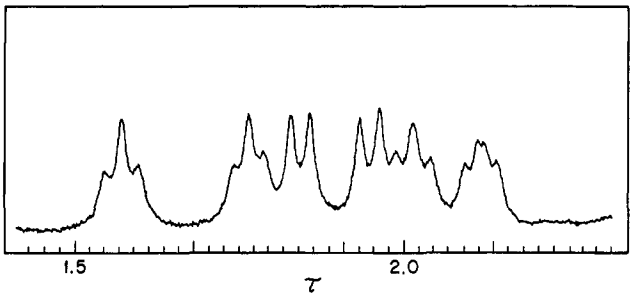

Figure 3. The NH region of the 220-MHz spectrum of [4Gly-2D-Ala] in DMSO-d_6 at 25°.

existence in solution in this type of solvent. Hence, one can say with some confidence and prior to conducting measurements in solution, that the crystalline and solution conformations of ⌐4Gly-2D-Ala¬ are most probably quiet different.

The data from a solution nmr study are coupled with approximate conformational energy calculations[9,10] in the determination of the conformational characteristics of ⌐4-Gly-2D-Ala¬ in solution. More specifically, the measured amide to α-proton coupling constants in each residue are related to the rotation angle φ about the N–C^{α} bond in each residue according to a "Karplus-like relation."[12,13] Low-energy cyclic conformations, whose individual residue conformations correspond to the measured couplings according to the "Karplus-like relation," are then searched for. The total intramolecular energy of each cyclic conformation generated is estimated by summing the independent residue energies obtained from previously calculated conformational energy maps,[9] and subtracting from this sum any stabilization due to the presence of intramolecular hydrogen bonds.[10]

Experimental Section

Nmr Study. The compound under study ⌐4Gly-2D-Ala¬ was synthesized and made available to us by Professor Yu. A. Ovchinnikov.[14,15] Spectra were recorded on a Varian Associates HR-220 nmr spectrometer. The sample was dissolved in Diaprep dimethyl-d_6 sulfoxide (100% D), dried over molecular sieves, to a concentration of 5 wt %. Tetramethylsilane was used as the internal reference. All spectra were recorded at 25° except the temperature dependence of the peptide NH protons whose chemical shifts were observed between 18 and 54°. Exchange of the amide protons with D_2O was followed as a function of time in DMSO-d_6 solution. D_2O was added in a 10:1 mole ratio of D to NH.

The peptide NH proton region of the nmr spectrum of ⌐4Gly-2D-Ala¬ in DMSO-d_6 is presented in Figure 3. The amide to α-proton coupling constants $J_{N\alpha}$ in both D-Ala residues, whose NH resonances are doublets at τ 1.94 and 1.83 (see Figure 3), are seen to be 6.5 Hz. Of the four glycine NH resonances, three exhibit a sum of amide to α-proton couplings (D and L α protons) in the range 10–12 Hz, while the fourth at τ 2.11 is sufficiently resolved to yield $J_{N\alpha}$ = 4.5 and 6.5 Hz.

The temperature dependence of the amide proton chemical shifts is presented in Figure 4. It is observed that the glycine NH resonance at τ 2.11 at 25° (see Figure 3) is shifted upfield with increasing temperature to a lesser degree than the five remaining amide protons. All six of the amide protons, however, exchange rapidly with D_2O, with each of the NH resonances reduced to half of their original areas within 4 min.

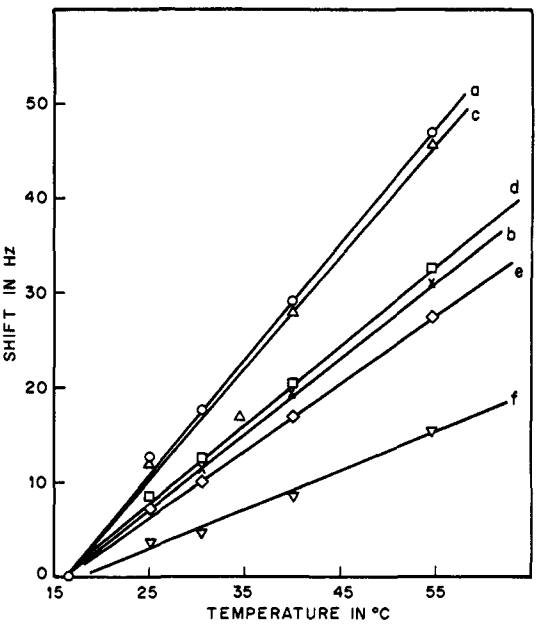

Figure 4. Temperature dependence of NH proton resonances; labelled a–f from lowest to highest field, respectively, as they appear in Figure 3.

Calculations

For polypeptides whose residues are separated by rigid and planar trans amide or imide bonds, the potential energy of rotation φ and ψ about the N–C^{α} and C^{α}–C backbone bonds in a given residue (see Figure 1) is independent[8,9] of the corresponding rotations in neighboring residues. Semiempirical potential functions,[8,9] which account for the threefold intrinsic torsional potentials about the N–C^{α} and C^{α}–C bonds, the nonbonded steric repulsions and London dispersion energies (6–12 potential), and the nonbonded electrostatic interactions (monopole–monople), have been used to estimate the conformational energies of various α-amino acid residues. These approximate intramolecular potential energy calculations[8–10,16–21]

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have been successful in describing the conformational characteristics of polypeptides in solution and in some cases in the crystal.

It has been shown[22,23] that averaging a "Karplus-like relation" connecting the vicinal nmr coupling $J_{N\alpha}$ and the dihedral angle φ' between N–H and C^{α}–H^{α} in a peptide residue ($\varphi' = |240° - \varphi|$) over all conformations found to be favorable by the approximate potential energy calculations[9] leads to the correct coupling constants observed for random coil polypeptides[22] and dipeptides[23] in solution. This agreement justifies extension of a "Karplus-like relation"[12,13] to the vicinal coupling between amide and α-protons in peptides and lends further support to the approximate conformational energy calculations. A combination of these two approximate theoretical tools, which has proved successful in the conformational analysis of other polypeptides (cyclolinopeptide A,[24] antamamide,[25] phalloidin,[26] α-amanitin,[27] cyclic (Pro-Ser-Gly)$_2$,[28] and cyclic (Pro-Gly-Ser)$_2$[28]), is used in conjunction with the information obtained from the nmr solution study to determine the conformational characteristics of ⌐4Gly-2D-Ala¬.

Cyclic conformations whose individual residue conformations are restricted by allowing only those values of φ and ψ which reproduce the measured vicinal couplings according to a "Karplus-like relation"

$$J_{N\alpha} = \begin{cases} 8.5 \cos^2 \varphi' & (0° \leq \varphi' \leq 90°) \\ 9.5 \cos^2 \varphi' & (90° \leq \varphi' \leq 180°) \end{cases} \quad (1)^{12}$$

or

$$J_{N\alpha} = 8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi' \quad (2)^{13}$$

and which correspond to energetically favorable residue conformations[9] are generated. All peptide bonds are assumed to be trans, and the usual[8] bond lengths and valence angles are adopted. Since both D-Ala residues have a measured $J_{N\alpha} = 6.5$ Hz, $\varphi_{D\text{-Ala}} = 260$ and 340° and $\psi_{D\text{-Ala}} = 0$, 120, 240, 270, 300, and 330° are selected for use in the generation of cyclic conformations (see eq 1 and 2). The measured sum of couplings for the L and D α-protons in each of the four glycine residues is found to be in the range of 10.0–12.0 Hz. Consequently, the following conformations are adopted for each glycine residue: $(\varphi, \psi)_{Gly}$ = 45, 240; 65, 240; 115, 90; 115, 120; 245, 0; 245, 30; 245, 60; 245, 240; 245, 270; 295, 120; and 315, 120°. These are the lowest energy conformations that are consistent with the measured vicinal couplings (see the conformational energy maps for the alanine and glycine residues in ref 9).

For each conformation, *i.e.*, for each of the sets of six pairs of rotation angles φ and ψ, the distance between the α-carbon atoms in Gly_3 and Gly_2 in the corresponding linear hexapeptide (Gly_3-Gly_4-D-Ala_2-Gly_1-Gly_2) terminated by these residues is calculated using the transformation of virtual bond vectors method.[8] If the calculated distance between $C_{Gly_2}^{\alpha}$ and $C_{Gly_3}^{\alpha}$ is between 3.7 and 3.9 Å, then the conformation under consideration is assumed to be cylic.[29] The intramolecular energy of each cyclic conformation generated is estimated by summing the independent residue energies obtained from their random coil energy maps,[9] which do not include energetic contributions made by intramolecular hydrogen bonds. A space-filling molecular model is constructed for each cyclic conformation generated in a search for the presence of intramolecular hydrogen bonds and steric overlaps longer in range than those considered in the conformational energy calculations. Intramolecular hydrogen bonding stabilization is evaluated following the method of Brant.[10]

Calculated Results and Discussion

Twenty-five low-energy cyclic conformations were generated in the manner described above for ⌐4-Gly-2D-Ala¬ and are listed in Table II. Each of these conformations is, according to eq 1 and 2, consistent with the observed amide to α-proton couplings, and each has nearly the same intramolecular energy. None of these

Table II. All-Trans Peptide Bond Cyclic Conformations Generated for ⌐4-Gly-2D-Ala¬

φ, ψ, deg						
Gly_1	Gly_2	Gly_3	Gly_4	D-Ala_1	D-Ala_2	E_{conf}[a]
315, 120	295, 120	245, 270	245, 240	260, 300	340, 300	8.5
115, 120	115, 120	245, 60	45, 240	260, 270	340, 300	6.9
245, 240	245, 240	295, 120	45, 240	340, 200	260, 300	7.5
115, 120	115, 120	115, 120	45, 240	340, 300	340, 330	6.5
115, 120	115, 120	115, 120	45, 240	340, 330	340, 330	6.4
245, 240	315, 120	115, 120	65, 240	340, 220	260, 330	7.3
245, 240	245, 240	315, 120	65, 240	340, 220	260, 330	7.3
245, 240	315, 120	115, 120	65, 240	340, 300	260, 300	7.5
245, 240	245, 240	315, 120	65, 240	340, 300	260, 300	7.5
245, 60	45, 240	295, 120	65, 240	340, 300	260, 330	8.5
245, 60	115, 120	315, 120	65, 240	340, 300	200, 330	7.5
245, 0	45, 240	65, 240	245, 240	260, 220	260, 270	8.2
245, 0	65, 240	45, 240	245, 240	260, 220	260, 270	8.2
245, 60	45, 240	65, 240	245, 240	260, 300	260, 300	7.2[b]
245, 60	65, 240	45, 240	245, 240	260, 300	260, 300	7.4[b]
245, 30	45, 240	65, 240	245, 240	260, 330	260, 270	6.8[b]
245, 30	65, 240	45, 240	245, 240	260, 330	260, 270	6.8[b]
245, 0	45, 240	65, 240	245, 240	260, 330	260, 330	7.3[b]
245, 0	65, 240	45, 240	245, 240	260, 330	260, 330	7.2[b]
245, 60	45, 240	65, 240	245, 240	260, 330	340, 270	7.1[b]
245, 60	65, 240	45, 240	245, 240	260, 330	340, 270	7.3[b]
115, 120	45, 240	65, 240	245, 240	340, 120	260, 300	8.4
115, 120	65, 240	45, 240	245, 240	340, 120	260, 300	8.4
245, 240	315, 120	65, 240	245, 240	340, 220	260, 300	7.8
245, 240	295, 120	45, 240	245, 240	340, 220	260, 300	7.8

[a] In kcal/mole of hexapeptide relative to the lowest energy, non-hydrogen-bonded, acyclic conformation. [b] Possesses a $(N\text{–}H)_{Gly_3}$ ---$(O{=}C)_{D\text{-}Ala_2}$ intramolecular hydrogen bond.

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(29) In polypeptides with the usual bond lengths and valence angles, the distance between the α-carbon atoms in adjacent residues is invariant to conformation and equals 3.8 Å when the peptide bonds are trans.[18]

conformations even remotely resembles the crystal structure[5] (Table I) as was predicted.[30] The 25 cyclic conformations generated here can be divided into those that possess a $(N-H)_{Gly_3}$ to $(O=C)_{D-Ala_2}$ intramolecular hydrogen bond and those that do not.

Each of the intramolecular $(N-H)_{Gly_3}\cdots(O=C)_{D-Ala_2}$ hydrogen bonds in the eight hydrogen-bonded, cyclic conformations generated is relatively weak (*ca.* −1.0 kcal/mol). By slightly varying the conformations of the four glycine residues from those listed in Table II for the eight hydrogen-bonded conformations, it is possible to improve the hydrogen bond to the extent that its energy is −3.0 kcal/mol. However, at the same time the sum of the conformational energies of the four glycine residues is increased by *ca.* 2.0 kcal/mol, resulting in total conformational energies which are very close to the energies presented in Table II for the hydrogen-bonded conformations. Hence, it is possible to strengthen the $(N-H)_{Gly_3}\cdots(C=O)_{D-Ala_2}$ hydrogen bond, but without simultaneously reducing the total intramolecular energy of the hydrogen-bonded conformation in question.

The temperature study of the amide proton chemical shifts indicated[31-34] that one of the glycine N–H's may be hydrogen bonded. The glycine residue with the smallest temperature coefficient of the amide proton chemical shift has observed amide to α-proton couplings of 4.5 and 6.5 Hz. None of the hydrogen-bonded, cyclic conformations generated here has a φ_{Gly_3} which corresponds to these couplings according to eq 1 and 2. However, if $J_{N\alpha}(L)_{Gly_3}$ and $J_{N\alpha}(D)_{Gly_3}$ are averaged[22,23] over all 25 cyclic conformations generated, then $\langle J_{N\alpha}(L)_{Gly_3}\rangle_{calcd}$ = 6.3 Hz and $\langle J_{N\alpha}(D)_{Gly_3}\rangle$ = 5.0 Hz in excellent agreement with the measured values.

This implies that both hydrogen-bonded and non-hydrogen-bonded conformations are in rapid dynamic equilibrium. Inspection of molecular space-filling models indicates the absence of any substantial steric barriers to the interconversion of hydrogen-bonded to non-hydrogen-bonded conformations. The nmr deuterium exchange studies can also be rationalized on the basis of this proposed dynamic equilibrium. All protons, including the glycine N–H whose chemical shift is nearly independent of temperature and appears to be intramolecularly hydrogen bonded,[31-34] should be able to exchange rapidly with the solvent in the non-hydrogen-bonded conformations generated here and listed in Table II. Only $(N-H)_{Gly_3}$ should be prohibited from exchange with solvent in the hydrogen-bonded conformations generated. However, since the cyclic hexapeptide is rapidly interconverting between conformations where $(N-H)_{Gly_3}$ can and cannot exchange with the solvent, $(N-H)_{Gly_3}$ should also exchange rapidly.

Thus, it appears that ⌐4Gly-2D-Ala¬ is a flexible molecule in solution rapidly interconverting between conformations with and without a $(N-H)_{Gly_3}$ to $(O=C)_{D-Ala_2}$ intramolecular hydrogen bond. Furthermore, none of the solution conformations in equilibrium even grossly approximates the crystalline structure observed[5] for this cyclic hexapeptide.

(30) Substitution of the values of the dihedral angles between the amide and α protons in the crystalline conformation (see Table I) into eq 1 or 2 leads to $J_{N\alpha}$(D-Ala$_1$) = 4.0 Hz and $J_{N\alpha}$(D-Ala$_2$) = 9.0 Hz. Hence it is clear that the crystalline conformation is not retained in solution, because $J_{N\alpha}$ = 6.5 Hz in DMSO-d_6 for both D-Ala residues (see Figure 3).

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