α-CD·H_2O while the higher homologues β-CD and γ-CD both occur, as "empty" hydrate complexes, in the "round", relaxed form with included water molecules displaying extensive statistical disorder.[15,16] As pointed out recently,[17] the main reason for these structural differences might reside in the average O(2)···O(3) distances which in α-CD are approximately 3.0 Å but are shortened to about 2.83 Å in both β-CD and γ-CD. These distances allow only relatively weak hydrogen bonds in α-CD but strong hydrogen bonds in β-CD and δ-CD and therefore contribute significantly to the stabilization of the "round" structure of the macrocycle in β-CD and γ-CD.

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Supplementary Material Available: Tables of bond lengths (Table IIIS), bond angles (Table IVS), torsion angles (Table VIIS), and observed and calculated structure factor amplitudes (Table XS) (27 pages). Ordering information is given on any current masthead page.

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Crystal Structure and Solution Studies of the Molecular Conformation of the Cyclic Hexapeptide *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)

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Abstract: The crystal structure of *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)·$3H_2O$ has been determined by single-crystal X-ray diffraction and refined by block-diagonal least squares to an *R* value of 0.053. The crystals are monoclinic, $P2_1$, with cell constants of a = 13.163 (1) Å, b = 15.632 (1) Å, c = 7.469 (1) Å, and β = 106.83 (1)°. The molecular conformation in the crystal includes a type I β turn at L-Ala-L-Tyr and a type II′ β turn at Gly-L-His, with 3.10- and 3.06-Å (4→1) hydrogen bonds, respectively. The conformation is very similar to that reported for crystalline *cyclo*-(Gly-L-Ala-Gly-L-Ala-L-Ala-Gly). The results of proton NMR studies of the peptide in solution suggest that the crystal conformation, if present in solution, is in rapid exchange with other conformations. β-turn conformations found in crystalline cyclic hexapeptides are reviewed.

Introduction

In 1958 Schwyzer proposed that cyclic hexapeptides adopt as a stable backbone conformation in antiparallel β structure, in which two chain reversals stabilized by ten-membered hydrogen-bonded rings (β turns) are connected by two extended residues.[2] Since then, evidence has been accumulating that two β-turn structures, where possible, are very much favored by cyclic hexapeptides in the crystal and in solution, although the transannular hydrogen bonds are not consistently present. Because this regularly appearing framework provides the possibility of generating well-defined structures that may be useful in the design of physiologically active peptides, it is of interest to establish what rules operate to position a sequence of six amino acid residues on that backbone. To that end it is still necessary to examine crystal and solution structures of additional cyclic hexapeptides.

We report here the crystal structure of *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly) (*c*-GHGAYG), which provides another example of the two-β-turn backbone. Because the sample from which the crystals were grown was mislabeled as *cyclo*-(Gly-L-His-Gly-L-Tyr-L-Ala-Gly) (*c*-GHGYAG), we reinvestigated[3] the nuclear magnetic resonance spectra of the substance as well. We discuss the likely solution conformations of *c*-GHGAYG and the rules so far evident for placing the hexapeptide sequence within the Schwyzer backbone.

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(2) (a) Schwyzer, R. *Rec. Chem Prog.* **1959**, *20*, 147. (b) Schwyzer, R.; Sieber, P. *Helv. Chim. Acta* **1958**, *41*, 2186–9.
(3) Kopple, K. D.; Go, Anita; Logan, R. H., Jr.; Savrda, J. *J. Am. Chem. Soc.* **1972**, *94*, 973–81.

Experimental Section

cyclo-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)·HBr from a bottle labeled-*cyclo*-(Gly-L-His-Gly-L-Tyr-L-Ala-Gly) was dissolved in methanol and the solvent was allowed to evaporate slowly. Thick, platelike crystals were obtained. The crystal used for data collection measured 0.3 × 0.2 × 0.2 mm. Examination of the reciprocal lattice showed systematic extinctions characteristic of space group $P2_1$. ($P2_1/m$ is not possible.) Cell constants, obtained by least-squares refinement of ±2θ values of 20 reflections using Cu Kα radiation, are a = 13.163 (1) Å, b = 15.632 (1) Å, c = 7.469 (1) Å, and β = 106.83 (1)°. The measured density of the crystal, by flotation in chloroform–cyclohexane, was 1.35 g/mL, and the calculated density is 1.374, assuming one peptide molecule and three water molecules in the asymmetric unit.

Three-dimensional intensity data were collected using nickel-filtered Cu Kα radiation to a 2θ maximum of 125°. A θ–2θ scan rate of 2° min^{-1}, with a variable scan width and 10-s background measurements at both extremities of the scan, was used to measure 2442 independent reflections. Throughout the data collection three standard reflections were remeasured every 50 reflections to monitor the decay and alignment of the crystal. A regular, periodic fluctuation of about 20%, arising probably from an instability of the X-ray source, was observed. The observed reflection intensities were therefore corrected as a function of time according to the fluctuation observed in standard reflections. The structure amplitudes and their estimated errors were calculated from the expressions $F_o = (QI_n)^{0.5}$ and $\sigma^2(F_o) = (Q/4I_n)[I_s + (t_s/t_b)^2 I_b + (0.02I_n)^2]$, where Q contains corrections for Lorentz polarization, ab-

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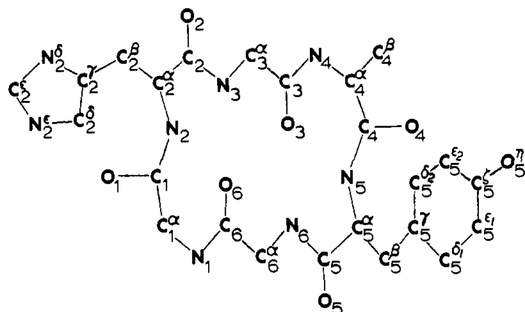

Figure 1. Atom labeling in *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly).

sorption, attenuation, and the systematic fluctuations, t_s and t_b are the scan and background times, and I_s, I_b, and I_n are the scan, background, and net intensities, repectively. The maximum difference in a ϕ scan at $\chi = 90°$ was 8% (linear $\mu = 9.1$ cm^{-1}). A total of 2368 reflections with $|F_o| > 3\sigma(F_o)$, representing 96% of the reflections collected, were considered observed and were used in structure determination and refinement.

The structure was solved by the direct methods program MULTAN 74.[4] A total of 300 reflections with $|E| > 1.47$ were used to generate 64 phase sets for tangent refinement. Two phase sets had the same lowest residue and ψ_0 and the largest combined figure of merit, although the absolute figure of merit ranked only 53rd and 54th. Electron density maps generated from these phase sets differed only in an origin shift along the y axis. Twenty-six peaks compatible with the expected geometry were used to calculate a new set of phases and to generate a new electron density map that contained all 39 nonhydrogen atoms in the peptide, plus three peaks corresponding to oxygen atoms of water molecules. At this point, the sequence of the peptide was found to be as is now reported.

The coordinates of the peptide and water molecules were refined by block-diagonal least squares, minimizing $\sum w(\Delta F)^2$, where $w = 1/\sigma^2$. Final refinement involved anisotropic temperature factors for 39 nonhydrogen atoms in the peptide and the three oxygen atoms in water molecules and isotropic temperature factors for 33 hydrogen atoms, located from difference electron density maps, in peptide and water molecules.

The final R value ($\sum|\Delta F|/\sum|F_o|$) is 0.053 and a final difference electron density map shows no peaks greater than 0.5 e/Å^3.

Proton NMR spectra of *c*-GHGAYG were obtained with the NT-C-360 spectrometer of the Purdue University Biochemical Magnetic Resonance Laboratory, using ca. 0.03 M solutions in dimethyl-d_6 sulfoxide and water. The pulse Fourier transform mode was used for solutions in deuterated solvents, and the rapid scan–correlation mode was used for solutions in ordinary water. Assignments of the doublet N–H proton resonances of the substituted residues were made on the assumption that the residue with the larger (15 Hz) geminal β-proton coupling is histidine and that that with the smaller (13.8 Hz) is tyrosine.[5] The resonances of the glycine residues were not assigned to specific positions in the sequence.

The free base form of the peptide was obtained by neutralization of an aqueous solution of the hydrobromide,[3] which caused the free base to crystallize. It was recrystallized from water.

Results

Crystal Structure. The final atomic coordinates for nonhydrogen atoms in *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)·3H_2O are given in Table I. Atom numbering is given in Figure 1. Temperature factors are available as supplementary material; there were no unusual values. Bond lengths and angles, listed in Table II, are in agreement with corresponding values observed in other peptides. A stereoscopic view of the peptide molecule is shown in Figure 2. The peptide backbone can be described as two β turns connected by two glycine residues, a type I turn at L-Ala-L-Tyr, with a 3.10-Å 4→1 N–H···O=C hydrogen bond, and a type II′ turn at Gly-L-His, with a 3.06-Å 4→1 hydrogen bond. (The residues named here and in later discussions are those commonly numbered $i + 1$ and $i + 2$.[6]) The two aromatic side chains are positioned with χ_1 near −60°.

(4) Germain, G.; Main, P.; Wolfson, M. M. *Acta Crystallogr., Sect. A* **1971**, *A27*, 368–76.
(5) We have regularly found this difference to occur. See also, for example: Bundi, A.; Wuthrich, K. *Biopolymers* **1979**, *18*, 285–97.
(6) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425–36.

Table I. Fractional Atomic Coordinates

atom	x	y	z
N_1	0.1272 (3)	0.4467 (3)	0.3695 (6)
C^{α}_1	0.2218 (4)	0.3936 (3)	0.4162 (8)
C'_1	0.2998 (3)	0.4279 (3)	0.5921 (7)
O_1	0.2733 (3)	0.4496 (3)	0.7287 (5)
N_2	0.4019 (3)	0.4333 (3)	0.5882 (5)
C^{α}_2	0.4852 (4)	0.4662 (3)	0.7462 (7)
C^{β}_2	0.5932 (4)	0.4377 (4)	0.7322 (7)
C^{γ}_2	0.6163 (4)	0.3446 (3)	0.7798 (6)
C^{δ}_2	0.5589 (4)	0.2852 (4)	0.8373 (7)
N^{δ}_2	0.7140 (3)	0.3109 (3)	0.7811 (6)
N^{ϵ}_2	0.6191 (4)	0.2121 (3)	0.8732 (6)
C^{ϵ}_2	0.7123 (4)	0.2318 (4)	0.8393 (7)
C'_2	0.4867 (4)	0.5646 (3)	0.7653 (7)
O_2	0.5472 (4)	0.5960 (3)	0.9024 (6)
N_3	0.4247 (3)	0.6083 (3)	0.6228 (6)
C^{α}_3	0.4258 (4)	0.7010 (3)	0.6263 (7)
C'_3	0.3268 (4)	0.7389 (3)	0.6626 (6)
O_3	0.2513 (3)	0.6951 (2)	0.6718 (5)
N_4	0.3276 (3)	0.8244 (3)	0.6759 (5)
C^{α}_4	0.2454 (4)	0.8700 (3)	0.7310 (6)
C^{β}_4	0.2637 (5)	0.9669 (4)	0.7258 (9)
C'_4	0.1347 (4)	0.8496 (3)	0.6063 (6)
O_4	0.609 (3)	0.8483 (2)	0.6800 (5)
N_5	0.1201 (3)	0.8356 (3)	0.4261 (5)
C^{α}_5	0.0143 (3)	0.8238 (3)	0.2961 (6)
C'_5	0.0052 (4)	0.8715 (3)	0.1129 (7)
C^{γ}_5	0.0226 (3)	0.9660 (3)	0.1455 (6)
$C^{\delta 1}_5$	0.1208 (4)	1.0033 (4)	0.1628 (8)
$C^{\delta 2}_5$	−0.0573 (4)	1.0183 (4)	0.1709 (7)
$C^{\epsilon 1}_5$	0.1393 (4)	1.0889 (4)	0.2026 (8)
$C^{\epsilon 2}_5$	−0.0402 (4)	1.1050 (4)	0.2110 (8)
C^{ζ}_5	0.0581 (4)	1.1406 (3)	0.2283 (7)
O^{η}_5	0.0805 (3)	1.2244 (3)	0.2695 (6)
C_5	−0.0184 (4)	0.7295 (3)	0.2606 (6)
O_5	−0.1094 (3)	0.7115 (3)	0.1739 (5)
N_6	0.0554 (3)	0.6709 (3)	0.3251 (6)
C^{α}_6	0.0321 (4)	0.5806 (3)	0.3007 (7)
C_6	0.1340 (3)	0.5288 (3)	0.3329 (6)
O_6	0.2180 (2)	0.5619 (2)	0.3249 (5)
O_{40}	0.6555 (3)	0.0310 (3)	0.9276 (5)
O_{41}	0.4458 (3)	0.3834 (3)	0.2348 (5)
O_{42}	0.3273 (4)	0.2512 (3)	0.0152 (9)

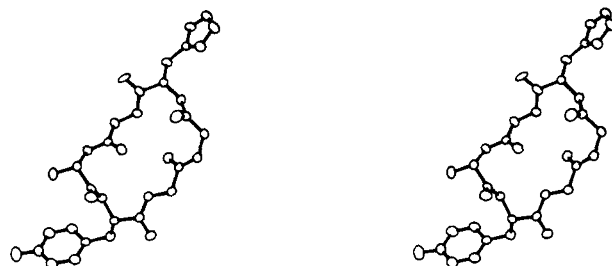

Figure 2. Stereoscopic drawing of the peptide molecule of *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly).

All peptide N–H and C=O groups, the tyrosine hydroxyl, and both nitrogens of the imidazole ring are involved in hydrogen bonds (Figure 3). Two of these are weak transannular 4→1 hydrogen bonds, one is an intermolecular N–H···O=C bond, and three others are intermolecular bonds between side-chain moieties and the backbone. The remaining nine hydrogen bonds involve water molecules. A polar cavity formed by four carbonyl oxygens, two amide protons, and the N_ϵ of imidazole surrounds three water molecules, which are themselves associated. The central oxygen is the proton donor in its two bonds to water, and the central O···O···O angle is 104°. The parameters of the hydrogen bonds are given in Table III. Details of the peptide bond hydration are given also in Table III. These are completely consistent with the statistics of our earlier survey,[7] particularly in that C=O··O_w

(7) Yang, C. H.; Brown, J. N.; Kopple, K. D. *Int. J. Pept. Protein Res.* **1979**, *14*, 12–20.

Table II. Bond Lengths (Å) and Bond Angles (Deg)

bond	Gly-1	His-2	Gly-3	Ala-4	Tyr-5	Gly-6	average
N_i–C^{α}_i	1.453 (6)	1.453 (6)	1.450 (7)	1.453 (6)	1.461 (6)	1.446 (7)	1.453
C^{α}_i–C_i	1.512 (7)	1.545 (7)	1.525 (7)	1.520 (7)	1.538 (7)	1.526 (7)	1.527
C'_i–O_i	1.218 (6)	1.205 (7)	1.225 (6)	1.247 (6)	1.219 (6)	1.239 (6)	1.225
C'_i–N_{i+1}	1.356 (6)	1.327 (7)	1.341 (6)	1.322 (6)	1.322 (6)	1.322 (7)	1.331
C^{α}_i–C_i		1.523 (7)		1.536 (8)	1.533 (7)		1.530
				Tyrosine Side Chain			
C^{β}_5–C^{γ}_5 = 1.504 (7)		$C^{\epsilon1}_5$–$C^{\delta1}_5$ = 1.378 (8)		$C^{\epsilon1}_5$–C^{η}_5 = 1.398 (8)		$C^{\delta1}_5$–C^{γ}_5 = 1.390 (7)	
$C^{\delta2}_5$–$C^{\epsilon2}_5$ = 1.394 (8)		$C^{\epsilon2}_5$–C^{ζ}_5 = 1.381 (8)		$C^{\delta2}_5$–C^{γ}_5 = 1.389 (7)		C^{ζ}_5–C^{η}_5 = 1.358 (7)	
av for C–C in benzene ring = 1.388							
				Histidine Side Chain			
C^{β}_2–C^{γ}_2 = 1.508 (7)		C^{γ}_2–C^{δ}_2 = 1.344 (7)		C^{δ}_2–N^{ϵ}_2 = 1.374 (7)		C^{γ}_2–N^{δ}_2 = 1.388 (6)	
C^{ϵ}_2–N^{δ}_2 = 1.315 (7)		C^{ϵ}_2–N^{ϵ}_2 = 1.358 (7)					
C'_{i-1}–N_i–C_i[a]	120.0 (4)	121.6 (4)	120.0 (4)	121.4 (4)	121.8 (4)	121.6 (4)	121.1
N_i–C^{α}_i–C'_i	109.0 (4)	114.5 (4)	112.8 (4)	112.6 (4)	113.7 (4)	110.7 (4)	112.2
C^{α}_i–C'_i–N_{i+1}	115.5 (4)	116.7 (4)	114.3 (4)	119.3 (4)	117.5 (4)	116.0 (4)	116.6
C^{α}_i–C'_i–O_i	122.7 (4)	118.1 (5)	122.8 (4)	117.6 (4)	119.8 (4)	121.9 (4)	120.5
N_{i+1}–C_i–O_i	121.8 (5)	125.0 (5)	122.9 (4)	123.0 (4)	122.7 (5)	121.9 (4)	122.9
C_i–C^{α}_i–C^{β}_i		108.1 (4)		108.8 (4)	111.6 (4)		109.5
N_i–C^{α}_i–C^{β}_i		109.9 (4)		110.0 (4)	110.2 (4)		110.0
				Tyrosine Side Chain			
C^{α}_5–C^{β}_5–C^{γ}_5 = 111.4 (5)		C^{γ}_5–$C^{\delta1}_5$–$C^{\epsilon1}_5$ = 122.0 (5)		$C^{\delta2}_5$–$C^{\epsilon2}_5$–C^{ζ}_5 = 120.0 (5)		C^{γ}_5–$C^{\delta2}_5$–$C^{\epsilon2}_5$ = 121.4 (5)	
C^{β}_5–C^{γ}_5–$C^{\delta1}_5$ = 121.4 (5)		$C^{\epsilon1}_5$–C^{ζ}_5–$C^{\epsilon2}_5$ = 119.3 (5)		C^{β}_5–C^{γ}_5–$C^{\delta2}_5$ = 121.0 (5)		$C^{\delta1}_5$–$C^{\epsilon1}_5$–C^{ζ}_5 = 119.8 (5)	
C^{ϵ}_5–C^{ζ}_5–O^{η}_5 = 117.7 (5)		$C^{\delta1}_5$–C^{γ}_5–$C^{\delta2}_5$ = 117.5 (5)		$C^{\epsilon2}_5$–C^{ζ}_5–O^{η}_5 = 123.1 (5)			
				Histidine Side Chain			
C^{α}_2–C^{β}_2–C^{γ}_2 = 113.3 (4)		N^{δ}_2–C^{γ}_2–C^{δ}_2 = 109.9 (4)		N^{δ}_2–C^{γ}_2–C^{β}_2 = 119.4 (4)		C^{δ}_2–C^{γ}_2–C^{β}_2 = 130.5 (5)	
N^{ϵ}_2–C^{δ}_2–C^{γ}_2 = 107.0 (5)		C^{ϵ}_2–N^{δ}_2–C^{γ}_2 = 104.8 (4)		C^{ϵ}_2–N^{ϵ}_2–C^{δ}_2 = 106.1 (5)		N^{ϵ}_2–C^{ϵ}_2–N^{δ}_2 = 112.2 (5)	

Table III. Hydrogen Bonds in *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)·$3H_2O$

A. Hydrogen Bond Distances and Angles

donor	acceptor	D-H---A dist, Å	H---A dist, Å	D-H---A angle, deg
N_1-H	O_4 $(-x, -1/2 + y, 1 - z)$	2.84	2.15	148
O_{40}-H	O_1 $(-x, -1/2 + y, 2 - z)$	2.77	1.96	170
N_2-H	O_{41} (x, y, z)	2.97	2.03	162
N_5-H	N^{δ}_2 $(1 - x, 1/2 + y, 1 - z)$	3.05	2.14	165
O_{42}-H	O_2 $(1 - x, -1/2 + y, 1 - z)$	2.90		
N_4-H	O_{41} $(1 - x, 1/2 + y, 1 - z)$	3.01	2.29	160
O^{η}_5-H	O_4 $(-x, 1/2 + y, 1 - z)$	2.79		
O_{42}-H	O_5 $(-x, -1/2 + y, -z)$	2.87		
O_{40}-H'	O_6 $(1 - x, -1/2 + y, 1 - z)$	2.90	1.87	167
N^{ϵ}_2-H	O_{40} (x, y, z)	2.88	2.12	129
O_{41}-H	O_{40} $(1 - x, 1/2 + y, 1 - z)$	2.76	1.86	157
O_{41}-H'	O_{42} (x, y, z)	2.81	1.77	158
N_3-H	O_6 (x, y, z)	3.06	2.21	147
N_6-H	O_3 (x, y, z)	3.10	2.49	148

B. C'=O---H-O_W Angles

acceptor	donor	C=O---O_W angle, deg	N-C'-O---O_W dihedral angle, deg
$C_1=O_1$	O_{40}	143	9
$C_6=O_6$	O_{40}	131	63
$C_2=O_2$	O_{42}	136	−31
$C_5=O_5$	O_{42}	154	176

C. C'-N-H---O_W Angles

donor	acceptor	C'-N---O_W angle, deg	C_i-C'_i-N_{i+1}---O_W dihedral angle, deg
C'_1-N_2	O_{41}	117	2
C'_3-N_4	O_{41}	108	−20

angles are significantly greater than 120° and in that most of the water oxygens do not lie very far out of the plane of the peptide bond.

Table IV. Dihedral Angles[a] of Crystalline *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)[b] and *cyclo*-(Gly-L-Ala-Gly-L-Ala-LAla-Gly)[c] (Deg)

	Gly	His	Gly	Ala	Tyr	Gly
ϕ	60.9	−77.3	−106.1	−56.0	−95.4	162.7
ψ	−135.2	−10.4	176.7	−35.4	−10.0	160.5
ω	179.1	−176.9	−172.9	−174.0	179.2	−175.3
$\chi^{1,1}$		−74.5			−60.0	
$\chi^{2,1}$		−177.1			97.3	
$\chi^{2,2}$		−2.5			−78.7	
	Gly	**Ala**	**Gly**	**Ala**	**Ala**	**Gly**
ϕ	84.2	−105.8	−96.4	−53.1	−84.2	139.2
ψ	−112.8	−9.4	173.0	−43.0	−0.2	158.5
ω	168	178.6	−175.7	−175.3	175.6	−176.6

[a] The standard convention (*Biochemistry* 1970, *9*, 3471–9) is used. [b] This work. [c] Reference 8.

The dihedral angles describing the peptide are given in Table IV. Table IV also includes those reported for *cyclo*-(Gly-L-Ala-Gly-L-Ala-L-Ala-Gly)$_2$·H_2O[8] (*c*-GAGAAG) for comparison.

Solution Data. It is surprising that the crystal grown for X-ray analysis was the neutral peptide, when the actual sample of peptide used was, by chemical test and spectroscopic analysis, the hydrobromide. This may be related to the apparent similarity of the solution conformations or conformational distributions of the neutral and protonated forms that is revealed by the proton NMR data. Spectra were obtained for the protonated and neutral forms in dimethyl sulfoxide and in water. Key data are given in Table V; results for the neutral form in water are lacking because exchange rendered the N–H protons resonances undetectable.

In Me_2SO the N–H chemical shift patterns of the two forms are similar, the side-chain and backbone coupling constants are similar, and in both forms the tyrosine N–H chemical shift is relatively temperature insensitive. There is a difference, in that a temperature-insensitive glycine N–H resonance present in the protonated form is absent in the neutral case. In water, the protonated form exhibits, like the Me_2SO solutions, a distinctly low value for the alanine H–N–C–H coupling, and it also has a

(8) Hossain, M. B.; van der Helm, D. *J. Am. Chem. Soc.* **1978**, *100*, 5191–8.

Table V. Proton NMR Data for *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)[a]

	Ala NH			His[b] NH				Tyr NH				Gly			Gly			Gly		
	δ	J	dδ/dT	δ	J	dδ/dT	$J_{\alpha\beta}$	δ	J	dδ/dT	$J_{\alpha\beta}$	δ	J	dδ/dT	δ	J	dδ/dT	δ	J	dδ/dT
·HBr, Me_2SO[b]	8.22	4.8	0.006	8.32	7.5[c]	0.0058	4.6, 8.5	7.82	8.0	0.002	4.4, 10.2	8.32	6.4, 5.0	0.0058	8.15	5.6, 5.2	0.003	8.08	6.1, 4.4	0.000
free base, Me_2SO[b]	8.18	≃5	0.0065	8.20	7	0.0065	5.0, 8.7	7.80	7.7	0.0028	4.2, 9.8	8.22[d]	7.5,[d] 5.5[d]	0.007	≃8.18[d]	5.8,[d] 5.0[d]	⩽0.005	8.10	6.5, 4.8	0.004
·HBr, H_2O[b]	8.60	4.2	0.0088	8.52	7.9	0.0077	6.9, 8.7[e]	7.61	7.1	0.0007	5.1, 6.3[f]	8.60		0.0072	8.39		0.0067	7.94		0.0008

[a] Chemical shifts given for 25 °C, temperature coefficients in ppm/degree upfield, J in Hz. [b] Imidazole C^2-H, C^4-H chemical shifts: HBr salt in Me_2SO, 8.36, 7.12; free base in Me_2SO, 7.52, 6.75; HBr salt in H_2O, 8.26, 7.16; free base in H_2O, 7.68, 6.94 ppm. [c] From 8:1 Me_2SO-water, where overlap does not occur. [d] Assignment of NH to H^α arbitrary in these two cases. [e] $J_{\alpha\beta}$ = 7.5, 8.8 Hz in free base. [f] $J_{\alpha\beta}$ = 5.4, 6.0 Hz in free base.

temperature-insensitive tyrosine N–H. In common with the protonated form in Me_2SO, it also shows one temperature-insensitive glycine N–H.

In a 1:1 mixture of Me_2SO and water, a mixture chosen to minimize overlaps, the protonated form did not exhibit any clear distinctions in the extent to which the N–H resonances were broadened by added nitroxyl radical.

Discussion

Crystal Structure. The dihedral angles given in Table IV show that the backbone conformation of crystalline *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly) is very similar to that of crystalline *cyclo*-(Gly-L-Ala-Gly-L-Ala-L-Ala-Gly) reported by Hossain and van der Helm,[8] although the hydration and hydrogen-bonding patterns in the two crystals are entirely different, and two aralkyl side chains, with additional hydrogen-bonding functions, replace two of the three methyl side chains. It seems likely that the stable backbone conformation is determined by intramolecular interactions of 1,3,4-tri-L-substituted *cyclo*-hexaglycine backbone and that crystal packing forces are secondary.

A parallel example of the dominance of intramolecular forces in cyclic hexapeptides can now be seen in a comparison of the reported structures of *cyclo*-(Gly-L-Pro-D-Ala)$_2$,[9] *cyclo*-(Gly-L-Pro-D-Phe)$_2$·$(CH_3)_2SO$,[10] *cyclo*-(L-Ala-L-Pro-D-Phe)$_2$·$5H_2O$,[11] *cyclo*-(L-Val-D-Phe-L-Pro)$_2$·$(CH_3)_2SO$·$2H_2O$,[12] and *cyclo*-(Gly-D-Leu-L-Leu)$_2$·$4H_2O$.[13] In the third and fourth of these crystals there are no interpeptide hydrogen bonds, and the last named lacks any constraining proline residues. Each of these, in spite of differences in packing and hydration, and whether or not there are proline residues, has a conformation in which two type II or II′ β turns at the L,D or D,L sequences are connected by two extended residues.

Although these observations are suggestive, they do not indicate that the conformations of cyclic hexapeptides are completely independent of the environment. All of the (L-L-D)$_2$ or (Gly-L-D)$_2$ peptides just cited are known to exist in two or more forms in solution, in ratios dependent on the solvent. It also seems likely, as will be discussed below, that a single conformation type does not dominate in solutions of the 1,3,4-tri-L-substituted cases. However, its consistent appearance in crystalline cyclic hexapeptides in the absence of covalent interresidue constraints does indicate that this backbone, two β turns and two extended connecting residues, is among the most stable for cyclic hexapeptides.

It would be desirable, from the standpoint of molecular design, to be able to predict the likely position of the turns in this stable backbone conformation along a cyclic hexapeptide sequence. Except for proline, which cannot adopt the extended conformation found in the connecting residues, no amino acid is excluded by intraresidue interactions from any position in the backbone. However, certain preferences seem to determine the conformations found in crystals to date.

a. In *cyclo*-(Gly-Xxx-Gly)$_2$, where Xxx is L-Tyr,[14] L-Leu,[15] or L-Pro,[16] type I and type II turns at Xxx-Gly coexist. Thus for Xxx-Gly type I and II turns are of comparable stability, and Xxx-Gly is preferred to Gly-Xxx.

b. In the 1,3,4-tri-L-substituted cases and the one 1,2,4-tri-L-substituted peptide *cyclo*-(Gly-L-Ala-Gly-Gly-L-Ala-L-Ala),[8] the backbone has one type I L-Xxx-L-Xxx turn and a Gly-Xxx or Xxx-Gly turn. Other conformations that include one or two of the substituted residues in the connecting positions, not obviously unstable a priori, do not occur. In *cyclo*-(Gly-Gly-Gly-D-Ala-D-

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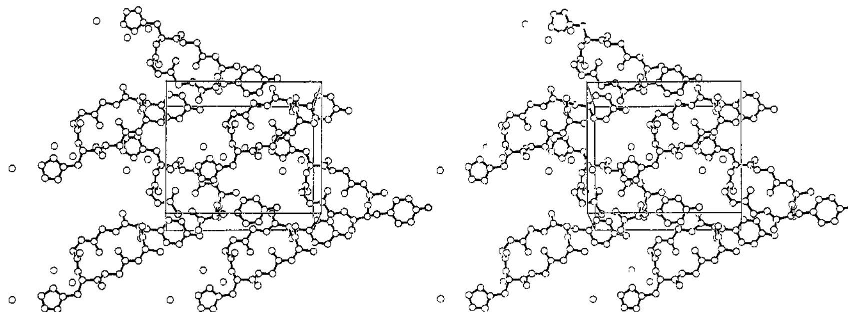

Figure 3. Stereoscopic drawing of the unit cell of *cyclo*-(Gly-L-His-Gly-L-Ala-L-Tyr-Gly)·$3H_2O$.

Ala-Gly) the backbone with a type I turn at Ala-Ala is also preferred to the conformations in which Ala is a connecting residue.[17]

c. In crystals of *cyclo*-(Gly-L-Xxx-D-Yyy)$_2$ the type II L-Xxx-D-Yyy turn is preferred to any form of Gly-Xxx or Yyy-Gly.

A consistent thread in these observations is that in glycine-containing cyclic hexapeptides, glycine is preferred in the extended connecting positions. However, the two-β turn backbone also occurs when glycine is not present, as in *cyclo*-(L-Ala-L-Pro-D-Phe). Here the L-Pro-D-Phe turn is preferred to the L-Ala-L-Pro turn, in part perhaps because there is interference between the side chain and the proline δ methylene in the otherwise likely type I L-Xxx-L-Pro turns. In solution, as in the crystal of *cyclo*-(L-Val-D-Phe-L-Pro)$_2$, *cyclo*-(L-Xxx-D-Phe-Pro)$_2$ peptides prefer the D-Phe-L-Pro type II′ turn to the L-Pro-L-Xxx type I turn. This suggests that the D,L sequence is a strong determinant for a turn in cases where the ring is fully substituted. Future cyclic hexapeptide structures may test whether the observed preference for glycine in the extended connecting positions can be taken to suggest that in a fully substituted peptide lacking such determinants as proline or D,L sequences the smallest side chains will tend to take the extended positions of the two-β turn backbone.

Solution Conformations. The accumulated experience with cyclic hexapeptides of the *cyclo*-(Gly-Xxx-Gly)$_2$ family leads to the prediction that the amide proton resonances of a well-defined *c*-GHGAYG backbone with turns at Gly-His and Ala-Tyr should show lines for two solvent-shielded glycine N–H protons and four other solvent-exposed protons. Resonances of the solvent-shielded protons would probably occur at the high-field end of the N–H region and exhibit reduced sensitivity to temperature or solvent variations, or to the line broadening effects of added nitroxyl. In addition, if the conformation is close to the crystal structure, the HNC$^\alpha$H coupling of the alanine residue should be low (J = 3–4 Hz, HNC$^\alpha$H angle $\simeq$115°) and those of the other substituted residues should be higher (6–8 Hz, 140–160°).

Some years ago, Portnova et al. reported N–H resonance data for a series of cyclic hexapeptides composed of glycine and alanine in various ratios and permutations.[18] The series included, among numerous others, *c*-GAGAAG, the analogue of the peptide reported on here, as well as two C_2-symmetric peptides that show clear evidence of a dominant two-β-turn backbone in dimethyl sulfoxide solution, *cyclo*-(Gly-L-Ala-Gly)$_2$ (*c*-GAGGAG) and *cyclo*-(L-Ala-D-Ala-L-Ala)$_2$ (*c*-A-D-AAA-D-AA). *c*-GAGGAG showed a glycine N–H at 7.55 ppm with zero temperature coefficient, and the other two N–H resonances 0.8–0.9 ppm downfield, at 8.39 and 8.46, with temperature coefficients of 0.004–0.006 ppm/degree. *c*-A-D-AAA-D-AA had an N–H resonance at 7.38 ppm with zero temperature coefficient, and two others at 8.20 and 8.56 ppm with temperature coefficients of 0.006–0.007. In contrast, all of the N–H resonances of *c*-(GAGAAG) occurred in a range of only 0.3 ppm, between 7.97 and 8.28, and the temperature coefficients were all between 0.003 and 0.0064, i.e., no N–H protons were clearly internal. The data in Table V indicate that this appears also to be the case for the free base of *c*-GHGAYG in dimethyl sulfoxide: the chemical shift range is 7.8–8.2 ppm, and there are no very small temperature coefficients. Considering that the numerous X-ray results show that two-β-turn backbones are likely, it is probably that the NMR observations are to be best explained by rapid exchange between two or more such conformers, so that no N–H proton is consistently internal.

The hydrobromide in water, on the other hand, appears to exhibit the expected N–H pattern. The amide proton resonances range over 1 ppm and can readily be divided into a group of two at higher field with small temperature coefficients (0.001 ppm/degree) and four at lower field with high temperature coefficients. However, the high-field, temperature-insensitive protons are those of the tyrosine residue and one glycine, and a two-β-turn structure with simultaneously internal glycine and tyrosine amide protons is not possible for the sequence GHGAYG.

The tyrosine N–H is at highest field and has a temperature dependence on the low side even in the dimethyl sulfoxide solution of the free base. It is possible that the benzyl side chain shields it sterically and electronically. Models suggest that this is possible with combinations of ϕ_{Tyr} and $\chi^1{}_{Tyr}$ near that of the crystal and is even more likely, given the crystal backbone, if $\chi^1{}_{Tyr}$ is +60°. Pachler analysis of the tyrosine side-chain coupling constants shows that the +60° rotamer accounts for about 15% of the rotamer population in dimethyl sulfoxide (free base or hydrobromide) but about 50% in water (also in both forms), where the proton in question is at higher field and less temperature sensitive.

With the above explanation for the tyrosine amide proton, one could speculatively point to the higher field glycine N–H's in the dimethyl sulfoxide solution of the hydrobromide as having low temperature dependences (one is 0.003 ppm/degree) and thus suggest agreement between crystal and solution structure in some circumstances, but the more prudent interpretation for the hydrobromide, as well as the free base, is that a single backbone is not dominant in the solutions examined.

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Supplementary Material Available: Tables listing anisotropic thermal parameters, hydrogen atom coordinates, and structure amplitudes (17 pages). Ordering information is given on any current masthead.

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