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Crystal Structure and Molecular Conformation of the Cyclic Hexapeptide *cyclo*-(Gly-Aib-Gly)$_2$

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We have synthesized and crystallized the cyclic peptide (Gly-Aib-Gly)$_2$. Its structure has been determined by conventional X-ray diffraction methods. In the crystal it adopts a conformation with one β-turn (type I) and its mirror image at the other side of the ring. All conformational angles are similar to those reported for these amino acid residues. In particular the Aib residue has a conformation intermediate between α- and 3_{10}-helical conformations. The ring is an adequate model for the β-turn conformation. A molecule of formic acid is found in the crystal which shows a very short hydrogen bond with one of the glycine carbonyl groups.

Keywords: Aminoisobutyric acid; glycine; cyclic peptides; X-ray diffraction; β-turns

INTRODUCTION

The conformation of cyclic peptides has attracted increasing attention in efforts to understand the structure–function relationship in biologically active compounds. Owing to their restricted conformational mobility the study of cyclic structures may provide models for more complicated structures. Added interest in these compounds comes from the fact that cyclopeptides have a tendency to form cylinders by ring stacking; therefore they may be used as models for channel-forming polypeptides in membranes [1].

Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine) is the prototype of C^{α}-tetrasubstituted α-amino acids. It is a naturally occurring residue in some microbial peptides, particularly in the 'peptaibols' [2]. The most striking property of several members of the peptaibol family is their ability to insert into membranes, forming voltage-dependent ion channels [3]. The presence of Aib in peptaibol sequences has been attributed to its role in constraining the peptide backbone. Descriptions of the structure of several linear peptides provide a firm experimental foundation for the incorporation of amino acids in which the α-proton has been replaced with a methyl group as a means of restricting the backbone conformation of the peptide at that residue [4–6]. Only few papers have dealt so far with the X-ray structure of cyclic peptides containing Aib [7,8].

The two methyl groups on the C^{α} impose a marked restriction on the available conformational space. In general, peptides containing Aib residues show either 3_{10} or α-helical structures, with average conformational angles $\varphi = \pm 55°$ in both cases and $\psi = \pm 30°$ for the 3_{10}-helix and $\psi = \pm 45°$ for the α-helix. Fluctuations of about 10° around these average values are found in different cases [2,6]. Similar values are found when Aib is part of a β-turn [9]. The chirality of the residues adjoining the Aib residue influences the sense of the helix. In spite of the small

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energetic difference between the two types of helix, from the analysis of molecular models, it is evident that unfavourable steric interactions are more significant in the α-helix of poly(Aib)$_n$ than in the 3_{10}-helix [10,11]. Thus, addition of Aib residues could become a powerful method in the engineering of peptides with specific turn and helical conformations. However, little is known about the preferred conformations when the residue adjoining Aib is the flexible glycine (Gly), which, because of its symmetric nature, occupies a unique place among the protein amino acids. Studies of the sequence Aib-Gly-Aib located at the N-terminal part of the antibiotic trichotoxin, and reported by Gessman *et al.* [9] and Gurunath and Balaram [12], offered the first opportunities to check if the specific position of Gly could be structurally important. Their observations suggest that non-helical conformations may be important in Gly-rich peptides containing Aib.

Recent studies on the structural properties of Aib-containing sequences have also been stimulated by the possibility of using C^{α}-tetrasubstituted amino acid residues as stereochemical directors of polypeptide folding. Even a single Aib residue, centrally positioned in a heptapeptide, can strongly stabilize helical folding [13–15]. Thus, replacement of C^{α} hydrogen atoms at strategic positions by methyl groups may prove a powerful way for inducing stability into helical structures.

MATERIALS AND METHODS

Synthesis

The cyclic dimer (Gly-Aib-Gly)$_2$ was obtained as a byproduct during the synthesis of copolymers of Gly and Aib by conventional peptide procedures. Polymerization of H-Gly-Aib-Gly-OPcP (PcP, pentachlorophenyl) was carried out in dimethylformamide and induced by triethylamine. After several days a mixture of products was obtained, dialysed against water and lyophilized. The fractions with lower molecular weights were analysed using HPLC methods and then separated by semi-preparative HPLC: column RP-18 Spherisorb ODS-2 25 × 0.4 cm; flow rate = 1 ml/min, eluent 50% water + 50% methanol. Sample concentration 1 mg/ml. The compound was detected at $t_r = 2.20$ min. Melting point: decomposes before melting. The ^{1}H-NMR (320 MHz, D_2O) spectrum of our product suggested a cyclic structure with two peaks (δ: 4.35, 4.49) belonging to methylene groups of different glycines and one peak corresponding to the methyl groups of Aib (δ:1.66).

X-ray Diffraction

Crystals suitable for X-ray diffraction were obtained from a formic acid/ethyl ether mixture (40:60) by the vapour diffusion method. Crystals grew in the form of thin rhomboid-shaped platelets. X-ray data were collected using an Enraf-Nonius CAD4 diffractometer. Lattice parameters were determined from least-squares analysis of the setting angles for 25 reflections with $2\theta < 60°$. As crystals are not quite stable, intensities were measured at 0°C using an ω-scan technique. Every hour three reflections were measured as an intensity control. No decay was observed. Intensity data were corrected for Lorentz and polarization effects, and absorption was disregarded. Cell dimensions are indicated in Table 1, together with other experimental parameters.

The structure was solved by direct methods using the SHELXS-86 [16] computer program package and refined by a full-matrix least-squares procedure (SHELXL-93) [17]. The E map revealed all the non-hydrogen atoms and showed an asymmetric unit composed of half cyclopeptide and a formic acid molecule. Cycles of refinement and difference Fourier synthesis showed all the H atoms in the structure. The H atoms bonded to the N atoms were placed in the position found in the difference Fourier map. No geometrical constraints were used. Anisotropic full-matrix refinement for non-H atoms and isotropic for

Table 1 Crystallographic Data for *Cyclo*(Gly-Aib-Gly)$_2$

Crystal size (mm^3)	0.4 × 0.3 × 0.2
Molecular formula	$C_8H_{13}N_3O_4.CH_2O_2$
Crystal system	monoclinic
Space group	$P2_1/c$
a (Å)	11.8542(12)
b (Å)	8.0702(11)
c (Å)	12.231(2)
β(deg)	96.840(12)
V (Å^3)	1161.8(3)
Z	4
Asymmetric unit	1/case cycle and 1 formic acid molecule
Calculated density (Mg/m^3)	1.236
Radiation (Å)	Mo Kα ($\lambda = 0.71069$)
Collected reflections	3559 ($2\theta < 60°$)
No. of refined parameters	215
R(int) = $\sum/F^2 - \{F\}^2_{mean}/\sum F^2$	0.0257
$R\{\sigma\} = \sum\sigma\{F^2\}/\sum F^2$	0.1476
Unique reflections	3382
	3345 $I > 2\sigma(I)$
R factor	0.0478

H atoms converged to a standard agreement factor $R = 0.0478$. The maximum and minimum heights in the final difference Fourier map were $+0.231$ and -0.181 $e.Å^{-3}$ respectively.

RESULTS AND DISCUSSION

A diagram of the molecule, based on the experimentally determined coordinates and thermal parameters, is shown in Figure 1. Atoms are labelled according to the IUPAC-IUB convention [18]. A stereoview of the unit cell is shown in Figure 2. Figures were drawn using the ORTEP [19] computer program. The average values of bond distances and angles are in agreement with the corresponding values observed in other small peptides, both cyclic and linear [20,21].

The geometry around the C^{α} atom of the Aib residues is asymmetric as in other peptides containing Aib. Following the nomenclature given by Paterson *et al.* [22] we designate as $C^{\beta L}$ and $C^{\beta D}$ the atoms in Aib which occupy the same position as the C^{β} and the H^{α} atom, respectively, in L-amino acids. For positive φ, ψ values of the Aib residue the N–C^{α}–$C^{\beta D}$ and C′–C^{α}–$C^{\beta D}$ angles are 108.2° and 107.1°, significantly smaller than the tetrahedral value (109.5°), while the N–C^{α}–$C^{\beta L}$ and C′–C^{α}–$C^{\beta L}$ angles are 110.8° and 110.0°, significantly larger. The opposite asymmetry is found for negative φ, ψ values. This deformation of bond angles, which requires little energy, may represent a discriminating factor in

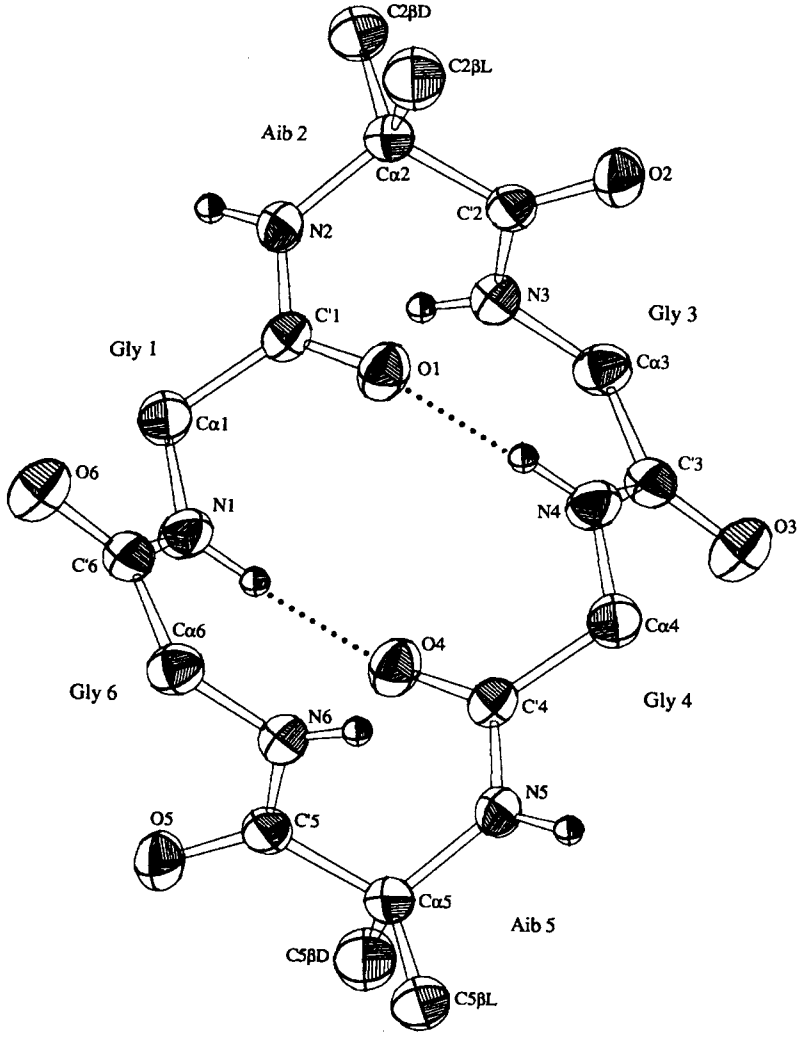

Figure 1 Observed conformation and atom labelling. The atoms are represented by thermal ellipsoids at 50% probability. Approximate projection onto the *bc* plane. Only intramolecular hydrogen bonds are indicated. Hydrogen atoms bound to carbon atoms have been omitted.

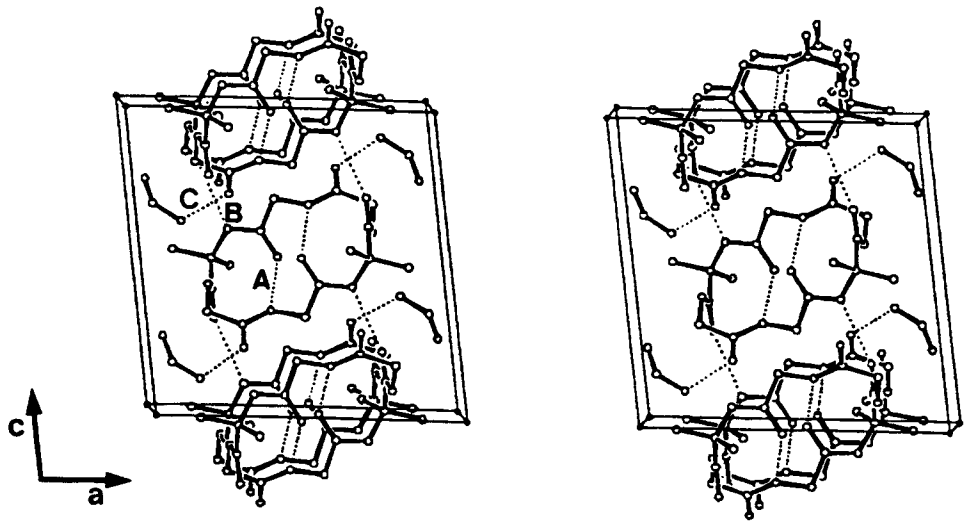

Figure 2 Stereoview of the structure showing the hydrogen bonding interactions. Three types of hydrogen bonds are present (**A**, **B** and **C**) and are discussed in the text. It should be noted that the rings are inclined with respect to the plane of the figure in such a way that the ring planes of neighbour molecules along *c* are approximately perpendicular.

fixing the overall conformation of the molecule [22,23].

The fact that the molecule has a centre of symmetry is reflected in identical values and opposite signs for torsional angles of the pairs $j=1$ and $4, j=2$ and 5 and $j=3$ and 6 (Table 2). All peptide units are in the *trans* conformation with a maximum deviation of ω_j from 180° about ± 10.7° for Gly 3 and Gly 6. Two glycines (1 and 4) have a *trans* conformation of the C_j^{α}–C_j' bond ($\psi_1 = 178.55°$), whereas the other two glycine are near the *cis* conformation ($\psi_3 = -2.69°$).

Table 2 Conformational Angles for *cyclo*(Gly-Aib-Gly)$_2$: (a) Range of Values for Other Six Cases with Aib in the First Position of β-turn I or I′, (b) Range of Values for Other Five Cases of Gly in the Second Position of β-turn I or I′

Residue	Angle	(Gly-Aib-Gly)$_2$	(a)	(b)
	Φ1	− 109.1 (0.23)		
Gly 4	Ψ1	− 178.5 (0.18)		
	ω1	− 180.0 (0.21)		
	Φ2	− 55.3 (0.24)	− 55/−51	
Aib 5	Ψ2	− 35.2 (0.23)	− 46/−35	
	ω2	− 178.4 (0.17)		
	Φ3	− 83.9 (0.24)		− 107/−84
Gly 6	Ψ	+2.7 (0.29)		17/5
	ω3	− 169.3 (0.19)		

(a) and (b) were compiled by Karle [32]

The φ, ψ angles for both types of glycine residues are common in proteins [24]. Observed values of φ and ψ for the Aib residues ± (55.3, 35.2) agree remarkably well with the predicted values for the low-energy form of an Aib residue ± (50, 42), and are midway between expected values for an α-helix and a 3_{10}-helix [4].

The overall conformation of the cyclic peptide consists of two β-turns stabilized by strong hydrogen bonds of the type $1 \leftarrow 4$ that connect NH and CO groups of glycine residues 1 and 4. These H-bonds encompass Aib and Gly residues whose φ and ψ torsional angles are fairly close to the characteristic values for β-turns of types I and I′ [25]. The methyl groups of Aib occupy the corners of turns as in other cyclic hexapeptides with bulky side chains.

It should be noted that a ring conformation practically identical to the one described here has been found in several other rings of the type *cyclo*(Gly-A-B)$_2$, where A and B correspond to other standard L or D amino acids, from which the structure has been determined. A and B are both Gly [26], Leu and Gly [27] or Leu and Phe [28]. The conformational angles of all these rings are very similar to those given in Table 2. It appears therefore that the strict conformational preferences of the Aib residue are not essential to establish this particular ring conformation. Most compounds of the type cyclo(Gly-A-B)$_2$ may adopt the same conformation.

The crystal of (Gly-Aib-Gly)$_2$ contains two molecules of formic acid per molecule of cyclohexapeptide.

Table 3 Hydrogen Bond Parameters in *cyclo*(Gly-Aib-Gly)$_2$

Label	Donor	Acceptor	Symmetry operation on donor	D-H (Å)	H...A (Å)	D-H...A length (Å)	D-H-A angle (deg)
A	N4	O1	$-x, -y, -z$	0.90 (0.026)	2.04	2.89	158.9
B	N2	O2	$x, 0.5-y, z+0.5$	0.86 (0.023)	2.00	2.84	166.5
C	OF	O3	$x, 0.5-y, 1.5+z$	0.80 (0.036)	1.77	2.56	172.6

An efficient scheme of hydrogen bonding exists which utilizes all the C=O and NH groups except N3, N6 and the carbonyl of formic acid. Of the eight hydrogen bonds per cycle, two of them involve two formic acid molecules (bond **C**), four are intermolecular (bond **B**), and two intramolecular bonds (bond **A**). Figure 2 illustrates all the hydrogen bonds and their parameters are listed in Table 3.

Intramolecular hydrogen bond lengths are 2.90 Å, somewhat shorter than the values observed for similar bonds in hexaglycyl and other cyclohexapeptides. The intermolecular hydrogen bonds are oriented along the **c**-axis direction. They directly link every ring with four adjacent rings. Formic acid only participates as a donor, as was predicted by Taylor *et al.* [29,30]. The hydrogen bond distance **C** is very short, in agreement with other bond distances found in the Cambridge Structural Database [31]. Other cases of carboxylic acids with short hydrogen bonds have been reported, but hydrogen bond **C** is the shortest bond found to link formic acid to an amide group.

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APPENDIX

Table of Atomic Coordinates ($\times 10^{-4}$) with e.s.d.s in Parentheses and Equivalent Isotropic Displacement Parameters ($Å^{-2} \times 10^{-3}$) for (Gly-Aib-Gly)$_2$. *U*(eq) is Defined as One-third of the Trace of the Orthogonalized U_{ij} Tensor

	x	*y*	*z*	*U*(eq)
O(1)	5337(1)	1998(2)	54(1)	35(1)
O(2)	7575(1)	2809(2)	2094(1)	39(1)
O(3)	6843(1)	−2309(2)	2860(1)	43(1)
OF	1571(2)	869(3)	1174(2)	58(1)
OF′	508(2)	1148(3)	2541(2)	70(1)
N(1)	5807(1)	−561(2)	1710(2)	34(1)
N(2)	6843(1)	2710(2)	−805(1)	32(1)
N(3)	7718(1)	704(2)	922(1)	30(1)
C(1)	5287(2)	1269(3)	−1857(2)	36(1)
C(1′)	5810(2)	2041(2)	−781(2)	28(1)
C(2)	7458(2)	3513(3)	167(2)	31(1)
C(2′)	7573(2)	2313(3)	1146(2)	29(1)
Cβ(2D)	8659(2)	3910(4)	−88(2)	49(1)
Cβ(2L)	6850(3)	5085(3)	460(2)	48(1)
C(3)	7866(2)	−535(3)	1778(2)	33(1)
C(3′)	6783(2)	−1203(3)	2154(2)	30(1)
CF	719(2)	532(4)	1716(3)	65(1)

Table of Hydrogen Coordinates ($\times 10^{-4}$) and Isotropic Displacement Parameters (Å$^{-2} \times 10^{-3}$).

	x	*y*	*z*	*U*(eq)
HN1	5835(21)	159(35)	1148(19)	53(8)
HN2	7143(20)	2681(29)	− 1410(19)	40(7)
HN3	7807(21)	466(32)	279(22)	50(8)
H(1)	5801(22)	433(34)	− 2088(21)	58(8)
H(1A)	5193(22)	2101(33)	− 2426(22)	52(7)
H(3)	8332(19)	− 113(31)	2446(19)	43(6)
H(3A)	8238(20)	− 1451(31)	1506(18)	41(6)
H(β2D)	9034(23)	2980(35)	− 382(22)	56(8)
H(β2DA)	9096(25)	4347(37)	539(24)	73(9)
H(β2DB)	8651(23)	4765(38)	− 710(25)	72(9)
H(β2L)	6789(24)	5792(37)	− 158(23)	67(9)
H(β2LA)	7244(25)	5528(37)	1094(25)	69(9)
H(β2LB)	6084(25)	4804(38)	643(23)	70(9)
HCF	262(28)	− 326(47)	1298(27)	96(11)
HOF	2027(30)	1503(44)	1468(27)	80(11)