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Chemistry

Parallel packing of α-helices in crystals of the zervamicin IIA analog Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe·2H_2O

(x-ray structure analysis/hydrogen bond/helix curvature/ionophore/membrane channel)

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ABSTRACT **An apolar synthetic analog of the first 10 residues at the NH_2-terminal end of zervamicin IIA crystallizes in the triclinic space group *P*1 with cell dimensions $a = 10.206 \pm 0.002$ Å, $b = 12.244 \pm 0.002$ Å, $c = 15.049 \pm 0.002$ Å, $\alpha = 93.94 \pm 0.01°$, $\beta = 95.10 \pm 0.01°$, $\gamma = 104.56 \pm 0.01°$, $Z = 1$, $C_{60}H_{97}N_{11}O_{13}\cdot 2H_2O$. Despite the relatively few α-aminoisobutyric acid residues, the peptide maintains a helical form. The first intrahelical hydrogen bond is of the 3_{10} type between N(3) and O(0), followed by five α-helix-type hydrogen bonds. Solution ^1H NMR studies in chloroform also favor a helical conformation, with seven solvent-shielded NH groups. Continuous columns are formed by head-to-tail hydrogen bonds between the helical molecules along the helix axis. The absence of polar side chains precludes any lateral hydrogen bonds. Since the peptide crystallizes with one molecule in a triclinic space group, aggregation of the helical columns must necessarily be parallel rather than antiparallel. The packing of the columns is rather inefficient, as indicated by very few good van der Waals' contacts and the occurrence of voids between the molecules.**

A number of naturally occurring peptides containing α-aminoisobutyric acid (Aib) residues form voltage-dependent channels across lipid bilayer membranes. Transmembrane channels are likely to be formed by close association of the peptide helices in the lipid bilayers, as already indicated by the structure of alamethicin (1). Individual 3_{10}- or α-helices have an inner pore too small for passage of ions. Therefore, channel formation requires the aggregation of peptide helices (2). The role of specific side chains in promoting helix association is being studied by means of synthetic analogs. The present study has begun with apolar analogs of zervamicin IIA:

Ac-Trp-Ile-Gln-Aib-$\overset{5}{\text{Ile}}$-Thr-Aib-Leu-Aib-$\overset{10}{\text{Hyp}}$-
-Gln-Aib-Hyp-Aib-$\overset{15}{\text{Pro}}$-Phe-ol.

(Hyp is 4-hydroxyproline; Phe-ol is phenylalaninol.) The decapeptide whose structure is reported in this paper is the apolar analog of the first 10 residues in which Gln-3, Thr-6, and Hyp-10 have been replaced with Ala, Val, and Pro, respectively; the NH_2 terminus has been blocked with *t*-butoxycarbonyl (Boc) rather than acetyl; and the COOH terminus has been esterified with methanol:

Boc-Trp-Ile-Ala-Aib-$\overset{5}{\text{Ile}}$-Val-Aib-Leu-Aib-$\overset{10}{\text{Pro}}$-OMe.

EXPERIMENTAL PROCEDURE

Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe was synthesized by conventional solution-phase procedures, and crystals were grown from dimethyl sulfoxide/H_2O in the form of triangular prisms. X-ray diffraction data were measured from a crystal that was 0.10 mm thick and 0.30 mm on each side of the triangular face. The crystal was sealed in a capillary with a small amount of mother liquor. The data were collected with a four-circle automated diffractometer using Cu Kα radiation and a graphite monochromator ($\lambda = 1.54178$ Å). The θ–2θ scan technique was used with a 2.0° scan, 15°/min scan rate, and $2\theta_{max} = 115°$, for a total of 5294 independent reflections and 4378 reflections with intensities $>3\sigma(F)$ to a resolution of 0.91 Å. Three reflections monitored after every 60 measurements remained constant within 3% during the data collection. Lorentz, polarization, and absorption corrections were applied to the data. The space group is *P*1 with $a = 10.206 \pm 0.002$ Å, $b = 12.244 \pm 0.002$ Å, $c = 15.049 \pm 0.002$ Å, $\alpha = 93.94 \pm 0.01°$, $\beta = 95.10 \pm 0.01°$, $\gamma = 104.56 \pm 0.01°$, $V = 1804.9 \pm 0.5$ Å^3, $Z = 1$, calculated density = 1.119 g/cm^3 based on a molecular weight of 1180.52 + 36.03 for $C_{60}H_{97}N_{11}O_{13}\cdot 2H_2O$, and one formula unit per cell.

The structure was solved by direct phase determination using the random-tangent formula procedure in the SHELXTL computer program‡. Initially, the positions of 36 atoms, mainly in the backbone, were found. The positions of the remainder of the C, N, and O atoms were found by means of partial structure development. Eight of the nine H atoms on amide groups were found in difference maps after partial least-squares refinement, and subsequently their coordinates and isotropic thermal factors were refined by least-squares. Hydrogen atoms for the two water molecules were not found. Although peaks for many other H atoms also were found in difference maps, idealized calculated positions were used for all the H atoms on C atoms. Least-squares refinement with anisotropic thermal factors for the C, N, and O atoms; isotropic thermal factors for eight amide H atoms; and 88 H atoms on C atoms kept fixed in idealized positions yielded an agreement factor of $R = 5.61\%$ for the 4378 reflections measured $>3\sigma(F)$. Fractional coordinates for the C, N, and O atoms and the amide H atoms are listed in Table 1. Bond lengths and angles are shown in Tables 2 and 3.§

RESULTS

Conformation of Molecule. The backbone of Boc-Trp-Ile--Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe, in which all the residues are nonpolar and hydrophobic, folds into an α-helix for most of its length. The helix is initiated by the Trp residue at the NH_2 terminus. There is a helix reversal at residue Aib-9

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Abbreviations: Aib, α-aminoisobutyric acid; Boc, *t*-butoxycarbonyl; Phe-ol, phenylalaninol; OBu^t, *t*-butoxy.

‡Sheldrick, G. M. (1981) SHELXTL, An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data (Univ. of Gottingen, F.R.G.).

§Supplementary material consisting of observed and calculated structure factors, anisotropic thermal factors, and coordinates for the H atoms are available from I.L.K.

Table 1. Atomic coordinates (× 10^4) and thermal factors (Å^2 × 10^3)

Atom†	Mean value (standard deviation)			
	x	*y*	*z*	U_{eq}‡
C(OBu^t)-5	10592 (8)	9053 (8)	4699 (5)	148 (4)
C(OBu^t)-4	10580 (10)	7379 (10)	5555 (5)	173 (6)
C(OBu^t)-3	10443 (9)	9193 (11)	6351 (5)	195 (6)
C(OBu^t)-2	10069 (9)	8425 (8)	5486 (5)	125 (4)
O(OBu^t)	8591 (4)	8129 (5)	5390 (3)	58 (2)
C′(0)	7861 (6)	7516 (5)	4696 (4)	87 (3)
O(0)	8259 (4)	6898 (4)	4159 (2)	94 (2)
N(1)	6549 (5)	7583 (4)	4599 (3)	74 (2)
Cα(1)	5545 (5)	6899 (4)	3902 (3)	63 (2)
C′(1)	5921 (5)	7064 (4)	2962 (3)	58 (2)
O(1)	5552 (3)	6294 (3)	2363 (2)	65 (1)
Cβ(1)	4122 (5)	7069 (4)	3998 (3)	66 (2)
Cγ(1)	4008 (5)	8236 (4)	3885 (3)	63 (2)
Cδ1(1)	4142 (6)	9102 (5)	4529 (4)	78 (2)
Nε1(1)	3992 (5)	10050 (4)	4163 (3)	85 (2)
Cε2(1)	3692 (6)	9816 (4)	3257 (4)	70 (2)
Cζ2(1)	3382 (7)	10503 (5)	2620 (5)	100 (3)
Cη2(1)	3120 (9)	10040 (6)	1755 (5)	116 (4)
Cζ3(1)	3100 (8)	8902 (7)	1505 (4)	110 (4)
Cε3(1)	3388 (7)	8225 (5)	2159 (4)	85 (3)
Cδ2(1)	3697 (5)	8683 (4)	3051 (4)	67 (2)
N(2)	6704 (4)	8085 (3)	2818 (2)	56 (1)
Cα(2)	7076 (5)	8282 (4)	1919 (3)	56 (2)
C′2	7916 (5)	7480 (4)	1614 (3)	59 (2)
O(2)	7858 (4)	7189 (3)	802 (2)	69 (1)
Cβ(2)	7773 (6)	9541 (4)	1855 (3)	72 (2)
Cγ1(2)	7888 (7)	9801 (5)	886 (4)	97 (3)
Cγ2(2)	9140 (7)	9916 (5)	2393 (4)	100 (3)
Cδ1(2)	6573 (9)	9580 (6)	275 (4)	129 (4)
N(3)	8715 (4)	7143 (3)	2225 (3)	57 (2)
Cα(3)	9511 (5)	6381 (4)	1969 (3)	64 (2)
C′(3)	8629 (5)	5241 (4)	1533 (3)	58 (2)
O(3)	8908 (4)	4756 (3)	864 (2)	71 (1)
Cβ(3)	10432 (6)	6226 (5)	2765 (4)	84 (2)
N(4)	7529 (4)	4788 (3)	1952 (2)	59 (2)
Cα(4)	6536 (5)	3712 (4)	1617 (3)	62 (2)
C′(4)	5974 (5)	3812 (4)	641 (3)	61 (2)
O(4)	5847 (4)	3062 (3)	37 (2)	78 (2)
Cβ1(4)	5353 (6)	3543 (4)	2200 (4)	81 (2)
Cβ2(4)	7172 (7)	2711 (4)	1663 (4)	83 (2)
N(5)	5596 (4)	4766 (3)	520 (3)	59 (2)
Cα(5)	4958 (6)	4926 (4)	−357 (3)	67 (2)
C′(5)	5927 (6)	5003 (4)	−1074 (4)	61 (2)
O(5)	5520 (4)	4511 (3)	−1830 (2)	80 (2)
Cβ(5)	4414 (7)	6010 (5)	−274 (4)	89 (3)
Cγ1(5)	3241 (12)	5827 (10)	287 (7)	156 (6)
Cγ2(5)	4089 (10)	6408 (7)	−1161 (6)	148 (5)
Cδ1(5)	2281 (11)	4930 (10)	308 (8)	205 (7)
N(6)	7208 (4)	5616 (3)	−854 (2)	62 (2)
Cα(6)	8162 (6)	5747 (4)	−1525 (3)	70 (2)
C′(6)	8421 (6)	4625 (5)	−1839 (4)	72 (2)
O(6)	8456 (4)	4359 (3)	−2632 (2)	86 (2)
Cβ(6)	9482 (7)	6661 (5)	−1180 (5)	102 (3)
Cγ1(6)	9211 (10)	7813 (6)	−1072 (5)	145 (4)
Cγ2(6)	10601 (8)	6614 (7)	−1793 (5)	141 (4)
N(7)	8571 (4)	3930 (4)	−1206 (3)	68 (2)
Cα(7)	8756 (6)	2796 (5)	−1429 (4)	77 (3)
C′(7)	7540 (7)	2084 (5)	−2064 (3)	85 (3)
O(7)	7715 (5)	1388 (4)	−2644 (3)	119 (2)
Cβ1(7)	8818 (7)	2234 (5)	−551 (3)	89 (3)
Cβ2(7)	10107 (7)	2879 (6)	−1830 (4)	109 (3)
N(8)	6306 (5)	2199 (3)	−1931 (2)	69 (2)
Cα(8)	5084 (7)	1536 (4)	−2489 (3)	77 (2)
C′(8)	4975 (7)	1915 (5)	−3436 (4)	83 (3)
O(8)	4382 (5)	1234 (3)	−4066 (2)	103 (2)
Cβ(8)	3818 (7)	1529 (5)	−2039 (3)	81 (2)
Cγ(8)	3648 (7)	877 (5)	−1200 (4)	88 (2)
Cδ1(8)	2431 (9)	1081 (8)	−767 (5)	146 (4)
Cδ2(8)	3516 (9)	−364 (5)	−1447 (5)	136 (4)
N(9)	5498 (6)	3021 (4)	−3512 (3)	89 (2)
Cα(9)	5637 (8)	3462 (5)	−4398 (3)	98 (3)
C′(9)	4312 (7)	3068 (4)	−5024 (3)	84 (3)
O(9)	4313 (4)	2625 (3)	−5788 (2)	89 (2)
Cβ1(9)	6061 (11)	4751 (6)	−4249 (4)	146 (4)
Cβ2(9)	6756 (8)	3053 (8)	−4805 (4)	139 (4)
N(10)	3118 (8)	3204 (5)	−4776 (4)	104 (3)
Cα(10)	1916 (8)	2787 (7)	−5427 (5)	117 (4)
C′(10)	2044 (7)	3398 (5)	−6267 (5)	97 (3)
O(10)	2573 (6)	4355 (3)	−6286 (3)	126 (2)
Cβ(10)	776 (11)	3062 (12)	−4927 (7)	228 (7)
Cγ(10)	1486 (16)	3673 (16)	−4093 (7)	294 (9)
Cδ(10)	2850 (12)	3650 (10)	−3898 (5)	184 (6)
O(OMe)	1403 (5)	2698 (4)	−6982 (4)	111 (2)
C(OMe)	1369 (9)	3185 (7)	−7822 (5)	132 (4)
W(1)§	7343 (7)	296 (5)	4057 (4)	177 (3)
W(2)§	7102 (11)	9915 (8)	5893 (5)	258 (6)
H(1)	6313 (42)	8016 (33)	4847 (25)	50 (11)¶
H(2)	6923 (41)	8745 (32)	3205 (25)	59 (11)¶
H(3)	8755 (53)	7425 (43)	2722 (32)	92 (15)¶
H(4)	7277 (4)	5126 (32)	2348 (24)	50 (11)¶
H(5)	5682 (56)	5284 (44)	899 (34)	90 (15)¶
H(6)	7416 (47)	6080 (37)	−366 (29)	65 (13)¶
H(7)	8565 (51)	4132 (41)	−621 (31)	96 (15)¶
H(8)	6177 (66)	2593 (52)	−1527 (39)	125 (18)¶

†The three equivalent C atoms of the *t*-butoxy (OBu^t) moiety of the Boc blocking group are numbered 3, 4, and 5; the central C atom is numbered 2. Atoms of the carbonyl moiety of Boc (residue 0) and of amino acid residues 1–10 are identified according to conventions recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (3); for instance, Cε3(1) is the ε3 carbon of Trp-1. Amide nitrogens are symbolized H(1) to H(8).

‡$U_{eq} = \frac{1}{3}\Sigma\Sigma U_{ij} a_i^* a_j^* (\mathbf{a}_i \cdot \mathbf{a}_j)$.

§W = water (molecule 1 or 2).

¶Isotropic value.

where the ϕ and ψ values change sign from negative to positive (Table 4). The breaking of the helix is coincident with the occurrence of the Pro residue.

The structure of the peptide molecule, viewed perpendicular to the helix, is displayed in Fig. 1. The conformational angles for the backbone and side chains are listed in Table 4, and the hydrogen bonds are listed in Table 5. The first hydrogen bond in the helix at the NH_2 terminus, from N(3)H to the carbonyl oxygen O(0) of the Boc group, is a 4→1 type appropriate for a 3_{10}-helix. The values of the ϕ and ψ angles for residues 1 and 2 are near the values (−60°, −30°) for an idealized right-handed 3_{10}-helix. The following five hydrogen bonds involving N(5)H to N(9)H are of the 5→1 type that constitute an α-helix. The experimentally determined values of the ϕ and ψ torsion angles for residues 3 to 8 have an average ϕ value somewhat larger in magnitude and an average ψ value somewhat smaller in magnitude than the values (−57°, −47°) for an idealized right-handed α-helix (4). The ϕ and ψ values for the α-helix in the present peptide are much closer to the average ϕ and ψ torsion angles of −65° and −41° reported by Chothia (5). The N(4)H group of Aib-4 is directed toward O(0) of the Boc group; however, the N(4)···O(0) distance is 3.96 Å, a value far outside the usual range of hydrogen bond lengths. The N(4)···O(1) distance of 3.13 Å is reasonable for a hydrogen bond, and perhaps O(1) is an acceptor for two hydrogen bonds, a 5→1 type from

Table 2. Bond lengths

Bond	Length,* Å										
	Boc-0	Trp-1	Ile-2	Ala-3	Aib-4	Ile-5	Val-6	Aib-7	Leu-8	Aib-9	Pro-10
N(*i*)—Cα(*i*)		1.451	1.456	1.438	1.471	1.466	1.453	1.470	1.459	1.477	1.458
Cα(*i*)—C′(*i*)		1.511	1.531	1.526	1.552	1.519	1.517	1.530	1.531	1.527	1.512
C′(*i*)—O(*i*)	1.233	1.220	1.241	1.225	1.221	1.234	1.220	1.234	1.223	1.236	1.164
C′(*i*)—N(*i* + 1)	1.358	1.348	1.330	1.351	1.339	1.333	1.344	1.333	1.343	1.351	1.336
Cα(*i*)—Cβ(*i*)		1.536	1.541	1.508	1.535	1.565	1.541	1.534	1.510	1.524	1.533
					1.529			1.535		1.517	
Cβ(*i*)—Cγ(*i*)		1.483	1.522			1.505	1.506		1.538		1.447
			1.495			1.491	1.541				
Cγ(*i*)—Cδ(*i*)		1.359	1.510			1.279†			1.519		1.404
		1.444							1.510		

*Estimated SD for backbone atoms, ≈0.007 Å; for side groups, 0.008–0.014 Å.
†Associated with a large thermal factor that indicates rotational disorder about the Cβ(5)–Cγ1(5) bond. The value of the bond length has a large error.

N(5)H and a 4→1 type from N(4)H. But if this is the case, then the value of the N(4)H···O(1) angle, 132°, indicates a rather bent hydrogen bond.

^{1}H NMR studies of the peptide in chloroform solutions, using solvent perturbation experiments, suggest the presence of seven solvent-shielded NH groups, with the Trp-1 and Ile-2 NH groups exposed to the solvent (unpublished results). These results are fully consistent with the observed solid-state conformation. However, in dimethyl sulfoxide, only four solvent-shielded NH groups have been established, based on the temperature-dependence of NH chemical shifts (unpublished data). Furthermore, inter-residue Cα(*i*)H---N(*i* + 1)H nuclear Overhauser effects have been observed for the four NH_2-terminal residues (unpublished results), suggesting an extended conformation for this segment.

A view of the molecule, directly into the helix, is shown in Fig. 2. In both Figs. 1 and 2 it can be seen that the large side chains—that is, in residues Trp-1, Ile-2, Ile-5, and Leu-8, as well as the segment of the backbone containing the Pro-10 residue—are directed toward one side of the helix (left side as drawn). This side of the helix is entirely buried by bulky, hydrophobic side chains. On the other hand, residues with small side chains—that is, Ala-3, Aib-4, and Aib-7—occur on the other side of the helix. In fact, in the direction between Ala-3 and Aib-7, the helical backbone is exposed to the exterior environment. In natural zervamicin IIA, of which the present compound is an apolar synthetic analog of the first 10 residues, Ala-3 becomes Gln-3 and Val-6 becomes Thr-6. If the conformation of residues 1–10 of zervamicin IIA is the same as in the analog, the presence of polar residues at positions 3 and 6 would impart a polar character to the right side of the helix, thus making the helix amphiphilic.

Curvature of α-Helix. The more than two turns of α-helix in the synthetic peptide appears to have a curvature with a radius of approximately 70 Å, similar to curvatures observed in amphiphilic helices found in proteins (6, 7). The hydrogen bonds N(6)···O(2) and N(9)···O(5) on the concave side of the curvature, the side with the bulky hydrophobic groups, have an average length of 2.99 Å, whereas the hydrogen bonds N(5)···O(1), N(7)···O(3), and N(8)···O(4) on the convex side, the side with the small hydrophobic groups (replaced by hydrophilic groups in zervamicin IIA), have an average length of 3.18 Å. These values are similar to those in curved amphiphilic α-helices (6, 8). However, the O=C′—N angles for residues 1–8 remain constant at 122.3 ± 0.3°, and the N—C′—C^α angles average 116.8 ± 1.1°, with those on the inner surface somewhat larger than those on the outer surface. This latter effect with the N—C′—C^α angles is opposite in direction from that observed in proteins. The curvature of the helix in the present peptide with Aib residues may perhaps be due to differences in torsional angles rather than differences in bond angles. For the Aib residues, the ϕ values are smaller in magnitude and the ψ values are larger in

Table 3. Bond angles

Bonds	Angle value,* degrees										
	Boc-0	Trp-1	Ile-2	Ala-3	Aib-4	Ile-5	Val-6	Aib-7	Leu-8	Aib-9	Pro-10
C′(*i* − 1)—N(*i*)—Cα(*i*)		122.3	127.1	120.8	123.0	120.1	119.7	122.0	121.6	121.4	117.1
N(*i*)—Cα(*i*)—C′(*i*)		114.1	110.6	112.5	108.2	112.2	111.7	110.1	112.7	112.0	111.9
Cα(*i*)—C′(*i*)—N(*i* + 1)	113.9†	117.4	119.0	115.0	114.8	117.6	116.7	117.8	116.3	121.9	110.3‡
Cα(*i*)—(C′(*i*)—O(*i*)	125.7§	121.1	118.9	122.8	122.5	120.2	121.3	119.8	120.0	120.1	124.9
N(*i* + 1)—C′(*i*)—O(*i*)	120.4¶	121.4	122.1	122.2	122.6	122.2	122.0	122.3	123.6	118.0	124.7‖
C′(*i*)—Cα(*i*)—Cβ(*i*)		110.0	113.6	110.9	108.4	110.8	112.9	108.8	111.7	110.0	110.0
					111.2			111.6		109.8	
N(*i*)—Cα(*i*)—Cβ(*i*)		111.3	111.3	110.4	108.0	109.4	110.5	107.3	111.2	107.7	102.9
					111.6			110.6		108.0	
Cα(*i*)—Cβ(*i*)—Cγ(*i*)		114.4	111.6			110.4	110.8		116.0		103.7
			112.4			112.7	110.2				
Cβ(*i*)—Cγ(*i*)—Cδ(*i*)		128.3	116.9			128.5			109.0		116.6
		126.5							110.1		

*Estimated SD for backbone atoms, ≈0.4°; for side groups, ≈0.6°.
†O(OBu′)—C′(0)—N(1)
‡Cα(10)—C′(10)—O(OMe)
§O(OBu′)—C′(0)—O(0)
¶N(1)—C′(0)—O(0)
‖O(10)—C′(10)—O(OMe)

Table 4. Torsion angles

Angle	Value,* degrees									
	Trp-1	Ile-2	Ala-3	Aib-4	Ile-5	Val-6	Aib-7	Leu-8	Aib-9	Pro-10
ϕ	−58.4†	−61.8	−61.4	−56.3	−65.6	−64.2	−58.9	−72.2	50.7	−62.7
ψ	−29.3	−31.0	−44.6	−47.0	−42.2	−41.8	−38.1	−32.1	52.2	147.1‡
ω	−179.3	179.4	178.1	−175.6	−178.0	176.8	−178.5	170.9	−179.5	176.2§
$\chi 1$	63.5	−167.7			−67.9	−66.7		−68.7		4.4
		67.6			164.7	167.4				
$\chi 2$	−94.1	60.2			−38.2			172.8		−11.9
	87.2							−63.2		
$\chi 3$										13.5
$\chi 4$										−10.0
Cδ, N, Cα, Cβ										3.6

The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, and ω) and about bonds of the amino acid side chains (χ) are described in ref. 3. For a right-handed α-helix, ideal values of ϕ and ψ are −57° and −47°. For a right-handed 3_{10}-helix, ideal values of ϕ and ψ are −60° and −30°.

*Estimated SD, ≈0.6°.

†C′(0), N(1), Cα(1), C′(1).

‡N(10), Cα(10), C′(10), O(OMe).

§Cα(10), C′(10), O(OMe), C(OMe).

magnitude than the ϕ and ψ values for the remainder of the residues in the helix.

Packing of Parallel Helices

Intermolecular Hydrogen Bonding. The helical molecules are connected head-to-tail by hydrogen bonds to form infinite columns. At the top of the helix, there are three NH groups, N(1)H, N(2)H, and the Nε1(1)H in the Trp-1 side chain, with all the N—H bonds directed upward. At the bottom of the helix, there are three nearly parallel carbonyl groups, CO(7), CO(8), and CO(9), with the C=O bonds directed downward.

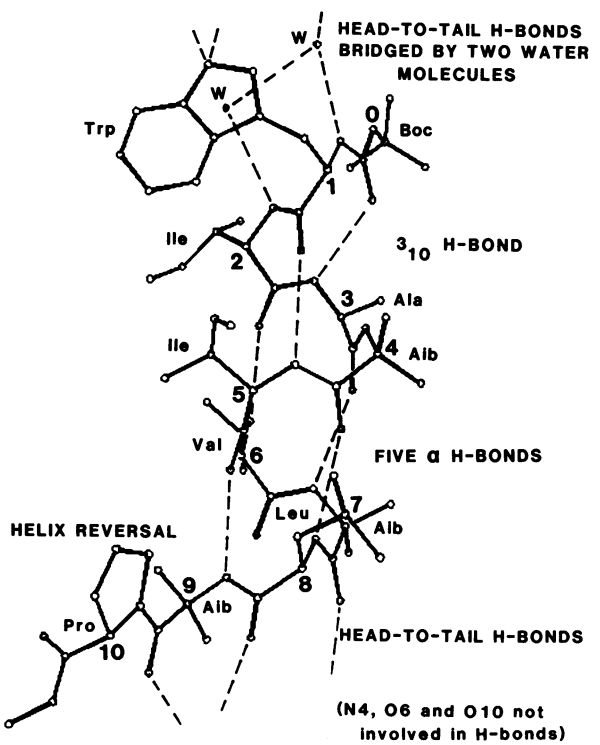

FIG. 1. View along the helix of the decapeptide and the two molecules of water (W) that bridge the head-to-tail hydrogen bonding. The C^{α} atoms are labeled 1–10. The number 0 is at the position of an oxygen atom in the Boc group attached to the NH_2 terminus. Hydrogen bonds are indicated by dashed lines.

There is a direct three-center bond¶ between Nε1(1)H and both O(8) and O(9) in an adjacent molecule translated by one cell length. Two water molecules [W(1) and W(2)], the only water molecules in the cell, are needed to bridge the space between N(1)H and N(2)H in one peptide molecule and O(7) in an adjacent peptide molecule. Atoms N(1) and N(2) donate their protons to form hydrogen bonds with W(2) and W(1), respectively. In turn, W(1) forms a hydrogen bond with W(2), and W(2) is a donor to a hydrogen bond with O(7) (see Fig. 1 and Table 5).

Carbonyl oxygens O(6) and O(10) do not participate in hydrogen bonding. There are no lateral hydrogen bonds.

Aggregation of Helices. Since the peptide crystallizes with one molecule in the triclinic cell, the only relationship between molecules is one of translation. The columns of head-to-tail-bonded helices must necessarily pack in a parallel fashion. This parallel packing of the α-helices is in contrast to the antiparallel packing generally observed in proteins and in other small α-helical structures (9–11).

In the packing scheme (Fig. 3), the columns of helices are translated with respect to each other so that the molecules are staggered in the adjacent columns. Voids are created between molecules. These voids traverse the crystal. The closest contacts between molecules in the lateral direction are O(10)···Cβ(1) = 3.28 Å and Cβ1(9)···N(1) = 3.91 Å near the bottom of one molecule and Cδ1(2)···Cδ2(8) = 3.89 Å near the top of the same molecule. All the other nearest approaches in the lateral directions are greater than 4.1 Å. In the center of the void, the closest contact is 4.92 Å between O(6) and C-4 of the Boc group. The parallel packing scheme has the appearance of inefficiency, particularly since there are only three normal van der Waals' approaches and all the others are very weak.

α-Helix Versus 3_{10}-Helix

The presence of one or more Aib residues in tri-, tetra- and pentapeptides induces the formation of a 3_{10}-helix containing 4→1-type hydrogen bonds (β-bonds). A review of crystal structures of 28 peptides shows the 3_{10}-helix or an incipient 3_{10}-helix in all but one (12).

Few crystal structures of longer peptides containing Aib residues are available. Those shown to form a 3_{10}-helix or an almost continuous 3_{10}-helix are Boc-(Val-Aib-)$_3$Val-OMe

¶Three-center bonds were previously referred to as bifurcated; see ref. 7, for example.

Table 5. Hydrogen bonds

Type	Donor	Acceptor	Length, Å	Angle, degrees (C=O···N)
Intermolecular*	W(2)	O(7)	2.671	
	W(1)	W(2)	2.854	
	Nε1(1)†	O(8)	2.890	
		O(9)	3.085	
	N(1)	W(2)	3.240	
	N(2)	W(1)	3.073	
Intramolecular				
3_{10}-helix (4→1)	N(3)	O(0)	3.010	130
α-helix (5→1)	N(5)	O(1)	3.247	159
	N(6)	O(2)	2.960	157
	N(7)	O(3)	3.170	155
	N(8)	O(4)	3.130	152
	N(9)	O(5)	3.012	158

The separations between N(4) and O(1) (3.130 Å) and between N(9) and O(6) (3.169 Å) are within possible hydrogen bond lengths; however, the geometry at the N atoms is not favorable for hydrogen bond formation.
*Head-to-tail region.
†Three-center bond (formerly called bifurcated).

(13), *p*BrBz-Aib$_8$-OBu^t (*p*BrBz, *p*-bromobenzoyl) (14), *p*BrBz-Aib$_3$-Val-Gly-Leu-Aib$_2$-OMe (14), and Boc-Aib-Pro--Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (15), all of which have an Aib content of 43–100%. The nonapeptide Boc-Leu--Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe-ol, representing the COOH terminus of alamethicin, forms a mixed helix with three 4→1 and three 5→1 hydrogen bonds (10), whereas the undecapeptide Boc-Ala-Aib-Ala-Aib-Ala-Glu(OBzl)-Ala-Aib-Ala-Aib-Ala-OMe (Bzl, benzyl) (9), a model of the NH_2 terminus of alamethicin, forms an α-helix nine residues long with six 5→1 hydrogen bonds and one 4→1 hydrogen bond. The preliminary report (1) on the structure of alamethicin with three molecules per asymmetric unit indicated mainly α-helical conformations with short interruptions of 3_{10}-helical regions. The present peptide also has an α-helical region eight residues long, and the helix is extended with a 4→1 bond to the Boc group. In the peptides forming α-helices rather than 3_{10} helices, the fraction of Aib residues is 0.30 to 0.35.

In this small sample of Aib-containing peptides with seven or more residues, the 3_{10}-helix has been formed when almost half or more residues are Aib, while the α-helix has been favored when the number of Aib residues is one-third of the total.

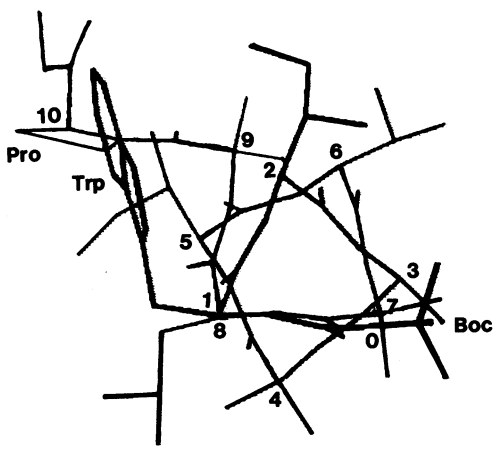

FIG. 2. View down the helix of the decapeptide. The C^α atoms are labeled 1–10.

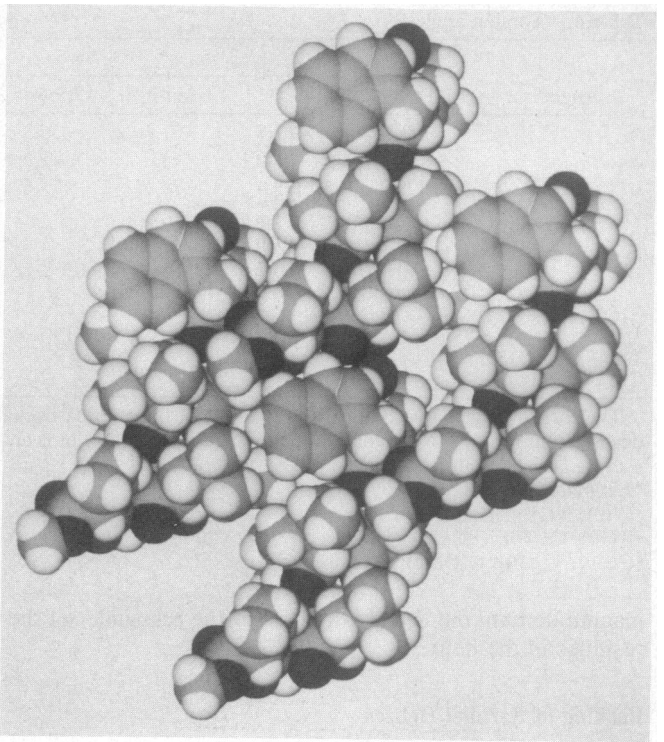

FIG. 3. Space-filling model with van der Waals' radii of four molecules of the decapeptide in four adjacent triclinic cells. The molecules are in the same orientation as in Fig. 1, with the two molecules in the middle of the diagram showing head-to-tail hydrogen bonding. Empty channels in the structure, perpendicular to the view of the diagram (e.g., see center right), are bounded by three peptide molecules.

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