Aqueous channels within apolar peptide aggregates: Solvated helix of the α-aminoisobutyric acid (Aib)-containing peptide Boc-(Aib-Ala-Leu)$_3$-Aib-OMe·2H$_2$O·CH$_3$OH in crystals

(apolar decapeptide/hydrogen bond/helix distortion/amphiphilic helix mimic/transmembrane peptide channel)

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Contributed by Isabella L. Karle, September 14, 1987

ABSTRACT Although the peptide Boc-Aib[1]-Ala[2]-Leu[3]-Aib[4]-Ala[5]-Leu[6]-Aib[7]-Ala[8]-Leu[9]-Aib[10]-OMe [with a *t*-butoxycarbonyl (Boc) blocking group at the amino terminus, a methyl ester (OMe) at the carboxyl terminus, and four α-aminoisobutyric (Aib) residues] has a 3-fold repeat of residues, the helix formed by the peptide backbone is irregular. The carboxyl-terminal half assumes an α-helical form with torsion angles ϕ and ψ of approximately $-60°$ and $-45°$, respectively, whereas the amino-terminal half is distorted by an insertion of a water molecule between the amide nitrogen of Ala[5] [N(5)] and the carbonyl oxygen of Ala[2] [O(2)]. The water molecule W(1) acts as a bridge by forming hydrogen bonds N(5)⋯W(1) (2.93 Å) and W(1)⋯O(2) (2.86 Å). The distortion of the helix exposes the carbonyl oxygens of Aib[1] and Aib[4] to the outside environment, with the consequence that the helix assumes an amphiphilic character despite having all apolar residues. Neighboring helices in the crystal run in antiparallel directions. On one side of a helix there are only hydrophobic contacts with efficient interdigitation of leucine side chains with those from the neighboring helix. On the other side of the helix there are hydrogen bonds between protruding carbonyl oxygens and four water molecules that separate two neighboring helices. Along the helix axis the helices bind head-to-tail with a direct hydrogen bond N(2)⋯O(9) (3.00 Å). Crystals grown from methanol/water solution are in space group $P2_1$ with $a = 15.778 \pm 0.004$ Å, $b = 11.228 \pm 0.002$ Å, $c = 18.415 \pm 0.003$ Å, $\beta = 102.10 \pm 0.02°$, and two formula units per cell for C$_{49}$H$_{88}$N$_{10}$O$_{13}$·2H$_2$O·CH$_3$OH. The overall agreement factor R is 7.5% for 3394 reflections observed with intensities $>3\sigma(F)$, and the resolution is 0.90 Å.

Transmembrane channels formed by alamethicin and related fungal peptides are likely to arise by close association of peptide helices in bilayer membranes (1, 2). The role of specific side chains in promoting helix association is being studied by means of synthetic analogues. It has been observed that α-aminoisobutyric (Aib) residues, common components of membrane-active peptides, promote the formation of 3$_{10}$-helices in short peptides (3, 4), and in longer peptides (>7 residues) if they comprise one-half or more of the peptide (ref. 5 and references therein). An α-helix has been favored in longer peptides when the Aib residues constitute one-third of the total. In the present study, the Aib content is 40%. The initial intent was to determine the type of helix formed and the mode of aggregation of these helices in the crystal. The serendipitous entry of a water molecule into the helix formed by the backbone atoms has produced unanticipated insights into the hydration and aggregation behavior of helices in peptides.

EXPERIMENTAL PROCEDURES

Boc-(Aib-Ala-Leu)$_3$-Aib-OMe (where Boc is *t*-butoxycarbonyl) was synthesized by conventional solution-phase procedures and crystals were grown from CH$_3$OH/H$_2$O solution in the form of thin hexagonal plates. A crystal of size 0.20 × 0.35 × 0.08 mm was sealed in a thin-walled glass capillary with a small amount of mother liquor, and x-ray diffraction data were collected on an automated diffractometer using Cu Kα radiation and a graphite monochromator ($\lambda = 1.54178$ Å). The θ–2θ scan technique was used with a scan of 2.0°, a variable scan rate between 3°/min and 15°/min, and $2\theta_{max} = 115°$, for a total of 4863 independent reflections and 3394 reflections with intensities $>3\sigma(F)$. Three reflections (4 0 0, 6 0 0, and 0 0 7) monitored after every 60 measurements remained constant within 4% during the data collection. Lorentz and polarization corrections were applied to the data. The cell parameters are $a = 15.773 \pm 0.004$ Å, $b = 11.228 \pm 0.002$ Å, $c = 18.415 \pm 0.003$ Å, and $\beta = 102.10 \pm 0.02°$ for space group $P2_1$. The calculated density is 1.138 g/cm^3, based on two formula units per cell ($Z = 2$), a cell volume $V = 3190$ Å^3, and a molecular weight of 1025.3 ± 68.1 for C$_{49}$H$_{88}$N$_{10}$O$_{13}$·2H$_2$O·CH$_3$OH.

Since the backbone was expected to be helical, a vector search procedure was used to solve the structure. Twenty-eight atoms in an α-helical backbone found for a different decapeptide (5) were used as a model to search the Patterson map of the present molecule, computed with EF values as coefficients [where the F quantities are the experimentally measured structure factors and the E quantities (7) are the corresponding normalized values], for the best orientation of the model. This was followed by a translation search to place the oriented model correctly with respect to an origin of the unit cell. The computer program PATSEE (6) in the SHELX84 package of programs[‡] was used. The vector search procedure did not succeed until the normalized |E_{hkl}| values (7) were obtained from the measured intensity data by processing with the XTAL86 program,[§] rather than the SHELX84 program.[‡] The differences between the coordinates of the 28-atom model and refined positions of equivalent atoms in the new molecule averaged 0.33 Å. The complete structure was subsequently derived from the 28-atom fragment by the partial structure procedure (8) and difference maps. Full-matrix least-squares refinement, initially with isotropic thermal factors and finally with anisotropic thermal factors for the C, N, and O atoms,

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Abbreviations: Aib, α-aminoisobutyric acid; Boc, *t*-butoxycarbonyl.
[‡]Micro VAX version of SHELXTL system of programs (Nicolet Analytical Instruments, Madison, WI).
[§]Refined with program CRYLSQ by R. Olthof-Hazekamp from the XTAL System of Crystallographic Programs, Technical Report 1364.2, University of Maryland, Computer Science Center, College Park, MD 20742, 3rd Ed., March 1986.

Table 1. Fractional atomic coordinates (x, y, z) and thermal factors $(U_{eq}, \text{Å}^2 \times 10^2)$

Atom*	Mean value (SD × 10⁴)			U_{eq}†	Atom*	Mean value (SD × 10⁴)			U_{eq}†
	x	y	z			x	y	z	
O(OBuᵗ)	0.7976 (6)	0.0346 (0)	−0.3373 (6)	7.8	Cα(6)	0.6461 (7)	0.3758 (15)	0.1311 (7)	5.4
C(OBuᵗ)-1	0.7310 (11)	−0.0399 (16)	−0.3128 (12)	10.2	C'(6)	0.6899 (8)	0.2704 (15)	0.1740 (6)	4.7
C(OBuᵗ)-2	0.7584 (14)	−0.0748 (20)	−0.2339 (14)	13.6	O(6)	0.6821 (5)	0.2501 (12)	0.2376 (4)	6.1
C(OBuᵗ)-3	0.6464 (11)	0.0250 (19)	−0.3278 (10)	10.2	Cβ(6)	0.5698 (9)	0.3400 (18)	0.0701 (8)	7.7
C(OBuᵗ)-4	0.7270 (14)	−0.1464 (19)	−0.3627 (14)	16.6	Cγ(6)	0.5026 (9)	0.2595 (21)	0.0949 (9)	10.0
C'(0)	0.8227 (9)	0.1388 (14)	−0.3047 (8)	5.8	Cδ1(6)	0.4413 (14)	0.2067 (31)	0.0289 (13)	17.6
O(0)	0.8014 (6)	0.1771 (11)	−0.2489 (5)	6.6	Cδ2(6)	0.4619 (13)	0.3109 (25)	0.1486 (13)	14.6
N(1)	0.8761 (7)	0.1947 (12)	−0.3410 (5)	5.1	N(7)	0.7405 (6)	0.2032 (12)	0.1388 (5)	4.6
Cα(1)	0.9276 (8)	0.2977 (14)	−0.3100 (6)	4.9	Cα(7)	0.7886 (10)	0.0991 (15)	0.1746 (8)	6.5
C'(1)	0.8702 (8)	0.3944 (14)	−0.2874 (6)	4.7	C'(7)	0.8458 (8)	0.1342 (16)	0.2481 (7)	5.5
O(1)	0.8934 (5)	0.4536 (11)	−0.2297 (4)	5.3	O(7)	0.8586 (7)	0.0685 (12)	0.3024 (5)	8.4
Cβ1(1)	0.9706 (8)	0.3506 (16)	−0.3690 (6)	6.0	Cβ1(7)	0.8430 (10)	0.0524 (15)	0.1235 (8)	6.8
Cβ2(1)	0.9975 (9)	0.2593 (17)	−0.2430 (7)	7.1	Cβ2(7)	0.7221 (10)	0.0010 (16)	0.1874 (9)	8.0
N(2)	0.7931 (6)	0.4137 (12)	−0.3333 (5)	4.3	N(8)	0.8884 (7)	0.2396 (13)	0.2488 (5)	5.5
Cα(2)	0.7358 (8)	0.5097 (15)	−0.3189 (6)	5.1	Cα(8)	0.9495 (8)	0.2802 (16)	0.3151 (7)	5.6
C'(2)	0.7050 (8)	0.4942 (15)	−0.2471 (7)	5.0	C'(8)	0.9071 (8)	0.3072 (15)	0.3786 (6)	5.0
O(2)	0.6768 (6)	0.5814 (11)	−0.2195 (5)	7.1	O(8)	0.9435 (6)	0.2851 (13)	0.4428 (5)	7.5
Cβ(2)	0.6604 (10)	0.5251 (18)	−0.3840 (7)	7.5	Cβ(8)	0.9981 (9)	0.3882 (18)	0.2972 (8)	8.2
N(3)	0.7049 (6)	0.3826 (13)	−0.2193 (5)	5.0	N(9)	0.8317 (6)	0.3670 (13)	0.3641 (5)	5.0
Cα(3)	0.6770 (8)	0.3582 (14)	−0.1511 (6)	4.8	Cα(9)	0.7922 (8)	0.4118 (15)	0.4222 (6)	5.6
C'(3)	0.7503 (8)	0.3391 (14)	−0.0849 (6)	4.5	C'(9)	0.7293 (8)	0.3262 (15)	0.4485 (6)	5.1
O(3)	0.7366 (5)	0.2993 (11)	−0.0260 (4)	5.6	O(9)	0.7257 (6)	0.3214 (12)	0.5126 (4)	6.9
Cβ(3)	0.6156 (8)	0.2506 (15)	−0.1593 (7)	5.5	Cβ(9)	0.7445 (9)	0.5293 (16)	0.3973 (7)	6.0
Cγ(3)	0.5318 (10)	0.2633 (19)	−0.2179 (8)	7.7	Cγ(9)	0.8061 (9)	0.6360 (16)	0.4010 (7)	7.0
Cδ1(3)	0.4775 (11)	0.1508 (21)	−0.2210 (11)	11.1	Cδ1(9)	0.8424 (12)	0.6727 (19)	0.4803 (10)	10.0
Cδ2(3)	0.4803 (12)	0.3702 (22)	−0.2058 (13)	12.4	Cδ2(9)	0.7606 (8)	0.7413 (25)	0.3565 (10)	11.3
N(4)	0.8299 (6)	0.3733 (12)	−0.0912 (5)	4.5	N(10)	0.6812 (8)	0.2616 (15)	0.3961 (6)	8.5
Cα(4)	0.9068 (7)	0.3561 (14)	−0.0307 (6)	4.4	Cα(10)	0.6161 (12)	0.1772 (21)	0.4123 (9)	9.6
C'(4)	0.8888 (8)	0.4017 (14)	0.0420 (6)	4.5	C'(10)	0.5574 (12)	0.2433 (24)	0.4537 (9)	9.5
O(4)	0.9133 (5)	0.3491 (11)	0.1008 (4)	5.4	O(10)	0.5340 (10)	0.3426 (20)	0.4446 (9)	14.1
Cβ1(4)	0.9284 (9)	0.2230 (15)	−0.0264 (7)	6.3	Cβ1(10)	0.5585 (16)	0.1388 (27)	0.3391 (10)	19.2
Cβ2(4)	0.9815 (8)	0.4259 (16)	−0.0499 (6)	6.0	Cβ2(10)	0.6635 (17)	0.0701 (22)	0.4557 (15)	16.9
N(5)	0.8495 (6)	0.5108 (12)	0.0402 (5)	4.5	O(OMe)	0.5325 (7)	0.1720 (15)	0.5031 (6)	11.3
Cα(5)	0.8399 (8)	0.5658 (14)	0.1087 (6)	4.8	C(OMe)	0.4702 (12)	0.2230 (29)	0.5428 (10)	16.4
C'(5)	0.7745 (8)	0.5032 (14)	0.1457 (6)	4.4	W(1)‡	0.2088 (8)	0.1663 (14)	0.0883 (5)	9.5
O(5)	0.7820 (6)	0.5055 (12)	0.2127 (4)	6.3	W(2)‡	0.0734 (8)	0.1927 (14)	0.1655 (7)	11.4
Cβ(5)	0.8128 (10)	0.6961 (16)	0.0953 (8)	7.5	O(M)§	0.9332 (10)	0.0982 (14)	0.5352 (6)	12.7
N(6)	0.7081 (6)	0.4467 (13)	0.1004 (5)	4.9	C(M)§	0.9034 (15)	0.0021 (21)	0.4936 (10)	12.1

*The three equivalent C atoms of the *t*-butoxy (OBuᵗ) moiety of the Boc blocking group are numbered 2, 3, and 4; the central C atom is numbered 1. 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 (9).

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

‡W = water (molecule 1 or 2).
§Atoms in CH₃OH solvent molecule.

resulted in an overall agreement factor $R = 0.075$ for 3394 data measured $>3\sigma(F)$. Hydrogen atoms on the 10 N atoms were found in difference maps and their coordinates were refined. The 78 H atoms attached to C atoms were placed in

Table 2. Bond lengths

Bond	Length,* Å									
	Boc⁰	Aib¹	Ala²	Leu³	Aib⁴	Ala⁵	Leu⁶	Aib⁷	Ala⁸	Leu⁹	Aib¹⁰
N(i)—Cα(i)		1.460	1.467	1.442	1.478	1.441	1.464	1.472	1.461	1.437	1.472
Cα(i)—C'(i)		1.528	1.510	1.509	1.514	1.523	1.509	1.513	1.495	1.531	1.511
C'(i)—O(i)	1.225	1.241	1.228	1.232	1.223	1.213	1.225	1.224	1.226	1.193	1.175
C'(i)—N(i + 1)	1.337	1.344	1.355	1.342	1.370	1.353	1.358	1.359	1.344	1.315	1.332†
Cα(i)—Cβ(i)		1.517	1.512	1.536	1.532	1.529	1.518	1.496	1.508	1.541	1.521
		1.533			1.517			1.572			1.547
Cβ(i)—Cγ(i)				1.527			1.533			1.535	
Cγ(i)—Cδ(i)				1.521			1.507			1.510	
				1.492			1.410‡			1.529	

*Estimated SD for backbone atoms, ≈0.012 Å; for side chains, 0.015–0.020 Å.
†C'(10)—O(11).
‡Associated with a large thermal factor; the value of the bond length has a large error.

Table 3. Bond angles

Angle	Boc^0	Aib^1	Ala^2	Leu^3	Aib^4	Ala^5	Leu^6	Aib^7	Ala^8	Leu^9	Aib^{10}
					Angle value,* degrees						
C'(i − 1)—N(i)—Cα(i)		122.3	121.0	122.0	122.2	119.5	120.6	121.7	121.2	122.0	121.7
N(i)—Cα(i)—C'(i)		110.8	113.4	114.1	110.3	113.5	111.0	110.5	113.1	115.3	108.2
Cα(i)—C'(i)—N(i + 1)	110.2†	116.9	117.6	117.5	116.6	116.7	115.9	115.8	117.9	115.3	110.5‡
Cα(i)—C'(i)—O(i)	125.3§	121.9	118.7	121.1	122.5	120.9	121.3	122.6	121.2	120.6	127.0
N(i + 1)—C'(i)—O(i)	124.5¶	121.2	123.5	121.3	120.7	122.4	122.7	121.4	120.6	124.1	122.5∥
C'(i)—Cα(i)—Cβ(i)		107.3	111.4	109.2	111.4	108.7	112.6	110.0	109.9	108.9	105.7
		110.3			109.3			109.9			113.7
N(i)—Cα(i)—Cβ(i)		109.0	110.7	111.4	107.8	110.3	110.4	107.8	110.2	109.8	108.4
		109.9			108.1			109.0			108.8
Cα(i)—Cβ(i)—Cγ(i)				115.5			115.2			112.9	
Cβ(i)—Cγ(i)—Cδ(i)				110.2			111.0			111.3	
				112.8			114.3			111.0	

*Estimated SD for backbone atoms, ≈0.8°; for side groups, ≈1.2°.
†O(OBuᵗ)—C(0)—N(1).
‡Cα(10)—C'(10)—O(OMe).
§O(OBuᵗ)—C(0)—O(0).
¶N(1)—C(0)—O(0).
∥O(10)—C'(10)—O(OMe).

Table 4. Torsion angles

Angle	Aib^1	Ala^2	Leu^3	Aib^4	Ala^5	Leu^6	Aib^7	Ala^8	Leu^9	Aib^{10}
					Angle value,* degrees					
φ(N—Cα)	−54.0†	−62.8	−101.8	−49.5	−69.9	−64.3	−55.1	−65.9	−90.2	53.0
ψ(Cα—C')	−40.3	−23.4	15.2	−45.6	−29.7	−49.3	−41.5	−42.9	−37.5	−145.6‡
ω(C'—N)	−176.0	179.8	−178.3	−172.6	174.9	178.7	−176.4	−171.0	−178.5	−175.5§
χ1(Cα—Cβ)			−60.5			176.8			−77.0	
χ2(Cβ—Cγ)			179.4			−167.1			−72.5	
			−56.3			61.3			164.2	

The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, ω) and about bonds of the amino acid side chains (χ) are described in ref. 9. For a right-handed α-helix, ideal values of ϕ and ψ are −65° and −41° (10). For a right-handed 3$_{10}$-helix, ideal values of ϕ and ψ are −60° and −30°.
*Estimated SD ≈ 0.9°.
†C(0), N(1), Cα(1), C'(1).
‡N(10), Cα(10), C'(10), O(OMe).
§Cα(10), C'(10), O(OMe), C(OMe).

idealized positions and included in the final cycles of refinement, although their parameters were held constant. Fractional coordinates for the C, N, and O atoms are listed in Table 1, bond lengths and bond angles are shown in Tables 2 and 3, and torsional angles are listed in Table 4.¶

RESULTS

Conformation of Molecule. A diagram of the peptide molecule, drawn by computer using the experimentally determined coordinates, is shown in Fig. 1. Although the Aib-Ala-Leu sequence is repeated three times in the decapeptide, the regularity in the sequence is not reflected in the helical backbone. The carboxyl-terminal half of the helix is a regular α-helix with four 5→1 hydrogen bonds: N(7)···O(3), N(8)···O(4), N(9)···O(5), and N(10)···O(6) (Table 5). The amino-terminal half of the helix is distorted considerably by the entry of water molecule W(1) between N(5) and O(2).

The consequences on the conformation are several: (*i*) Hydrogen bonds N(5)···W(1) and W(1)···O(2) are formed. (*ii*) The separation between N(5) and O(2) is enlarged to 5.05 Å. (*iii*) The helix axis is bent in the vicinity of W(1), mostly by

Table 5. Hydrogen bonds

Type	Donor	Acceptor	Length, Å	Angle, degrees (C=O···N)
Intramolecular				
4→1	N(3)	O(0)	2.879	134
	N(4)	O(1)	3.065	118
	N(6)	O(3)	2.966	125
5→1	N(7)	O(3)	3.208	169
	N(8)	O(4)	3.089	154
	N(9)	O(5)	3.145	148
	N(10)	O(6)	2.925	166
Intermolecular				
Head-to-tail	N(2)	O(9)*	3.000	147
	N(1)	O(M)*†	2.835	
	O(M)†	O(8)	2.727	
Insertion	N(5)	W(1)	2.930	
	W(1)	O(2)	2.859	
Lateral	W(1)	W(2)	2.818	
	W(2)	O(1)	2.936	
	W(2)	O(4)‡	3.101	

O(7) and O(10) do not participate in hydrogen bonding.
*x, y, −1 + z (transformation of coordinates in Table 1 in order to obtain coordinates of acceptor atom that is in a symmetry-related molecule; see also footnote ‡).
†O(M) is the oxygen atom in the CH_3OH solvent molecule.
‡$2 - x$, $\frac{1}{2} + y$, −z.

¶Supplementary material consisting of observed and calculated structure factors, anisotropic thermal factors, and coordinates for H atoms is available from I.L.K.
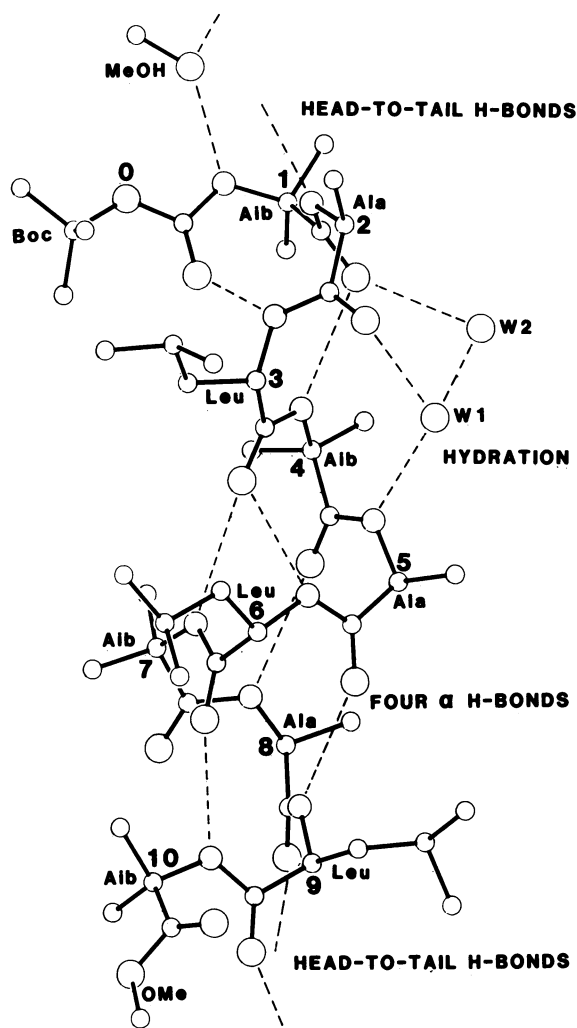
FIG. 1. View along the helix of Boc-(Aib-Ala-Leu)₃-Aib-OMe. Included are the CH₃OH molecule that bridges the head-to-tail hydrogen bonding, and the two water molecules that bind to backbone atoms. Water W(1) is inserted into the helical backbone. The Cα atoms are labeled 1–10. The number 0 is at the position of an oxygen atom in the Boc group attached to the amino terminus. Hydrogen bonds are indicated by dashed lines. Carbonyl oxygens O(7) and O(10) do not participate in hydrogen bonding.

the unusual torsion at Cα(3), where $\phi = -102°$ and $\psi = 15°$. A plot of the torsion angles ϕ and ψ (Fig. 2) shows the large deviation of ϕ and ψ for Leu[3] from the values observed for the other residues that fall near the ideal values for 3_{10}- or α-helices. (iv) The remaining intramolecular hydrogen bonds, N(3)···O(0), N(4)···O(1), and N(6)···O(3) are of the 4→1 type, with the first and third stabilizing β-bends having ϕ and ψ angles typical for a type III bend. The Ala²-Leu³ segment has ϕ and ψ angles more compatible with a type I β-bend. (v) The bend in the helix causes the carbonyl oxygen of Aib¹ to protrude out of the helix and form a hydrogen bond to an additional water molecule, W(2)···O(1). (vi) Water molecule W(1) participates in a third hydrogen bond by being a donor to W(2). The two water molecules create a mini solvation region in very close contact with the peptide helix.

The bulky side groups of Leu³ and Leu⁶, as well as the *tert*-butyl moiety of the Boc blocking group, are directed toward one side of the helix. On the other side, where the water molecule W(1) resides, there are only the Aib and Ala residues and, due to the bend in the helix axis, the CH₃ groups of these residues are directed away from the area of W(1) and W(2). The closest contacts between a C atom in a side chain and the water molecules are 3.34 Å for W(1) and Cβ(5) and
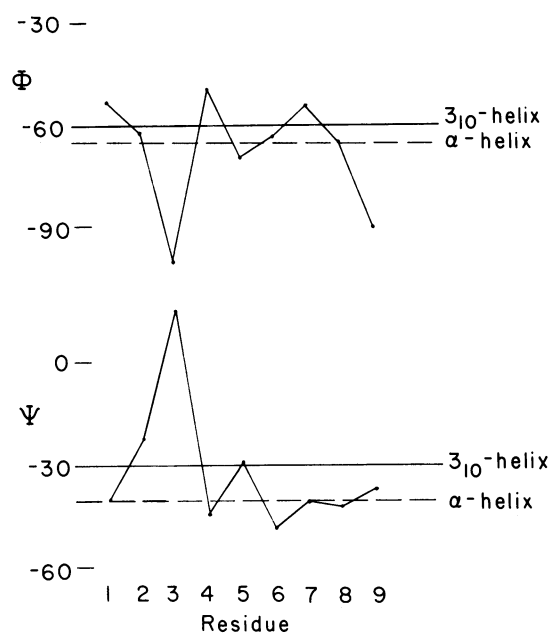

FIG. 2. Deviations of ϕ values (torsions about N—Cα) (upper plot) and ψ values (torsions about Cα—C') (lower plot) from ideal values for 3_{10}-helix ($\phi = -60°$, $\psi = -30°$) and for α-helix ($\phi = -65°$, $\psi = -41°$) [according to Chothia (10)].

3.67 Å for W(2) and Cβ2(4). All other such contacts are >3.8Å.

The torsion angles for the side chains in Leu³ and Leu⁹ have the preferred values ($\chi 1 \approx -60°$; $\chi 2 \approx -60°$, 180°) usually observed for peptides or proteins (11), whereas Leu⁶ has $\chi 1 = 177°$ and $\chi 2 = +61°$, $-167°$ (Table 3). The Ala-Leu-Aib sequence is the same for all three Leu residues.
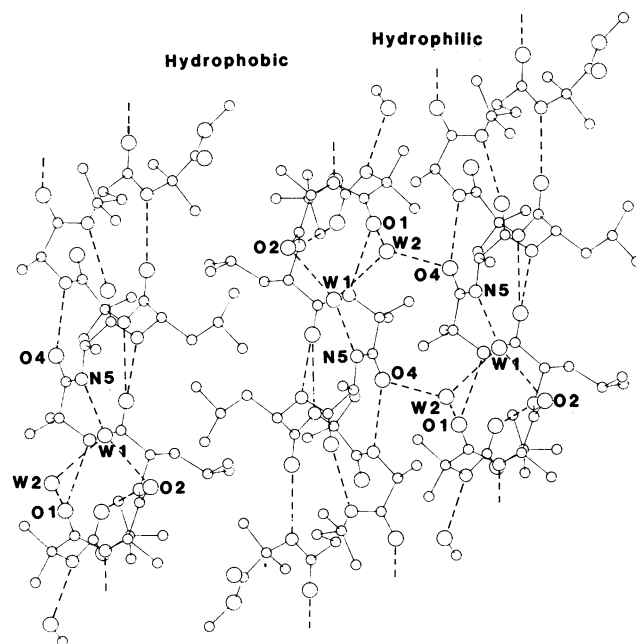

FIG. 3. Antiparallel packing of helical molecules in crystal (three peptide molecules shown). View projected down the *b* axis; the *c* axis (at the left edge and between the center and right molecules) is parallel to the helix axes. The left and center molecules have only hydrophobic contacts. The center and right molecules have hydrophilic contacts in the form of hydrogen bonds to water molecules. In the direction of the helices, there is head-to-tail hydrogen bonding. Hydrogen bonds are indicated by broken lines.
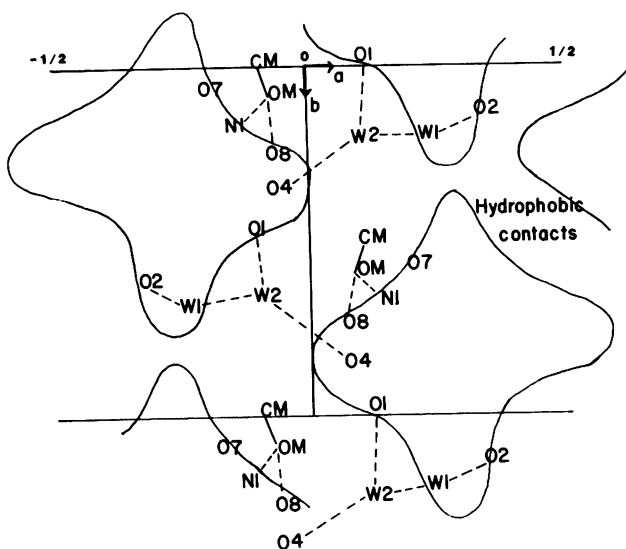
FIG. 4. A schematic drawing of the packing of helices looking down (on the left) and up (on the right) the helix axes. The projection is along the c axis. The locations of the solvent molecules [W(1), W(2), and CH_3OH, where CH_3OH is denoted by CM—OM] and some carbonyl oxygens are indicated. A continuous, undulating solvent channel is formed parallel to the helices. The lower molecules are related to the upper molecules by one cell translation along the b direction. The molecules on the left are related to those on the right by a 2-fold screw operation along the b axis. The broken lines denote hydrogen bonds.

There is a reversal in the sign of the ϕ value for Aib[10], which is not unusual for a terminal Aib residue (4, 5).

Crystal Packing. In the $P2_1$ cell, the helix of the peptide is parallel to the c axis (Fig. 3). Along the helical direction, there is head-to-tail hydrogen bonding between successive molecules. Direct hydrogen bonding occurs between N(2)H and O(9), and the OH group in CH_3OH acts as a bridge between N(1)H and O(8). The hydrogen bond lengths are listed in Table 5.

Due to the 2-fold screw symmetry operation, neighboring helices run in antiparallel directions. Along the middle of the cell, as shown in Fig. 3, between the left and center molecules four leucine side chains, two from each molecule, interdigitate to form very hydrophobic contacts. The hydrophobic contacts are continued along the length of the helices by contacts between $C\beta1(10)$ of one helix and $C\beta(2)$ of another helix, as well as between $C\beta2(7)$ and $C\delta2(3)$. Except for the contact between $C\beta2(7)$ and $C\delta2(3)$, 3.60 Å, all other nearest approaches in the hydrophobic region are ≥3.9 Å.

Along the edge of the cell, as shown in Fig. 3, between the center and right molecules, antiparallel helices make *polar* contacts mediated by the presence of W(1), W(2), and the symmetry-related W(1)' and W(2)' $(2 - x, \frac{1}{2} + y, 1 - z)$. As described above, W(1) is inserted between, and makes hydrogen bonds with, N(5) and O(2) and is the donor to a third hydrogen bond to W(2). In turn, W(2) donates its protons to hydrogen bonds with O(1) and also O(4) of the antiparallel molecule. The carbonyl oxygen of Aib[1] protrudes to the exterior environment by the bending of the helix at Leu[3], and the carbonyl oxygen of Aib[4] is not shielded by neighboring side groups.

Polar Channels. The occurrence of hydrophobic contacts on one side of the helix and polar contacts in the form of hydrogen bonds between backbone atoms and water molecules on the other side gives rise to a helix with *amphiphilic* character, even though all the residues have apolar side chains.

A view of the packing down the c axis (that is, down the helix axes of neighboring peptide molecules; Fig. 4) shows an undulating solvent channel that is continuous in the b and c directions. The channel contains W(1), W(2), and CH_3OH, and is lined with carbonyl oxygens O(1), O(4), O(7), and O(8) and with O(Boc). If the CH_3OH were replaced with a water molecule—that is, if the CH_3 moiety were missing—then an oval channel of 4.5×6.5 Å could be inserted between the centers of the water molecules. The existence in this structure of a solvent channel containing water molecules (and CH_3OH) and being surrounded by intruding carbonyl oxygens is suggestive for a mode of ion transport through membranes by an aggregation of largely hydrophobic membrane-active peptides. A metal ion, upon stimulation from an applied voltage, could successively displace water molecules along its path in such a channel and still participate in transient ligands to carbonyl oxygens or other water molecules at various levels in the channel. The channel found in the present structure is not isolated, but extended and multiple, so that presumably many ions could be accommodated in parallel.

DISCUSSION

The expectation was that Boc-$(Aib$-Ala-$Leu)_3$-Aib-OMe would form an α-helix, or perhaps a mixed 3_{10}-helix/α-helix, like other apolar deca- and undecapeptides that contain several Aib residues (5, 12). The fortuitous insertion of a water molecule into the helix of the present peptide gives rise to a stable, static model of solvation of a helix backbone. The transition from an apolar helix to a potentially amphiphilic structure is brought about by deviations in the backbone conformational angles at a single residue. This structure may perhaps serve as a model for helix–coil transitions (13), solvation-energy calculations (14), or the dynamics of hydrogen exchange (15). Furthermore, the water site may be an attractive region for binding ions and/or participating in ion transport. Voltage gating of helical transmembrane peptide channels by electric field-induced backbone conformational changes, with attendant effects on hydration, must also be considered.

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