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Apolar Peptide Models for Conformational Heterogeneity, Hydration, and Packing of Polypeptide Helices: Crystal Structure of Hepta- and Octapeptides Containing α-Aminoisobutyric Acid

Isabella L. Karle,[1] Judith L. Flippen-Anderson,[1] K. Uma,[2] and P. Balaram[2]
[1]*Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D. C. 20375-5000, and*
[2]*Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*

ABSTRACT **The crystal structures of two helical peptides Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe (VALU-8) have been determined to a resolution of 1.0 and 0.9 Å, respectively. Both the seven and eight residue peptides crystallize with two conformers per asymmetric unit. The VALU-8 conformers are completely helical and differ only at the C-terminus by a sign reversal of the ϕ, ψ angles of the last residue. One of the VALU-7 conformers occurs as a normal α-helix, whereas in the other, the N(7)—-O(3) α-type hydrogen bond is ruptured by the entry of a water molecule (W) into the helix, which in turn makes hydrogen bonds N(7)···W = 2.97 Å and W···O(3) = 2.77 Å. The other side of the water molecule is surrounded by a hydrophobic pocket. These two conformers give a static representation of a step in a possible helix unwinding or folding process. In the VALU-8 crystal the helices aggregate in a parallel mode, whereas the aggregation is antiparallel in the VALU-7 crystal. The crystal parameters are VALU-7, $P2_1$, a = 10.203 (3) Å, b = 19.744 (6) Å, c = 22.561 (6) Å, β = 96.76°, Z = 4, $C_{38}H_{69}N_7O_{10}\cdot 0.5\ H_2O$, R = 6.65% for 3674 reflections observed >3σ(F); and VALU-8, $P2_1$, a = 10.593 (4) Å, b = 27.57 (6) Å, c = 17.745 (5) Å, β = 95.76 (3)°, Z = 4, $C_{42}H_{76}N_8O_{11}\cdot 0.25\ CH_3OH$, R = 6.63% for 4701 reflections observed >3σ(F).**

Key words: hydrophobic α-helices, water insertion into helix, water in hydrophobic pocket, helix unfolding, helix folding, parallel packing

INTRODUCTION

Hydrophobic helical peptides containing α-aminoisobutyric acid (Aib), a naturally occurring residue in peptides such as zervamicin and alamethicin, have been the focus of several investigations,[1–9] Since they serve as models for helical aggregates, which have been implicated in the functioning of alamethicin and related Aib-containing membrane channel-forming polypeptides.[10–14] Recent studies on the structural properties of Aib-containing sequences have also been stimulated by the possibility of using α,α-dialkylated residues as stereochemical directors of polypeptide folding,[15] thereby providing a route to the de novo design of an appropriately folded synthetic protein.[16] Crystal structures of relatively long Aib peptides have provided structural information on 3_{10} and α-helical conformations at atomic resolution and have yielded new and surprising insights into the nature of helix packing in crystals, helix hydration, and conformational heterogeneity in peptide structures.[4–9,16] Parallel helix packing, considered extremely unlikely on the basis of estimates of electrostatic interactions between helix macrodipoles[17–19] has often been observed in crystals of these peptides. The serendipitous discovery of the invasion of a water molecule into the backbone of an apolar helical peptide in Boc-Aib-(Ala-Leu-Aib)$_3$-OMe and the concomitant transformation of a completely hydrophobic sequence into an amphipathic helix in crystals[6] stimulated a search for other apolar sequences where a similar hydration event may occur. In order to provide a systematic base for analysis of helical conformations, helix packing, and hydration, the crystal structure analysis of four peptides, containing a repetition of the -Val-Ala-Leu-Aib- (VALU) tetrad of residues has been completed. The crystal structures of the peptides Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe (VALU-8), both of which contain two independent molecules in the asymmetric unit, are described in this paper. The structures illustrate the effect of Aib residues on helix formation, the facile transition between 4→1 and 5→1 hydrogen bonds near the helix terminii, occurrence of

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Address reprint requests to Isabella L. Karle, Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C., 20375-5000.

Abbreviations used: Aib, α-aminoisobutyric acid (one letter code U); Boc, *tert*-butyloxycarbonyl; -OMe, methyl ester.

TABLE I. Crystal and Diffraction Parameters

	VALU-7	VALU-8
Empirical formula	$C_{38}H_{69}N_7O_{10}\cdot 1/2H_2O$	$C_{42}H_{76}N_8O_{11}\cdot 1/4CH_3OH$
Crystal habit	Colorless plate	Striated plate
Crystal size (mm)	0.8 × 0.4 × 0.1	1.0 × 1.0 × 0.2
Space group	$P2_1$	$P2_1$
Cell parameters (Å)		
a	10.203(3)	10.593(4)
b	19.744(6)	27.571(6)
c	22.561(6)	17.745(5)
β	96.76(2)°	95.76(3)°
Volume (Å^3)	4513(2)	5156(3)
Z	4	4
Molecules/asymmetric unit	2	2
Molecular weight	783.0 + 9.0	869.1 + 8.0
Density (g/cm)	1.166	1.130
F(000)	1720	1906
Radiation (Å)	MoK_α ($\lambda = 0.71069$)	CuK_α($\lambda = 1.54184$)
Temperature (K)	203	273
Resolution (Å)	1.04	0.91
Scan type	2θ-θ	2θ-θ
Scan speed	Variable	Variable
Independent reflections	4385	5525
Observed reflections ($\|F\|>3\sigma(F)$)	3674	4701
Final *R* indices:		
Observed data	R = 6.65%, R_w = 6.68%	R = 6.63%, R_w = 6.45%
All data	R = 8.38%, R_2 = 6.82%	R = 8.21%, R_w = 6.72%
Data/parameter ratio	7.4/1	8.5/1

parallel (VALU-7) and antiparallel (VALU-8) helix packing modes, hydration and conformational distortion (VALU-7), and conformational stability among the family of peptides containing -(Val-Ala-Leu-Aib)$_n$- sequences with n = 1–3.

MATERIALS AND METHODS

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7) and Boc-(Val-Ala-Leu-Aib)$_2$-OMe (VALU-8) were synthesized by conventional solution-phase procedures using a fragment condensation approach.[20] For both peptides, crystals were grown by slow evaporation from a CH_3OH/H_2O solution. For each peptide, X-ray diffraction data were collected from a dry crystal on an automated four-circle diffractometer with a graphite monochromator. Three reflections used as standards, monitored after every 97 measurements, remained constant within 3% in both data collections. Pertinent parameters concerning the data collection and the crystal of each peptide are listed in Table I. At room temperature, crystals of UV-7 scattered only to a limited scattering angle. The intensities at higher scattering angles improved considerably when the crystal was cooled to −70°C by a cold stream of nitrogen.

The structures were solved by a vector search procedure in the PATSEE computer program[21] contained in the SHELX84 package of programs (MicroVAX version of SHELXTL system of programs, Siemens Analytical X-ray Instruments, Madison, Wisconsin). The model used for the search was based on the fragments of the backbone and C$^\beta$ atoms in the Boc-Aib-(Val-Ala-Leu-Aib)$_2$-OMe structure[8] that are in common with the VALU-7 and VALU-8 molecules. After rotation and translation to a correct position for one of the two conformers in each crystal, the remainder of the atoms, including those in the second conformer, were found with the partial structure procedure.[22] In the crystals of both VALU-7 and VALU-8 there are two independent peptide molecules per asymmetric unit, therefore coordinates for twice as many nonhydrogen atoms had to be determined, that is 110 atoms for VALU-7 and 122 atoms for VALU-8.

Full-matrix, anisotropic least-squares refinement was performed on the C, N, and O atoms before hydrogen atoms were added in idealized positions and allowed to ride with the C or N atom to which each was bonded. The least-squares refinement was executed in alternating blocks, each block consisting of the coordinates and thermal parameters for one peptide molecule. One water molecule (for two peptide molecules) was found at full occupancy in the VALU-7 structure. In the VALU-8 crystal, a CH_3OH molecule was found at about one-half occupancy (per two peptide molecules).

Fractional coordinates for C, N, and O atoms are listed in Tables II and III for VALU-7 and VALU-8, respectively.* Torsional angles are listed in Table IV.

*Supplementary material consisting of bond lengths, bond angles, anisotropic thermal parameters, and coordinates for hydrogen atoms will be deposited with the Cambridge Crystallographic Data File. Observed and calculated structure factors are available from I.L.K. or J.F.A.

TABLE II. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$) (VALU-7)

Molecule	x	y	z	U(eq)*
A				
C(1)	2767(11)	7095(6)	3629(5)	42(5)
C(2)	2748(11)	7848(5)	3700(5)	55(5)
C(3)	1374(11)	6836(6)	3456(5)	63(5)
C(4)	3666(11)	6871(6)	3187(4)	52(5)
O	3313(6)	6877(3)	4229(3)	32(3)
C(5)	3413(11)	6222(7)	4362(5)	35(5)
O(0)	2974(7)	5740(4)	4054(3)	47(3)
N(1)	4128(8)	6134(4)	4902(4)	36(3)
$C^{\alpha}(1)$	4369(10)	5457(5)	5137(5)	35(4)
C′(1)	3186(10)	5112(6)	5327(4)	34(4)
O(1)	2955(7)	4513(6)	5231(3)	40(3)
$C^{\beta}(1)$	5511(10)	5478(6)	5680(4)	38(4)
$C^{\gamma}(12)$	6752(11)	5786(8)	5486(6)	91(7)
$C^{\gamma}(11)$	5776(13)	4773(6)	5916(5)	74(6)
N(2)	2393(8)	5497(4)	5620(3)	30(3)
$C^{\alpha}(2)$	1220(11)	5194(6)	5841(5)	43(4)
C′(2)	257(11)	4909(6)	5318(5)	38(5)
O(2)	−210(7)	4345(4)	5338(3)	49(3)
$C^{\beta}(2)$	502(10)	5704(6)	6196(4)	46(4)
N(3)	17(8)	5304(4)	4827(4)	31(3)
$C^{\alpha}(3)$	−879(10)	5038(5)	4322(4)	35(4)
C′(3)	−218(12)	4451(6)	4013(5)	39(5)
O(3)	−914(7)	3961(4)	3801(3)	37(3)
$C^{\beta}(3)$	−1279(10)	5608(5)	3872(5)	40(4)
$C^{\gamma}(3)$	−2406(11)	5423(5)	3392(4)	41(4)
$C^{\delta}(32)$	−2605(12)	6020(6)	2959(5)	62(5)
$C^{\delta}(31)$	−3672(10)	5246(6)	3626(5)	62(5)
N(4)	1104(8)	4493(4)	3985(3)	31(3)
$C^{\alpha}(4)$	1809(10)	3955(6)	3703(4)	33(4)
C′(4)	1635(9)	3274(6)	3989(5)	33(4)
O(4)	1615(9)	2751(4)	3707(3)	67(4)
$C^{\beta}(41)$	3285(9)	4122(6)	3812(4)	42(4)
$C^{\beta}(42)$	1357(10)	3935(6)	3040(4)	53(5)
N(5)	1564(8)	3269(4)	4595(4)	36(3)
$C^{\alpha}(5)$	1443(11)	2604(5)	4892(4)	40(4)
C′(5)	−2(11)	2381(6)	4873(4)	31(4)
O(5)	−260(7)	1801(4)	5003(3)	46(3)
$C^{\beta}(5)$	2132(12)	2604(6)	5550(5)	57(5)
$C^{\gamma}(51)$	1518(13)	3082(6)	5959(5)	65(6)
$C^{\gamma}(52)$	3598(11)	2751(7)	5531(6)	88(7)
N(6)	−902(9)	2849(5)	4694(3)	38(4)
$C^{\alpha}(6)$	−2298(11)	2678(6)	4589(5)	40(5)
C′(6)	−2721(12)	2320(6)	4000(5)	39(5)
O(6)	−3485(8)	1853(5)	3959(3)	68(4)
$C^{\beta}(6)$	−3166(11)	3318(6)	4633(5)	61(5)
N(7)	−2138(9)	2582(5)	3545(4)	48(4)
$C^{\alpha}(7)$	−2526(13)	2326(7)	2920(5)	66(6)
C′(7)	−1351(23)	2110(11)	2638(7)	147(10)
O(7)	−1695(12)	1705(8)	2198(6)	159(7)
$C^{\beta}(7)$	−3199(11)	2901(6)	2537(5)	54(5)
$C^{\gamma}(7)$	−4550(12)	3128(6)	2722(5)	55(5)
$C^{\delta}(71)$	−5017(13)	3771(6)	2373(6)	80(6)
$C^{\delta}(72)$	−5599(13)	2581(7)	2670(7)	98(7)
O(8)	−327(10)	2058(7)	2891(5)	102(5)
C(8)	810(12)	1820(7)	2649(5)	75(6)
B				
C(1)	8486(11)	4737(6)	1275(5)	45(5)
C(2)	8697(16)	3988(6)	1187(6)	103(7)
C(3)	9424(12)	5002(8)	1800(5)	88(6)
C(4)	7096(12)	4878(7)	1377(6)	78(6)
O	8805(7)	5041(4)	720(3)	50(3)

(*continued*)

TABLE II. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$) (VALU-7) (*Continued*)

Molecule	*x*	*y*	*z*	*U*(eq)*
C(5)	8705(10)	5718(6)	620(5)	30(5)
O(0)	8280(8)	6117(4)	951(3)	50(3)
N(1)	9090(8)	5850(4)	91(4)	33(3)
C^{α}(1)	9180(10)	6552(5)	−107(5)	37(4)
C′(1)	7807(11)	6890(7)	−245(5)	35(5)
O(1)	7592(8)	7465(4)	−83(3)	54(3)
C^{β}(1)	9921(11)	6594(5)	−656(4)	40(4)
C^{γ}(11)	9990(14)	7309(6)	−885(5)	79(6)
C^{γ}(12)	11348(12)	6317(7)	−506(6)	74(6)
N(2)	6866(9)	6522(5)	−571(3)	42(4)
C^{α}(2)	5621(11)	6831(6)	−779(5)	47(5)
C′(2)	4921(12)	7007(7)	−243(5)	43(5)
O(2)	4346(8)	7557(4)	−218(3)	57(3)
C^{β}(2)	4821(12)	6393(6)	−1218(5)	65(5)
N(3)	4897(8)	6556(4)	195(4)	33(3)
C^{α}(3)	4253(11)	6711(5)	722(5)	38(4)
C′(3)	5011(14)	7257(6)	1092(5)	44(5)
O(3)	4359(7)	7703(4)	1333(3)	46(3)
C^{β}(3)	4134(11)	6075(6)	1078(5)	42(5)
C^{γ}(3)	3386(13)	6152(6)	1632(5)	53(5)
C^{δ}(31)	3396(13)	5486(6)	1960(5)	70(6)
C^{δ}(32)	2007(11)	6420(7)	1481(6)	75(6)
N(4)	6351(9)	7250(4)	1147(3)	37(4)
C^{α}(4)	7094(11)	7748(5)	1527(4)	37(4)
C′(4)	6706(10)	8471(6)	1354(5)	34(4)
O(4)	6664(8)	8919(4)	1730(3)	51(3)
C^{β}(41)	6923(12)	7621(6)	2183(4)	64(5)
C^{β}(42)	8579(12)	7664(6)	1424(5)	63(5)
N(5)	6478(8)	8596(4)	767(4)	31(3)
C^{α}(5)	6238(9)	9290(5)	546(4)	27(4)
C′(5)	4775(12)	9461(7)	475(5)	43(5)
O(5)	4429(7)	10055(4)	311(3)	49(3)
C^{β}(5)	6854(11)	9423(6)	−28(5)	47(5)
C^{γ}(51)	6193(12)	9019(6)	−561(5)	62(5)
C^{γ}(52)	8317(11)	9306(6)	59(6)	67(6)
N(6)	3940(9)	9015(5)	619(4)	39(4)
C^{α}(6)	2512(10)	9160(5)	566(4)	38(4)
C′(6)	2171(12)	9640(6)	1033(5)	40(5)
O(6)	1245(8)	10031(4)	932(3)	62(3)
C^{β}(6)	1773(11)	8483(6)	601(5)	65(5)
N(7)	2843(9)	9605(4)	1587(4)	40(4)
C^{α}(7)	2526(10)	10043(6)	2082(5)	43(4)
C′(7)	3774(13)	10342(7)	2411(6)	51(6)
O(7)	3756(9)	10838(5)	2720(5)	87(4)
C^{β}(7)	1760(10)	9657(6)	2525(4)	46(4)
C^{γ}(7)	371(10)	9412(6)	2272(5)	43(4)
C^{δ}(71)	−166(12)	8988(6)	2744(5)	73(6)
Cδ(72)	−530(11)	10000(7)	2085(5)	62(5)
O(8)	4815(9)	10002(6)	2342(4)	90(4)
C(8)	6082(12)	10235(8)	2653(6)	108(8)
W	4302(24)	8535(11)	2320(9)	324(15)

*Equivalent isotropic *U* defined as one-third of the trace of the orthogonalized U_{ij} tensor.

RESULTS AND DISCUSSION

Hydration of VALU-7 Peptide

Diagrams of the two conformers of UV-7, drawn by computer using the experimentally determined coordinates, are shown in Figure 1 superimposed with a least-squares fit of backbone atoms N(1) to C^{α}(5). The two conformers are essentially identical from the Boc terminus to C^{α}(5) with a rms deviation of 0.09 Å for the backbone atoms. Beginning with C^{α}(5), the α-helix present in conformer A is spread open in conformer B with the rupture of the N(7)H···O(3) hydrogen bond and the accommodation of a water molecule, W. In conformer B, the water molecule participates in strong hydrogen bonding as an acceptor from N(7)H and a donor to O(3) and O(4). The hydrogen bond lengths are tabulated in TAble V.

It is interesting to note that aside from the hydro-

TABLE III. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$) (VALU-8)

Molecule	x	y	z	U(eq)*
A				
C(1)	1416(11)	8001(3)	9827(5)	76(5)
C(2)	583(11)	7594(4)	9423(6)	117(6)
C(3)	1643(10)	7897(3)	10636(5)	91(5)
C(4)	746(11)	8489(3)	9688(6)	123(6)
C(5)	3466(11)	7714(3)	9389(5)	59(4)
O	2615(7)	8064(2)	9469(3)	73(3)
O(0)	3303(6)	7279(2)	9532(3)	63(2)
N(1)	4469(7)	7892(2)	9095(3)	50(3)
$C^{\alpha}(1)$	5327(9)	7563(2)	8768(4)	49(3)
C′(1)	5929(8)	7191(2)	9322(4)	48(3)
O(1)	6040(6)	6769(2)	9140(3)	61(2)
$C^{\beta}(1)$	6375(8)	7847(3)	8414(4)	53(3)
$C^{\gamma}(12)$	5806(9)	8168(3)	7758(4)	79(4)
$C^{\gamma}(11)$	7359(9)	7507(3)	8120(5)	78(4)
N(2)	6373(7)	7351(2)	10034(3)	60(3)
$C^{\alpha}(2)$	6975(9)	7007(3)	10562(4)	62(4)
C′(2)	6083(12)	6601(3)	10756(4)	55(4)
O(2)	6539(6)	6183(2)	10893(3)	81(3)
$C^{\beta}(2)$	7559(10)	7262(3)	11267(5)	97(5)
N(3)	4917(9)	6712(2)	10804(3)	54(3)
$C^{\alpha}(3)$	3999(10)	6329(3)	10939(4)	54(4)
C′(3)	3816(9)	5981(3)	10269(4)	56(4)
O(3)	3818(6)	5537(2)	10373(3)	65(2)
$C^{\beta}(3)$	2735(10)	6568(3)	11083(5)	65(4)
$C^{\gamma}(3)$	1652(13)	6217(4)	11197(6)	96(5)
$C^{\delta}(31)$	1905(12)	5916(4)	11873(6)	134(7)
$C^{\delta}(32)$	447(12)	6493(4)	11214(7)	138(7)
N(4)	3600(6)	6183(2)	9583(3)	50(3)
$C^{\alpha}(4)$	3377(10)	5901(3)	8877(4)	55(4)
C′(4)	4414(12)	5505(3)	8870(4)	53(4)
O(4)	4122(6)	5083(2)	8704(3)	69(2)
$C^{\beta}(41)$	2091(11)	5665(3)	8850(5)	92(5)
$C^{\beta}(42)$	3408(9)	6251(3)	8220(4)	67(4)
N(5)	5598(9)	5663(2)	8995(3)	53(3)
$C^{\alpha}(5)$	6668(10)	5330(3)	8908(5)	64(4)
C′(5)	6786(9)	4955(3)	9539(5)	60(4)
O(5)	7204(7)	4548(2)	9399(3)	95(3)
$C^{\beta}(5)$	7913(11)	5594(3)	8822(6)	88(5)
$C^{\gamma}(52)$	7766(12)	5927(3)	8144(6)	119(6)
$C^{\gamma}(51)$	8434(12)	5859(4)	9477(7)	132(7)
N(6)	6500(7)	5077(2)	10231(3)	52(3)
$C^{\alpha}(6)$	6613(9)	4730(3)	10862(4)	57(3)
C′(6)	5613(11)	4340(2)	10792(4)	51(4)
O(6)	5799(6)	3957(2)	11122(3)	66(2)
$C^{\beta}(6)$	6591(10)	4996(3)	11600(4)	81(4)
N(7)	4521(8)	4438(2)	10379(3)	49(3)
$C^{\alpha}(7)$	3478(10)	4087(3)	10281(4)	54(4)
C′(7)	3552(9)	3751(3)	9595(4)	53(4)
O(7)	2884(6)	3381(2)	9555(3)	63(2)
$C^{\beta}(7)$	2206(9)	4339(3)	10234(4)	55(4)
$C^{\gamma}(7)$	1883(12)	4600(4)	10945(7)	89(5)
$C^{\delta}(72)$	2007(13)	4306(4)	11637(6)	141(7)
$C^{\delta}(71)$	588(11)	4824(4)	10782(7)	119(6)
N(8)	4221(7)	3903(2)	9048(3)	57(3)
$C^{\alpha}(8)$	4250(9)	3691(3)	8305(4)	50(3)
C′(8)	4872(9)	3202(3)	8379(5)	63(4)
O(8)	5265(6)	2990(2)	8923(3)	82(3)
$C^{\beta}(81)$	5070(11)	4027(3)	7873(5)	105(5)
$C^{\beta}(82)$	2952(9)	3662(3)	7881(4)	75(4)
O(9)	4920(8)	3015(2)	7667(3)	102(3)
C(9)	5671(13)	2580(3)	7618(5)	135(7)
B				
C(1)	1614(13)	8372(3)	6807(6)	87(5)
C(2)	2575(12)	8373(4)	6298(6)	134(7)
C(3)	1702(11)	7915(3)	7274(5)	108(5)
C(4)	1725(13)	8826(3)	7256(6)	133(6)
C(5)	−115(11)	8081(3)	5873(5)	68(4)

(*continued*)

TABLE III. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$) (VALU-8) (*Continued*)

Molecule	*x*	*y*	*z*	*U*(eq)*
O	424(7)	8421(2)	6312(3)	84(3)
O(0)	325(7)	7665(2)	5826(3)	74(3)
N(1)	−1158(8)	8241(2)	5472(3)	59(3)
C^{α}(1)	−2070(9)	7903(3)	5117(4)	59(4)
C′(1)	−1512(10)	7574(3)	4544(5)	60(4)
O(1)	−1813(6)	7144(2)	4516(3)	80(3)
C^{β}(1)	−3280(9)	8192(3)	4771(5)	69(4)
C^{γ}(12)	−3945(10)	8433(4)	5406(6)	109(5)
C^{γ}(11)	−4140(11)	7873(4)	4268(6)	107(5)
N(2)	−813(7)	7774(2)	4047(3)	61(3)
C^{α}(2)	−392(10)	7462(3)	3454(4)	70(4)
C′(2)	549(11)	7074(3)	3785(6)	86(5)
O(2)	644(9)	6705(3)	3437(4)	146(4)
C^{β}(2)	177(12)	7748(3)	2873(4)	101(5)
N(3)	1268(7)	7179(2)	4430(4)	61(3)
C^{α}(3)	2079(9)	6795(3)	4799(5)	61(4)
O(3)	1428(6)	5985(2)	5103(3)	72(3)
C^{β}(3)	3068(9)	7020(3)	5383(5)	72(4)
C^{γ}(3)	3965(9)	6669(3)	5815(6)	72(4)
C^{δ}(31)	4754(10)	6392(3)	5292(7)	109(6)
C^{δ}(32)	4791(11)	6910(4)	6457(6)	119(6)
N(4)	376(7)	6603(2)	5584(4)	55(3)
C^{α}(4)	−437(10)	6284(3)	5999(5)	65(4)
C′(4)	−1054(10)	5903(3)	5467(5)	72(4)
O(4)	−1153(7)	5471(2)	5658(3)	91(3)
C^{β}(41)	−1505(9)	6604(3)	6286(5)	78(4)
C^{β}(42)	389(9)	6050(3)	6654(5)	77(4)
N(5)	−1554(7)	6050(2)	4781(4)	73(3)
C^{α}(5)	−2297(13)	5719(4)	4283(6)	106(5)
C′(5)	−1471(15)	5308(4)	3966(6)	93(6)
O(5)	−1929(10)	4915(3)	3778(5)	152(5)
C^{β}(5)	−3146(18)	6033(8)	3676(13)	219(13)
C^{γ}(51)	−2289(14)	6143(5)	3046(7)	142(7)
C^{γ}(52)	−4089(19)	5773(6)	3425(9)	262(15)
N(6)	−273(12)	5396(3)	3980(4)	85(4)
C^{α}(6)	622(13)	5042(3)	3745(5)	93(5)
C′(6)	979(10)	4653(3)	4323(5)	71(4)
O(6)	1467(7)	4267(2)	4157(3)	86(3)
C^{β}(6)	1874(14)	5277(4)	3540(6)	161(8)
N(7)	875(7)	4775(2)	5041(3)	60(3)
C^{α}(7)	1358(10)	4469(3)	5687(4)	67(4)
C′(7)	301(12)	4208(3)	6042(4)	63(4)
O(7)	491(7)	3973(2)	6289(3)	78(3)
C^{β}(7)	2138(10)	4752(3)	6281(4)	64(4)
C^{γ}(7)	3285(12)	5001(4)	5999(6)	99(5)
C^{δ}(72)	4162(12)	4721(5)	5528(6)	148(7)
C^{δ}(71)	4086(11)	5284(3)	6633(6)	104(5)
N(8)	−767(9)	4457(2)	6094(4)	76(4)
C^{α}(8)	−1817(11)	4264(3)	6474(6)	72(4)
C′(8)	−1436(12)	4051(4)	7234(6)	80(5)
O(8)	−1885(8)	3694(2)	7501(4)	105(3)
C^{β}(81)	−2529(12)	3899(4)	5978(6)	129(6)
C^{β}(82)	−2714(9)	4690(3)	6593(6)	106(5)
O(9)	−549(8)	4329(3)	7609(4)	98(3)
C(9)	−221(12)	4175(5)	8415(6)	146(7)
C′(3)	1227(11)	6427(3)	5170(5)	69(4)
OM†	5838(28)	4515(8)	4033(18)	264(18)
CM†	4651(61)	4523(17)	3466(21)	556(33)

*Equivalent isotropic *U* defined as one-third of the trace of the orthogonalized U_{ij} tensor.

gen bands and O(4) and O(OMe), which are 2.98 and 2.94 Å, respectively from W, all on one side of the water molecule, the remainder of the pocket containing the water molecule is very hydrophobic, lined with methyl groups from Leu-3, Aib-4, and Leu-7 (Figs. 1 and 2). The closest approaches to W are from C^{β}(4) at 3.27 Å and from C^{β}(7) at 3.48 Å. The water pocket is closed with the hydrophobic ter-

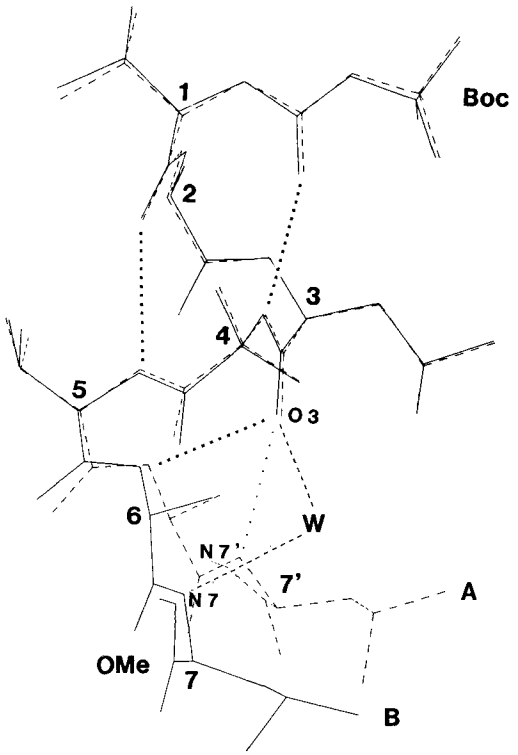

Fig. 1. The two cocrystallized conformers of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7) are shown superimposed with a least-squares fit of backbone atoms N(1) to $C^{\alpha}(5)$, conformer A (dashed line) and conformer B (solid line). The C^{α} atoms are labeled 1–7. Heavy dotted lines indicate NH···OC hydrogen bonds common to both conformers. The light dotted line indicates an N(7)···O(3) hydrogen bond (α-helix type) in conformer A which is broken by the invasion of a water molecule W into conformer B. The dashed lines indicate hydrogen bonds W···O(3) and N(7)···W in conformer B.

tiary butyl group from a neighboring molecule of conformer A, where the closest distances to W from C(2) and C(4) of Boc are 3.90 and 3.92 Å (Fig. 2).

Thus, the water molecule has not only penetrated into the helix, but has done so in a highly hydrophobic environment. The intimate association of the anhydrous, α-helical conformer A and the hydrated conformer B in the same crystal suggests a relatively facile conversion from one form to the other. The hydrated form may be an intermediate in the denaturing process or in the folding process. An examination of the torsional angles for both conformers of VALU-7 shows that the average values of φ and ψ for residues 1, 2, 3, 4, and 6 are −67° and −40°, respectively. These values compare closely with the idealized values for a right-handed α-helix, −65° and −41°.[23] The complete conformational change for the insertion of water occurs solely at $C^{\alpha}(5)$ where the φ and ψ values become −91° and +2°, respectively. However, in the unhydrated conformer A, the φ and ψ values for residue 5, −87° and −11°, are already distorted from the idealized values [the N(6)···O(2) distance is 3.33 Å, as compared to ~3.0 Å for normal 5→1 hydrogen bonds], as if in preparation for the invasion of water. Helix hydration by interposing a water molecule between CO(i) and NH($i+3$) has been observed also in the sequences Boc-Aib-(Ala-Leu-Aib)$_3$-OMe (6) and Boc-(Ala-Leu-Aib)$_2$-OMe.[24]

Conformational Stability in Crystals

The VALU-8 peptide differs in sequence from the VALU-7 only by the addition of an Aib residue at the C terminus. The two conformers of VALU-8 occurring in the crystal are shown in Figure 3. The conformations are quite similar, as manifested by a comparison of their torsional angles (Table IV), except for the helix reversal at $C^{\alpha}(8)$. Such a helix reversal at an Aib residue near the terminus has been noted previously both for short peptides, up to 5 residues,[1,25] and for longer peptides.[4]

In order to study the conformational effect of length of peptide and the role of Aib residues next to the Boc or OMe end groups, crystal structures were determined of the following series of peptides:

(VALU-13) Boc*Aib*ValAlaLeu*Aib*ValAlaLeu*Aib*ValAlaLeu*Aib*---OMe[16]

(VALU-9) Boc*Aib*ValAlaLeu*Aib*ValAlaLeu*Aib*----------OMe[8]

(VALU-8) Boc-----ValAlaLeu*Aib*ValAlaLeu*Aib*----------OMe

(VALU-7) Boc-----ValAlaLeu*Aib*ValAlaLeu----------OMe

Each of the peptides is helical, primarily having 5→1 type hydrogen bonds. The closeness of conformational fit is illustrated in Figure 4A–C, where the pairs of superpositions of VALU-13/VALU-9, VALU-13/VALU-8A,and VALU-13/VALU-7A are shown, respectively. The diagrams were made with a least-squares fit of backbone atoms with terminal groups excluded. The least-squares fit of backbone atoms common to both peptides has a rms deviation of 0.21 Å for VALU-13/VALU-9, 0.22 Å for VALU-13/VALU-8 (conformer A), and 0.33 Å for VALU-13/VALU-7 (conformer A). As discussed above, VALU-8 conformers A and B are very similar except for helix reversal at $C^{\alpha}(8)$ and VALU-7 conformers A and B are almost identical for residues 1–5 (Fig. 1). Side chains were not included in the least-squares fit; nevertheless, their conformations also are quite similar in the various structures. Shortening the sequence or stripping Aib groups at either or both ends has little effect on the helical conformation of this series of peptides in the solid state.

TABLE IV. Torsional Angles (Degree)*,†

Residue	Angle	VALU-7A	VALU-7B	VALU-8A	VALU-8B
Val-1	ϕ	−71	−70	−60	−60
	ψ	−41	−45	−44	−47
	ω	−178	−172	−179	−174
	χ^1	−179	178	175	169
		−57	−61	−63	−65
Ala-2	ϕ	−61	−65	−62	−66
	ψ	−45	−45	−36	−28
	ω	179	179	175	174
Leu-3	ϕ	−71	−68	−66	−73
	ψ	−35	−38	−49	−48
	ω	180	−177	−179	−176
	χ^1	−169	−176	177	180
	χ^2	61	57	64	62
		−176	−177	−172	−172
Aib-4	ϕ	−57	−56	−48	−51
	ψ	−35	−40	−52	−45
	ω	−178	−173	−172	−172
Val-5	ϕ	−87	−91	−70	−70
	ψ	−11	2	−34	−21
	ω	173	179	179	176
	χ^1	64	67	66	85
		−61	−58	−59	−157
Ala-6	ϕ	−78	−73	−72	−78
	ψ	−41	−36	−25	−26
	ω	−175	−177	−179	−171
Leu-7	ϕ	−127	−134	−89	−105
	ψ	10	−22	−22	−38
	ω	176	−178	−169	−175
	χ^1	−67	−65	−66	−59
	χ^2	171	174	180	−179
		−62	−63	−52	−47
Aib-8	ϕ			−68	48
	ψ			−178	41
	ω			170	173

*The torsion angles for rotation about bonds of the peptide backbone (ϕ,ψ,ω) and about bonds of the side chains (χ) follow the conventions suggested by the IUPAC-IUB Commission on Biochemical Nomenclature.[32]
†Estimated SD ~0.6°.

Hydrogen Bonds

Both VALU-7 and VALU-8 crystallize with columns of helical peptides formed by head-to-tail hydrogen bonds directly between NH and C=O moieties (Fig. 3). Each column contains conformers of only a single type. Values for the intermolecular hydrogen bonds are shown in Tables V and VI.

Intramolecular hydrogen bonds in the helices are very sensitive to small changes in conformational angles that produce changes in N···O and NH···O distances and in C=O···N angles. The helix in VALU-7 conformer A is primarily an α-helix. The aberration in the ϕ and ψ angles at $C^{\alpha}(5)$, preliminary to hydration, vide supra, has increased the N(6)···O(2) distance to 3.33 Å, a long value for the expected 5→1 type hydrogen bond and has brought the N(6)···O(3) distance to 2.98 Å to form a 4→1 type bond. After hydration in conformer B, the N(6)···O(2) distance is increased even further to 3.49 Å, whereas the 4→1 type bond between N(6)···O(3) remains with a distance of 3.05 Å (see Table V).

VALU-8 conformer A is also primarily an α-helix. In addition to five 5→1 type hydrogen bonds, there may be a 4→1 type bond between N(3) and O(0). The carbonyl O(0) already is an acceptor for a 5→1 type bond from N(4). The N(3)H···O(0) distance is rather long with a value of 2.55 Å if the N-H bond is fixed at 0.96 Å (Table VI). In addition, the N(8)···O(4) distance is also rather long at 3.31 Å. In conformer B of VALU-8, there is some hydrogen bond switching as compared to conformer A. Two of the 5→1 type bonds have become 4→1 type, that is N(6)···O(3) [instead of N(6)···O(2) which is quite long in conformer A] and N(7)···O(4) instead of N(7)···O(3). The N(8)···O(4) distance in conformer B has improved with a value of 2.92 Å.

There is one molecule of CH_3OH cocrystallized per two molecules of VALU-8. It seems to play the role of filling a void. The OH of the methanol forms a hydrogen bond with O(5) in conformer B.

Parallel Versus Antiparallel Packing of Helices

In the present series of peptides, the helices of VALU-13[16] and VALU-7 (Fig. 2) associate so that the helix axis directions are antiparallel to each

TABLE V. Hydrogen Bonds in Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7)

Type	Donor	Acceptor	N···O (Å)	H†···O (Å)	Angle (deg.) C=O···N
			Conformer A*		
Head-to-tail	N(1)	0(6)‡	3.074	2.48	119
	N(2)	0(6)‡	3.011	2.09	116
	N(3)	O(5)‡	2.987	2.14	161
5→1	N(4)	O(0)	3.107	2.24	145
5→1	N(5)	O(1)	3.104	2.28	157
4→1	N(6)	O(3)	2.978	2.27	111
5→1	N(7)	O(3)	3.022	2.15	165
			Conformer B*		
Head-to-tail	N(1)	O(6)§	2.806	1.98	122
	N(2)	O(5)§	3.266	*2.65*	151
	N(3)	O(5)§	3.279	2.40	151
5→1	N(4)	O(0)	3.045	2.18	149
5→1	N(5)	O(1)	3.236	2.36	155
4→1	N(6)	O(3)	3.053	2.15	114
Water insertion	N(7)	W	2.972	2.17	
	W	O(3)	2.774		145**

*O(2) and O(4) do not participate in hydrogen bonding.
†NH bond length fixed at 0.96 Å.
‡Symmetry operation $-x, +1/2+y, 1-z$.
§Symmetry operation $1-x, -1/2+y, -z$.
**C=O···W angle.

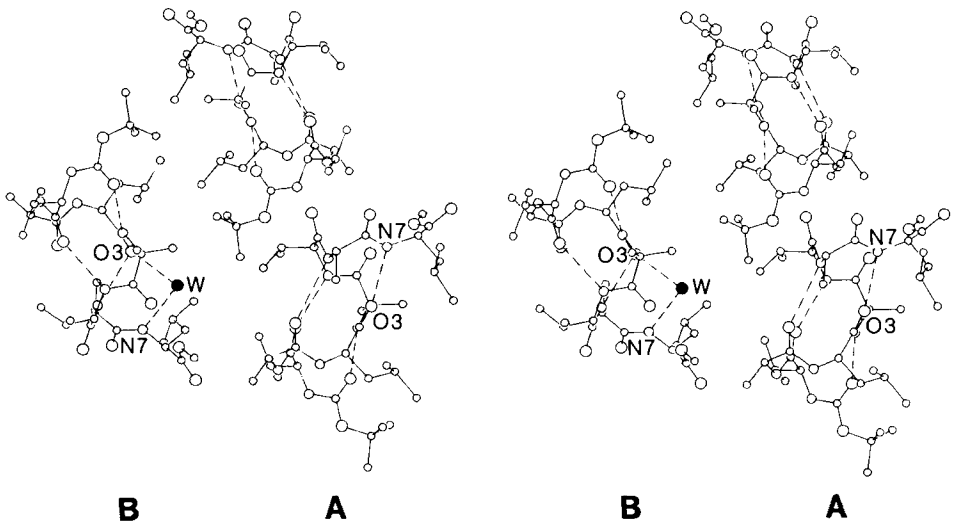

Fig. 2. Stereoview of hydrophobic packing environment around water molecule W in VALU-7. Two molecules of conformer A related by a vertical 2-fold screw axis are shown. Helices of all conformer A molecules are directed approximately antiparallel to helices of all conformer B molecules.

other. By contrast, in VALU-9[8] and VALU-8 (Fig. 3) the helix axis directions of all the peptides in the cell are parallel. Peptide helices possess appreciable macrodipole moments[26–28] and antiparallel helix packing in proteins has been rationalized by invoking helix dipole–dipole interactions as an important determinant in protein folding.[27] Electrostatic energy calculations for helical peptides packed in crystals have led to the conclusion that antiparallel helix packing is overwhelmingly favored.[19] However, recent crystal structure determinations of hydrophobic peptide helices have provided several examples of parallel packing of helices in crystals.[4,5,7,8] It is pertinent to note that a recent theoretical analysis of assembling a four-helix bundle with antiparallel orientations of adjacent helices suggests that such an arrangement may indeed be *destabilized* by helix dipole interactions.[29]

Solution Conformations

In solution, NMR studies suggest that both peptides adopt helical conformations in chloroform but

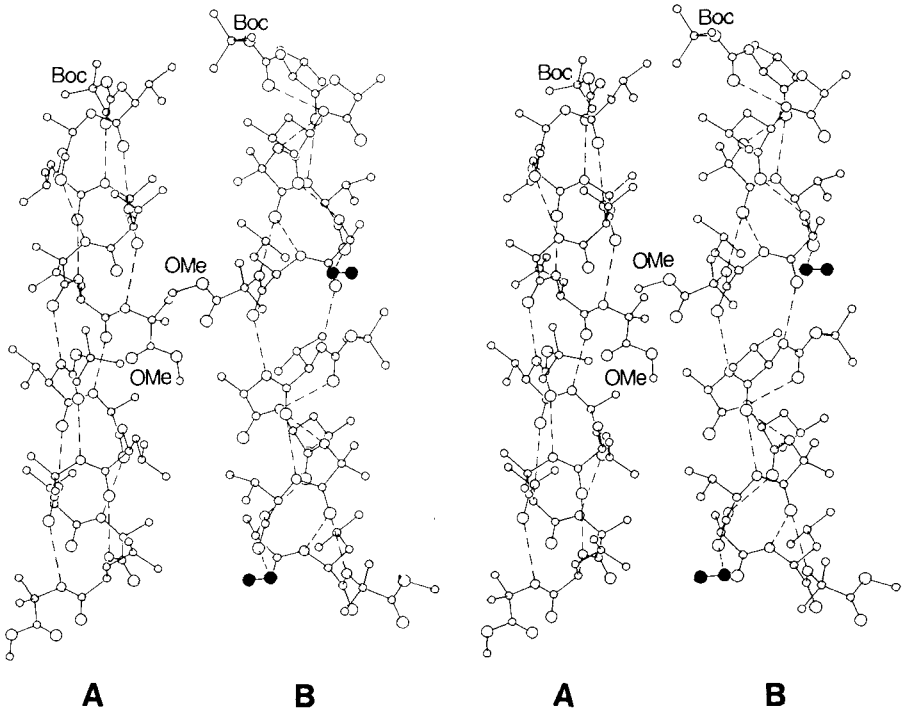

Fig. 3. Crystal packing of two cocrystallized conformers of Boc-(Val-Ala-Leu-Aib)$_2$-OMe (VALU-8). Conformer A forms columns in the *b* direction (vertical) by head-to-tail hydrogen bonding. Conformer B forms similar columns that alternate with columns of conformer A. All the helices pack in a parallel mode. The cocrystallized CH_3OH molecule is shown shaded.

TABLE VI. Hydrogen Bonds in Boc-(Val-Ala-Leu-Aib)$_2$-OMe (VALU-8)

			Conformer A*		
Type	Donor	Acceptor	N···O (Å)	H†···O (Å)	Angle (deg.) C=O···N
Head-to-tail	N(1)	O(6)‡	2.973	2.07	142
	N(2)	O(7)‡	3.018	2.14	129
4→1	N(3)	O(0)	3.112	*2.55*	124
5→1	N(4)	O(0)	3.039	2.08	162
5→1	N(5)	O(1)	3.091	2.16	161
5→1	N(6)	O(2)	3.266	2.39	142
5→1	N(7)	O(3)	3.121	2.32	163
5→1	N(8)	O(4)	*3.308*	*2.67*	152
			Conformer B§		
Head-to-tail	N(1)	O(6)**	2.930	2.05	136
	N(2)	O(7)**	2.898	1.99	140
4→1	N(3)	O(0)	3.070	2.46	128
5→1	N(4)	O(0)	2.960	2.01	159
5→1	N(5)	O(1)	3.059	2.16	158
4→1	N(6)	O(3)	3.023	2.26	119
4→1	N(7)	O(4)	3.158	2.30	114
5→1	N(8)	O(4)	2.920	1.98	167
Solvent-peptide	OM	O(5)	2.689		130††

*O(5) does not participate in hydrogen bonding.
†NH bond length fixed at 0.96 Å.
‡Symmetry operation $1-x, 1/2+y, 2-z$.
§O(2) and O(8) do not participate in hydrogen bonding.
**Symmetry operation $-x, 1/2+y, 1-z$.
††C=O···OM (methanol) angle.

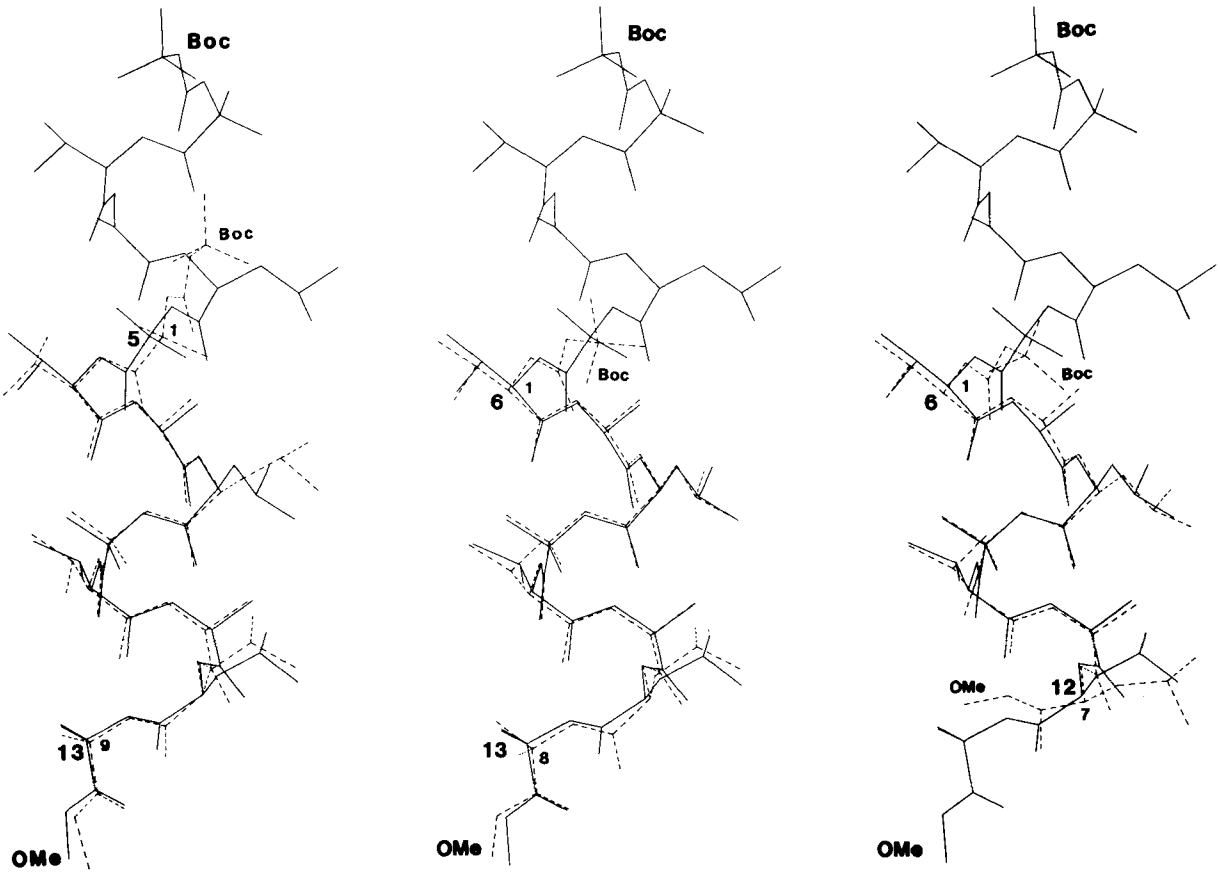

Fig. 4. Comparisons of conformations of VALU-13[16] (solid line) with VALU-9[8] (dashed line) in (A), with VALU-8 conformer A (dashed line) in (B), and with VALU-7 conformer A (dashed line) in (C). The pairs of peptides were superimposed by a least-squares fit of backbone atoms common to both peptides.

the helix in VALU-7 is appreciably more fargile in dimethyl sulfoxide as compared to VALU-8 (Balaram and Uma, to be published). Greater backbone distortions from ideal helical conformations are also noted for VALU-7 in crystals at the Val(5) residue.

CONCLUSIONS

The crystal structures of the peptides Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (VALU-7) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-OMe (VALU-8) provide a glimpse at high resolution of the helical conformations favored by these apolar sequences. The presence of both hydrated and unhydrated molecules in the VALU-7 crystal may be viewed as a static representation of a step in the helix unwinding or folding process. One conformer is completely helical while the other opens its helix by scission of a hydrogen bond and permits entry of a water molecule into the distorted helix. The coexistence in the crystal of both hydrated and normal helical forms indicates the approximate equality of the stability of both forms, thus pointing to a possible pathway for helix unfolding. The structures reiterate the important role of Aib residues in nucleating helical structures.[6,8–10,15,30] More importantly, the conformation of VALU-7 in crystals suggests that even a single Aib residue, centrally positioned in a heptapeptide, can strongly stabilize helical folding. Replacement of C^{α} hydrogen atoms at strategic positions in a polypeptide chain by methyl groups may thus prove a powerful way of engineering stability into helical structures. Incorporation of α,α-dialkyl amino acids into proteins appears to be an attractive possibility. While chemical synthesis appears to be the only readily available route to such a goal at present, the development of methodology for the "genetically coded" incorporation of unnatural (nonprotein) amino acids holds great promise for the future.[31]

ACKNOWLEDGMENTS

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