

S. Aravinda¹
N. Shamala¹
Chittaranjan Das²
P. Balaram²

¹ Department of Physics,
Indian Institute of Science,
Bangalore- 560 012, India

² Molecular Biophysics Unit,
Indian Institute of Science,
Bangalore- 560 012, India

Received 30 October 2001;
accepted 16 April 2002

Structural Analysis of Peptide Helices Containing Centrally Positioned Lactic Acid Residues

Abstract: The effect of insertion of lactic acid (Lac) residues into peptide helices has been probed using specifically designed sequences. The crystal structures of 11-residue and 14-residue depsipeptides Boc-Val-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (**1**) and Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (**3**), containing centrally positioned Lac residues, have been determined. The structure of an 11-residue peptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (**2**), analog of a which is an amide previously determined Lac-containing depsipeptide, Boc-Val-Ala-Leu-Aib-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe I. L. Karle, C. Das, and P. Balaram, *Biopolymers*, Vol. 59, (2001) pp. 276–289], is also reported. Peptide **1** adopts a helical fold, which is stabilized by mixture of 4→1 and 5→1 hydrogen bonds. Peptide **2** adopts a completely α -helical conformation stabilized by eight successive 5→1 hydrogen bonds. Peptide **3** appears to be predominately α -helical, with seven 5→1 hydrogen bonds and three 4→1 interaction interspersed in the sequence. In the structure of peptide **3** in addition to water molecules in the head-to-tail region, hydration at an internal segment of the helix is also observed. A comparison of five related peptide helices, containing a single Lac residue, reveals that the hydroxy acid can be comfortably accommodated at interior positions in the helix, with the closest C=O...O distances lying between 2.8 and 3.3 Å. © 2002 Wiley Periodicals, Inc. *Biopolymers* 64: 255–267, 2002

Keywords: 4→1/5→1 hydrogen bonds; depsipeptides; α -helix; lactic acid; x-ray crystal structures

INTRODUCTION

The insertion of α -hydroxy acids into peptide sequences provides a method for evaluating the impor-

tance of specific hydrogen bonds in the stabilization of peptide secondary structures. The field of polypeptide stereochemistry has long been influenced by Pauling's successes in formulating α -helices and

Correspondence to: P. Balaram, (email: pb@mbu.iisc.ernet.in); or N. Shamala, (email: shamala@physics.iisc.ernet.in)

Contract grant sponsor: Council of Scientific and Industrial Research, and Department of Biotechnology, Government of India
Biopolymers, Vol. 64, 255–267 (2002)

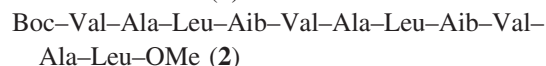
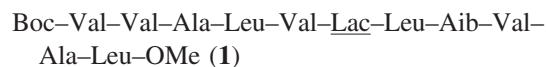
© 2002 Wiley Periodicals, Inc.

Table I Crystal and Diffraction Parameters for Peptides 1–3

	Peptide 1	Peptide 2	Peptide 3
Empirical formula	C ₅₇ H ₁₀₂ N ₁₀ O ₁₅ · H ₂ O	C ₅₆ H ₁₀₁ N ₁₁ O ₁₄ · 3H ₂ O	C ₇₀ H ₁₂₅ N ₁₃ O ₁₈ · 2H ₂ O
Crystal habit	Clear rectangular shaped	Clear rectangular shaped	Clear
Crystal size (mm)	0.3 × 0.2 × 0.15	0.4 × 0.23 × 0.075	0.25 × 0.2 × 0.075
Crystallizing solvent	Acetonitrile/water	Isoproponal/water	Isoproponal/water
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁	P2 ₁
Cell parameters			
<i>a</i> (Å)	9.893(3)	9.938(3)	9.814(2)
<i>b</i> (Å)	20.7694(11)	34.342(3)	19.953(5)
<i>c</i> (Å)	33.839(5)	10.434(2)	22.018(2)
α (deg)	90.00(1)	90.00	90.00
β (deg)	90.01(2)	93.99(2)	93.89(2)
γ (deg)	90.05(2)	90.00	90.00
Volume (Å ³)	6952.8(24)	3552.3(13)	4301.7(13)
<i>Z</i>	4	2	2
Molecules/asym unit	1	1	1
Cocrystallized solvent	One water molecule	Three water molecule	Two water molecule
Molecular weight	1185.50	1206.53	1472.86
Density (g/cm ³) (cal)	1.133	1.128	1.137
<i>F</i> (000)	2576	1312	1600
Radiation	CuK α (λ = 1.5418 Å)	CuK α (λ = 1.5418 Å)	CuK α (λ = 1.5418 Å)
Temperature (°C)	21	21	21
2 θ Range (°)	136	130	130
Scan type	ω -2 θ	ω -2 θ	ω -2 θ
Scan speed	Variable	Variable	Variable
Independent reflection	6892	6159	7540
Observed reflection	5309	5158	3688
[$ F > 4\sigma(F)$]			
Final <i>R</i> (%)	6.15	4.3	10.3
Final <i>wR</i> ₂ (%)	16.46	11.19	27.87
Goodness-of-fit (<i>S</i>)	1.05	1.07	1.11
$\Delta\rho_{\max}$ (e Å ⁻³)	0.46	0.23	0.43
$\Delta\rho_{\min}$ (e Å ⁻³)	-0.35	-0.16	-0.57
Data-to-parameter ratio	7:1	6.8:1	3.9:1

β -sheets as stable conformations for polypeptides, an insight driven largely by the recognition that cooperative hydrogen bond formation between backbone CO and NH groups is the critical element in determining the folded structures.^{1,2} How much do hydrogen bonds really contribute to the stability of the helical polypeptide structure? In the case of apolar peptides the implicit assumption is made that hydrogen bonds significantly stabilize peptide helices in poorly solvating media. As a part of a program to investigate the effect of hydrogen-bond deletion on the structure of well-characterized helical sequences, we have embarked on a systematic study of the structural characteristics of linear peptides containing a single substitution of lactic acid (Lac) for alanine (Ala) in designed peptide helices. We have previously described

structures of three lactic acid containing depsipeptides of length 10, 11, and 14 residues.³ Ohya and co-workers have recently described the crystal structure of two depsipeptides each containing three Lac residues, of length 11 and 15 residues.^{4,5} In this report, we describe the crystal structure of 11 and 14 residue peptides, containing a centrally positioned Lac residue and also the structure of an all amide analog of peptide. Structures are reported for the following sequences:



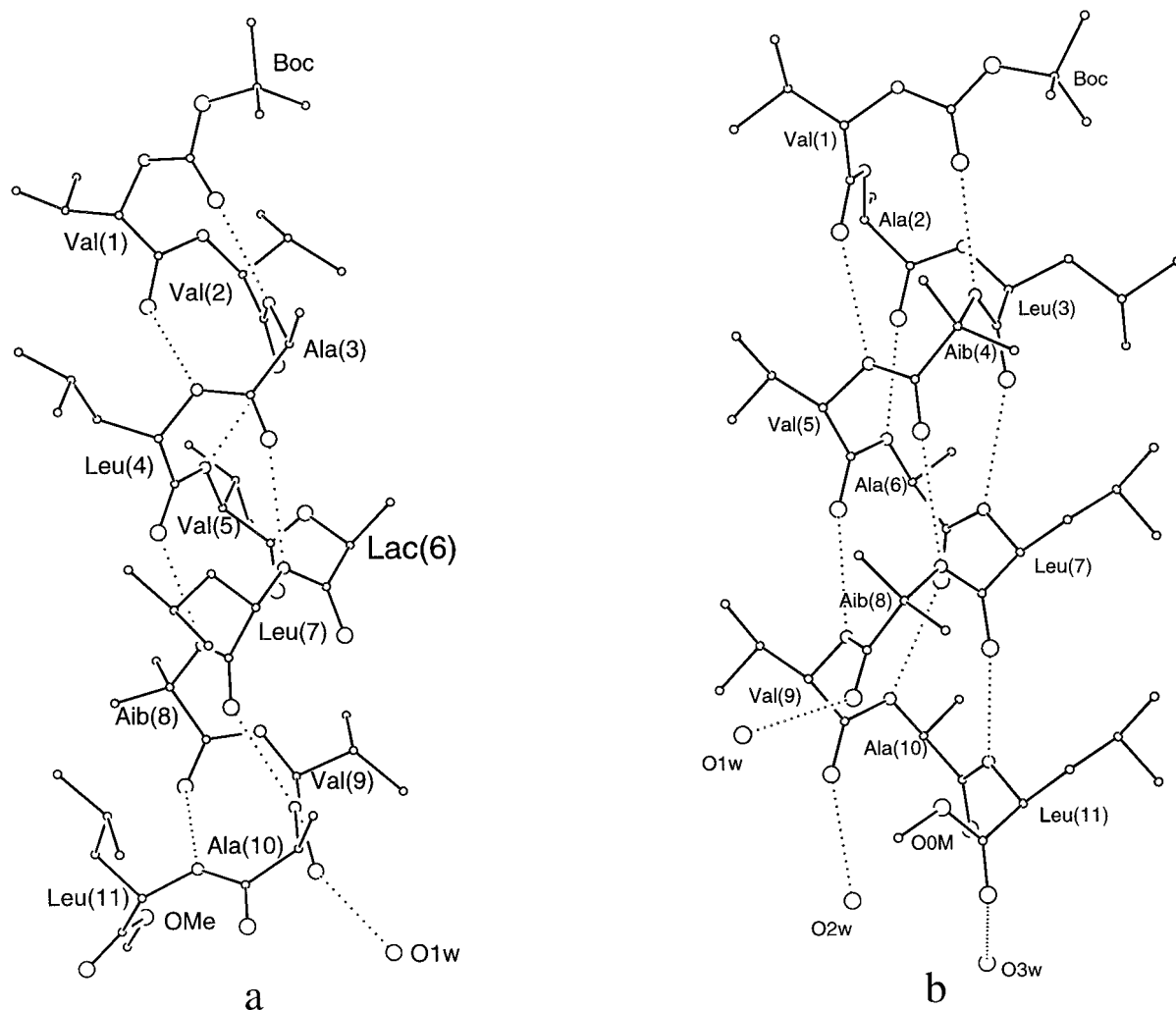


FIGURE 1 Molecular conformation of peptide **1** (a) and **2** (b) in crystals. Hydrogen bonds are shown by dotted lines.

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (**3**)

A comparison with the other reported depsipeptide helices is presented. The results suggest that the replacement of an amide NH group by an ester oxygen atom in the center of the peptide helix can be tolerated with the minor adjustment of the $3_{10}/\alpha$ -helical hydrogen bond pattern near the site of replacement.

EXPERIMENTAL PROCEDURE

Peptides **1–3** were synthesized by conventional solution phase methods by using a fragment condensation strategy. The *t*-butyloxycarbonyl (Boc) group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Deprotections were performed using 98% formic acid or sa-

ponification for N- and C-terminus, respectively. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt), and in case of coupling involving the hydroxy group of lactic acid (for ester formation), DCC and dimethylaminopyridine (DMAP) were employed.³ The final peptides were purified by reverse phase, medium pressure chromatography (MPLC) (C_{18} , 40–60 μ) and by high performance liquid chromatography (HPLC) on a reverse phase C_{18} column (5–10 μ , 7.8 \times 250 mm) using methanol–water gradients. The purified peptides were characterized by electrospray mass spectrometry on a Hewlett-Packard HP-1100 LC-MSD mass spectrometer and were fully characterized by 500 MHz ^1H NMR.

X-ray Diffraction

Crystals of peptides **1–3** were grown by slow evaporation of the solutions of the peptides in acetoni-

Table II Torsion Angles^a of Peptide 1

Residues	ϕ (deg)	ψ (deg)	ω (deg)	χ^1 (deg)	χ^2 (deg)
Val (1)	-61.5 ^b	-21.1	174.5	56.6, -74.4	
Val (2)	-53.3	-32.3	179.7	-75.1, 154.6	
Ala (3)	-64.2	-18.7	175.8		
Leu (4)	-70.2	-23.6	177.5	-64.6	-72.3, 165.5
Val (5)	-75.0	-34.8	173.4	-51.6, -177.3	
Lac (6)	-66.6	-40.9	176.8		
Leu (7)	-60.9	-41.4	-173.5	176.4	57.2, -177.1
Aib (8)	-52.6	-40.3	-175.4		
Val (9)	-58.9	-26.9	178.9	-67.3, 164.9	
Ala (10)	-80.1	-12.9	-173.4		
Leu (11)	-111.6	-20.5 ^c	-178.6 ^d	-55.2	-57.1, -178.9

^a Lac: lactic acid. 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^1 , χ^2) as suggested by the IUPAC-IUB Commission on Biochemical Nomenclature. *Biochemistry*, 1970, Vol. 9, pp. 3471–3479.

^b C'(0)—N(1)—C α (1)—C'(1).

^c N(10)—C α (10)—C'(10)—OMe.

^d C α (10)—C'(10)—O(OMe)—C(OMe).

Table III Hydrogen-Bond Parameters of Peptide 1 (Boc-Val-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe)

Type	Donor	Acceptor	N···O (Å)	H···O (Å)	C=O···H (deg)	C=O···N (deg)	O···HN (deg)
Intermolecular							
^c	N(1) ^a	O(10)	2.955	2.188	117.2	126.0	148.4
^c	N(2) ^a	O1W	3.286	2.434			167.5
^c	O1W	O11 ^b	2.942				
Intramolecular							
4→1 ^c	N(3)	O(0)	2.959	2.143	134.0	137.2	158.4
4→1 ^c	N(4)	O(1)	2.896	2.099	118.6	125.5	153.9
4→1 ^c	N(5)	O(2)	3.040	2.259	115.1	122.0	150.9
4→1	N(7)	O(4)	3.430	3.065	77.1	90.2	108.0
4→1	N(8)	O(5)	3.339	2.841	85.4	98.1	118.6
4→1	N(9)	O(6)	3.420	2.740	105.0	113.2	136.9
4→1 ^c	N(10)	O(7)	2.929	2.158	122.8	130.4	149.1
4→1 ^c	N(11)	O(8)	3.017	2.183	115.9	120.5	163.3
5→1	N(4)	O(0)	4.441	3.803	128.6	136.4	133.7
5→1	N(5)	O(1)	4.075	3.409	140.6	148.8	136.4
5→1 ^c	N(7)	O(3)	3.035	2.215	142.0	147.4	159.5
5→1 ^c	N(8)	O(4)	2.909	2.171	131.4	140.3	143.8
5→1	N(9)	O(5)	3.576	2.812	141.1	144.8	148.8
5→1	N(10)	O(6)	4.192	3.491	135.7	142.6	140.7
5→1	N(11)	O(7)	3.816	3.305	140.6	151.2	120.7
Solvent ^c	O1W	O(9)	2.885				

^a Symmetrically related by $x, y - 1, z$.

^b Symmetrically related by $x + 1, y, z$.

^c These are acceptable hydrogen bonds.

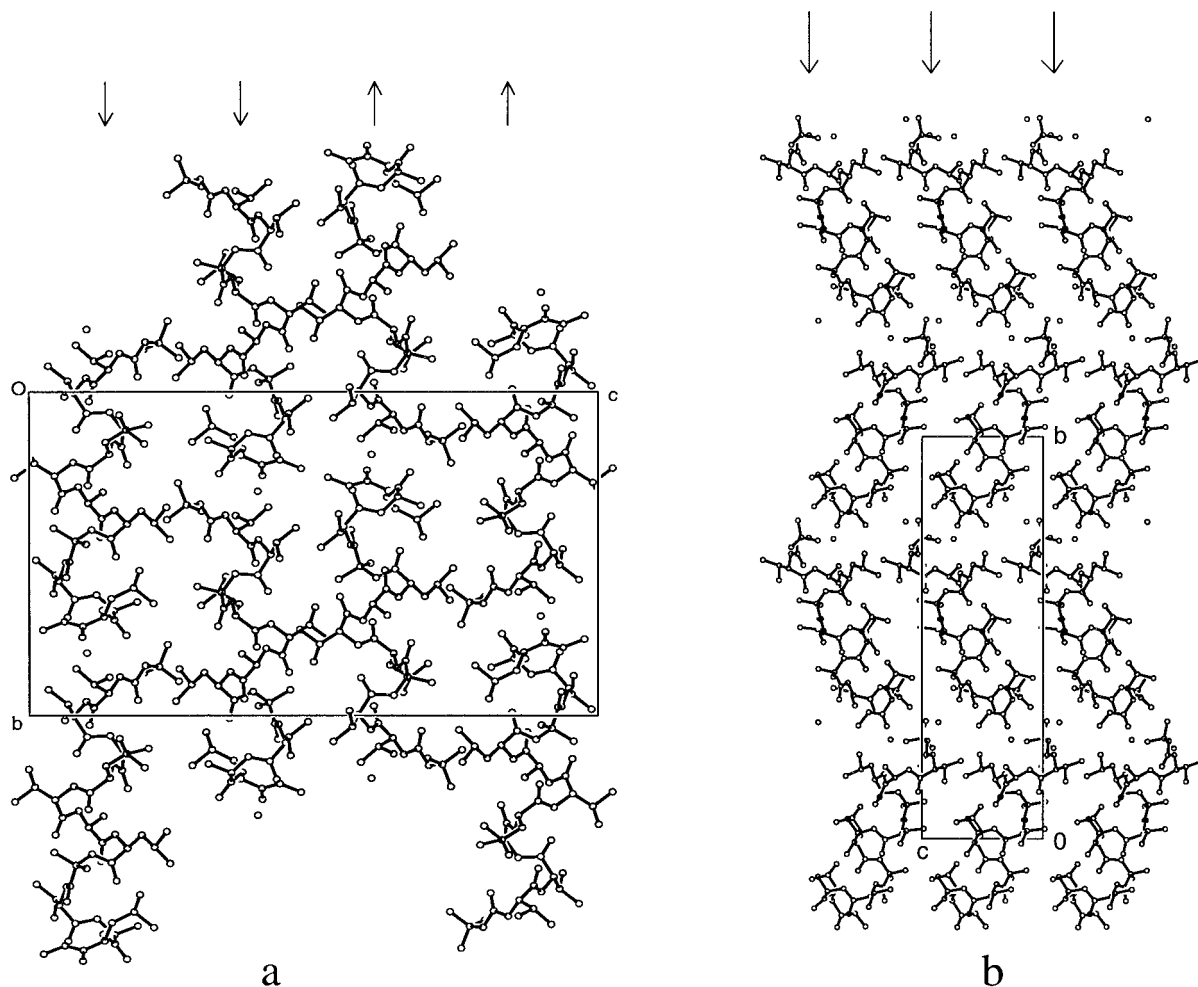


FIGURE 2 Packing of the molecules in peptide **1** (a), and **2** (b). The arrows indicate the direction of helix axes and mode of aggregation.

trile/water (for peptide **1**), and isopropanol/water (for peptides **2** and **3**), respectively. X-ray diffraction data for the peptide crystals were collected at room temperature, 21°C, on an automated four-circle diffractometer with $\text{CuK}\alpha$ ($\lambda = 1.5418 \text{ \AA}$) radiation. Unit cell parameters were obtained and refined by least-squares fit of the angular setting of 25 accurately determined reflections in the range $0^\circ < \theta < 25^\circ$. Intensity data were collected up to $2\theta = 136^\circ$ for peptide **1** and 130° for peptides **2** and **3** using ω - 2θ scans, with variable speed. Diffraction quality was much poorer for **3** as compared to **1** and **2**. Two reflections used as standards, monitored every hour, remain constant within 3%. Lorentz, polarization, and absorption corrections were applied ($\mu = 0.68 \text{ mm}^{-1}$ for peptide **1**, $\mu = 0.63 \text{ mm}^{-1}$ for peptide **2**, and $\mu = 0.68 \text{ mm}^{-1}$ for peptide **3**).

Structure Solution and Refinement

The structures of peptides **1** and **2** were determined by direct phase determination method using SHELXS-97.⁶ For peptide **3**, we used the Shake-and-Bake method, which uses Minimal-Function Phase Refinement and Fourier filtering procedure.⁷ This gave a fragment containing 81 atoms. Remaining atoms were located from difference Fourier maps. Refinement for all the three structures were carried out with a full matrix anisotropic least-squares method using SHELXL-97.⁸ The hydrogen atoms were fixed geometrically in the idealized position, and refined as riding over the heavier atom to which they were bonded. The final R factor was 6.15% ($wR_2 = 16.46\%$) for peptide **1**, 4.3% ($wR_2 = 11.19\%$) for peptide **2**, and 10.34% ($wR_2 = 27.87\%$) for peptide **3**. All the relevant crystallographic data collection pa-

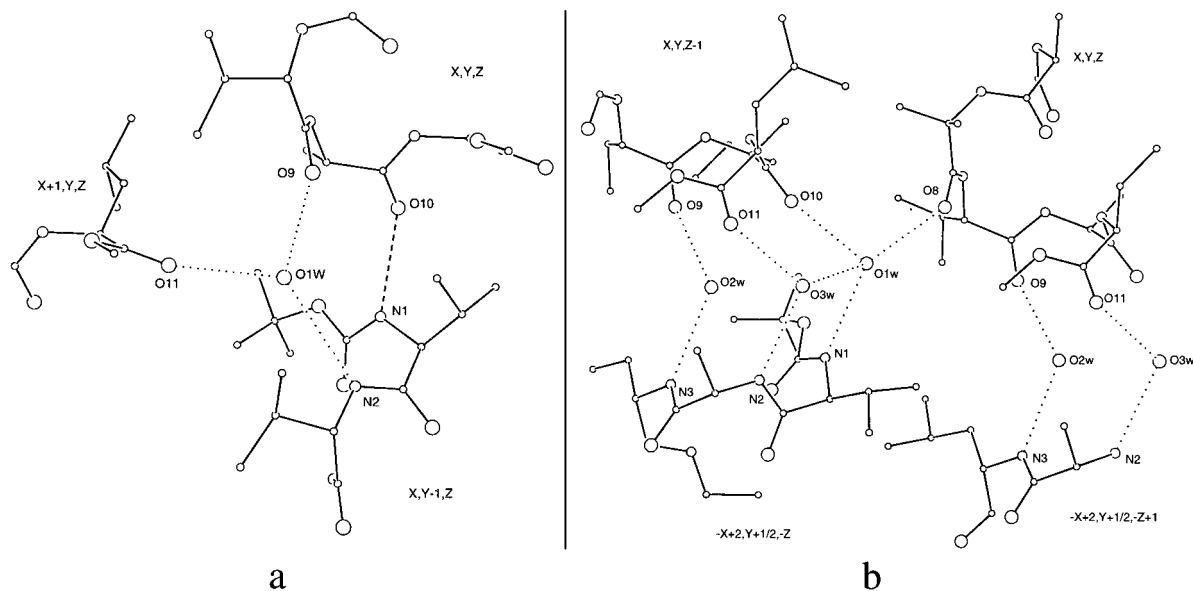


FIGURE 3 Environment of water molecules in peptide **1** (a) and **2** (b). The intermolecular hydrogen bonds are shown in dotted lines.

rameters and structure refinement details for the three peptides are summarized in Table I.

RESULTS

Structure of Peptide 1 (Boc-Val-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe)

Figure 1a shows a view of the molecular conformation in crystals. The relevant backbone and side-chain torsion angles are listed in Table II and hydrogen-bond parameters in Table III. In view of the extended discussion in the literature on 3_{10} - and α -helices in peptides,^{9–13} we have provided the parameters for both $4 \rightarrow 1$ and $5 \rightarrow 1$ interactions in the cases of all the molecules reported, to facilitate the choice between various hydrogen-bonding possibilities.^{14–19} Inspection of the potential hydrogen-bonding pattern in Table III suggests that residues 1–4 form a 3_{10} -helical segment, stabilized by three successive $4 \rightarrow 1$ hydrogen bonds. The segment spanning residues 5–7 is better described as a part of an α -helical turn, with two successive $5 \rightarrow 1$ hydrogen bonds [N(7) \cdots O(3) and N(8) \cdots O(4)]. The polypeptide chain tightens again to a 3_{10} -helical segment for residues 8–10, with two successive $4 \rightarrow 1$ hydrogen bonds [N(10) \cdots O(7) and N(11) \cdots O(8)].

The aggregation of helices into infinite columns is unexceptional with molecules in a column stabilized by head-to-tail hydrogen bonding, between exposed NH and CO groups.^{19–21} There is a lone water mol-

ecule providing an additional intermolecular hydrogen bond. Within the crystal helical columns run parallel and antiparallel in pairs ($\uparrow\uparrow \downarrow\downarrow \uparrow\uparrow$) as indicated by the arrows in the Figure 2a. Figure 3a provides a view of the environment of the water molecule.

Structure of Peptide 2 (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe)

A view of the molecular conformation in crystals is shown in Figure 1b. The relevant torsion angles and potential hydrogen-bond parameters are listed in Tables IV and V, respectively. The peptide backbone adopts an almost perfect α -helical conformation with eight successive $5 \rightarrow 1$ hydrogen bonds (Table V). The helices in crystals are packed in parallel fashion as indicated by arrows in Figure 2b, with as many as three water molecules entrapped. Figure 3b shows a view of the intermolecular hydrogen-bond network mediated by water molecules.

Structure of Peptide 3 (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe)

Figure 4a shows the molecular conformation of the peptide in crystals. The relevant torsion angles and potential hydrogen bond parameters are listed in Tables VI and VII, respectively. The structure appears to be predominately α -helical, with seven $5 \rightarrow 1$ hydro-

Table IV Torsion Angles^a of peptide 2

Residues	ϕ (deg)	ψ (deg)	ω (deg)	χ^1 (deg)	χ^2 (deg)
Val (1)	-63.2 ^b	-53.4	-173.8	-65.9, 170.0	
Ala (2)	-64.3	-43.1	-179.6		
Leu (3)	-68.2	-40.7	176.2	-178.4	60, -177.1
Aib (4)	-51.8	-51.5	-176.9		
Val (5)	-64.5	-46.6	-179.2	-68.1, 167.4	
Ala (6)	-55.5	-46.4	-179.1		
Leu (7)	-67.2	-47.9	-174.1	-70.0	-70, 168.1
Aib (8)	-59.8	-34.0	-176.0		
Val (9)	-92.0	-42.7	-178.2	-52.4, -175.2	
Ala (10)	-71.0	-37.7	173.6		
Leu (11)	-80.3	-47.2 ^c	176.9 ^d	-64.0	-66.3, 170.6

^a As in Table II.^b C'(0)—N(1)—C α (1)—C'(1).^c N(10)—C α (10)—C'(10)—OMe.^d C α (10)—C'(10)—O(OMe)—C(OMe).

Table V Hydrogen-Bond Parameters of Peptide 2 (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe)

Type	Donor	Acceptor	N···O (Å)	H···O (Å)	C=O···H (deg)	C=O···N (deg)	O···HN (deg)
Intermolecular							
^d	N(1) ^a	O1W	2.991	2.143			169.3
^d	N(2) ^b	O3W	2.908	2.071			164.7
^d	N(3) ^b	O2W	3.010	2.192			159.1
^d	O1W	O(10) ^c	2.804				
^d	O3W ^c	O1W	2.920				
Intramolecular							
4→1	N(3)	O(0)	3.601	3.210	98.4	110.4	110.4
4→1	N(4)	O(1)	3.384	3.029	92.9	106.3	107.2
4→1	N(5)	O(2)	3.361	2.947	92.2	104.8	111.7
4→1	N(6)	O(3)	3.253	2.907	90.6	104.7	106.2
4→1	N(7)	O(4)	3.492	3.050	92.7	104.4	114.2
4→1	N(8)	O(5)	3.313	2.945	93.2	106.9	108.0
4→1	N(9)	O(6)	3.635	3.015	92.4	102.1	130.7
4→1	N(10)	O(7)	3.543	3.191	91.0	103.9	107.3
4→1	N(11)	O(8)	4.583	4.193	68.4	78.0	111.8
5→1 ^d	N(4)	O(0)	2.971	2.146	154.8	160.2	160.8
5→1 ^d	N(5)	O(1)	3.058	2.217	153.4	157.0	166.0
5→1 ^d	N(6)	O(2)	2.900	2.060	151.5	155.8	165.1
5→1 ^d	N(7)	O(3)	2.989	2.138	156.7	158.6	170.1
5→1 ^d	N(8)	O(4)	3.012	2.177	150.1	154.8	163.5
5→1 ^d	N(9)	O(5)	3.046	2.260	153.8	158.5	152.0
5→1 ^d	N(10)	O(6)	2.960	2.141	145.9	151.9	159.0
5→1 ^d	N(11)	O(7)	2.943	2.134	143.0	149.8	156.0
Solvent ^d	O1W	O(8)	2.775				
Solvent ^d	O2W	O(9)	2.872				
Solvent ^d	O3W	O(11)	2.842				

^a Symmetrically related by $-x + 2, y + \frac{1}{2}, -z$.^b Symmetrically related by $-x + 2, y + \frac{1}{2}, -z + 1$.^c Symmetrically related by $x, y, z - 1$.^d Acceptable hydrogen bonds.

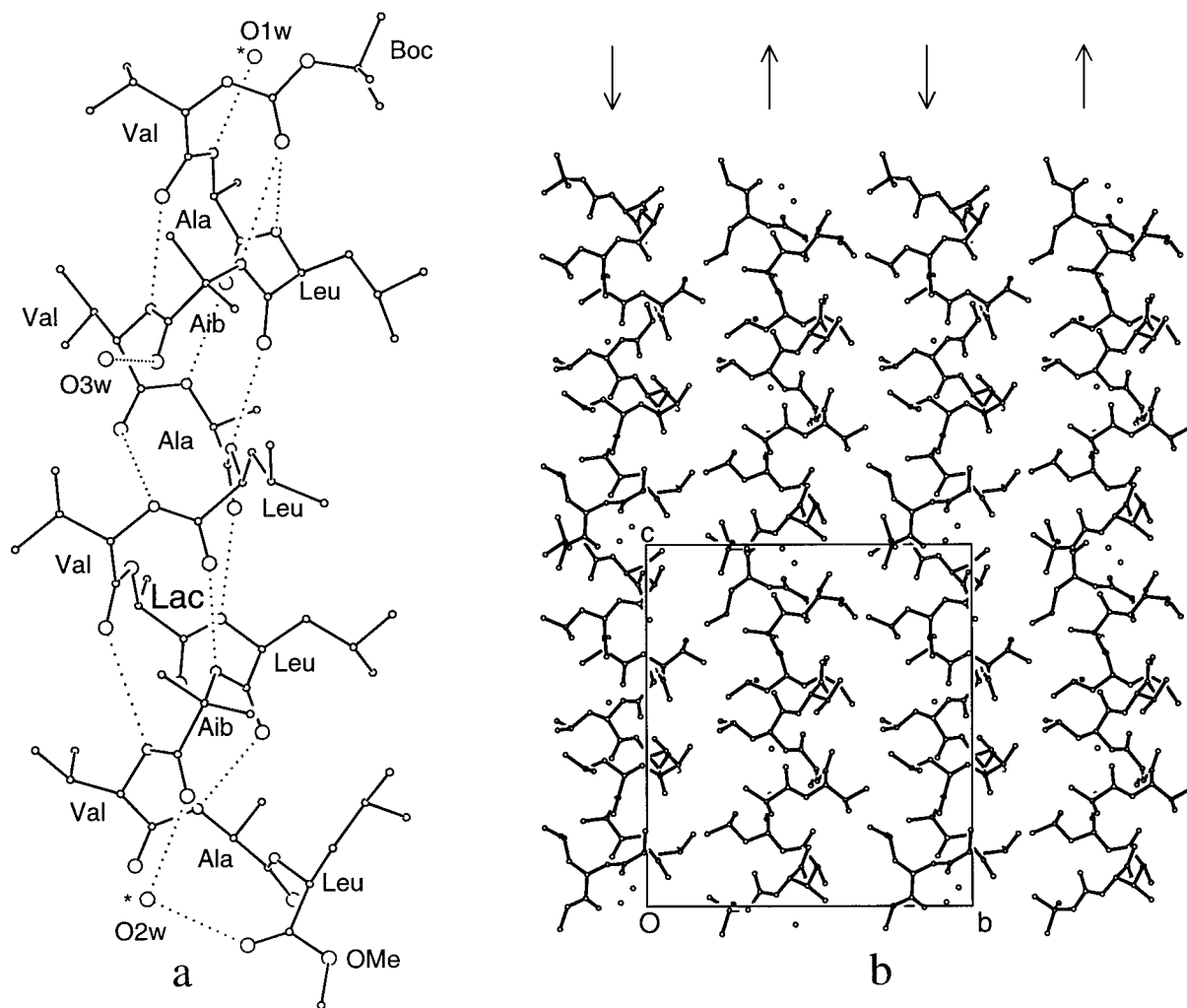


FIGURE 4 (a) Molecular conformation of peptide 3. (b) Packing of the molecule arrows indicating the direction of helix axes and mode of aggregation.

Table VI Torsion Angles^a of Peptide 3

Residue	ϕ (deg)	ψ (deg)	ω (deg)	χ^1 (deg)	χ^2 (deg)
Val(1)	-57.2 ^b	-32.4	-178.4	73.6, -161.5	
Ala(2)	-61.5	-38.4	177.8		
Leu(3)	-65.0	-45.1	-179.5	179.8	62.5, -175.3
Aib(4)	-55.1	-41.9	-177.4		
Val(5)	-70.7	-42.7	179.9	170.4, -66.6	
Ala(6)	-65.3	-23.5	177.5		
Leu(7)	-65.4	-35.0	178.9	-172.7	65.3, -170.3
Val(8)	-61.8	-43.6	178.4	-54.0, -178.6	
Lac(9)	-68.2	-37.6	175.6		
Leu(10)	-59.0	-43.9	-168.0	-176.3	64.4, -170.2
Aib(11)	-61.1	-38.1	-175.9		
Val(12)	-81.3	-4.7	172.5	68.8, -55.4	
Ala(13)	-93.9	-16.6	-177.4		
Leu(14)	-89.5	168.4 ^c	172.8 ^d	-64.3	-52.1, -175.2

^a As in Table II.

^b C'(0)—N(1)—C^α(1)—C'(1).

^c N(14)—C^α(14)—C'(14)—O(OMe).

^d C^α(14)—C'(14)—O(OMe)—C(OMe).

Table VII Hydrogen-Bond Parameters of Peptide 3 (Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe)

Type	Donor	Acceptor	N···O (Å)	H···O (Å)	C=O···H (deg)	C=O···N (deg)	O···H—N (deg)
Intermolecular							
^d	N(1)	O(13) ^a	2.946	2.093	127.7	130.3	170.98
^d	N(2)	O2W ^{b,e}	3.029	2.410			129.23
^d	O1W ^c	O(11) ^b	2.952				
^d	O1W ^c	O(13) ^a	2.810				
^d	O3W ^c	O(6)	3.071				
Intramolecular							
4→1 ^d	N(3)	O(0)	3.176	2.573	110.0	119.3	128.0
4→1	N(4)	O(1)	3.257	2.865	87.4	101.2	109.7
4→1	N(5)	O(2)	3.469	2.948	86.3	97.3	120.9
4→1	N(6)	O(3)	3.081	2.675	97.6	112.1	110.3
4→1	N(7)	O(4)	3.554	2.915	89.9	100.0	132.6
4→1 ^d	N(8)	O(5)	3.099	2.391	111.4	120.1	139.9
4→1	N(10)	O(7)	3.309	2.938	80.5	94.0	108.1
4→1	N(11)	O(8)	3.251	2.695	88.8	101.1	123.7
4→1	N(12)	O(9)	3.451	2.834	99.5	108.8	130.1
4→1 ^d	N(13)	O(10)	2.904	2.145	108.1	117.4	147.0
4→1	N(14)	O(11)	3.482	2.683	100.3	106.0	155.3
5→1 ^d	N(4)	O(0)	3.433	2.604	144.1	148.4	162.2
5→1 ^d	N(5)	O(1)	3.110	2.297	148.3	152.4	157.5
5→1 ^d	N(6)	O(2)	3.086	2.270	138.4	144.1	158.5
5→1 ^d	N(7)	O(3)	2.969	2.226	156.9	164.8	144.5
5→1 ^d	N(8)	O(4)	3.879	3.166	132.4	138.6	141.8
5→1 ^d	N(10)	O(6)	3.036	2.213	145.5	150.9	160.1
5→1 ^d	N(11)	O(7)	2.889	2.157	136.5	145.3	142.8
5→1 ^d	N(12)	O(8)	3.432	2.644	144.7	148.6	152.9
5→1	N(13)	O(9)	3.552	2.975	134.9	146.0	126.3
5→1	N(14)	O(10)	3.327	2.741	153.0	164.5	126.6
Solvent ^d	N(2)	O1W ^c	2.917	2.062			172.1
Solvent ^d	O2W ^c	O(11)	2.869				
Solvent ^d	O2W ^c	O(14)	2.992				
Solvent ^d	O3W	O(4)	2.861				

^a Symmetrically related by (x+1, y, z-1).^b Symmetrically related by (x, y, z-1).^c Symmetrically related by (x-1, y, z).^d Acceptable hydrogen bonds.^e The occupancy of O1W = O2W = 0.5.

gen bonds (Table VII). Three 4→1 interaction are interspersed in the sequence. The 4→1 hydrogen bonds N(3)···O(0) and N(13)···O(10) lie at the N- and C-termini of the helix. The observation of a single 3_{10} -helical turn at helix termini is fairly common in proteins and peptides.^{22,23} Interestingly, a single 4→1 hydrogen bond N(8)···O(5) is observed at the center of the helix. The placement of the Lac residue at position 9 precludes formation of an N(9)···O(5) interaction. The molecules are packed together in

crystals with adjacent helical columns running anti-parallel in pairs as indicated by arrows in Figure 4b. Three water sites are observed in crystals in the interhelical space between the head and tail regions of the adjacent helical column. There are two water sites O1w and O2w, both of which show half occupancy. The proximity of the sites precludes the simultaneous occupation of both positions. Interestingly, a water molecule (O3w) is observed in the space between the adjacent columns, which forms a hydrogen bonded

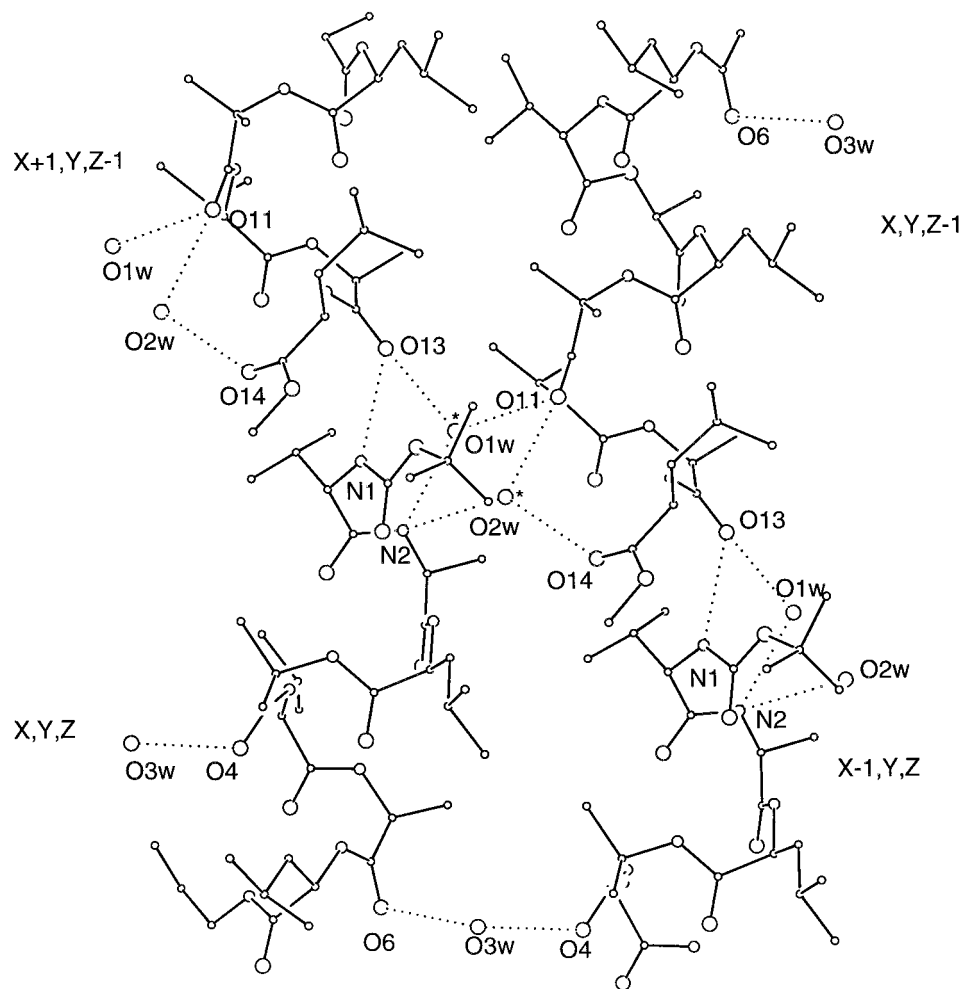


FIGURE 5 Environment of water molecules in peptide **3**. The intermolecular hydrogen bonds are shown in dotted lines. (Asterisk indicates the occupancy of O1w and O2w = 0.5.)

link between O(6) of Ala(6) CO and O(4) of Aib(4)CO of symmetry-related molecules. The entrapment of water in the hydrophobic spaces between adjacent helical columns is a relatively rare occurrence.^{24–27} The presence of a Lac residue at position 9 presumably results in perturbation of hydrogen-bond patterns facilitating backbone hydration in the middle of the helix. Figure 5 shows the water-mediated intermolecular hydrogen-bonding patterns.

DISCUSSION

The determination of the crystal structures of peptides **1–3** provides an opportunity for comparing the effect of Lac residue incorporation into peptide helices and the effect of positioning of the residue along the sequence. A comparison of these structures with the structures of three lactic acid containing helices **4–6**

reported earlier³ is presented below. For the purpose of the present analysis, the peptides may be divided into two groups.

Boc-Val-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (peptide **1**)

Boc-Val-Ala-Leu-Aib-Val-Lac-Leu-Aib-Val-Leu-OMe (peptide **4**)

Boc-Val-Ala-Leu-Aib-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (peptide **5**)

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (peptide **2**)

and

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Lac-Leu-Aib-Val-Ala-Leu-OMe (peptide **3**)

Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Val-Ala-Leu-Aib-Val-Lac-Leu-OMe (peptide **6**)

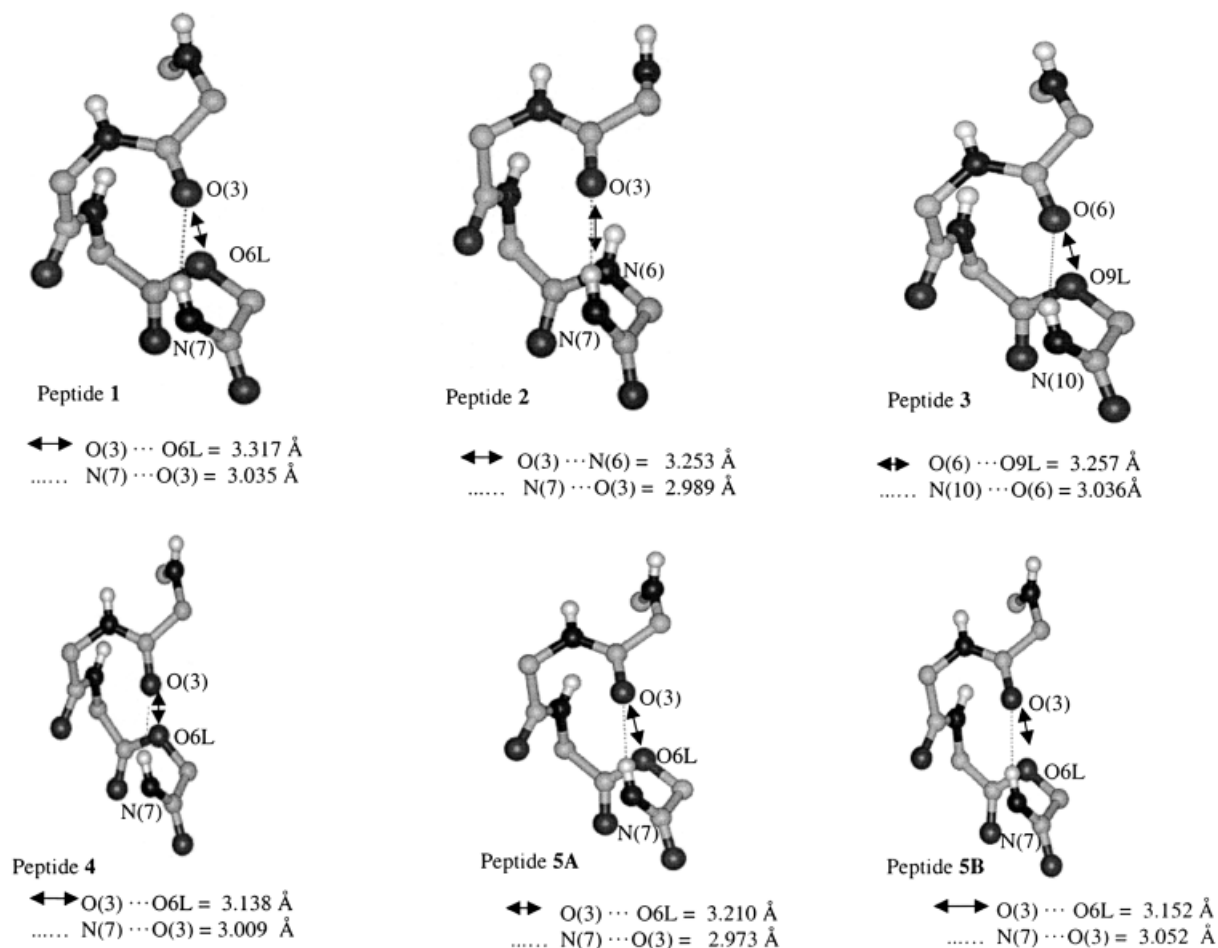


FIGURE 6 Environment of the Lac(6), ester oxygen atom O(6) in peptides 1, 3, 4, and 5 (A, B) compared with the surroundings of the Ala(6) NH group in peptide 2.

In the first group all the four peptides are 10–11 residues in length with peptides 1, 4, and 5 containing lactic acid at position 6. Peptide 2 is the analog of peptide 5 in which the Lac(6) residue is replaced by an Ala residue (Peptide 2 is the amide analog of depsipeptide 5). The two 14-residue peptides 3 and 6 differ only in the position of Lac residue. In peptide 3 the Lac residue is at position 9 in the potential helical segment. In peptide 6 the Lac residue is at position 13, the C-terminus of the helix.

Effect of Lac Residues in the Center of Helical Segments

Peptide 1 and 3–5 provide examples of Lac residues accommodated comfortably into the helical peptide segment, without major distortion. Figure 6 illustrates the environment of the Lac residue in five examples where it is located in the interior of the helix (note in peptide 5 there are two independent molecules in the

asymmetric unit). For comparison, a corresponding environment of Ala(6) in the all-amide analog is also shown. In the helical peptide 2 (all-amide analog), the N(6) \cdots O(3), N(7) \cdots O(3) distances are 3.2 and 2.9 Å, respectively. It is observed that in all the five cases where the Lac residue is inserted the corresponding O(3) \cdots O(6)Lac, distances are between 3.1 and 3.3 Å [note in peptide 3 the Lac residue is at position 9 and the relevant distance is O(6) \cdots O(9)Lac]. The N(7) \cdots O(3) distance [N(10) \cdots O(6) in peptide 3] lies between 2.9 and 3.1 Å.

Thus, there is very little difference locally at the site of lactic acid insertion with the oxygen atom of the hydroxy acid comfortably accommodated in place of the NH group within the body of peptide helix. Similar observations have been made in the case of two depsipeptides [Boc-(Leu-Leu-Lac)₃-Leu-Leu-OEt⁵ and Boc-(Leu-Leu-Ala)₂-(Leu-Leu-Lac)₃-OEt⁴] in which the former contains three lactic acid and the later contains two lactic acid residues. The

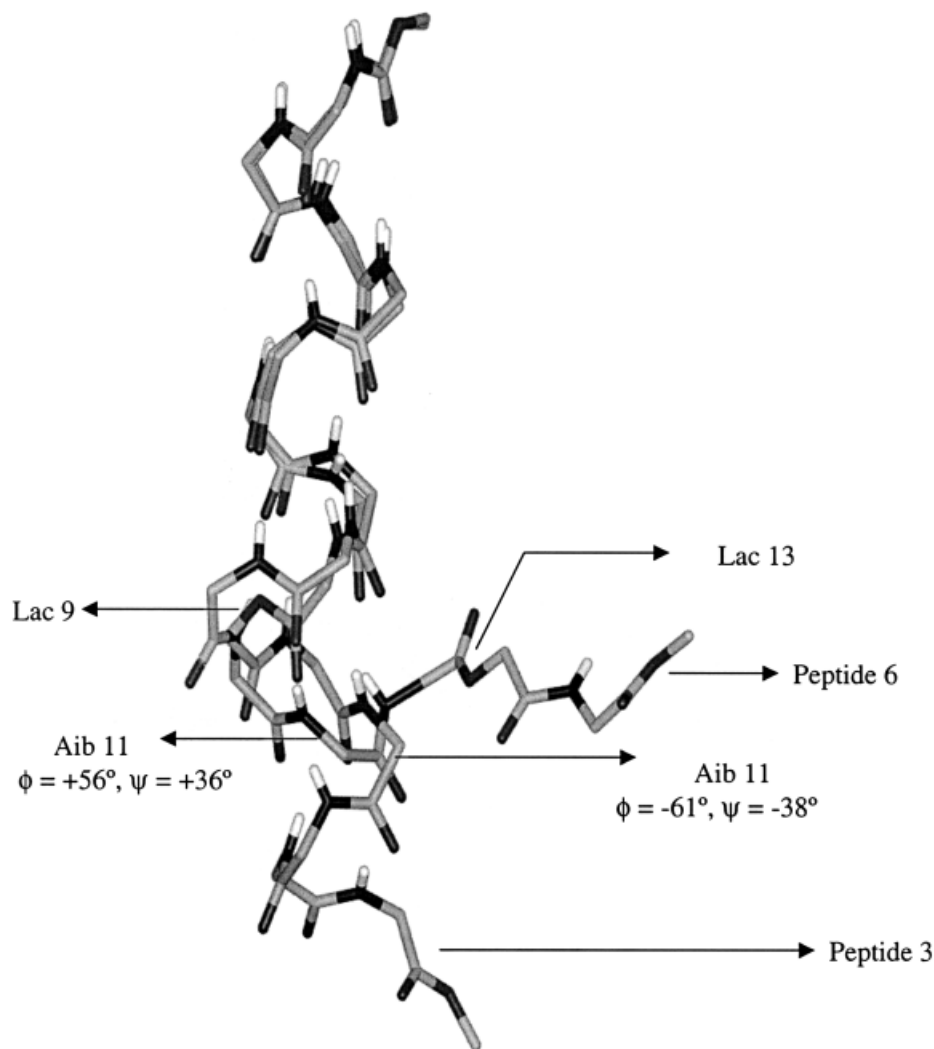


FIGURE 7 Superposition of peptides **3** and **6** to emphasize the difference in conformation arising due to helix termination by a Schellman motif in peptide **6**.

reported C=O...O distances in the 11-residue peptide are 3.47 and 3.24 Å, while in the 15-residue peptide the corresponding distances are 3.81 and 3.87 Å. It is noteworthy that in both peptides containing multiple Lac residues the reported O...O distances are significantly greater although the overall helical fold is not perturbed.

The most significant effect of lactic acid insertion appear to be a readjustment in the $3_{10}/\alpha$ -helical hydrogen-bonding patterns found in helical peptides. In retrospect, it is not surprising that peptide helices are able to accommodate the loss of a potential hydrogen bond by minor readjustment to allow a transition between 5→1 and 4→1 interactions, and also by bifurcation of hydrogen-bond interactions.

A comparison of the 14-residue peptides **3** and **6** (Figure 7) suggests that the Lac positioning may be used

to facilitate helix termination in designed sequences. While Lac(9) is comfortably accommodated into a helical segment in peptide **3**, Lac(13) in peptide **6** lies outside the helix. Notably, in the structure of peptide **6** there is an achiral Aib residue located at position 11. The loss of the hydrogen-bonding NH group at position 13 in a potentially continuous helix appears to be sufficient to induce the energetically accessible left-handed helical conformation (α_L) at Aib11. This in turn provides helix termination in a Schellman motif stabilized by a strong 6→1 hydrogen bond.

CONCLUSIONS

The structure determination of two peptide helices containing a single internal Lac residue and the all-

amide analog of a previously determined depsipeptide has provided an opportunity to examine the structural consequences of replacing an NH group by an oxygen atom in helical peptides. The substitution of an α -amino acid by a α -hydroxy acid is isosteric. The observation of helical peptide folds accommodating Lac residues in internal positions of a helix in as many as six independent structures, including two determined in the present study, suggests that deletion of a single hydrogen bond can be easily compensated by readjustments of the backbone, resulting in local transition between 4 \rightarrow 1 and 5 \rightarrow 1 interactions. The short C=O...O distances of 2.8–3.3 Å observed in all cases of single lactic acid containing peptides suggests that proximity between two oxygen atoms is not a major destabilizing factor. The example of the 14 residue peptides **3** and **6**, suggests that the placement of lactic acid at the C-terminal end of the helices may provide a signal for termination, particularly an achiral residue capable of adopting left-handed α -helical conformation is positioned two residue ahead of Lac residue. Indeed, a similar pattern has been observed for the hydrogen-bond interrupting Pro residue in proteins, where the actual site of helix termination occurs at two residues preceding proline.²⁸ Interestingly, the comparison of depsipeptide **5** and its all-amide analog peptide **2** reveals that the oxygen atom of the Lac(6) hydroxyl group in **5** sits snugly in a position similar to that of the corresponding NH group in peptide **2**.

This research was supported by a grant from the Council of Scientific and Industrial Research, and the “Drug and Molecular Design” Program of the Department of Biotechnology, Government of India.

REFERENCES

- Pauling, L.; Corey, R. B.; Branson, H. R. *Proc Natl Acad Sci USA* 1951, 37, 205–211.
- Pauling, L.; Corey, R. B. *Proc Natl Acad Sci USA* 1951, 37, 251–256.
- Karle, I. L.; Das, C.; Balaram, P. *Biopolymers* 2001, 59, 276–289.
- Ohyama, T.; Oku, H.; Hiroki, A.; Maekawa, Y.; Yoshida, M.; Katakai, R. *Biopolymers* 2000, 54, 375–378.
- Ohyama, T.; Oku, H.; Hiroki, A.; Yoshida, M.; Katakai, R. *Biopolymers* 2001, 58, 636–642.
- Sheldrick, G. M. *Acta Cryst* 1990, A46, 467–473.
- Miller, R.; Gallo, S. M.; Khalal, H. G.; Weeks, M. C. *J Appl Cryst* 1994, 27, 613–621.
- Sheldrick, G. M. Program for the Refinement of Crystal Structures, Universität Göttingen, Germany.
- Dehner, A.; Planker, E.; Gemmecker, G.; Broxterman, Q. B.; Bisson, W.; Formaggio, F.; Crisma, M.; Toniolo, C.; Kessler, H. *J Am Chem Soc* 2001, 123, 6678–6686.
- Topal, I. A.; Burt, S. K.; Deretey, E.; Tang, T.; Perczel, A.; Rashin, A.; Csizmadia, I. G. *J Am Chem Soc* 2001, 123, 6054–6060.
- Mammi, S.; Rainaldi, M.; Bellanda, M.; Schievano, E.; Peggion, E.; Broxterman, Q. B.; Formaggio, F.; Crisma, M.; Toniolo, C. *J Am Chem Soc* 2000, 122, 11735–11736.
- Paul, H.; Martinez, G.; Millhauser, G. *J Am Chem Soc* 1996, 118, 271–272.
- Basu, G.; Kitao, A.; Hirata, F.; Gö, N. *J Am Chem Soc* 1994, 116, 6307–6315.
- Prasad, B. V. V.; Balaram, P. *CRC Crit Revs Biochem* 1984, 16, 307–347.
- Toniolo, C.; Benedetti, E. *Trends Biochem Sci* 1991, 16, 350–353.
- Toniolo, C.; Benedetti, E. *ISI Atlas Sci Biochem* 1988, 1, 225–230.
- Toniolo, C.; Benedetti, E. *Macromolecules* 1991, 24, 4004–4009.
- Datta, S.; Shamala, N.; Banerjee, A.; Balaram, P. *J Peptide Res* 1997, 49, 604–611.
- Karle, I. L.; Balaram, P. *Biochemistry* 1990, 29, 6747–6756.
- Karle, I. L.; *Acta Cryst* 1992, B48, 341–356.
- Karle, I. L. *Biopolymers (Peptide Sci)* 1996, 40, 157–180.
- Barlow, D. J.; Thornton, J. M. *J Mol Biol* 1988, 201, 601–619.
- Baker, E. N.; Hubbard, R. E. *Progr Biophys Mol Biol* 1984, 44, 97–179.
- Karle, I. L.; Flippen-Anderson, J.; Uma, K.; Balaram, P. *Biopolymers* 1989, 28, 773–781.
- Karle, I. L.; Flippen-Anderson, J.; Uma, K.; Balaram, P. *Int J Peptide Protein Res* 1994, 44, 491–498.
- Karle, I. L.; Flippen-Anderson, J.; Uma, K.; Balaram, P. *Proc Natl Acad Sci USA* 1988, 85, 299–303.
- Karle, I. L.; Flippen-Anderson, J.; Uma, K.; Sukumar, M.; Balaram, P. *J Am Chem Soc* 1990, 112, 9350–9356.
- Gunasekaran, K.; Nagarajaram, H. A.; Ramakrishnan, C.; Balaram, P. *J Mol Biol* 1998, 275, 917–932.