Unfolding of an α-Helix in Peptide Crystals by Solvation: Conformational Fragility in a Heptapeptide

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SYNOPSIS

The structure of the peptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe has been determined in crystals obtained from a dimethylsulfoxide–isopropanol mixture. Crystal parameters are as follows: $C_{38}H_{69}N_7O_{10} \cdot H_2O \cdot 2C_3H_7OH$, space group $P2_1$, a = 10.350(2) Å, b = 26.084(4) Å, c = 10.395(2) Å, β = 96.87(12), Z = 2, R = 8.7% for 2686 reflections observed > 3.0 $\sigma(F)$. A single 5 → 1 hydrogen bond is observed at the N-terminus, while two 4 → 1 hydrogen bonds characteristic of a 3_{10}-helix are seen in the central segment. The C-terminus residues, Ala(6) and Leu(7) are extended, while Val(5) is considerably distorted from a helical conformation. Two isopropanol molecules make hydrogen bonds to the C-terminal segment, while a water molecule interacts with the N-terminus. The structure is in contrast to that obtained for the same peptide in crystals from methanol–water [I. L. Karle, J. L. Flippen-Anderson, K. Uma, and P. Balaram (1990) *Proteins: Structure, Function and Genetics, Vol.* 7, pp. 62–73] in which two independent molecules reveal an almost perfect α-helix and a helix penetrated by a water molecule. A comparison of the three structures provides a snapshot of the progressive effects of solvation leading to helix unwinding. The fragility of the heptapeptide helix in solution is demonstrated by nmr studies in $CDCl_3$ and $(CD_3)_2SO$. A helical conformation is supported in the apolar solvent $CDCl_3$, whereas almost complete unfolding is observed in the strongly solvating medium $(CD_3)_2SO$. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

The α-helix is a major structural component of globular proteins.[1,2] The features of α-helical structures have been extensively analyzed using available protein structures, determined at high resolution.[3] The mean length of helical segments in proteins is only 10–12 residues.[3,4] However, peptides of this length do not generally fold into helical conformations in solution. A considerable body of work has addressed the issue of stabilizing helical conformations of oligopeptides in aqueous solutions,[5–13] with recent attempts focusing on the use of metal ion complexation[14,15] and covalent bridging[16] as a means of promoting helix formation. The strong solvating abilities of water provide a means of minimizing the stabilizing influence of intramolecular NH—OC hydrogen bonds, resulting in a lowered tendency for short peptides to fold into monomeric helices. Indeed, recent molecular dynamics studies provide a picture of helix unfolding in water by mechanisms involving helical hydrogen-bond breakage and invasion of the backbone by water molecules.[17,18] Crystal structures of peptides[19–21] and proteins[22] have yielded a static view of the modes of hydration of helix backbones.

The stabilization of helices in short peptides is readily achieved by the incorporation of α-aminoisobutyryl (Aib) residues, which are constrained to adopt a limited range of ϕ, ψ values, as a consequence of the presence of the additional methyl group at the C^α atom.[23,24] Extensive studies of Aib-containing peptides emphasize the role of this residue in promoting β-turn and 3_{10}-helical conformations in short peptides[25,26] and 3_{10}- and α-helical structures in larger sequences.[27,28] Interestingly, even a single centrally placed Aib residue in 6–9 residue peptides

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Table I Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U (eq)[a]
C (1)	6327 (14)	6476 (7)	673 (13)	62 (6)
C (2)	5622 (14)	6973 (7)	701 (13)	85 (7)
C (3)	7561 (12)	6522 (8)	20 (12)	86 (7)
C (4)	5455 (14)	6055 (8)	110 (14)	94 (8)
O	6863 (8)	6339 (6)	2000 (8)	67 (4)
C (5)	6118 (14)	6257 (7)	2950 (14)	65 (6)
O (0)	4891 (8)	6302 (6)	2843 (8)	82 (4)
N (1)	6853 (9)	6125 (6)	4030 (9)	58 (4)
C^{α} (1)	6278 (13)	5936 (7)	5151 (12)	58 (5)
C′ (1)	5659 (14)	6343 (7)	5902 (12)	55 (5)
O (1)	4678 (9)	6262 (6)	6398 (10)	86 (4)
C^{β} (1)	7312 (16)	5607 (6)	5972 (13)	81 (7)
C^{γ} (11)	6760 (21)	5412 (8)	7172 (16)	142 (11)
C^{γ} (12)	7866 (21)	5169 (8)	5243 (16)	154 (12)
N (2)	6251 (10)	6805 (6)	5993 (10)	57 (4)
C^{α} (2)	5703 (13)	7212 (7)	6614 (14)	68 (6)
C′ (2)	4526 (13)	7462 (7)	5831 (15)	58 (6)
O (2)	3561 (8)	7578 (6)	6362 (9)	67 (4)
C^{β} (2)	6711 (13)	7610 (7)	7124 (15)	86 (7)
N (3)	4628 (10)	7551 (6)	4613 (10)	58 (4)
C^{α} (3)	3535 (14)	7790 (7)	3860 (13)	64 (6)
C′ (3)	2259 (16)	7465 (6)	3763 (13)	62 (6)
O (3)	1195 (9)	7660 (6)	3676 (10)	84 (4)
C^{β} (3)	3797 (19)	7915 (7)	2494 (17)	99 (8)
C^{γ} (3)[b]	3137 (29)	8361 (10)	1757 (28)	127 (18)
C^{δ} (31)[b]	3581 (43)	8363 (14)	414 (30)	110 (15)
C^{δ} (32)[b]	3319 (46)	8875 (15)	2441 (37)	116 (16)
C^{γ} (3)′[b]	4599 (25)	8392 (8)	2359 (27)	97 (14)
C^{δ} (31)′[b]	4098 (42)	8874 (12)	2951 (32)	111 (13)
C^{δ} (32)[b]	4547 (44)	8424 (14)	893 (27)	123 (15)
N (4)	2470 (9)	6954	3845 (9)	51 (4)
C^{α} (4)	1337 (13)	6584 (6)	3749 (14)	63 (6)
C′ (4)	515 (13)	6719 (7)	4870 (13)	54 (5)
O (4)	−645 (9)	6606 (6)	4690 (9)	84 (4)
C^{β} (41)	542 (13)	6602 (8)	2446 (11)	91 (7)
C_{β} (42)	1922 (13)	6050 (7)	4094 (14)	80 (7)
N (5)	1061 (9)	6927 (6)	5970 (10)	50 (4)
C^{α} (5)	369 (12)	7031 (7)	7062 (12)	59 (6)
C′ (5)	160 (12)	7608 (7)	7245 (17)	67 (6)
O (5)	−132 (10)	7779 (6)	8270 (11)	100 (5)
C^{β} (5)	889 (14)	6779 (8)	8300 (15)	79 (7)
C^{γ} (51)	2175 (15)	6917 (10)	8731 (15)	159 (12)
C^{γ} (52)	817 (19)	6197 (9)	8180 (17)	142 (11)
N (6)	312 (9)	7916 (6)	6241 (12)	62 (5)
C^{α} (6)	148 (12)	8466 (7)	6307 (15)	66 (6)
C′ (6)	−1214 (13)	8628 (7)	5579 (15)	55 (6)
O (6)	−1782 (9)	8398 (5)	4672 (9)	67 (4)
C^{β} (6)	1172 (13)	8733 (8)	5616 (19)	116 (10)
N (7)	−1652 (10)	9066 (6)	6063 (11)	69 (5)
C^{α} (7)	−2784 (12)	9326 (7)	5369 (14)	64 (6)
C′ (7)	−2364 (19)	9679 (8)	4337 (17)	79 (8)
O (7)	−1333 (11)	9831 (7)	4241 (13)	120 (6)
C^{β} (7)	−3421 (15)	9646 (7)	6334 (15)	81 (7)
C^{γ} (7)	−4014 (18)	9341 (8)	7468 (17)	93 (8)

Table I (*Continued*)

	x	y	z	U (eq)[a]
C$^{\delta}$ (71)	−5127 (17)	9015 (9)	6833 (20)	141 (11)
C$^{\delta}$ (72)	−4430 (20)	9739 (10)	8324 (20)	161 (12)
O (8)	−3416 (12)	9764 (6)	3472 (13)	105 (6)
C (8)	−3154 (18)	10119 (8)	2503 (18)	134 (10)
W (1)	7413 (8)	7439 (6)	3851 (8)	76 (4)
W (2)[c]	1750 (39)	3693 (43)	9224 (49)	344 (57)
O (B)[d]	290 (24)	3600 (7)	10119 (16)	168 (12)
C (1B)[d]	600 (18)	3476 (8)	8841 (14)	139 (11)
C (2B)[e]	1574 (31)	3839 (10)	8436 (26)	41 (9)
C (3B)[e]	1101 (38)	2946 (8)	8805 (29)	79 (14)
C (2B)′[e]	675 (41)	3950 (10)	8069 (23)	83 (14)
C (3B)′[e]	1853 (34)	3197 (17)	8932 (30)	95 (28)
O (A)	−353 (11)	9440 (6)	8512 (11)	117 (6)
C (1A)[f]	502 (21)	9846 (8)	9022 (26)	140 (19)
C (1A)′[g]	693 (24)	9806 (12)	8696 (31)	164 (33)
C (2A)′[g]	1413 (46)	9752 (20)	10009 (51)	189 (26)
C (3A)′[g]	186 (60)	10335 (9)	8519 (63)	210 (30)
C (2A)[c]	1877 (16)	9683 (16)	9070 (45)	72 (14)
C (3A)[c]	228 (48)	9995 (25)	10337 (43)	175 (36)
C (2A)″[c]	1236 (43)	10070 (16)	8022 (37)	75 (15)
C (3A)″[c]	−236 (45)	10255 (17)	9600 (52)	98 (18)

[a] Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.
[b–g] Occupancy (approximately); b = 0.5, c = 0.3; d = 0.7, e = 0.35, f = 0.6, g = 0.4.

facilitates helix formation.[29,30,*] The ready crystallizability of Aib-containing peptides permits precise determination of these structures by x-ray diffraction. We describe in this report the characterization in crystals of a partially unwound helix in a heptapeptide, Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (I) and compare the structure (referred to as molecule C) with that determined earlier in another crystal form (molecules A and B).[29] The results provide a snapshot of progressive unwinding of an α-helix with increasing solvation.

EXPERIMENTAL PROCEDURES

Crystal Structure

The heptapeptide was synthesized by conventional solution phase procedures using a fragment-condensation approach and purified by reverse phase high performance liquid chromatography on a C_{18} column.[31] Crystals were grown by slow evaporation from a DMSO/isopropanol solution. The x-ray diffraction data were measured on a dry crystal at room temperature with an automated four-circle diffractometer equipped with a graphite monochromator. The $\theta/2\theta$ scan mode was used with a 2.0° + $2\theta(\alpha_1 - \alpha_2)$ scan width, 8°/min scan, and $2\theta_{max}$ = 112° (0.93 Å resolution). Three reflections, monitored after every 97 measurements, lost about 12% of their intensity in the course of the data collection. An appropriate correction was made to the measured intensities. The crystal data are $C_{38}H_{69}N_7O_{10} \cdot H_2O \cdot 2C_3H_8O$, mol. wt. 784.0 + 138.2, space group $P2_1$, cell parameters a = 10.350(2) Å, b = 26.084(4) Å, c = 10.395(2) Å, β = 96.87(1)°, V = 2786.2(9) Å^3, Z = 2, calculated d = 1.099 g/cm^3.

The structure was solved by direct phase determination using the programs in the SHELX84 package (micro VAX version of the SHELXTL system of programs, Siemens Instruments, Madison, WI). A positional disorder in the side chain of Leu(3) as well as disorders in the two cocrystallized isopropanol molecules required an alternation of difference map calculations with cycles of least-squares calculations and distance constraints for the disordered groups. Full-matrix anisotropic least-squares were performed on the C, N, and O atoms

* The crystal structure of Boc-Val-Ala-Leu-Phe-Aib-Val-Ala-Leu-Phe-OMe reveals a helical backbone conformation of the nanopeptide (unpublished results).

Table II Torsional Angles (deg)[a]

Residue	ϕ	ψ	ω	χ^1	χ^2
Val (1)	−75[b]	−36	177	179, −56	
Ala (2)	−76	−44	−179		
Leu (3)	−62	−29	−179	−151	−178, 55[c]
				−79	−53 ,−175[c]
Aib (4)	−59	−28	−176		
Val (5)	−109	+17	−180	60, −63	
Ala (6)	−102	153	169		
Leu (7)	−86	159[d]	176[e]	−63	−65, 174

[a] 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) are described in Ref. 41.
[b] C′ (0), N (1), C$^\alpha$ (1), C′ (1).
[c] Side-chain disordered, with two conformations.
[d] N (7), C$^\alpha$ (7), C′ (7), O (OMe).
[e] C$^\alpha$ (7), C′ (7) O (OMe), C (OMe).

in the peptide, after which H atoms were placed in idealized positions, with C—H = 0.96 Å, and allowed to ride with the C or N atom to which each was bonded for the final cycles of refinement. The occupancy for the two positions of the Leu(3) side chain is near 0.5 each. Water molecule W(1) is fully present. The O(A) atom of one isopropanol molecule (labeled A) is fully occupied; however, there is considerable rotational disorder about the C—O bond (almost unhindered rotation) as well as two distinct positions for the C bonded to O(A). The second isopropanol molecule (labeled B) also has rotational disorder about the C—O bond. Moreover, the second isopropanol molecule B appears to occupy its sites only for 70% of the time. The remainder of the time the site may be occupied by a water molecule labeled W(2). After taking into account the various disorders, the agreement factor R for the least-squares refinement is 8.7% for 2686 reflections observed $> 3.0\ \sigma(F)$.

Fractional coordinates for the C, N, and O atoms are listed in Table I. Bond lengths and bond angles (estimated ESD ~ 0.02 Å for bonds and ~ 1.2° for angles) do not show significant or systematic dif-

Table III Hydrogen Bonds[a]

Type	Donor	Acceptor	O···O or N···O (Å)	H···O[b] (Å)	Angle (deg) C=O···N
Interpeptide	N (1)	O (4)	2.886	2.09	167
Peptide–water	N (2)	W (1)	3.127	2.51	
Peptide–water	N (3)	W (1)	3.093	2.24	
5 → 1	N (4)	O (0)	3.299	2.54	144
4 → 1	N (5)	O (2)	3.081	2.25	121
4 → 1	N (6)	O (3)	2.995	2.12	114
Peptide–Ipr	N (7)	O (A)	2.901	2.01	
Peptide–water	W (1)	O (4)[c]	3.016		
Peptide–water	W (1)	O (6)[c]	2.741		
Ipr–Ipr	O (A)	O (B)[d]	2.610		
Ipr–Peptide	O (B)[d]	O (5)	2.734		

[a] Carbonyl oxygen atoms O (1) and O (7) do not participate in any hydrogen bonding. Hydroxyl oxygen O (A) and O (B) are in two isopropanol solvent molecules (Ipr).
[b] The H atoms were placed in idealized positions with the N—H distance equal to 0.96 Å.
[c] Symmetry equivalent $1 + x, y, z$ to coordinates listed in Table I.
[d] Symmetry equivalent $-x, 1/2 + y, 2 - z$ to coordinates listed in Table I.

ferences from expected values. Torsional angles are listed in Table II and hydrogen bond values are in Table III.

NMR Studies

Two hundred seventy MHz ^{1}H-nmr spectra were obtained on a Bruker WH-270 spectrometer at the Sophisticated Instruments Facility, Bangalore. Spectra were recorded at peptide concentrations of 10 mg/mL in $CDCl_3$ or $(CD_3)_2SO$.

RESULTS AND DISCUSSION

Figure 1 shows a stereoview of the structure of peptide I in crystals obtained from DMSO–isopropanol mixtures (molecule C). Although the crystals were grown from a solution that was predominantly DMSO, the cocrystallized solvent molecules are water and isopropanol. The backbone torsion angles listed in Table II show that the four N-terminal residues adopt classical helical conformations, while Val(5) falls in the "bridge region" of the Ramachandran map.[4,32] Such distorted conformations are generally observed at the C-terminal end of helical segments in proteins.[33] Ala(6) and Leu(7) adopt completely nonhelical ϕ,ψ values, with both residues lying in the β-strand region of ϕ,ψ space. Two good 4 → 1 hydrogen bonds are observed, viz., Ala(2) CO—HN Val(5) and Leu(3) CO—HN Ala(6) (Table III). The only interaction that may be considered as a 5 → 1 hydrogen bond, albeit weak, is between the Boc CO and Aib(4) NH groups. However, the N(4)—O(0) distance is rather long, 3.30 Å.

Solvation and Packing

The three solvent molecules in the crystal form several hydrogen bonds to the peptide backbone. The Leu(7) NH and Val(5) CO groups hydrogen bond to two different isopropanol hydroxyl groups (Figure 2). The pair of solvent molecules are also held together by a O(A)H—O(B) hydrogen bond. The water molecule in this structure does not invade the helix but mediates hydrogen bonding between two peptide molecules that repeat by translation along the *a* axis (Figure 2). The water molecule accepts hydrogen bonds from N(2)H and N(3)H of one peptide molecule and donates hydrogen bonds to carbonyl O(4) and carbonyl O(6) of a neighboring molecule.

There is also a direct hydrogen bond N(1)H—O(4) between the same two peptide molecules. Molecule C is no longer a sufficiently good helix to pack with head-to-tail hydrogen bonding, forming long helical columns as is common in crystals of helical peptides.[27] In this case, the hydrogen bonding occurs only laterally between peptide molecules. In contrast, head-to-tail hydrogen bonding is a feature

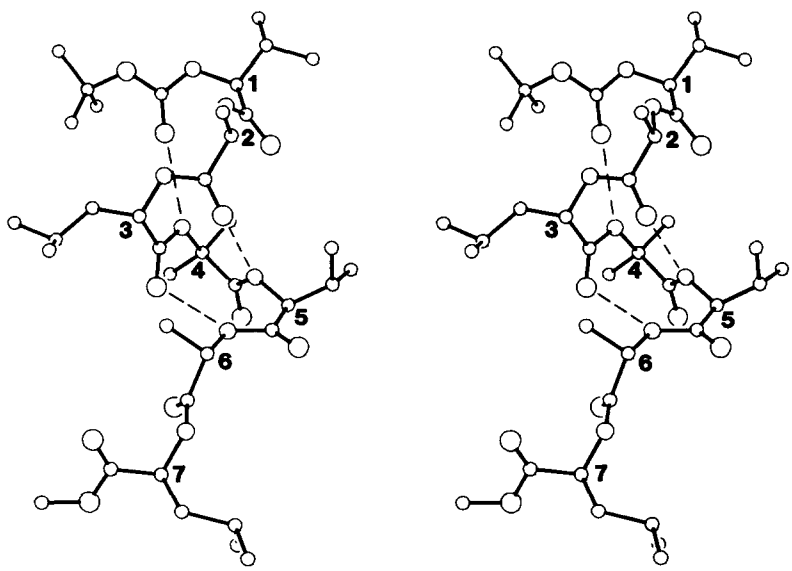

Figure 1. Stereoview of the structure of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (I) in crystals from DMSO–isopropanol.

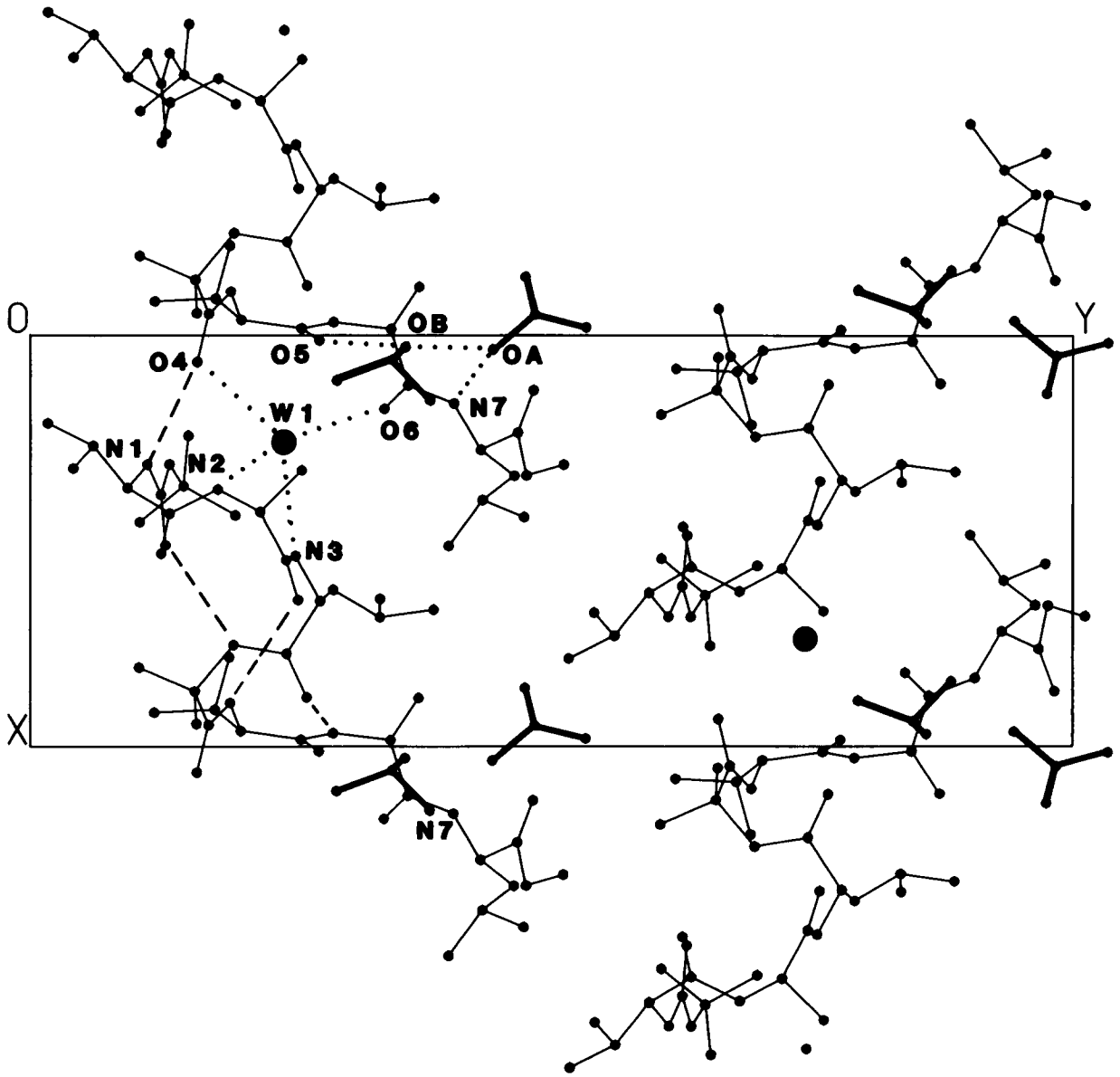

Figure 2. Packing diagram (projected down the *c* axis) of I crystallized from DMSO–isopropanol. The water molecule is denoted by a heavy dot, while the isopropanol molecules are shown in heavy lines. Only one of the conformations in the disorder in the isopropanol molecules and the side chain in Leu (3) is shown for clarity. Dashed lines represent NH · · · O=C hydrogen bonds, while dotted lines show hydrogen bonds to solvent molecules.

present in columns of molecules A and B in the crystals from methanol–water.[29]

Comparison of Crystal Forms

The structure of peptide I has been determined earlier in crystals grown from methanol–water, which also belonged to the space group $P2_1$ with two independent molecules in the asymmetric unit.[29] Figure 3 compares the structures of the three conformations observed in crystals. The backbone torsion angles ϕ,ψ are compared in Figure 4. Molecule A (Figure 3a) is an almost complete α-helix, with three 5 → 1 and one 4 → 1 hydrogen bonds. The N—O distances for the 5 → 1 hydrogen bonds range from 3.02 to 3.10 Å, while the N—O=C angle has values between 145° and 165°. For the 4 → 1 hydrogen bond between Leu(3) CO and Ala(6) NH groups,

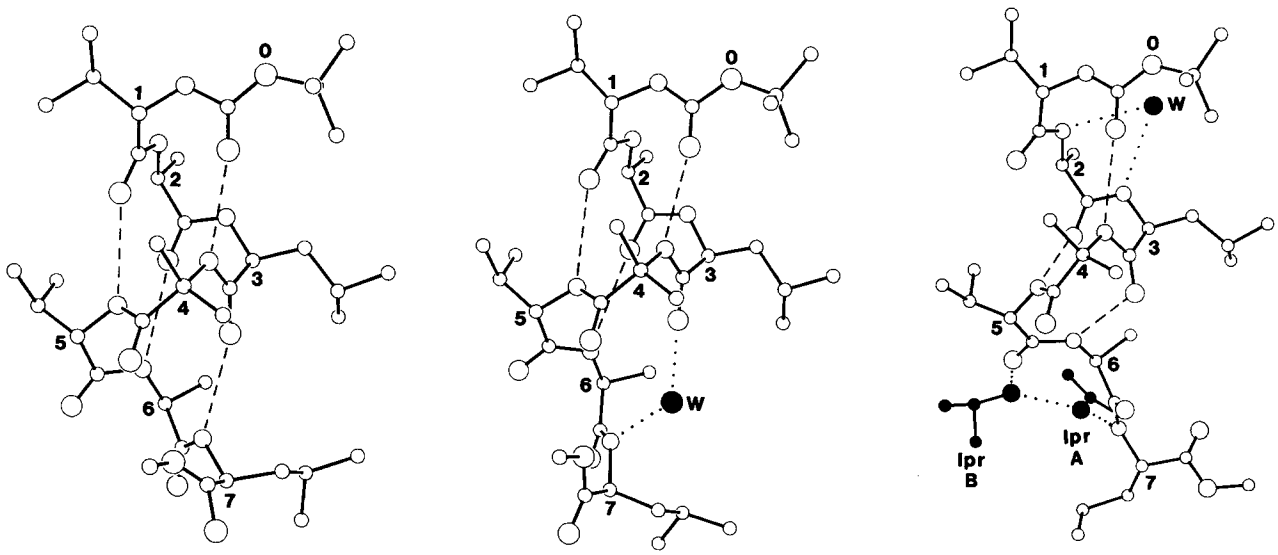

Figure 3. Unfolding of α-helix in Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe. (a) α-Helix in crystal grown from MeOH/H_2O (molecule A). (b) Insertion of water molecule W into helix backbone molecule B. Conformers A and B are side by side in same crystal.[29] (c) Unwinding of helix by two isopropanol molecules in crystal grown from DMSO–IprOH (molecule C). C^{α} atoms are numbered 1–7. Letter O denotes O atom of Boc group. Dashed lines indicate NH · · · O=C hydrogen bonds and dotted lines indicate hydrogen bonds in which solvent molecules participate. Only one position is shown for the disordered atoms in (c), which includes the side chain in Leu (3) and the methyl groups in both isopropanol molecules.

the corresponding values are 2.98 Å and 111°. The sole distortion from an ideal α-helix is the large N—O distance of 3.33 Å corresponding to an Ala(2) CO—HN Ala(6) hydrogen bond. The N—O=C angle in this case is 149°. Such long 5 → 1 distances have been observed a number of times in peptide helices containing Aib residues.[27] In A, this distortion is a consequence of slightly aberrant ϕ,ψ values (−87°, −11°) at Val(5), which differ significantly from the standard values of an α-helix and even more from those of a 3_{10}-helix.[26] The NH groups of Ala(6) and Leu(7) simultaneously hydrogen bond to the CO group of Leu(3).

In molecule B from methanol–water (Fig. 3b), a water molecule has broken the Leu(3) CO—HN Leu(7) hydrogen bond, and forms two hydrogen bonds that bridge N(7) and O(3). The ϕ,ψ values for Val(5) undergo further distortion (−91°, 2°). However, Ala(6) remains in the helical region (−73°, −36°) (Figure 4). The consequences of the rotation in Val(5) have been to increase the N(6)—O(2) distance to 3.49 Å and the N(7)—O(3) distance to 4.13 Å. The remaining two 5 → 1 and one 4 → 1 hydrogen bonds are retained as in molecule Å.

Greater damage to the helix occurs in the crystal form from DMSO–isopropanol (Molecule C, Figure 3c). Although the ϕ,ψ values for residues 1–4 have changed relatively little (Figure 4) from the conformations in Figure 3 a,b, the N(5)—O(1) separation has increased to 4.10 Å. In fact, the only resemblance to a 5 → 1 type hydrogen bond in Figure 3c is the N(4)—O(0) pair with a rather long distance of 3.30 Å. The carbonyl group of Val(1) does not participate in any hydrogen bonding. Further the ϕ,ψ values for Val(5) continue to diverge from helical values. The conformational change at the Ala(6) and Leu(7) residues is dramatic. At both residues there is an almost complete flip of 180° about ψ, with smaller changes of ϕ (Figure 4). The unraveling of the backbone at the C-terminus in molecule C is facilitated by peptide–solvent hydrogen bonds involving two isopropanol molecules.

Figure 3 demonstrates progressive unwinding of

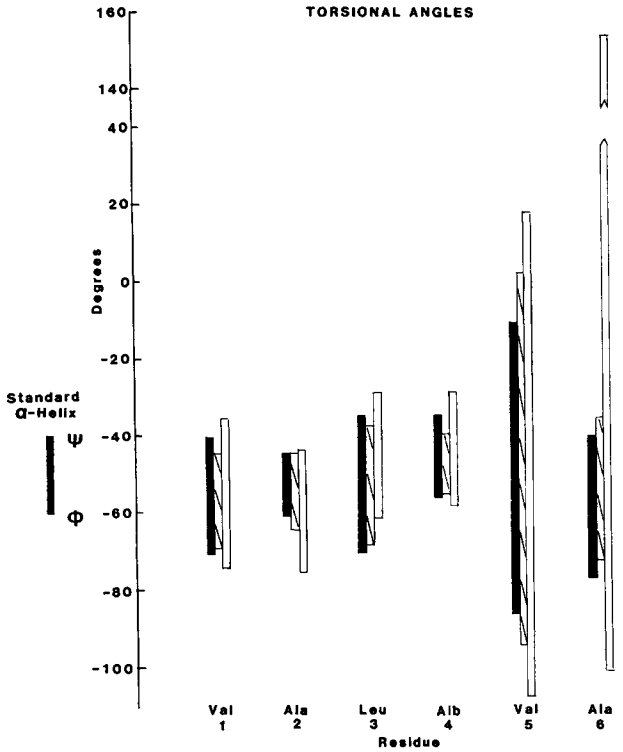

Figure 4. A comparison of the torsional angles ϕ ($N_i - C_i^\alpha$) and ψ ($C_i^\alpha - C_i'$) in the three crystalline conformations of I shown in Figure 3: solid bar, helix A; hashed bar, hydrated helix B; open bar, partially unfolded helix C. The bottom of the bar represents the ϕ value and the top of the bar represents the ψ value. A bar showing ϕ and ψ values for a "standard" α-helix is shown at the far left. The comparisons for residue 7 (not illustrated) show a typical effect for helix termination.

the heptapeptide helix in I, with increasing solvation. Interestingly, this 7-residue sequence has been used as a "helical module" in the synthetic construction of a peptide mimic of an α,α-supersecondary structural motif. Crystal structure analysis of the peptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-Acp-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (Acp, ϵ-aminocaproic acid) reveals that both segments are completely helical, with the central Acp residue serving as a linking spacer.[34]

Solution Conformation

In solution the fragility of the helix in I has been noted in nmr studies. Assignments of spin systems were made using the two-dimensional correlated spectroscopy spectrum shown in Figure 5. Val(1) and Ala(2) NH groups could be unambiguously assigned to the upfield NH resonances in $CDCl_3$ following earlier studies.[35] The lone singlet is the Aib(4) NH resonance. The only ambiguous assignment is that of the two Leu NH groups. One-dimensional difference NOE experiments were unsuccessful in resolving this assignment. Relevant nmr parameters for amide resonances are summarized in Table IV. Solvent titration experiments[35] in $CDCl_3 - (CD_3)_2SO$ mixtures (Figure 6) establish that only two NH groups, assigned to Val(1) and Ala(2), move appreciably downfield on addition of the strongly hydrogen-bonding solvent, $(CD_3)_2SO$, to solutions in the apolar solvent, $CDCl_3$. Clearly, in solutions containing up to 30% $(CD_3)_2SO$, the remaining five NH groups are shielded from solvent,[35] strongly supporting a completely helical conformation involving the NH groups of residues 3–7 in intramolecular hydrogen bonds. A completely 3_{10}-helical stretch with five 4 → 1 hydrogen bonds is consistent with the data. However, structures involving both 4 → 1 and 5 → 1 hydrogen-bonding patterns, with the Boc CO group accepting hydrogen bonds from both Leu(3) and Aib(4) NH groups

Table IV Nmr parameters

	$CDCl_3$		$(CD_3)_2SO$		
Resonance	δ_{NH} (ppm)	$^3J_{HNC^\alpha H}$ (Hz)	δ_{NH} (ppm)	$^3J_{HNC^\alpha H}$ (Hz)	$d\delta/dT$ (ppb/K)
Val (1) NH	5.34	3.3	6.76	8.6	6.8
Ala (2) NH	6.78	3.5	8.0	7.5	5.0
Leu (3) NH[a]	7.30	7.4	7.91	9.2	5.0
Aib (4) NH	7.49	—	8.08	—	4.8
Val (5) NH	6.87	6.8	7.07	8.2	1.3
Ala (6) NH	7.65	7.9	7.98	6.6	5.8
Leu (7) NH[a]	7.27	8.8	7.88	7.8	4.0

[a] Assignment of these resonances is based on a comparison of their coupling constants and torsion angles ϕ in the crystal structure.

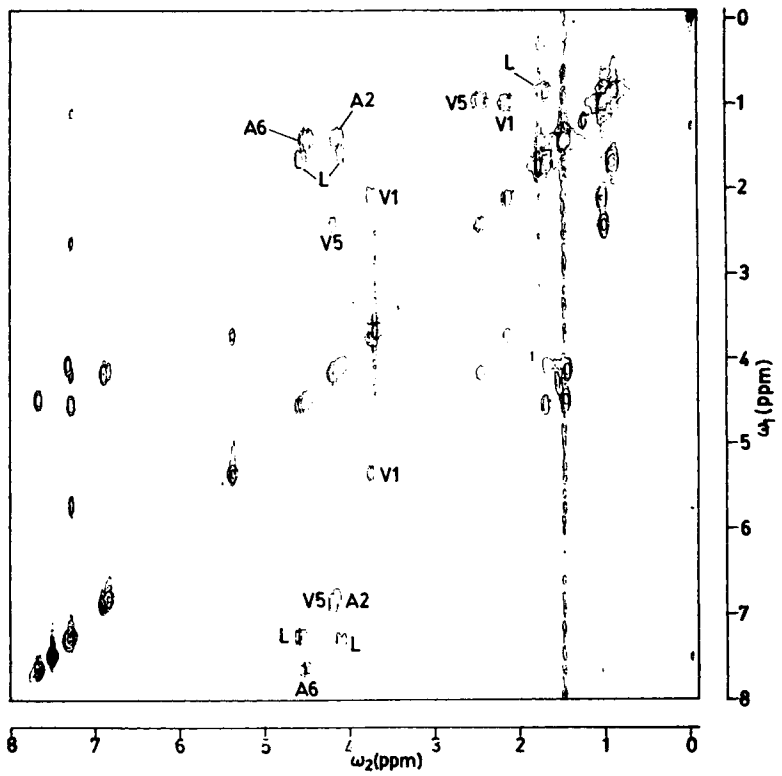

Figure 5. Two-dimensional correlated spectroscopy (^{1}H, 270 MHz) spectrum of Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe (I) in $CDCl_3$. Peptide concentration ~ 10 mg/mL. Assignments are marked against the NH-C$^\alpha$H cross peaks using a one-letter code (V, Val; A, Ala; L, Leu).

cannot be ruled out. Such hydrogen-bonding patterns with a mixed $3_{10}/\alpha$-helical turn are often found at the N-termini of α-helical peptides.[27]

An interesting feature of the nmr data in $(CD_3)_2SO$ is the relatively high value of the temperature coefficients ($d\delta/dT$) of all the NH groups (> 4 ppb/K), with the exception of Val(5) NH, which has a value of 1.3 ppb/K (Table IV). This suggests a completely nonhelical conformation in DMSO, with an isolated Leu(3)-Aib(4) β-turn, stabilized by a 4 → 1 hydrogen bond between the Ala(2) CO and Val(5) NH groups. Such X-Aib β-turns are a common feature in small Aib-containing peptides.[36,37] The $J_{HNC^\alpha H}$ values in $CDCl_3$ are small at the N-terminus, characteristic of helical conformations. The values for the C-terminal residues are larger (6–7.5 Hz), but still within limits seen in completely helical peptides.[35] In $(CD_3)_2SO$, the J values for all residues are uniformly high, with values > 8 Hz for Val(1), Leu(3), and Val(5) residues, which are characteristic of extended conformations ($\phi > 100°$).[38] The nmr data is thus supportive of a helical structure in $CDCl_3$, which is completely disrupted in the more strongly solvating medium, $(CD_3)_2SO$.

CONCLUSIONS

Peptide I, with a single centrally placed Aib residue, appears to be a good model for examining transitions from a helical structure to more disordered conformations. In crystals the various structures determined provide a snapshot of the effects of solvation on unwinding the helix. The fragility of the structure appears to be more pronounced at the C-terminus. The nmr studies reinforce the conclusion that the helix in heptapeptide I is finely poised with respect to unfolding. The crystal structure analysis of polymorphic forms of peptides with inherently fragile secondary structures may provide useful structural information on intermediate solvated forms, which may be valuable in picturing the folding–unfolding process. Specific hydration may indeed direct helical folding as noted for very short peptides[39] and could be an important feature of helices in proteins even

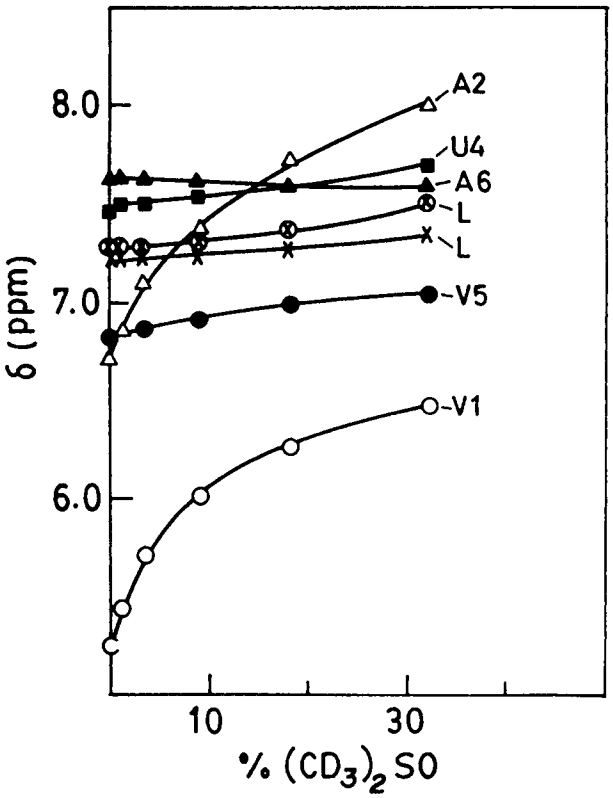

Figure 6. Solvent dependence of NH chemical shifts in peptide I $CDCl_3$ – $(CD_3)_2SO$ mixtures of varying composition. Peptide concentration ~ 10 mg/mL. Assignments to specific residues are marked using a one-letter code (V, Val; A, Ala; L, Leu; U, Aib).

in solution, as noted in a recent nmr study involving localization of bound water molecules.[40]

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Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients and H atom coordinates will be deposited with the Cambridge Crystallographic Data File; observed and calculated structure factors are available from the authors.

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