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A Change in the 3_{10}- to α-Helical Transition Point in the Heptapeptides Containing Sulfur and Selenium

Published as part of a virtual special issue on Structural Chemistry in India: Emerging Themes

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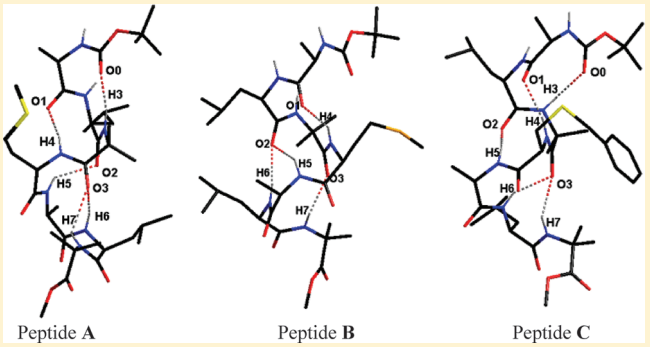
Supporting Information

ABSTRACT: Crystal structures of three heptapeptides Boc-Ala-Leu-Aib-XXX-Ala-Leu-Aib-OMe (where XXX = methionine in peptide **A**, selenomethionine in peptide **B**, and *S*- benzyl cysteine in peptide **C**) reveal mixed 3_{10}-/α-helical conformations with *R* factors of 6.94, 5.79, and 5.98, respectively. All the structures were solved in the $P2_12_12_1$ space group. 3_{10}- to α-helical transitions are observed in all of these peptides. The helices begin as a 3_{10}-helical segment at the N-terminus and then transit for peptides **A** and **C** at residue Aib(3) carbonyl (O(3)), while for peptide **B** the transition occurs at residue Leu(2) carbonyl oxygen (O(2)). There are water molecules associated in the crystal of each of these peptides and they form different types of hydrogen bonding patterns in each crystal. The observations suggest that 3_{10}- to α-helical transition is sequence dependent in these short heptapeptide sequences.



INTRODUCTION

The 3_{10}-helix, the α-helix, and the π-helix are three frequently occurring monomeric helical secondary structures found in globular proteins. They differ only in the pattern of their C═O···HN intramolecular hydrogen bonding. This hydrogen bonding scheme in a 3_{10}-helix is from the *i*th to *i* + 3rd amino acid residue (assuming that the numbering begins at the nitrogen for a residue and ends at the carbonyl after the Cα next to it), whereas it is from the *i*th to *i* + 4th residue in an α-helix and from *i*th to *i* + 5th residue in case of a π- helix. 3_{10}- and α-helices are the most abundantly occurring helices in proteins.[1,2] In proteins, the 3_{10}-helices are significantly shorter than the α- helix.[1] There are 3.0 residues per turn in a 3_{10}-helix as compared to 3.6 residues per turn in the case of α-helices.[1] Although the 3_{10}-helix is less stable than the α-helix, it exists as approximately 15−20% of all the helices present in the proteins.[1] In fact, water inserted 3_{10}-helices have been looked upon to be snapshots during the folding of α-helices by Sundaralingam and Shekharudu.[3] 3_{10}-helices are biochemically important as *peptaibol* antibiotics of fungal origin contain a significant amount of these helices.[4−7] The 3_{10}-helix is most commonly formed and stabilized in the presence of α-aminoisobutyric acid.[8−12] In the literature, considerable effort has been devoted to 3_{10}-/α-helical transitions that were shown to depend upon the chain length and the amount of Aib residues in the peptides.[2,13] The Aib containing peptides were shown to crystallize in the form of a α-helix, a 3_{10}-helix, or mixed 3_{10}-/α-helices[10] making them suitable for such model studies. Mixed 3_{10}-/α-helices are reported in Aib containing peptides where a structural helical transition from 3_{10}- to α-helix is observed quite frequently.[13] Peptide sequences with five residues or less are favored to form 3_{10}-helices,[14] and sequences with more than seven residues tend to form mixed 3_{10}-/α- or α-helices.[9,10] The energy of this transition has been predicted by theory to have a low conformational energy barrier.[15−18] So it is important to study 3_{10}- to α-helical transitions in peptides in terms of both sequence and chain length. The Ala-Leu-Aib segment has been characterized well in the literature to yield nice helices in crystals.[10] In that context, we synthesized and crystallized three Aib containing heptapeptides Boc-Ala-Leu-Aib-XXX-Ala-Leu-Aib-OMe where XXX is methionine in peptide **A** selenomethionine in peptide **B** and a protected cysteine in peptide **C**. Our design of seven residue peptides was made with the rationale that this well characterized Ala-Leu-Aib segment should now nest the guest residues to nucleate the helix. The purpose was to study the effect of bulky side chain atoms on helix transitions.

These model peptides allow an investigation into the structural presence of sulfur and selenium in 3_{10}-/α-helical transitions. Herein we show that mixed 3_{10}-/α-helices are indeed observed in

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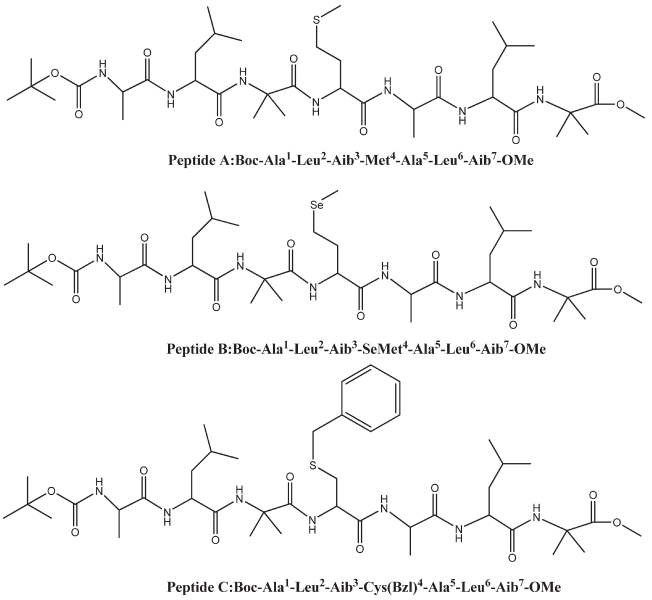

Figure 1. Chemical structures and the amino acid sequences of the protected peptides **A**, **B**, and **C**. The numbers reflect the position of amino acids in the sequence.

these short peptides with a structural transition from 3_{10}- → α-helix at Leu(2) to residue Aib(3) when the residue at position four is changed from selenomethionine to either methionine or a protected cysteine.

EXPERIMENTAL SECTION

Peptide Synthesis Strategy. All amino acids barring Aib were of the L-configuration. The amino acids were procured from SD Fine Chemical, Sigma Chemicals, and Loba Chemicals. Selenomethionine was a gift from Dr. Majeed of Sami Laboratories India. All the peptides were synthesized by solution phase synthesis methods using a fragment condensation strategy using previously published protocols[19]

The N-terminus was protected as a tertiary butyloxy carbonyl (Boc) and the C-terminus was protected as a methyl ester. Deprotection of Boc group was performed using 98% formic acid, and deprotection of methyl ester group was carried out by 4(N) NaOH in 50% methanol. Peptides were coupled using dicyclohexyl carbodiimide/1-hydroxy benzotriazole.[20] Intermediate peptides were used without purification after ascertaining that there was no racemization during the synthesis by high field proton NMR. The final peptides were purified by column chromatography and eventually by high performance liquid chromatography (HPLC) in a C18 column (5 μ) using a methanol−water gradient. Purified peptides were further characterized by ESI-MS and 500 MHz ^{1}H NMR by 1D, 2D (COSY, ROESY) in $CDCl_3$.

X-ray Crystallography. Single crystals of the three peptides were obtained by slow evaporation from methanol −water using batch protocols. Single-crystal X-ray data were collected using graphite-monochromated Mo-*K*α radiation (λ = 0.71073 Å) on "Bruker SMART APEX CCD" diffractometer at temperature 293 K. The data integration and reduction were processed with SAINT+.[21] The structures were solved by direct methods using Sir92.[22] The structures were refined using full matrix least-squares on F^2 (SHELX 97).[23] All the non-hydrogen atoms refinement was performed anisotropically. The hydrogen atoms were refined isotropically as riding atoms except for some of the NH and water hydrogen atoms which were located on difference Fourier maps and were constrained to those positions. DIAMOND

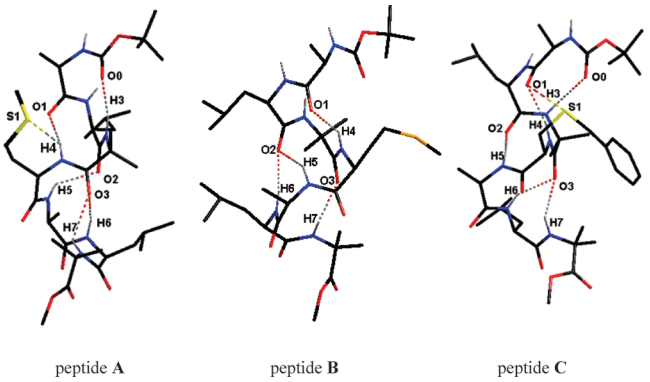

Figure 2. Crystal structures of the three peptides **A** (methionine at position 4), **B** (selenomethionine at position 4), and **C** (S-benzyl cysteine at position 4). The residues are numbered from 1 to 7. The Boc protecting group is numbered as zero and the methyl ester group as 8 in all the peptides. Some of the intramolecular hydrogen bonds used to characterize the helical types together with the short contacts in peptide **A** and peptide **C** are also shown. Only the amide hydrogens in all the peptides are displayed.

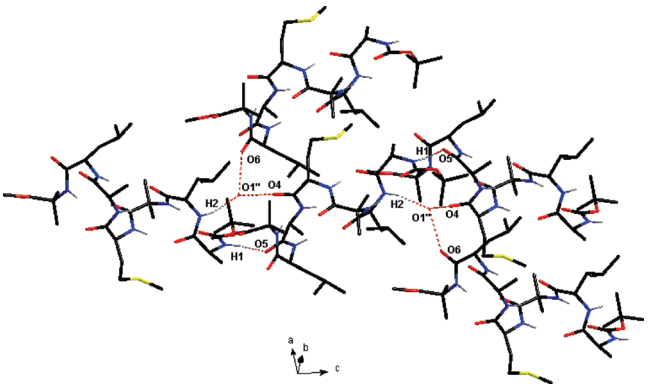

Figure 3. Intermolecular hydrogen bonding pattern observed in peptide **A** showing involvement of water molecule in connecting adjacent unit cells to form a complete network.

(version 3.0) and Mercury (version 1.4.1) were used to view and draw the structures.

RESULTS AND DISCUSSION

The peptide **A** helix begins as a 3_{10}-helical segment with four successive 1 ← 4 hydrogen bonds which involves Aib(3)NH with Boc carbonyl, Met(4)NH with Ala(1) carbonyl, Ala(5)NH with Leu(2) carbonyl, and Leu(6)NH with Aib(3) carbonyl groups. Aib(3) carbonyl oxygen also participates in 1 ← 5 hydrogen bonding with Aib(7)NH permitting a helical transition from 3_{10}- → α-helix (N(7)H···O(3)). Sulfur atom (S1) of Met(4) residue is in contact with H(4) and the S(1)···H(4) distance is 3.2 Å. Molecules in the crystal are aligned in a head-to-tail manner aided by intermolecular hydrogen bonding. There is one direct intermolecular hydrogen bonding between N(1)H···O(5). The water molecule in the crystal is inserted into the head-to-tail region. This water molecule O(1″) forms a trifurcated hydrogen bond (O(1″)···O(4), O(1″)···O(6), N(2)H···O(1″)) connecting three different peptide molecules.

Peptide **B** helix also starts with a 3_{10}-helical segment with two successive 1 ← 4 hydrogen bonds involving SeMet(4) and

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Table 1. Crystallographic Parameters

molecule	A	B	C
formula	$C_{37}H_{67}N_7O_{10}S$, H_2O	$C_{37}H_{67}$ N_7O_{10} Se, $3H_2O$	$C_{42}H_{69}N_7O_{10}S$, H_2O
formula weight	820.05	902.98	882.12
temperature/K	293(2)	293(2)	293(2)
crystal system	orthorhombic	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a/Å	9.253(4)	11.939(2)	22.410(2)
b/Å	21.321(10)	16.031(3)	9.218(3)
c/Å	23.611(12)	24.640(3)	23.588(2)
α/°	90.000	90.000	90.000
β/°	90.000	90.000	90.000
γ/°	90.000	90.000	90.000
V/Å^3	4658.05(4)	4716.0(13)	4872.7(17)
Z	4	4	4
D_c/g cm^{-3}	1.169	1.272	1.202
μ/mm^{-1}	0.128	0.860	0.128
F (000)	1776	1928	1904
reflections collected	25981	26440	27388
independent reflections	9081	9257	9543
parameters	536	586	600
goodness-of-fit on F^2	0.942	1.008	1.040
R_1, wR_2(obs) [$I > 2\sigma(I)$]	0.0694, 0.1322	0.0579, 0.1207	0.0598, 0.1281
R_1, wR_2(all data)	0.1641, 0.1752	0.0822, 0.1351	0.0893, 0.1601

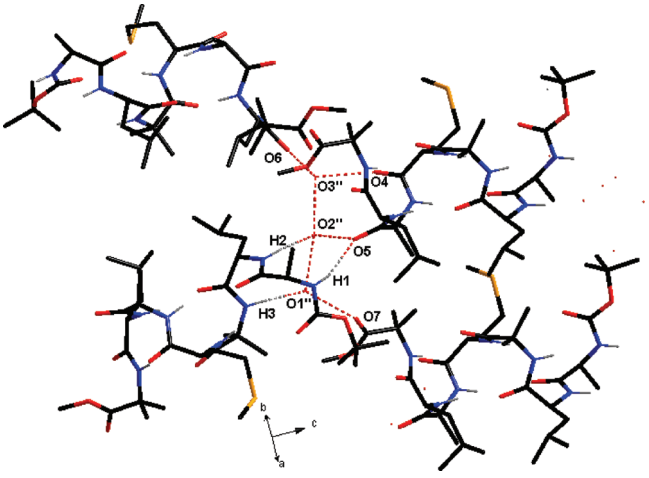

Figure 4. Intermolecular hydrogen bonding pattern observed in peptide **B** showing involvement of three water molecules in connecting neighboring unit cells to form a complete network.

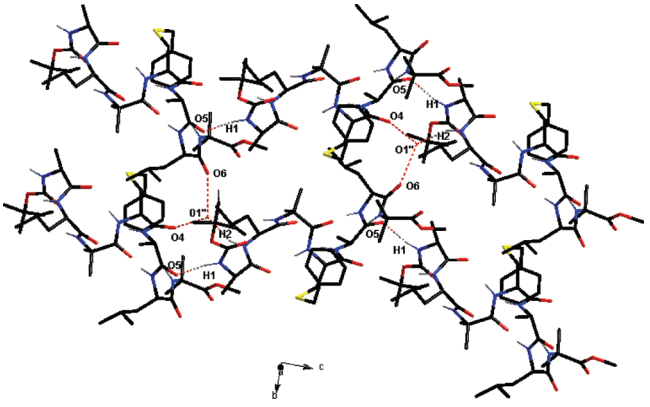

Figure 5. Intermolecular hydrogen bonding pattern observed in peptide **C** showing involvement of a water molecule (O1′′) in connecting adjacent unit cells to form a complete network.

Ala(5) NH groups hydrogen bonded with Ala(1), Leu(2) carbonyl oxygen atoms respectively (N(4)H···O(1), N(5)H···O(2)). Leu(2) carbonyl oxygen accepts another hydrogen from Leu(6)NH (N(6)H···O(2)) which permits a helical transition from 3_{10}- → α-helix as a 1 ← 5 hydrogen bond. Then the helix ends as an α- helix which involves hydrogen bonding between Aib(7)NH and Aib(3) carbonyl group(N(7)H···O(3)). There are three hydrogen bonded water molecules O(1′′), O(2′′), and O(3′′)) in this crystal. Molecules are arranged in a head-to-tail packing of helices in the crystal. In the crystal packing, there is one direct hydrogen bond between two molecules (N(1)H···O(5)). Water molecules O(1′′), O(2′′), and O(3′′) form hydrogen bonding to each other (O(1′′)···O(2′′)···O(3′′)). O(1′′) is further hydrogen bonded with O(7) and N(3)H, while O(2′′) is hydrogen bonded with N(2)H and O(5) and O(3′′) is hydrogen bonded with O(4) and O(6) to different peptide molecules inside the crystal lattice. Three water molecules connect a total of four peptide molecules in the crystal to form a complicated intermolecular hydrogen bonding pattern.

The peptide **C** helix begins as a 3_{10}-helical segment. Again there are four successive 1 ← 4 hydrogen bonds which involve Aib(3)NH with Boc carbonyl, Cys(Bzl)(4) NH with Ala(1) carbonyl, Ala(5) NH with Leu(2) carbonyl and Leu(6)NH group with the carbonyl groups of Aib(3). O(1) of Ala(1) also has a short contact with the sulfur (S1) of Cys(Bzl)(4) side chain.

Table 2. Hydrogen Bonding Distances Observed in Peptides A, B, and C[a]

			peptide A		peptide B		peptide C	
type	donor	acceptor	N···O/O···O (Å)	H···O (Å)	N···O/O···O (Å)	H···O (Å)	N···O/O···O(Å)	H···O (Å)
1 ← 4 transition	N3	O0	2.95(6)	2.14	**3.73**	**3.37**	2.96(4)	2.22(4)
	N4	O1	3.12(6)	2.32	2.93(4)	2.25	3.12(4)	2.21(4)
	N5	O2	2.91(6)	2.24	2.95(4)	2.30(3)	2.97(4)	2.42(6)
	N6	O3	3.09(6)	2.52(4)	**3.51**	**3.09**	2.95(4)	2.15(3)
1 ← 5 transition	N6	O2	**3.46**	**2.78**	2.96(5)	2.09(2)	**4.02**	**3.34**
	N7	O3	3.04(6)	2.50	2.93(5)	2.30(4)	3.01(4)	2.27(3)
head to tail	N1	O5	2.82(6)[b]	2.00(5)	2.96(5)[f]	2.14(3)	2.85(4)[k]	1.96(3)
solvent	O1″	O4	2.81(6)[c]	1.98(6)			2.77(4)[k]	1.93(4)
	O1″	O6	2.87(6)[d]	2.04(5)			2.83(4)[l]	1.99(3)
	O1″	O7			2.96(5)[g]	2.19(6)		
	O1″	O2″			2.84(5)[c]	2.08(5)		
	O2″	O5			2.72(5)[h]	1.90(6)		
	O2″	O3″			2.74(5)[h]	1.89(4)		
	O3″	O4			2.81(5)	1.98(3)		
	O3″	O6			2.80(4)[i]	1.98(3)		
water bridge	N2	O1″	2.98(6)[e]	2.15			2.93(4)	2.12(4)
	N2	O2″			2.88(5)[j]	2.02(3)		
	N3	O1″			2.92(5)[f]	2.07(2)		

[a] The distances in bold are between atoms that have potential tendencies to form either 3_{10}- or α-helix type H bonds. These distances are clearly more that the ones involved in actual hydrogen bonds. [b] $3/2 - x, 1 - y, 1/2 + z$ (symmetry operations). [c] $1 - x, -1/2 + y, 1/2 - z$. [d] $2 - x, -1/2 + y, 1/2 - z$. [e] $1/2 + x, 1/2 - y, 1 - z$. [f] $3/2 - x, -y, -1/2 + z$. [g] $-1 + x, y, z$. [h] $1 - x, 1/2 + y, 1/2 - z$. [i] $2 - x, 1/2 + y, 1/2 - z$. [j] $1/2 + x, 1/2 - y, -z$. [k] $1/2 - x, 1 - y, 1/2 + z$. [l] $1/2 - x, -y, 1/2 + z$.

Table 3. Comparison of Torsion Angles in the Three Peptides[a]

peptide **A**	ϕ	ψ	ω	peptide **B**	ϕ	ψ	ω	peptide **C**	ϕ	ψ	ω
Ala(1)	−60	−30	−166	Ala(1)	−78	−49	−169	Ala(1)	−63	−31	−163
Leu(2)	−67	−16	−179	Leu(2)	−56	−40	−180	Leu(2)	−67	−28	−180
Aib(3)	−49	−41	173	Aib(3)	−52	−38	−179	Aib(3)	−56	−28	178
Met(4)	−70	−24	−174	SelMet(4)	−67	−35	−177	Cys(S-Bzl)(4)	−57	−32	180
Ala(5)	−70	−28	175	Ala(5)	−80	−34	−180	Ala(5)	−70	−20	180
Leu(6)	−95	−17	−176	Leu(6)	−98	−3	−175	Leu(6)	−103	−15	−178
Aib(7)	−56	−36	−168	Aib(7)	−45	−44	−180	Aib(7)	−57	−38	−165

[a] The estimated standard deviation is around 3.0°.

The S(1)···O(1) distance is 3.28 Å. Aib(3) carbonyl oxygen O(3) accepts two hydrogen bonds (N(6)H···O(3), N(7)H···O(3)) which allows for the helical transition from 3_{10}-helix to α-helix. There is one water molecule associated with this crystal as well. In this peptide molecules are packed in a head-to-tail fashion as has been observed in the other two reported cases. In the crystal packing, there is one direct intermolecular hydrogen bond N-(1)H···O(5) connecting two different peptide molecules. The water molecule O(1″) forms a trifurcated hydrogen bond (N(2)H···O(1″), O(1″)···O(4), O(1″)···O(6)) as observed in the case of peptide **A**. Three molecules of peptides are hydrogen bonded by this single water molecule in peptide **C**.

Helices can also be characterized based on the values of torsion angles (Table 3). In fact the 3_{10}- and the α-helix differ very slightly in their torsion angles. According to Toniolo and Benedetti analysis for peptides the (ϕ, ψ) values are −63°, −42° for α- and −57°, −30° for 3_{10}- helix. Our values indicate that these angles are significantly deviated in the case of Leu(6) because of the bifurcated hydrogen bond in all three peptides. All the other (ϕ,ψ) values in the three peptides lie within acceptable ranges advanced in the literature for such helices by earlier workers.[1,2,24]

Previously correlations have been made between peptide chain length and the type of helix formed in Aib containing peptides.[13] In this study, the composition of Aib is two in seven (28.6%). These are predicted to be 3_{10}-helices for this composition. It is therefore surprising that a mixed 3_{10}-/α-helix with a change in the transition point be observed with just a slight alteration of residue 4 from methionine or *S*-benzyl cysteine to selenomethionine. These can be explained by means of the short contacts observed in the two peptides **A** and **C** illustrated in Figure 2. In peptide **A** the side chain S atom interacts with N−H(4) the distance being 3.2 Å, while in peptide **C** the side chain S atom is at a distance of 3.3 Å from O(1). Both these interactions are indeed absent in peptide **B** and selenium is far away from any meaningful interaction. This means that the extra stabilization offered by these interactions could now tip the energy balance in favor of the 3_{10}-helix in peptides **A** and **C**.

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CONCLUSION

From the crystal structures of the three hepta peptides, we have seen that each of them underwent 3_{10}-/α-helical transition but at different residues. While the 3_{10}-/α-helical transition is established in residue 2 in peptide **B**, it occurs at Aib (3) in peptides **A** and **C**. The only difference in these peptides seems to be the presence of a comparatively bulkier side chain containing selenium in peptide **B** or S-benzyl protecting group in peptide **C**. In the literature while considerable effort has been devoted to 3_{10}-/α-helical transitions,[25−31] no effort has been made in determining/predicting transition points between 3_{10}- → α-helix types. Data analysis[3] by Sundaralingam and Shekharudu in 1989 had led to the suggestion that the 3_{10}-helices are initially formed in the folding process and that these get converted later into stable α-helices. Indeed, elegant experiments subsequently done by Millhauser have only added credence to this belief. Efforts have previously concentrated on use of chiroptical techniques to monitor 3_{10}-/α- transitions by tuning the nature of the solvent.[27,28,32] However, no one has to date suggested the parameters important for the location of this transition. Even though considerable theoretical calculations done by Pengo et al.[27] have shown that the 3_{10}-/α-helical equilibrium is affected by the solvent polarity parameter E_T^N, there is limited evidence of this in sequences of the peptides examined by others.[28,32] Indeed, prediction of such transition points are important for both theory and experiments in folding processes in which 3_{10}-helices must nucleate first only to be transformed into α-helices later. Our results demonstrate the importance of including side chain interactions as they also affect the transition point. In the crystal structure reported here, the water molecules also play an important role in connecting the peptide molecules by forming water mediated hydrogen bonds.[33] Thus, interactions of side chains could be exploited to stabilize alpha helices in extremely short peptides as well.

ASSOCIATED CONTENT

Supporting Information. Scheme and description of synthesis of the peptides, HPLC conditions; NMR, COSY, ROESY, and mass spectra; HPLC profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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