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Peptide α/3_{10}-Helix Dimorphism in the Crystal State

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The two helical structures most frequently found in peptides and proteins, the α- and the 3_{10}-helix, can be visualized as the successions of C═O···H−N intramolecularly H-bonded pseudocyclic structures, the $i \leftarrow i+4$ and $i \leftarrow i+3$ forms, respectively.[1] In these two helices, also the number of residues per turn, the pitch, and the ϕ,ψ backbone torsion angles are different, although not dramatically. About 10% of all helical residues in globular proteins are 3_{10}-helical.[2] The 3_{10}-helices are typically short (3−4 residues) and observed at the N- or C-terminus of α-helices. The 3_{10}-helical structure has been suggested as an intermediate in α-helix folding and melting processes.[3] However, the 3_{10}-helix has been authenticated at atomic resolution mainly in model peptides based on C^{α}-tetrasubstituted α-amino acids. In particular, the N^{α}-acylated homooligomers from α-aminoisobutyric acid -$(Aib)_{11}$- and -$(Aib)_{10}$- represent the longest 3_{10}-helical peptide sequences so far investigated by X-ray diffraction.[4] Although transitions from α-helix to random coil or to β-sheet structure have been extensively investigated, only limited attention has been paid to the transition between α- and 3_{10}-helices, despite the fact that this could form the first step toward a molecular switch based on these two conformational states. By comparing *different*, but related, peptides it has been experimentally shown that the factors involved in shifting the conformational preference from 3_{10}- to α-helix include the decreasing percentage of C^{α}-tetrasubstituted α-amino acids in the sequence and the increasing length of the peptide.[5] More subtle influence can also be exerted by the amino acid sequence and the nature of the terminal protecting (blocking) groups. Moreover, by spectroscopically analyzing the *same* peptide, based on a combination of C^{α}-tetrasubstituted and protein (C^{α}-trisubstituted) α-amino acids, under different experimental conditions (solvent, temperature), a fast and reversible transition between the α- and 3_{10}-helical states was observed, although in a very limited number of extremely different cases.[6]

Sometime ago, we decided to investigate *systematically* the equilibrium between α- and 3_{10}-helices beginning from simplified peptide sequences formed *exclusively* by the *same* C^{α}*-tetrasubstituted* α-amino acid. From our previous studies it was already known that among the chiral residues of this class the β-branched C^{α}-methyl-L-valine [L-(αMe)Val] is that with the most pronounced bias toward the right-handed 3_{10}-helix.[5b,7] Therefore, our first target in this area was an N-acylated L-(αMe)Val homo-octapeptide *tert*-butyl ester which was shown to undergo an intriguing phenomenon, namely a *slow* and *irreversible* conversion from 3_{10}- to α-helix, the rate of which is particularly enhanced by high solvent polarity (e.g., in 1,1,1,3,3,3-hexafluoropropan-2-ol, HFIP).[8a,b] However, more recently we have unambiguously demonstrated that in HFIP solution a *slow*, unexpected, acidolysis of the *tert*-butyl ester functionality does take place, *irreversibly* affording the corresponding octapeptide free acid, which in turn rapidly folds into the α-helix conformation, possibly due to the increased (by one) number of H-bonding donors in its sequence.[8c,d]

On the basis of the above observations, in the present study we focused on an N-acylated, *chemically stable*, L-(αMe)Val homopeptide with the same number of H-bonding donor NH groups as the unstable *tert*-butyl ester described above, namely the N^{α}-acylated homoheptapeptide alkylamide Ac-$[L\text{-}(\alpha Me)Val]_7$-NH*i*Pr, where Ac is acetyl and NH*i*Pr is isopropylamino (Supporting Information).

Circular dichroism (CD) experiments on Ac-$[L\text{-}(\alpha Me)Val]_7$-NH*i*Pr clearly showed that it undergoes a *fast*, solvent-driven, *reversible* α-helix/3_{10}-helix equilibrium, thus behaving as a *molecular spring*. More specifically, according to the CD patterns[9] this peptide is overwhelmingly folded in the α-helix conformation in HFIP solution, whereas it essentially adopts the 3_{10}-helix conformation in the less polar methanol (MeOH) solution. Repeated cycles of helix-to-helix conversion can be carried out, highlighting inter alia the chemical stability of the peptide under the experimental conditions used in this work (Supporting Information).

The X-ray diffraction structure of an impressive number of oligopeptides containing C^{α}-tetrasubstituted α-amino acids, from five to twenty residues in length, have been solved and found to be either fully developed 3_{10}- or α-helical, or mixed 3_{10}/α-helical. This Communication describes in detail an example of an unambiguous α/3_{10}-helix dimorphism in an N^{α}-acylated heptapeptide amide, Ac-$[L\text{-}(\alpha Me)Val]_7$-NH*i*Pr, crystallized from two different solvents. All of the numerous homopeptides based on C^{α}-tetrasubstituted α-amino acids, the 3D-structure of which have been solved so far by X-ray diffraction (except the terminally protected, α-helical octapeptide reported by Tanaka, Suemune, and their co-workers)[10] have been found to adopt the 3_{10}-helical structure in the crystal state.

The conformations of the three independent molecules (**A**, **B**, and **C**) in the asymmetric unit of the unsolvated Ac-$[L\text{-}(\alpha Me)Val]_7$-NH*i*Pr crystallized from MeOH solution, where it is 3_{10}-helical, **1**, are very similar in that all are regular, right-handed, 3_{10}-helices spanning the entire sequence (Figure 1). The average ϕ,ψ backbone torsion angles for the seven residues are −55.2°, −30.3° (molecule **A**), −55.3°,−33.5° (molecule **B**), and −54.9°,−34.3° (molecule **C**), very close to those typical for a peptide 3_{10}-helix.[1a] In each of the three peptide molecules all six, consecutive, $i \leftarrow i+3$ (peptide) C═O···H−N (peptide or amide) intramolecular H-bonds are of normal strength for these types of interactions, the range of O···N distances being 2.910(9)−3.258(9) Å.[11] The major conformational differences among the three molecules are seen in the $\chi^{1,1}$, $\chi^{1,2}$ side-chain torsion angles of the seven L-(αMe)Val residues. While three sets of angles are the same in the three molecules (t,g^- at positions

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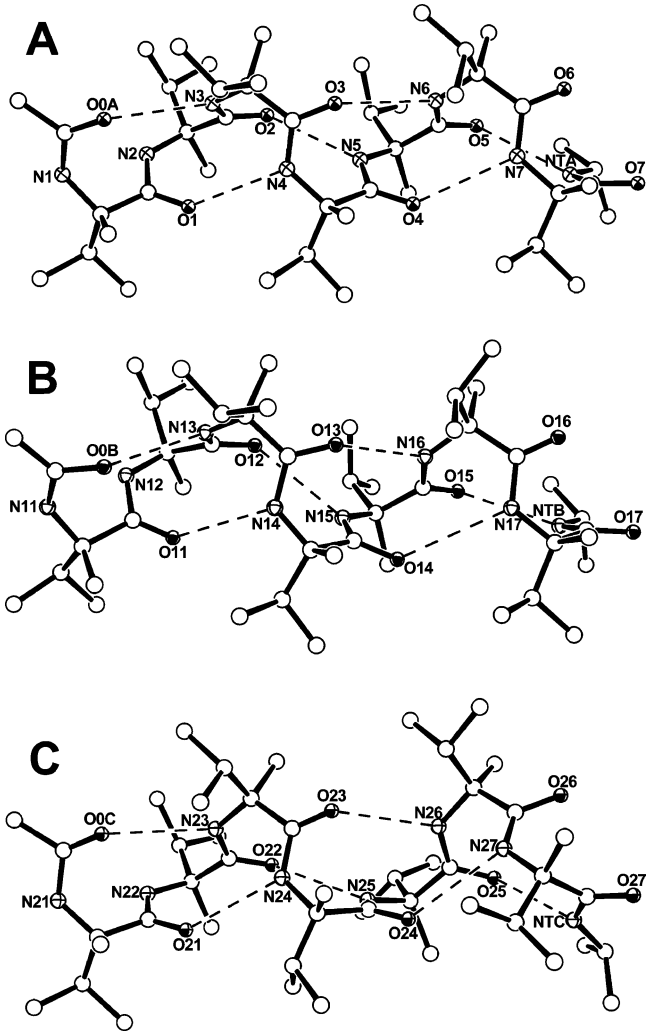

Figure 1. X-ray diffraction structures of the three independent molecules (**A**, **B**, and **C**) in the asymmetric unit of unsolvated Ac-[L-(αMe)Val]$_7$-NH*i*Pr (**1**) (crystals grown from a MeOH solution). H-atoms have been omitted for clarity. Dashed lines represent intramolecular C═O···H−N H-bonds.

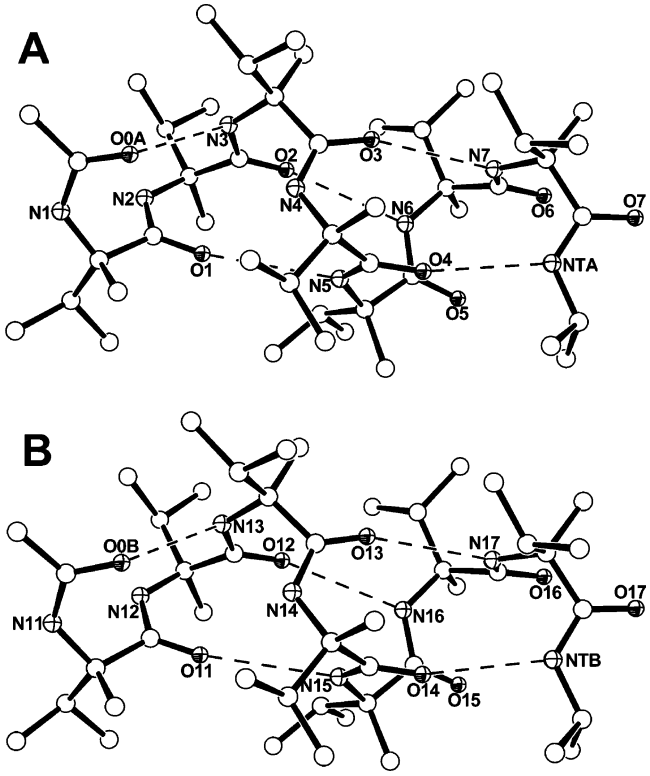

Figure 2. X-ray diffraction structures of the two independent peptide molecules (**A** and **B**) in the asymmetric unit of Ac-[L-(αMe)Val]$_7$-NH*i*Pr bis-HFIP solvate (**2**) (crystals grown from an HFIP solution). H-atoms have been omitted for clarity. Dashed lines represent intramolecular C═O··· H−N H-bonds.

1 and 7, and g^+,t at position 2), different combinations of angles (t,g^-; g^+,g^-; g^+,t) characterize the positions from 3 to 6. Overall, the t,g^- set prevails over the g^+,t and g^+,g^- sets.[7,12]

A significant modification was observed in the 3D-structure of the same peptide when the crystals were grown from an HFIP solution, where it is α-helical (heptapeptide bis-HFIP solvate, **2**). The two independent peptide molecules (**A** and **B**) in the asymmetric unit are almost identical, the corresponding ϕ,ψ backbone torsion angles not differing more than 5° (Figure 2). Both are folded in right-handed helical structures. The average ϕ,ψ torsion angles for the seven residues are −54.9°,−50.8° (molecule **A**) and −55.1°,−50.4° (molecule **B**). Interestingly, the ψ values are much closer to those expected for an α-helix (−42°) than for a 3_{10}-helix (−30°).[1a] Also the L-(αMe)Val side-chain dispositions are remarkably the same for molecules **A** and **B**, all sets of $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles being t,g^- except those of residue 2 (g^+,t).[7,12] Finally, no difference has been found in the intramolecular H-bonding schemes: an $i \leftarrow i+3$ hydrogen bond (indicative of a 3_{10}-helix) at the N-terminus is followed by four, consecutive, $i \leftarrow i+4$ hydrogen bonds (typical of an α-helix). The range of O···N separations is 2.941(11)−3.262(10) Å.[11] The development of a fully formed α-helical structure from an initial 3_{10}-helical nucleus is not surprising as it is characteristic of a large number of α-helices in peptides and globular proteins.[1,2,5]

Figure 3 shows the interactions between the cocrystallized HFIP molecules and the three C-terminal carbonyls of each of the peptide molecules **A** and **B**. The details of H-bonding are not strictly equivalent. In both complexes the −OH group of one HFIP molecule is H-bonded to the C-terminal carbonyl and the (HFIP) C−H group is H-bonded to the penultimate carbonyl. Only in the complex with molecule **A** is the −OH group of this same HFIP molecule additionally H-bonded to the penultimate carbonyl, thus generating two three-center H-bond motifs. In both complexes the −OH group of each of the two sites of the second HFIP molecule is H-bonded to the last but two carbonyl. Interestingly, this is the carbonyl which would be involved in an intramolecular H-bond with the C-terminal amide N−H group if the peptide would be 3_{10}- instead of α-helical. In all four HFIP molecules of the two complexes the O−H and C−H bonds are syn periplanar, as reported for the X-ray diffraction structure of the fluoroalcohol itself.[13a] There are no H-bond interactions between HFIP molecules. Conversely, the shortest F···F separations between HFIP neighbors are 3.013(21) and 3.096(20) Å, respectively, in the two complexes. It has been extensively demonstrated since 1964 that HFIP is a strong H-bonding donor, thus being able to solvate and dissolve peptides, proteins, and synthetic polyamides.[13b−e] H-bonding interaction between cocrystallized HFIP and peptide molecules have been previously reported only for two cyclic dimers.[14] It is also worth recalling that a single 2,2,2-trifluoroethanol (TFE) molecule cocrystallized with Ac-[L-(αMe)Val]$_8$-OH is not able to displace this homo-octapeptide free acid from the fully 3_{10}-helical conformation.[15]

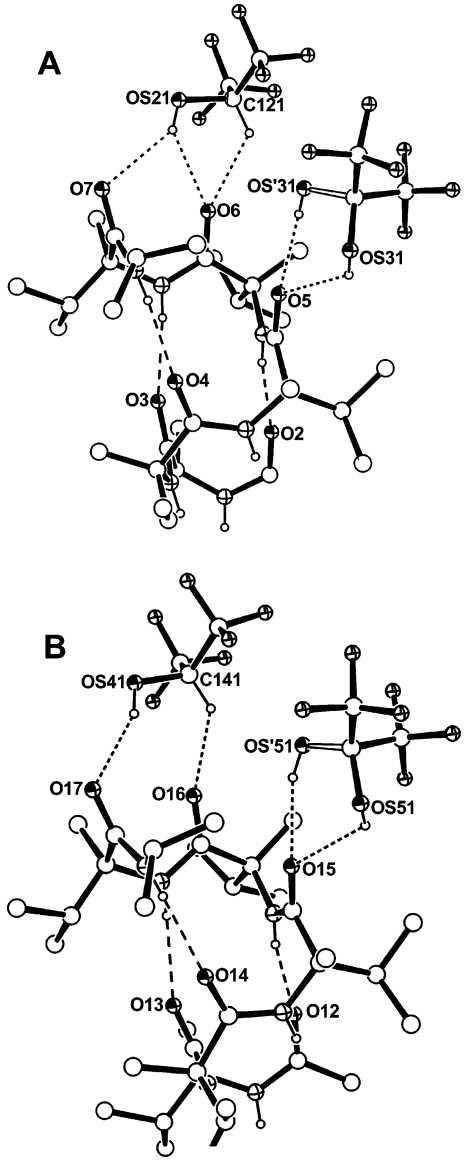

Figure 3. HFIP molecules bound to the carbonyl oxygen atoms in the C-terminal region of the two independent peptide molecules (**A** and **B**) in the asymmetric unit of the α-helical Ac-[L-(αMe)Val]$_7$-NH*i*Pr (**2**). Peptide N−H and HFIP hydrogen atoms are shown (all other hydrogen atoms have been omitted for clarity). Dashed lines represent intramolecular C═O···H−N hydrogen bonds, while dotted lines represent (HFIP) O−H···O═C (peptide) and (HFIP) C−H···O═C (peptide) hydrogen bonds. Major and minor occupancy sites for the hydroxyl group of one of the two HFIP molecules bound to peptide **A** or **B** are indicated by solid and open C−O bonds, respectively.

In conclusion, we have described an example of a solvent-driven α/3_{10}-helix dimorphism for a peptide molecule in the *crystalline* state. The fully C^{α}-methylated homo-peptide Ac-[L-(αMe)Val]$_7$-NH*i*Pr is completely 3_{10}-helical when its crystals are grown from a MeOH solution. By contrast, it is folded in the α-helical conformation when crystallized from HFIP, an alcohol of high polarity. In this latter case, two cocrystallized solvent molecules bind to the three C-terminal peptide (or amide) carbonyl functions not involved in the C═O···H−N intramolecular H-bonding network. Both O−H···O and C−H···O types of H-bonds participate in the solvation. The conformations of the peptide in the two crystals strictly mirror those occurring in the two solvents. Interestingly, Karle, Balaram, and their co-workers have already reported the X-ray diffraction structures of the same, $C^{\alpha,\alpha}$-di-*n*-propylglycine containing, heptapeptide sequence (having a different N-terminal group) in the 3_{10}- and α-helix conformations despite being crystallized from the *same* solvent (the 3_{10}-helical structure is monohydrated).[16] The present investigation highlights that the interconversion between α- and 3_{10}-helices might be allowed even in peptides exclusively composed by C^{α}-tetrasubstituted α-amino acids and provides clues for a deeper understanding of the interactions of HFIP with helical peptides.

Supporting Information Available: Preparative procedures and characterization data; CD spectra; X-ray diffraction details, including crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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