Solvent-Driven Helix Equilibria

Quantitative Correlation of Solvent Polarity with the α-/3_{10}-Helix Equilibrium: A Heptapeptide Behaves as a Solvent-Driven Molecular Spring**

Paolo Pengo, Lucia Pasquato, Stefano Moro, Alessandro Brigo, Federico Fogolari, Quirinus B. Broxterman, Bernard Kaptein, and Paolo Scrimin**

A considerable amount of converging evidence indicates that the 3_{10}-helical conformation is a thermodynamic intermediate in α-helix folding.[1–3] The two helices differ for the relative position of the C=O and NH groups involved in hydrogen-

[*] Prof. Dr. L. Pasquato[+]
CNR IMT, Padova Section
via Marzolo, 1–35131 Padova (Italy)
Prof. Dr. P. Scrimin, Dr. P. Pengo
Department of Organic Chemistry and
CNR IMT, Padova Section
University of Padova
via Marzolo, 1-35131 Padova (Italy)
Fax: (+39) 049-827-5239
E-mail: paolo.scrimin@unipd.it
Dr. S. Moro, A. Brigo
Department of Pharmaceutical Sciences
University of Padova, via Marzolo, 15-35131 Padova (Italy)
Dr. F. Fogolari
Scientific and Technological Department
University of Verona
Strada Le Grazie, 15—Cà Vignal, 37134 Verona (Italy)
Dr. Q. B. Broxterman, Dr. B. Kaptein
DSM Research
DFC Advanced Synthesis and Catalysis
6160 MD Geleen (The Netherlands)
[+] Current address: University of Trieste
Department of Chemical Sciences
via L. Giorgieri, 1-34127 Trieste (Italy)
Fax: (+39) 040-558-3903
E-mail: pasquato@dschsun3.univ.trieste.it

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bond formation ($i \leftarrow i+3$ and $i \leftarrow i+4$ in 3_{10} and α helices, respectively). Consequently the helical pitch is different (5.7 and 6.3 Å for the α and 3_{10} helix, respectively) and the relative position of functional groups in the lateral chains varies when switching from one conformation to the other.[4] The number and intrinsic energy content of the individual hydrogen bonds, as well as the interaction between the functional groups in the lateral chains and the surrounding environment, are among the parameters that account for the stabilization of one or the other of the two conformations. In proteins the α helix largely prevails,[3] but the two conformations appear equally probable as the length of the peptide becomes shorter.[3a] Experimental as well as theoretical studies on the $\alpha/3_{10}$ equilibrium have not yet provided conclusive information on the parameters that favor one or other conformation;[5] these studies have focused on the polarity of the solvent,[6] the temperature,[7] and the formation of aggregates.[8]

We present here the outstanding case of a peptide made up of seven amino acids that is not only highly folded but also shows the unique property that the type of helical conformation adopted is dependent on the polarity of the solvent. The peptide sequence Ac-[Aib-L-(αMe)Val-Aib]$_2$-L-His-NH$_2$ (**1**) comprises six C^{α}-tetrasubstituted amino acids (four achiral Aib, α-aminoisobutyric acid amino acid, and two chiral (αMe)Val, C^{α}-methylvaline) and one histidine.[9] We have selected these noncoded amino acids because they are known to induce helical conformations even in very short oligomers[10] as a result of the conformational constraint imposed by the substituents at the α carbon atom. The conformational stability that **1** shares with other oligopeptides composed of similar C^{α}-tetrasubstituted amino acids renders **1** an excellent probe for testing the parameters that affects its conformation. The surrounding environment (namely, the solvent) is, clearly, one of the most important of these parameters.[6] For this reason we have studied the conformation of **1** by circular dichroism (CD) in a series of solvents of different polarity (Figure 1). These spectra show a striking dependence on the nature of the solvent. Thus, in methanol (MeOH) or isopropanol (*i*PrOH) the spectrum signature is that of a right-handed 3_{10} helix:[8,11,12] the spctrum shows a negative Cotton band centered at about 208 nm ($\pi \rightarrow \pi^*$) with a shoulder at 222 nm ($n \rightarrow \pi^*$, $\theta_{222}/\theta_{208}$ ca. 0.3/1).[13] In hexafluoroisopropanol (HFIP), however, the $n \rightarrow \pi^*$ band becomes dominant and the $\theta_{222}/\theta_{208}$ ratio is 1.4:1. Although this ratio is typically reported to be close to 1/1 for α-helical polypeptides, recent results by Kemp et al.[14] have shown that it may become as large as 1.3:1–1.4:1. This phenomenon has been attributed to the formation of very short hydrogen bonds.[15] Thus, it can be safely stated that **1** prevailingly adopts a α-helical conformation in HFIP. The series of spectra shown in Figure 1 hence indicates that **1** converts from a 3_{10}-helical conformation in *i*PrOH into an α-helical one in HFIP. The interconversion does not show any kinetic dependence[8,16] and occurs instantaneously and reversibly upon dissolution of **1** in the appropriate solvent (Figure 1, inset).

We have used the E_T^N polarity scale introduced by Reichardt and Dimroth[18] to correlate the position of the α-/3_{10}-helix equilibrium with a parameter describing the property of the solvent. This scale correlates linearly the

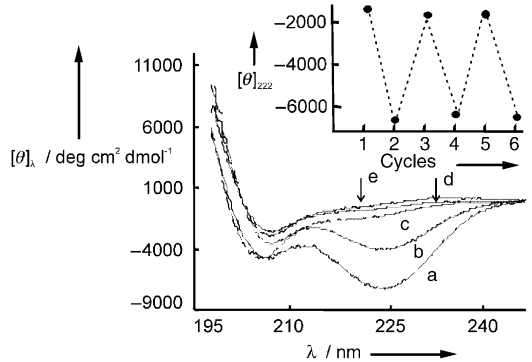

Figure 1. CD spectra of peptide **1** in the 200–250 nm region and in different solvents (a: HFIP; b: TFE; c: MeOH; d: EtOH; e: *i*PrOH). The concentration of **1** was 1 mM.[17] Inset: Change in the ellipticity at 222 nm on repeated cycles of dissolution in *i*PrOH (odd numbers) and HFIP (even numbers) followed, each time, by drying.

Gibbs free energy (ΔG^0) of reaction equilibria with the polarity of the solvent, and has been successfully applied also for predicting solvent effects on reaction rates, solute-solvent interactions, and spectra absorptions. A plot of the mean residue molar ellipticity at the wavelength of the two $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions (208.5 and 222 nm, respectively) against E_T^N reveals the profiles shown in Figure 2 a.

We were able to obtain values for the equilibrium constants ($K_{\alpha/3_{10}}$) in each solvent by using the experimental $[\theta]_\lambda$ value at these two wavelengths in the different solvents (see Supporting Information for the details). Furthermore, by applying the Reichardt and Dimroth approach to the α-/3_{10}-

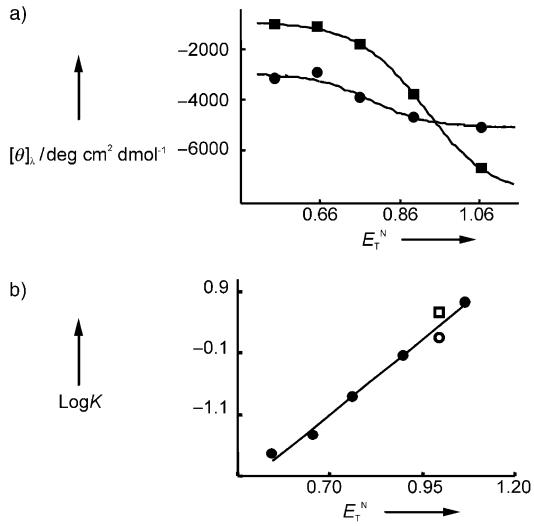

Figure 2. a) Plot of the mean residue ellipticity of peptide **1** at 208.5 (circles) and 222 nm (squares) as a function of the empirical solvent polarity parameter E_T^N. The solid lines represent the best fitting of the points (see Supporting Information for details). b) Linear correlation between the α-/3_{10}-helix equilibrium constants ($\log K_{\alpha/3_{10}}$) and E_T^N. The correlation coefficient is >0.99. Open symbols are the extrapolated values for water from water/MeOH (square) and water/*i*PrOH (circle) mixtures. The linear correlation was performed omitting these two points.

helix equilibrium, we could fit the experimental points and obtained the two curves shown in Figure 2a. These calculations require that the peptide is mostly folded (as indicated by all experimental evidences (CD and NMR spectroscopy) as well as calculations (see below)) or that the random-coil fraction, if any, does not change as **1** switches from one helix to the other.[19] A plot of $\log K_{\alpha/3_{10}}$ against E_T^N provides the excellent linear free energy correlation shown in Figure 2b. These results suggest that in the case of peptide **1** the α- to 3_{10}-helix equilibrium is governed by the polarity of the solvent, and by increasing E_T^N from 0.546 (*i*PrOH) to 1.068 (HFIP) the $\alpha/3_{10}$-helix ratio changes from 2:98 to 85:15.

In the set of solvents considered, there is the notable absence of water, the typical natural solvent. The reason for this is that **1** is sparingly soluble in this solvent. To overcome this problem we have examined solvent mixtures of different water/alcohol composition using *i*PrOH and MeOH as cosolvents and extrapolated the data to 100% water. The dependence of the α-/3_{10}-helix equilibrium from the solvent composition with both alcohols gives double straight lines with break points at low alcohol:water ratios (at a mole fraction of 0.1 and 0.2 of *i*PrOH and MeOH, respectively, see Supporting Information), as reported for equilibria involving other species.[20] This behavior is likely a consequence of molecular segregation of the alcohol[21] with selective solvation[22] of the peptide. The extrapolation places the $\log K_{\alpha/3_{10}}$ value, as indicated in Figure 2b (open symbols), in fairly good accord with the value expected for the $E_T^N = 1$ of water. Thus, the prevailing conformer in water is the α helix, similar to the situation in 2,2,2-trifluoroethanol (TFE) and HIFP, and there is no evidence of any "structuring effect" of peptide **1** by the fluorinated alcohols,[23,24] clearly because the peptide is already folded in a helical conformation.

Further support for this finding comes from molecular dynamics (MD) simulations in explicit water, a tool that has proven to be quite useful for studying the process of reversible peptide folding. In the case of **1**, we have explored the conformational evolution of the peptide starting from both the α and 3_{10} helix canonical states. All simulations were carried out by using the CHARMm 27 suite,[25] at 300 K and using the explicit TIP3P model for water.[26,27] They show that the helical structure is stable for extended periods on the nanosecond time scale (average root-mean-square deviation, (RMSD) = 0.98 Å), on the basis of the secondary structure analysis implemented by the program MOLMOL.[28] Hydrogen-bonding distance matrix and Ramachandran plots of all seven residues are consistent with a stable α-helical conformation (data not shown). In addition, the 3_{10}-helical conformation does not seem to be thermodynamically stable in the aqueous medium and, in fact, a 3_{10}- to α-helical transition occurs during the first 40 ps of equilibration of the dynamic run (Figure 3a) and evidenced by analyzing the RMSD value during the dynamics run (average RMSD = 2.75 Å). After the 3_{10}- to α-helical transition, the helical structure is stable for extended periods on the nanosecond time scale (Figure 3a, inset). Consistently, hydrogen-bonding-distance matrix and Ramachandran plots are coherent with this observation. This fact further supports the clear preference for the α-helical conformation in water with a shift of the

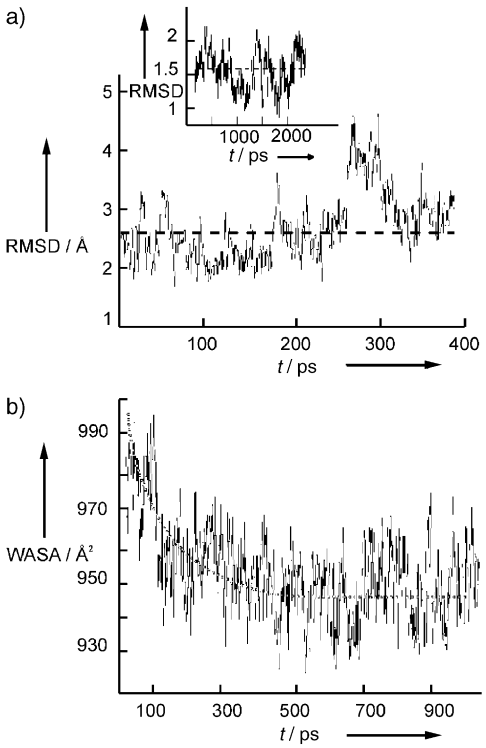

Figure 3. a) Backbone atom positional root-mean-square deviation (RMSD) of peptide **1** for all residues starting from the 3_{10}-helical conformation during the first 400 ps and the following 3000 ps (inset). Dashed lines correspond to the average RMSD with reference to the 3_{10}-helical conformation and the α-helical one, respectively. b) Variation of the calculated water-accessible surface area (WASA) of peptide **1** during the dynamic simulation.

C=O···HN hydrogen bonds from $i \leftarrow i+3$ to $i \leftarrow i+4$, as expected for a 3_{10}- to α-helix transition.

As a result of the way in which the E_T^N values[18] are determined, they reflect all the nonspecific intermolecular forces between solvent and solute molecules occurring in the process, particularly the H-bonding or hydrophobic interactions, but they do not provide any information on the prevailing driving force for the shift of the equilibrium. In particular, when the 3_{10} helix is converted into a α helix the maximum number of intramolecular hydrogen bonds in sequence **1** decreases by one unit (from 6 to 5) while the number of relative interactions between the lateral chains of the amino acids changes because of the decrease in the pitch of the helix. Careful examination of the water-accessible surface area in the MD simulations during the helix conversion reveals that this parameter decreases significantly (Figure 3b). Accordingly, the calculations suggest that a major contribution to the conformational change in aqueous solution is hydrophobic in nature. This result means also that the lateral chains of amino acids in the *i*- and $i+4$-positions in the sequence get closer and interact less with the solvent. Indeed, the peptide shortens from 17 to 15 Å in the course of the transition (Figure 4), thus behaving like a molecular spring whose length and pitch are controlled by the polarity of the solvent. Thus the toothed sawlike profile of the graph in the inset of Figure 1 in fact shows the fully reversible process

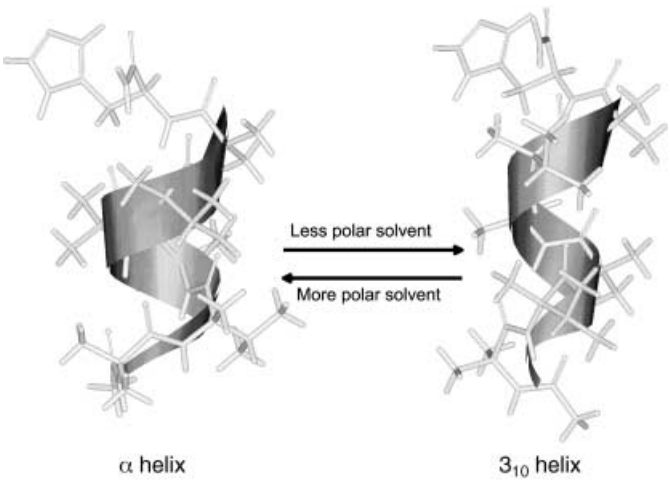

Figure 4. Representation of peptide **1** in its two helical conformations in equilibrium according to the polarity of the solvent. The two helices are shown with the canonical dihedral angles ($\phi = -63°$, $\psi = -41°$ for the α helix and $\phi = -60°$, $\psi = -30°$ for the 3_{10} helix).

of stretching and compressing of the molecular spring represented by peptide **1**.

In conclusion, we have discovered an acylated heptapeptide amide comprising six C^{α}-tetrasubstituted amino acids and one histidine group that adopts two helical conformations according to the polarity of the solvent: a α or 3_{10} helix in higher or lower polarity solvents, respectively. We were able to correlate the position of the α-/3_{10}-helix equilibrium with the empirical solvent polarity parameter E_T^N and also able to show that hydrophobic interactions between the side arms of the amino acids provide a major driving force for the stabilization of the α helix in aqueous solution. As shown by the molecular calculations, switching from one conformation to the other elongates (3_{10} helix) or shortens (α helix) the peptide, thus it behaves like a solvent-driven molecular spring.

Since, in converting the peptide from the 3_{10}- into a α-helical conformation, we trade an intramolecular hydrogen bond for a discrete decrease in the solvent-exposed hydrophobic surface, these data indicate that, by keeping the hydrophobic contribution of each individual amino acid in the folded oligopeptides constant, longer sequenches will prevailing adopt a α-helical conformation in water while shorter ones will favor 3_{10} helices. This finding is in full accord with results found by other research groups.[29,30]

Experimental Section

Synthesis: Peptide **1** was synthesized by solution methods using the acylfluoride to activate the carboxylate group in the coupling process up to the tripeptide z-Aib-L-(αMe)Val-AibO*t*Bu. This was subsequently dimerized to the hexapeptide, z-[Aib-L-(αMe)Val-Aib]$_2$-OtBu, by converting the carboxylate into the oxazolone and treating it with the free amine. His-NH_2 was eventually connected (3-(3-dimethylaminopropyl)-1-ethylcarbodiimide/7-aza-1-hydroxy-1*H*-benzotriazole (EDC/HOAt)) to the hexapeptide to obtain the final protected heptapeptide. Conversion into **1** was performed following standard protocols.

CD studies: The CD spectra of peptide **1** were recorded on a Jasco model J-715 dichrograph by using cylindrical, fused-quartz cells of 1- or 0.1-mm path lengths. The data are expressed in terms of mean residue molar ellipticity (eight amide bonds). The accuracy of the concentrations of the peptide stock solutions were determined by quantitative amino acid analysis.

Calculations: Molecular dynamics simulations were performed using SGI workstations and Intel PIV PCs computers as platforms. Ideal α-helix ($\phi = -63°$, $\psi = -41°$) and 3_{10}-helix ($\phi = -60°$, $\psi = -30°$) conformations for the sequence Ac-[Aib-L-(αMe)Val-Aib]$_2$-L-His-NH_2 (peptide **1**) were constructed by using the BUILD molecular editor module implemented in CHARMm 27. An equilibrated box of solvent was stacked around the α-helix conformation to form a cubic box with 3.5-nm sides. By contrast, the 3_{10}-helix conformation was placed in a slightly longer box (4.0-nm sides) of water to accommodate the longer peptide. Both conformations were introduced into the corresponding boxes, and all water molecules with any atom within 0.28 nm of the peptide were removed. The conformational behavior of the peptide was calculated by using the program CHARMm and the CHARMm 27 force field. The TIP3P water model was used for the solvent. A dielectric permittivity ($\varepsilon = 1$) was assumed and the van der Waals interactions were cut off at 0.8 nm. The SHAKE algorithm[31] (tolerance 0.0005 Å) was applied to all bonds, a 2-fs time step was used, and a temperature of 300 K was maintained through Berendsen temperature coupling. The water-accessible surface area was calculated by using the Molecular Operating Environment (MOE, ver.2002.03).[32]

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