Solvent Effects on the 3_{10}-/α-Helix Equilibrium in Short Amphipathic Peptides Rich in α,α-Disubstituted Amino Acids

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Received August 16, 1996

Prediction of peptide secondary structure (α-helix, *β*-sheet, 3_{10}-helix, etc.) *a priori* from amino acid sequence is a primary goal of *de novo* protein design and protein folding studies.[1] Relative to the α-helix and *β*-sheet, the factors favoring formation of the 3_{10}-helix (*i, i* + *3* hydrogen bonding; constituting ∼10% of protein structure[2]) are only beginning to be understood.[3] Herein, we report isomeric decapeptides Pi-10 and Ipi-10, putative amphipathic α- and 3_{10}-helices (Figure 1), which exhibit their designed secondary structure in aqueous SDS micelles (Figure 2, panel A). In organic−aqueous solvent mixtures, Pi-10 exhibits the expected α-helix to coil transition as water content is increased (0−50%; Figure 2, panel B; Table 1) while Ipi-10 converts from a 3_{10}-helical to α-helical and coil structure as water content is increased (0−50%; Figure 2, panel C; Table 1). These results show that amphipathy is a design tool for *both* α-helical and 3_{10}-helical structures, even in very short peptides rich in α,α-disubstituted amino acids (ααAAs). Additionally, the amphipathic design is primary in controlling secondary structure, overriding other factors such as the number of ααAA residues[4] and the order of α-amino acids and ααAAs in the sequence,[5] which are often thought to be key in controlling the 3_{10}/α-helix equilibrium. We have been able to confirm experimentally what had previously been calculated,[6] *viz.* that at least some peptides rich in ααAAs are more stable as 3_{10}-helices in organic solvents than in aqueous environments.

The 3_{10}/α-helix equilibrium is being intensely studied[3−6] because the 3_{10}-helix is a likely protein folding intermediate to the α-helix conformation. In addition, short stretches of 3_{10}-helix occur frequently in globular proteins,[2] and protein recognition steps may involve facile transitions between the α-helix and 3_{10}-helix.[7] Experiments probing the 3_{10}/α-helix equilibrium have focused almost exclusively on short hydrophobic peptides composed of several α,α-disubstituted amino acids (ααAAs), such as oligomers of α-aminoisobutyric acid (Aib, Figure 1A).[8] Studies of these peptides have been limited to spectroscopic measurements in organic solvents [e.g., trifluoroethanol (TFE), acetonitrile (CH_3CN), dimethyl sulfoxide, methanol, etc.] or X-ray structure determinations of peptides crystallized from organic solvents.[4,9]

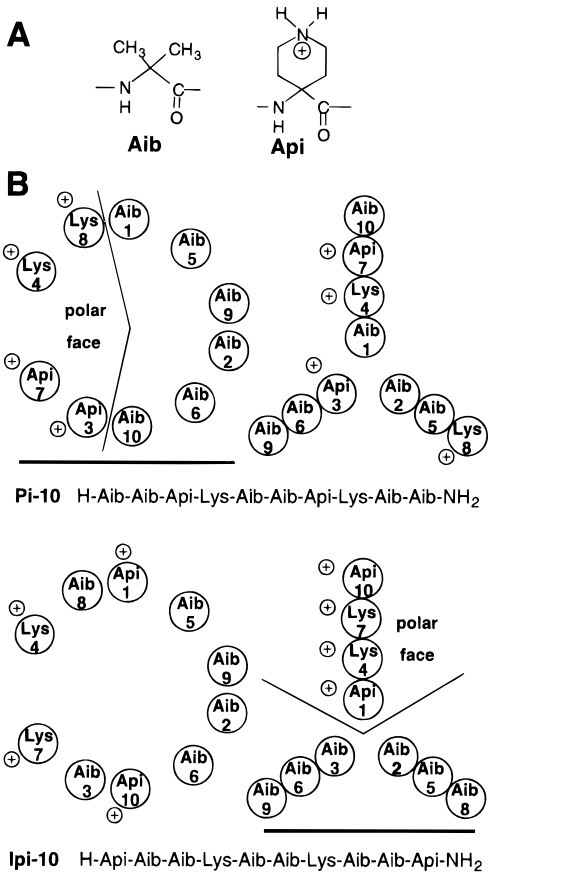

Figure 1. (A) Structure of α-aminoisobutyric acid (Aib) and 4-aminopiperidine-4-carboxylic acid (Api) residues. (B) The α- and 3_{10}-helical wheel diagrams and sequences for Pi-10 and Ipi-10 peptides. Pi-10 can be perfectly amphipathic as an α-helix (left side of figure, underlined); the alternative 3_{10}-helix is not amphipathic. Ipi-10 can be perfectly amphipathic as a 3_{10}-helix (right side of figure, underlined); the alternative α-helix is poorly amphipathic with hydrophobic Aib residues interrupting the hydrophilic face.

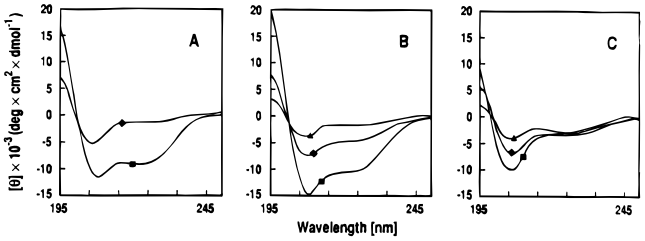

Figure 2. CD spectra of 0.2 mM peptide: panel A, Pi-10, ■, and Ipi-10, ◆; in buffered SDS (25 mM) micelles; panel B, Pi-10 in 1:1 CH_3CN-H_2O, ▲, 9:1 CH_3CN-H_2O, ◆, and 9:1 CH_3CN−TFE, ■; and panel C, Ipi-10 in 1:1 CH_3CN-H_2O, ▲, 9:1 CH_3CN-H_2O, ◆, and 9:1 CH_3CN−TFE, ■.

Peptides Pi-10 and Ipi-10 contain two L-Lys residues and 8 achiral ααAAs − six Aib residues and two 4-aminopiperidine-4-carboxylic acid[10] (Api, Figure 1A) residues. The L-Lys residues are included to induce a right-handed helix (detectable by CD) and are well separated and near the middle of the sequences to have maximal effect.[5] The lysine-like ααAA Api can be incorporated readily into Pi-10 and Ipi-10 by solid-phase

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S0002-7863(96)02875-2 CCC: $14.00

Table 1. Circular Dichroism Data and Derived Structural Parameters for Peptides Pi-10 and Ipi-10

	Pi-10				Ipi-10			
solvent	$[\theta]_{\pi\rightarrow\pi^*}$[a,b]	$[\theta]_{n\rightarrow\pi^*}$[a,c]	R[d]	α-helicity (%)[e]	$[\theta]_{\pi\rightarrow\pi^*}$[a,b]	$[\theta]_{n\rightarrow\pi^*}$[a,c]	R[c]	3_{10}-helicity (%)[f]
25 mM SDS	−11860	−9605	0.81	32	−5316	−1750	0.32	25
9:1 CH_3CN−TFE	−14647	−10299	0.72	34	−9916	−3145	0.33	45
9:1 CH_3CN-H_2O	−7382	−4785	0.65	16	−6740	−3605	0.54	*g*
1:1 CH_3CN-H_2O	−3818	−1899	0.50	6	−4204	−3118	0.74	*h*

[a] Units for $[\theta]$ are deg cm^2 dmol^{-1}. [b] The minimum for the $[\theta]_{\pi\rightarrow\pi^*}$ band is observed in the range from 205−209 nm. [c] The minimum for the $[\theta]_{n\rightarrow\pi^*}$ band is observed in the range from 222 to 225 nm. [d] $R = [\theta]_{n\rightarrow\pi^*}/[\theta]_{\pi\rightarrow\pi^*}$. [e] The α-helical content was calculated according to ref 15. [f] The amount of 3_{10}-helix present was estimated according to the equation in ref 16. [g] This peptide is likely a mixture of α-helical, 3_{10}-helical, and coil structures. According to ref 15, % α-helix is estimated at 12%; according to ref 16, % 3_{10}-helix is estimated at 31%. [h] This peptide is α-helical as indicated by $R = 0.74$ and has ∼10% α-helix according to the equation in ref 15.

methods[10] and acts as a helix-promoting, water-solubilizing, and amphipathic design element in the peptide sequences. The peptides are designed to form amphipathic helices (see Figure 1B) with charged Lys and Api residues forming a hydrophilic face and nonpolar Aib residues forming a hydrophobic face.[11] Pi-10 is designed to form an amphipathic α-helix and Ipi-10 is designed to form an amphipathic 3_{10}-helix; both peptides are less amphipathic in the alternative helical forms (Figure 1B).

Negative CD bands at ∼222 nm ($n\rightarrow\pi^*$) and ∼207 nm ($\pi\rightarrow\pi^*$) are diagnostic of helical peptide structures. The ratio R of the intensity of these bands, where $R = [\theta]_{n\rightarrow\pi^*}/[\theta]_{\pi\rightarrow\pi^*}$, has been used as a parameter to distinguish α-helical and 3_{10}-helical secondary structure: $R \approx 1$ for α-helix, $R \leq 0.4$ for 3_{10}-helix.[12−14] Table 1 shows the CD minima, percent α-helicity, and R for Pi-10 and Ipi-10 in SDS micelles and in aqueous−organic solvents. Treatment of Pi-10 peptide with SDS (25 mM) micelles induces transition to a typical α-helix CD spectrum with $R = 0.81$ and percent α-helix = 32%.[15] The CD spectrum of Ipi-10 in the presence of SDS micelles has an $R = 0.32$ indicating a 3_{10}-helical structure. The 3_{10}-helicity of Ipi-10 is estimated at 25%.[16,17] Additionally, the positive CD band centered near 195 nm is much weaker for Ipi-10 than for the α-helical Pi-10, which has been noted in other studies of 3_{10}-helical peptides[13,14] and is predicted by theory.[12] The relatively low absolute helicities for both Pi-10 and Ipi-10 can be accounted for in part by helix end effects[18] and by incomplete micelle binding.[19,11b]

CD spectra of Pi-10 and Ipi-10 were taken in organic/aqueous solvent mixtures (Figure 2, panels B and C; Table 1). In the aqueous/organic solvent mixtures (9:1 CH_3CN−TFE,[20] 9:1 CH_3CN-H_2O, 1:1 CH_3CN-H_2O), Pi-10 behaves as a normal α-helical peptide, exhibiting a clear cooperative helix/coil transition. An isodichroic point appears at 201 nm and helicity decreases with decreasing organic solvent composition. The shift of the α-helix/coil equilibrium toward helix as organic composition increases agrees with what is known for monomeric α-helices.[1] However, as Pi-10 contains 80% ααAAs and has no two α-amino acids together, both Karle and Balaram[4] and Kuki[5] would have predicted this sequence to be 3_{10}-helical. According to calculations, Pi-10 should have been increasingly 3_{10}-helical as organic solvent content increased.[6] Thus, amphipathic design is more important than the mere percentage of ααAAs or order of α-amino acids and ααAAs in the sequence. In contrast, Ipi-10 displays strong 3_{10}-helical character in 9:1 CH_3CN-TFE ($R = 0.32$; 45% 3_{10}-helicity) and exhibits a conversion to an α-helical and coil structure in 9:1 CH_3CN-H_2O and 1:1 CH_3CN-H_2O. The lack of an isodichroic point in this series of CD spectra suggests a non-cooperative transition indicative of multiple equilibria such as 3_{10}-helix/α-helix, 3_{10}-helix/coil, and α-helix/coil. These results support predictions that in peptides in which a 3_{10}/α-helix equilibrium exists, increasing polarity of the solvent will favor α-helix formation.[6] Similar trends have been noted in our studies of the *N*-terminal acetylated peptides, Pi-10-ac and Ipi-10-ac (see Supporting Information).

This work shows that amphipathic design is an effective way to influence the balance of 3_{10}-helical and α-helical structure in a peptide and that significant 3_{10}-helicity can be achieved in aqueous milieu. Utilization of the positively charged ααAA Api as a helix-promoting, amphipathic design element in Pi-10 and Ipi-10 was key to these findings. We are currently exploring other structural features such as self-aggregation and salt bridging to stabilize 3_{10}-helices in water to gain further experimental insights into factors controlling the 3_{10}/α-helix equilibrium.

Acknowledgment. We greatly appreciate the assistance of Ms. Martha M. Juban, Mr. Christopher L. Wysong, and Ms. Rebekah L. Gundry. We thank the National Science Foundation and the Louisiana Education Quality Support Fund for support of this research via the NSF/EPSCoR Grant [(RII/EPSCoR)LEQSF(1992-96)-ADP-01].

Supporting Information Available: CD spectra and listings of spectral data for all compounds (19 pages). See any current masthead page for ordering and Internet access instructions.

JA962875C

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(15) The % α-helix was estimated using the following equation: % α-helix = (100%)$[\theta]_{\pi\rightarrow\pi^*}/[-40000(1 - 2.5/n)]$, where n is the number of amide bonds (including the *C*-terminal amide). Taken from: Scholtz, J. M.; Marqusee, S.; Baldwin, R. L.; York, E. J.; Stewart, J. M.; Santoro, M.; Bolen, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2854−2858.

(16) We have used as a model 3_{10}-helix the CD spectrum of H-(Leu-Arg-Leu)$_8$-OH in diphosphatidylcholine liposomes, where $[\theta]_{\pi\rightarrow\pi^*} = -21500$ deg cm^2 dmol^{-1} is defined as 100% 3_{10}-helix (see ref 13); % 3_{10}-helix = (100%)$[[\theta]_{\pi\rightarrow\pi^*}/-21500]$, see ref 17.

(17) Currently there are no accepted models for estimation of the % 3_{10}-helix by CD mainly because of the lack of a large structural database as is available for α-helix and β-sheet. Also, theoretical calculations (ref 12) suggest that the absolute intensity of CD bands of 3_{10}-helical peptides will be highly dependent on the ϕ and ψ torsion angles in the peptide backbone. It is known that 3_{10}-helical ααAA-containing peptides have different ϕ and ψ angles than 3_{10}-helical peptides having only proteinogenic amino acids (see refs 4, 9, and 12). In light of this, the peptide Ac-(αMeVal)-O*t*Bu, recently prepared and studied by Toniolo and co-workers,[14a] may be a better 3_{10}-helical model for Ipi-10. Assuming Ac-(αMeVal)$_8$-O*t*Bu is 100% helical in TFE with $[\theta]_{\pi\rightarrow\pi^*} = -9000$ deg cm^2 dmol^{-1}, the calculated 3_{10}-helicity for Ipi-10 in SDS micelles and 9:1 CH_3CN−TFE would be 60% and 110%, respectively. Clearly, more work is needed on the correlation of solid-state structure with solution structure (CD and NMR) of 3_{10}-helical peptides before helical content of such peptides can be accurately estimated.

(18) For the 10-mer peptide Pi-10 in a totally α-helical conformation, a maximum number of 7 hydrogen bonds are possible. This leaves the three *N*-terminal NH and the three *C*-terminal C=O without internal hydrogen bonding partners. For Ipi-10 in a totally 3_{10}-helical conformation, a maximum number of 8 hydrogen bonds are possible. This leaves the two *N*-terminal NH and the two *C*-terminal C=O without internal hydrogen bonding partners. In either peptide both termini may achieve non-ideal structures at the termini to interact with solvent. For an example of this see: Bindra, V. A.; Kuki, A. *Int. J. Peptide Prot. Res.* **1994**, *44*, 539−548.

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