3_{10}-Helix stabilization *via* side-chain salt bridges[1]

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Decapeptides, Sb-10 (H-Api-Aib-Aib-Glu-Aib-Aib-Glu-Aib-Aib-Api-NH_2) and Ipi-10 (H-Api-Aib-Aib-Lys-Aib-Aib-Lys-Aib-Aib-Api-NH_2), where Aib is α-aminoisobutyric acid and Api is 4-aminopiperidine-4-carboxylic acid, are designed to be amphipathic as 3_{10}-helices; Sb-10 is the first example of a 3_{10}-helical peptide stabilized by side-chain salt bridging or ion pairing.

The 3_{10}-helix (*i*, *i* + 3 hydrogen bonding pattern) comprises 10% of all recognized helical structures in proteins.[2] These helices are commonly found in short stretches of proteins, often as the terminating segment of an α-helix.[2] Interest in the 3_{10}-helix arises because the 3_{10}-helix is thought to participate in receptor binding and may also be a protein folding intermediate to the α-helix.[2,3] *De novo* design studies examining the 3_{10}-helix have primarily focused on peptides rich in α,α-disubstituted amino acids (ααAAs).[4,5] The ααAAs are used extensively because the ϕ and ψ angles of these residues are restricted to those favoring helical structures.[4] Many factors have been reported for the selective formation of the 3_{10}-helix over the α-helix including: placement of ααAAs, percentage of ααAAs, peptide length, and peptide design.[5–7] Unfortunately, most studies exploring these factors have focused on hydrophobic peptides in organic media.[4] The extensive library of methods available for the stabilization of α-helices in aqueous media such as amphipathy, side-chain intra- and inter-peptide salt bridges, and side-chain covalent linkages[8–10] provides an excellent starting point for the stabilization of 3_{10}-helical peptides in aqueous media. The Marqusee–Baldwin peptides cleanly show α-helix stabilization from intra-helical, side-chain ion-pairing that is reduced at high salt concentrations.[9a] The *i*, *i* + 4 positioning of Glu and Lys residues stabilize the α-helix while *i*, *i* + 3 positioning does not. Additional combinations of salt-bridging residues (*i*, *i* + 4) also stabilize α-helical conformations.[9b]

We have reported the synthesis and characterization of a 3_{10}-helical decapeptide with 80% ααAAs (Fig. 1; **Ipi-10**).[7] The high percentage of ααAAs in **Ipi-10** promotes helicity and the peptide was designed to be most amphipathic as a 3_{10}-helix. Herein we report the synthesis and characterization of an analogous amphipathic 3_{10}-helical peptide containing two intra-peptide side-chain salt bridges (**Sb-10**), which are designed to further stabilize the 3_{10}-helix.

Sb-10 has the same sequence as **Ipi-10** with the exception of glutamic acids replacing the lysines. The peptide sequences are: **Sb-10**, H-Api-Aib-Aib-Glu-Aib-Aib-Glu-Aib-Aib-Api-NH_2 and **Ipi-10**, H-Api-Aib-Aib-Lys-Aib-Aib-Lys-Aib-Aib-Api-NH_2, where Aib is α-aminoisobutyric acid and Api is 4-aminopiperidine-4-carboxylic acid. The design results in an amphipathic 3_{10}-helix that places the two Api residues and the two glutamic acids on the same face (*i*, *i* + 3) of the 3_{10}-helix (Fig. 1). The *i*, *i* + 3 placement of the Glu and Api residues potentially introduces ionic interactions that can provide additional 3_{10}-helix stability relative to the alternative α-helix conformations, since *i*, *i* + 3 salt bridges do not stabilize α-helices.[9a]

Sb-10 was synthesized *via* a combination of manual and automated solid-phase peptide synthesis. The first three C-terminal residues were coupled to PAL-PEG-PS (PerSeptive Biosystems) by refluxing the preformed fluoren-9-ylmethoxycarbonyl (Fmoc)-amino acid fluorides (8 equiv.) and Pr^i_2NEt (3 equiv.) in CH_2Cl_2 until quantitative Fmoc cleavage tests[11] showed at least 90% coupling for each step.[12] The remainder of the peptide was synthesized on a PerSeptive Biosystems 9050 peptide synthesizer using preformed Fmoc-acid fluorides.[12] The peptide was purified by RP-HPLC on a C4 column with a H_2O–MeCN–0.5% TFA gradient. Peptide purity was greater than 95% according to an analytical RP-HPLC using C18 column and a similar gradient. Molecular weight was verified by MALDI-MS and amino acid analysis gave the expected amino acid content. Peptide concentrations for circular dichroism (CD) studies were determined by quantitative amino acid analysis.

α- and 3_{10}-Helical peptides have minima centered about 222 (n→π*) and 207 nm (π→π*) in the CD spectra. The ratio, *R*, of the n→π* band over the π→π* band differentiates the α- and 3_{10}-helix.[13] The ratio is near 1 for the α-helix and is approximately 0.4 for a 3_{10}-helix.[13]‡

The CD spectra of **Sb-10** and **Ipi-10** for comparison were taken in 50–100% aqueous–organic solvent mixtures [1:1 MeCN–H_2O, 9:1 MeCN–H_2O, 9:1 MeCN–trifluoroethanol (TFE)]. **Ipi-10** has a CD spectra indicative of a weak 3_{10}-helix only in 100% organic solvent [Fig. 2(A)].§ **Sb-10** exhibits moderate α-helicity in 1:1 MeCN–H_2O and begins to show 3_{10}-helical character in 9:1 MeCN–H_2O. In 9:1 MeCN–TFE,

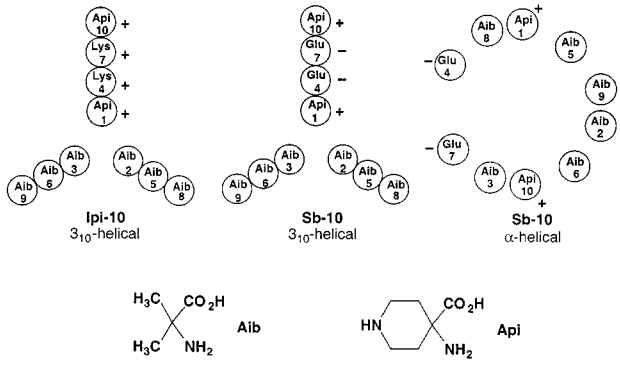

Fig. 1 Helical wheel diagrams of **Ipi-10** and **Sb-10**

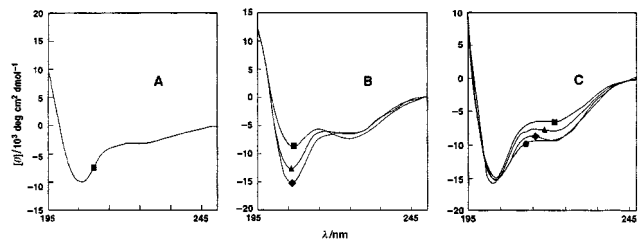

Fig. 2 Circular dichroism spectra with 0.2 mM peptide: (A) **Ipi-10** in 9:1 MeCN–TFE (■); (B) **Sb-10** in 1:1 MeCN–H_2O (■), 9:1 MeCN–H_2O (▲) and 9:1 MeCN–TFE (◆); (C) **Sb-10** with 0.1 M TMAT in 9:1 MeCN–TFE (■), 0.01 M TMAT in 9:1 MeCN–TFE (▲), 0.001 M TMAT in 9:1 MeCN–TFE (●) and 9:1 MeCN–TFE (◆)

Table 1 Circular dichroism data and derived structural parameters for **Sb-10**

Solvent	$[\theta]_{\pi\rightarrow\pi^*}$[a,b]	$[\theta]_{n\rightarrow\pi^*}$[a,c]	*R*	Helicity[d] (%)
9:1 MeCN–TFE	−15230	−6579	0.43	71 (3_{10})
9:1 MeCN–H_2O	−12749	−6461	0.50	*e*
1:1 MeCN–H_2O	−8673	−7433	0.85	31 (α)
0.1 M TMAT in 9:1 MeCN–TFE	−15774	−9576	0.61	*f*
0.01 M TMAT in 9:1 MeCN–TFE	−15038	−9235	0.61	*g*
0.001 M TMAT in 9:1 MeCN–TFE	−15333	−6531	0.43	71 (3_{10})

[a] Units for $[\theta]$ are deg cm^2 dmol^{-1}. [b] The minimum for the $[\theta]_{\pi\rightarrow\pi^*}$ band is in the range from 205–209 nm. [c] The minimum for the $[\theta]_{n\rightarrow\pi^*}$ band is in the range from 222–225 nm. [d] The percentage of α-helix is estimated using the equation: α-helix (%) = $-100([\theta]_{n\rightarrow\pi^*}+ 3000)/33000$ and the percentage of 3_{10}-helix is estimated using the equation: 3_{10}-helix (%) = $-100([\theta]_{\pi\rightarrow\pi^*})/21\ 500]$. [e] This peptide probably forms a mixture of α-helical, 3_{10}-helical and coil structures. The percentage of α-helix is estimated at 29% and the percentage of 3_{10}-helix is estimated at 59%. [f] This peptide probably forms a mixture of α-helical, 3_{10}-helical and coil structures. The percentage of α-helix is estimated at 57% and the percentage of 3_{10}-helix is estimated at 73%. [g] This peptide probably forms a mixture of α-helical, 3_{10}-helical and coil structures. The percentage of α-helix is estimated at 54% and the percentage of 3_{10}-helix is estimated at 70%.

the CD spectrum of **Sb-10** has strong 3_{10}-helical character (71% 3_{10}-helicity). The transition of **Sb-10** from an α-helix to a 3_{10}-helix as the solvent dielectric is decreased agrees with the theoretical calculations of the solvent effects on the 3_{10}/α-helix equilibrium.[14] Marshall's calculations predict Aib-rich peptides should favor the α-helix in water and the 3_{10}-helix in less polar media.[14]

The percent 3_{10}-helicity of **Sb-10** is estimated to be higher than that of **Ipi-10** (71 *vs.* 45%[5]) suggesting that the two salt bridges significantly stabilize the 3_{10}-helical conformation. The CD spectral changes upon titration of **Sb-10** with an organic solution (9:1 MeCN–TFE) of tetramethylammonium trifluoroacetate¶ (TMAT, 0.0 to 0.1 M) are consistent with side-chain ionic interactions stabilizing 3_{10}-helical conformation. **Sb-10** makes a transition from a 3_{10}-helix to a partial α-helical structure as the TMAT concentration is increased. The decreasing 3_{10}-helix stability of **Sb-10** as the salt concentration is increased parallels what has been observed for α-helical peptides stabilized by side chain ionic interactions.[9a]

Ipi-10 and **Sb-10** have the same number of residues, the same percentage of ααAAs, and the same amphipathic design. The α-helix forming propensities of lysine and glutamic acid are similar[15] so the additional 3_{10}-helicity of **Sb-10** compared to **Ipi-10** must result from side-chain interactions. The decreasing stability of the 3_{10}-helical conformation of **Sb-10** as salt concentration is increased also supports the idea that *i*, *i* + 3 salt bridging or ion pairing stabilize 3_{10}-helices.

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Notes and References

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‡ This intrepetation of ratio (*R*) of the n→π* and π→π* bands of the CD spectrum has been disputed by Andersen *et al.* (N. H. Andersen, Z. Liu and K. S. Prickett, *FEBS Lett.*, 1996, **399**, 47). Their study used much longer peptides (22 residues) containing low percentages (1/22–5/22) of Aib, which can form highly amphipathic α-helices (but not amphipathic 3_{10}-helices). They find that comparison of NMR (NOE) and CD evidence suggests that these peptides are a mixture of α-helix, 3_{10}-helix (in one case) and random coil. When the CD was corrected for 'disordered' residues, a peptide containing 5/22 Aib residues had $R > 1$, which they argue goes against the findings of Toniolo (ref. 13). We are confident in the case of **Ipi-10** and **Sb-10** that $R \sim 0.4$ reflects a 3_{10}-helix and not some contribution from a random coil spectrum as the CD of **Ipi-10** and **Sb-10** in water or phosphate buffer are essentially baseline ($-[\theta]_{\pi\rightarrow\pi^*} < 50$). This is apparently due to a very weak random coil ('disordered') spectrum of these peptides resulting from the lack of stereogenic amino acids (only 2 L-Lys or 2 L-Glu resides).

§ **Sb-10** shows no concentration dependence of the CD spectrum in 9:1 MeCN–TFE over a 10-fold concentration range (0.02–0.3 mM): $[\theta]_{\pi\rightarrow\pi^*} = -15\ 000 \pm 750$; $R = 0.42 \pm 0.02$. This suggests that **Sb-10** is monomeric under these conditions.

¶ A highly organic-soluble salt, such as TMAT, is required for titration of the **Sb-10** side-chains because strong 3_{10}-helix character is only present in 100 % organic media.

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