Fine Tuning of β-Peptide Foldamers: a Single Atom Replacement Holds Back the Switch from an 8-Helix to a 12-Helix

Amandine Altmayer-Henzien, Valérie Declerck, Jonathan Farjon, Denis Merlet, Régis Guillot, and David J. Aitken*

Abstract: Cyclic homologated amino acids are important building blocks for the construction of helical foldamers. Naminoazetidine-2-carboxylic acid (AAzC), an aza analogue of trans-2-aminocyclobutanecarboxylic acid (tACBC), displays a strong hydrazino turn conformational feature, which is proposed to act as an 8-helix primer. tACBC oligomers bearing a single N-terminal AAzC residue were studied to evaluate the ability of AAzC to induce and support an 8-helix along the oligopeptide length. While tACBC homooligomers assume a dominant 12-helix conformation, the aza-primed oligomers preferentially adopt a stabilized 8-helix conformation for an oligomer length up to 6 residues. The (formal) single-atom exchange at the N terminus of a tACBC oligomer thus contributes to the sustainability of the 8-helix, which resists the switch to a 12-helix. This effect illustrates atomic-level programmable design for fine tuning of peptide foldamer architectures.

boldamers are unnatural oligomers that adopt well-defined folded patterns, and several factors that govern their conformational preferences have been identified.^[1] β-Peptides were amongst the first foldamers to be studied and they provide benchmark helix-forming manifolds,^[2,3] the diversity of which has been enlarged through the study of α/β -, β/γ -, and other mixed peptides.^[3,4] Oligomers of cyclic β -amino acids adopt stabilized helices where the pitch depends largely on the backbone torsional angle θ (N-C^{β}-C^{α}-C(=O)), which in turn is determined by the ring size and its stereochemistry. Robust 14-helix^[5,6] and 12-helix^[7] foldamers can be constructed rationally from appropriate cyclic trans-\beta-amino acids, while the alternating 10/12-helix^[8] and regular 6ribbon strands^[9] are observed for oligomers of cyclic *cis*-βamino acids. Nonetheless, helical folding may be influenced by other factors, such as side-chain interactions,^[10] the steric bulk of the monomer,^[11] solvent/concentration effects,^[12] or the oligomeric environment into which the monomer is

[*] Dr. A. Altmayer-Henzien, Dr. V. Declerck, Prof. Dr. D. J. Aitken CP3A Organic Synthesis Group, ICMMO UMR 8182 Université Paris Sud 15 Rue George Clemenceau, 91405 Orsay cedex (France) E-mail: david.aitken@u-psud.fr
Dr. J. Farjon, Prof. Dr. D. Merlet LRMN, ICMMO UMR 8182, Université Paris Sud 15 Rue George Clemenceau, 91405 Orsay cedex (France) Dr. R. Guillot Services Communs, ICMMO UMR 8182, Université Paris Sud 15 Rue George Clemenceau, 91405 Orsay cedex (France)
Is Rue George Clemenceau, 91405 Orsay cedex (France)
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201504126. placed.^[13] Further exploration of such modulating factors could therefore enable the fine tuning of programmable helical folding.

Oligomers of *trans*-2-aminocyclobutanecarboxylic acid (*t*ACBC) can adopt a 12-helix conformation both in solution and in the solid state.^[14] However, the preferred conformer of a *t*ACBC dipeptide is an 8-membered hydrogen-bonded ring (C8),^[15] and it has been suggested that a *t*ACBC tetrapeptide might display three consecutive C8 structural features.^[16] This corresponds to an 8-helix, which is a rarity for β -peptides.^[17,18] The 12-helix preference of *t*ACBC oligomers raises the question of the sustainability of an 8-helix (Figure 1);



Figure 1. 8- vs. 12-membered H-bonded rings in *t*ACBC oligomers and the Hz-turn conformation of an AAzC residue.

indeed, in previous cases where 12-helix conformers compete, the 8-helix was only observed for small (\leq 4-mer) oligomers.^[17] *N*-Aminoazetidine-2-carboxylic acid (AAzC)^[19] is an aza analogue of *t*ACBC and is characterized by a strong tendency to form a bifurcated C8/5-ring H-bonded structure known as a hydrazino (Hz) turn (Figure 1).^[20,21] We therefore examined the ability of a single AAzC residue to behave as an "8-helix primer" when employed as the N-terminal residue in an oligo-*t*ACBC sequence.

Four oligopeptides (1–4; Figure 2) were prepared (see the Supporting Information) in order to compare their conformational behavior with that of the corresponding homooligomers $Boc(tACBC)_nOMe$ (5–8; n=2, 4, 6, 8, respectively).^[14] The two series are identical with the exception of the formal



Figure 2. Structures of peptides 1-4.

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Figure 3. IR absorption spectra (in $CHCl_3$) of AAzC peptides 1–4 (top) and tACBC oligomers 5–8 (bottom).

single-atom exchange (N for C) at the β -position of the N-terminal residue in the former series.

In CHCl₃ solution, the N–H stretch absorptions in the IR spectra were useful for tracking conformational changes with increasing peptide length in the two series of peptides (Figure 3). In the reference tACBC oligomer series, **5** showed an H-bond absorption band at 3290 cm⁻¹ consistent with a C8 conformer. This absorption appeared with progressive red-shift in **6** and **7**, although the spectrum of the latter showed the presence of a second Hbonded conformer. This second conformer was predominant in the spectrum of **8**. These data are consistent with a predominant 8-helix conformation for **6**, a 12-helix conformer for **8**, and the coexistence

of these two conformers for 7. In the AAzC peptide series 1– 4, the diagnostic small N–H absorbance at 3360 cm⁻¹ indicated the presence of an Hz-turn in all cases.^[20] Peptides 1–3 showed a strong H-bond absorbance, which became progressively red-shifted from 3300 to 3240 cm⁻¹ with increasing peptide length. The spectrum of 4 featured an additional strong H-bond absorption at 3300 cm⁻¹ and an absorption corresponding to free N–H at 3440 cm⁻¹. Comparison of the two series clearly suggests that in CHCl₃ solution, the 12-helix is already prevalent in the *t*ACBC hexamer and predominant in the octamer, while for the AAzC peptides, an Hz-turn-promoted 8-helix is predominant up to the hexapeptide and remains a significant conformer for the octapeptide; the second conformer is not clearly identified at this stage.

These observations were corroborated by NMR analysis of the AAzC peptides 2-4 in CDCl₃ solution. In each case, the N⁴H ¹H NMR signal appeared at $\delta \approx 6.5$ ppm and a group of strongly deshielded signals at $\delta \approx 9.0$ ppm was observed for the other amide groups, thus suggesting the latter to be involved in strong hydrogen bonds. The intramolecular nature of the H-bonds in 2 and 3 was confirmed by the absence of a significant variation in chemical shift with concentration, which is consistent with an 8-helix structure. For 4, only the $N^{10}H$ signal ($\delta = 8.99$ ppm) was strongly deshielded and was uninfluenced by concentration, thus suggesting the presence of an Hz-turn. Other amide signals were moderately deshielded ($\delta = 7.86-8.70$ ppm) and showed moderate concentration dependence, thus suggesting the presence of both H-bonded and non-H-bonded conformations, which is in agreement with a conformational equilibrium for 4. The N⁴H signal was an exception, with significant concentration and isotopic exchange effects suggesting that it remains essentially non-H-bonded in 4.

Further evidence for the H-bond networks in peptides 2–4 in CDCl₃ was acquired by using fast-pulsing high-field SOFAST HMBC experiments.^[22] The diagnostic N¹⁰H···N⁵ interaction of an Hz-turn was ascertained for all three peptides by ¹H^N₋¹⁵N SOAFST HMBC. A ¹H^N₋¹³CO SOFAST HMBC experiment for **3** revealed a series of $(i \rightarrow i-2)$ H-bonds, which together with the Hz-turn, constitute an uninterrupted 8-helix (Figure 4). This is entirely consistent



Figure 4. Hydrogen bonding in peptides **3** (in $CDCl_3$) and **4** (in $[D_5]$ pyridine) observed by SOFAST HMBC NMR experiments.

with the IR data. A CDCl₃ solution of **4** failed to give usable 2D NMR data, probably owing to the coexistence of two interconverting conformers. NMR studies were carried out in $[D_5]$ pyridine solution, thus allowing the identification of the second conformer of **4**. The Hz-turn was confirmed by ¹H-¹⁵N SOFAST HMBC, while ¹H-¹³CO SOFAST HMBC revealed four $(i \rightarrow i-3)$ H-bonds that define a 12-helical segment involving all seven *t*ACBC residues (Figure 4). Peptide **4** thus adopts a unique hybrid conformation in pyridine, starting with an 8-membered Hz-turn and switching to a 12-helix for the rest of the sequence. This conformer coexists with the 8-helix for a solution of **4** in CHCl₃.

The low solubility of peptides 1-4 in protic solvents (< 5 mM in CD₃OH) precluded similar NMR studies in such media. However, the CD spectra were recorded in dilute



Figure 5. CD spectra (in MeOH) of AAzC peptides 1–4 (top) and *t*ACBC oligomers 5–8 (bottom).

MeOH solution (0.2 mM) and compared with those of the *t*ACBC oligomers **5–8** (Figure 5). Comparison of AAzC peptides **3** and **4** with oligomers **6** and **7**, respectively, is revealing. While the contribution from a 12-helix (max. 207 nm, min. 227 nm)^[14] is already significant in tetramer **6**, this structure is only partially in evidence for hexapeptide **3**. Tetrapeptide **2** displayed a different curve (max. 203 nm, min. 223 nm): prevalence of an 8-helix conformation is an attractive hypothesis but this should be treated with caution since the CD signal of the β -peptide 8-helix has not been calculated. All CD curve intensities were only partially diminished over the temperature range 5–55 °C, with no detectable conformer change.^[23]

A hybrid MCMM conformational search was carried out on peptides 2-4 in vacuum by using MacroModel 10.6^[24] and the MMFF94s force field without restraints. Low-energy families were subjected to ab initio geometrical optimization at the B3LYP 6-31G** level of theory in CHCl₃ and in MeOH using GAUSSIAN09.^[25] The Hz-turn of AAzC dominated in low-energy conformers, regardless of the other conformational features. Only two low-energy conformer families emerged: the 8-helix and the Hz-turn/12-helix hybrid (Figure 6). In CHCl₃, the 8-helix was the most stable conformer for 2 and 3, whereas for 4, the hybrid conformer was only 0.7 kJ mol⁻¹ higher in energy. These results are in complete agreement with the experimental data, thus suggesting the predominance of the 8-helical conformer up to six residues, and the presence of two conformers for octapeptide 4. In MeOH, the 8-helix is the lowest energy conformer for tetrapeptide 2 by at least 8.6 kJ mol⁻¹. This would explain the low CD ellipticity of 2, which can now be considered more



Figure 6. Calculated low-energy conformers of peptides 2-4.

confidently as the signature of an 8-helix. For **3**, the 8-helix (**3**-**I**) and the hybrid Hz-turn/12-helix (**3**-**II**) have closer energies and for **4**, the hybrid structure **4-II** is more stable. These observations account qualitatively for the increasing "12-helix-like" contribution to CD data with increasing peptide length.

Inspection of the backbone torsional angles (ϕ , θ , ψ) revealed the remarkable simplicity of the helix pitch switch: the AAzC residues in **4-II** and **4-II** are virtually superimposable, with a ψ value (ca. 4.0°) that is amenable to both 8-helix and 12-helix progressions. In each helix type, the *t*ACBC residues have similar values for ϕ (range 87° to 100°) and θ (range -95° to -103°); only the ψ values differ significantly (ca. 35° in **4-II**).

A single crystal of **4** was obtained through slow evaporation of a CH₂Cl₂/MeOH solution and was analyzed by X-ray diffraction (Figure 7).^[26] The main characteristics of the hybrid Hz-turn/12-helix conformer **4-II** were in evidence: the N-terminal AAzC shows the three-centered H-bonded Hz-turn, while the *t*ACBC segment adopted a 12-helix structure into which a molecule of methanol had been inserted between N³⁴H (residue 3) and O=C²¹ (residue 6), with minor distortion of the helical axis.^[27]

From these studies, it emerges that replacement of the Nterminal residue of a *t*ACBC oligomer by AAzC, conceptually a single-atom substitution, has the effect of enhancing 8-helical folding prompted by the strong Hz-turn of AAzC. The 8-helices described herein are the longest described to date for β -amino acid oligomers. In CHCl₃, the 8-helix is the



Figure 7. X-ray diffraction structure of peptide **4**: side view (left) and view along the axis (right).

main conformer up to an octapeptide, whereas the corresponding *t*ACBC oligomers largely exist in 12-helical conformations.^[14] In MeOH, the 8-helix makes a significant contribution up to a 6-residue sequence when primed by AAzC. When the 8-helix prompt is not followed, the *t*ACBC segment folds into a 12-helix, giving a hybrid Hz-turn/12-helix conformer. These results contribute to our understanding of the subtle factors that may govern the preferences for 8- or 12helix folding, and this understanding could be employed advantageously to the design of polymorphic molecular architectures.

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