Helices and Other Secondary Structures of β- and γ-Peptides

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Abstract: The principal secondary structural motifs adopted by peptides assembled from β-amino acid units are discussed: the 14-, 12-, 10-, 12/10-, and 8-helices, as well as the hairpin turn, extended structures, stacks, and sheets. Features that promote a particular folding propensity are outlined and illustrated by structures determined in solution (NMR) and in the solid-state (x-ray). The N-Cβ-Cα-CO dihedral angles from molecular dynamics simulations, which are indicative of a particular secondary structure, are presented. A brief description of a helix and a turn of γ-peptides is also given. © 2005 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 84: 23–37, 2006

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INTRODUCTION

In 1995, our group embarked on a research project defined by the question: what happens when each amino acid residue in a peptide, with the natural proteinogenic side chains, is homologated by insertion of one or two CH₂ groups? The most important answers, as far as structural changes are concerned, are summarized in Figure 1.

(i) The oligomers of the homologated l-amino acids fold to helices in solution, with chain length of as few as four residues (NMR analysis in MeOH,
MeOH/H2O or pyridine). (ii) On progressing from α- to β- to γ-peptides, the stability of the helices increases, although the number of hydrogen bond donors and acceptors per chain atom decreases (one of each in 3, 4, and 5 chain atoms, respectively). (iii) The helicity reverses upon each homologation step: (P) or right-handed for the 3.613- and 3.10-α-, (M) or left-handed for the 3.14-β-, and (P) again for the 2.614-γ-helical peptides. (iv) The direction of the helix macrodipole also reverses with homologation: it points from N- to C- in α-, from C- to N- in β-, and again from the N- to C-terminus in γ-peptidic helices. (v) From α- to β- to γ-peptides, there is an alternating switch of helix stabilities of terminally protected (RO2C-N, C-OR) versus unprotected derivatives: the degree of helicity decreases upon deprotection of α- and γ-peptides1,2 and increases when β-peptides are deprotected.3,4 This effect can be correlated with pole-charge interactions, a kind of intrinsic "capping" effect5 in the β-peptidic helix. (vi) The helices of β1- and β2-peptides have opposite chirality, (M) versus (P), and the helix consisting of β2-amino acids is less stable. (vii) In the NMR-solution structures of β-peptide helices, the C-terminus is less structurally ordered and unwinding occurs.

Another fundamental difference between α- and β-peptidic helices was uncovered when the NMR-solution structure in methanol was determined in an eicosapeptide carrying the 20 proteinogenic side chains (Figure 2). Despite the large resulting macrodipole this peptide folds to the 14-helix over the full length—this would not have been observed for a corresponding α-peptide (cf. the single-domain proteins studied by Baldwin et al.5). Thus, β-peptides not only fold to 314-helices with as few as six, but also with as many as 20 residues! For detailed discussions with ample citation we refer to an extensive review article6 and a recent full paper7 by Seebach et al.

THE HELICES OF β-PEPTIDES

Five distinct helices have so far been identified in the field of β-peptides: the 14-3,4,7,21 a 12-,22–29 a 10-,30,31 the 12/10-,3,32–35 and an 8-helix,36,37 which are defined by the sizes of 8-, 10-, 12-, and 14-membered hydro-
gen-bonded rings. Extended arrangements, namely parallel and antiparallel pleated sheets (the latter being inherently part of a hairpin turn),\textsuperscript{38–45} a non-aggregating linear structure,\textsuperscript{46} and stacks\textsuperscript{47–49} have also been identified. NMR spectroscopy has emerged as the most useful tool in determining the secondary structures of $\beta$-peptides. Samples suitable for single-crystal or powder x-ray diffraction structure determination have, so far, only been obtained from $\beta$-peptides consisting of the conformationally restricted trans-2-aminocyclopentanecarboxylic acid (ACPC)\textsuperscript{29} and trans-2-aminocyclohexanecarboxylic acid (ACHC)\textsuperscript{18,50} units and from sheet- and stack-forming $\beta$-peptides.\textsuperscript{41,45,47,49}

The 14-Helix. This secondary structure has emerged as the best documented of the folding structures of $\beta$-peptides.\textsuperscript{6} There are 14-membered hydrogen-bonded rings between $\text{N} \cdots \text{C} = \text{O}$ $(i)$ and $\text{C} = \text{O}$ $(i + 2)$ in a three-residue repeating arrangement. As can be seen from the structure of the eicosamer (Figure 2),\textsuperscript{7} there is a deviation from an idealized $(M)$-14-helix; substituents in the $(i)$- and $(i + 3)$ positions are not exactly positioned on top of each other, but offset by ca. 15° in a right-handed direction (a “3.2-helix”). For steric reasons only $\text{H}$, $\text{OH}$, or $\text{F}$ are allowed in the axial positions (Figure 3), a methyl group is not. Thus, homologs of Aib ($\beta^2$- and $\beta^3$hAib, homoaminoisobutyric acid) are helix-breaking in the “$\beta$-regime,”\textsuperscript{4} while Aib itself is strongly $3_{10}$-helix-inducing in the “$\alpha$-world.”\textsuperscript{51} $\beta^{2,3}$ Amino acid residues of (S,S)- or l-configuration\textsuperscript{52} strongly favor the synclinal\textsuperscript{53} conformation and stabilize the 14-helix,\textsuperscript{3} and (S,S)-trans-2-ACHC moieties lock it.\textsuperscript{17,18,50,54,55}

The 12/10-Helix. Since an l-$\beta^3$- and an (S)-$\beta^2$ amino acid fit in the left-handed 14-helix (the side chains of both are in allowed lateral positions, green in Figure 3), peptides containing alternatively $\beta^2$- and $\beta^3$-residues of these configurations were expected to fold to the $3_{14}$-helix, as do all-l-$\beta^3$- and all-(S)-$\beta^2$-peptides.\textsuperscript{3,7,13,56,57} In contrast, they were found to form a peculiar, right-handed 2.7$\textsubscript{12,10}$-helix (Figure 4) con-
sisting of alternating 12- and 10-membered hydrogen-bonded rings (Figure 4B). The dimer segment containing an amide bond with no adjacent substituents (NH-CHR\textsubscript{CO}CH\textsubscript{2}CO/NH-CHR\textsubscript{CO}CH\textsubscript{2}CO) is part of the 12-membered ring, the one with substituents (NH-CHR\textsubscript{CO}CH\textsubscript{2}CO/NH-CHR\textsubscript{CO}CH\textsubscript{2}CO) being part of the 10-membered ring.

As in the 14-helices, the conformation around the C(2)–C(3) ethane bonds is (\(\mp\))-synclinal in the \(\beta\text{-}2\) and in the \(\beta\text{-}3\) amino acid residues. The distribution of the substituents on the surface of the helix is “irregular” (Figure 4C). Most notably, the 12/10-helix has no resulting macrodipole; the C=O (and the N–H) bonds have an alternating up/down direction with respect to the helix axis (Figure 4A). For the delicate balance between 14- and 12/10-helices, see discussions in the literature and under Modeling Studies, below.

**The 12-Helix.** When the substituents in the 2- and 3-positions of the \(\beta\)-amino acid become part of a five-membered ring (cyclopentane, pyrrolidine), the accessible dihedral angle range in the trans-substituted ring is restricted to values above ca. 85°. The oligomers of such non-protein-derived amino acids have been found to fold to 2.6 12-helices in the crystalline state, as well as in solution (Figure 5). For other \(\beta\)-peptidic 10-membered hydrogen-bonded ring systems, we refer to the section on turn structures below.

**An 8-Helix?** Like the 10-helix, the 8-helix has not been observed in any type of oligomer composed of homologated proteinogenic amino acids (see section 8 in Seebach et al.\textsuperscript{5}). Two remarkable examples are, however, shown in Figure 6. Structural parameters from x-ray diffraction of crystalline \(\beta\text{-di-}, \beta\text{-tri-},\) and \(\beta\)-tetrapeptides consisting of achiral 1-aminoethylcyclopropane carboxylic acid residues were used to model a longer oligomer (Figure 6A), which has a stair-like arrangement where each step consists of a folded eight-membered hydrogen-bonded ring; as expected, the C=O bond bisects the three-membered ring, having a rigidifying effect on the backbone. A twisted form of the stair of A, i.e., a \((P)\text{-}2\text{\textsubscript{a}}\)-helix B was derived from an NMR analysis in methanol solution of an oligomer composed of \((2R,3S)\text{-}2\text{-...
hydroxy-3-aminocarboxylic acid moieties with Val, Ala, and Leu side chains. MD simulations, however, suggested a preference for this β-peptide to form 12-membered H-bonded rings: it was pointed out that the measured NMR data are equally compatible with a (P)-2.512-helix structure (Figure 6C). 3-Aza and 3-oxa-β-amino-acid oligomers have also been found to fold to 8-helices.

β- and γ-Peptide Secondary Structures

FIGURE 4 The (P)-2.712/10-helix of β3/β2-mixed peptides. (A) NMR structure in MeOH solution of the β-nona-peptide Boc-β3hVal-β2hAla-β3hLeu-β3hVal-β3hAla-β3hLeu-β3hVal-β3hAla-β3hLeu-OBn. (B) Schematic presentation of the 12- and 10-membered hydrogen-bonded rings. (C) View along the axis of the R-β3hVal-β2hAla-β3hLeu-β3hVal-β3hAla-β3hLeu-OR' helix (NMR structure in MeOH). (D) Newman projection along the Cβ-Cα bond. For nomenclature see also Figure 3 and Seebach et al.6

L-β3hXaa : R2 = H
(S)-β2hXaa : R3 = H
(+)-synclinal

(β3hPro)n: A helix without intramolecular hydrogen bonds? Polyproline [Poly(Pro)n] and polyhydroxyproline [Poly(Hyp)n], where Hyp is 4-hydroxyproline, play an important role in nature. They fold to helices and triple helices (collagen) without backbone stabilization by intramolecular hydrogen bonding. Oligomers of homologated proline (β3hPro or (S)-pyrrolidin-2-yl acetic acid and β3hPro or (R)-piperidin-3-carboxylic acid).
acid) have also been prepared. From crystal structure data of \((\beta^3\text{hPro})_2\) derivatives a higher oligomer was modeled, which is, indeed, a helix (Figure 7).

**OTHER SECONDARY STRUCTURES OF \(\beta\)-PEPTIDES**

Besides helical arrangements, \(\beta\)-peptides, like the \(\alpha\)-peptidic prototypes, can attain non-aggregating extended-chain structures, can assemble to pleated sheets, fold to hairpin turns, and stack in crystals. Keeping in mind certain simple rules, which we have learned in 10 years of \(\beta\)-peptide research and/or which are known from the age-old world of \(\alpha\)-peptides, we can now construct most of these arrangements by design.

Thus, a \(\beta\)-peptide made of \(\mu\)-2-methyl-3-aminocarboxylic acids units ((2R,3S)- or (2S,3R)-configuration) cannot possibly fold to a 14-helix (rule: *no alkyl substituent in an axial position!*). Such a \(\beta^{2,3}\)-peptide is therefore forced to stay in an extended form, with an antiperiplanar (ap) conformation of the amino acid residues, with all C=O bonds pointing in one direction and all N—H bonds in the opposite direction and with the side chains approximately perpendicular to the amide planes—an ideal structure for assembly of parallel (Figure 8A) or antiparallel (cf. also Figure 10 below) pleated sheets.

Sheet formation can be prevented by remembering another rule: ‘*no substituent other than hydrogen may be pointing inwards of the ring held together by two hydrogen bonds in a pleated sheet.*’ A \(\beta\)-peptide consisting of 2,2-dimethyl-3-aminocarboxylic acid \((\beta^{2,2}\text{hAib})\) is thus ‘condemned’ to stay in an extended arrangement and not assemble to a pleated sheet (Figure 8B).
A hairpin turn can be constructed by incorporating turn-inducing (cf. the 12/10 helix, above) or turn-enforcing structural elements, and, attaching to them sheet-favoring appendices (Figures 9 and 10).38,40–44,69–72 The amide group in the 10-membered hydrogen-bonded ring of the \( \beta_2/\beta_3 \) section ((S)-\( \beta_2hXaa-(L-\beta_3hXaa-)) \) and the adjacent side chains actually have the same geometry found in an \( \alpha \)-peptidic \( \beta_II \) turn, built from an (S)- and an (R)-Xaa amino acid; this allowed mimicking of the somatostatin peptide receptor interaction with a – metabolically stable73,74 – \( \beta \)-peptide.75,76 Turns are structurally inherent to cyclic peptides. Cyclo-\( \beta \)-tri-, -tetra-, and -hexapeptides have been prepared.6 The simple tri-
FIGURE 8 (A) Parallel pleated sheet of a β-tripeptide from u-2-methyl-3-amino acids. Compounds of this type are extremely insoluble; in contrast to an α-peptidic sheet all C=O bonds point in the same direction, as do the N–H bonds, rendering the β-peptidic sheet polar.41,68 (B) In a similar backbone conformation as in A, geminal dimethyl-substitution prevents the N–H group from engaging in hydrogen bond donation (a kind of A1,3 effect). Compounds of this type have good solubility in organic media; the structure shown in B has been derived from solid-phase NMR experiments (REDOR technique) with a magnetically labeled derivative.46 For a sheet structure with sc-conformation of βhGly residues see Chung et al.69

FIGURE 9 Simple β-peptidic turn structures. (A) Preferred conformation of the β-dipeptide derivative (S,S)-N-acetyl-β2hVal-β3hPhe-NHCH3 (NMR in MeOH, and MD simulation).71 (B) A geminally disubstituted β2,2-amino acid as a turn stabilizer? (crystal structure).43 (C) β2hPro as turn-inducing residues (crystal structure).69
FIGURE 10 A β-hexa- and β-octapeptide folded to hairpin turns. (A) Combination of a turn-inducing \( \beta^2/\beta^3 \)-segment with two linearly enforced \( \beta^{2,3} \)-dimer segments (NMR-structure in MeOH).\(^{41} \) (B) Zn\(^{2+} \)-enforced turn structure (NMR-structure in H\(_2\)O) of an otherwise 14-helical-β-peptide.\(^{70} \)

FIGURE 11 (A) Solid-state structure (powder x-ray diffraction) of cyclo(β\(^3\)hAla)\(_4\). The molecules stack through four intermolecular C=O⋯H—N hydrogen bonds, to form columns, which are arranged in an antiparallel manner;\(^{47,49} \) the compound is extremely insoluble. (B) A cyclo-β-peptide consisting of two \( \beta^2 \)- and two \( \beta^3 \)-amino acids (NMR-solution structure in MeOH): there is a transannular hydrogen bond creating a bicyclic system, which contains a 10- and a 12-membered hydrogen-bonded ring;\(^{78,80} \) cf. the turn structures (Figure 9) and the 10-, 12-, and 12/10-helices, above.
and tetrapeptides stack to highly insoluble solids (Figure 11A), although soluble derivatives can be obtained by introducing functionalized side chains. In the solution structures of the cyclo-β-tetrapeptides there is a transannular hydrogen bond; the structures may therefore be considered as a pair of fused 10- and 12-membered turns (Figure 11B).

**HELIX AND TURN OF γ-PEPTIDES**

Double homologation of proteinogenic amino acids in peptides leads to γ2-, γ3-, and γ4-peptides. Of these most simple derivatives an NMR-solution structure has only been found for a γ4-hexapeptide, which was shown to be a 2.614-helix (cf. Figure 1). More heavily side-chain–substituted derivatives, such as γ2,4- and γ2,3,4-peptides, can also fold to a 2.614-helix (Figure 12A). The configuration around both C—C ethane bonds (N—Cγ—Cβ—Cα and Cγ—Cβ—Cα—CO) is sc in these helices.

Depending upon the relative configuration of the γ2,4-residues, turn motifs can also be constructed (Figure 12B). For both secondary structures to be observed in solution, the required chain length can be even shorter than in the case of β-peptides: four residues for the helix and two for the turn.

**FIGURE 12 γ-Peptidic helix and turn.** The γ-amino acids with three substituents in the tetrapeptide A, n = 2, can be assigned d-configuration, and the helix is (M) or left-handed. L-γ4-Residues give rise to a (P)-helix (Figure 1, far right). The γ-dipeptide (top right) forms a nine-membered hydrogen-bonded ring. Turns as the one shown in B can be used as scaffolds for α-peptidic turn mimics, given the proper side chains adjacent to the peptide bond.
The alkyl chain backbone takes control, while the number of hydrogen bonds per chain atom decreases! The structural diversity of \( \gamma \)-amino acids and \( \gamma \)-peptides has not been elucidated nearly as well as that of \( \beta \)-peptides: it is expected to be richer.

**MOLECULAR DYNAMICS SIMULATIONS OF \( \beta \)-PEPTIDE STRUCTURES**

NMR-solution structures provide time and ensemble averages of individual conformations: the time-scale ("exposure time") of NMR spectroscopy is in the order of \( 10^{-7} \) s, very long in comparison with the rate of conformational changes, which is in the range of \( 10^{-12} \) s. Furthermore, the NMR analysis uses \( ^J \) values and NOE distance bounds, and the NOE between non-neighboring atoms is not subject to quantitative analysis (weak/medium/strong) and drops with distance in the power of \( r^{-6} \). A structure present in low abundance may thus "disappear," while a conformation giving rise to pronounced NOEs will be grossly overrepresented in the NMR analysis, even in the presence of a predominant form that gives much weaker or, indeed, no NOEs. X-ray-crystal structures, on the other hand, are snapshots showing a single, solely present conformation in the solid state.

In contrast, MD simulations offer a picture at atomic resolution and can give insight into dynamic processes, such as those maintaining the folding/unfolding equilibrium of a peptide. Using the GROMOS simulation package with its thermodynamically calibrated force field, several \( \beta \)-peptides have been analyzed in methanol solution, with simulation periods of up to 250 ns and at temperatures of 298 and 340 K. Depending on the sampling frequency (for instance every 10 ps), a large number of structures can be extracted from such a long simulation. Relative populations ("thermodynamic stability") and average lifetimes ("kinetic stability") can be calculated.

In a most fruitful collaboration between the van Gunsteren and Seebach groups at the ETH, it was established that the GROMOS force field is able to describe the folding of all \( \beta \)-peptidic secondary structures that were experimentally determined by NMR spectroscopy: the 14-helix, the 12/10-helix, and the hairpin turn (Figure 13).

At 298 K the \( \beta \)-heptapeptide with a central, helix-stabilizing, L-(2Me)-\( \beta \)hAla residue (Figure 13A) is found to preferentially form a 14-helix (53% populated in the simulation starting from a fully extended conformation). Under the same conditions the 12/10-helix constitutes 52% of the conformations of the \( \beta^2/\beta^2 \)-nonapeptide (Figure 13B) and the \( \beta \)-hexapeptide shown in Figure 13C is folded to a hairpin structure in 20% of the conformations. From simulation data, the \( \beta \)-heptapeptide lacking Val and Ile side chains and having two positively charged Lys side chains, a \( (P) \)-12-helix structure (ca. 5% of the population) could be extracted (Figure 13D). Clearly, the 14- and the 12/10-helices, as well as the turn-part of the hairpin, have (+)-sc dihedral angles close to the ideal one of staggered ethane (60°), while in the 12-helix the values are nearer to +90° (cf. Figure 5). Also, in the antiparallel sheet section of the turn, angles near the ideal 180° are obtained. Besides folding to the major secondary structures A–C in Figure 13, these peptides sample overlapping conformational spaces: in addition to the turn, there are 12/10- helical structures and together with the 12/10-helix there are also 14-helical structures, and, of course, there are numerous partially folded helices and structures with single 10-, 12-, and 14-membered hydrogen-bonded rings. These MD simulations are compatible with the conclusion from temperature-dependent NMR and CD measurements, namely that the folding/unfolding process of \( \beta \)-peptides and of \( \gamma \)-peptides is non-cooperative.

The MD simulations also confirm that the effect of secondary alkyl side chain groups (Val and Ile) on the 14-helix’s stability is probably more due to a destabilizing effect of the ap conformation of \( \beta \)-peptidic residues with such side chains-after all, the fully helical \( \beta \)-eicosapeptide shown in Figure 2 contains only one \( \beta \)Val and one \( \beta \)Ile. Subtle structural differences caused by terminal protection/deprotection of \( \beta \)-peptides and effects of charged side chains on folding rate and helix stability of \( \beta \)-peptides (cf. hydrogen-bridges between Lys-\( \omega \)-NH\(_{3} \) and backbone carbonyl oxygens were also investigated by MD simulation, providing further insights into the folding/unfolding equilibrium at atomic detail.

The MD simulations of \( \beta \)-peptides have helped to deepen our understanding and permit questioning of some generally accepted "facts" of \( \alpha \)-peptide chemistry, as is evident from publication titles such as: "Peptide Folding: When Simulation Meets Experiment,..."

"Molecular Dynamics Simulations of Small Peptides: Can One Derive Conformational Preferences from ROESY Spectra?,..." and "The Key to Solving the Protein-Folding Problem Lies in an Accurate Description of the Denatured State."
Besides molecular dynamics simulations, ab initio calculations of $\beta$, $\gamma$, and $\delta$-peptides have been conducted primarily by the groups of Hofmann and Wu. It is beyond the scope of this article to describe the results obtained: remarkably, the folded structures of $\beta$-peptides can be derived from the structure of their monomeric building blocks, and the 12-helix of a $\beta$-peptide is more stable than the 14-helix, according to these calculations.

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