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Differential Impact of β and γ Residue Preorganization on $\alpha/\beta/\gamma$-Peptide Helix Stability in Water

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Supporting Information

ABSTRACT: Cyclic constraints have proven to be very effective for preorganizing β-amino acid residues and thereby stabilizing β- and α/β-peptide helices, but little is known about possible preorganization effects among γ residues. Here we assess and compare the impact of cyclic preorganization of β and γ residues in the context of a specific $\alpha/\beta/\gamma$-peptide helix. The results show that β residue preorganization is critical for helix stability but that γ residue preorganization is less important.

The surfaces of folded proteins often bear identifying information that is encoded in specific side chain arrangements and recognized by other proteins.[1] Oligomers that can mimic these molecular messages while displaying superiority to conventional peptides in other aspects (e.g., diminished susceptibility to enzymatic degradation, enhanced conformational stability) can be useful as antagonists of specific protein−protein associations or agonists of polypeptide-activated receptors.[2] We have previously shown that α/β-peptides generated from a prototype sequence by replacing 25−33% of the original α-amino acid residues with β-amino acid residues, at sites that are dispersed along the backbone, can adopt a conformation that is very similar to the α-helix and bind tightly to recognition surfaces that evolved to bind natural α-helices.[3] In some cases, affinity can be enhanced by use of cyclic β residues, presumably because the extra backbone bond introduced with each $\alpha\rightarrow\beta$ replacement raises the entropic cost of folding unless that bond is appropriately constrained.[3,4] The effective α-helix mimicry manifested by these α/β-peptides is somewhat surprising because they contain at least one additional backbone atom per helical turn relative to a true α-helix.

Recently, we proposed that the recognition surface of a natural α-helix could be mimicked by replacement of α heptads, which form two full helical turns, with $\alpha\gamma\alpha\alpha\beta\alpha$ hexads (Figure 1a).[5] The β and γ residues, collectively, contain the same number of backbone atoms as the three α residues they replace. This design strategy built upon pioneering studies of the folding of γ residue-containing oligomers by other groups.[6−8] The $\alpha\gamma\alpha\alpha\beta\alpha$ arrangement we developed allows the α residues to define one face of the helix. We showed via 2D NMR data that $\alpha/\beta/\gamma$-peptide 12-mer **1** in aqueous solution adopts a conformation resembling an α-helix, and that this conformation is considerably more stable than the true α-helix formed by analogous α-peptide 14-mer **2**.[5] We originally postulated that this high conformational stability in water arises from the backbone preorganization of both the β and γ residues provided by the five- and six-membered rings, respectively. Here we test this hypothesis and show it to be only partially correct.

To evaluate the contribution of β residue and γ residue preorganization independently, we prepared three $\alpha/\beta/\gamma$-peptide analogues of **1** (Figures 1c and 2a). In **3**, the γ residue constraints have been relaxed, because γ^4-hAla occurs in place of the two cyclohexane-based residues of **1**. In **4**, the β residue constraints have been relaxed, because β^3-hGln and β^3-hPhe replace the (*S*,*S*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) residues. In **5**, the β and γ residues constraints are relaxed simultaneously. Circular dichroism was used for an initial comparison of new $\alpha/\beta/\gamma$-peptides **3**−**5** with **1** in aqueous buffer (Figure 2). The strong minimum at 204 nm seen for **1** was previously assigned to the α-helix-like conformation because 2D NMR data reveal a network of

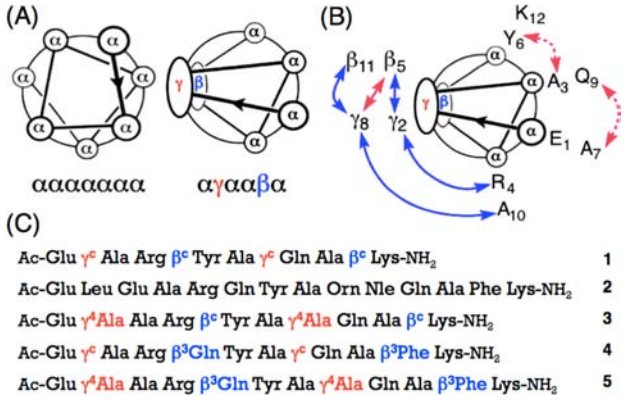

Figure 1. (a) Helical wheel diagrams for the $\alpha\alpha\alpha\alpha\alpha\alpha\alpha$ heptad and the $\alpha\gamma\alpha\alpha\beta\alpha$ hexad. (b) NOEs observed between nonadjacent residues for $\alpha/\beta/\gamma$-peptide **3** in CD_3OH (blue and pink, peptide concentration 6 mM) or in aqueous solution (blue, peptide concentration 2 mM) at 10 °C. Medium- and weak-intensity NOEs are shown as solid and dashed arrows, respectively, on the helical wheel diagram. (c) Sequences of $\alpha/\beta/\gamma$-peptides **1** and **3**−**5** and α-peptide **2**. Superscript "c" indicates a ring-constrained residue; the structures of β^c and γ^c are shown in Figure 2. All stereocenters are *S*.

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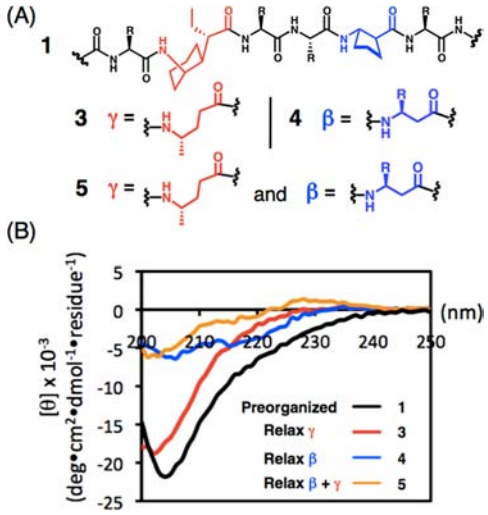

Figure 2. (a) Backbones of $\alpha\gamma\alpha\alpha\beta\alpha$ hexads from $\alpha/\beta/\gamma$-peptides **1** and **3**−**5**; β and γ residues are shown in blue and red, respectively. (b) Circular dichroism data for $\alpha/\beta/\gamma$-peptides **1** and **3**−**5** measured in PBS buffer, pH 7.5, at 20 °C. Peptide concentration is 0.1 mM for all spectra.

medium-range NOEs consistent with this conformation under the same conditions.[5] For $\alpha/\beta/\gamma$-peptide **5**, the very limited CD intensity suggests little or no helix formation, as we expected for a backbone lacking β or γ residue constraints. Similar behavior is observed for **4**, which indicates that the β residue constraint provided by the ring in ACPC is important for helical folding; this observation is consistent with previous demonstrations that incorporation of ACPC or similar cyclic β residues is crucial for helical folding of α/β-peptides in aqueous media.[3a,b,4,9] The CD data for **3**, however, were surprising: the strong minimum near 202 nm implies a significant population of the helical conformation, despite the absence of γ residue constraints.

NMR analysis was undertaken to probe the behavior of $\alpha/\beta/\gamma$-peptide **3** in more detail (Figure 1b and SI), because CD can provide only low-resolution insight on molecular structure. Previously we observed that fully preorganized $\alpha/\beta/\gamma$-peptide **1** displays numerous NOEs between protons from residues with i,i+2 and i,i+3 sequence relationships in both aqueous buffer and methanol; these medium-range NOEs were all consistent with an α-helix-like conformation.[5] For **3**, in which the cyclic γ residues have been replaced by unconstrained γ^4-hAla residues, a smaller set of i,i+2 and i,i+3 NOEs was observed in methanol relative to **1** in methanol, and an even smaller set of medium-range NOEs was detected for **3** in aqueous solution. However, the NOEs observed for **3** in water included both of the possible γ residue CγH(i)---β residue NH(i+3) NOEs and both γ residue CγH(i)---α residue NH(i+2) NOEs, which provides strong evidence for a significant population of the helical state. Thus, the 2D NMR data indicate that removing the γ residue constraints, to generate **3**, causes some diminution in helix population relative to fully preorganized **1**, but that the unconstrained γ^4-hAla residues nevertheless manifest a significant helical propensity, more so than unconstrained β^3 residues.

In order to gain higher-resolution insight regarding accommodation of the $\alpha\gamma\alpha\alpha\beta\alpha$ motif in an α-helix-like conformation, we designed oligomers intended to provide crystallographic data. GCN4-pLI is a 33-residue α-peptide derived from the dimerization domain of the yeast transcriptional regulator GCN4.[10] The pLI version forms a very stable tetrameric α-helix bundle, while the native sequence prefers lower oligomerization states.[11] Previously we found that many α/β-peptides derived from GCN4-pLI crystallize in four-helix bundle assemblies that are remarkably similar to the quaternary structure of GCN4-pLI itself.[9a,12] We therefore prepared a series of $\alpha/\beta/\gamma$-peptides based on this sequence, containing varying numbers of $\alpha\gamma\alpha\alpha\beta\alpha$ hexads in different locations; two of these oligomers provided high-quality crystals from aqueous solutions (Figure 3). The resulting structures show that an $\alpha\beta\alpha\alpha\gamma\alpha\alpha\beta\alpha$ unit can indeed adopt an α-helix-like conformation, whether the γ residue has the cyclic constraint (**6**) or not (**7**).

The H-bonding pattern among backbone amide groups within the $\alpha/\beta/\gamma$ segments of **6** and **7** is somewhat more complex than the H-bonding pattern of the canonical α-helix, which features i,i+4 C═O---H—N H-bonds (13-atom rings).[13] All backbone carbonyl groups in the $\alpha/\beta/\gamma$ segments of **6** and **7** form i,i+3 C═O---H—N H-bonds, which occur in 10-, 11- or 12-atom rings, depending upon the types of residues between the amide groups.[13] Such H-bonds are typically identified based on O---N distances, with 3.3 Å as a standard upper limit;[14] in the $\alpha/\beta/\gamma$ segments of **6** and **7**, a few i,i+3 O---N distances exceed this limit (3.5−3.9 Å). Some backbone carbonyls in **6** and **7** participate in bifurcated H-bonds, involving i,i+4

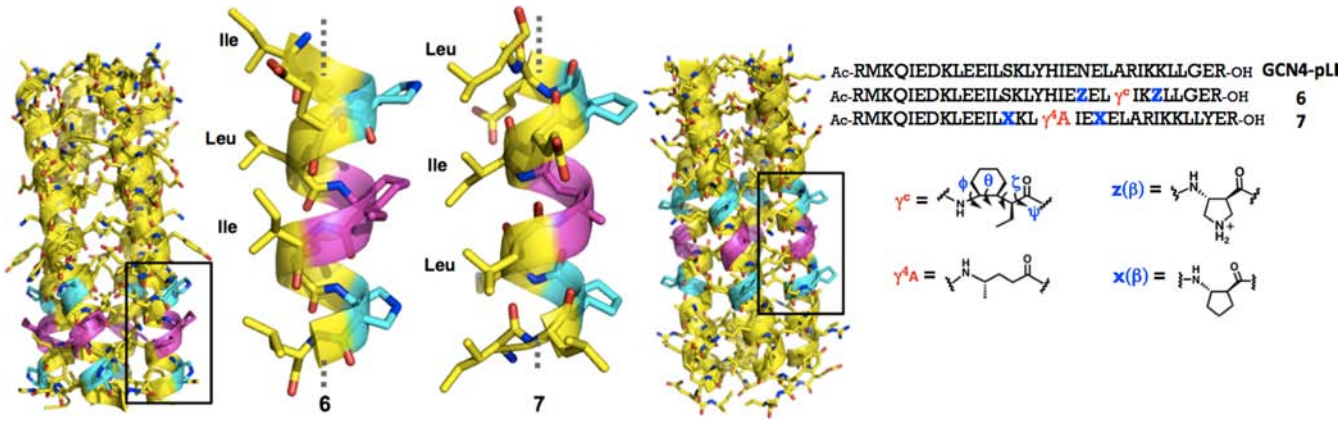

Figure 3. Four-helix bundles formed by **6** (left, PDB 4HJB) and **7** (right, PDB 4HJD), as determined via X-ray crystallography; β and γ residues are highlighted in purple. Also shown are sequences of GCN4-pLI and α + $\alpha/\beta/\gamma$-chimeric peptides **6** and **7** and structures of β and γ residues.

C═O---H—N interactions (14-atom rings) in addition to the *i*,*i*+3 interactions.

The crystallographic data show that NOEs attributed to the α-helix-like conformation are indeed associated with pairs of backbone hydrogen atoms that are brought into proximity in this folded state, including the two types of medium-range NOE mentioned above for **3** in aqueous solution (Figure 1b and SI). In **6** the distance between the γ residue CγH (position 24) and the β residue NH at position 27 is 3.0 Å, while in **7** the distance between the γ residue CγH (position 18) and the β residue NH at position 21 is 2.9 Å (H---H distance). In **6**, the distance between the γ residue CγH (position 24) and the α residue NH at position 26 is 2.5 Å, and the same separation is observed in **7** between the γ residue CγH (position 18) and the α residue NH at position 20. We note, however, that some comparable backbone *i*,*i*+2 or *i*,*i*+3 H---H distances in the crystal structures do not give rise to NOEs for **3** in aqueous solution,[13] perhaps because this conformation is only partially populated.

The crystal structures allow a detailed comparison of α/β/γ-peptide hexads with the α heptads they are meant to replace. In addition, the crystallographic data for **6** and **7** enable comparisons with several recently reported crystal structures of peptidic oligomers that contain γ residues and display C═O(*i*)---H—N(*i*+3) H-bonded helical secondary structures. Table 1 compares these standard helix parameters determined for the αβαaγα hexads in **6** and **7** with analogous parameters for the α-helix (as represented by GCN4-pLI; PDB 1GCL), for a canonical 3_{10}-helix,[14] for α-helix-mimetic α/β-peptides (PDB 3O43, 3F87, and 3C3H), and for various oligomers containing the cyclic γ residue we have employed[15] or γ^4 residues.[16,17] Although all of these helices have generally similar geometries, the αβαaγα unit does best at matching the α-helix radius. Table 2 compares the four backbone torsion angles of cyclic γ residues and γ^4 residues in various helical contexts. As might be expected, the cyclohexane-based γ residue generally prefers gauche torsion angles (~60°) about the backbone Cα−Cβ and Cβ−Cγ bonds.[15] The α-helix-like conformation observed in α/β/γ-peptide **6**, however, seems to require a somewhat smaller Cβ-Cγ torsion angle (45.9°) in the cyclohexyl ring. Crystal structures of helical α/γ-peptides containing γ^4 residues that have recently emerged from the Gopi[17] and Balaram[16] laboratories show that in these cases the Cβ-Cγ torsion angles are frequently in the range of 50°, but an even smaller torsion angle is observed for the corresponding backbone bond in the γ^4-hAla residue of **7** (38.9°).

Table 1. Helical Parameters for α/β/γ-, γ-, α/β-, and α-Peptides[a]

peptide	pattern	rise/turn, ρ (Å)	radius, r (Å)
α/β/γ-**6**, **7**	αβαaγα	5.7	2.3
$\gamma^{c\ b}$	γ	5.5	2.9
$\alpha/\gamma^{c\ c}$	αγ	6.1	2.5
$\alpha/\gamma^{4\ d}$	αγ	5.3	2.1
$\beta^c/\gamma^{c\ e}$	βγ	5.4	2.5
α/β^f	αaβ, αaβαaaβ	5.2−5.4	2.4−2.5
α^g	GCN4-pLI	5.4	2.3
	3_{10}-helix	5.8	2.0

[a]Helical parameters were calculated as previously described.[15a] [b]Ref 15a. [c]Ref 15c. [d]Refs 16 and 17. [e]Ref 15b. [f]PDB 3O43, 3F87, and 3C3H. [g]PDB 1GCL.

Table 2. Average Backbone Torsion Angles (degrees) for γ Residues in Various Peptides

peptide	ϕ	θ	ζ	ψ
$\alpha/\beta^c/\gamma^c$-**6**	−135.5	45.9	56.9	−118.6
$\alpha/\beta^c/\gamma^4$Ala-**7**	−132.9	38.9	64.7	−107.3
$\gamma^{c\ a}$	−154.5	60.2	59.5	−126.8
$\alpha/\gamma^{c\ b}$	143.7	-56.6	-52.5	111.7
α/γ^4Val ^{c}	−125.4	52.6	61.2	−118.7
α/γ^4Phe ^{d}	−125.8	50.8	61.9	−118.9
$\beta^c/\gamma^{c\ e}$	−134.7	60.1	59.8	−121.0

[a]Ref 15a. [b]Ref 15c. [c]Ref 16a. [d]Ref 17a. [e]Ref 15b.

The results reported here suggest that β^3- and γ^4-amino acid residues, which bear a single side chain and are therefore homologous to α-amino acid residues, differ in their intrinsic tendencies to adopt helical secondary structure, with γ^4 residues displaying the higher propensity. Previous work has shown that short peptidic oligomers containing β^3 residues or γ^4 residues, either exclusively or in combination with α residues, can adopt helical conformations in the crystalline state or in organic solvents.[6−8,16−19] Aqueous solution represents a more challenging environment in terms of peptidic secondary structure formation,[20] and we have used comparisons in water to show that cyclic β residues display substantially higher helical propensities than do β^3 residues.[4,9,21] Against this background, the relatively large helical propensity of γ^4 residues revealed by our comparisons among α/β/γ-peptides **1** and **3**−**5** in aqueous solution is unexpected. The helical propensity of the cyclic γ residue in **1** appears to be somewhat higher than that of γ^4-hAla, but the relatively small θ backbone torsion angles documented crystallographically for the γ residues in **6** and **7** raise the possibility that the cyclohexyl-constrained γ backbone may not be ideally suited to the α-helix-mimetic conformation. We are currently exploring the hypothesis that alternative types of γ residue constraint could lead to improved stability of the α-helix-like conformation available to α/β/γ-peptides.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, including polypeptide characterization, CD and NMR spectra, and X-ray crystallography. This material is available free of charge via the Internet at http://pubs.acs.org. Model coordinates and structure factors have been deposited in the Protein Data Bank as entries 4HJB (α/β/γ-peptide **6**) and 4HJD (α/β/γ-peptide **7**).

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Notes

The authors declare no competing financial interest.

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