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Structural Mimicry of the α-Helix in Aqueous Solution with an Isoatomic α/β/γ-Peptide Backbone

Tomohisa Sawada and Samuel H. Gellman*

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, United States

Supporting Information

ABSTRACT: Artificial mimicry of α-helices offers a basis for development of protein−protein interaction antagonists. Here we report a new type of unnatural peptidic backbone, containing α-, β-, and γ-amino acid residues in an αγααβα repeat pattern, for this purpose. This unnatural hexad has the same number of backbone atoms as a heptad of α residues. Two-dimensional NMR data clearly establish the formation of an α-helix-like conformation in aqueous solution. The helix formed by our 12-mer α/β/γ-peptide is considerably more stable than the α-helix formed by an analogous 14-mer α-peptide, presumably because of the preorganized β and γ residues employed.

Unnatural oligomers that can reproduce the three-dimensional (3D) shapes and side-chain projection patterns characteristic of natural polypeptides offer a basis for rational development of protein−protein interaction antagonists.[1] Considerable effort has been devoted to mimicry of individual helices, which frequently mediate information transfer in biological systems.[2] Many alternatives to the α-helical scaffold have been examined, including oligomers based on unnatural peptidic backbones (e.g., β-peptides[3]) and entirely nonpeptidic oligomers (e.g., oligophenyls[4]). Evaluation of these new designs has typically focused on α-helices two to four turns in length (e.g., mimicry of the p53 N-terminal domain in binding to hDM2,[5] or mimicry of a BH3 domain in binding to Bcl-2-family proteins[6]); however, epitopes of this size can be effectively mimicked by small molecules.[7] We have recently shown that the recognition properties of a 10-turn α-helix, the CHR domain of HIV protein gp41, can be recapitulated with α/β-peptide oligomers in which two of every seven among the original α-amino acid residues are replaced by analogous β-amino acid residues (Figure 1).[8] Oligomers with the ααβαααβ heptad repeat can adopt a helical secondary structure in which each turn contains one extra backbone carbon relative to an α-helix. This backbone alteration confers significant resistance to proteolytic degradation while allowing reasonably good mimicry of the side-chain projection pattern along one side of the helix. However, this mimicry is not perfect: the resulting α/β-peptides have lower affinity for the target surface than does the original α-peptide.

Here we introduce a new foldamer design containing α-, β-, and γ-amino acid residues in an αγααβα hexad repeat pattern, which is intended to mimic an αααααααα heptad without additional backbone atoms (Figure 1a). Both the α/β/γ hexad and the all-α heptad correspond to two helical turns.[9] The helical wheel juxtaposition in Figure 1b shows that the αγααβα repeat potentially allows a direct correspondence between the α residues of this hexad and four of the α residues in a standard peptide heptad. In the parlance of coiled coil-forming sequences, the common α residues could correspond to positions *a*, *d*, *e*. and *g* of an all-α heptad repeat, which define a large and continuous surface that runs along one side of the helix.[10]

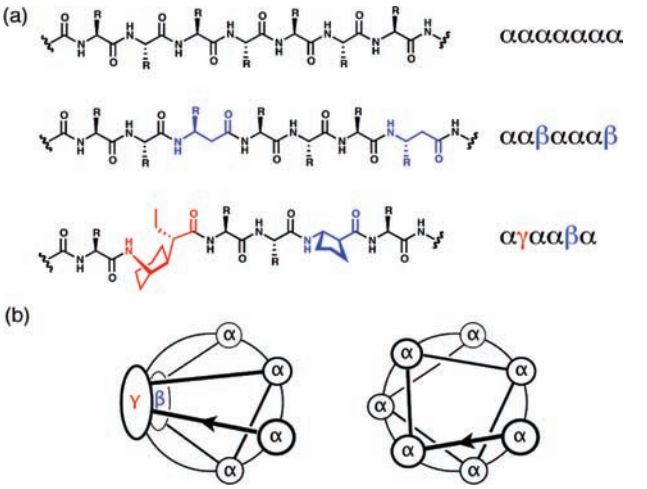

Figure 1. (a) Three backbones that correspond to approximately two helical turns: αααααααα, ααβαααβ, αγααβα; (b) helical wheels for the αγααβα hexad and the αααααααα heptad.

In order to test the α-helix mimicry hypothesis outlined above, we prepared α/β/γ-peptide 12-mer **1** via solid-phase methods. All of the α residues in **1** are derived from proteinogenic amino acids. The helix-forming properties of β-amino acid residues[11] and γ-amino acid residues[12] have been widely studied, and based on these precedents we employed conformationally preorganized β and γ residues that seemed likely to maximize the propensity of **1** to adopt an α-helix-like conformation (Figure 2). The β residues are derived from (*S*,*S*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC), which promotes helical α/β-peptide conformations that strongly resemble the α-helix,[13] including the gp41 CHR-mimetic oligomers mentioned above.[8] The γ residues in **1** are derived from α-ethyl-*cis*-2-aminocyclohexaneacetic acid (EtACHA; **3**), which has recently been shown to participate in helical conformations.[14]

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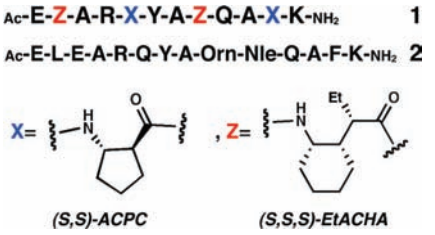
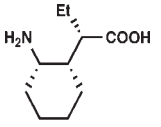


Figure 2. Sequences for α/β/γ-peptide **1** and α-peptide **2**. Proteinogenic α residues are indicated with the conventional single-letter code; for nonproteinogenic α residues, Orn = ornithine and Nle = norleucine; the β and γ residue structures are shown.



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The α- and β-amino acid residues of **1** were incorporated via microwave-assisted solid-phase synthesis employing common reagents.[15] Coupling of Fmoc-EtACHA to the growing polypeptide proved to be challenging, because of intrinsically limited reactivity (perhaps resulting from steric hindrance) and a tendency toward epimerization, presumably at the α-position of the γ-amino acid backbone. The best method for addition of Fmoc-EtACHA proved to be the use of 4 equiv of HOAt and EDCI in DMF for 6 h at room temperature (no microwave irradiation).

α/β/γ-Peptide **1** is highly water-soluble and displayed sufficient ^{1}H NMR resonance dispersion to enable 2D NMR analysis in aqueous solution (8 mM **1**, 9:1 H_2O/D_2O, 100 mM acetate buffer, pH 3.8, 10 °C). Resonances from backbone amides and side chains (6.5−9.0 ppm) were monitored between 0.02 and 14 mM **1**; no concentration-dependent variations in chemical shift were observed, which suggests that **1** does not self-associate under these conditions. Numerous NOEs were detected between protons on sequentially nonadjacent residues ($i \rightarrow i+2$ or $i \rightarrow i+3$; Figure 3a,b), all of which could be accommodated by a single helical conformation. The expected alignment of the β and γ residues along one side of this helix was indicated by characteristic NOE patterns: EtACHA $C_\gamma H$ (i) → ACPC NH ($i+3$) and ACPC $C_\beta H$ (i) → EtACHA NH ($i+3$). These NOEs between β and γ residues were detected even at 50 °C, which implies that the helical conformation of α/β/γ-peptide **1** is quite robust. Further evidence of α/β/γ-peptide helix stability was obtained from H/D exchange studies. When **1** was dissolved in D_2O at room temperature, some NH resonances disappeared completely within 4 min (the backbone NH of Glu 1 and the side-chain NH resonances of Lys, Arg, and Gln), but most of the backbone NH resonances could be detected even after 1 h. α-Peptide 14-mer **2** was prepared for direct comparison with **1**, but the ^{1}H NMR spectrum of **2** displayed poor dispersion, which precluded resonance assignments and 2D NMR analysis.

NOE-restrained molecular dynamics calculations for α/β/γ-peptide **1** in aqueous buffer were carried out with the CNS program.[16] Only $i \rightarrow i+1$, $i \rightarrow i+2$ and $i \rightarrow i+3$ NOEs were used for these calculations. Good overlap among backbone atoms was observed for the 10 best among 1000 calculated structures (rmsd = 0.86 ± 0.24 Å; Figure 3c). Figure 3d shows superimposition of the average of these 10 helical structures for **1** on a canonical α-helix.[17] Of particular interest is the overlap between the six central α residues of **1** and the corresponding residues of the α-helix (the terminal α residues of **1** were excluded from this comparison because of expected "fraying" effects). For overlay of the two sets of six α-carbons, rmsd = 0.98 ± 0.50 Å.[18] Inspection

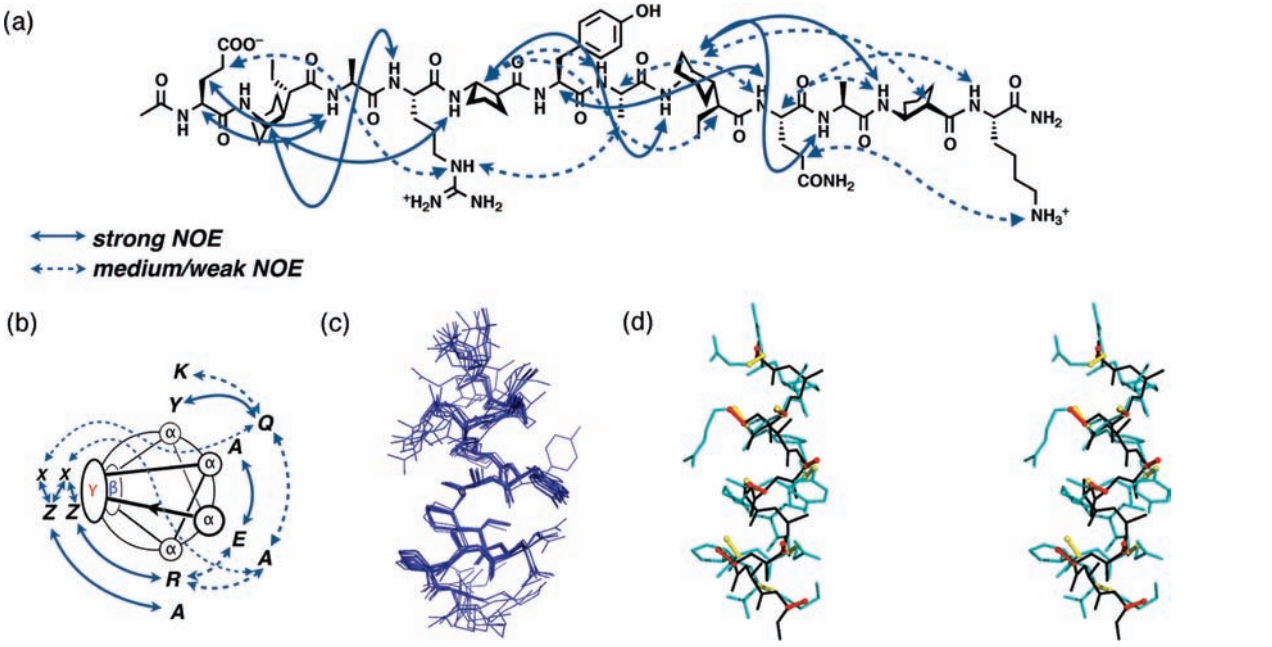

Figure 3. (a) Structure of α/β/γ-peptide **1** with NOEs observed in aqueous buffer between nonadjacent residues indicated by curved arrows (8 mM peptide in 100 mM acetate buffer, pH 3.8); (b) NOEs indicated on the α/β/γ-peptide helix wheel; (c) overlay of the 10 best conformations generated via NOE-restrained dynamics (see text for details); (d) stereoview of the average of the 10 structures from the NOE-strained dynamics simulations (blue backbone) overlaid on a canonical α-helix (black backbone).

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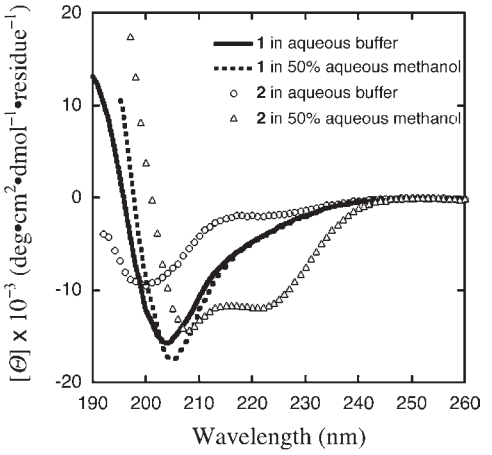

Figure 4. Circular dichroism data for α/β/γ-peptide **1** and α-peptide **2** in 10 mM aqueous acetate buffer, pH 3.8, or 50 vol % aqueous methanol (one volume of methanol added to the aqueous buffer). The concentration of peptides is 0.2 mM.

of the stereoview in Figure 3d shows that the C_α-to-side chain vectors for these two sets of α residues are largely coincident.

We turned to circular dichroism (CD) to compare α/β/γ-peptide **1** and α-peptide **2**, because the latter could not be studied via 2D NMR. Figure 4 shows far-UV CD data for **1** and **2** in aqueous buffer and in 50 vol % aqueous methanol. In aqueous solution, the CD signature of α-peptide **2** suggests a largely unfolded state. In the presence of 50 vol % methanol **2** manifests a typical α-helical CD signature, with minima at ~208 and ~222 nm; the pronounced helix-promoting impact of the organic cosolvent is typical for short α-peptides. The behavior of α/β/γ-peptide **1** is quite different in that the CD signature is not strongly affected by changing the solvent. In both cases a single strong minimum is observed (~204 nm in aqueous buffer, ~205 nm in aqueous methanol), with only a minor intensity difference between solvents. Since NMR analysis indicates substantial population of an α-helix-like conformation by **1** in aqueous buffer, we assign the CD signature observed for **1** to this conformation. A similar far-UV CD signature has been established for α-helix-like conformations adopted by α/β-peptides;[13] it is unclear why these heterogeneous peptidic backbones fail to manifest a second minimum in the helical state. The fact that similar CD signatures are observed for **1** in aqueous buffer and in 50% aqueous methanol suggests that the α-helix-like conformation of this α/β/γ-peptide is highly populated even in a fully aqueous environment, an extent of folding that would be unusual for a linear α-peptide of comparable length. Further evidence of the high stability of the α/β/γ-peptide **1** helix is provided by variable-temperature CD: the minimum at ~204 nm becomes less intense on heating from 10 to 90 °C, as expected from thermally induced unfolding, but at 90 °C this minimum retains ~70% of the intensity observed at 10 °C.[19]

We have introduced a new type of heterogeneous peptidic foldamer and shown via 2D NMR that this system supports a helical conformation in aqueous solution. The αγααβα hexad pattern we designed leads to mimicry of the side-chain display along one side of an α-helix, despite the presence of nonproteinogenic backbone components. Direct comparison with a conventional α-peptide reveals that the α/β/γ-peptide has a much stronger folding propensity, a feature that should facilitate the development of biologically active examples. The high stability of the new α/β/γ-peptide helix is attributed to the use of appropriately preorganized β and γ residues. Additional studies will be required to determine whether other αααα+β+γ hexads with different subunit ordering give rise to stable helical conformations. To our knowledge, this study represents the first example of high-resolution structural analysis of a γ-amino acid-containing foldamer in aqueous solution. It will now be important to determine whether the α/β/γ-peptide design introduced here can support functional mimicry of biological α-helices.

■ ASSOCIATED CONTENT

Supporting Information. All experimental procedures and data; complete ref 7b. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
gellman@chem.wisc.edu

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■ REFERENCES

(1) (a) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252–262. (b) Hecht, S., Huc, I., Eds. *Foldamers: Strucutre, Properties, and Applications*; Wiley-VCH: Weinheim, Germany, 2007.

(2) (a) Jochim, A. L.; Arora, P. S. *ACS Chem. Biol.* **2010**, *5*, 919–923. (b) Jochim, A. L.; Arora, P. S. *Mol. BioSyst.* **2009**, *5*, 924–926.

(3) (a) Werder, M.; Hauser, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774–1783. (b) Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2004**, *126*, 9468–9469. (c) English, E. P.; Chumanov, R. S.; Gellman, S. H.; Compton, T. *J. Biol. Chem.* **2006**, *281*, 2661–2667.

(4) (a) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382–5383. (b) Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D. *J. Am. Chem. Soc.* **2002**, *124*, 11838–11839. (c) Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326–334. (d) Hu, X.; Sun, J.; Wang, H.-G.; Manetsch, R. *J. Am. Chem. Soc.* **2008**, *130*, 13820–13821. (e) Shaginian, A.; Whitby, L. R.; Hong, S.; Hwang, I.; Farooqi, B.; Searcey, M.; Chen, J.; Vogt, P. K.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 5564–5572. (f) Marimganti, S.; Cheemala, M. N.; Ahn, J.-M. *Org. Lett.* **2009**, *11*, 4418–4421. (g) Plante, J. P.; Burnley, T.; Malkova, B.; Webb, M. E.; Warriner, S. L.; Edwards, T. A.; Wilson, A. J. *Chem. Commun.* **2009**, 5091–5093. (h) Campbell, F.; Plante, J. P.; Edwards, T. A.; Warriner, S. L.; Wilson, A. J. *Org. Biomol. Chem.* **2010**, *8*, 2344–2351.

(5) Murray, J. K.; Gellman, S. H. *Biopolymers* **2007**, *88*, 657–686.

(6) Youle, R. J.; Strasser, A. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 47–59.

(7) (a) Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844–848. (b) Oltersdorf, T.; et al. *Nature* **2005**, *435*, 677–681. (c) Lessene, G.; Czabotar, P. E.; Colman, P. M. *Nat. Rev. Drug Discov.* **2008**, *7*, 989–1000.

(8) Horne, W. S.; Johnson, L. M.; Ketas, T. J.; Klasse, P. J.; Lu, M.; Moore, J. P.; Gellman, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 14751–14756.

(9) (a) For crystal structures showing that a β-alanine-γ-aminobutyric acid dipeptide segment can replace a tri-α-peptide segment in short helical oligomers, see: Karle, I. L.; Pramanik, A.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. *J. Am. Chem. Soc.* **1997**, *119*, 9087–9095. (b) For related work, see: Araghi, R. R.; Jäckel, C.; Cölfen, H.; Salwiczek, M.; Völkel, A.; Wagner, S. C.; Wieczorek, S.; Baldauf, C.; Koksch, B. *ChemBioChem* **2010**, *11*, 335–339. Araghi, R. R.; Koksch, B. *Chem. Commun.* **2011**, 3544–3546.

(10) (a) Woolfson, D. N. *Adv. Protein Chem.* **2005**, *70*, 79–112. (b) Grigoryan, G.; Keating, A. E. *Curr. Opin. Struct. Biol.* **2008**, *18*, 477–483.

(11) (a) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (b) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366–1375.

(12) (a) Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569–8570. (b) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983–1002. (c) Seebach, D.; Hook, D. F.; Glättli, A. *Biopolymers* **2006**, *84*, 23–37.

(13) (a) Horne, W. S.; Price, J. L.; Keck, J. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180. (b) Horne, W. S.; Price, J. L.; Gellman, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9151–9156. (c) Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2010**, *132*, 12378–12387.

(14) (a) Guo, L.; Chi, Y.; Almeida, A. M.; Guzei, I. A.; Parker, B. K.; Gellman, S. H. *J. Am. Chem. Soc.* **2009**, *131*, 16018–16020. (b) Guo, L.; Almeida, A. M.; Zhang, W.; Reidenbach, A. G.; Choi, S. H.; Guzei, I. A.; Gellman, S. H. *J. Am. Chem. Soc.* **2010**, *132*, 7868–7869. (c) For related γ-amino acid building blocks, see: Nodes, W. J.; Nutt, D. R.; Chippindale, A. M.; Cobb, A. J. A. *J. Am. Chem. Soc.* **2009**, *131*, 16016–16017.

(15) (a) Murray, J. K.; Gellman, S. H. *Org. Lett.* **2005**, *7*, 1517–1520. (b) Murray, J. K.; Gellman, S. H. *Nat. Protoc.* **2007**, *2*, 624–631.

(16) (a) Brünger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. *Acta Crystallogr.* **1998**, *D54*, 905–921. (b) Brunger, A. T. *Nat. Protoc.* **2007**, *2*, 2728–2733.

(17) The canonical α-helix structure was built by molecular modeling using *HyperChem*, 8.0.6.

(18) Superimposition of two helices was carried out by using the *pair fitting* function in *PyMol*, 1.3.

(19) See Supporting Information.