

## Helix Macrodipole Control of $\beta^3$ -Peptide 14-Helix Stability in Water

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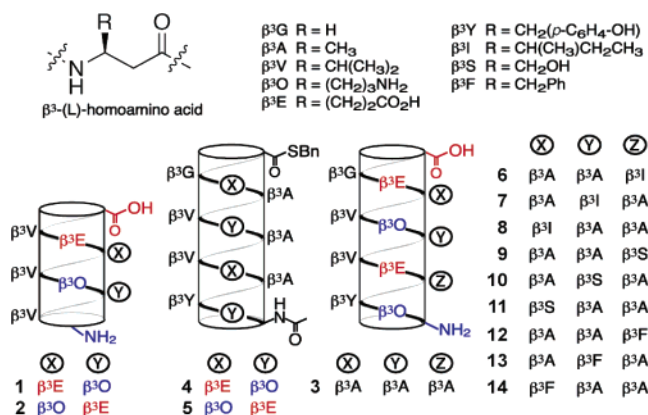
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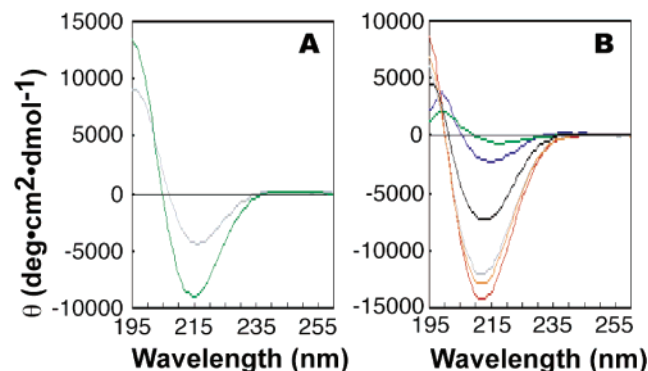
$\beta$ -Peptides have attracted considerable attention by virtue of their ability to populate helical secondary structures, even in the absence of stabilizing tertiary interactions.<sup>1–3</sup> The left-handed 14-helix<sup>4</sup> formed by  $\beta^3$ -L-amino acid oligomers is characterized by H-bonds between the backbone carbonyl oxygen of residues  $i$  and the amide proton of residues  $i - 2$ . The 14-helix possesses three distinct helical faces, with side chains aligned at 120° intervals when viewed down the helix axis.<sup>2</sup> Until recently,  $\beta^3$ -peptide 14-helices had been observed only in methanol, not in water. Recently, Seebach<sup>5,6</sup> and DeGrado<sup>7</sup> reported  $\beta^3$ -peptides with oppositely charged residues positioned to form two or four stabilizing intramolecular salt bridges, respectively, between residues at the  $i$  and  $i + 3$  positions along two of three helical faces. Indeed, these studies produced the first known  $\beta^3$ -peptides with significant 14-helix structure in water and nicely complement work of Gellman on water-stable, helical oligomers of (cyclo) $\beta^2$ - $\beta^3$ -amino acids.<sup>8</sup> More recently, DeGrado reported a disulfide-bridged homodimer of 14-helices.<sup>9</sup> Here we show that stabilization of the 14-helix macrodipole alleviates the requirement for multiple  $\beta^3$ -Glu/ $\beta^3$ -Lys or  $\beta^3$ -Glu/ $\beta^3$ -Orn salt bridges on two of three helical faces. We apply this principle to design stable, monomeric  $\beta^3$ -peptides with salt bridges on only one helical face and significant side chain heterogeneity on the two others.

A primary consideration in de novo  $\alpha$ -helix design is the  $\alpha$ -helix macrodipole, which places a partial positive charge at the N-terminus and a partial negative charge at the C-terminus.<sup>10–13</sup>  $\alpha$ -Helix stability is enhanced significantly by negatively charged side chains near the N-terminus, by positively charged side chains near the C-terminus,<sup>10</sup> and by neutralizing charge associated with free N- and C-termini.<sup>11</sup> Because of its unique H-bonding pattern, the 14-helix macrodipole should be oriented in the opposite direction, with partial positive charge at the C-terminus and partial negative charge at the N-terminus.<sup>2</sup> This orientation predicts that 14-helix stability should be enhanced by positively charged side chains near the N-terminus and negatively charged side chains near the C-terminus, and by preserving the charge associated with free N- and C-termini. These design principles, the reverse of well-established rules for stabilizing isolated  $\alpha$ -helices, seemed an ideal starting point from which to investigate 14-helix stabilization.

To probe the effect of the helix macrodipole on 14-helix stability in water, we synthesized heptamers **1** and the previously synthesized **2**<sup>5,6</sup> (Figure 1). **1** and **2** differ only by the orientation of a single  $\beta^3$ -Glu/ $\beta^3$ -Orn salt bridge. This salt bridge is positioned to interact favorably with the 14-helix macrodipole in **1** but not in **2**. The CD signature of a  $\beta^3$ -peptide 14-helix is characterized by ellipticity maxima and minima near 195–198 nm and 213–215 nm, respectively.<sup>2</sup> The 14-helix content of **1** and **2** in aqueous buffer (pH 7, 25 °C) was determined by circular dichroism (CD) spectroscopy at 214 nm ( $\theta_{214}$ ). The mean residue ellipticity of peptide **1** was



**Figure 1.** Helical net diagrams of  $\beta^3$ -peptides described herein. Amino acid codes refer to  $\beta^3$ -(L)-homoamino acids as indicated above. Acidic residues and C-termini are in red; basic residues and N-termini are in blue.



**Figure 2.** CD spectra illustrating the mean residue ellipticity of (A) **1** (green) and **2** (gray) and (B) **3** (black), **4** (blue), **5** (green), **6** (orange), **7** (red), and **8** (gray). All spectra were acquired at room temperature in PBC buffer (1 mM Na phosphate, 1 mM Na borate, 1 mM Na citrate (pH 7)); [peptide] = 200  $\mu$ M.

twice that of peptide **2** ( $\theta_{214} = -8,930$  and  $-4,370$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>, respectively) (Figure 2A). The CD spectra of both peptides were independent of concentration between 25 and 200  $\mu$ M. This dramatic difference in structure emphasizes the significant effect of salt bridge orientation on 14-helix stability in short  $\beta^3$ -peptides.

Next we asked whether electrostatic interactions with the 14-helix macrodipole would stabilize longer, mixed sequence  $\beta^3$ -peptides. In particular, we were interested in  $\beta^3$ -peptides with salt bridges on only *one* 14-helix face. Undecapeptide **3**, with two correctly oriented  $\beta^3$ -Glu/ $\beta^3$ -Orn salt bridges on one face, three  $\beta^3$ -Ala residues on the second face, and either  $\beta^3$ -Val,  $\beta^3$ -Tyr, or a glycine equivalent ( $\beta$ -alanine) on the third face (Figure 1) displayed significant, concentration-independent 14-helix structure at pH 7 and 25 °C ( $\theta_{214} = -7577$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>) (Figure 2). The presence of 14-helix structure in **3** was supported further by high-resolution NMR measurements.<sup>14</sup> Peptide **3** is monomeric under these condi-

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tions as determined by sedimentation equilibrium,<sup>14</sup> indicating that the helix content of this molecule is not due to interhelical interactions. In contrast to the 14-helicity of **3**, undecapeptides **4** and **5** were significantly (**4**) or completely (**5**) unstructured ( $\theta_{214} = -2500$  and  $-661$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>, respectively). As for short  $\alpha$ -helices, 14-helix stabilities are influenced significantly by modifications that neutralize the positive and negative charge of unmodified N- and C-termini, respectively;<sup>11</sup> these rules are simply reversed in the context of a  $\beta^3$ -peptide. These comparisons demonstrate that the presence of free (that is, uncapped) N- and C-termini and the locations of charged residues are critical parameters in the design of 14-helices that are well folded in water.

We also explored the extent to which the 14-helix structure of **3** could be modulated by other  $\beta^3$ -amino acids. We chose three  $\beta^3$ -amino acids with diverse side-chain functionality ( $\beta^3$ -Ile,  $\beta^3$ -Ser, and  $\beta^3$ -Phe) and substituted each one singularly in place of  $\beta^3$ -Ala at positions 3, 6, or 9 of **3** (Figure 1). The 14-helix content of each peptide, as determined at pH 7 and 25 °C by the magnitude of  $\theta_{214}$ , spanned a wide range, with values from  $-4833$  to  $-14\,250$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>. Interestingly, the branched residue  $\beta^3$ -Ile was stabilizing relative to  $\beta^3$ -Ala at all positions; undecapeptides **6**, **7**, and **8** all exhibited significantly greater 14-helical structure than that of **3**, with  $\theta_{214}$  values of  $-11\,970$ ,  $-14\,250$ , and  $-12\,920$ , respectively (Figure 2B).  $\beta^3$ -Ser was destabilizing relative to  $\beta^3$ -Ala at position 3, stabilizing at position 6, and neutral at position 9, with  $\theta_{214}$  values for **9**, **10**, and **11** of  $-5890$ ,  $-11\,020$ , and  $-7790$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>, respectively. Like  $\beta^3$ -Ser,  $\beta^3$ -Phe was destabilizing at position 3 but neutral at positions 6 and 9, with  $\theta_{214}$  values for **12**, **13**, and **14** of  $-5890$ ,  $-8360$ , and  $-9310$  deg·cm<sup>2</sup>·dmol<sup>-1</sup>.<sup>14</sup> Further work will be necessary to deconvolute the structural and electronic factors contributing to the position-dependent effects of  $\beta^3$ -Ser and  $\beta^3$ -Phe side chains. These data emphasize the unrecognized impact of  $\gamma$ -branched residues on the stability of short  $\beta^3$ -peptides in water. Whereas  $\alpha$ -Ala is the most stabilizing amino acid in  $\alpha$ -helices,<sup>15</sup> the rules for 14-helix formation perhaps once again diverge from those of  $\alpha$ -helix formation since  $\gamma$ -branched residues may be preferred within 14-helices.

Previous studies have shown that amphiphilic  $\beta^3$ -peptides can form aggregates,<sup>16</sup> and substitution of isoleucine for leucine in  $\alpha$ -peptide coiled-coils alters helix bundle stoichiometry.<sup>17,18</sup> Peptides **6–14** are amphiphilic, and we observed a strong increase in 14-helicity upon  $\beta^3$ -Ala/ $\beta^3$ -Ile substitutions. It thus seemed plausible that interhelical interactions could be responsible for a portion of the 14-helix stability in certain cases. However, CD analysis revealed that the  $\theta_{214}$  signal of peptides **6–14** remained constant between 50 and 200  $\mu$ M,<sup>14</sup> and peptide **7**, like **3**, was monomeric at concentrations between 10 and 200  $\mu$ M as determined by sedimentation equilibrium.<sup>14</sup>

We have demonstrated that favorable interactions with the 14-helix macrodipole stabilize the 14-helix in water, alleviating the need for multiple salt bridges on two of three helical faces. Moreover, our results suggest that 14-helix stability withstands—and in some cases is enhanced by—a variety of chemically diverse  $\beta^3$ -amino acids. The most structured molecules we report are highly heterogeneous at the primary sequence level; peptides **6–14** contain

seven different  $\beta^3$ -amino acids within an 11 residue sequence. We anticipate that molecules **6–14** will facilitate the design of well-folded 14-helices that explore the interactions between  $\beta^3$ -peptides and biological macromolecules *in vitro* and *in vivo*.

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**Note Added In Proof.** Gellman and co-workers recently reported the stabilization of 14-helix structure by branched side chains in methanol, supporting our observation that  $\beta^3$ -Ile residues stabilize 14-helix structure in water: Raguse, T. L.; Lai, J. R.; Gellman, S. H. *Helv. Chim. Acta.* **2002**, 4154–4164.

**Supporting Information Available:** Experimental procedures, CD data for **3**, **6–11**, and **13–14**, NMR data for **3**, and sedimentation equilibrium data for **3** and **7** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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