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Relationship between Salt-Bridge Identity and 14-Helix Stability of β^3-Peptides in Aqueous Buffer

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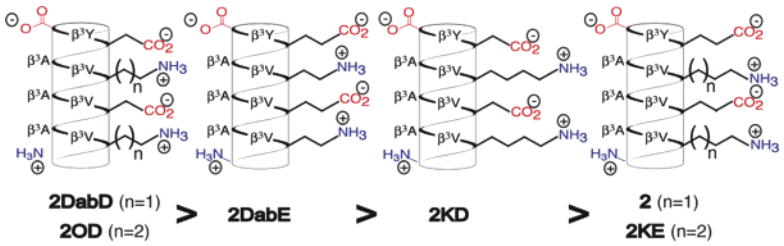
ABSTRACT



We report a systematic analysis of the relationship between salt bridge composition and 14-helix structure within a family of model β-peptides in aqueous buffer. We find an inverse relationship between side-chain length and the extent of 14-helix structure as judged by CD. Introduction of a stabilizing salt bridge pair within a previously reported β-peptide ligand for hDM2 led to changes in structure that were detectable by NMR.

β-Peptides adopt a diverse array of secondary structures including a variety of helices, pleated-sheets, and tubes.[1−5] β-Peptides composed of β^3-amino acids often assemble into a unique helical form called the 14-helix that is characterized by a defined set of long-range hydrogen bonds and three distinct faces.[6] Although the majority of published work in the β^3-peptide field describes molecules folded in organic solvents,[5] in 2001 Seebach and DeGrado reported independently that β^3-peptides containing an alternating pattern of oppositely charged side chains at positions i and $i+3$ on two of three helical faces displayed moderate levels of 14-helix structure in aqueous buffer.[7−9] We subsequently demonstrated that the requirement for stabilizing salt bridges on two helical faces could be lifted by introducing side chains that stabilize the 14-helix macrodipole.[10,11] We established that β-peptide 14-helices stabilized in this way tolerate a vast

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array of proteinogenic side chains[10] and can be modified to generate molecules that bind with moderate affinity to the proteins hDM2[12] and HIV gp41.[13] Here, we describe experiments that identify the charged side chain partners that best stabilize the 14-helix as judged by circular dichroism (CD) spectroscopy. We demonstrate that β-peptides containing β^3-HAspartate and either (*S*)-2,4-Homodiaminobutyric acid (β^3-HDab, Figure 1) or β^3-HOrnithine along one helical face

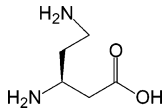

Figure 1. (*S*)-2,4-Homodiaminobutyric acid (β^3-HDab).

provide the greatest level of 14-helix stabilization. Introduction of the 14-helix stabilizing β^3-HOrnithine/β^3-HAspartate salt bridge into the previously reported hDM2 ligand, β**53-1**, led to changes in 14-helix structure that could be detected by 2D-NMR spectroscopy.

We studied a set of six β-dodecamers to evaluate the effect of side-chain identity on 14-helix stability in water (Figure 2). All six molecules are derivatives of the previously

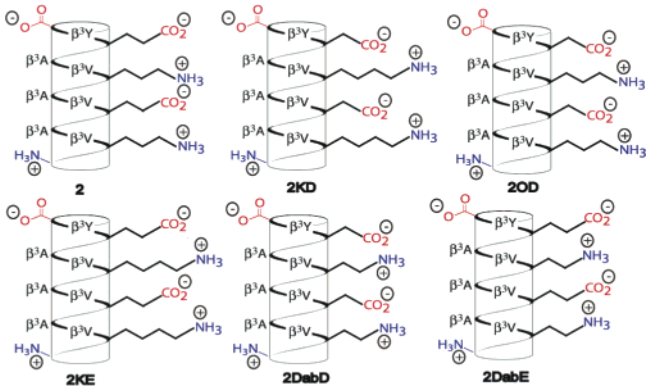

Figure 2. Helical net diagrams of β^3-peptides studied herein.

reported β-peptide **2**,[10,11] in which β^3-HOrnithine (O) or β^3-HDab (Dab) replace β^3-HLysine (K) and β^3-HAspartate (D) replaces β^3-HGlutamate (E). All six β-dodecamers contain helix-promoting[10,11,14] aliphatic β^3-HValine residues at positions 2, 5, 8, and 11 along one face of the putative 14-helix and β^3-HAlanine residues at positions 3, 6, and 9 along a second face. Each molecule also contained a β^3-HTyrosine residue to simplify spectrophotometric concentration determination. The β^3-peptides were synthesized using standard Fmoc solid-phase methods,[15−17] purified using reverse phase HPLC, and their sequences confirmed using MALDI-TOF mass spectrometry.[18] All six molecules are monomeric at 80 μM as determined by analytical ultracentrifugation.

We used circular dichroism (CD) spectroscopy to monitor the extent of 14-helix structure in each β-peptide at 25 °C. While CD data on β-peptides must be interpreted carefully,[19] it is reasonable to assume that, for β^3-peptides in particular, changes in intensity of the 14-helical signature correlate to changes in 14-helical population.[3,12,19,20] The CD spectra of all six molecules are consistent with a 14-helix structure, with ellipticity minima between 211 and 214 nm, ellipticity maxima between 195 and 198 nm, and a crossover between negative and positive ellipticity between 200 and 202 nm (Figure 3a, Table 1).[1,5,6] The maximal values of negative

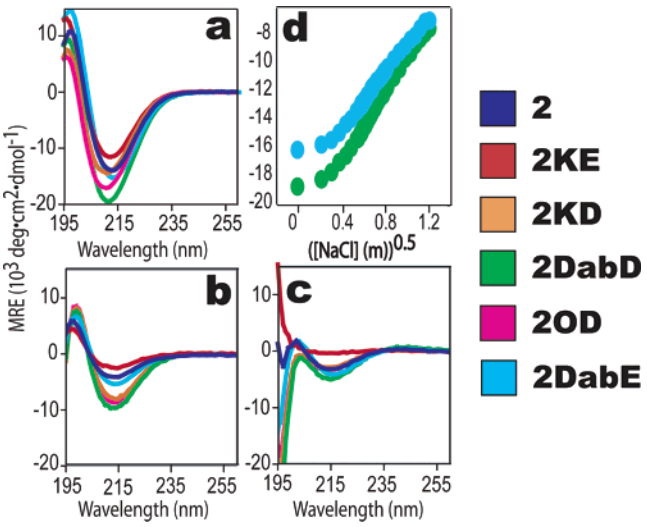

Figure 3. CD spectra of **2**, **2KE**, **2OD**, **2KD**, **2DabD**, **2DabE** at 80 μM (PBC buffer) at: (a) pH 7 (b) pH 2 (c) pH 12. (d) MRE_{214} of 100 μM **2DabE** and **2DabD** *vs* $[NaCl]^{0.5}$.

ellipticity range from −11500 deg·cm^2·dmol^{-1} to −19500 deg·cm^2·dmol^{-1}, representing a change of greater than 40%. The CD data suggest that the level of 14-helix structure among the six molecules is, from greatest to least: **2DabD** > **2OD** > **2DabE** > **2KD** > **2** > **2KE**.

Table 1. Minimum MRE (deg·cm^2·dmol^{-1}·residue^{-1}) for β-Peptides Studied Herein at 80 μM and 25 °C

	$-\theta_{min}$ (pH 7)	$-\theta_{min}$ (pH 2)	$-\theta_{min}$ (pH 12)	%Δ (pH 2/7)	%Δ (pH 12/7)
2DabD	19500	9690	4970	50	74
2OD	17100	8710	3370	49	80
2DabE	15200	5350	4110	65	73
2KD	14400	8040	2880	44	80
2	13900	4130	3190	70	77
2KE	11500	2570	376	78	97

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Several trends emerge when the relative stabilities of the six β-peptides are compared. First, molecules containing β^3-HAsp display higher levels of 14-helix structure than otherwise identical molecules containing β^3-HGlu (compare **2DabD** vs **2DabE**, **2OD** vs **2**, and **2KD** vs **2KE**). Second, molecules containing β^3-HDab display higher levels of 14-helix structure than otherwise identical molecules containing β^3-HOrn (compare **2DabD** vs **2OD**, and **2DabE** vs **2**). Finally, molecules containing β^3-HOrn display higher levels of 14-helix structure than otherwise identical molecules containing β^3-HLys (compare **2OD** vs **2KD**, and **2** vs **2KE**). These trends suggest that the level of 14-helix structure in β-peptides related to **2** correlates inversely with side chain length: shorter side chains are better. Interestingly, solvent exposed salt bridges often contribute minimally to protein stability,[21] and glutamate, not aspartate, is the preferred partner for intra-α-helical salt bridges in proteins of known structure.[22]

The 14-helix stabilities of **2DabD** and **2DabE** were examined further by monitoring their CD spectra as a function of NaCl concentration at pH 7 in PBC buffer (Figure 3d). Both **2DabD** and **2DabE** become significantly less 14-helical as the NaCl concentration increases from 0 to 1.5 M as judged by the change in MRE_{214}. In both cases the dependence of MRE_{214} on NaCl concentration is approximately sigmoidal with a midpoint at 0.5 M NaCl; the plateaus observed at low salt suggest the formation of a stable conformation under these conditions. These CD data are highly reminscent of those reported by DeGrado for a 15-residue β-peptide containing β^3-HLys/β^3-HGlu salt-bridges on two 14-helical faces and a C-terminal D-Asp; this molecule also showed a sigmoidal dependence of MRE_{214} on NaCl concentration with a midpoint of 0.4 M NaCl.[8]

β53-1[12] is a structurally well-characterized[23] β-peptide that binds the oncoprotein hDM2 (Figure 4a). Based on the CD spectra of **2** and **2OD**, we hypothesized that substitution of β^3-HAsp for both β^3-HGlu residues in **β53-1** would lead to differences in structure observable by NMR. As expected, the ROESY spectrum of **β53-1D** at 10 °C in CD_3OH showed multiple (ten of thirteen possible) long-range ROEs characteristic of the 14-helix conformation: five of seven possible $C_\alpha H(i) \rightarrow C_\beta H(i+3)$ ROEs and five of six possible $C_N H(i) \rightarrow C_\beta H(i+3)$ ROEs (Figure 4b). Additional backbone ROEs

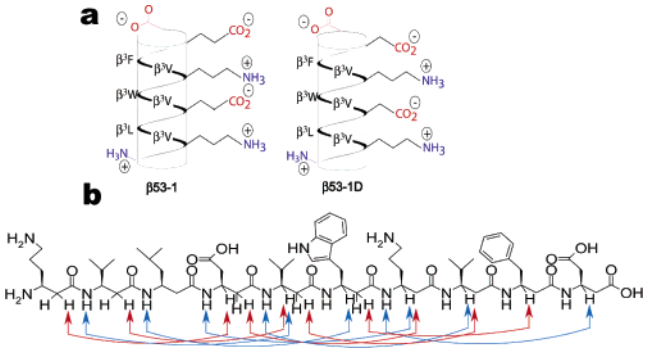

Figure 4. (a) Helical net diagrams depicting **β53-1** and **β53-1D**. (b) Backbone ROEs observed in the ROESY spectrum of **β53-1D**; $C_\alpha H(i) \rightarrow C_\beta H(i+3)$ ROEs are in red, $C_N H(i) \rightarrow C_\beta H(i+3)$ ROEs are in blue.

may have been present but were obscured by resonance overlap, as was true for **β53-1**.[12] No backbone ROEs inconsistent with the 14-helix were observed. Overall, the ROESY spectrum of **β53-1D** closely matched that of **β53-1**, further supporting the conclusion that **β53-1D** assembles into a 14-helix.

Interestingly, aliphatic ^{13}C-HSQC[18] and TOCSY[24,25] spectra revealed that the vicinal protons in the γ position of β^3-HOrn at position 1 were clearly resolved in the NMR spectrum of **β53-1D** but not **β53-1** (Figure 5a,c). The portion

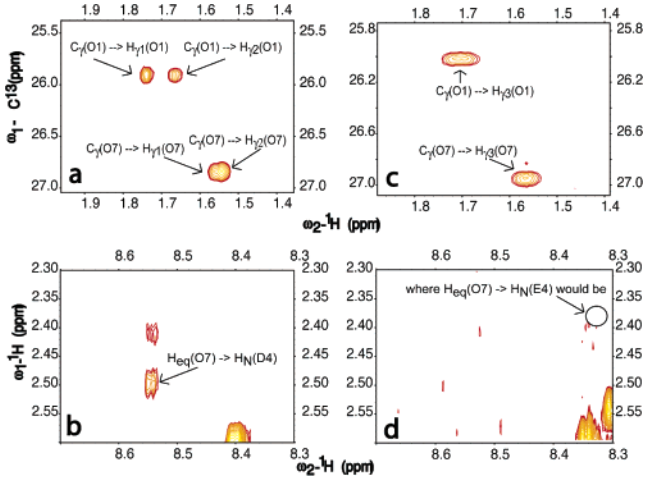

Figure 5. Differences in the 2D-NMR spectra of **β53-1D** (a, b) and **β53-1** (c, d). Regions of the ^{13}C-HSQC spectra are shown in a and c; differences in the ROESY spectra are shown in b and d.

of the aliphatic ^{13}C-HSQC NMR spectrum shown in Figure 5 identifies the interactions between the γ protons on β^3-HOrn1 and 7 and the corresponding γ carbon within **β53-**

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1D (Figure 5a) and β**53-1 (**Figure 5c). In the case of β**53-1**, the γ protons of both β^3-HOrn1 and 7 were broadened due to exchange and the exact positions of the two peaks could not be fully defined. In the case of β**53-1D**, however, the γ protons of β^3-HOrn1 were narrower and resolved. The differences between β**53-1D** and β**53-1** were also seen in the ROESY spectra (Figure 5b,d). In the case of β**53-1D**, we observed six long-range ROEs between protons on β^3-HOrn and those on proximal β^3-HAsp residue(s). These ROEs include three—those between protons of β^3-HAsp4 and β^3-HOrn7—that were not observed in the ROESY spectrum of β**53-1** (Figure 5b,d). The ROESY spectra of β**53-1D** and β**53-1** also differed in terms of the distribution of long-range ROEs throughout the sequence: the spectrum of β**53-1D** showed comparably fewer unambiguous ROEs between β^3-HOrn1 or β^3-HOrn7 and β^3-HAsp4 and 10 but comparably greater ROEs between β^3-HOrn7 and β^3-HAsp4. Overall, although the CD spectra of β**53-1** and β**53-1D** are nearly identical, the NMR data implies a subtle increase in the order of the salt-bridge side-chains in β**53-1D** when compared with β**53-1**.

In summary, here we provide evidence that salt bridge identity exerts an influence on 14-helix stability and identify the β^3-HDab/β^3-HAsp pair as the most stabilizing of those salt bridges studied. With this information in place, we can now apply the structure-stabilizing salt-bridge effects to the design of other biologically active β-peptides, thereby further assessing the delicate connection between β-peptide structure and function.

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Supporting Information Available: β^3-Peptide synthesis, purification, CD spectroscopy, and NMR spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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