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COMMUNICATIONS

Published on Web 03/14/2003

Helix Macrodipole Control of β^3-Peptide 14-Helix Stability in Water

Scott A. Hart,[†] Adilah B. F. Bahadoor,[†] Erin E. Matthews,[†] Xiaoyan J. Qiu,[†] and Alanna Schepartz*[,†,‡]

Departments of Chemistry and Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8107

Received December 21, 2002; E-mail: alanna.schepartz@yale.edu.

β-Peptides have attracted considerable attention by virtue of their ability to populate helical secondary structures, even in the absence of stabilizing tertiary interactions.[1−3] The left-handed 14-helix[4] formed by β^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.[2] Until recently, β^3-peptide 14-helices had been observed only in methanol, not in water. Recently, Seebach[5,6] and DeGrado[7] reported β^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 β^3-peptides with significant 14-helix structure in water and nicely complement work of Gellman on water-stable, helical oligomers of (cyclo)β^2-β^3-amino acids.[8] More recently, DeGrado reported a disulfide-bridged homodimer of 14-helices.[9] Here we show that stabilization of the 14-helix macrodipole alleviates the requirement for multiple β^3-Glu/β^3-Lys or β^3-Glu/β^3-Orn salt bridges on two of three helical faces. We apply this principle to design stable, monomeric β^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 α-helix design is the α-helix macrodipole, which places a partial positive charge at the N-terminus and a partial negative charge at the C-terminus.[10−13] α-Helix stability is enhanced significantly by negatively charged side chains near the N-terminus, by positively charged side chains near the C-terminus,[10] and by neutralizing charge associated with free N- and C-termini.[11] 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.[2] 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 α-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**[5,6] (Figure 1). **1** and **2** differ only by the orientation of a single β^3-Glu/β^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 β^3-peptide 14-helix is characterized by ellipticity maxima and minima near 195−198 nm and 213-215 nm, respectively.[2] 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 (θ_{214}). The mean residue ellipticity of peptide **1** was

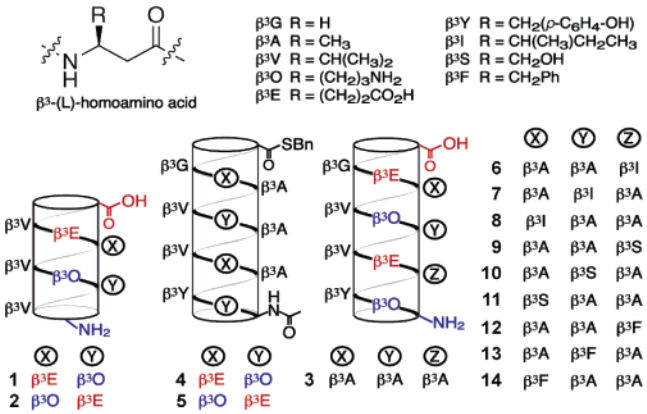

Figure 1. Helical net diagrams of β^3-peptides described herein. Amino acid codes refer to β^3-(L)-homoamino acids as indicated above. Acidic residues and C-termini are in red; basic residues and N-termini are in blue.

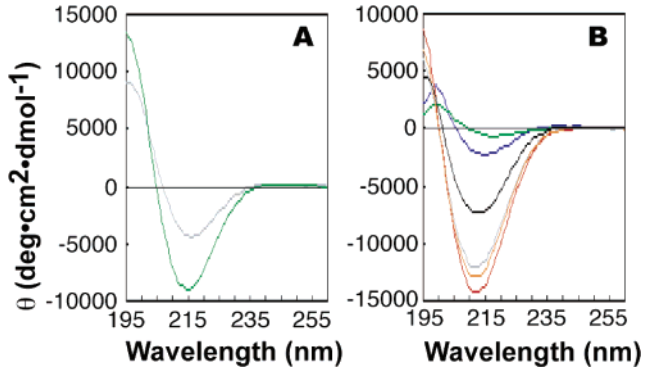

Figure 2. CD spectra illustrating the mean residue ellipticity of (A) **1** (green) and **2** (gray) and (B) **3** (black), **4** (blue), **5** (green), **6** (orange), **7** (red), and **8** (gray). All spectra were acquired at room temperature in PBC buffer (1 mM Na phosphate, 1 mM Na borate, 1 mM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

twice that of peptide **2** ($\theta_{214} = -8{,}930$ and $-4{,}370$ deg•cm²•dmol^{-1}, respectively) (Figure 2A). The CD spectra of both peptides were independent of concentration between 25 and 200 μM. This dramatic difference in structure emphasizes the significant effect of salt bridge orientation on 14-helix stability in short β^3-peptides.

Next we asked whether electrostatic interactions with the 14-helix macrodipole would stabilize longer, mixed sequence β^3-peptides. In particular, we were interested in β^3-peptides with salt bridges on only *one* 14-helix face. Undecapeptide **3**, with two correctly oriented β^3-Glu/β^3-Orn salt bridges on one face, three β^3-Ala residues on the second face, and either β^3-Val, β^3-Tyr, or a glycine equivalent (β-alanine) on the third face (Figure 1) displayed significant, concentration-independent 14-helix structure at pH 7 and 25 °C ($\theta_{214} = -7577$ deg•cm²•dmol^{-1}) (Figure 2). The presence of 14-helix structure in **3** was supported further by high-resolution NMR measurements.[14] Peptide **3** is monomeric under these condi-

[†] Department of Chemistry.
[‡] Department of Molecular, Cellular and Developmental Biology.

10.1021/ja029868a CCC: $25.00

tions as determined by sedimentation equilibrium,[14] indicating that the helix content of this molecule is not due to interhelix 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^2·dmol^{-1}, respectively). As for short α-helices, 14-helix stabilities are influenced significantly by modifications that neutralize the positive and negative charge of unmodified N- and C-termini, respectively;[11] these rules are simply reversed in the context of a β^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 β^3-amino acids. We chose three β^3-amino acids with diverse side-chain functionality (β^3-Ile, β^3-Ser, and β^3-Phe) and substituted each one singularly in place of β^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 θ_{214}, spanned a wide range, with values from -4833 to $-14\,250$ deg·cm^2·dmol^{-1}. Interestingly, the branched residue β^3-Ile was stabilizing relative to β^3-Ala at all positions; undecapeptides **6**, **7**, and **8** all exhibited significantly greater 14-helical structure than that of **3**, with θ_{214} values of $-11\,970$, $-14\,250$, and $-12\,920$, respectively (Figure 2B). β^3-Ser was destabilizing relative to β^3-Ala at position 3, stabilizing at position 6, and neutral at position 9, with θ_{214} values for **9**, **10**, and **11** of -5890, $-11\,020$, and -7790 deg·cm^2·dmol^{-1}, respectively. Like β^3-Ser, β^3-Phe was destabilizing at position 3 but neutral at positions 6 and 9, with θ_{214} values for **12**, **13**, and **14** of -5890, -8360, and -9310 deg·cm^2·dmol^{-1}.[14] Further work will be necessary to deconvolute the structural and electronic factors contributing to the position-dependent effects of β^3-Ser and β^3-Phe side chains. These data emphasize the unrecognized impact of γ-branched residues on the stability of short β^3-peptides in water. Whereas α-Ala is the most stabilizing amino acid in α-helices,[15] the rules for 14-helix formation perhaps once again diverge from those of α-helix formation since γ-branched residues may be preferred within 14-helices.

Previous studies have shown that amphiphilic β^3-peptides can form aggregates,[16] and substitution of isoleucine for leucine in α-peptide coiled-coils alters helix bundle stoichiometry.[17,18] Peptides **6**−**14** are amphiphilic, and we observed a strong increase in 14-helicity upon β^3-Ala/β^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 θ_{214} signal of peptides **6**−**14** remained constant between 50 and 200 μM,[14] and peptide **7**, like **3**, was monomeric at concentrations between 10 and 200 μM as determined by sedimentation equilibrium.[14]

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 β^3-amino acids. The most structured molecules we report are highly heterogeneous at the primary sequence level; peptides **6**−**14** contain seven different β^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 β^3-peptides and biological macromolecules *in vitro* and *in vivo*.

Acknowledgment. We are grateful to Lynne Regan and Andrew Hamilton for use of AVIV 202 and AVIV 215 CD spectrometers, respectively, Donald M. Engelman for use of a Beckman Optima XL-A analytical ultracentrifuge, Loretta Yang for acquisition of mass spectra, and William DeGrado for advise on the analysis of sedimentation equilibrium data. S.A.H. thanks the NIH for a postdoctoral fellowship. This work was supported by grants from the NIH to A.S. (GM 65453) and DME (GM 54160) and in part by a grant to Yale University, in support of A.S., from the Howard Hughes Medical Institute.

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 β_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.

References

(1) Hill, J. H.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011.
(2) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232.
(3) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(4) The term 14-helix does not on its own denote helical handedness. In the case of L-β^3-amino acids, the helix is left-handed. Other nomenclature systems refer to this structure as the 3_{14}, L_{+2}, 2L, or (M) 3_{14} helix.
(5) Arvidsson, P. I.; Rueping, M.; Seebach, D. *Chem. Commun.* **2001**, 649−650.
(6) Seebach, D.; Schreiber, J. V.; Arvidsson, P. I.; Frackenpohl, J. *Helv. Chim. Acta* **2001**, *84*, 271−279.
(7) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 5162−5163.
(8) Oligomers of (cyclo)β^2-β^3-amino acids can form well-defined 14-[16] and 12-[19−22] helices in water.
(9) Cheng, R. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 11564−11565.
(10) Armstrong, K. M.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11337−11340.
(11) Fairman, R.; Shoemaker, K. R.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Proteins* **1989**, *5*, 1−7.
(12) Lockhart, D. J.; Kim, P. S. *Science* **1993**, *260*, 198−202.
(13) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563−567.
(14) See Supporting Information.
(15) Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 211−222. O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646−651.
(16) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963−3966.
(17) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401−1407.
(18) Harbury, P. B.; Kim, P. S.; Alber, T. *Nature* **1994**, *371*, 80−83.
(19) Lee, H. S.; Syud, F. A.; Wang, X. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 7721−7722.
(20) Appella, D. H.; Barchi, J. J.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309−2310.
(21) Wang, X. F.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4821−4822.
(22) LePlae, P. R.; Fisk, J. D.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 6820−6821.

JA029868A