ORGANIC LETTERS

2008
Vol. 10, No. 9
1799−1802

Distinctive Circular Dichroism Signature for 14-Helix-Bundle Formation by β-Peptides

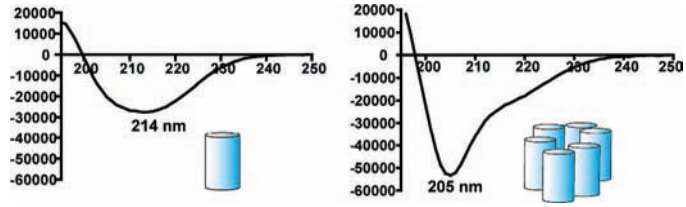
William C. Pomerantz, Tami L. R. Grygiel, Jonathan R. Lai, and Samuel H. Gellman*

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

gellman@chem.wisc.edu

Received March 17, 2008

ABSTRACT



We identify a distinctive circular dichroism (CD) signature for self-assembled 14-helical β-peptides. Our data show that self-assembly leads to a mimimum at 205 nm, which is distinct from the well-known minimum at 214 nm for a monomeric 14-helix. The onset of assembly is indicated by $[\theta]_{205}/[\theta]_{214} > 0.7$. Our results will facilitate rapid screening for self-assembling β-peptides and raise the possibility that far-UV CD will be useful for detecting higher-order structure for other well-folded oligoamide backbones.

The development of unnatural oligomers that can adopt specific folding patterns ("foldamers")[1] represents a profound challenge in molecular design. Considerable success has been achieved at the level of secondary structures, especially for helices, and attention has begun to turn toward tertiary structures.[2] A helix-bundle tertiary structure, in which two or more helices associate with their long axes approximately aligned, is common among globular proteins and constitutes an attractive goal for foldamer-based efforts. Folding in water can be driven by burial of stripes of hydrophobic side chains that are projected from one side of each helix.[3] The design of α-peptide sequences that adopt globally amphiphilic α-helical conformations and assemble to form helix-bundle quaternary structure has proven to be a useful step toward de novo protein (i.e. tertiary structure) design,[4] and several groups have sought to recapitulate this approach with foldamers.[2] We reported the first example of β-peptide helix-bundle quaternary structure formation in aqueous solution.[2a] Subsequent studies by the groups of DeGrado[2b] and Schepartz[2c−g] have advanced this field. Each of these efforts has involved the β-peptide 14-helix, which is defined by backbone i,i-2 C═O−H−N H-bonds (14-atom ring). Progress

(1) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180. (b) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232. (c) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4011. (d) Goodman, C. M.; Choi, S.; Shandler, S.; Degrado, W. F. *Nature Chem. Biol.* **2007**, *3*, 252–262.

(2) (a) Raguse, T. L.; Lai, J. R.; LePlae, P. R.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 3963–3936. (b) Cheng, R. P.; Degrado, W. F. *J. Am. Chem. Soc.* **2004**, *124*, 11564–11565. (c) Qiu, J. X.; Petersson, J.; Matthews, E. E.; Schepartz, A. *J. Am. Chem. Soc.* **2006**, *128*, 11338–11339. (d) Daniels, D. S.; Petersson, E. J.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 1532–1533. (e) Goodman, J. L.; Petersson, E. J.; Daniels, D. S.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 14746–14751. (f) Petersson, E. J.; Craig, C. J.; Daniels, D. S.; Qiu, J. X.; Schepartz, A. *J. Am. Chem. Soc.* **2007**, *129*, 5344–45. (g) Petersson, E. J.; Schepartz, A. *J. Am. Chem. Soc.* **2008**, *130*, 821–823. (h) Burkoth, T. S.; Beausoleil, E.; Kaur, S.; Tang, D.; Cohen, F. E.; Zuckermann, R. N. *Chem. Biol.* **2002**, *9*, 647–654. (i) Lee, Y. C.; Zuckermann, R. N.; Dill, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10999–11009. (j) Delsuc, N.; Leger, J.-M.; Massip, S.; Huc, I. *Angew. Chem., Int. Ed.* **2007**, *46*, 214–217. (k) Horne, W. S.; Price, J. L.; Keck, J. L.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180. (l) Price, J. L.; Horne, W. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 6376–6377. (m) Bradford, V. J.; Iverson, B. L. *J. Am. Chem. Soc.* **2008**, *130*, 1517–1524.

(3) Crick, F. H. C. *Acta Crystallogr.* **1953**, *6*, 689–697.

(4) (a) Woolfson, D. N. *Adv. Protein Chem.* **2005**, *70*, 79–112. (b) Lupas, A. N.; Gruber, M. *Adv. Protein Chem.* **2005**, *70*, 37–78.

10.1021/ol800622e CCC: $40.75
Published on Web 04/09/2008

toward helix-bundle formation has been achieved with other foldamer backbones as well.[2g–m]

Here we report that formation of a helix-bundle quaternary structure by 14-helical β-peptides is accompanied by a characteristic change in the circular dichroism (CD) signature relative to a monomeric 14-helix. Discovery and optimization of α-peptides or foldamers that adopt a specific tertiary or quaternary structure are facilitated if formation of the target structure can be detected spectroscopically. CD provides a convenient method for monitoring helix-bundle quaternary assembly among α-peptides, which are typically unfolded as monomers in aqueous solution. Self-assembly leads to a dramatic concomitant increase in α-helicity that can be detected by CD and provides an indirect indication of assembly.[5] In our initial studies of β-peptide quaternary structure formation, we concluded that CD was not useful for detecting self-assembly of 14-helices because the β-peptides we employed were already 14-helical in the monomeric state.[2a] This high level of secondary structure formation was achieved by using sequences rich in *trans*-2-aminocyclohexanecarboxylic acid (ACHC) residues, which strongly promote 14-helicity.[6]

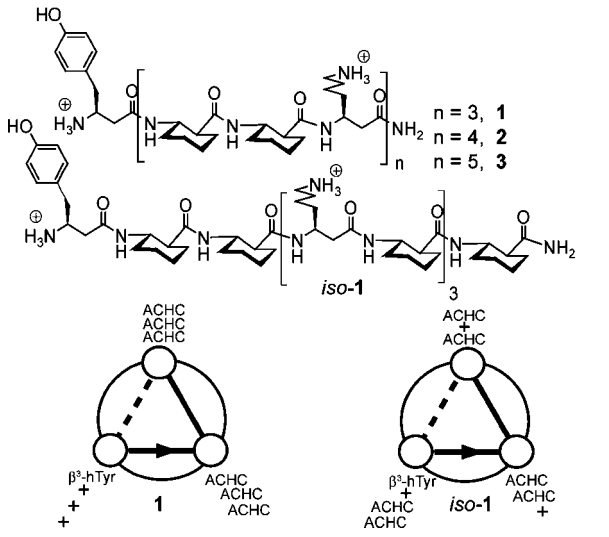


Figure 1. Sequence and helical wheel diagrams of self-assembling β-peptides **1**−**3** and nonassociating sequence isomer *iso*-1.

The isolated 14-helix gives rise to a strong minimum around 214 nm, as established in many studies.[1,7] Here, we show that a specific 14-helix quaternary structure is signaled by a distinctive minimum around 205 nm. This characteristic CD signature is observed whether or not the self-assembling β-peptides contain ACHC. Our findings rationalize previously unexplained features in reported CD data of β-peptides that form helix bundles.[2c–g]

We examined three β-peptides (**1**−**3**), which are designed to self-assemble in aqueous solution. These ACHC-rich β-peptides should adopt the 14-helical conformation, which has approximately three residues per turn. The lipophilic-lipophilic-hydrophilic triads common to **1**−**3** lead to segregation of the lipophilic ACHC residues on one side of the 14-helix and the hydrophilic β^3-hLys residues on the other side (Figure 1). The global amphiphilicity of these 14-helices favors the assembly of 14-helices with lipophilic ACHC residues at the core. Previous work showed that 10-mer **1** begins to form discrete assemblies, in equilibrium with the monomeric state, at concentrations above ∼1 mM in aqueous solution; analytical ultracentrifugation (AU) indicated a preference for either hexameric or tetrameric assembly depending upon pH.[2a] We expected 13-mer **2** and 16-mer **3** to have a higher propensity to self-associate than does 10-mer **1** because α-peptide self-association is known to increase as the hydrophobic surface area increases.[8] Indeed, AU of **1** mM solutions of either **2** or **3** in aqueous buffer indicated that these β-peptides are both highly self-associated, while 1 mM **1** is mostly monomeric under similar conditions. β-Peptides **2** and **3** form much larger assemblies than does **1**: for 1 mM solutions of **2** or **3**, we estimate the average assembly to contain ∼20 or ∼40 molecules, respectively.[9] ^{1}H NMR data support the conclusion that lengthening the globally amphiphilic 14-helix increases the degree of self-association. For 10-mer **1**, as previously reported, amide proton resonances are sharp below 1 mM (100 mM acetate buffer, pH 3.8), but the resonances broaden at or above 1.4 mM which is consistent with the onset of self-association.[9] In contrast, for 0.1 mM 13-mer **2** in aqueous solution, amide proton resonances are broadened beyond the point of detectability.[9]

The far-UV CD spectra of 0.1 mM aqueous solutions of **2** or **3**[10] differ considerably from the canonical 14-helical signature (Figure 2A), and these differences can be shown to arise from a high degree of self-association. Both samples display an intense minimum at 205 nm, along with a shoulder at 214 nm (isolated 14-helices give rise to a minimum at 214 nm). Comparison of CD and AU data suggests that the $[\theta]_{205}/[\theta]_{214}$ ratio is a useful indicator for self-association of 14-helices (Figure 2, Table 1). $[\theta]_{205}/[\theta]_{214} \approx 0.7$ for 10-mer **1** under conditions that do not support self-association according to AU (0.1 mM in aqueous Tris buffer, pH 7). A similar $[\theta]_{205}/[\theta]_{214}$ value is observed for ***iso*-1**, an isomeric 10-mer that has been shown via AU not to self-associate.[2a] Therefore, we conclude that $[\theta]_{205}/[\theta]_{214} \approx 0.7$ is the signature for a β-peptide that is 14-helical but does not form helix bundles. β-Peptide 13-mer **2** (0.1 mM) displays $[\theta]_{205}/[\theta]_{214} \approx 0.7$ in pure methanol, a solvent expected to disfavor

(5) (a) Degrado, W. F.; Wasserman, Z. R.; Lear, J. D. *J. Am. Chem. Soc.* **1989**, *243*, 622–628. (b) Degrado, W. F.; Lear, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7684–9.

(6) (a) Appella, D. H.; Barchi, J. J., Jr.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309–2310. (b) Lee, M.-R.; Raguse, T. L.; Schinnerl, M.; Pomerantz, W. C.; Wang, X.; Wipf, P.; Gellman, S. H. *Org. Lett.* **2007**, *9*, 1801–1804.

(7) Kritzer, J. A.; Tirado-Rives, J.; Hart, S. A.; Lear, J. D.; Jorgensen, W. L.; Schepartz, A. *J. Am. Chem. Soc.* **2005**, *127*, 167–178.

(8) Lau, S. Y. M.; Taneja, A. K.; Hodges, R. S. *J. Biol. Chem.* **1984**, *259*, 13253–13261.

(9) Please see Supporting Information.

(10) For comparison, the molar ellipticity of **3** was normalized to the intensity of **2** at 214 nm to account for difficulties in accurately determining the concentration of **3** by UV.

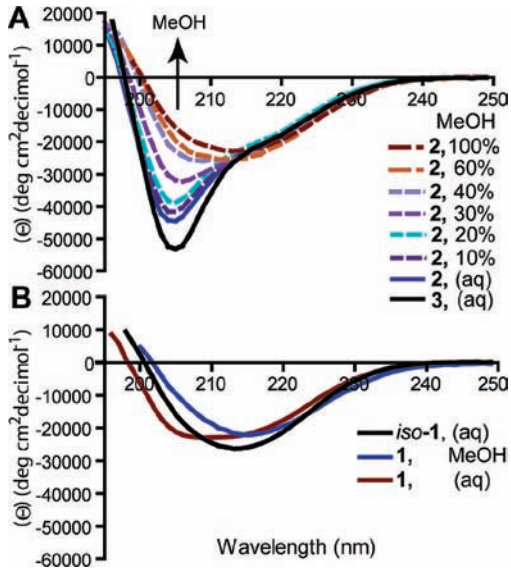


Figure 2. CD spectra of **1**, **2**, **3**, and ***iso*-1** under various solution conditions. (A) 0.1 mM, Tris pH 7.3, (**3**, black[10] and **2**, blue), and 10−100% MeOH (**2**, purple to red, dashed lines); (B) ***iso*-1**, 1.6 mM, Tris pH 8.0 (black) **1**, 1.6 mM MeOH (blue), **1**, and 1.6 mM, Tris pH 8.0 (red).

self-association. However, 0.1 mM **2** shows $[\theta]_{205}/[\theta]_{214} \approx 1.8$ in aqueous Tris buffer, pH 7.3, conditions demonstrated by AU to promote extensive self-assembly. Under similar conditions, $[\theta]_{205}/[\theta]_{214} \approx 2.0$ for β-peptide 16-mer **3**. We conclude that these large $[\theta]_{205}/[\theta]_{214}$ values represent a high degree of self-association. Previously reported AU data for 1.6 mM 10-mer **1** in Tris buffer, pH 8, indicated partial self-assembly,[2a] and this sample displays $[\theta]_{205}/[\theta]_{214} \approx 0.95$, a value between the extremes.

Table 1. $[\theta]_{205}/[\theta]_{214}$ Ratios of β-Peptides **1**−**3** and ***iso*-1** under Various Solution Conditions

β-peptide	solvent	$[\theta]_{205}/[\theta]_{214}$
1	1.6 mM, Tris pH 8.0	0.95
1	0.1 mM, Tris pH 7.3	0.75 ± 0.03
***iso*-1**	1.6 mM, Tris pH 8.0	0.62 ± 0.01
2	0.1 mM, Tris pH 7.3	1.78 ± 0.08
2	0.1 mM, MeOH	0.70 ± 0.02
3	0.1 mM, Tris pH 7.3	2.04 ± 0.05

CD data indicate that the associated state of 13-mer **2** can be disassembled, without disruption of 14-helicity, by addition of methanol (Figure 2A). This cosolvent is known to promote helical secondary structure, relative to pure water, in both α- and β-peptides;[6b,11] however, methanol disfavors tertiary and quarternary structure among proteins, because this cosolvent diminishes the drive for burial of hydrophobic surfaces.[12] Figure 3 shows how $[\theta]_{205}/[\theta]_{214}$ changes when

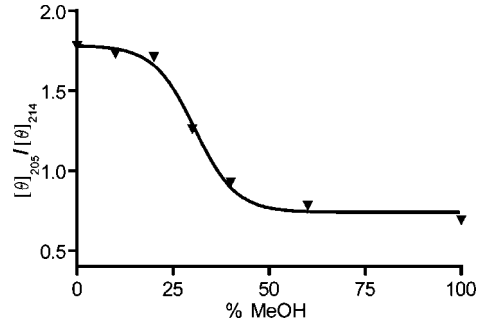


Figure 3. Plot of $[\theta]_{205}/[\theta]_{214}$ of **2** as a function of MeOH concentration.

an increasing proportion of methanol is added to an aqueous solution of 0.1 mM **2**. Small methanol proportions exert little effect, but self-assembly appears to be completely suppressed for >50 vol % methanol. The midpoint for the transition from self-assembled to nonassembled states occurs near 30 vol % methanol, which is similar to the methanol content necessary to denature small globular proteins such as α-chymotrypsinogen.[12c] NMR data support the conclusion that methanol prevents the self-association, because the amide resonances are sharp for 0.1 mM **2** in pure CD_3OH, while, as noted above, these resonances are extensively broadened for 0.1 mM **2** in aqueous solution. Similar behavior was observed for **1** at higher concentrations.[9]

Our results show that a shift in CD minimum from 214 to 205 nm is correlated with a shift from monomeric to self-assembled states of β-peptides, but is there evidence to indicate that 14-helical folding persists upon self-assembly? Addressing this question is critical, because an alternative explanation for the CD shift is that self-assembly causes a change in β-peptide secondary structure. Indirect evidence for persistence of 14-helical folding in the self-assembled state may be found in recent work of Schepartz et al.,[2d] who observed a CD minimum at 205 nm for the self-associated state of a β-peptide containing exclusively β^3-residues; this β-peptide crystallizes as a bundle of eight 14-helical molecules. To gain direct evidence against induction of alternative folding patterns upon self-association in solution, we conducted two-dimensional NMR studies of 10-mer **1** at 2.3 mM in aqueous buffer, conditions that allow partial self-association but that provide sufficiently resolved NMR resonances to enable conformational analysis. All backbone proton resonances of **1** could be assigned, and we could observe medium-range NOEs characteristic of the 14-helix (Figure 4, Table S2, Supporting Information). The strongest such NOEs occur between $C_\beta H(i)$ and $C_\beta H(i+3)$. Four of the seven possible NOEs of this type could be assigned for **1**; the other three may have been present but were ambiguous because of resonance overlap. NH(i) → $C_\beta H(i+2)$ NOEs are

(11) (a) Conio, G.; Patrone, E.; Brighetti, S. *J. Biol. Chem.* **1970**, *245*, 3335–3340. (b) Gung, B. W.; Zou, D.; Stalcup, A. M.; Cottrell, C. E. *J. Org. Chem.* **1999**, *64*, 2176–2177.

(12) (a) Bianchi, E.; Rampone, R.; Tealdi, A.; Ciferri, A. *J. Biol. Chem.* **1970**, *245*, 3341–3345. (b) Herskovitz, T. T.; Gadegbeku, B.; Jaillet, H. *J. Biol. Chem.* **1970**, *245*, 2588–98. (c) Kamatari, Y. O.; Konno, T.; Kataoka, M.; Akasaka, K. *J. Mol. Biol.* **1996**, *259*, 512–523.

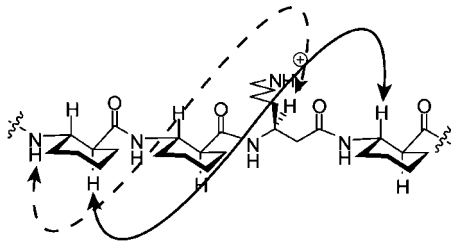


Figure 4. Representative 14-helical NOEs: NH(i) → C_βH(i+2); dashed line, C_αH(i) →C_βH(i+3) solid line.

characteristic of the 14-helix as well, although they are weaker than the C_αH(i) →C_βH(i+3) NOEs (Figure 4).[13] Three of the seven possible NH(i) → C_βH(i+2) NOEs were assigned, and two others were ambiguous. Additionally, one weak 14-helical NH(i) → C_βH(i+3) was assigned. Most importantly, no NOEs inconsistent with 14-helical folding were detected, which suggests that self-association does not cause the population of a different secondary structure.

Our findings indicate that self-assembly of 14-helical β-peptides causes a diagnostic change in CD signature relative to monomeric 14-helices. This change seems to be characteristic of a specific mode of 14-helix assembly, because the formation of liquid crystalline phases by 14-helical β-peptides, which involves assembly into nanofibers,[14] does not cause the characteristic shift from 214 to 205 nm. Schepartz et al. have identified β-peptides that do not contain ACHC but that form helical bundles in aqueous solution.[2d,f,g] These β-peptides display a CD minimum at 205 nm in the self-assembled state, which was attributed to 14-helix formation. However, our results show that the 205 nm minimum is not reporting on secondary structure, but instead is a distinctive indicator of helix bundle self-assembly. The structural changes that give rise to this CD shift appear to be relatively subtle, since 14-helicity is maintained. Lau and Hodges made related CD observations with α-helical coiled-coils.[8] The ratio of the n−π * (∼220 nm) and π−π * (∼209 nm) transition minimum intensities is 0.8 for a monomeric α-helix, but after α-helical coiled-coil formation $[\theta]_{220}/[\theta]_{209} = 1.0$. This α-peptide precedent supports our hypothesis that the electronic transitions underlying β-peptide far-UV CD are affected in a characteristic manner by 14-helix-bundle formation.

Our results will facilitate the screening of new β-peptide sequences for the propensity to form either a quaternary or tertiary structure because CD is a more rapid technique for initial analysis than is NMR or AU. In addition, these results raise the possibility that far-UV CD will be useful for detecting higher-order structure in other oligoamide backbones that are preorganized to display a high degree of secondary structure.

Acknowledgment. This research was supported by the UW-Madison NSEC (NSF DMR-042588) and NIH grant GM56414. T.L.R.G. was supported in part by a graduate fellowship from the National Science Foundation, and J.R.L. was supported in part by a PGS B scholarship from the Natural Sciences and Engineering Research Council (NSERC) of Canada. We thank PepTech for providing Fmoc β^3-amino acids at discounted prices.

Supporting Information Available: NMR, CD, and AU data in addition to experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800622E

(13) Appella, D. H.; Barchi, J. J., Jr.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309–2310.

(14) Pomerantz, W. C.; Yuwono, V. M.; Pizzey, C. L.; Hartgerink, J. D.; Abbott, N. L.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 1241–1244.