

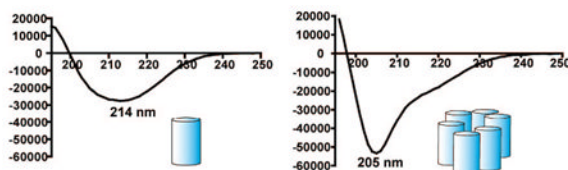
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Distinctive Circular Dichroism Signature for 14-Helix-Bundle Formation by β -Peptides

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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 minimum 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 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

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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>.

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

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 ¹H 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 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.

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 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 CD₃OH, 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. $NH(i) \rightarrow C_{\beta}H(i+2)$ NOEs are characteristic of the 14-helix as well, although they are weaker than the $C_{\alpha}H(i) \rightarrow C_{\beta}H(i+3)$ NOEs (Figure 4).¹³ Three of the seven possible $NH(i) \rightarrow C_{\beta}H(i+2)$ NOEs were assigned, and two others were ambiguous. Additionally, one weak 14-helical $NH(i) \rightarrow C_{\beta}H(i+3)$ 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 $n-\pi^*$ (~220 nm) and $\pi-\pi^*$ (~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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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.

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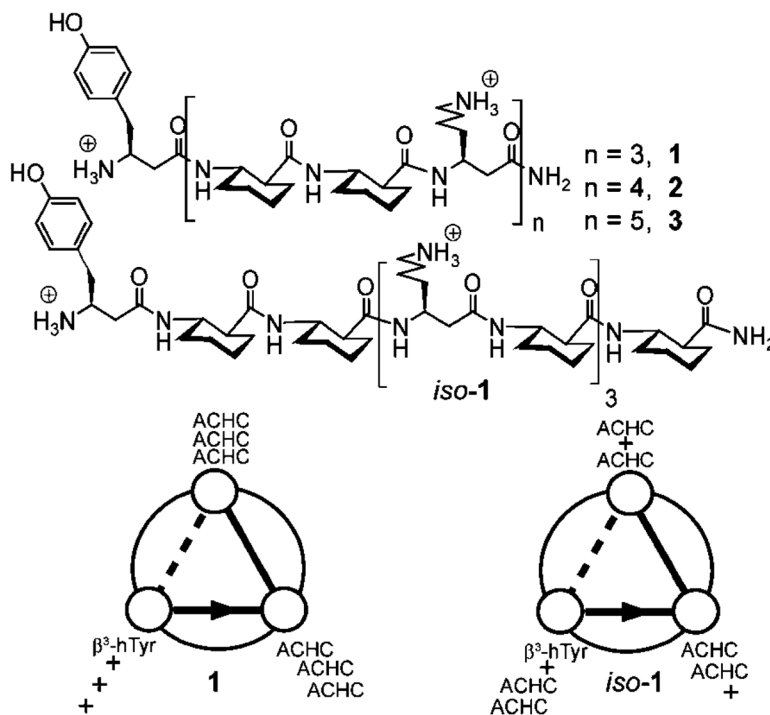


Figure 1. Sequence and helical wheel diagrams of self-assembling β -peptides **1–3** and nonassociating sequence isomer *iso-1*.



Figure 2. CD spectra of **1**, **2**, **3**, and *iso-1* under various solution conditions. (A) 0.1 mM, Tris pH 7.3, (**3**, black¹⁰ 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).

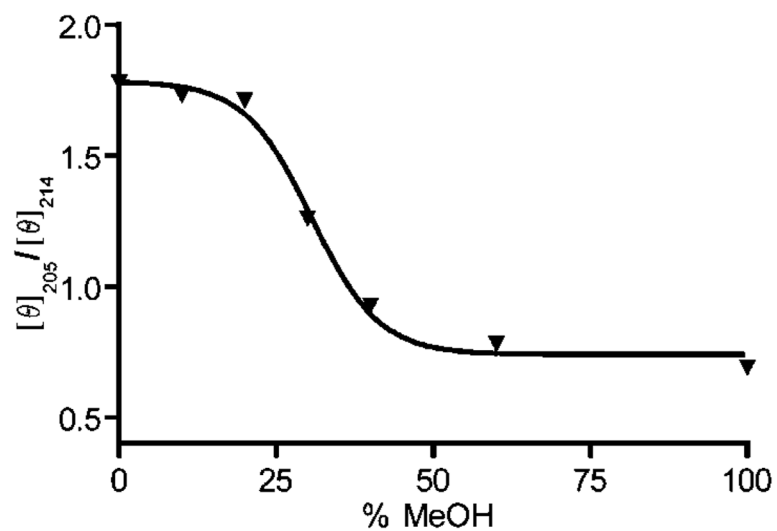


Figure 3. Plot of $[\theta]_{205}/[\theta]_{214}$ of **2** as a function of MeOH concentration.

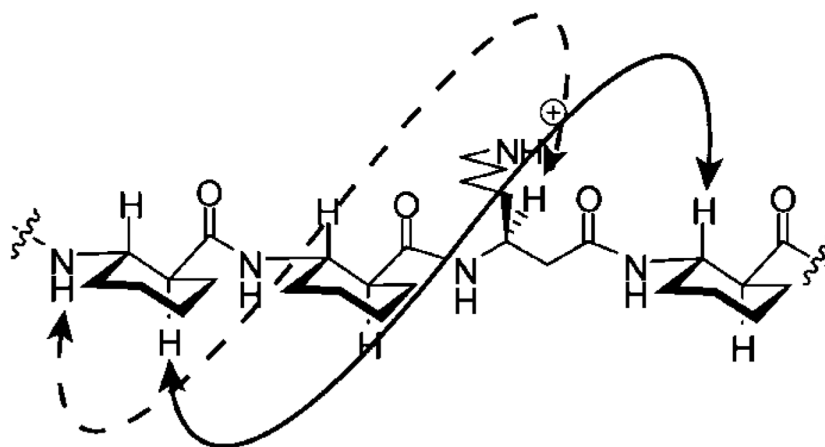


Figure 4. Representative 14-helical NOEs: $NH(i) \rightarrow C_{\beta}H(i+2)$; dashed line, $C_{\alpha}H(i) \rightarrow C_{\beta}H(i+3)$ solid line.

Table 1[θ]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 \pm 0.03
<i>iso-1</i>	1.6 mM, Tris pH 8.0	0.62 \pm 0.01
2	0.1 mM, Tris pH 7.3	1.78 \pm 0.08
2	0.1 mM, MeOH	0.70 \pm 0.02
3	0.1 mM, Tris pH 7.3	2.04 \pm 0.05