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Environment- and Sequence-Dependence of Helical Type in Membrane-Spanning Peptides Composed of β^3-Amino Acids

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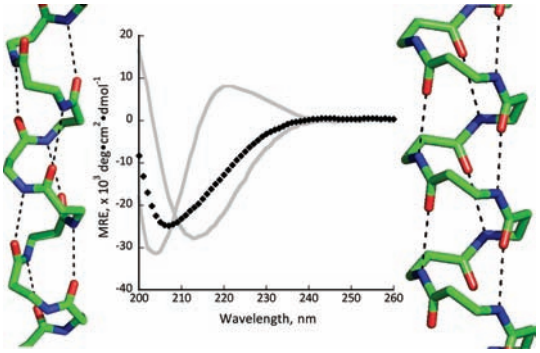
ABSTRACT



Transmembrane (TM) β-peptides comprised of acyclic β^3-amino acids demonstrate equilibrium between 12- and 14-helical structures in an environment- and sequence-dependent manner. Circular dichroism (CD) spectra of TM β^3-peptides may be described as linear combinations of the 12- and 14-helical CD spectra. The apparent malleability of β^3-substituted acyclic β-peptides has practical implications for foldamer design, as it suggests that both the 14-helix and 12-helix might be reasonable platforms for molecular recognition.

Foldamers are polymers comprised of non-natural monomeric units that fold into well-defined structures, mimicking the secondary and tertiary structures of natural proteins and oligosaccharides. In the past decade sequence-specific foldamers have been designed to address fundamental questions concerning molecular recognition and the molecular mechanisms by which proteins fold into their native structures.[1−6] Foldamers can also have beneficial properties relative to natural peptides, including smaller size, protease resistance, and improved pharmacokinetic properties, and antimicrobial foldamers are now being evaluated in humans as potential antibiotics.[7] Peptides composed of β^3-substituted amino acids are a well-studied class of foldamers, providing a useful platform for morphing from the world of natural peptides and proteins to smaller abiological foldamers and ultimately small molecules.[7,8] While many studies have focused on β-peptides that bind to water-soluble targets, membrane surfaces, or even membrane proteins, the design of transmembrane (TM) β-peptides that are sufficiently long to span a biological membrane has not been reported. Here we describe the synthesis and conformational evaluation of a series of β-peptides designed to target the TM helix of the α-subunit of the integrin $\alpha_{IIb}\beta_3$. CD studies suggest they exist in an environment-sensitive equilibrium between a 12-helical and a 14-helical conformation.

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Most detailed conformational work on hydrophobic β-peptides has focused on relatively short five- to seven-residue peptides. Previous studies with α-peptides of this length had showed a complex multiconformational behavior in which turn-like conformations, 3_{10}-helical, and α-helical conformations are in a dynamic equilibrium. In a similar manner, short β-peptides also can adopt multiple turn-like and helical forms, although the helical forms have somewhat greater stability than the corresponding helical forms of α-peptides.[2,5,6,9] The most stable helix for peptides formed from β^3-substituted amino acids is generally a 14-helix. In both the α-helix of α-peptides and the 14-helix of β-peptides, as the chain is elongated, the number of internally hydrogen-bonded amides (with amide acceptors and donors both above and below in the helix) increases, and end effects decrease, leading to an increase in helix stability. Moreover, the addition of hydrophobic solvents to aqueous solutions of α- and β-peptides generally increases the stability of the α-helix and 14-helix, respectively.[10−12] Thus, we were surprised to observe a strong sequence and environmental variability in the apparent helical type of the 26-residue β-peptides studied here, prompting the current evaluation. To explore their 12- and 14-helical contents, we extended established methods for evaluation of the secondary structure of α-peptides to probe β-peptides.

Table 1. Sequences of the β-CHAMP Peptides[a]

***β*-CHAMP**	KKKVVLVVIFGILGLLGVLVWLVKKK
***β*-CHAMP G14I**	KKKVVLVVIFGILILLGVLVWLVKKK
***β*-CHAMPscr**	KKKVVVIVGIVLVFLGLVLWLGLKKK

[a] One letter code refers to the corresponding β^3-amino acid.

The sequences of the β^3-peptides evaluated in this work are shown in Table 1. The β^3-peptides longer than approximately 10 residues in length can be difficult to synthesize, and both the synthesis and purification of very hydrophobic TM peptides are often notoriously problematic. Nevertheless, a modification of the microwave methods[13] allowed preparation of the target sequences (Supporting Information).

The far UV CD spectra of an amide shows bands associated with $\pi-\pi^*$ and $n-\pi^*$ electronic transitions. In helices the $\pi-\pi^*$ band near 200 nm splits into two excitonically coupled transitions of opposite sign.[14,15] Theoretical methods accurately predict that the higher wavelength component will occur near 205 to 212 nm, for a variety of helical types in both α- and β-peptides.[15] Although the sign and intensity of the transitions depend on the helical type and length, their positions generally lie within this range. The $n-\pi^*$ transition also contributes a feature to the spectrum near 215−225 nm. Thus, the presence of both a $\pi-\pi^*$ and $n-\pi^*$ transition gives rise to the double minimum near 208 and 222 nm classically observed for the α-helix.

The CD spectrum of the 12-helix has been well characterized,[16,17] and the $\pi-\pi^*$ transition has been treated theoretically.[18] The higher-wavelength $\pi-\pi^*$ band occurs at 205 nm and is of opposite sign from the $n-\pi^*$ transition near 220 nm, allowing easy resolution of the two bands above 200 nm. By contrast, in the 14-helix the higher-wavelength $\pi-\pi^*$ transition is of the same sign as the $n-\pi^*$ transition, resulting in only one broad band being observed near 215 nm in the classically observed spectrum for a 14-helix in solution.[16,19,20] An exception occurs in relatively long 14-helices within helical bundles, in which case the $\pi-\pi^*$ component can shift to slightly shorter wavelengths and increase in intensity leading to a double-minimum appearance.[21] Similarly, the 10/12-helix formed by β^2/β^3 mixed peptides shows one broad band with a maximum below 210 nm.[22]

The secondary structural content of proteins is often estimated by analyzing their CD spectra in terms of linear combinations of basis spectra.[15] While modern methods use basis sets obtained from a large database of proteins of known three-dimensional structures, this structural database was not available to early workers, who instead used experimental spectra of amino acid polymers in various conformations.[23]

In a similar manner, we use CD spectra of well characterized 12- and 14-helices to fit the spectra of novel peptides (Figure 1). As a 14-helix basis spectrum, we used a 18-residue antimicrobial peptide.[19] This peptide was chosen because it is relatively long and comprised of exclusively β^3-substituted amino acids and its spectra were available in dodecylphosphatidylcholine (DPC) micelles, matching the medium used in the present investigation. Its spectrum is very similar to that of a shorter, structurally characterized 14-helical peptide, comprised mainly of acyclic β^3-amino acids.[11] As an example of a 12-helical peptide, we used a well-characterized peptide composed primarily of the conformationally restrained amino acid, *trans*-2-aminocyclopentanecarboxylic acid.[24] We also

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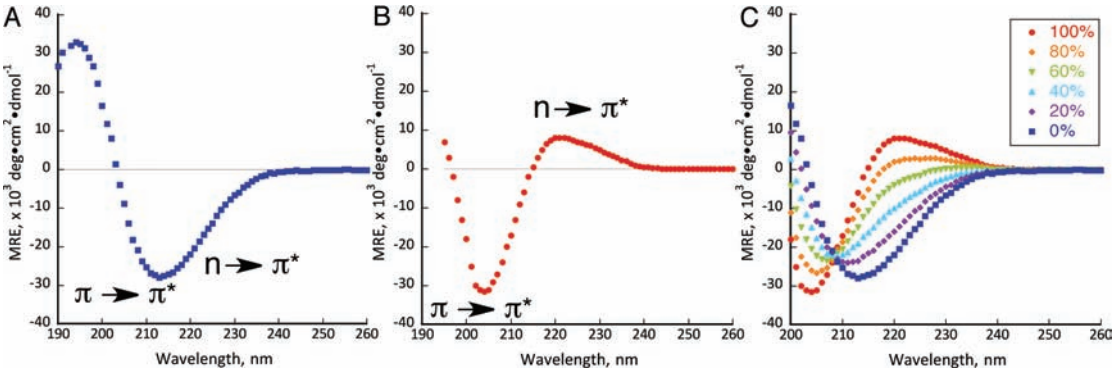

Figure 1. CD spectra of the 14-helix β-peptide (A), the 12-helix β-peptide (B) and the linear combinations (the inset legend shows fraction of the 12-helix) of the two spectra (C).

have measured the spectra of short unstructured β-peptides to model the random coil state and have found that they do not contribute significantly to the spectrum between 200 and 260 nm and, hence, are not included in the present analysis. It should be also noted that short partially folded conformer ensembles were shown by molecular dynamics simulations to contribute to CD spectra of β-peptides.[12] Determining exact contribution of such species to CD spectra of long β-peptides would require high resolution structural information. Figure 1C shows linear combinations of the 12- and 14-helix spectra. Beginning with the 14-helix spectrum, as the fraction of the 12-helix increases, the minimum at 215 nm shifts in a monotonic fashion to lower wavelengths. The maximum associated with the n$-\pi^*$ transition of the 12-helix becomes observable only when the fraction of this helix reaches approximately 80%; at lower fractions the strong negative feature from the 14-helix obscures this component. Thus, the appearance of a positive feature near ca. 222 nm would appear diagnostic of a large fraction of 12-helix and is helpful to distinguish between ensembles that are rich in 12-helix versus bundles of 14-helices, both of which can show bands near 205 nm.

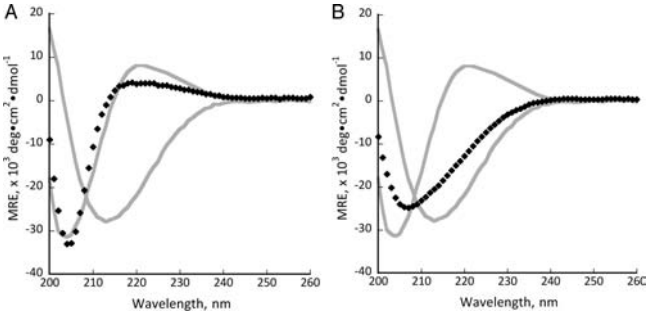

Figure 2. CD spectra of ***β*-CHAMP** (◆) in DPC micelles (A) and TFE (B). CD spectra of 14- and 12-helices shown in gray.

The CD spectrum of ***β*-CHAMP** in DPC micelles is compared with the spectrum in trifluoroethanol (TFE) in Figure 2A and 2B; the 12-helical and 14-helical standards are also included for reference. The solvent has a large effect on the spectra. In DPC, the maximum at 215 nm and minimum at 205 nm are characteristic of a 12-helical conformation, and the overall spectrum resembles the theoretical curve generated from a mix containing at least 80% of the 12-helix (Figure 1C). The experimental spectrum is well described by a linear combination of the 12- and 14-helix basis sets. Least square analysis (Supporting Information) showed the spectrum was optimally described as 88% 12-helix and 5% 14-helix (Table 2). The spectra of ***β*-CHAMP** in TFE and the other peptides under all conditions had less 12-helix character and increased 14-helix character (Figure 3). The overall spectra could be reproduced to within a few percent (near the experimental error of recording the spectrum) with the same outcome by either (1) a linear combination of the 12-helix and the 14-helix spectrum or (2) combining the 14-helix spectrum with that of the ***β*-CHAMP** in DPC.

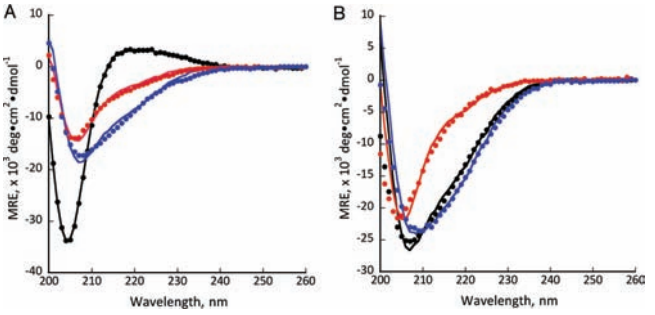

Figure 3. CD spectra of ***β*-CHAMP** (black), ***β*-CHAMPscr** (blue) and ***β*-CHAMP G14I** (red) in DPC micelles (A) and TFE (B). The corresponding fits using ***β*-CHAMP** as the 12-helix standard are shown as solid lines. Fit parameters are given in Table 2.

Least square analysis of the ***β*-CHAMP** in TFE predicts 43% 12-helix and 57% 14-helix content (here and in the subsequent discussion the relative percentages of the

Table 2. Least Squares Fit Parameters for the β-CHAMP Peptides Using Different Basis Sets[a]

peptide	f_{14} (**β-CHAMP** basis set)	f_{12} (**β-CHAMP** basis set)	R^2	f_{14} (acpc basis set)	f_{12} (acpc basis set)	R^2
β-CHAMP (DPC)	0	1	1	0.05	0.88	0.942
β-CHAMPscr (DPC)	0.25	0.33	0.996	0.27	0.29	0.950
β-CHAMPG14I (DPC)	0.33	0.47	0.995	0.30	0.49	0.982
β-CHAMP (TFE)	0.70	0.53	0.899	0.72	0.47	0.901
β-CHAMPscr (TFE)	0.30	0.57	0.908	0.32	0.52	0.914
β-CHAMP G14I (TFE)	0.76	0.38	0.956	0.78	0.33	0.952

[a] The sums of f_{14} and f_{12} should be near 1.0, although they frequently deviate significantly from unity in protein secondary structure determination.[25,26]

helices are normalized to 1 for proper comparison). Even small changes in the amino acid sequence of the peptide had large effects on the 12- versus 14-helical contents. The **β-CHAMPscr** peptide, in which the sequence in the TM region is scrambled, showed the 12-helix content changed to 66% in DPC and 51% in TFE. Furthermore, a point mutant in which hGly14 was converted to hIle shows 50% 12-helical character in DPC and 33% in TFE.

This analysis is further supported by early observations of Gellman et al.,[27] where they showed that amphiphilic peptides comprised of exclusively β^3-amino acid residues can switch between 12- and 14-helical structures in a very environment- and sequence-dependent manner. These authors also showed that CD spectra of peptides in the intermediate cases may be described as linear combinations of the 12- and 14-helical CD spectra. The lack of perfect reference spectra results in the sum of the weight coefficients deviating from 1.0. Using these deviations as a proxy metric we can estimate that the error of population measurement to be on the order of $\pm 10-20\%$.

In summary, this work shows large environmental effects associated with even long β-peptides, as well as a method to analyze their spectra. While high-resolution structures by NMR or X-ray crystallography would be desirable for this set of peptides, they are well beyond the lengths for which structures have been determined in the past, and their hydrophobic natures provide additional impediments to structural studies. While CD does not unequivocally define the structure of peptides, it has been a good guide for determining the approximate secondary structure of proteins and is most successful when applied to helical proteins. It also is often the method of choice for measuring conformational changes in proteins, particularly when considered in conjunction with other spectroscopic or crystallographic methods. Thus, the extension of methods of CD structural analysis to the analysis of β-peptides should be a welcome addition.

A surprising aspect of the present work is the finding of significant apparent 12-helix content in long peptides composed of β^3-amino acids, which generally have a much greater propensity for 14-helix versus 12-helix, due in part to unfavorable 1−5 strain associated with the side chain of the amino acids and their backbone carbonyl. This interaction would be minimized for hGly residues that lack a side chain, and there may be some cooperativity associated with placing them in regular spacing at three-residue increments as in **β-CHAMP** (but not in the other sequences), stabilizing the 12-helix. Also, the environment clearly plays a role. Overall, however, it is difficult to explain the difference with confidence, because the energetic difference between the conformations is very small, particularly on a per-residue basis. The apparent malleability of β^3-substituted β-peptides can also have practical implications for design, as it suggests that the 14-helix as well as the 12-helix might be a reasonable platform for molecular recognition, if the long-range specific interactions are sufficiently strong and sequence specific to drive selection of the 12-helical conformation.

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Supporting Information Available. Description of optimized synthetic procedures and CD spectra analysis along with MALDI and HPLC data for β-peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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