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Directional Assembly of α -Helical Peptides Induced by Cyclization

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S Supporting Information

ABSTRACT: Effective stabilization of short peptide chains into a helical structure has been a challenge in the fields of chemistry and biology. Here we report a novel method for α -helix stabilization of short peptides through their confinement in a cyclic architecture. We synthesized block peptides based on a short peptide and a flexible linker as linear precursors. Subsequent cyclization of the peptide precursors resulted in a conformational change of the peptide unit from a random coil to an α -helix. The incorporation of hydrophobic residues into the peptide unit led to a facially amphiphilic conformation of the molecular cycle. The resulting amphiphilic peptide self-assembled into undulated nanofibers through the directional assembly of small oblate micelles.

Many important biological functions originate from molecular recognition events of proteins. For many proteins to bind various biomolecules, well-defined secondary structures are placed in the recognition domains.^{1,2} The α -helix is a common motif in the secondary structure of proteins, especially existing vastly in the recognition domains of various protein–protein or nucleic acid–protein interactions.³ Inspired by biological systems, many studies have been focused on the development of stable α -helices to mimic the interactions between the original proteins.⁴ However, folding of short peptides into an α -helical structure in solution is limited because stabilizing interactions and the enthalpy gain from hydrogen bonds between amides on adjacent helical turns are not sufficient to compensate for the entropic cost involved in the folding of the peptide chain.⁵

To date, several approaches to increase the helical content of peptides have been reported. For example, more hydrophobic environments such as the plasma membrane, the presence of a cosolvent such as 2,2,2-trifluoroethanol (TFE), or isolation from solvent into the gas phase cause the α -helix to become a more stable structure.⁶ Another approach is stapling, which involves covalent cross-linking of amino acids in the same face of the helix.^{7,8} In this approach, the α -helical structure can be stabilized by the entropic disadvantage of an unfolded state when tethers containing rigid aromatic groups are used as cross-linkers that hold the sequence tightly. In this system, the peptide is based on a hydrophilic sequence because of the poor solubility of the rigid conjugated aromatic tether in aqueous solution. This approach has successfully increased the helical content of the peptide sequence in a simple way. However, many biologically active epitope sequences are rather hydrophobic to provide enhanced binding forces in aqueous environments.⁸ In the aspect of applications, it is rather difficult

to achieve biologically effective concentrations from the previous approaches because of the solubility problems of both hydrophobic peptides and tethers. Another tether-type approach is the incorporation of a β -sheet segment as a tether. The hydrophilic β -sheet linkers aggregate into small micelles, constraining the hydrophilic peptide units to the micellar surfaces.^{7c} Consequently, the hydrophilic peptide sequences adopt folded structures driven by aggregation of the β -sheet peptides.

Herein we report a novel approach to make short peptides adopt a stable α -helical structure through macrocyclization of their linear precursors. When more hydrophobic amino acid residues are incorporated into the peptide block, the helical structure forces the cyclic molecules to adopt a facially amphiphilic conformation (Figure 1b). The resulting amphiphilic folding of the cyclic molecule leads to the formation of undulated nanofibers through directional assembly of discrete micelles.

We synthesized small cyclic diblock molecules composed of a peptide and a flexible linker. Cycle 1 as a model compound for

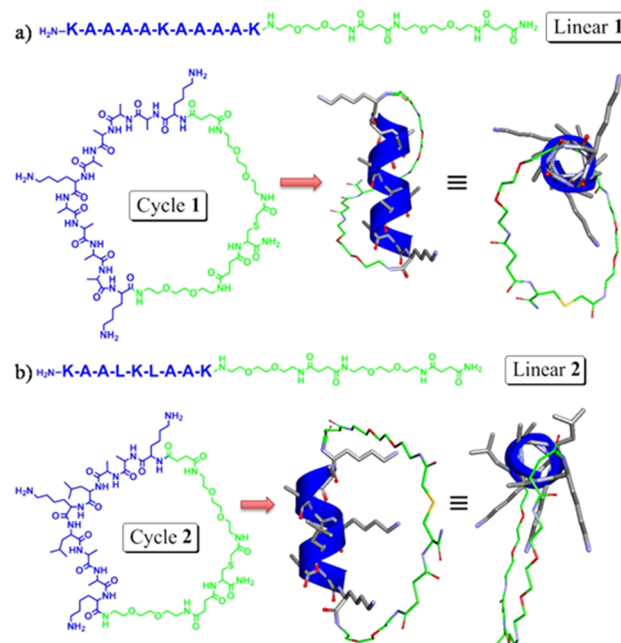


Figure 1. Molecular structures of cyclic and linear peptides and schematic illustrations of α -helical structures: (a) KAAAAKAAAK sequence; (b) KAALKLAALK sequence.

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the molecular-level helix stabilization is based on a (KAAAA)_n repeat sequence, which has been reported to form a monomeric helix in aqueous solution.⁹ A perpendicular view of the peptide helix axis (Figure 1a) shows that three amino residues from lysine side chains are equally spaced to minimize the electrostatic repulsion between the amino groups, resulting in the same polarity around the helix surface. The effect of molecular cyclization was examined by comparing linear **1** and cycle **1**, which are composed of the short peptide segment KAAAAKAAAAK and a flexible linker. In addition to the stabilization of the monomeric small peptide helix, we designed cycle **2** based on the sequence that can form a facially amphiphilic helix (Figure 1b). Cycle **2** has a KAALKLAAK sequence, and the three amino groups in the molecule are placed in the same face of the helix when the helical structure is formed. Moreover, the leucine residues, which are known to interact with each other through hydrophobic interactions, can be utilized to provide a hydrophobic surface on one side of the helix.¹⁰ The facial amphiphilicity of this design is easily seen in the view from the perpendicular helix axis (Figure 1b). An ethylene glycol-based linker segment, *N*-(Fmoc-8-amino-3,6-dioxaoctyl)succinic acid, was introduced as a flexible linker unit.¹¹ The cyclization reaction was performed with an on-resin cyclization method to achieve high synthetic efficiency (Figure S1 in the Supporting Information). Each molecule was characterized by MALDI mass spectroscopy (see the SI).

We investigated the peptide secondary structures of cycle **1** and linear **1** to confirm the effect of macrocyclization on α -helix stabilization using circular dichroism (CD) spectroscopy in the universally used electrolyte condition, aqueous KF solution, and pure water (Figure 2a).¹² An α -helix was successfully formed in

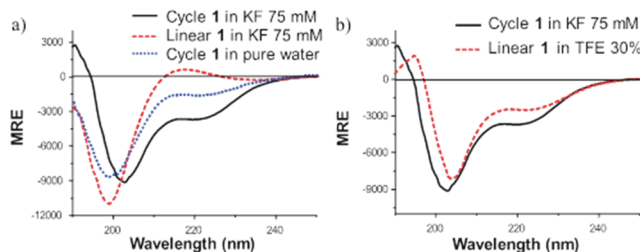


Figure 2. CD spectra of cyclic and linear peptides: (a) cycle **1** and linear **1** in 75 mM aqueous KF solution (black solid and red dashed lines, respectively) and cycle **1** in pure water (blue dotted line); (b) cycle **1** in 75 mM aqueous KF solution (black solid line) and linear **1** in 30% TFE (red dashed line).

cycle **1**, in contrast to the completely random coil structure of its linear counterpart. In the CD spectra, cycle **1** showed negative bands at 204 and 222 nm and a positive band at 190 nm, indicative of a typical α -helix. Dangling amide bonds at the linker position that adopts a free conformation would contribute a little amount to the deviation from the perfect helix band (208 nm negative band). It should be noted that the CD signals remained unaltered upon heating to 60 °C, indicating that the helical structure is stable within our experimental temperature range. In great contrast, the linear **1** peptide showed a typical random coil structure with a strong negative band at 196 nm and no signals at 222 nm. Figure 2b shows CD spectra comparing the helix stabilization effect of the cyclization with that of the well-known helix-stabilizing agent TFE (30% solution). Indeed, the peak intensity at 222 nm that is indicative of helicity is larger for the cyclic system than for the

TFE-stabilized linear peptide system, indicating that the helix stabilization effect in the cyclic system is even greater than that of TFE. These results demonstrate that the cyclization is an effective method for stabilizing small α -helices in a monomeric fashion in typical aqueous salt solutions. This result can be explained by considering the confinement of the peptide segment to a cyclic structure, in which its entropy penalty is much less because of its constrained nature. The degree of freedom of the cyclic peptide chain is much reduced compared with its linear peptide chain, resulting in the achievement of an enthalpically favorable helix conformation.

Because this system is based on a peptide and a hydrophilic flexible coil segment, amphiphilic characteristics could be induced by incorporating more hydrophobic residues into the peptide segment. With this in mind, we investigated whether the cycle **2** molecule would form a helical structure to induce an amphiphilic feature. Similar to cycle **1**, cycle **2** also adopted a helical conformation different from its linear counterpart (Figure 3a). This was rather surprising because the three

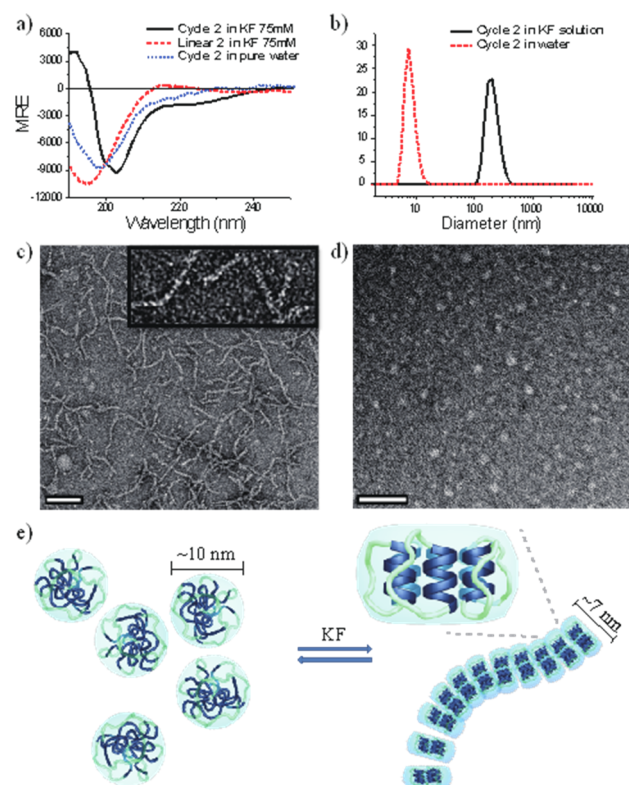


Figure 3. (a) CD spectra of the peptides based on KAALKLAAK sequence: cycle **2** and linear **2** in 75 mM aqueous KF solution (black solid and red dashed lines, respectively) and cycle **2** in pure water (blue dotted line). (b) Size distribution graph from DLS measurements of aqueous solution: cycle **2** in KF solution (black solid line) and cycle **2** in water without salt (red dashed line). (c, d) Negative-stain TEM images of cycle **2** in (c) KF solution and (d) pure water (scale bars = 100 nm). (e) Schematic representation of the transformation between micelles and undulated nanofibers.

amino groups in cycle **2** are even more closely spaced in the helix. In contrast to its linear counterpart, which did not show apparent aggregation behavior, the cyclic peptide self-assembled into a fibrous structure while maintaining an α -helical conformation of the hydrophobic peptide unit. The transmission electron microscopy (TEM) image (Figure 3c) shows

the formation of unique nanofibers with regular undulation along the fiber axis that have diameters of 6–7 nm and lengths of a few hundred nanometers. Closer examination of the samples showed the individual objects along the fiber axis to be oblate rather than spherical (Figure 3c inset), suggesting that the undulation arises from the micellar stacking.

To gain insight into the mechanism for the formation of the undulated nanofibers, we investigated the aggregation behavior in pure water without any electrolytes. Interestingly, the CD signal drastically shifted toward lower wavelengths (Figure 3a), indicating that the helical structure of the peptide segments was transformed predominantly into a random coil conformation. This conformational change was accompanied by a structural transformation from the elongated fibers to spherical micelles. Dynamic light scattering (DLS) analysis showed a drastic reduction in the hydrodynamic diameter from several hundred nanometers to ~10 nm upon removal of KF salt (Figure 3b). The TEM image shows spherical micelles with an average diameter of ~10 nm, which is larger than the fiber width (Figure 3d). These results suggest that the leucine–leucine interactions are destabilized in a random coil conformation, resulting in looser packing between the peptide segments in pure water than in KF solution. Consequently, the looser packing of the hydrophobic peptide segments with a random coil conformation gives rise to the spherical micelles with a larger diameter than the fiber width.

From these observations, induction of the α -helical structure of the peptide segment seems to be the main driving force for the formation of the undulated nanofibers. In the KF solution, which provides a more hydrophobic environment because of the salting-out effect, peptide chains would favor being folded into α -helices. The general effect of salting out in helix stabilization was observed from cycle 1 folding upon removal of the KF salt (Figure 2a). The helicity of the peptide decreased, as determined from the size of 222 nm band and the much-shifted negative minimum. With aid of this salting-out effect, the resulting helical peptides, cycle 2, are oriented parallel to each other to form oblate micelles in which the hydrophobic leucine residues are located on the inside and lysine units together with the hydrophilic linkers are on the exterior. This anisotropic packing arrangement of the helical peptides results in oblate micelles with more hydrophobic tops and bottoms resulting from the α -helical core. To reduce the exposure of the hydrophobic parts of the micelles in a water environment, the oblate micelles stack on top of each other to form undulated nanofibers (Figure 3e).

In conclusion, we have demonstrated that cyclization of block peptides leads to a conformational transition of the peptide segment from random coil to α -helix, which is important for many biological applications of small epitopes. When amphiphilicity was introduced into our cyclic system by elaborate modification of the peptide sequence, the helical conformation of the peptide forced the molecular cycle to be facially amphiphilic. The resulting facial amphiphiles self-aggregated into unique undulated nanofibers originating from one-dimensional stacking of oblate micelles through directional interactions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, synthesis of peptides, MALDI-TOF data, HPLC data, and TEM images. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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