Supramolecular Assembly

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Use of an Octapeptide–Guanidiniocarbonylpyrrole Conjugate for the Formation of a Supramolecular β-Helix that Self-Assembles into pH-Responsive Fibers

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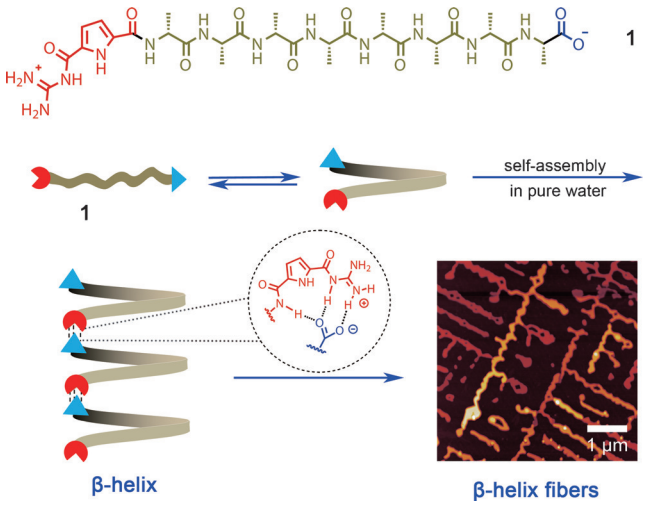
Abstract: *Peptides that adopt β-helix structures are predominantly found in transmembrane protein domains or in the lipid bilayer of vesicles. Constructing a β-helix structure in pure water has been considered difficult without the addition of membrane mimics. Herein, we report such an example; peptide* ***1*** *self-assembles into a supramolecular β-helix in pure water based on charge interactions between the individual peptides. Peptide* ***1*** *further showed intriguing transitions from small particles to helical fibers in a time-dependent process. The fibers can be switched to vesicles by changing the pH value.*

Short peptides that adopt helical structures under physiological conditions have profound impacts on biological systems. Typical helical structures include α- and β-helices. One current goal of supramolecular chemistry is to design artificial self-assembling systems that could mimic these structures under biomimetic conditions.[1] Several assembling strategies were developed based on amphiphilic peptides, amyloid and coiled-coil peptides, or cyclic peptides.[2] Peptide assembly represents a highly interesting approach for a variety of applications including stimuli-responsive materials, tissue engineering, and regenerative medicines.[3] While peptide assembly aiming to resemble α-helix structure has been demonstrated by several studies, reports on β-helix mimics are rare.[4] In comparison to the abundance of α-helix structures, there are very few peptides that form β-helices, so that these in general are less well studied.[5] Nevertheless, it has been demonstrated that β-helix peptides are important in the function of transmembrane ion channels.[6]

Gramicidins are a natural example of structures that can form transmembrane ion channels.[7] They have been used extensively as model peptides to study the functions of transmembrane channels.[8] Natural gramicidin is a mixture of peptides consisting mainly of gramicidin A, a linear pentadecapeptide constructed from alternating D and L amino acids. It can easily insert into a cell membrane as most amino acids of gramicidin A are quite hydrophobic.[9] As a consequence, this peptide is generally believed to form a unique head-to-head dimer of two single-stranded β-helices (the channel form), but only within a membrane.[10] In organic solvents in free solution, it exists in a totally different structure, namely as a double-strand intertwined helix (the non-channel form). It has been reported that structural motifs similar to that of gramicidin A can be realized with artificial non-peptidic systems such as aromatic hydrazide oligomers and synthetic triazole compounds.[11] To the best of our knowledge, a strategy for a self-assembling peptide forming a β-helix without the need for a stabilizing membrane environment has not yet been reported.

We previously introduced a strong anion-binding motif, the guanidiniocarbonylpyrrole (GCP) cation.[12] This cation has high binding affinity toward carboxylates and other oxoanions in polar organic solutions (K_a(DMSO): $>10^{10}\,M^{-1}$).[13] The rigid and planar conformation of GCP allows hydrogen-bond-assisted ion pairing with carboxylates (Scheme 1).[14] Herein, we report that functionalization of the N-terminus of an alanine octamer with alternating D and L amino acids with our GCP cation leads to peptide **1** (Scheme 1), which forms a supramolecular β-helix in pure



Scheme 1. Peptide **1** self-assembles into a supramolecular β-helix held together by ion pair formation between the guanidiniocarbonylpyrrole cation on the N-terminus of one peptide and the free C-terminus of another.

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water without the need for a stabilizing membrane environment.

Peptide **1** was designed to mimic gramicidin A, but using alanine as the basic building block. Alanine has a strong preference to form helix structures, but it is also a weak β-sheet former.[15] It has moderate hydrophobicity, which makes it suitable to be applied to aqueous conditions. The GCP moiety was coupled to the N-terminus of the alanine octamer. As comparison, peptide **2** without GCP modification was also synthesized.

Circular dichroism (CD) was first applied to study the structures of **1** and **2** in water at pH 5.0. Both peptides exist as zwitterions at this pH in water, which is ideal for self-assembling (GCP: $pK_a \approx 7$; carboxylate: $pK_a \approx 3$–4). The CD spectrum of **1** clearly showed the characteristic signals for a β-helix (positive signal at 218 nm and negative signal at 229 nm), which were in excellent agreement with the CD spectrum of gramicidin A in vesicles[16] (for the CD spectrum of gramicidin A, see the Supporting Information, Figure S3). A weaker positive signal around 242 nm was also found (Figure 1 A). Gramicidin A is known to adopt a β-helix conformation only within membranes, whereas the CD spectrum in organic solvents is quite different as no β-helix is formed.[10b,17] In sharp contrast to gramicidin A, peptide **1** was able to adopt this structure even without the need for the presence of vesicles. Moreover, peptide **2** does not show any CD signals that are characteristic of β-helices. In line with this finding, peptide **1** exhibited an additional absorbance band at 1638 cm^{-1}, a frequency commonly observed for other β-helix proteins,[18] in contrast to **2** in FTIR measurement (Figure 1 B). Thus, to our knowledge peptide **1** represents the first example of a supramolecular self-assembled β-helix in pure water. Compared to peptide **2**, the strong interaction between GCP cation and carboxylate most likely stabilized the formation of β-helix.

Indeed, molecular modelling (Macro Model, OPLS2005 force-field, conformational search within an energy window of 5 kJ mol^{-1}) suggested that the GCP groups incorporated in peptide **1** can interact with the carboxylate group of another peptide, leading to the formation of a supramolecular β-helix (Figures 1 C and S7). Furthermore, the top view of the solvent accessible surface area of the modelled helix of **1** clearly showed the existence of a central pore which is characteristic of a β-helix in comparison to a traditional α-helix (Figure 1 D).[19]

We further studied the structure of peptides **1** and **2** by atomic force microscopy (AFM) and transmission electron microscopy (TEM). Peptide **2** did not form any ordered structures in water (Figure S4). Surprisingly, we observed that peptide **1** exhibited an aging effect in water. For freshly prepared samples of peptide **1**, only small particles with sizes around 20 nm were found in both AFM and TEM (Figure 2 A). After 8 days incubation of the same sample solution at room temperature, fibrous structures with length of several

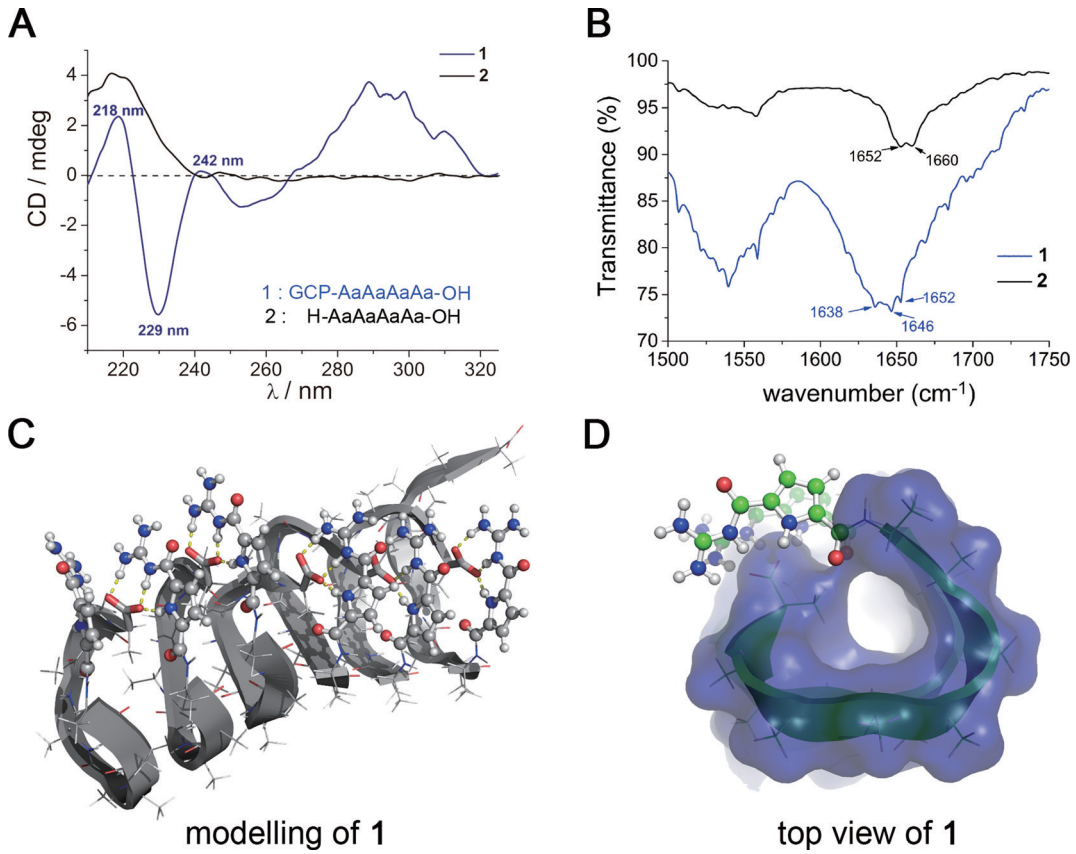

Figure 1. β -helix confirmation. A) CD spectra of 0.1 mM peptides **1** and **2** at pH 5.0. B) FTIR spectra of peptides **1** and **2**. C) Molecular modelling of an oligomer of peptide **1**. D) Top view of the solvent–accessible surface area (1.4 Å radius for water) of **1**.

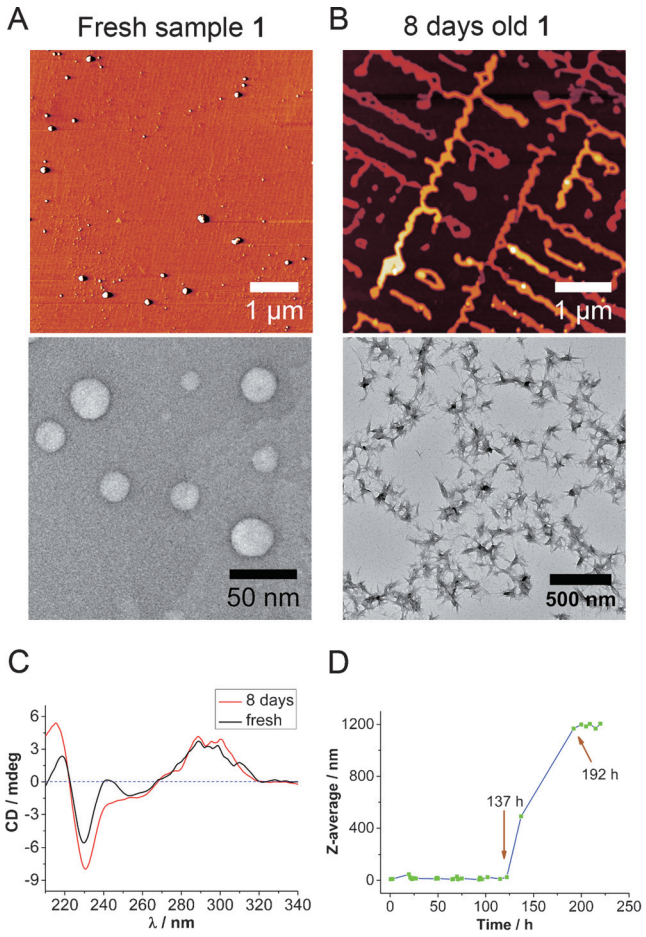

Figure 2. Growth process of 0.1 mM peptide **1** at pH 5.0. A) Freshly prepared and B) 8 days old samples of peptide **1**. Lower channel: TEM images (negative stain, carbon grid); Upper channel: AFM images (mica surface). C) CD spectrum of peptide **1** in water. Black curve: freshly prepared sample; red curve: same sample solution after standing for 8 d at room temperature. D) Hydrodynamic diameters of peptide **1** in water obtained with DLS.

micrometers were observed (Figure 2B). The aging effect was also observed in the CD spectrum (Figure 2C). For the aged sample, the overall shape of the CD curve was similar to the freshly prepared sample, which suggested that the β-helix structure of peptide **1** did not change over time. The intensity of both signals at 219 nm and 230 nm was significantly enhanced, and the signal at 242 nm changed into a shoulder. At the same time, the positive signal at 280–320 nm was unaffected. The enhancements in the CD signal most likely reflected the changes in the aggregation state of peptide **1** in water. Notably, gramicidin A also exhibited a time-dependent change in the CD spectrum, but in this case the CD spectrum completely changed as a transition from a helical dimer to a double-stranded intertwined helix occurs.[10a,20]

We also studied the aggregation process of **1** in pH 5.0 water by DLS (Figure 2D), which correlated well with the AFM and TEM results. The sizes of the aggregates remained unchanged for the first 137 h. Afterwards, rapid growth of the aggregates formed by peptide **1** was observed. Such behavior is characteristic of a nucleation–elongation mechanism.[21]

Heating an aged sample of peptide **1** demonstrated a typical cooperative disassembling curve, which further confirmed the results obtained with DLS (Figure S6).

Additionally, the fibers formed by peptide **1** were responsive towards pH changes. When the pH of the aged sample of **1** was adjusted to 10, the fibers were immediately transformed into vesicles (Figure 3A). This was not surprising because the

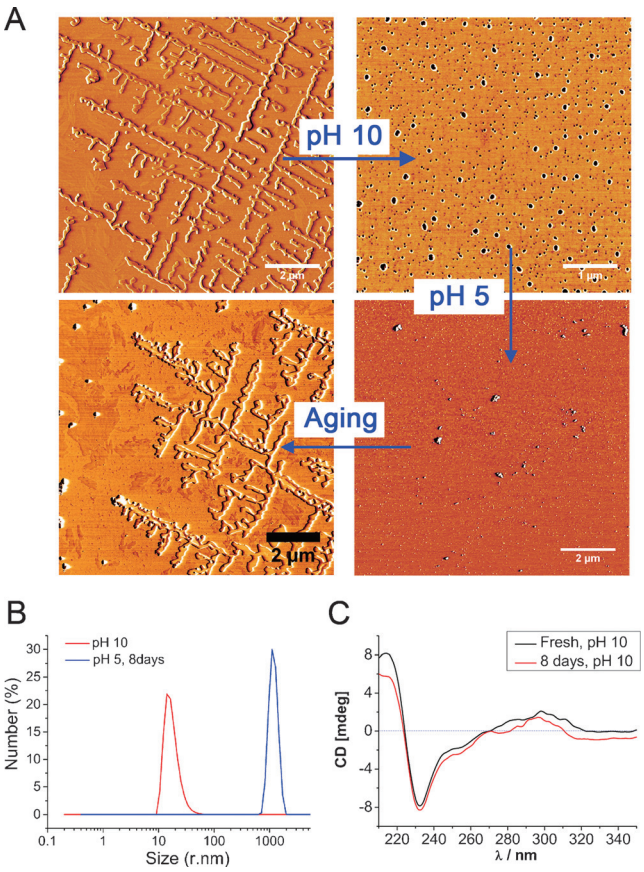

Figure 3. A) AFM phase images of peptide **1** (0.1 mM) at different pH in water. B) DLS number distribution of a solution (0.1 mM) of peptide **1** at different pH. C) CD spectrum of freshly prepared and 8 days old sample of peptide **1** (0.1 mM) at pH 10.

pK_a of the GCP moiety is around 7. Peptide **1** at basic pH is a simple anionic amphiphile that accordingly forms vesicles with sizes around 25 nm. After adjusting the pH of the solution back to 5, the small particles were again found in AFM and then grew into long fibers in the same aging process. Similarly, DLS results showed that the sizes of the aggregates formed by peptide **1** in water changed from approximately 1000 nm at pH 5 to 25 nm at pH 10 (Figure 3B).

No aging effect was observed when peptide **1** was dissolved in water at pH 10. As shown in Figure 3C, the differences in the CD spectrum of peptide **1** were negligible after incubation for 8 days. However, the intensity, mainly of the GCP-attributed CD band at 295 nm, was much weaker. This negligible CD band suggested that the GCP moiety is not uniformly oriented relative to the chiral peptide helix, which again pointed to the importance of protonation of GCP-

guanidine at pH 5 for the formation of the supramolecular β-helix.

In conclusion, to the best of our knowledge, peptide **1** is the first example for a peptide that self-assembles into a supramolecular β-helix in pure water. Furthermore, this work highlights the importance of tailor-made artificial recognition motifs for controlling the secondary structure of peptides. Without the GCP cation, no supramolecular β-helix is formed. The pH-dependent switch of peptide **1** between fibers and vesicles also showed interesting potential in constructing responsive materials.

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Keywords: ion pair formation · molecular recognition · peptide fibers · peptide structures · self-assembly

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