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RAPID REPORT

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# Self-Assembly of Short Collagen-Related Peptides into Fibrils via Cation−$\pi$ Interactions

Chia-Ching Chen, Wei Hsu, Tang-Chun Kao, and Jia-Cherng Horng*

Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30013, ROC

Supporting Information

**ABSTRACT:** Introduction of a cationic residue at the N-terminus and an aromatic residue at the C-terminus of a collagen-related peptide can generate favorable cation−$\pi$ interactions between the termini of collagen triple helices. The experimental results indicate that such cation−$\pi$ interactions can promote the self-assembly of collagen triple helices into a higher-order structure in a head-to-tail manner. Our current work shows that cation−$\pi$ interactions can serve as an effective force in preparing collagen-related biomaterials.

Collagen, the most abundant protein in mammals, is composed of three parallel polyproline type II (PPII) helices and exists as the most predominant component of the extracellular matrix and the major component of connective tissue.[1,2] Its high natural abundance and excellent biocompatibility have driven the development of collagen as a valuable biomaterial.[3] Although natural collagen has been shown to be a good biomaterial and useful in various applications, potential problems such as deleterious immunogenic responses still occur after implantation.[4,5] In addition, it is relatively difficult and infeasible to make site-specific covalent modifications to the collagen structure. Therefore, to resolve these problems, a myriad of strategies have recently been developed to assemble small synthetic collagen-related peptides into a higher-order structure.[6] These strategies include the use of cysteins knots,[7−10] electrostatic interactions,[11] native chemical ligation,[12] aromatic $\pi$−$\pi$ stacking,[13,14] aromatic−proline interactions,[15] and metal−ligand coordination,[16−19] producing favorable interactions to assist in the self-assembly of small collagen-related peptides into large-scale collagen fibrils. More recently, it has also been shown that incorporating short interrupted amino acid sequences into the collagen repeating triplets can promote the formation of supramolecular structures.[20,21] Among these studies, the structure of collagen-related peptides was essentially manipulated to facilitate the higher-order organization of collagen triple helices.

To explore more effective and feasible means for assembling collagen triple helices, we investigated the possibility of organizing short collagen-related peptides into a large-scale structure, fibrils, via cation−$\pi$ interactions. The cation−$\pi$ interaction belongs to one noncovalent force, which is regarded as an electrostatic attraction between a positive charge and the quadrupole moment of the aromatic ring,[22] and has garnered an increasing level of attention because of its important role in protein stability and protein−ligand interactions.[23−27] Recently, we have also used peptide models to show that cation−$\pi$ interactions can stabilize the collagen triple helix.[28] Thus, we first chose Arg-Phe as the cationc−aromatic pair and designed and synthesized a collagen-related peptide, RG$(POG)_{10}$F, in which one Arg is attached to the N-terminus of Pro-Hyp-Gly triplets and one Phe is attached to the C-terminus of the peptide. As shown in Figure 1, we infer that the individual collagen triple helices will proceed to assemble in a head-to-tail manner via the favorable cation−$\pi$ interactions between Arg and Phe. To show that the proposed cation−$\pi$ interactions between the ends of RG$(POG)_{10}$F are the main force for the assembly, we also prepared $(POG)_{10}$, RG$(POG)_{10}$, and G$(POG)_{10}$F as control peptides. Circular dichroism (CD) measurements show that the $T_m$ value of RG$(POG)_{10}$F is 67.4 °C, which is higher than that of all control peptides (Table 1), indicating that RG$(POG)_{10}$F forms a more stable collagen triple helix than these peptides. Although $(POG)_{10}$ has been previously shown to be capable of self-assembly into higher-order structures at an optimal temperature and pH 7.0, its self-assembly rate is relatively slow.[29] To examine the rate of self-assembly, we performed the turbidity measurements for all the peptides in 20 mM phosphate buffer (pH 7.0) at their optimal temperature. The optimal temperature was chosen in accord with the finding of Brodsky et al. that the collagen-related peptide can have a fast fibril growth rate at a temperature slightly below its $T_m$ value.[15,29] As shown in Table 1 and Figure S4 of the Supporting Information, the $t_{1/2}$ of self-assembly determined by turbidity measurements for RG$(POG)_{10}$F is less than 4 min at a concentration of 1.0 mg/mL and is even shorter with an increase in peptide concentration. At a concentration of 3.0 mg/mL, $(POG)_{10}$ has a $t_{1/2}$ of more than 12 h, concurring with the previous study,[29] whereas RG$(POG)_{10}$F has a much shorter $t_{1/2}$ of ∼1.7 min, indicating that RG$(POG)_{10}$F can self-assemble into a higher-order structure much more efficiently than $(POG)_{10}$. With regard to the other two control peptides, the $t_{1/2}$ is ∼13.3 min for G$(POG)_{10}$F and no turbidity was observed for RG$(POG)_{10}$ within 1 h. The results strongly suggest that it requires both Arg and Phe at the ends of the peptide for rapid self-assembly. In addition, the self-assembly of RG$(POG)_{10}$F is very slow at lower temperatures (4, 37, and 58 °C), indicating that the temperature is critical for this process.

Dynamic light scattering (DLS) spectroscopy was used to monitor the growth of fibrils for RG$(POG)_{10}$F. As shown in Figure 2A, at a concentration of 1.0 mg/mL, its hydrodynamic

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**Table 1. Melting Temperatures ($T_m$) for the Peptides and Their Self-Assembly Times at the Optimal Temperature**

| | | $t_{1/2}$ of self-assembly[a] | | |
|---|---|---|---|---|
| peptide | $T_m$ (°C) | 1.0 mg/mL | 2.0 mg/mL | 3.0 mg/mL |
| $(POG)_{10}$ | 59.6 | —[b] | —[b] | >12 h |
| $RG(POG)_{10}$ | 65.9 | >1 h | —[b] | —[b] |
| $G(POG)_{10}F$ | 59.6 | ~13.3 min | —[b] | —[b] |
| $RG(POG)_{10}F$ | 67.4 | ~3.6 min | ~2.3 min | ~1.7 min |
| $(POG)_8$ | 49.6 | —[b] | —[b] | —[b] |
| $RG(POG)_8F$ | 54.1 | —[b] | —[b] | —[b] |

[a] $t_{1/2}$ values for the self-assembly process are the times taken to reach the half-values of the maximal turbidities. The $t_{1/2}$ values were determined at 58 °C for $(POG)_{10}$ and $G(POG)_{10}F$, 64 °C for $RG(POG)_{10}$, and 65 °C for $RG(POG)_{10}F$. [b] Not determined.

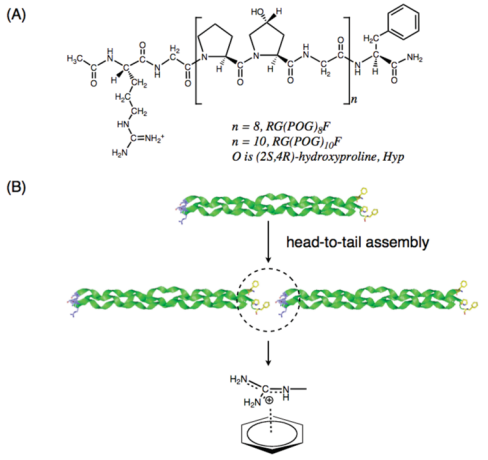


**Figure 1.** Self-assembly of collagen-related peptides. (A) Designed peptide sequences of $RG(POG)_{10}F$ and $RG(POG)_8F$. (B) Illustration of the head-to-tail assembly of collagen triple helices via cation−$\pi$ interactions. Arg residues are colored blue and Phe residues yellow.

diameter ($d$) increases from ~350 to ~1400 nm when its incubation time is extended from 0 to 24 h. In fact, the hydrodynamic diameter is up to 1200 nm after a 20 min incubation, exhibiting a rapid self-assembly process (Figure S5 of the Supporting Information). When the peptide concentration is increased to 3.0 mg/mL, the peptides can partly congregate into large fibrils without incubation (Figure 2B), and the size becomes much larger ($d$ ~ 1700 nm) after 24 h. In contrast, the DLS measurements of $(POG)_{10}$, $G(POG)_{10}F$, and $RG(POG)_{10}$ show that their hydrodynamic diameters are all less than 150 nm upon being dissolved in solution and increase more slowly than that of $RG(POG)_{10}F$ (Figure S6 of the Supporting Information). From DLS analysis, it is evident that the end-to-end cation−$\pi$ interactions play an important role in the process. We further performed transmission electron microscopy (TEM) to assess the topology and size of self-assembled collagen fibrils. As shown in Figure 3A and Figure S7 of the Supporting Information, linear fibrils can be visualized by TEM for $RG(POG)_{10}F$ with a very short incubation at a concentration of 1.0 mg/mL, consistent with the DLS measurements. Panels B and C of Figure 3 indicate that the fibrils become larger and denser when the incubation time or the peptide concentration is increased, and the fibrils can

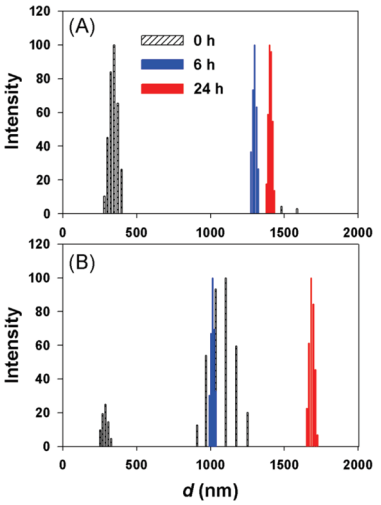


**Figure 2.** Dynamic light scattering measurements for $RG(POG)_{10}F$ at concentrations of (A) 1.0 and (B) 3.0 mg/mL after different incubation times (0, 6, and 24 h) in 20 mM phosphate buffer (pH 7.0). The measurements were taken at 25 °C (0 h incubation) or 65 °C (6 and 24 h incubations). The $x$-axis ($d$) represents the hydrodynamic diameter.

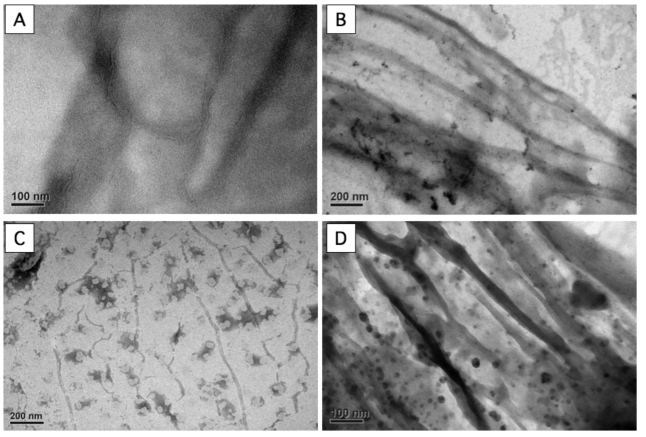


**Figure 3.** TEM images of (A) 1.0 mg/mL $RG(POG)_{10}F$ without incubation, (B) 1.0 mg/mL $RG(POG)_{10}F$ after incubation for 24 h, (C) 3.0 mg/mL $RG(POG)_{10}F$ after incubation for 6 h, and (D) 3.0 mg/mL $RG(POG)_8F$ after incubation for 24 h. The scale bar is 100 nm in panels A and D and 200 nm in panels B and C.

be more than 1.0 $\mu$m long. Moreover, under a physiological condition, $RG(POG)_{10}F$ can also form fibrils (Figure S7D of the Supporting Information) even though the self-assembly process is slower than that at 65 °C. The results suggest that $RG(POG)_{10}F$ can rapidly self-assemble into micrometer-scale fibrils via cation−$\pi$ interactions. Meanwhile, linear fibrils are the predominant structures observed by TEM. This observation demonstrates that the peptides should form higher-order structures mainly via a head-to-tail assembly, although the possibility of assembling the peptides via side-by-side packing of antiparallel triple helices cannot be completely excluded.

To further investigate whether cation−$\pi$ interactions can drive even shorter collagen-related peptides to proceed via a similar self-assembly, we prepared and characterized a shorter peptide, $RG(POG)_8F$, which contains only eight Pro-Hyp-Gly triplets. CD measurements show that $RG(POG)_8F$ can form a


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more stable collagen triple helix than $(POG)_8$ and its $T_m$ increases by 4.5 °C (Table 1). When a 3.0 mg/mL $RG(POG)_8F$ solution was incubated for 24 h, the fibrillar structure could be observed by TEM. As shown in Figure 3D, the fibrils formed by the self-assembly of $RG(POG)_8F$ are ~700 nm long. In contrast, at a similar concentration and incubation time, $(POG)_8$ cannot form the same type of fibrillar structures (Figure S8A of the Supporting Information), showing that the end-to-end cation−$\pi$ interactions between collagen triple helices serve as a critical force in aiding the self-assembly of $RG(POG)_8F$. At a higher concentration of 7.0 mg/mL, $RG(POG)_8F$ can also form a micrometer-scale structure as observed by TEM (Figure S8B of the Supporting Information). To the best of our knowledge, this is the first example to show that a collagen-related peptide with fewer than 30 amino acids can self-assemble into large-scale fibrillar structures.

In conclusion, we have used short synthetic peptides to demonstrate that cation−$\pi$ interactions can serve as a very effective force to assist the self-assembly of small collagen-related peptides. Our results show that incorporating cationic and aromatic residues into the termini of each collagen-related peptide can facilitate the formation of cation−$\pi$ interactions between collagen triple helices and promote the fabrication of fibrils. Because the preparation of such modified peptides simply employs natural amino acids and can be easily achieved by solid phase syntheses, we anticipate that the morphology of fibrils may be manipulated by implanting cationic and aromatic amino acids into the specific positions of the peptide. Our work provides a new and simple strategy for building up high-order collagen structures from small synthetic peptides, which can be applied to the preparation of various collagen-related biomaterials.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Experimental details, HPLC chromatographs, mass spectra, CD thermal unfolding curves, DLS spectra, turbidity measurements, and additional TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Department of Chemistry, National Tsing Hua University, Taiwan, ROC. Phone: +886-3-5715131, ext. 35635. Fax: +886-3-5711082. E-mail: jchorng@mx.nthu.edu.tw.

### Author Contributions

C.-C.C. and W.H. contributed equally to this work.

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