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Self-Assembly of Collagen Mimetic Peptides

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Introduction

Collagen is a widely used biomaterial, with applications in skin and bone replacement, engineered tissues, and culture media [1,2]. Collagen mimetics have shown promise as biomaterials for cell adhesion and proliferation [3,4], but active structures have been limited to the length of synthetic peptides. Here, we use sticky-end–directed assembly [5] of collagen peptides to generate long synthetically tunable structures that have potential as peptide-based collagen substitutes.

The unique structure of collagen comprises three strands folded into a triple helix [6]. Each strand possesses XaaYaaGly repeats, with ProHypGly (where Hyp is (2*S*,4*R*)-4-hydroxyproline) being the most abundant triplet [6]. For this study, helicogenic collagen sequences $(ProProGly)_n$ and $(ProHypGly)_n$ were tethered through a simplified cystine knot [7] to afford trimers **1** and **2** (Fig. 1a). In these trimers, the identical α1 and α1′ strands are constrained to be parallel to the α2 strand by a $(ProYaaGly)_3$ intramolecular helix. This helix organizes the trimers for intermolecular assembly (Fig. 1b).

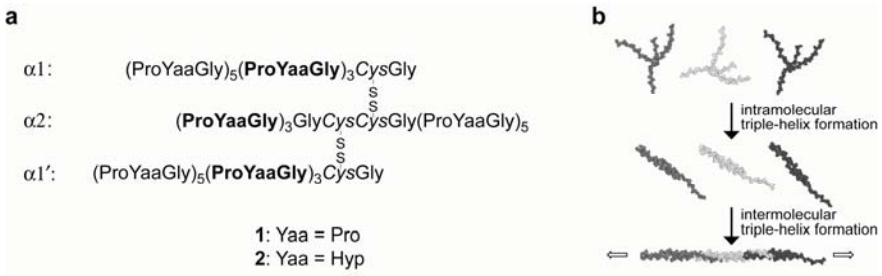

*Fig. 1. (a) Trimers **1** and **2**. (b) Representation of the self-assembly process.*

Results and Discussion

Circular dichroism (CD) spectra of trimer assemblies in 50 mM HOAc at pH 2.9 were characteristic of a triple helix (Fig. 2a), having a positive peak near 225 nm and a strong negative peak at 200–210 nm. Heat caused a cooperative change in the molar ellipticity at 226 nm (Fig. 2b), which is characteristic of triple-helix denaturation. Assembly $(\mathbf{2})_n$ had a larger T_m value than did assembly $(\mathbf{1})_n$, as expected from the stability imparted by Hyp in the Yaa position [6]. Both trimers assembled with a concentration-dependent rate (Fig. 2c), indicative of an intermolecular process. Assembly $(\mathbf{2})_n$ formed more rapidly than did assembly $(\mathbf{1})_n$, a result likely due to its greater preorganization as well as the rapid *cis–trans* isomerization of its Pro–Hyp peptide bonds [6].

The size of assemblies $(\mathbf{1})_n$ and $(\mathbf{2})_n$ in 50 mM HOAc at 10 °C was estimated by using dynamic light scattering. Hydrodynamic radii were measured to be 3.1 nm for $(\mathbf{1})_n$ and 4.0 nm for $(\mathbf{2})_n$. Using the Broersma relations [8], lengths were calculated to be 16 nm for $(\mathbf{1})_n$ and 22 nm for $(\mathbf{2})_n$, indicating that the average size of the

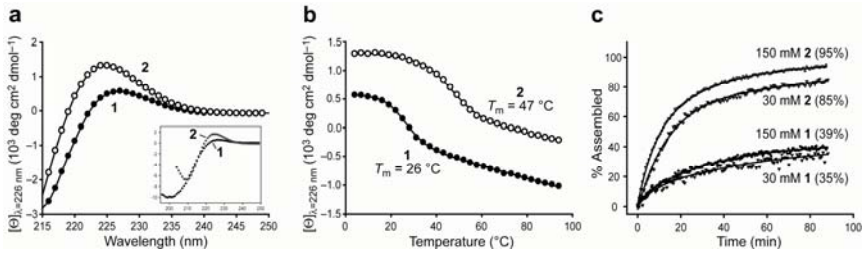

Fig. 2. (a) CD spectra, (b) thermal denaturation curves, and (c) folding rates of $(\mathbf{1})_n$ *and* $(\mathbf{2})_n$.

assemblies was 2–4 monomer units. This size is consistent with sedimentation equilibrium data (not shown).

The morphology of $(\mathbf{1})_n$ and $(\mathbf{2})_n$ was determined by atomic force microscopy (AFM) and transmission electron microscopy (TEM). AFM images of $(\mathbf{1})_n$ (Fig. 3a,b) and $(\mathbf{2})_n$ showed 20–120 nm long fibrils with diameters of 0.5–1.0 nm, which are similar to those of natural collagen. Rotary-shadowed TEM images of $(\mathbf{1})_n$ (Fig. 3c) and $(\mathbf{2})_n$ showed fibrillar structures 30 nm to >400 nm in length that resemble TEM images of natural collagen. The AFM and TEM data indicate that trimers **1** and **2** can self-assemble into one-dimensional fibrils that are similar in length to natural collagen.

This work is the first step towards the development of synthetic collagen-based biomaterials that could serve as bona fide collagen substitutes as well as templates for a variety of applications in nanotechnology.

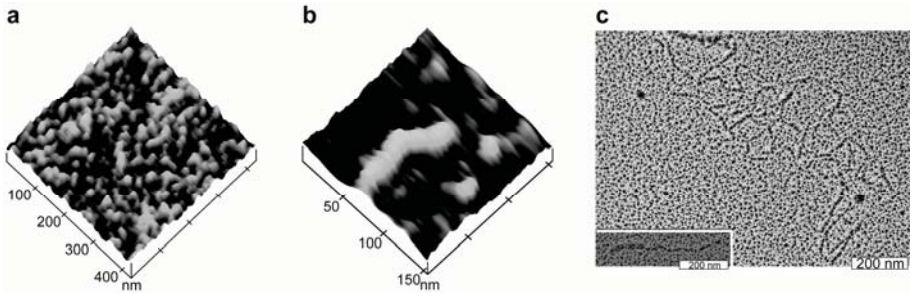

Fig. 3. (a,b) AFM and (c) rotary-shadowed TEM images of $(\mathbf{1})_n$.

Acknowledgments

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References

1. Lee, C. H., *et al. Int. J. Pharm.* **221**, 1–22 (2001).
2. Ramshaw, J. A. M., *et al. Biotechnol. Genet. Eng. Rev.* **13**, 335–382 (1996).
3. Johnson, G., *et al. J. Biomed. Mater. Res.* **51**, 612–624 (2000).
4. Fields, G. B., *et al. Biopolymers* **47**, 143–151 (1998).
5. MacPhee, C. E. and Woolfson, D. N. *Curr. Opin. Solid State Mater. Sci.* **8**, 141–149 (2004).
6. Jenkins, C. L. and Raines, R. T. *Nat. Prod. Rep.* **19**, 49–59 (2002).
7. Ottl, J. and Moroder, L. *J. Am. Chem. Soc.* **121**, 653–661 (1999).
8. Claire, K. and Pecora, R. *J. Phys. Chem. B* **101**, 746–753 (1997).