JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Metal-Mediated Tandem Coassembly of Collagen Peptides into Banded Microstructures

Marcos M. Pires, David E. Przybyla, Charles M. Rubert Pérez, and Jean Chmielewski*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, United States

Supporting Information

ABSTRACT: The ability to recapitulate the features of natural collagen at the micro- and nanoscale with novel biopolymers has the potential to lead to improved biomaterials. Herein we describe stimuli-responsive collagen-based peptides (**IdaCol** and **HisCol**) that together form higher order assemblies in the presence of added metal ions. SEM and TEM imaging of these assemblies revealed microscale petal-like and intertwined fiber morphologies, each with periodic banding on the nanometer scale. The observed banding is consistent with tandem coassembly of alternating **IdaCol** and **HisCol** triple helical blocks that may laterally associate either in or out of register to form higher order structures, and mimics the banding found in natural collagen fibers.

The controlled assembly of protein- and peptide-based copolymers into novel functional biomaterials has the potential to lead to significant technological advances.[1] The modular nature of such biopolymers allows for the construction of distinct assemblies whose shape, size, and structure can be programmed into the primary peptide sequence. In particular, our goal has been to build collagen-like materials that are both stimuli-responsive and fully reversible to facilitate their translation into biomedical applications. We envisioned that we could use a block-like assembly process with tandem repeats of collagen peptides, thus expanding the repertoire of accessible collagen peptide-based architectures.[2] We also postulated that the alternating coblock assembly could provide a means to synthetically control the periodicity of synthetic collagen materials.[2h] Herein, we describe a tandemly repeating design composed of collagen-like peptides that assemble specifically in the presence of metal ions to form periodically banded microstructures.

With metal promoted coassembly in mind, we constructed peptides around two principle features. First, the central core of the collagen-like peptides was designed to contain repeating units of the tripeptide Pro-Hyp-Gly to endow higher thermal stability to the triple helical blocks of peptide (Figure 1a).[3,4] Second, two peptides were envisioned, each individually containing identical metal binding units at both termini, thereby allowing the linkage of one peptide block with the other by the addition of divalent metal ions (Figure 1b). Specifically, one peptide, **HisCol**, contains two histidines at both N- and C-termini of a monomeric strand (Figure 1a), whereas the other peptide, **IdaCol**, contains an iminodiacetic acid (Ida) moiety incorporated onto the side chain of lysine at both the N- and C-termini. The Ida ligand has previously been used with His-tags in immobilized metal affinity chromatography.[5] Triple helix formation with the **HisCol** peptide would produce a grouping of His-metal binding units at each terminus that is complementary to the Ida grouping of ligands at the termini of the **IdaCol** trimer in the presence of metal ions (Figure 1b).

Figure 1. (a) Sequences of the collagen-based peptides **IdaCol** and **HisCol** containing metal binding ligands at each termini. (b) Schematic representation of the block-like tandem assembly of alternating trimers of **IdaCol** and **HisCol** upon the addition of metal ions.

According to the design, the individual folding of either **IdaCol** or **HisCol** into a collagen triple helical conformation is a prerequisite for the proper organization of the metal binding ligands at their respective termini. Circular dichroism (CD) data for each peptide displayed a maximum at 225 nm, a profile consistent with a collagen polyproline type II helix (see Supporting Information). Also, cooperative thermal unfolding was observed by CD indicating that **IdaCol** and **HisCol** each adopt a stable triple helix with melting temperatures (T_m) of approximately 52 and 54 °C, respectively (see Supporting Information). It is possible for fraying at the ends of the triple helices due to buildup of charge at the termini of **IdaCol**, or a loss of register of the triple helix. We investigated, therefore, the effect of increased ionic strength on the ability of **IdaCol** to form a triple helix. The addition of up to 250 mM of NaCl had no effect on the T_m of the triple helix of **IdaCol** (see Supporting Information). These findings indicate that the installation of termini modifications on the collagen-like peptides did not preclude the central POG

Received: May 9, 2011
Published: August 24, 2011

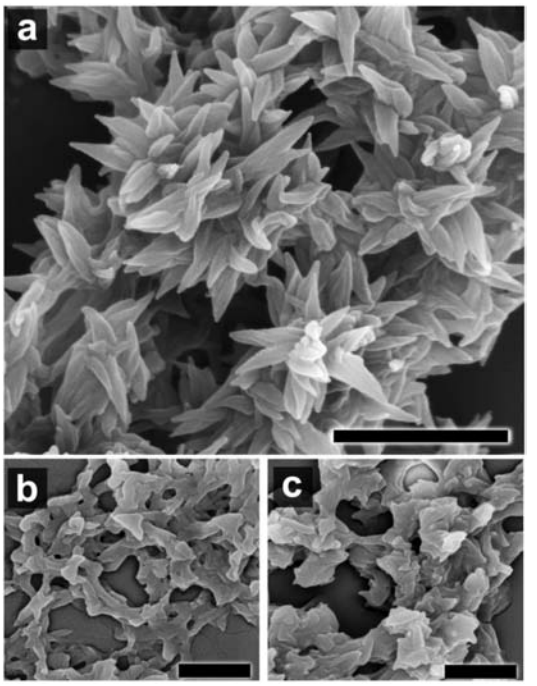

Figure 2. SEM imaging of assemblies formed from **IdaCol** (1 mM) and **HisCol** (1 mM) in MOPS pH 7.4 buffer with added (a) Ni(II) (2 mM), scale bar = 1 μm; (b) Zn(II) (2 mM), scale bar = 500 nm; and (c) Cu(II) (2 mM), scale bar = 500 nm.

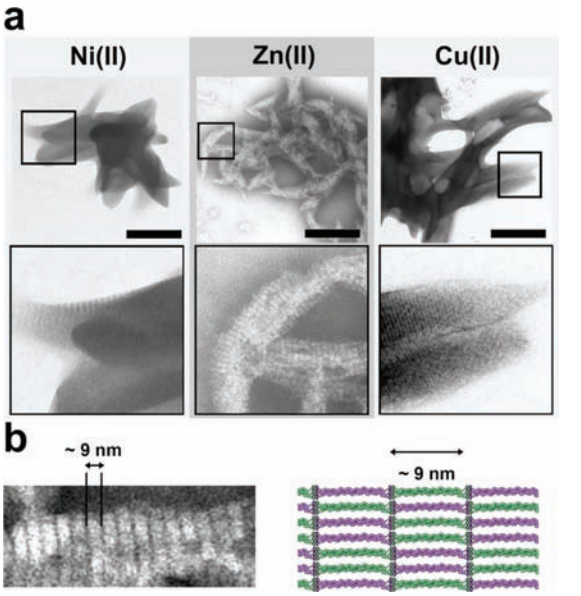

Figure 3. (a) TEM imaging of assemblies formed from **IdaCol** (1 mM) and **HisCol** (1 mM) in MOPS pH 7.4 buffer in the presence of [left] Ni(II) (uranyl acetate stain, scale bar 700 nm), [center] Zn(II) (phosphotungstic acid stain, scale bar 225 nm), and [right] Cu(II) (uranyl acetate stain, scale bar 200 nm) with an increased magnification within the inset boxes. (b) Comparison of the periodic banding pattern observed for the Zn(II)-assembled structures and the predicted length of a single collagen triple helix within the predicted coblock assembly (note: heterogeneous lateral assembly is shown).

core from homotrimerization, a result that has also been observed with collagen peptides containing three carboxylate moieties.[6] More significantly, these data indicated that the metal binding ligands should be suitably positioned to participate in a trivalent interblock interaction at room temperature.

Initially, the ability of various divalent metal ions to trigger the assembly of the peptide blocks **IdaCol** and **HisCol** was monitored by visualizing solution turbidity and by dynamic light scattering (DLS). Solutions composed of either **IdaCol** or **HisCol** alone (1 mM) did not become turbid upon incubation at room temperature in the absence of metal ions during monitoring time (>1 week), and no aggregates over the size of that expected for the collagen triple helix were observed by DLS (approximately 2−3 nm radii; see Supporting Information). Likewise, coincubation of **IdaCol** and **HisCol** (1 mM each) at room temperature in the absence of metal ions over a similar time period led to no solution turbidity and no change in the DLS data as compared to those obtained for the individual peptides. However, the inclusion of 2 mM Cu(II), Zn(II), or Ni(II) to the same mixed equimolar peptide solution led to the formation of a highly visible precipitate in less than 1 h, with no smaller aggregates observed by DLS. These data are in stark contrast to those observed with the individual peptides with the same metal ions, namely no visible precipitation and more limited aggregation as found by DLS (see Supporting Information)

The observed precipitation with the equimolar peptide mixture and added metal ions was found to be reversible by the addition of a metal chelator. The addition of EDTA (10 mM) to the highly turbid solution led to an almost instantaneous clarification of the solution. These findings not only indicate that metal ions play a key role in the association of the peptides but also, more significantly, demonstrate the reversibility afforded by the described system.

Scanning electron microscopy (SEM) was first used to determine the morphology of the assemblies formed from the equimolar mixture of **IdaCol** and **HisCol** in the presence of metal ions. SEM imaging of the Ni(II)-promoted assembly revealed an exquisite petal-like self-assembly (Figure 2a). Each individual petal varied in length from 100 to 500 nm, with clustering and outward growth. The Cu(II)- and Zn(II)-assembled materials displayed interconnected units at the microscale, although the overall morphology appeared less organized when visualized by SEM (Figure 2b-c). Together, these images reveal the morphological properties at the micrometer scale of the peptide blocks assembled by metal ions.

Following the discovery that the addition of metal ions to a solution containing both **IdaCol** and **HisCol** formed defined microsized materials, we sought a more detailed understanding of the assemblies at the nanometer scale. The same materials that had been studied by SEM were subjected to transmission electron microscopy (TEM) analysis. With each set of metal-promoted materials we observed clearly visible and periodic banding at the nanometer scale (Figure 3a). For instance, TEM visualization of the Ni(II)-promoted petal-like structures showed that each petal was distinctly banded, whereas the Zn(II)-mediated assemblies were composed of approximately 200 nm fiber clusters also with banding. By TEM the Cu(II) material was found to have more in common with the petal-like structures observed with Ni(II), and a staggered banding pattern was observed within each segment. Furthermore, the distance between the banding gaps for all three metal-assembled structures was found to be approximately 9 to 12 nm (see Supporting Information). This length is in agreement

with the computed average length for a triple helical peptide with nine repeating units of POG and the additional flexible termini modifications (Figure 3b). Therefore, it is conceivable that the periodic banding observed is a product of linear assembly of alternating **IdaCol** and **HisCol** blocks that laterally associate either in or out of register to form higher order structures (Figure 3b, out of register shown). From these results, we demonstrate that tandem block-type assembly of collagen peptides may achieve consistent and easily discernible banding patterns at the nanometer scale within microstructures.

The assembly of the individual blocks of **IdaCol** and **HisCol** was based on orthogonal metal binding ligands that are segregated within each homotrimer. The importance of this segregation was probed by heating a solution containing both **IdaCol** and **HisCol** (1 mM each) above their respective melting temperatures (95 °C) for 10 min and allowing them to cool back to room temperature. This process was intended to induce strand exchange between **IdaCol** and **HisCol** due to their similar Pro-Hyp-Gly regions and charge complementarity.[7] Following a 48 h equilibration period at 4 °C, the thermally annealed solution was treated with the same metal ions (2 mM). Interestingly, no solution turbidity was observed with added metal ions, in contrast to what had been observed with the two peptides before heating, and no significant higher order aggregates were observed by DLS (see Supporting Information). The scrambling of the strands should result in heterotrimers whose terminal moieties would display charge-paired His and Ida ligands within the same triple helices, a process that may preclude extensive metal-promoted interblock association. Alternatively, if metal ion binding did occur at the heterotrimeric ends, a satisfied metal ion complex at the termini may result, essentially capping off the triple helix for further association.

In conclusion, we have developed a set of two distinct collagen-based peptides, each displaying different metal binding units, with the potential for metal-promoted tandem coassembly. We have demonstrated that the addition of various divalent metal ions to the combined peptides leads to assembled microstructures with periodic banding at the nanoscale. This exquisite control of higher order coassembly mimics the banding observed in natural collagen fibers, although resulting through an alternative assembly pattern. Interestingly, the mussel byssus is also known to contain a number of His-rich collagen proteins that bind to metal ions, potentially to cross-link the load-bearing proteins of this structure.[8] The ability to introduce chemical diversity[2d,9] into collagen peptide assemblies will be pursued in the future to control the orientation and spacing of functional units within these collagen peptide microstructures.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures; synthetic schemes; characterization, CD, DLS, and TEM data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
chml@purdue.edu

■ ACKNOWLEDGMENT

We are grateful to the NSF (0848325-CHE) and the TRASK Foundation for support of this research and to D. Sherman for assistance with SEM and TEM.

■ REFERENCES

(1) (a) Rabotyagova, O. S.; Cebe, P.; Kaplan, D. L. *Biomacromolecules* **2011**, *12*, 269. (b) Branco, M. C.; Schneider, J. P. *Acta Biomater.* **2009**, *5*, 817. (c) Vandermeulen, G. W. M.; Klok, H. A. *Macromol. Biosci.* **2004**, *4*, 383.

(2) (a) Chen, C. C.; Hsu, W.; Kao, T. C.; Horng, J. C. *Biochemistry* **2011**, *50*, 2381. (b) Przybyla, D. E.; Chmielewski, J. *J. Am. Chem. Soc.* **2010**, *132*, 7866. (c) Pires, M. M.; Chmielewski, J. *J. Am. Chem. Soc.* **2009**, *131*, 2706. (d) Pires, M. M.; Przybyla, D. E.; Chmielewski, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 7813. (e) Kar, K.; Ibrar, S.; Nanda, V.; Getz, T. M.; Kunapuli, S. P.; Brodsky, B. *Biochemistry* **2009**, *48*, 7959. (f) Krishna, O. D.; Kiick, K. L. *Biomacromolecules* **2009**, *10*, 2626. (g) Przybyla, D. E.; Chmielewski, J. *J. Am. Chem. Soc.* **2008**, *130*, 12610. (h) Rele, S.; Song, Y.; Apkarian, R. P.; Qu, Z.; Conticello, V. P.; Chaikof, E. L. *J. Am. Chem. Soc.* **2007**, *129*, 14780. (i) Cejas, M. A.; Kinney, W. A.; Chen, C.; Leo, G. C.; Tounge, B. A.; Vinter, J. G.; Joshi, P. P.; Maryanoff, B. E. *J. Am. Chem. Soc.* **2007**, *129*, 2202. (j) Kotch, F. W.; Raines, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3028. (k) Koide, T.; Homma, D. L.; Asada, S.; Kitagawa, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5230. (l) Paramonov, S. E.; Gauba, V.; Hartgerink, J. D. *Macromolecules* **2005**, *38*, 7555.

(3) Brodsky, B.; Thiagarajan, G.; Madhan, B.; Kar, K. *Biopolymers* **2008**, *89*, 345.

(4) Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem.* **2009**, *78*, 929.

(5) Porath, J.; Carlsson, J.; Olsson, I.; Belfrage, G. *Nature* **1975**, *258*, 598.

(6) Lee, J.; Chmielewski, J. *Chem. Biol. Drug. Des.* **2010**, *75*, 161.

(7) Gauba, V.; Hartgerink, J. D. *J. Am. Chem. Soc.* **2007**, *129*, 2683.

(8) Zhao, H.; Waite, H. *Biochemistry* **2006**, *45*, 14223.

(9) Woolfson, D. N.; Mahmoud, Z. N. *Chem. Soc. Rev.* **2010**, *39*, 3464.