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Hierarchical and Helical Self-assembly of ADP-ribosyl Cyclase into Large-scale Protein Microtubes

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Abstract

Proteins are macromolecules with characteristic structures and biological functions. It is extremely challenging to obtain protein microtube structures through self-assembly as proteins are very complex and flexible. Here we present a strategy showing how a specific protein, ADP-ribosyl cyclase, helically self-assembles from monomers into hexagonal nanochains and further to highly ordered crystalline microtubes. The structures of protein nanochains and consequently self-assembled superlattice were determined by X-ray crystallography at 4.5 Å resolution and imaged by Scanning Electron Microscopy. The protein initially forms into dimers that have a fixed size of 5.6 nm, and then, helically self-assembles into 35.6 nm long hexagonal nanochains. One such nanochain consists of six dimers (12 monomers) that stack in order by a pseudo P₆₁ screw axis. Seven nanochains produce a series of largescale assemblies, nanorods, forming the building blocks for microrods. A proposed aging process of microrods results in the formation of hollow microstructures. Synthesis and characterization of large scale self-assembled protein microtubes may pave a new pathway, capable of not only understanding the self-assembly dynamics of biological materials, but also directing design and fabrication of multifunctional nanobuilding blocks with particular applications in biomedical engineering.

Keywords

Protein self-assembly; X-ray crystallography; nanochain; nanorod; nanobuilding block; microtube

INTRODUCTION

Self-assembly of molecular proteins into tubular structures is a process of intermolecular non-covalent aggregation without human intervention, assembling macromolecules into a series of functional entities^{1–5}. Discovery of enhanced or collected properties of biological building blocks at a wide range of scales motivates scientists and engineers to develop a variety of self-assembly strategies that could be utilized to fabricate various multi-functional building blocks with promising applications.^{6–13} Achievements are witnessed in syntheses of a series of nanobuilding blocks with controlled size, shape and morphology^{6–13}.

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SUPPORTING INFORMATION AVAILABLE

Description of experimental methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

However, current exploration for design and fabrication still relies on simple molding and patterning of pre-existing building blocks that originate mostly from static self-assembly^{6–10}. In nature, proteins are self-assembled functional biological macromolecules of polypeptide chains, and display typical sizes, shapes, and dynamics. These macromolecules are not only of intrinsic structural complexity but also have biological functions. Understanding the self-assembly mechanism in such a complex biological system will certainly pave an effective pathway that assists the design and synthesis of a series of functional nanobuilding blocks used in medicine and bioengineering.

Current protein overexpression techniques are capable of producing large quantities of protein that can serve as templates for fabrication of expected nanobuilding blocks¹⁴. However, it is still difficult and challenging to synthesize large scaled and highly ordered crystals with featured structure, shape and morphologies. This letter reports the fabrication of micron-sized protein tubes through three-leveled nanometric scale self-assemblies of a specific protein, called ADP-ribosyl cyclase¹⁵, and proposes a feasible model describing the underling mechanism of tube formation.

RESULTS AND DISCUSSION

Self-assembled protein microtubes were obtained by hanging-drop vapor diffusion methods at room temperature. The formation of microtubes was achieved from a precipitation process in which supersaturated protein precipitates against a reservoir that contains 50 mM imidazole, pH 7.5, and 12% polyethylene glycol (PEG) 4000. The proteins tubes obtained are of micrometric scale and display a typical hollow hexagonal morphology (Figure 1a). The tube length and width range 100–800 μm and 5–100 μm , respectively. The tubes are open at both ends and the tube walls are either sealed or unsealed. For some preparations, we observed broken tube structures with only half of the tubes remaining in the solution (Figure 1b). It appears that those broken tubes derive from the dissolving of sealed tubes shown in Figure 1a. Scanning electron microscopy (SEM) was used to characterize the surface morphologies and stacking characteristics of small needles with diameters of 5–50 μm as shown in Figure 1c. Hollow tube structures with highly ordered hexagonal shapes can be clearly discerned. The ends of these small tubes are also open, indicative of tube structures formation at micro-scale. The formation of tubular structures is both protein concentration and PEG 4000 concentration sensitive. Only protein of about 5 mg/mL and PEG 4000 of about 12% lead to a spontaneous development of self-assembled microtubes. Higher concentration protein, for example 10 mg/mL, or higher concentration of PEG 4000, for example 20%, result in the formation of regular protein crystals that are absent of any hollow structures.

Polarizing optical microscopic observations demonstrate that microtubes display strong anisotropic properties, indicative of forming a highly ordered crystalline lattice. The observed hexagonal shape implies that an internal six-fold axis directs the growth of tube structure. Correlating numerous inorganic nanotubes and nanorods to this study, the occurrence of a hexagonal shape suggests that self-assembly of nano-size proteins into large scale hexagonal tubes is most likely through a hexagonal stacking strategy starting from protein nanobuilding blocks.

X-ray diffraction measurements were conducted at the A1 station of CHESS to study the internal structure of the protein tubes. One single microtube of 120 μm width and 800 μm length was utilized for X-ray diffraction measurement (Figure 2a). The protein tube displays a strong diffracting ability, thus generating a group of highly separated diffraction spots (Figure 2b). A total number of 360 frames were collected on a Quantum 210 CCD detector with a 1° oscillation angle to cover the greatest possible reciprocal Ewald sphere. The X-ray

diffraction pattern shows that the microtube is a single crystal and capable of diffracting X-rays to ~ 4.0 Å. The collected diffraction pattern (Figure 2b) can be indexed in a primitive monoclinic symmetry. The unit cell has a total volume of $956,454 \text{ Å}^3$ and detailed unit cell parameters are presented in Figure 2c. Statistics of the complete data set up to 4.5 Å are listed in Table S1.

Previous studies show that ADP-ribosyl cyclase exists as a dimer¹⁵ in solution with each monomer having 251 amino acid residues. The atomic structure of the microtube was thus determined by a molecular replacement method implemented in the program Phaser¹⁶ with a dimer as a searching model. The refined structure at 4.5 Å resolution contains six molecules (monomers) with hierarchically self-assembled structure in a crystallographic asymmetric unit (Figure 3a, 3b, and 3c). The monomeric structure of ADP-ribosyl cyclase consists of two domains: N-term head domain and C-term tail domain (Figure 3a). Two monomers interact in a head-to-head and tail-to-tail fashion with a local 2-fold axis to form a dimer (Figure 3b). Each dimer displays a rectangular geometry, serving as a bottom-up building unit, and periodically three dimers self-assemble into a hexamer. The hexamer in a crystallographic asymmetric unit forms through a tail-to-head attachment of three dimers in order of AB-CD-EF (Figure 3c). The three dimers of AB, CD, and EF have an intermolecular accessible surface area of 1609 Å^2 , 1624 Å^2 , and 1632 Å^2 , respectively. The protein-protein interactions between the dimers are weaker as the inter-dimer accessible surface area reduces to 953 Å^2 (between dimers AB and CD) and 934 Å^2 (between dimers CD and EF), respectively (Figure 3c). A series of self-assemblies of dimers into a hexamer go through a pseudo $P6_1$ screw axis. Dimer AB rotates 60° around b-axis and translates 5.95 nm downward to form dimer CD; and dimer CD similarly rotates 60° around b-axis and translates 5.95 nm downward to form dimer EF.

The structure of a unit cell can be derived from the symmetry operation of the hexamer in the crystallographic asymmetric unit cell (Figure 3c) by a 180° rotation around the b-axis followed by a translation of half of one unit cell (17.84 nm) along the b-axis (Figure 3d). The two symmetry related hexamers (six dimers or 12 monomers) in the unit cell form a nanochain that has three dimensional scale of $5.6 \times 5.6 \times 35.6$ nm. The nanochain has a closed hexagonal packing of monomers as viewed along the b-axis (top view), and a helical self-assembly as viewed perpendicularly to the b-axis (side view) (Figure 3d). A pseudo $P6_1$ screw axis intrinsic to the six dimers guides the formation of the structure in the unit cell. As viewed along the long axis, each dimer rotates 60° around the b-axis and translates 5.95 nm along the b-axis relative to the previous one, and consequently results in the formation of a helically self-assembled hexagonal nanochain.

The crystal structure derived from X-ray diffraction allows proposing a feasible model of how protein nanochains (Figure 3d) self-assemble into a higher order hexagonal superlattice. Such high order assembly results in a nanorod that consists of seven protein nanochains as shown in Figure 4a. One nanochain is enclosed by six nanochains that stack in order by following a 6-fold rotational symmetry. The nanorod is also of hexagonal morphology with a diameter of ~ 15 nm. A typical SEM image taken from the cross-section of a tube (top view) shows protruded bulges having a wide range of sizes (Figure 4b). All bulges consist of minimal discernible particles of a fixed size of 15 nm, identical to the diameter of the nanorod. Certainly, the protruded bulge is indicative of a further assembly of nanorods.

Based on the structure of a nanorod, the self-assembly of nanorods into a micrometric scale structure should end up with a solid microrod, rather than a hollow microstructure. However, it is quite common that microtubes form in our experiments. To address the mechanism of microtube formation, we compared SEM images from three different portions of the microtube, e.g. from cross-section (Figure 4b), from the exterior side wall (Figure 4c),

and from the intersection between the cross-section and the side wall (Figure 4d). The comparison of these surface images indicates that protein nanorods and bulges (nanobuilding blocks) pack tightly on the surfaces of the side wall and the intersection, but loosely attach on the surface of the cross-section. This phenomenon suggests that protein nanobuilding blocks are likely to move away from the middle of the cross-section than that from elsewhere in the microtube. Therefore the preferential dissociation of nanobuilding blocks from the cross-section of microrods may finally lead to the hollow structures we describe in this study.

This mechanism of tube formation was further evaluated by careful analyses by SEM of hundreds of newly formed microtubes characterized. As shown in Figure 4e, we can observe the formation of a solid microrod. Noticeably, many craterlets appear on the cross-section of the microrod, but not on the side wall or the intersection. Compared with a typical hollow structure (Figure 4f), the formation of craterlets supports the concept that the tube formation starts from the dissociation of protein nanobuilding blocks from the cross-section.

During our experiments, we also noticed that after microtube formation in solution, extended incubation time of microtubes in solution consequently decreased their quality in terms of surface smoothness, anisotropy under polarizing microscopic observation, and tube completeness. Figure 1a and 1b show microtubes that were left in solution for an extended time of six days after tube formation. Compared with the newly formed microtubes in Figure 1c, microtubes in Figure 1a and 1b have undergone a dissolving process in which part or half of the tubes disappeared. Therefore, the microtubes are unstable in solution, and will dissolve following their formation. It is also possible that tube formation itself is part of the dissolving process in which protein nanobuilding blocks start to move away from the cross-section of microrods. For this later explanation, the dissolving process begins specifically from the cross-section of microrods, produces tubes as intermediates, and finishes with broken tubes.

That the tube formation is closely related to the dissociation of protein specifically from the cross-section allows us to propose a feasible mode of an aging process to illustrate tube formation (Figure 4g). In this mode, nanobuilding blocks first self-assemble into solid microrods following the crystallographic lattice (Figure 4g, left panel). Upon accomplishment of such a self-assembly process, an aging process starts at the cross-section of the microrod. The aging process includes the dissociation of nanobuilding blocks from the cross-section of the microrod, leading to the formation of craterlets which will continuously turn bigger and deeper (Figure 4g, middle panel). The aging process then continues to drill microrods to form hollow structures (Figure 4g, right panel). The aging process may proceed further to decrease the wall thickness, finally destroying tube structures. Some broken microtubes, shown in Figure 1a and 1b, are most likely the outcome of such an aging process. In other words, microrods aggregated from nanobuilding blocks are not stable in solution and will undergo an aging process which involves the development of microtube intermediate structures before they finally dissolve.

The synthesized protein tubes display excellent mechanical properties and have promise for significant applications. As long as protein tubes are fixed by dehydration using various buffers, their unique architectures are still preserved well. Upon external force loading, for example gravity during the dehydration process in this study, protein tubes do not break, implying excellent elasticity (Figure 1c). Therefore, incorporation of a series of hard materials into protein microtubes is highly capable of making strong building blocks that behave like human bone having improved hardness and enhanced toughness^{18,19}.

CONCLUSION

In conclusion, the synthesis of crystalline protein microtubes and the proposed assembling model provide an excellent example showing how hollow crystalline structures form from a specific protein at micrometric scales. The characterization of hollow cylindrical structures is capable of not only providing an understanding of the tuning mechanism of protein nanobuilding blocks, but also providing a structural basis for using protein engineering to generate functional protein architectures at different scales. Most importantly, crystalline protein microtubes can be further used as templates for effectively designing and fabricating a series of nanobuilding blocks that assemble inorganic materials into biological organisms with potential applications in biosensor and biomedical engineering. Upon assemblies of certain nanoparticles into protein microtubes, it will be feasible to fabricate a variety of tubular building blocks with expected electronic, optical, and other physical properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES

1. Chen C, Daniel MC, Quinkert ZT, De M, Stein B, Bowman VD, Chipman PR, Rotello VM, Kao CC, Dragnea B. *Nano Letters*. 2006; 6:611. [PubMed: 16608253]
2. Sleytr UB, Sara M, Pum D, Schuster B. *Progress in Surface Science*. 2001; 68:231.
3. Nogales E, Wang HW. *Current Opinion in Cell Biology*. 2006; 18:179. [PubMed: 16495041]
4. Yip CK, Kimbrough TG, Felise HB, Vuckovic M, Thomas NA, Pfuetzner RA, Frey EA, Finlay BB, Miller SI, Strynadka NCJ. *Nature*. 2005; 435:702. [PubMed: 15931226]
5. Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM. *Science*. 2004; 306:1040. [PubMed: 15528446]
6. Alivisatos AP, Johnsson KP, Peng XG, Wilson TE, Loweth CJ, Bruchez MP, Schultz PG. *Nature*. 1996; 382:609. [PubMed: 8757130]
7. Jacobs HO, Tao AR, Schwartz A, Gracias DH, Whitesides GM. *Science*. 2002; 296:323. [PubMed: 11951039]
8. Terfort A, Bowden N, Whitesides GM. *Nature*. 1997; 386:162.
9. Xia YN, Whitesides GM. *Annual Review of Materials Science*. 1998; 28:153.
10. Yang PD, Deng T, Zhao DY, Feng PY, Pine D, Chmelka BF, Whitesides GM, Stucky GD. *Science*. 1998; 282:2244. [PubMed: 9856944]
11. Ghadiri MR, Granja JR, Milligan RA, Mcree DE, Khazanovich N. *Nature*. 1993; 366:324. [PubMed: 8247126]
12. Rajagopal K, Schneider JP. *Current Opinion in Structural Biology*. 2004; 14:480. [PubMed: 15313243]
13. Martin CR, Kohli P. *Nature Reviews Drug Discovery*. 2003; 2:29.
14. Whitesides GM, Boncheva M. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:4769. [PubMed: 11959929]
15. Munshi C, Baumann C, Levitt D, Bloomfield VA, Lee HC. *Biochimica Et Biophysica Acta-Protein Structure and Molecular Enzymology*. 1998; 1388:428.

16. McCoy AJ. Acta Crystallographica Section D-Biological Crystallography. 2007; 63:32.
17. Vayssieres L, Keis K, Hagfeldt A, Lindquist SE. Chemistry of Materials. 2001; 13:4395.
18. Mershin A, Cook B, Kaiser L, Zhang SG. Nature Biotechnology. 2005; 23:1379.
19. Anderson DG, Burdick JA, Langer R. Science. 2004; 305:1923. [PubMed: 15448260]

Figure 1.

Micro-scale tube structures self-assembled from ADP-ribosyl cyclase. a) Micro-scale protein tubes with either wall sealed (black arrow) or unsealed (red arrows). Tubular ends are open and have hexagonal shapes. b) Micro-scale protein tubes with almost half their tube walls (red arrows) having disappeared. Both a) and b) are imaged under light microscope 6 days after tubes formation. c) Scanning electron microscopy image showing small protein tubes at ranges of 5 ~ 50 μm in diameter. The image was taken 1 day after tubes formation.

Figure 2.

X-ray diffraction analysis of protein microtubes. a) Morphology of the microtube used for X-ray diffraction experiment. b) A representative diffraction pattern. The resolution rings mapped on the image show the diffracting ability of the microtube. The region marked by a magenta rectangular box is zoomed in, showing a group of tightly packed diffraction spots. c) Unit cell parameters derived from the X-ray diffraction experiment by indexing.

Figure 3.

Mechanism of the hierarchical and helical assembly of ADP-ribosyl cyclase into a nanochain. a) Structure of a monomer, shown as ribbon representation and colored in green. “N” and “C” denote NH₂-terminus and COO-terminus of the protein, respectively. b) Two views of dimeric assembly from monomers. Each monomer is differently colored. c) Structure of the hexamer (three dimers) in the crystallographic asymmetric unit. Six individually colored molecules are shown as ribbons. The accessible surface areas representing intermolecular interactions are labeled between adjacent monomers. These values were calculated by the protein-protein interaction server (<http://www.biochem.ucl.ac.uk/bsm/PP/server>). d) Views of two hexamers (six dimers) in one unit

cell from b-axis (top panels) and from a-axis (bottom panels). Along a pseudo $P6_1$ screw axis, six dimers possess a hexagonal conformation (top view) and a helical conformation (side view). The six dimers form a nanochain with three dimensions of 5.6 nm by 5.6 nm by 35.6 nm.

Figure 4.

Proposed mechanism for the microtube formation. a) Top view of a nanorod assembly from seven nanochains. The formed hexagonal nanorod has one dimensional length of ~ 15 nm. b) SEM 12 image showing the cross-section morphology of a microtube crystal (top view). Each discernible particle has one dimensional length of ~ 15 nm. c) SEM image showing the side wall morphology of a microtube (side view). d) SEM image showing the intersection of the cross-section and side wall of a microtube (rim view). e) SEM image showing many shallow craterlets on the cross-section of a newly formed microrod. f) SEM image showing a fully developed microtube. g) A proposed model describing the formation of hollow structures. The aggregation of nanorods forms solid microrods. Then a preferential aging

process involving the dissociation of nanobuilding blocks begins from the cross-section of microrods. The dissociation process leads to the ultimate formation of hollow structures.