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HIGHLIGHT

Toroidal β-barrels from self-assembling β-sheet peptides

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Controlling nanostructural morphologies has been the subject of intensive research as nanostructures can display significantly different bioactivities depending on their physicochemical parameters. Toroidal proteins perform numerous important functions in biological systems. Recent studies show that toroidal nanostructures can be constructed by rationally designed self-assembling peptide building blocks.

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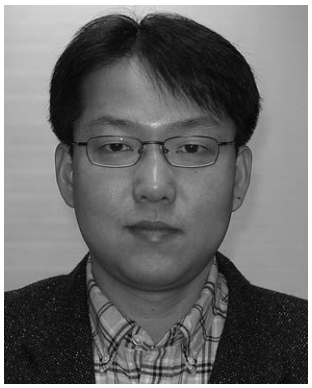
Introduction

Self-assembled nanostructures can possess many different morphologies including spherical micelles, cylindrical micelles, vesicles, bilayers, tubes, ribbons, helices, sheets, belts, networks, and toroids. Studies have shown that nanostructures, depending on their physical parameters such as shape and size, can display remarkably different bioactivities.[1–7] Moreover, it has been reported that nanostructures can display size and shape-dependent cytotoxicity profiles.[8–13] Importantly, the functional properties of nanostructures can be controlled by their size and shape.

Among the many different morphologies, toroid or nanoring structures have unique symmetrical and annular shapes. When compared to the more common cylindrical micelle nanostructures,



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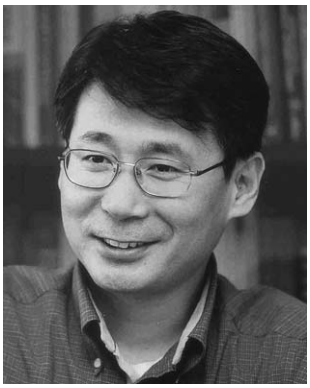

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Myongsoo Lee received a bachelor degree in Chemistry from Chungnam National University, Korea, in 1982 and his Ph.D. degree in Macromolecular Science from Case Western Reserve University in 1993. After a short postdoctoral appointment at University of Illinois at Urbana-Champaign, he joined the Faculty of Chemistry at Yonsei University (1994) and then moved to Seoul National University in 2009, where he is presently Fellow Professor of Chemistry. In 2002, he became a director of National Creative Research Initiative Center for Supramolecular Nano-Assembly. He received the PSK–Wiley Polymer Science Award for Young Scientist (2001), Yonsei Academy Award (2003), Scientist Award of This Month, Korea Ministry of Science & Technology (2006), Samsung Polymer Science Award (2008), and KCS Academy Award (2009). His main research interests include self-assembling molecules, controlled supramolecular structures, and peptide assembly.

relatively few examples of toroidal nanostructures have been reported to date. Despite the relatively small numbers of studies, reports have shown that various types of self-assembling molecules can form toroidal nanostructures. Self-assembled toroidal nanostructures have been found in amphiphilic block copolymers,[14–17] surfactants,[18,19] DNAs,[20,21] rod-coil amphiphiles,[22–25] peptides,[26–29] proteins,[30–32] and other building blocks.[33,34] In recent years, the amount of literature concerning the observation of self-assembled toroidal nanostructures, either serendipitously found or rationally designed, has increased. This is probably due to advanced nanostructural characterization techniques, widely available electron microscopes, and the growing interest in self-assembled nanostructures.

Toroidal protein structures found in nature

Folded protein structures similar to toroids are ubiquitous in nature. Among them, two of the most important protein folds are β-barrels and α/β proteins. β-barrels are protein folds in which the first β-strand is connected to the last β-strand by hydrogen bonds, forming closed circular structures (Fig. 1a).[28,35] There are a variety of functions that β-barrel proteins can perform and their functional categories are still expanding. α/β Proteins are also closed circular structures, where β strands form the inner wall and α helices form the outer wall of toroids (Fig. 1b). Typically, α/β proteins are referred to as TIM barrels, whose name is derived from triosephosphateisomerase, the first example of a α/β protein.[36] The canonical TIM barrel is composed of 8 modular units, each consisting of a β-strand and a α-helix that are connected by a βα-loop. TIM barrels with more than 8 units can also be found in nature.[37,38] The α/β protein is the most common fold among protein catalysts, appearing in approximately 10% of all known enzyme structures.[39] It is seen in many different enzyme families, catalysing completely unrelated reactions.

Formation of toroids from the self-assembly of peptides

When it comes to developing proteins as low cost and industrially manageable functional materials, certain restrictions must be overcome. First, most proteins, except some thermostable proteins, lose their functionality at high temperature. Second, it is difficult to bulk produce proteins consisting of hundreds of amino acids by the expression from cells. Third, the tailor-made construction of proteins with desired functionality and structure sometimes requires chemical modification of specific amino acid side chains, which can be difficult.

A peptide is a molecule consisting of amino acids linked by peptide bonds. They have the same chemical structure as proteins, but are shorter in length. Recently, self-assembled nanostructures based on peptide building blocks have been increasingly investigated. One of the fundamental advantages of peptide-based building blocks is that their constituent amino acids are bio-derived biocompatible materials. Additionally, compared to supramolecular building blocks based on synthetic polymers, another important characteristic feature of peptide building blocks is their monodisperse molecular weight distribution. Due to their monodisperse property, lot-to-lot variation can be minimized between different preparations. When appropriately designed either as a pure peptide molecule or as a peptide conjugated with other types of molecules (*e.g.*, peptide-fatty acid conjugate or peptide-polymer conjugate), a peptide can be made to self-assemble, thereby creating self-assembled peptide nanostructures or artificial nano-proteins.

Self-assembled peptide β barrel nanostructures

With the aim of fabricating self-assembled peptide nanostructures similar to naturally occurring toroidal proteins in terms of structure, we recently developed several rational approaches to construct toroidal nanostructures from relatively simple peptides. β-Barrel proteins are found in two different forms in nature, cytoplasmic and transmembrane. Cytoplasmic β-barrel proteins are water-soluble whereas transmembrane β-barrel proteins are water-insoluble amphiphiles. In order to devise the general form of peptide building blocks that would make the construction of both forms of β-barrels possible, we designed an organic/peptide hybrid T-shape building block that consisted of a β-sheet forming peptide and a oligoether dendron (Fig. 2).[28]

Despite the fact that the peptide building block has a relatively low molecular weight compared to that of a natural β-barrel protein, it is able to form discrete β-barrel nanoring structures. The bulky and hydrophilic dendron in the middle part of the peptide creates curvature at the interface between each building block due to the steric repulsion between the dendritic chains. When the critical force balance between the self-assembly driving force and the opposing force is satisfied, nanorings are formed. The nanoring diameter is highly uniform (~11 nm), thereby indicating that there is a preferred geometrical packing requirement for nanoring formation. The nanorings are composed of a single layer of building blocks, with the β strands oriented perpendicular to the plane of the nanoring to be accommodated into a highly bent structure. The FT-IR results show the presence of amide I contours for the β-sheet doublet at 1,631 and 1,691 cm^{-1}, revealing that the β-sheet structure is in an anti-parallel conformation within the nanorings. Considering that natural β-barrel proteins consist of single but very long polypeptide chains, it is remarkable that simple and relatively small peptide building blocks can mimic natural β-barrel protein folds through a pure non-covalent self-assembly process.

The unique feature of this system is the formation of discrete water-soluble β-barrel nanoring structures that are highly similar to natural β-barrel proteins in terms of structure and composition, which can then be transformed into transmembrane β-barrel pores by simple manipulation of their molecular structure. As the thickness of the nanoring (~4 nm) is large enough to span common lipid bilayer membranes(2–4 nm),[40] the transmembrane β barrels, if successfully formed, may act as pores or channels. To enable the outer surface of β barrels to interact with the inner hydrophobic space of a lipid bilayer membrane, a T-shaped building block was designed to have a hydrophobic hydrocarbon dendron instead of the hydrophilic oligoether dendron. Experiments showed that the hydrophobic building blocks can be incorporated into the lipid bilayer while forming β sheets. The black lipid

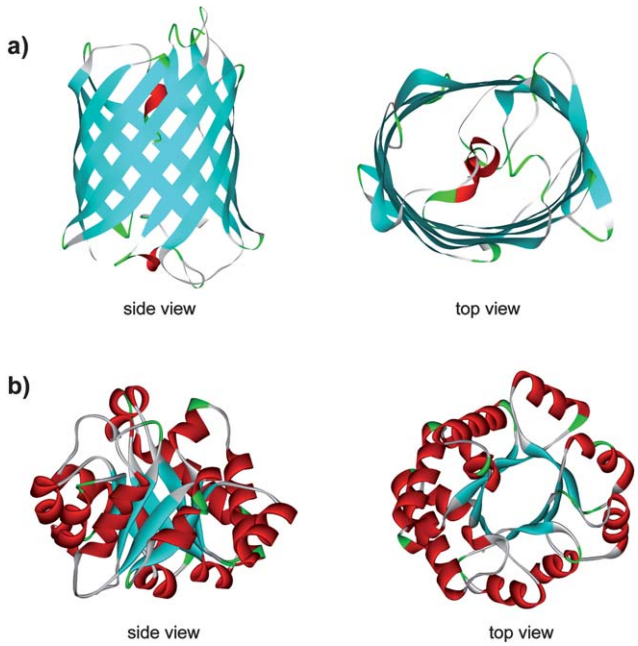

Fig. 1 (a) Typical structure of β-barrel proteins. Here, the structure of green fluorescent protein (GFP) is presented. PDB accession code: 3ADF. (b) ATIM barrel from triosephosphateisomerase. PDB accession code: 2X2G.

membrane experiment revealed the ion transport behavior of the incorporated structure, manifesting the formation of β-barrel pores.

The molecular arrangement of amino acid side chains significantly affected the ion transport behavior of the membrane incorporated β-barrels. When the β-barrel formed with hydrophobic side chains at the inner surface, it showed positive conductivity, thus indicating the flow of positively charged ions thorough the pore. In contrast, the β-barrel displayed negative conductivity when the inner surface consisted of hydrophilic amino acid side chains. These interesting results suggest that polarity and ionization state of the inner surface influence the ion selectivity of β-barrels. Therefore, this unique β-barrel system can be developed as functional membranous nanostructures that can selectively transport molecules depending on their charge.

By using small synthetic peptides as building blocks, the study shows that control of the polarities of the inner and outer surfaces in the self-assembled β-barrel nano-proteins is possible. Hence, this study lays a foundation for the development of versatile and biocompatible β-barrel protein mimic that can displace the diverse biological functions of natural β-barrel proteins with enhanced properties or with functions unprecedented in nature.

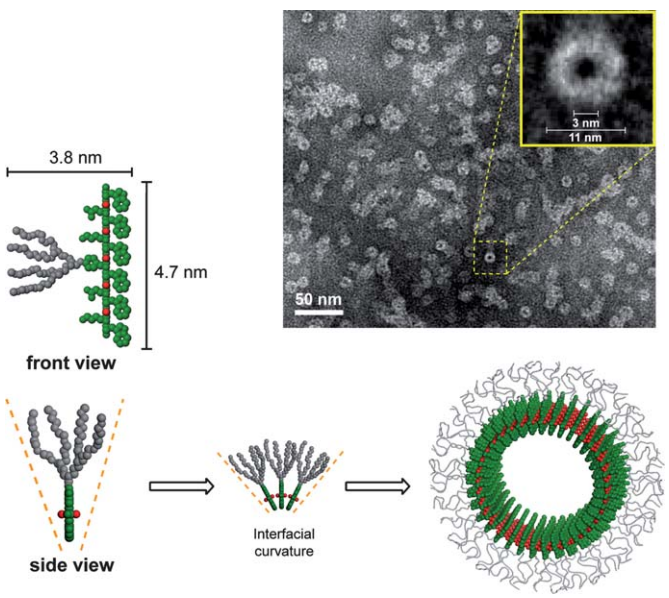

Fig. 2 Self-assembly of T-shaped peptides into toroidal β-barrel nanostructures. Reproduced in part from ref. 28. 2011 Wiley-VCH Verlag Gmb H & Co. KGaA. Used with permission.

Self-assembled peptide α/β protein nanostructures

Self-assembled peptide nanostructures similar to α/β proteins could be constructed by using macrocyclic peptides.[29] The peptide building block was designed in such a way that both an α-helical peptide segment and a self-assembling segment were located within a single macrocyclic structure (Fig. 3). The peptide macrocycle was designed based on the hypothesis that (1) a cyclic structure will partially stabilize the helical structure by decreasing the conformational entropy of the unfolded state and (2) a self-assembly induced coil-to-rod transition in the β-sheet segment will further constrain and stabilize the helical structure.

The self-assembly of the peptide macrocycles induced the helical structure stabilization followed by the formation of a spherical barrel-like structure. Considering the fact that the α-helix is bulkier than the β-strand, helices packed side-by-side on a sheet would be rotated with respect to each other, thus promoting the formation of spherical objects. In artificial α/β nano-proteins, hydrophobic Trp residues on one side of the β tape are likely to form a hydrophobic core of the spherical objects. The self-assembly of the macrocyclic peptide into the α/β barrel-like nanoring structure due to the bulkiness of the α-helical segment is reminiscent of the self-assembly behavior of the T-shape peptide (*vide ante*). Therefore, the induction of crowdedness can be a general means of preparing β-barrel-like nanostructures.

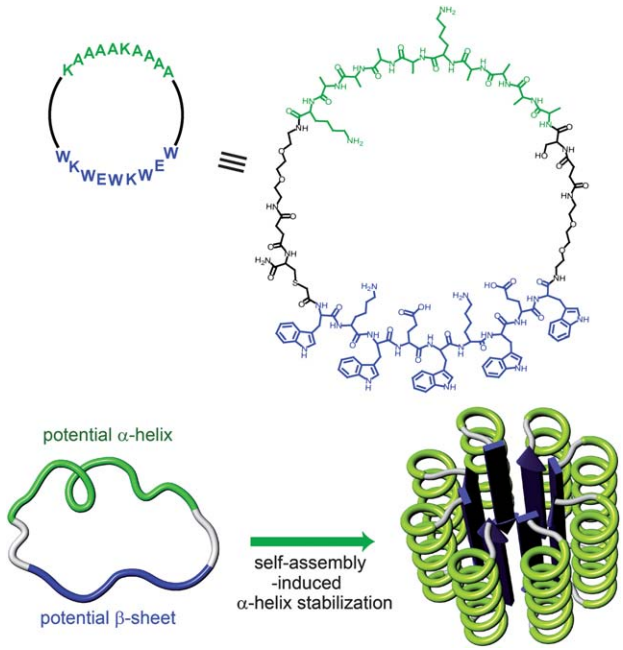

Fig. 3 Self-assembly of macrocyclic peptides into an α-helix-decorated artificial α/β nano-protein. Reproduced in part from ref. 29. 2009 Wiley-VCH Verlag Gmb H & Co. KGaA. Used with permission.

The α-helix, the best known and most common secondary structural unit in proteins, plays an important role in determining the protein structure and function. The α helix is an essential secondary structural motif in proteins. In particular, α helices at the outer surface of proteins plays an important role in specific biomolecular recognition events, such as in protein–DNA, protein–RNA, and protein–protein interactions. Given this premise, the macrocyclic design principle can provide a good starting point for developing artificial nano-proteins that can modulate α-helix-mediated molecular recognition occurring in a multi-valent fashion.[41–43]

Self-assembled β-sheet peptide nanorings

The β-sheet fibrils are organized in such a way that each β-strand runs perpendicular to the fibril axis (cross-β structure). Regardless of the natural or artificial origin of β-sheet fibrils, the cross-β structure has mostly resulted in the formation of one-dimensional (1D) filamentous nanostructures. However, it has been found that toroidal structures can be formed as discrete intermediates during the fibrillization of β-amyloid and α-synuclein.[44]

Cylindrical micelles collapse to form toroids *via* end-to-end or end-to-body connections when there is a fine balance between the conformational entropy and the end-cap energy. A large proportion of the reported toroidal structures has been observed in synthetic amphiphiles with charged hydrophilic blocks, where the presence of ionic species (*e.g.*, salts) plays an important role in the toroid formation.[14–16]

Based on these facts, we questioned whether β-sheet peptides can be made to self-assemble into toroidal nanostructures by adopting molecular structural requirements for toroid formation from synthetic amphiphiles. We envisioned a β-sheet peptide-based block molecule conjugated with a highly charged and flexible hydrophilic block, anticipating that the self-assembly behavior may be similar to that of the synthetic amphiphiles with charged hydrophilic blocks in aqueous, salt-containing solutions. With this in mind, a block copolypeptide was designed in which a charged hydrophilic block of poly (L-arginine) is attached to a β-sheet forming block (Fig. 4a).[27] The multiple positively charged arginine residues are placed between flexible oligo(ethylene glycol) linkers. It has been shown that when coupled to a β-sheet peptide, oligo (ethylene glycol) or poly(ethylene glycol) inhibits the formation of higher aggregates and enhances the solubility of peptide nanostructures in an aqueous solution.[45,46]

The self-assembly behavior of the block copolypeptide was investigated in phosphate buffered saline (PBS), a physiological buffer, by using circular dichroism (CD) spectroscopy, FT-IR, TEM, AFM, dynamic light scattering (DLS), and a zeta potential (ζ) analyser. As shown in Fig. 4b, discrete toroidal nanostructures were found to coexist with β-ribbon nanostructures under optimal conditions. The toroidal diameter was highly uniform(9.8 ± 0.8 nm), indicating that there is a preferred geometrical packing mode for toroid formation. The coexistence of toroids and β-ribbons, together with the uniform toroid sizes, suggests that toroids are likely to form *via* end-to-end connections of β-ribbons (Fig. 4c). To confirm that the toroids are not formed during a solvent evaporation process or by staining reagent while preparing TEM samples on the grid, cryo-TEM and DLS experiments, which are solution-based techniques, were performed. The results from these experiments were in clear accordance with the negative-stain TEM data, indicating that toroids were formed in the bulk solution.

The N-terminal part of the block copolypeptide could be modified to contain carbohydrates such as mannose and glucose, without compromising the ability to form toroidal nanostructures. In order to see if multiple copies of carbohydrates in the toroidal nanostructures could enhance the affinity of carbohydrate–protein interactions *via* the "glycosidic cluster effect", lectin agglutination assays were performed.[2,47] Upon addition of lectin (fluorescein-labelled Concanavalin A) to the nanostructure, the solution became turbid due to aggregate formation and the subsequent aggregate precipitation over time, indicating multivalent interactions between the toroids and the lectins. These results imply that the carbohydrate-decorated toroids can be used as multi-valent ligands with unique circular nanogeometry.

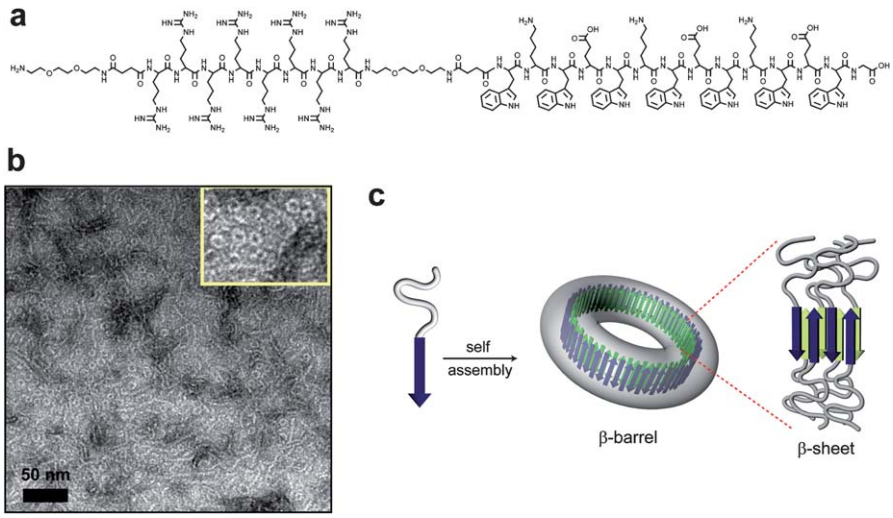

Fig. 4 (a) The representative chemical structure of a block copolypeptide based on poly(L-arginine) and β-sheet peptide. (b) Negative-stain TEM image of the block copolypeptide. (c) Schematic representation of a toroidal nanostructure from the block copolypeptide. Reproduced in part from ref. 27. 2011 Wiley-VCH Verlag Gmb H & Co. KGaA. Used with permission.

Conclusions

Nanostructures are intermediately sized between molecular structures and micrometre-sized objects. Nanostructures and nanomaterials have recently garnered significant attention due to their unique and valuable properties compared to those of conventional bulk materials. Nanostructures can display significantly different properties depending on their physicochemical parameters such as size, morphology, and stability. Peptides, as building blocks for bottom-up self-assembly, can become very useful biomaterials due to their biocompatibility, structural diversity, and similarity to proteins. The toroidal peptide nanostructure represents a novel class of peptide nanostructures and should be useful in fabricating tailor-made materials for displaying structurally stabilized and multiple peptide-decorated nanostructures, modulating biological multivalent interactions, fabricating smart membrane transport systems in artificial and live cell membranes.

Acknowledgements

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