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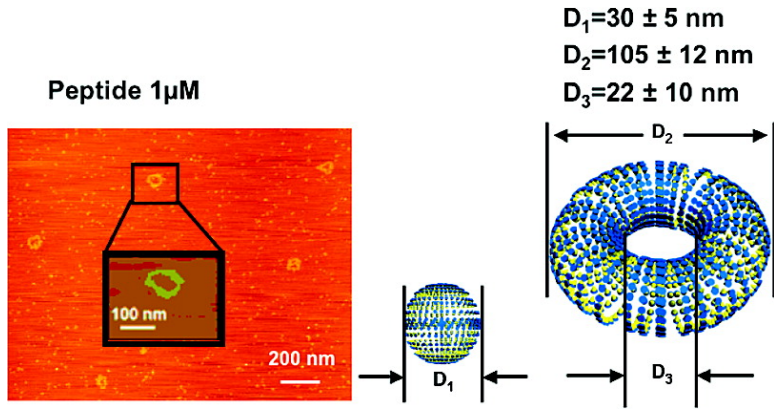

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Self-Assembly of Nanodonut Structure from a Cone-Shaped Designer Lipid-like Peptide Surfactant†

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We report here the donut-shaped nanostructure formation from the self-assembly of a designer lipid-like amphiphilic cone-shaped peptide. The critical aggregation concentration was measured using dynamic light scattering in water and phosphate-buffered saline. The dynamic self-assembly of the peptide was also studied using atomic force microscopy. We have studied numerous peptides over 17 years, and this is the first time that we have ever observed the nanodonut structure from cone-shaped peptides. We propose a plausible self-assembling pathway of the nanodonut structure that was self-assembled through the fusion or elongation of spherical micelles. Furthermore, the bending of the nanostructure gives rise to the nanodonut structures as a result of the tension originating from the interaction of the cone-shaped peptide side chains. Our observations may be useful for further fine tuning the geometry and shape of a new class of designer peptides and their self-assembled supramolecular materials for diverse uses.

1. Introduction

Designer peptides and proteins have become one of the most versatile natural building blocks for the nanostructure fabrication of materials because of their chemical, conformational, and functional diversities.[1] These nanostructured biomaterials from the self-assembly of DNA, peptides, and proteins have demonstrated potential applications in biosensors, controlled-released medicine, 3D cell cultures, reparative and regenerative medicine, as scaffold to organize metal nanocrystals and fabricate metal nanowires, and more.[2,3]

The self-assembly of designer peptides has attracted considerable attention from researchers in various fields. The reason is that designer peptides can be considered to be a simplified model for gaining a better understanding of the protein self-assembly mechanism.[1] Also, some amyloid diseases are related to peptide self-assembly, including Alzheimer's disease, Parkinson's disease, Huntington's disease, type II diabetes, and prion disorders.[4]

In our previous reports, several types of biomaterials including nanofibers,[5−11] nanocoatings,[12] and other nanostructures[13] have been developed using the self-assembly of several classes of designer peptides. These peptide nanostructures have demonstrated applications in (1) scaffold hydrogels for 3D cell cultures, (2) scaffolds for tissue repair and tissue regeneration, (3) tissue engineering, (4) stabilizing membrane proteins, (5) scaffold carrier for small molecules and protein slow releases, and (6) artificial transmembrane channels.[14−17]

Two classes of designer self-assembling peptides have been reported on the basis of both electrostatic and hydrophobic interactions: "peptide lego" for peptide nanofiber hydrogel scaffold and "peptide surfactant" for membrane protein stabilization. Besides the interactions, the dimensions and shapes of the supramolecular structures also depend on other factors such as the geometry of the polar headgroup and the geometrical constraints of the peptide itself.[18,19] Thus, it is possible to fine tune the supramolecular structures with expanded functionalities of peptide surfactants by introducing different shapes and structures.

Here we report the design of a cone-shaped amphiphilic peptide Ac-GAVILRR-NH_2 and studies on its self-assembling behavior using dynamic light scattering (DLS) and atomic force microscopy (AFM). The critical aggregation concentrations (CAC s) of the cone-shaped peptide in water and in buffer solution were measured using the DLS method. The self-assembled nanodonut structure and the dynamic formation mechanism as were also studied using AFM techniques because a detailed understanding and knowledge of the mechanism of the well-formed nanostructures is valuable for the design and controlled fabrication of nanostructured biomaterials. We also propose a plausible self-assembling pathway of the nanodonut structure. The nanodonut structure

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was formed through the fusion or elongation of spherical micelles, and the bending of the nanopipe gives rise to the formation of the nanodonut structures as a result of the tension originating from the interaction of the cone-shaped peptide side chains.

2. Materials and Methods

2.1. Peptide Design and Preparation. The cone-shaped lipid-like peptide Ac-GAVILRR-NH_2 has a hydrophilic head with two positive charges and a relatively large size and a hydrophobic tail with decreasing hydrophobicity and side-chain size. The peptide was synthesized and purified by SynBioSci, Livermore, CA (www.synbiosci.com). The peptide was received as a powder and was dispersed in Milli-Q water to a concentration of 5 mg/mL. The suspension was sonicated for 10 min and then incubated in a water bath at 55 °C for 10 min. Then the pH value of the solution was adjusted to 12.71 using a 1 M NaOH solution for the solubilization of the peptide. Finally, the peptide solution was kept at room temperature in a small centrifuge tube for DLS and AFM measurements.

2.2. DLS Sample Preparation. The peptide solutions were serially diluted twice to each concentration by adding Milli-Q water or phosphate-buffered saline, PBS (100 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4). An aliquot of 200 μL of the peptide solutions in various concentrations was used to perform DLS experiments using PDDLS/batch. The scattered light was collected at 90°, and the number of the photons falling on an avalanche photodiode was detected as the intensity of the scattered light. All of the DLS data were acquired and displayed by using the Precision Deconvolve program. These experiments were repeated two or three times for each sample, and the intensity was an average of 50 data points.

2.3. AFM Sample Preparation. To experimentally observe the dynamic self-assembly behavior of the lipid-like cone-shaped peptide Ac-GAVILRR-NH_2, AFM was used to follow its structure. AFM is a useful tool for observing structure in fine detail with minimal sample disturbance because there is no sample treatment except loading it onto the mica surface.[11] The peptide solutions were diluted to a desired concentration using Milli-Q water, and then a droplet of solution (3 μL) was deposited onto a freshly cleaved mica surface. After the solvent was completely evaporated, the AFM measurements were performed with a Nanoscope IIIa system and a Dimension 3100 system (Veeco Metrology) operated in tapping mode under ambient conditions. The cantilevers used in AFM imaging are silicon cantilevers with a typical spring constant of 30 $N \cdot m^{-1}$ and a resonance frequency of 280 kHz. Images are flattened for better illustration.

3. Results and Discussion

3.1. Structural Features of the Cone-Shaped Ac-GAVILRR-NH_2 Peptide. Seven-residue peptide Ac-GAVILRR-NH_2 is an amphiphilic molecule with a hydrophobic tail and a hydrophilic head. Two cationic amino acids (arginine) are chosen as the hydrophilic head, which are positively charged at neutral pH and are relatively large. The hydrophobic tail has five residues with successively decreasing hydrophobicity and amino acid size so that peptide Ac-GAVILRR-NH_2 has a cone shape as shown in Figure 1A. The dimensions of the peptide molecule are measured using Chem3D-Pro with an energy minimization approximation. The length is around 2.3 nm, which is similar to the length of the phospholipid molecules. The diameter of the hydrophilic head is around 1.2 nm, and the diameter of the hydrophobic tail was gradually decreased according to the molecular structures. A schematic model was proposed to illustrate the cone-shaped molecular model of Ac-GAVILRR-NH_2 (Figure 1B). The blue cap stands for the hydrophilic head, the positively charged arginine, with longer side chains, and the yellow pillar stands for the hydrophobic tail with shorter side chains. This unique cone-shaped geometry would not only add to the repertoire of the self-assembling nanostructures but also deepen the understanding of the mechanisms of the peptide self-assembly.

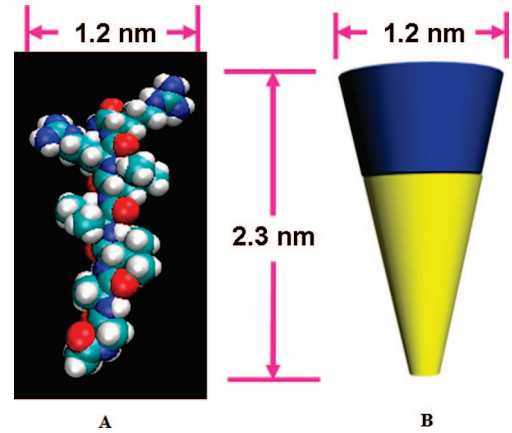

Figure 1. (A) Molecular model of Ac-GAVILRR-NH_2. The peptide length is approximately 2.3 nm, and the width is 1.2 nm. Color code: hydrogen, white; carbon, cyan; oxygen, red; and nitrogen, blue. (B) Cone-shaped model stands for the outer shape of the Ac-GAVILRR-NH_2 molecule. The blue part indicates the positively charged hydrophilic region, and the yellow part indicates the hydrophobic region.

3.2. CAC Measured Using Dynamic Light Scattering. The CAC, also commonly referred to as the critical micelle concentration (CMC), is a value at which the energetically favorable release of water molecules around the hydrophobic regions of the detergents becomes more significant, causing the molecules to self-assemble into micelles. Light scattering has been proven[20] to be a powerful tool for determining the CAC value because the number of photons scattered by the solution is dependent on the number and size of the surfactant aggregates.[21] When the concentration is lower than the CAC's, the surfactants are mostly monomers and/or small clusters, so the light scattering intensity is negligible. When the concentration is above the CAC, the peptides begin to self-assemble into dynamic nanostructures, which causes the intensity as detected by DLS to undergo an exponential increase as a function of concentration. Thus, the CAC can be obtained by the intersection of two lines, which constitute a linear fit of intensities in the two concentration ranges. As shown in Figure 2A, the CAC in water is 0.82 mM, which is highlighted by a red line at the intersection of the two black solid lines. Ac-GIVILRR-NH_2 has a lower CAC in PBS buffer solution, ~0.45 mM as shown in Figure 2B. The lower CAC value in PBS solution indicates a readiness to self-assemble at lower peptide concentration. This phenomenon is well known and is explained below. The charge-screening effect of the salt can reduce the electrostatic repulsion between the hydrophilic heads for better aggregation according to the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory.[22,23] Furthermore, salt ions interact with water, thus reducing the number of water molecules available to hydrate peptides.[24] The CAC in buffer solution is very important in biochemical experiments because nearly all buffer solutions contain salts, including PBS solution. The CAC in buffer solution is more important for the solubilization and stabilization of membrane proteins and is usually measured at or slightly above the CMC of the detergents.

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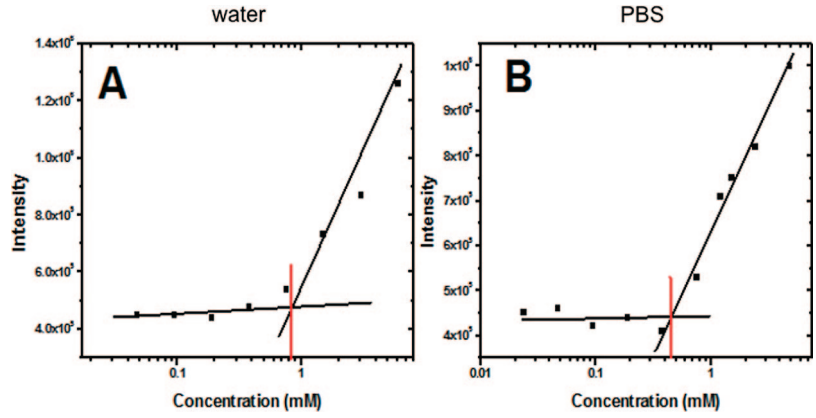

Figure 2. Critical aggregation concentration in water and phosphate-buffered saline (PBS, 100 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) measured by the dynamic light scattering method. The CAC was estimated using the intersection line of the two linear regression lines. (A) In water, the CAC is 0.82 mM. (B) In PBS, the CAC is 0.45 mM.

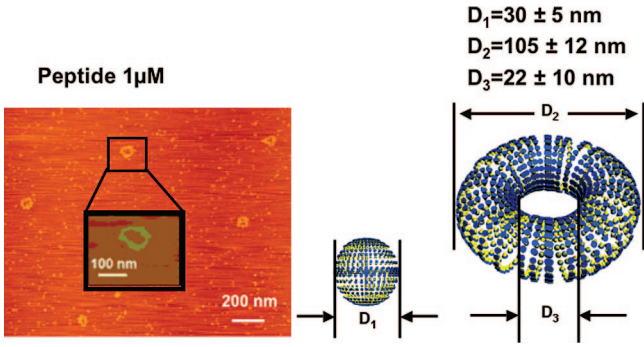

Figure 3. (A) AFM image for the aggregation structures of Ac-GAVILRR-NH_2 in water at concentration of 1 μM. The AFM image indicates the coexistence of nanodonut structures and spherical micelles; the scale bar is 1 μm. The inset shows an enlarged image of the donut-shaped structure; the scale bar is 200 nm. (B) Schematic illustration of the structure of the spherical micelles and the nanodonuts. The average diameter of the micelles (D_1 = 30 ± 5 nm) and the outer and inner diameters of the nanodonut structures (D_2 = 105 ± 12 nm, D_3 = 22 ± 10 nm) are obtained on the basis of a section analysis of tens of nanodonuts in different AFM images.

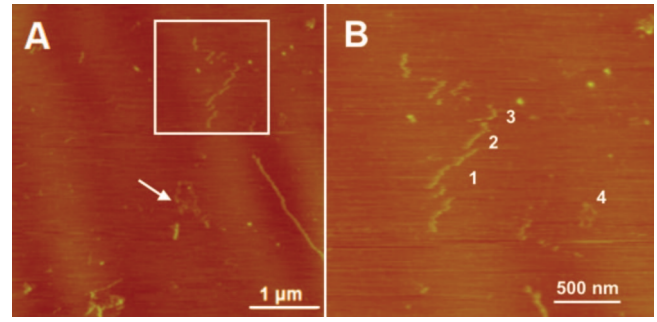

Figure 4. (A) AFM image of aggregation structures at 2 μM in water. Nanopipes with different bending angles indicate the assembly mechanism of nanodonut structures. The white arrow points to the larger nanodonut structures. (B) Enlarged image of the aggregation structures corresponding to the area highlighted by a white square in part A. Numbers 1−4 indicate different bending angles from smaller to larger, and structure 4 exhibits a very similar nanodonut structure with small openings.

3.3. Nanodonut Structure Formation Observed by Atomic Force Microscopy. To study the assembly of cone-shaped peptide Ac-GAVILRR-NH_2, AFM was used to examine images of the peptide assemblies on a freshly cleaved mica surface. The AFM image in Figure 3A shows typical nanostructures of cone-shaped peptide Ac-GAVILRR-NH_2 at a concentration of 1 μM. Many donut-shaped nanostructures were observed to coexist with smaller spherical particles, perhaps micelles. These small spherical nanoparticles are evenly distributed on the mica surface with a diameter of D_1 = ~35 nm. Taking into account the AFM tip broadening effect, the diameters of the particles are ~11 nm assuming the curvature radius of the tip to be ~10 nm, whereas the height of the particles is ~0.5−1 nm. Considering the length of the cone-shaped peptide (2.3 nm), these nanostructures might be a kind of self-assembled monolayer arising from the opening and rearrangement of the micelles when contacting the mica surface. This nanostructures could be a monolayer with hydrophilic heads adsorbed on the mica surface and the hydrophobic tails aligned perpendicular to the surface with a certain tilt angle, which is similar to the previous report.[25] Before contacting the mica surface, the spherical particles in solution may perhaps be micelles of Ac-GAVILRR-NH_2 with an outer layer of cationic heads RR and an inner core of hydrophobic tails GAVIL. A schematic structure of the spherical micelle is depicted on the left side of Figure 3B.

The dimensions of the donut-shaped assemblies were measured using the section analysis. The measured outer diameter is D_2 = ~105 nm, and the inner diameter is D_3 = ~22 nm. The average thickness of the donut-shaped structures is around ~41.5 nm, which is similar to the diameter of the spherical nanoparticles. The height of the nanodonuts is around 0.5−1 nm, which is also similar to that of the deformed micelles on the mica surface. These observations suggest that the nanodonut structures may be evolved from micelle assemblies. According to the micelle structures, the schematic illustration of the nanodonut structure is presumed to be the elongated micelle and is curved to be a closed ring (right panel of Figure 3B).

The formation of the nanodonut structure could be a direct micelle assembly or an indirect transformation through an intermediate structure. Interestingly, AFM observations of some short nanopipe structures with different bending angles are captured in a single image (Figure 4A). These features could be the intermediate states for the transformation of micelles into nanodonut structures. Figure 4B shows the enlarged image of Figure 4A over the selected area indicated by the white square. Numbers 1−4 in Figure 4B show different bending angles from smaller to larger, and some of them exhibit very similar nanodonut structure with small openings (Figure 4B). Because of the local concentration fluctuation on the surface and the low possibility of the occurrence of nanopipes, the correlation between micelles,

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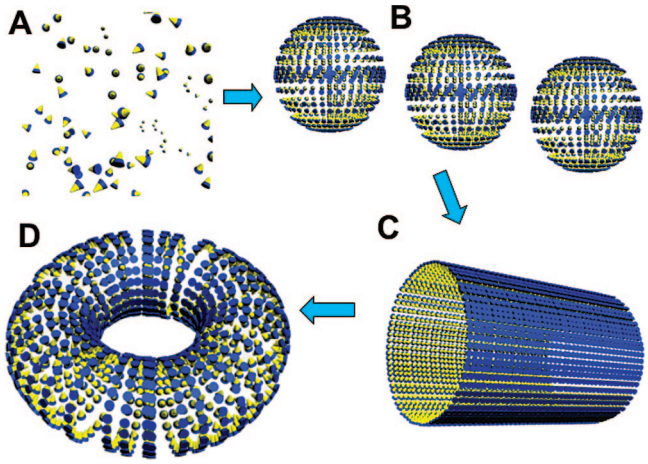

Figure 5. Proposed plausible self-assembly process of the nanodonut structure. (A) Randomly oriented and distributed peptides at low concentration. (B) Micelle formation above the CAC concentration. (C) Fusion or elongation of the micelles for the formation of a nanopipe. (D) Bending of the nanopipe for the formation of a nanodonut structure.

nanopipes, and nanodonuts could be interpreted in two possible ways. One is the fusion of the micelles that leads to the formation of a nanopipe structure and eventually the nanopipe bends into a donut-shaped structure. The other interpretation could be the reverse process in which micelle assembly gives rise to the donut-shaped structure and then the opening of the nanodonut leads to nanopipe structure formation. The occurrence of a larger donut-shaped structure in Figure 4A indicated by the white arrow suggests the possibility of the former pathway. We also observed that more donut-shaped structures are stacked together at higher concentration, which also supports the former pathway of the bending of nanopipes into nanodonuts (data not shown).

3.4. Proposed Plausible Nanodonut Structural Formation. On the basis of the AFM observations, we propose a plausible assembling pathway for the assembly process of the nanodonut structure in Figure 5. In the proposed model, the peptide geometry is simplified to be a cone-shaped structure where blue represents the positively charged head and yellow represents the hydrophobic tail. At lower concentration well below the CAC value, the Ac-GAVILRR-NH_2 peptides are randomly oriented and distributed as monomers or smaller assemblies in solution (Figure 5A). Similar to the common surfactants, the spherical micelles could be formed in the solution at a concentration above the CAC value (Figure 5B). At a higher concentration, the short nanopipe could be formed that could be presumed to be a fusion or elongation of the spherical micelles (Figure 5C). Because of the geometry restrictions of the cone-shaped peptide, the nanopipe tends to bend gradually to form the nanodonut structure in order to minimize the surface free energy (Figure 5D). The impact of the geometry of the peptide on the assembled structures has been reported by Israelachvili, Mitchell, and Ninham,[19] in which the concept of the surfactant packing parameter is illustrated as v/a_0l_C, where v is the hydrophobic chain volume, a_0 is the area per hydrophilic headgroup, and l_C is the length of the hydrophobic tails. When $v/a_0l_C = {}^1/_3$, the assembled structure is spherical, and when $v/a_0l_C = {}^1/_2$, the assembled structure is cylindrical. Because of the gradually smaller side chains in our peptide, the volume of the cone-shaped peptide would be larger than that of the common surfactants, which gives rise to cylindrical packing geometry. Because of the close-packing tendency of the hydrophobic tails, the nanodonut structure is formed by nanopipe bending to increase the interaction between hydrophobic tails, which is a kind of packing geometry between spherical and cylindrical structure. This interesting nanodonut structure from the assembly of cone-shaped peptides might be beneficial for a new class of nanostructured biomaterials for controlled drug-release, artificial transmembrane channels, and more.

We have studied numerous peptides since 1991,[2,5−11,13−17,20,23,24] and this is the first time that we have observed the nanodonut structure self-assembled from cone-shaped peptides. Furthermore, we have also studied several similar lipid-like peptide surfactants without cone-shaped geometry, and no nanodonut structure was ever observed that can be used as a control experiment for the confirmation of the important role of the cone shape in nanodonut formation.

4. Conclusions

From designing a cone-shaped peptide, we open a new way to design peptides and further fine tune the shape of supramolecular structures. The donut-shaped nanostructure from the self-assembly of a cone-shaped peptide was studied using DLS and AFM methods. The CAC values for the Ac-GAVILRR-NH_2 cone-shaped peptide are 0.82 mM in water and 0.45 mM in PBS buffer solution. We proposed a plausible assembly pathway for the nanodonut structure based on AFM observations. The nanopipe was formed by the fusion or elongation of spherical micelles, and the bending of the nanopipe gives rise to the nanodonut structures as a result of the tension originating from the interaction of the cone-shaped side chains. This donut-shaped structure might be useful for stabilizing membrane proteins because it is hypothesized that the peptide surfactant forms a "corset" around the proteins that is similar to donut geometry.[16] This also might be beneficial for the design a new class of nanostructured biomaterials for controlled drug release, artificial transmembrane channels, and beyond.

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