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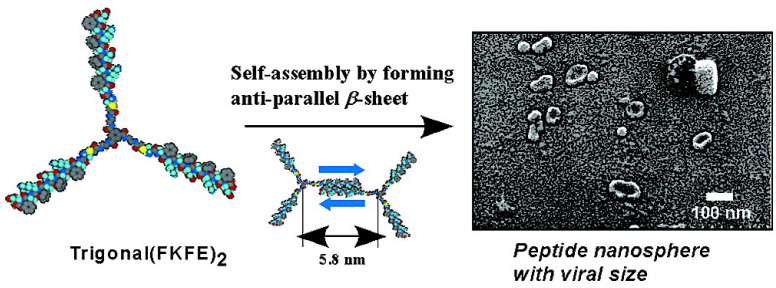

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Artificial Peptide-Nanospheres Self-Assembled from Three-Way Junctions of β-Sheet-Forming Peptides

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Self-assembly of proteins such as formation of viruses, flagella, microtubules, actin fibers, and amyloid fibrils plays pivotal roles in biology.[1] Rational design of such protein nano-assemblies will give new applications not only in biotechnology but also in nanotechnology.[2] To date, fibrous peptide assemblies have been designed based on the formation of β-sheets[3] and leucine zippers.[4] On the other hand, formation of spherical assemblies are limited to the oil-/water-templated protein aggregates,[5] self-assemblies of amphiphilic peptides,[6] aromatic dipeptides,[7] and β-amyloid 1−40 peptides.[8] These spherical assemblies are less exquisite compared to the self-assembly of symmetric capsids in spherical viruses.[1] For example, icosahedral internal skeleton of tomato bushy stunt virus is self-assembled from 60 C_3-symmetric β-sheet subunits.[9] Clathrin lattice, which participates in receptor-mediated endocytosis, is a polyhedral assembly of C_3-symmetric triskelions formed in the presence of Mg^{2+}.[10] The viral and clathrin's self-assembly is thus based on C_3-symmetric architectures with directed intermolecular interactions. This strategy would be generally applicable to the design of artificial, nanosized polypeptide assemblies. We have recently demonstrated self-assembly of DNA three-way junctions with self-complementary sticky ends into mesoscopic spherical DNA assemblies.[11] In this paper, we extend the three-way component design to polypeptides, and show a first example of artificial C_3-symmetric peptide conjugates that self-assemble into viral-sized peptide nanospheres.

As shown in Figure 1, the artificial C_3-symmetric conjugate **Trigonal(FKFE)$_2$** bears three β-sheet-forming peptides FKFEFKFE.[3a] When these peptide arms form antiparallel β-sheets in water, spontaneous self-assembly of peptide networks is expected. **Trigonal(FKFE)$_2$** was prepared by coupling of thiol groups of CFKFEFKFE peptides with C_3-symmetric iodoacetamidated core molecule **1** (Figure 1). The C_3-symmetric **1** was synthesized by condensation of trimesoyl chloride with N-Boc-ethylenediamine, followed by deprotection and iodoacetylation. The 9-mer H-CFKFEFKFE-OH peptide was prepared by standard solid-phase synthesis using Fmoc chemistry (for FKFEFKFE). It was then coupled with Boc-Cys(Trt)-OH and purified by reversed-phase HPLC. The **Trigonal(FKFE)$_2$** conjugate was prepared by coupling the 9-mer peptides with **1** in the presence of DIPEA (in degassed DMF at −20 °C).[12] The crude product was purified by reversed-phase HPLC eluting with a linear gradient of acetonitrile/water (0/100 to 60/40 over 60 min) containing 0.1% TFA (the isolated yield: 8%). Isolation of the compound was confirmed by MALDI-TOF-MS (m/z = 4127 ([M + H]$^+$)). The **Trigonal(FKFE)$_2$** was soluble in acidic water but was hardly soluble in neutral and alkaline water. Thus, the following experiments were conducted in acidic aqueous dispersions (pH 3.3).

The circular dichroism (CD) spectrum of the **Trigonal(FKFE)$_2$** (24 μM) in aqueous HCl (pH 3.3) showed negative peak at 219 nm, indicating the formation of β-sheet structures (Figure 2A).

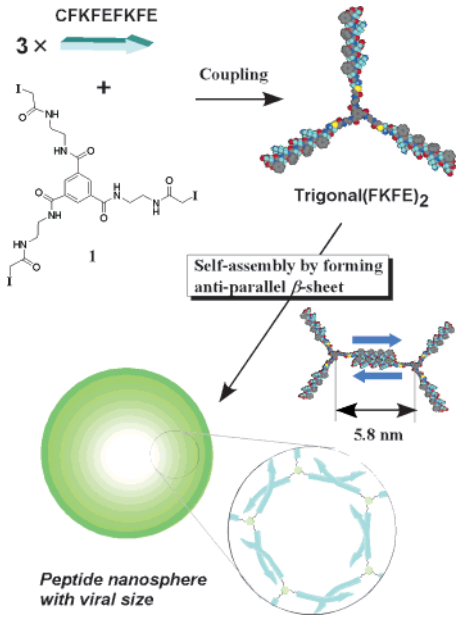

Figure 1. Synthesis of a C_3-symmetric β-sheet peptide conjugate and schematic illustration of the self-assembly.

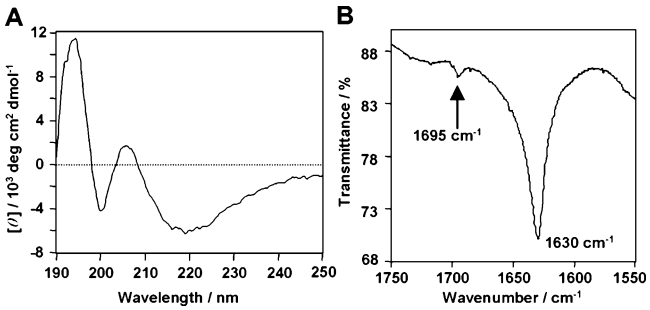

Figure 2. (A) CD spectrum of **Trigonal(FKFE)$_2$** (24 μM) in aqueous HCl solution (pH 3.3) at 25 °C and (B) FT-IR transmission spectrum of **Trigonal(FKFE)$_2$** cast from the acidic aqueous dispersion (24 μM, pH 3.3). Substrate, BaF_2.

Another negative peak at 200 nm is probably ascribed to an induced CD for the benzene ring or amide bonds of the core molecule, since such a CD peak was not observed for the component peptide FKFEFKFE alone. FT-IR transmission spectrum of **Trigonal(FKFE)$_2$** cast on BaF_2 plate from the acidic aqueous solution (24 μM, pH 3.3) showed a strong peak at 1630 cm^{-1} and a weak peak at 1695 cm^{-1} (Figure 2B), which are characteristic of antiparallel β-sheet structures.[13] These results clearly indicate that **Trigonal(FKFE)$_2$** forms hydrogen-bond-mediated intermolecular assemblies, since it is not possible for a single **Trigonal(FKFE)$_2$** molecule to form β-sheets in an antiparallel manner.

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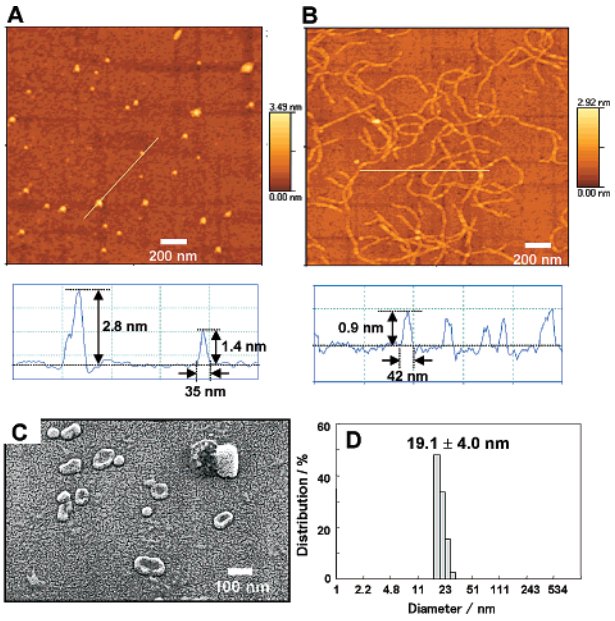

Figure 3. AFM images (noncontact mode) of **Trigonal(FKFE)$_2$** (A) and peptide CFKFEFKFE (B) adsorbed on mica substrate from the aqueous HCl solution (0.1 mg/mL, pH 3.3). (C) SEM image of **Trigonal(FKFE)$_2$** sample (A) coated with Pt (thickness, ca. 3 nm). (D) Size-distribution obtained from DLS of the aqueous HCl (pH 3.3) dispersion of **Trigonal-(FKFE)$_2$** (96 μM, at 25 °C).

Specimens for atomic force microscopy (AFM) of **Trigonal-(FKFE)$_2$** were prepared by placing a drop (10 μL) of the acidic aqueous solution (24 μM, pH 3.3) on a freshly cleaved mica substrate. The droplet was kept for 30 s, and the substrate was rinsed with deionized water. In AFM, many domed structures with *z*-height of 2.3 ± 0.4 nm and diameter of 35−70 nm are abundantly seen (Figure 3A). On the other hand, the component peptide CFK-FEFKFE afforded fibrils[3a] with a *z*-height of ca. 1.0 nm and width of ca. 40 nm (Figure 3B). Scanning electron microscopy (SEM) of **Trigonal(FKFE)$_2$** also showed the presence of spherical nanostructures with the size of 22−34 nm and concave structures with the size of 50−100 nm (Figure 3C). These concave structures might be formed by collapse of the spherical assemblies on mica substrate. Even when the concentration of **Trigonal(FKFE)$_2$** was increased to 96 μM, there was little change in the size and morphology of the assemblies. The average diameter of nanospheres as determined by dynamic light scattering (DLS) was 19.1 ± 4.0 nm (Figure 3D),[14] which nearly matches with the actual size of spherical nanostructures (ca. 16−28 nm) observed in SEM observations by considering the thickness of Pt coating (ca. 3 nm). The *z*-height of the collapsed assemblies of **Trigonal(FKFE)$_2$** on mica observed in AFM corresponds to twice that of peptide CFKFEFKFE fibrils, suggesting that **Trigonal(FKFE)$_2$** might form hollow nanospheres in acidic solution.

When two **Trigonal(FKFE)$_2$** molecules form an antiparallel β-sheet, the distance between two core benzene rings is estimated to be about 5.8 nm from the molecular model. Assuming that the distance is a side of the polyhedron, the diameter of the assembly is calculated to be ca. 26 nm for a soccer ball-like structure and ca. 16 nm for a dodecahedron structure. These estimated diameters are comparable to the average diameter of the nanospheres observed by DLS (19.1 ± 4.0 nm). The absence of fibrous β-sheet assemblies from **Trigonal(FKFE)$_2$**s indicates that the three-armed **Trigonal-(FKFE)$_2$** molecules self-assemble into a finite set of closed aggregates, most likely a soccer ball- or a dodecahedron-like superstructure which is composed of antiparallel hydrogen bonding.[15]

In conclusion, supramolecular peptide-nanospheres are spontaneously self-assembled from artificial C_3-symmetric β-sheet peptides. The present C_3-symmetric strategy would be widely applied to the design of spherical bio-nanoassemblies. As the present C_3-symmetric peptide can be chemically modified with desired functional groups, we envisage their use in many fields such as gene carriers[16] and as nanosized reactors.[17]

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Supporting Information Available: Experimental details, HPLC chart, and MALDI-TOF-MS. This material is available free of charge via the Internet at http://pubs.acs.org. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Since it was difficult to obtain sufficient light-scattering intensity at [**Trigonal(FKFE)$_2$**] = 24 μM, we conducted DLS measurement at the concentration of 96 μM.
(15) Since the size of assemblies was minimally affected by concentration of the components and concave structure of the assemblies were observed in SEM, dendritic or hyperbranched structures might be excluded from candidates.
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