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Molecular assembly composed of a dendrimer template and block polypeptides through stereocomplex formation†

Hisato Matsui, Motoki Ueda, Akira Makino and Shunsaku Kimura*

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A 2nd generation polyamideamine (PAMAM) dendrimer bearing right-handed helices to its eight terminals was shown to accommodate eight left-handed helices *via* stereocomplex formation to generate molecular assemblies of disk structure with 13–14 nm diameter and 6 nm thickness.

Self-assemblies of spherical micelles or vesicles have attracted much attention in the field of drug delivery systems as nanocarriers for imaging probes and drugs.[1–5] Especially, nanocarriers in the size range from 10 nm to 100 nm have been shown to accumulate selectively in solid tumors by the so-called enhanced permeability and retention (EPR) effect.[6–10] The nanocarriers can leak out of the loosened blood tube walls in tumor regions and stay there due to the immature lymphatic draining system. There is, however, a demand for nanocarrier technology for precise size control and facile surface modification with biologically active ligands. One resolution was recently proposed by Tirrell *et al.* to use a 5th generation dendrimer bearing a hydrophobic exterior of alkane chains, which provides an environment to accommodate biologically active peptides modified with alkane chains.[11] But this method has a drawback of poor stoichiometric control because formation of the supramolecular structure is based on hydrophobic interaction and alkyl chain packing. The peptides of 500 molecules thus finally remained in one dendrimer at a feed ratio of 20 : 1 peptide : dendrimer. In the present study, we prepared a molecular assembly using a 2nd generation dendrimer as a template, and amphiphilic peptides were assembled into the dendrimer based on stereocomplex formation between right-handed and left-handed helices. This strategy improves the stoichiometric control of the supramolecular assembly.

Helical peptides of a 12-mer alternating sequence of L-leucine (Leu) or D-leucine (leu) and 2-aminoisobutylic acid (Aib) were synthesized, and a poly(sarcosine) block was extended from the N-terminal of the helical peptides. A mixture of these amphiphilic block polypeptides was shown to self-assemble into planar sheets, which were transformed into vesicles upon heating.[12,13] The driving force of the molecular assembly process is explained by stereocomplex formation between right-handed and left-handed helices in the hydrophobic core region of the sheets due to the convexo-concave fitting between their surfaces. On the basis of these results, we introduced the amphiphilic block peptide with the right-handed helix as a hydrophobic block to eight terminals of a 2nd generation dendrimer (**8RD**). We studied here supramolecular assembly of **8RD** with the amphiphilic block peptide with the left-handed helix as a hydrophobic block due to stereocomplex formation (Fig. 1a).

A hydrophobic dendrimer core was synthesized using 1,3-diaminopropane as a nucleus and (Boc–NH–$(CH_2)_3)_2$–N–CH_2COOH as branches (compound **5**) (Scheme S1, ESI†). After synthesizing the 2nd generation dendrimer, 4-pentynoic acid with an alkyne group was condensed to eight terminals of the 2nd generation dendrimer (Fig. 1b). On the other hand, 5-azido pentanoic acid was connected to the C-terminal of the amphiphilic right-handed block peptide (**RP** in Fig. 1). The click chemistry was used for preparation of **8RD** (Schemes S2 and S3, ESI†).

The supramolecular assembly was analysed by dynamic light scattering (DLS) measurements and transmission electron

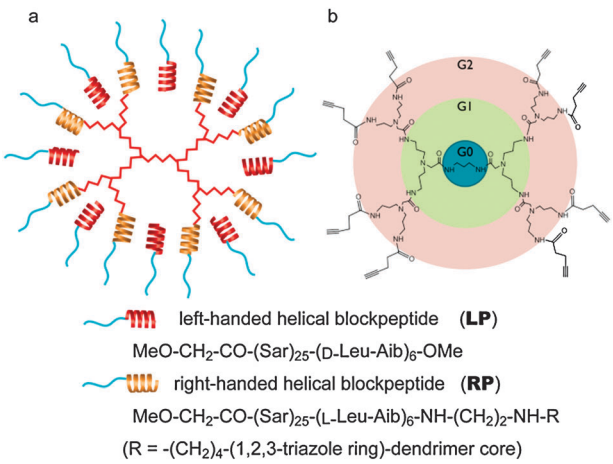

Fig. 1 The diagrams of molecular design: (a) the cartoon-like diagram of a co-assembling 2nd generation dendrimer template bearing amphiphilic right-handed block peptides (yellow helix) with amphiphilic left-handed block peptides (red helix), (b) the schematic diagram of the 2nd generation dendrimer core emphasized on the generation number.

Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto, 615-8510, Japan. E-mail: shun@scl.kyoto-u.ac.jp; Fax: +81 75-383-2401; Tel: +81 75-383-2400

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microscope (TEM) observations by varying the feed molar ratios of the amphiphilic left-handed block peptide (**LP** in Fig. 1) and **8RD**. The molecular assemblies were prepared by the injection method. An ethanol solution of **8RD** and **LP** at the specified feed ratio was injected into 1 mL of a 10 mM Tris buffer solution (pH 7.4, added 0.15 M NaCl). For example, the molecular assemblies at 8 : 1 **LP** : **8RD** were prepared by injecting an ethanol solution of 2.50 μL of **LP** (0.05 mg μL^{-1}) and 1.41 μL of **8RD** (0.1 mg μL^{-1}) into 1 mL of the buffer solution.

The hydrodynamic diameters of the self-assemblies have a minimum value of 42 nm at the feed molar ratio of 8 : 1 **LP** : **8RD**, suggesting that the assembly occurs in a stoichiometric manner of 1 : 1 association between the right-handed helices of **8RD** and the left-handed helices of **LP**. Below the **LP** : **8RD** feed molar ratio of 8 : 1, lowering the ratio further resulted in an increase in the hydrodynamic diameter up to a maximum of 150 nm, the value for pure **8RD**. **8RD** by itself therefore could form self-assemblies (sheets of 100 nm), whose proportion in the total self-assemblies however decreased with the addition of **LP**. **LP** therefore associates with **8RD** to generate smaller self-assemblies (disks of 50 nm). On the other hand, at the feed molar ratios higher than 8 : 1, the hydrodynamic diameters also increased because self-assemblies of pure **LP** (hydrodynamic diameter of 200 nm; curved sheets of 200 nm) increased their proportion in the total self-assemblies (Fig. S1 in ESI†).[12] The fractions of the sheets of 100 nm in the self-assemblies of 200 images shown in the TEM images decreased in the order 4 : 1 **LP** : **8RD** (163 : 200) > 8 : 1 **LP** : **8RD** (13 : 200) > 16 : 1 **LP** : **8RD** (8 : 200). The fractions of the disks of 50 nm shown in the TEM images increased in the order 4 : 1 **LP** : **8RD** (37 : 200) < 8 : 1 **LP** : **8RD** (187 : 200) < 16 : 1 **LP** : **8RD** (190 : 200). On the other hand, the curved sheets of 200 nm were not detected with 4 : 1 **LP** : **8RD** (0 : 200) and 8 : 1 **LP** : **8RD** (0 : 200), but appeared at 16 : 1 **LP** : **8RD** (2 : 200). One molecule of **8RD** is therefore considered to accommodate eight **LP** molecules probably in a stoichiometric manner.

The supramolecular assembly of **LP** with **8RD** was analysed by TEM observations. The TEM micrographs of molecular assemblies prepared at the feed molar ratios of 0 : 1, 8 : 1, and 32 : 1 (**LP** : **8RD**) are shown in Fig. 2a–c. **8RD** self-assembled into highly curved sheets, and the sizes of self-assemblies (the average size of 50 nm assemblies in the micrographs) decreased to 67 nm and 44 nm upon increasing the feed ratios to 2 : 1 and 4 : 1, respectively (Fig. S2a–d, ESI†). The added **LP** therefore acted as an inhibitor for **8RD** association for itself due to **LP** insertion into **8RD**. The self-assemblies were transformed into small planar sheets at the feed molar ratio of 8 : 1 (Fig. 2b and Fig. S2e, ESI†). These planar sheets remained as they were at the feed molar ratios of 16 : 1, 24 : 1, and 32 : 1, whilst the amounts of curved sheets increased (Fig. 2c and Fig. S2f–h, ESI†). The excessively added **LP** to more than 8 equivalents of **8RD** therefore self-assembled into curved sheets by themselves. The TEM observations also support the interpretation of DLS results that eight molecules of **LP** and **8RD** associate in a stoichiometric way. Simple calculation of surface areas of **8RD** (about 2.6 nm diameter) and cross-section area of **LP** (α-helix of about 1.2 nm diameter)

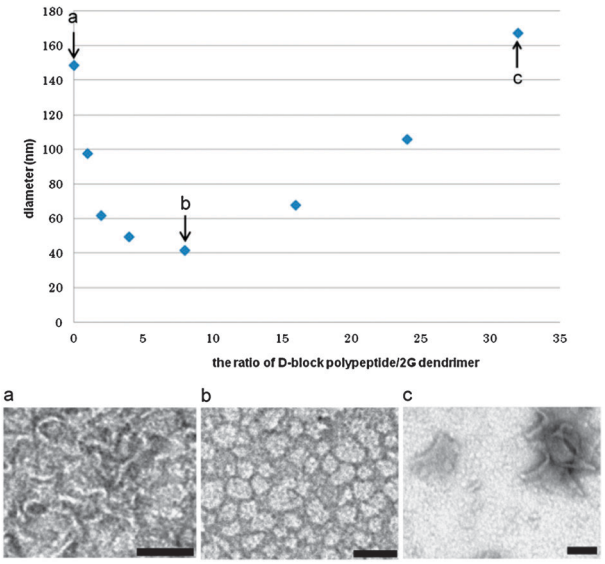

Fig. 2 Hydrodynamic diameters of self-assemblies prepared by varying the feed molar ratios of **LP** : **8RD**. Negative staining TEM micrographs at the feed molar ratios of (a) 0 : 1, (b) 8 : 1, and (c) 32 : 1. Each scale bar represents 50 nm.

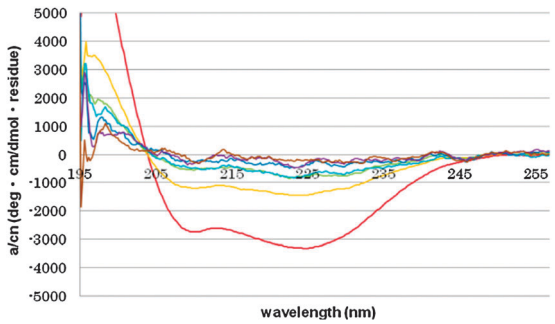

Fig. 3 Circular dichroism spectra of self-assemblies prepared by varying the feed molar ratios of **LP** : **8RD** after purification by using size-exclusion chromatography and a cut-off filter. Each curve shows 0 : 1 (red), 2 : 1 (yellow), 4 : 1 (green), 8 : 1 (sky blue), 16 : 1 (blue), 24 : 1 (purple), 32 : 1 (brown).

indicates that **8RD** has a space to accommodate 10–11 **LP** molecules, which is also agreeable with the interpretation.

The self-assemblies were analysed by circular dichroism (CD) measurements (Fig. 3). The self-assemblies of pure **8RD** showed double minima at 208 and 222 nm indicating α-helical structure.[14] The self-assemblies prepared at the feed molar ratios of 2 : 1 and 4 : 1, which were purified by a PD-10 column (packed Sephadex G-25, GE Healthcare), decreased the intensities of the Cotton effects upon increasing the amounts of **LP**, and no signal was observed with the self-assemblies prepared at the feed molar ratio of 8 : 1. The self-assemblies prepared at the feed molar ratios of 16 : 1, 24 : 1, and 32 : 1, which were purified by a PD-10 column and a cut-off filter of 0.1 μm to obtain self-assemblies of several tens of nm, show no signal at all. These results also support the interpretation that no more than eight molecules of **LP** are inserted into **8RD** even in the presence of excess amounts of **LP**.

The molecular assemblies prepared at the feed molar ratio of 8 : 1 were sonicated for 10 seconds to obtain relatively homogeneous disk-shaped assemblies (Fig. 4). The dimensions

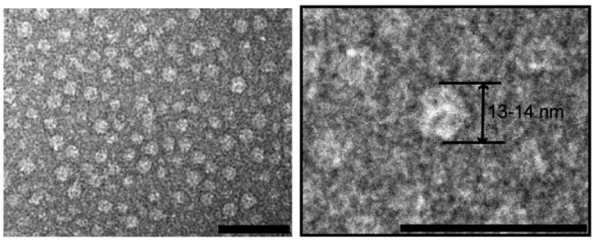

Fig. 4 Negative staining TEM micrographs of self-assemblies prepared at the feed molar ratio of 8 : 1 with sonication for 10 seconds. The right micrograph is the magnified one of the left micrograph. Each scale bar represents 50 nm.

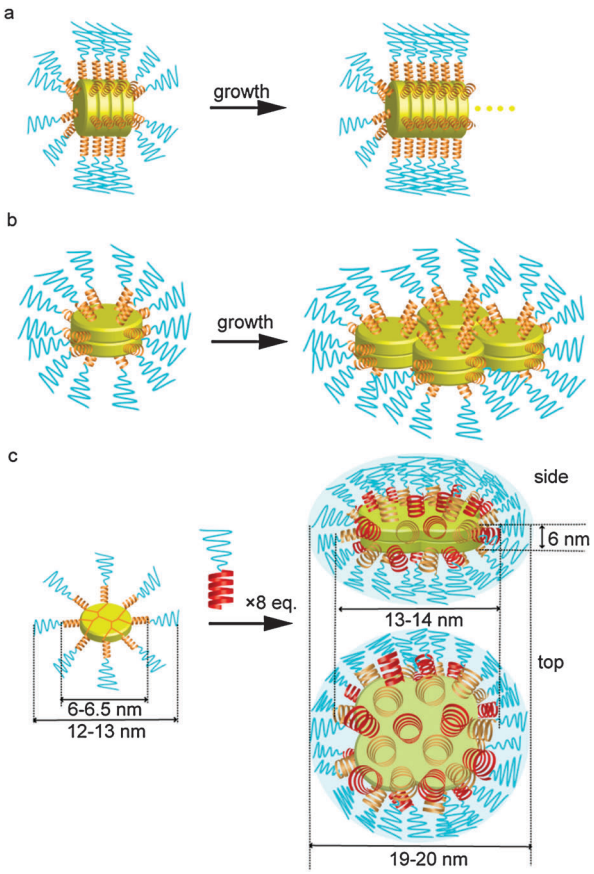

Fig. 5 The model structures of self-assemblies prepared at the feed molar ratio of 8 : 1 (a, b). The yellow and the blue regions represent the hydrophobic core and the hydrophilic poly(Sar) chains, respectively. (c) The unit model structure for the smallest self-assemblies prepared at the feed molar ratio of 8 : 1 with 10 seconds sonication. The noted sizes show the results from DLS and TEM.

were reduced down to 13–14 nm diameter and 6 nm thickness. The molecular structure of this smallest disk-shape assembly is discussed under the assumption that the dendritic core with eight hydrophobic helices at the terminals is hydrophobic with a planar structure in the assemblies. This planar structure is generally allowed for 2nd generation dendrimers.[15] Consequently, two structural models are considered, as shown in Fig. 5a and b, where the yellow and blue regions represent the hydrophobic core and hydrophilic poly(Sar) chains, respectively.

In the model shown in Fig. 5a, the assembly of eight molecules of **LP** and **8RD** takes a planar structure on the whole, which stacks continuously to yield worm-like micelles. The worm-like shape shown in Fig. 5a however cannot explain the disk shape observed by TEM. In the other model shown in Fig. 5b, eight poly(Sar) chains of eight **LP** molecules and eight poly(Sar) chains of **8RD** are extended to one face of the dendritic core plane, and the two dendritic core planes associate face-to-face. This unit may grow in one direction *via* contact between plane edges where poly(Sar) chain density is low. The model shown in Fig. 5b fits to the results of TEM observations as shown in Fig. 5c.

In summary, eight left-handed helices were incorporated into a 2nd generation dendrimer bearing eight right-handed helices at the terminals *via* stereocomplex formation. This supramolecular assembly occurred stoichiometrically through 1 : 1 association of the right-handed helix and the left-handed helix. With this strategy of stereocomplex formation, dendrimers can be designed as a host platform for accommodating a defined number of guest molecules with a specified size. A 3rd generation dendrimer is under research as a template for sixteen helices.

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