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<th>Transformation of peptide nanotubes into a vesicle via fusion driven by stereo-complex formation.</th>
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Two types of peptide nanotubes, one is prepared from an amphiphilic peptide having a right-handed helix segment and the other from that having a left-handed helix segment, are shown to transform the morphology to vesicle by membrane fusion due to stereo-complex formation between these chiral segments.

Helical peptides are easily designed to take either helicity, right-handed or left-handed, by using L- or D-residues in the sequence. When the right-handed helix is mixed with the left-handed helix, they are expected to form a stereo-complex probably due to the convexo-concave fitness between their surfaces. With using stereo-complex formation of these helical peptides, we demonstrate here vesicle formation from two types of peptide nanotubes by membrane fusion.

Several morphologies in the shapes of micelle, rod-shaped micelle, sheet, tube and vesicle have been prepared in solution by the current self-assembling techniques.\cite{1-4} We have reported on molecular assemblies of amphiphilic peptide molecules especially with using hydrophobic helical peptides at the hydrophobic core of the molecular assemblies.\cite{5-9} Helical peptides have a good ability to be packed regularly in the molecular assembly as shown by frequent observation of helix bundles in nature.\cite{10,11} Indeed, several hydrophobic helical peptides with attachment of suitable hydrophilic groups formed vesicular assemblies with a diameter of ca. 100 nm in water, which are named “peptosome”.\cite{5} Further, peptide nanotube with diameter of ca. 60 nm and length of ca. 200 nm was obtained from amphiphilic block polypeptide with a hydrophobic helix, (Sar)_{27-b-(L-Leu-Aib)}_{6}OMe.\cite{12} In the latter case, the block polypeptide initially formed a curved square sheet assembly, which was transformed quantitatively to nanotube morphology upon heating at 90 °C for 10 min. The transformation mechanism from the curved sheet to the nanotube is just to stick two opposing hydrophobic sides of the square sheet, which is unique from other reports on nanotube formation, where twisted long sheet or helix ribbon generally fused the edges together to grow into nanotubes.\cite{13-17} On the other hand, in our nanotubes, the size of the nanotube is determined by the initial size of the curved square sheet, which has an advantage of a very narrow size distribution of the produced nanotubes. The reason for the sheet curving is considered to be due to the regular packing of the right-handed helices in the hydrophobic core, similarly to the recent studies on molecular assemblies with chiral molecules.\cite{18-31}

Poly(sarcosine) is used here as a hydrophilic segment, because it is as hydrophilic as poly(ethylene glycol), and sarcosine is biodegradable by endogenous sarcosine dehydrogenase. We have applied the amphiphilic peptide micelles made of (Sar)_{27-b-(Glu-OMe)}_{6} or (Sar)_{27-b-(lactide)}_{6} for in vivo tumor imaging with using near-infrared fluorescence labelling probe. These poly(sarcosine) conjugates are shown to be highly biocompatible. The (Sar)_{27} block is here attached to the N-terminal of the hydrophobic helical block, (L- or D-Leu-Aib)$_{6}$, via polymerization of sarcosine N-carboxy anhydride (NCA) to obtain SLL or SDL (syntheses and identification in supporting information (SI)).

Morphologies of molecular assemblies are studied by transmission electron microscope (TEM) with negative staining or cryogenic freezing. As previously reported, SLL as injected in buffer takes a homogeneous nano curved-sheet morphology.\cite{12} The same nano curved-sheet assembly is also obtained from SDL.

These nano curved-sheet assemblies are transformed to nanotubes by heating the molecular assembly solution at 90 °C for 10 min. Circular dichroism (CD) measurements show that SLL and SDL in the molecular assemblies take right-handed and left-handed α-helix, respectively. Further, the Cotton effect at 222 nm is slightly stronger than that at 208 nm, indicating that the helices form a tightly packed bundle structure (Fig. S2 in SI).

The important point of these nano curved-sheet and nanotube is that they are metastable, where upon heating transformation from the nanosheet to the nanotube occurs quantitatively, and upon further heating elongation of nanotube to double and triple was observed.\cite{13} Even though the hydrophobic edges of the nanosheet and the nanotube are stabilized by a shielding effect of poly(sarcosine) chains nearby, the edges can be fused with other hydrophobic surfaces upon heating.

Nanotubes are prepared separately from SLL and SDL, and two types of the nanotubes are mixed at an equimolar ratio. The mixture is heated at 90 °C for 50 min, and the time course of morphology change is analyzed by TEM (Figures 1A–D). Before heat treatment, nanotubes with diameter of ca. 60 nm and length...
of ca. 200 nm are homogeneously formed (Figure 1A). Upon heating for 10 min, planar square sheets with ca. 1 μm side appear in TEM image (Figure 1B). Then, the sheets are gradually transformed to vesicles (Figures 1C and 1D). Transformation of the sheet to the vesicle is completed within 50 min at 90 °C. With SLL or SDL alone, no such change was observed but just elongation of the nanotubes partially. It is thus speculated that the SLL nanotube and the SDL nanotube should fuse together to transform the nanotube morphology to the planar square sheet. Indeed, the observation of a planar sheet can be reasonably explained by mixing of enantiomeric polypeptides SLL and SDL with stereo-complex formation to generate the achiral planar sheet. Whereas, SLL or SDL alone forms the curved sheet due to hydrophobic edges at the open mouths of the nanotubes. To close the planar sheet with 1.5 μm side, the planar sheet should be torn off to generate the most stable size of the vesicle. This size distribution of the vesicles is very narrow around 200 nm diameter. Since this size is smaller than the expected size by closing the planar sheet with 1.5 μm side, the planar sheet should be torn off to generate the most stable size of the vesicle. This size distribution of the vesicles is very narrow around 200 nm diameter. Since this size is smaller than the expected size by closing the planar sheet with 1.5 μm side, the planar sheet should be torn off to generate the most stable size of the vesicle. This size distribution of the vesicles is very narrow around 200 nm diameter. Since this size is smaller than the expected size by closing the planar sheet with 1.5 μm side, the planar sheet should be torn off to generate the most stable size of the vesicle.

Upon heating at 50 °C, the peptide nanotube fusion process proceeds slowly compared with that at 90 °C. TEM observations reveal that upon heating at 50 °C for the initial 1–2 h, longer nanotube assemblies connecting two or more tubes are observed (Figure 2C). Hydrophobic edges at the open mouths of the nanotubes therefore trigger the association of the nanotubes. Interestingly, some bunchy connecting regions of two nanotubes are observed (Figure 2C and S3 in SI). This observation strongly indicates the mixing of SLL and SDL takes place to generate a vesicle-like morphology at this connecting region. After heating for 7 h at 50 °C, the fused nanotubes are broken up and transformed to a planar square sheet with ca. 1.5 μm side (Figure 2D). These results indicate that the polypeptide nanotubes are connected randomly at the initial stage (association). When two types of the peptide nanotubes are connected, the right- and the left-handed helices diffuse through the connecting moieties to induce membrane fusion, because the stereo-complex is thermodynamically stable. The mixing proceeds with time, and finally the tubular structure breaks up to take the planar square sheet. It takes more than 7 h to form the planar sheet (Figures 2D). Upon heating at 90 °C, transformation to vesicle is attained. The morphology changes are followed by DLS similarly as changes of hydrodynamic diameters (Figure 2A). Importantly no vesicular assembly transformation from the planar sheet was observed at 50 °C, suggesting that the transformation from the planar sheet to the vesicle requires higher energy.

We confirm the vesicle formation due to the stereo-complex formation by using another preparative method. An equimolar mixture of SLL and SDL in ethanol is injected into a buffer solution. The morphology of the self-assembly is a planar-sheet (Figure 3A), which is different from the curved sheet prepared from the single component. Further, upon heating at 90 °C for 1 h, vesicles are quantitatively formed (Figure 3B, cryo-TEM). The size distribution of the vesicles is very narrow around 200 nm diameter. Since this size is smaller than the expected size by closing the planar sheet with 1.5 μm side, the planar sheet should be torn off to generate the most stable size of the vesicle. This should be the reason for the narrow distribution of the vesicle diameter.

We have shown two different preparation methods for the same vesicle morphology, which confirms our interpretation of vesicle formation due to the mixing of the right- and the left-handed helices in the molecular assembly, which is
thermodynamically induced by the stereo-complex formation. Indeed, electron diffraction pattern and FTIR spectrum from the vesicle are different from those of the nanotube, indicating tight molecular packing of SLL and SDL in the vesicle due to the stereo-complex formation (Figure 4, S4 and S5).

In conclusion, we demonstrate a new method for vesicle preparation by a novel fusion mechanism, which is driven by the stereo-complex formation of the helical amphiphiles. Morphology transformation process from the nanotube to the vesicle is composed of four steps; i) nanotube association, ii) mixing of right- and left-handed helices, iii) break-up of the tubular structure to the planar sheet structure, and iv) closing to the vesicular structure. This fusion mechanism will pave the way to construct more complex morphologies which is now under investigation.

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Notes and references