In-Water Truly Monodisperse Aggregation of Gear-Shaped Amphiphiles Based on Hydrophobic Surface Engineering

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Abstract: Exactly six gear-shaped amphiphiles self-assemble into a highly stable, water-soluble, box-shaped capsule, in which indented hydrophobic surfaces of the components mesh with each other like gears. A water-soluble, tetrahedron-shaped capsule was also constructed from four gear-shaped amphiphiles with a template guest. These findings provide a guideline for creating aggregates with a given number of amphiphiles based on hydrophobic surface engineering.

Conventional amphiphiles, consisting of a hydrophilic head and hydrophobic tail(s), form self-aggregates such as micelles and bilayers in water.[1] Much effort has been made to control the morphology of the resulting aggregates by modification of both the hydrophobic and hydrophilic parts.[2] However, in most cases, the number of components exhibits a wide distribution. Thus, dispersion has been recognized as the inherent nature of aggregation of amphiphiles. A driving force of the aggregation in water is the hydrophobic effect.[3] In other words, hydrophobic parts get together to minimize the hydrophobic surface areas exposed to water. Consequently, the stereoscopic complementarity of the hydrophobic surfaces of amphiphiles is a dominant factor determining the aggregate structure. It is therefore a challenging task to precisely design the hydrophobic surfaces of amphiphiles so as to assemble into an aggregate consisting of a defined number of components. As pioneering examples of discrete aggregates, dimeric structures[4,5] have been constructed by the hydrophobic effect. More recently, we reported a novel gear-shaped amphiphile (**2**) having an indented hydrophobic surface and three hydrophilic parts (Figure 1). The amphiphiles **2** were found to mesh with each other like gears in a 3:1 mixed solvent of CH_3OH and H_2O so as to reduce the area of water-exposed hydrophobic surface to form a monodisperse box-shaped hexamer[6] or a tetrahedron-shaped tetramer.[7] Herein we demonstrate that exactly six gear-shaped amphiphiles, **1**·I_2, self-assemble into a box-shaped hexameric capsule with extremely high stability in water. Furthermore, a tetrahedron-shaped tetrameric capsule was also constructed from four gear-shaped amphiphiles, **4**·I_3, and a template guest molecule.

The design of **1**·I_2 is based on the structure of hexameric capsule **2**$_6$, in which hydrophilic pyridyl nitrogen atoms are put outward while the indented hydrophobic surface of the hexaphenylbenzene part is buried deep.[6] Replacement of the pyridyl groups of **2** with N-methylpyridinium groups is expected to make the capsule
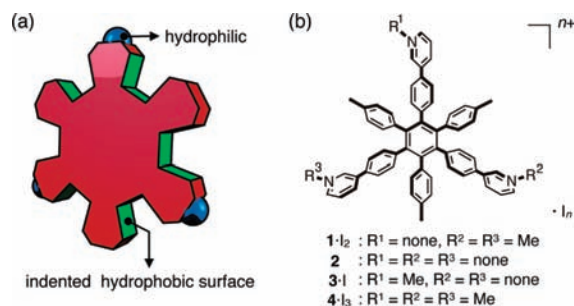

Figure 1. (a) Schematic representation of a gear-shaped amphiphile. (b) Chemical structures of gear-shaped amphiphiles.

remarkably soluble in water. Furthermore, when two of the three pyridyl groups of **2** are replaced with pyridinium groups, the resulting box-shaped aggregate **1**$_6$·I_{12} should become more stable due to the electrostatic interactions between positively charged pyridinium (colored cyan in Figure 2) and electron-rich pyridyl (colored yellow) nitrogen atoms in the triply stacked aromatic rings.

Association behaviors of **1**·I_2 in solution were examined by NMR spectroscopy. ^{1}H NMR spectra of **1**·I_2 varied markedly with solvents. The spectrum in CD_3OD showed a simple pattern for monomeric **1**·I_2 with C_{2v} symmetry (Figure 2a), whereas that in D_2O was rather complicated (Figure 2b). Several proton signals were observed with significant upfield shifts compared with those in CD_3OD, suggesting that gear-shaped molecules would mesh with each other to form an aggregate. The hexameric capsule **1**$_6$·I_{12} potentially has many structural isomers, depending on the position of pyridine rings in the triply stacked aromatic rings. Among them, the most symmetric structure is the one in which every pyridine ring is placed in the middle of the triply stacked aromatic rings, as illustrated in Figure 2. In this case, all the six components, **1**·I_2 with C_1 symmetry, are chemically equivalent. In the ^{1}H NMR spectrum, the signals for three inequivalent methyl groups of p-tolyl groups *i* and two inequivalent N-methyl groups *j* were observed. This result indicates that a highly symmetric box-shaped capsule structure was exclusively formed in D_2O, which was expected to be thermodynamically most stable.

The detailed structure of the box-shaped capsule **1**$_6$·I_{12} was determined as an inclusion complex [**1**$_6$⊃**5**$_2$]I_{12} (**5** = 2,4,6-tribromomesitylene) by X-ray analysis. As a result, it was revealed that six molecules of **1**·I_2 mesh with each other to form a box-shaped capsule (for the encapsulation of **5** in solution, vide infra). Every pyridine ring (colored yellow in Figure 3c) was placed between two pyridinium rings (colored cyan), thus establishing the highly symmetric structure demonstrated by ^{1}H NMR spectroscopy (Figure 2b).

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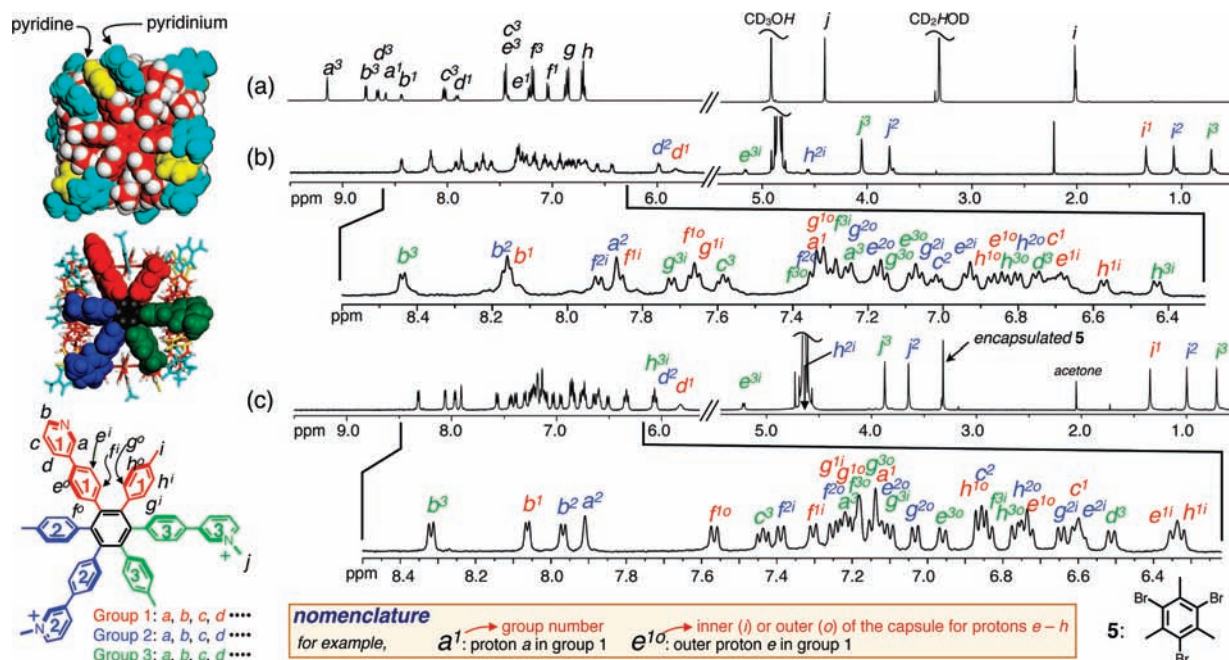|| Present address: Department of Basic Science, Graduate School of Arts and Sciences, The University of Tokyo.

Figure 2. ^{1}H NMR spectra of gear-shaped amphiphiles and their aggregates; 500 MHz, 293 K. Color labels in the top-left structure: red, carbon; white, hydrogen; cyan, *N*-methyl-3-pyridinium ring; yellow, 3-pyridine ring. A nomenclature for protons in $1_6 \cdot I_{12}$ is shown in the square frame. (a) $1 \cdot I_2$ in CD$_3$OD, $[1^{2+}] = 3.0$ mM. (b) $1_6 \cdot I_{12}$ in D$_2$O, $[1^{2+}] = 3.0$ mM. (c) $[1_6 \supset 5_2]I_{12}$ in D$_2$O, $[1^{2+}] = 2.0$ mM.
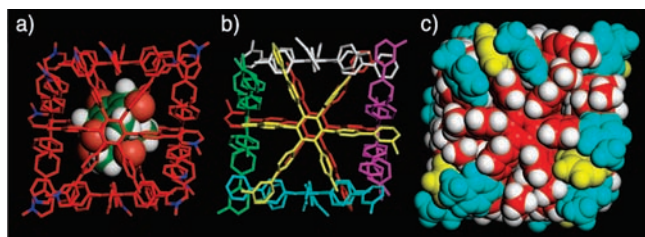

Figure 3. Crystal structure of $[1_6 \supset 5_2]I_{12}$. (a,b) 1^{2+} and **5** are shown by cylinder and space-filling models, respectively. Hydrogen atoms in the capsule are omitted for clarity. Color labels in (a): red, carbon in the capsule; blue, nitrogen; green, carbon in **5**; brown, bromine. In (b), two molecules of **5** are omitted, and each molecule is color-coded. (c) Space-filling model. Color labels: red, carbon; white, hydrogen; cyan, *N*-methyl-3-pyridinium ring; yellow, 3-pyridine ring.

All the proton signals of $1_6 \cdot I_{12}$ were fully characterized by ^{1}H$-^1$H COSY and ^{1}H$-^1$H NOESY measurements.[8] Characteristic chemical shift changes observed for the signals of protons, d^2, d^1, e^{3i}, and h^{2i}, which reside in close proximity to the clefts of other gear-shaped amphiphiles, indicate structural coincidence in solution and in the solid state. The formation of a monodisperse hexameric aggregate was also confirmed by pulse-field gradient spin$-$echo

(PFGSE) measurement.[8] A log D value (D, in m^2 s^{-1}, is the diffusion coefficient) of -9.90 for $1_6 \cdot I_{12}$ in D$_2$O was well consistent with the 2-nm-sized box-shaped capsule.[9]

Next we investigated the stability of the capsule in solution. Upon heating a solution of $1_6 \cdot I_{12}$ in D$_2$O ($[1^{2+}] = 3.0$ mM) to 353 K, ^{1}H NMR signals became rather sharper compared with those at 293 K, without any signals for dissociated monomeric $1 \cdot I_2$.[8] In contrast, the hexameric aggregate 2_6 was mostly dissociated into monomers in a 3:1 mixed solvent of CD$_3$OD and D$_2$O at 333 K. These results indicate that the water-soluble capsule $1_6 \cdot I_{12}$ is thermally far more stable than 2_6, mainly due to the stronger hydrophobic effect in water than in aqueous methanol. The association property of $1 \cdot I_2$ was also monitored by UV absorption spectrophotometry. A solution of $1 \cdot I_2$ in CH$_3$OH showed typical absorption bands at 265 and 287 nm for monomeric species. On the other hand, with an increase in the fraction volume of H$_2$O, their peak absorbance significantly decreased due to the stacking of the aromatic rings in the hexameric aggregate $1_6 \cdot I_{12}$.[8] To examine the concentration effect on the capsule formation, UV absorption of $1_6 \cdot I_{12}$ in H$_2$O was measured in the concentration range from 100 down to 1 μM. As a result, no hypochromism was observed at all in this concentration
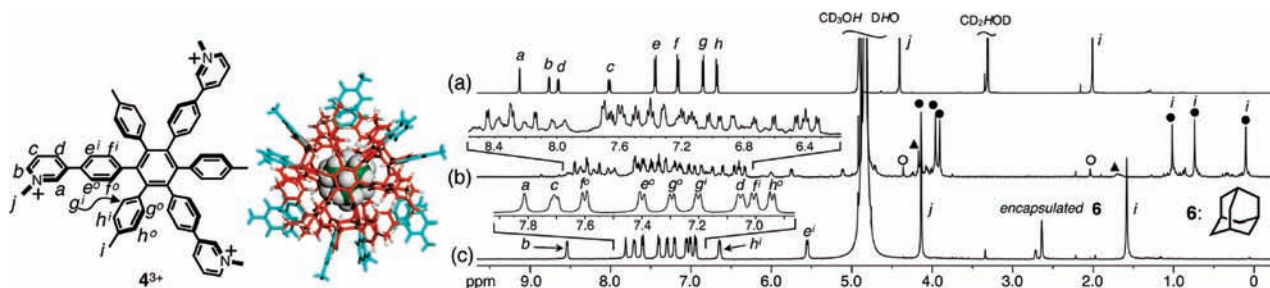

Figure 4. ^{1}H NMR spectra of $4 \cdot I_3$ and their aggregates; 500 MHz, $[4^{3+}] = 2.0$ mM, 293 K. A cylinder model denotes the structure of $[4_4 \supset 6]^{12+}$. Color labels: red, carbon in 4^{3+}; white, hydrogen; cyan, *N*-methyl-3-pyridinium ring; green, carbon in **6**. (a) $4 \cdot I_3$ in CD$_3$OD. (b) $4 \cdot I_3$ in D$_2$O. Filled circles, filled triangles, and open circles denote $4_6 \cdot I_{18}$, $4_4 \cdot I_{12}$, and $4 \cdot I_3$, respectively. (c) $[4_4 \supset 6]I_{12}$ in D$_2$O.

range.[8] This indicates that the critical concentration of the capsule $1_6 \cdot I_{12}$ is considerably lower than 1 μM.

As demonstrated by the crystal structure, the box-shaped aggregate has a hydrophobic inner space for encapsulating hydrophobic molecules. The 0.8×0.8 nm inner space of $1_6 \cdot I_{12}$ is large enough to accommodate two guest molecules **5**. Upon the addition of **5** to a solution of $1_6 \cdot I_{12}$ in D_2O, though **5** is insoluble in D_2O, two molecules of **5** were encapsulated inside $1_6 \cdot I_{12}$. A signal for methyl groups of **5** appeared at $\delta = 3.49$ ppm, which is downfield shifted compared with that of free **5** in CD_3OD ($\Delta\delta = +0.85$ ppm) (Figure 2c).

Formation of the hexameric aggregate depends substantially on the number of *N*-methylpyridinium groups introduced into amphiphiles. In the case of amphiphile $3 \cdot I$ with one *N*-methylpyridinium group, hexameric aggregates with several structural isomers were formed in aqueous methanol[8] but were insoluble in water. On the other hand, in the case of $4 \cdot I_3$ with three *N*-methylpyridinium groups, a water-soluble tetrahedron-shaped capsule $[4_4 \supset 6]I_{12}$ was built up from four gear-shaped amphiphiles $4 \cdot I_3$ and a template guest molecule **6** (Figure 4). A sharp ^{1}H NMR spectrum of $[4_4 \supset 6]I_{12}$ in D_2O was consistent with a highly symmetric structure of the tetrahedron-shaped capsule (Figure 4c, see also the Supporting Information for a previously reported tetrameric capsule,[8] $2_4 \supset 6$). The tendency of $4 \cdot I_3$ to form a stable tetrameric capsule originates from its own aggregation behavior. In a ^{1}H NMR spectrum of $4 \cdot I_3$ in D_2O, the signals for a tetrameric capsule $4_4 \cdot I_{12}$ were observed in addition to those of a hexameric capsule $4_6 \cdot I_{18}$ ($4_4 \cdot I_{12}:4_6 \cdot I_{18} =$ 1:9, Figure 4b),[8] in clear contrast to the fact that no tetrameric capsules were formed from the other gear-shaped amphiphiles $1-3 \cdot I$ without template guests. The relative stability of tetrahedron-shaped capsule $4_4 \cdot I_{12}$ is well explained by the electrostatic repulsion among three pyridinium groups of $4 \cdot I_3$.[8] That is, different discrete capsule structures can be constructed by utilizing electrostatic interactions between pyridinium (and pyridyl) rings incorporated into the gear-shaped amphiphiles.

In conclusion, the construction of truly monodisperse aggregates was achieved in pure water using purposefully designed gear-shaped amphiphiles. The studies of both the solution and the crystal structures of the hexameric capsule $1_6 \cdot I_{12}$ revealed that the amphiphiles $1 \cdot I_2$ engage one another so as to reduce the area of water-exposed hydrophobic surfaces, and that the meshing structure gives rise to extremely high stability in water. Furthermore, the tetrameric aggregate $[4_4 \supset 6]I_{12}$ with another amphiphile, $4 \cdot I_3$, was selectively formed by the hydrophobic effect as well as the template effect. Alternative aggregation structures would be possible based on the precise design of hydrophobic surface of amphiphiles.

Supporting Information Available: Experimental details, ^{1}H NMR and UV spectra, and X-ray structure data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) See the Supporting Information.

(9) No further aggregation of $1_6 \cdot I_{12}$ capsule was observed, probably due to the electrostatic repulsion that would occur between the positive charges on the outer surface of the capsules.

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