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Controllable self-assemblies of β-cyclodextrin-calix[4]arene couples

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Amphiphilic cyclodextrin-calixarene conjugates **1** and **2** were synthesized from "click chemistry", and their self-assembly behaviors were investigated by transmission electron microscopy, and atomic force microscopy. The results obtained show that **1** and **2** can self-assemble to form distinctly different aggregations, and the morphology of aggregations critically relies on the polarity and hydrophilicity of the solvent.

cyclodextrin, calixarene, self-assembly

1 Introduction

One of the major challenges in developing new technological application is to build deformable patterns on the surface [1]. So far, the most common way to obtain patterned surface is to use the top-down lithography [2]. Another way is the bottom-up supramolecular assembling on the surface [3], especially construction of supramolecular assemblies with deformable patterns under predetermined conditions, which would have wide applications in industry in the future [4]. In this way, amphiphilic molecules are good candidates for forming patterned aggregations in interface or surface. Besides many amphiphilic polymers [5], supramolecular amphiphilic molecules, which have advantages of including guest molecules, assembling with hydrogen bonding [6], π-π staking and so on, would have potential abilities of assembling in some different patterns with proper design. Giving predetermined agitation, such as solvent changing [7], temperature varying [8], or guest inducing [9], the pattern of assemblies would reform respectively.

Amphiphilic cyclodextrin (CD) derivatives had been widely investigated as liquid crystals, unimolecular micelles, nanoparticles, monolayers and bilayer vesicles [9, 10]. Substitution of primary or secondary or both faces of CD with long alkyl chain or analogues is the most feasible way introducing the hydrophobic moieties. However, appending rigid moieties covalently to CDs, such as calixarenes (CAs), is quite difficult. Certain reaction conditions either need to protect/deprotect the hydroxyl groups of CDs which increased steps to obtain the final products [9, 11] or have very low yield [12]. Recently, Cravotto *et al*. [13] and our group [14] had successfully set up the general method independly for synthesizing cyclodextrin derivatives through click chemistry, making the synthesis of CA-CD conjugates much accessiable. Herein, we synthesized two CA-CD conjugates (**1** and **2**) through the reaction of 6-N_3-*β*-CD with 1,3-bis(propargyl)-*p*-*tert*-butyl-calix[4]arene. Further, different self-assemblies formed by the two amphiphilic CD-CA conjugates were investigated on the surface. In addition, the aggregation behavior and the crucial role of solvent on pattern changing were examined in detail.

2 Results and discussion

The solubilities of compounds **1** and **2** are quite limited in pure water (less than 1 μM) because of introducing CA

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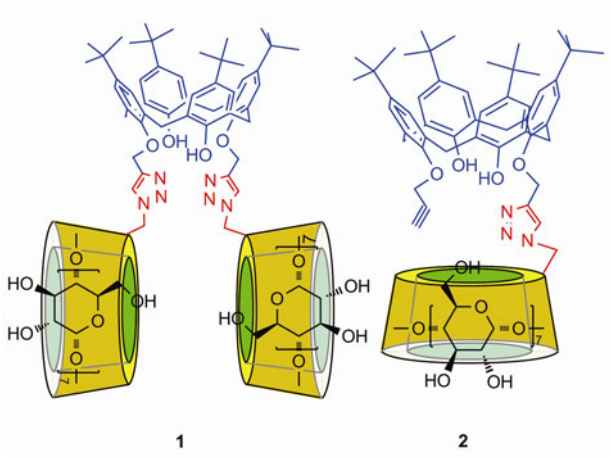
mosieties. Thus, we prepared the saturated solution of **1** and **2** in pure water for AFM analysis. As shown in Figure 1, the sheet-like structures were observed on mica surface due to self-assembly of compound **1**. The hight is ca. 1.3 nm, and the size is consistent well with the diameter of the secondary face of β-CD moiety, indicating that the sheet-like aggregation is the monolayer of **1**.

Detailed analysis of every section of this self-assembly shows an undulating hill like waveform. As the radius of the probe of AFM is too large to distingue each molecule composing this assembly, a method similar to the famous Millikan oil-drop experiment [15] was used to calculate the size of **1**. Assuming each molecule occupies a spherical space including its size and van der Waals distance, the horizontal distance of every neighborhood peak should be integral times of each sphere's diameter, which is quite simialr to that each oil drop contains integral times of elementary charge in Millikan experiment. Cutting each section with different angle (0–180°, 15° each, in order to reduce the error) of the sheet-like assembly and picking out all the horizontal distance on each section analysis waveform gives over 1000 samples for statistical analysis. The results obtained show that the spherical equivalent diameter of **1** is about 2.6 nm (Figure 1), which accords well with the MD calculation of the molecular size of compound **1** including Van der Waals distance. Combining the spherical equivalent size of **1** and the microstructure of assembly, it is suggested that each **1** molecules are arrayed next to each other tightly to form a monolayer sheet-like assemblies in pure water. Further, the number of **1** would be estimated easily through measuring the area of aggregation formed.



Scheme 1 Structure of **1** and **2**.

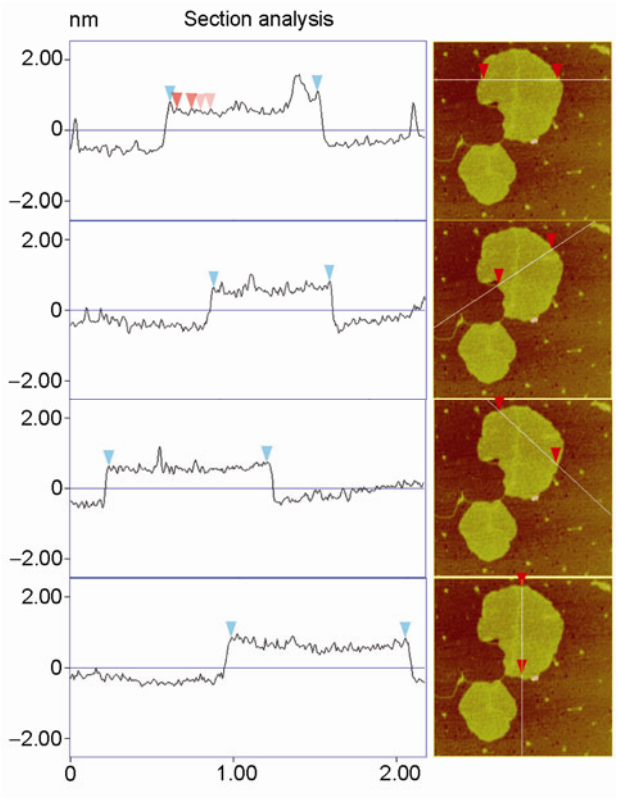


Figure 1 Section analysis of random parts of assemblies formed by **1** in pure water.

The following data were collected in the same way as shown in Figure 1. The horizontal distances between each peak were recorded as Group X: D0, D1, D2,…, D*n*. Then, the data were changed to (D1-D0), (D2-D1),…, (D*n*-D(*n*-1)) in order to reduce the error. According to our assumption, these data should be integral times of a static number A_0.

Thus, $Dn = kA_0$ $\quad k > 0, k \subset Z;$

$Dn - D(n\text{-}1) = mA_0,$ $\quad k > m > 0.$

With enough data we could get a linear fitting line from these data, and the slope of the fitting line should be A_0 (As shown in Figure 2).

Data (part, nm):

Group 01: 28.139, 51.162, 74.186, 104.88, 135.58, 166.28, 191.86, 235.35, 263.49, 322.32, 363.25, 391.39, 416.97, 465.58, 544.88, 570.46, 624.18, 675.34, 769.99, 800.69, 846.74;

Group 02: 40.93, 69.069, 86.976, 115.12, 140.70, 186.74, 222.56, 268.60, 304.42, 350.46, 396.51, 432.32, 468.14, 503.95, 560.23, 588.37, 613.95, 662.55, 713.72, 741.86,

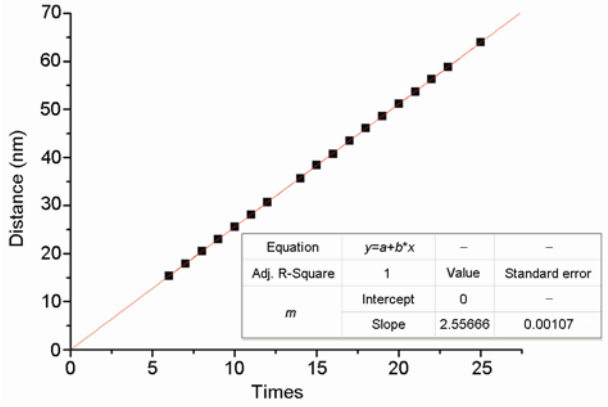


Figure 2 Final fitting line of mean of each group D*n*–D(*n*-1) vs. *m*.

777.67, 813.48, 841.62, 885.11, 923.48, 959.30, 987.43, 1013.00;

Group 03: 28.139, 81.860, 110.00, 145.81, 163.72, 209.77, 235.35, 263.49, 291.63, 317.21, 355.58, 393.95, 424.65, 452.79, 473.25, 496.28, 537.21, 562.79;

Group 04: 33.256, 63.953, 110.00, 133.02, 161.16, 225.11, 243.02, 317.21, 353.02, 388.83, 406.74, 425.65, 460.46, 488.60, 514.18, 534.65, 550.00;

Group 05: 51.162, 91.209, 120.23, 143.25, 168.84, 186.74, 214.88, 268.60, 286.51, 304.42, 340.23, 404.18, 440.00, 496.28, 511.62, 557.67, 639.53, 657.44, 683.02, 711.16, 764.88, 785.34, 800.69, 839.06, 856.97, 877.44, 900.46, 928.60, 956.74, 992.55.

In sharp contrast, conjugate **2** forms linear or dots like assemblies with the height of ca. 1.2 nm in pure water (Figure 3(d)). Due to the relative small size and the maldistribution, it is difficult to obtain the size of **2** using the same method as **1**.

Furthermore, we investigated the assembly behavior of **1** and **2** in DMSO-H_2O (*v*/*v* = 1:9). In contrast to those behaviors in pure water, both **1** and **2** (0.01 mM) showed surfactant properties in the mixed solution, which indicates they are surfactant active (Figure 4). AFM images of **1** in DMSO-H_2O solution give several bundles of sticks with the length ranging from 500 nm to 3.5 μm and the height of from 2.8 to 33.2 nm (Figure 3(b)). Some fine structure can be found on the edge of each bundles. TEM images of **1** provide the information of the microscopic structure of the bundles. As can be seen from Figure 5(a), there are many sticks in a bundle, and the width of each stick is ca. 5 nm. Since the size of **1** is around 2.5 nm, one could reasonably deduce that each stick is composed of a bilayer of **1**.

Significantly, **2** forms typical vesicles in DMSO-H_2O solution (Figure 3(e)). Its TEM images further support the formation of vesicles (highlighted by white rings in Figure 5(b)). The driving force of forming vesicles is the amphiphilic nature of **2**. Since the upper rim of CA has similar size with the diameter of secondary face of CD, the CA moiety in **2** can not be included in the cavity of CD. The only possible structure is that compound **2** is placed next to each other to form a vesicular bilayer.

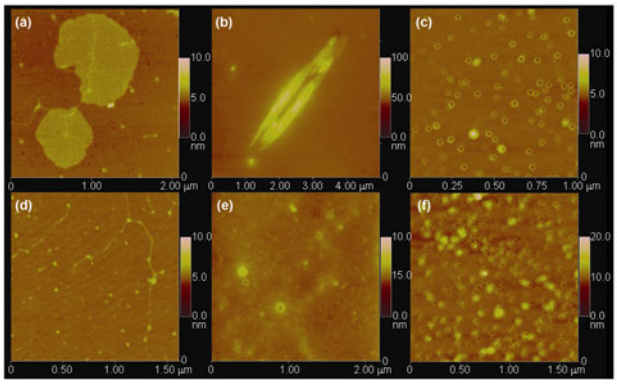


Figure 3 AFM images of **1** and **2** in pure water ((a) and (d)), DMSO-H_2O solution ((b) and (e)), $(CH_3)_2CO$-H_2O solution ((c) and (f)), respectively.

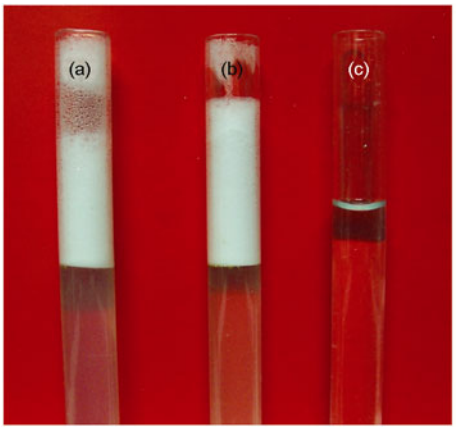


Figure 4 Amphiphilic molecules of **1** (0.05 mM), **2** (0.05 mM) and β-CD (1 mM) in water/DMSO solution after pipetting air into the solution: (a) **2**, (b) **1** and (c) β-CD.

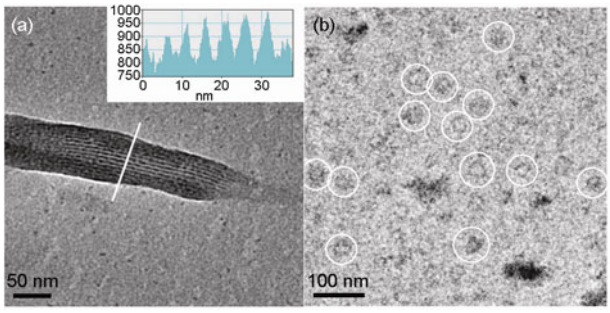


Figure 5 TEM images of **1** (a) and **2** (b) in DMSO-H_2O solution.

Instead of more polar DMSO with less polar acetone, we found that the morphology of self-assembly of **2** (0.01 mM) in $(CH_3)_2CO$-H_2O (*v*/*v* = 1:9) solution remains to some extent. AFM image shows the deformed vesicles on mica surface (Figure 3(f)). However, self-assemblis of **1** (0.01 mM) change from bundle-like structure in DMSO-H_2O solution to typical vesicles in $(CH_3)_2CO$-H_2O solution (Figure 3(c)). Many vesicles with the diameter around 50 nm can be found in its AFM image. That is, both **1** and **2** self-aggregate to form vesicles in the lower polar $(CH_3)_2CO$-H_2O solution.

Take a overview of **1** and **2** in different aqueous solutions, their self-assembled patterns critically rely on the solvent polarity. Conjugate **1** changes from sheet-like to bundle-like aggregations and further to a typical vesicle on mica surface, while **2** does from dots and linear aggregations to vesicles. Comparing the structures of **1** and **2**, they both are amphiphilic molecules which were composed of hydrophilic CD moieties and hydrophobic CA moieties. The only difference is the number of CD moieties on the backbone. Combining their structure difference and self-assemble behaviors in different solvents, a possible explanation is as follows. For compund **1**, the hydroxyl groups on the secondary face of two CD moieties have strong hydrogen bonding interaction [16] with the neighbourhood CD moie-

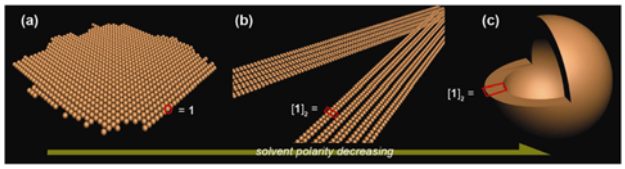


Figure 6 Representative scheme of **1** in (a) pure water, (b) DMSO-H_2O solution and (c) acetone-H_2O solution.

ties in pure water. The network hydrogen bonding also stabilizes CA moieties of **1**. Upon the addition of DMSO, the hydrogen bonding interaction is weaken, which could not support a wide spread network but only linear interactions between two molecules. Thus, the amphiphilic nature of **1** results in a bilayer stick-like assembly, in which CD moieties locate outside bilayer and CA does inside. The interfacial tension made these sticks aggregate as a bundle. To replace DMSO with acetone completely destroys the hydrogen bonding interactions. For this reason, only the vesicle structure is stable under interfacial tensions for **1**. The schematic representation of the presumed modes for **1** in different solvents is illustrated in Figure 6. For compund **2**, only possessing one CD moiety, this compound could not form any network hydrogen bonding interactions. In order to defense the interfacial tension, micelle or vesicle would be the most probable structure for **2** assembled.

3 Conclusion

In summary, two cyclodextrin-calixarene conjugates have been synthesized via "click chemistry", and their self-assemble behavior investigated in different aqueous solution. With decreasing polarity of solvent, they prefer to aggregate in a less interfacial tensed pattern from sheet-like to bundle-like aggregations, and then to vesicles. This observation provides a good model for understanding supramolecular superficial behavior. Further studies to test the compatibility of these assemblies with some guest molecules and their application in drug delivery are in progress.

4 Experimental

4.1 Reagents and instruments

All chemicals used were reagent grade unless noted. β-CD of reagent grade was recrystallized twice from water and dried in vacuo at 95 °C for 24 h prior to use. Propargyl bromide solution (80 wt% in toluene) was purchased from Acros Organics. Sodium ascorbate was purchased from Amresco Company. DMSO was distilled from calcium hydride. ^{1}H NMR and FT-IR spectra were recorded on a bruke Mercury VX400 spectrometer and a Shimadzu Bio-Rad FTS 135 instrument, respectively.

Samples were dissolved in water/DMSO or acetone mixed with the same volume ratio (*v*/*v* = 9:1). Then, the solution was sonicated for 30 min at room temperature and kept for 1 h except especially mentioned. The sample for TEM measurement was prepared by dropping the solution of **1** and **2** onto a copper grid. In AFM measurements, a drop of sample solution (1.0×10^{-6} M) was dropped onto newly clipped mica and then air-dried, which was examined using an AFM (Veeco Company, Multimode, Nano IIIa) in tapping mode in the air at room temperature. In DLS measurements, the sample was prepared by filtering solution through a 450 nm Millipore filter into a clean scintillation vial, which were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (Turbo Corr.) at a scattering angle of 90°.

4.2 Synthesis of 1 and 2

Compound 6-azide-deoxy-β-CD was prepared by a reported method and recrystallized before using. 1,3-Bis(propargyl)-*p-tert*-butylcalix[4]arene was prepared by *p-tert*-butylcalix[4]arene and propargyl bromide using potassium carbonate in aceton [17]. Calix[4]arene-couples-cyclodextrin was synthesized through 1,3-dipolar cycloaddition of 6-azo-deoxy-β-CD with 1,3-bis(propargyl)-*p-tert*-butylcalix[4]arene. In general, 1,3-bis(propargyl)-*p- tert*-butylcalix[4]arene was dissolved in 20 mL THF, and a solution of 6-azo-deoxyl-β-CD (20 mL) and $CuSO_4\cdot5H_2O$ was added in with strring. Later, an aqueous solution of sodium ascorbate (10 mL) was added to the mixure. The solution turned brown quickly and then turn to dark yellow in 30 min. The mixture was kept at 60 °C with vigorous stirring under nitrogen atmosphere. After 48 h heating, the mixture was cooled down and dried under reduce pressure. The residue was dissolved in 10 mL DMF and insolubles was removed by filtration. Concentrated the DMF solution to 5 mL and dropped to 300 mL aceton to form a precipitate. The solid was collected by filtration dissolved in 5mL DMF again and dropped to 300 mL aceton to wash impurities. The resulting precipitate was collected by filtration and dried. The crude product was then recrystallizaion in aceton/water (*v*/*v* = 1:9) twice to get a light yellow product.

1: 6-Deoxy-azido-β-CD (800 mg, 0.69 mM), 1,3-bis(propargyl)-*p-tert*-butylcalix[4]arene (167 mg, 0.23 mM), $CuSO_4\cdot5H_2O$ (173 mg, 0.69 mM), sodium ascorbate (410 mg, 2.07 mM). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.06 (s, 2H), 7.65 (s, 2H), 7.08 (d, 4H), 6.96 (d, 4H), 5.68 (m, 28H), 5.02 (d, 4H), 4.83 (m, 14H), 4.51–4.32 (m, 18H), 3.95 (d, 4H), 3.64–3.30 (m, 68H), 1.19 (s, 18H), 1.03 (s, 18H). IR (KBr) ν : 3746, 3671, 3338, 2951, 1698, 1653, 1558, 1483, 1417, 1363, 1299, 1239, 1202, 1154, 1079, 1033, 943, 871, 755, 704, 581, 530, 488, 457 cm^{-1}. Anal. calcd for $C_{134}H_{198}O_{72}N_6\cdot11H_2O$: C 49.63%, H 6.84%, N 2.59%; found C 49.22%, H 6.72%, N 2.72%. HRMS: 3067.199 $[M+Na]^+$.

2: 6-Deoxy-azido-β-CD (500 mg, 0.43 mM), 1,3-bis(propargyl)-*p-tert*-butylcalix[4]arene (942 mg, 1.3 mM),

$CuSO_4$ $5H_2O$ (108 mg, 0.43 mM), sodium ascorbate (256 mg, 1.29 mM). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.08 (s, 1H), 7.82–7.75 (t, 2H), 7.11–7.05 (m, 8H), 5.72 (m, 14H), 5.07 (t, 4H), 4.83 (m, 7H), 4.47 (m, 7H), 4.21(m, 4H), 3.95 (4H), 3.64–3.34 (m, 34H), 2.50 (1H, hide in the solvent peak), 1.17 (d, 18H), 1.07 (d, 18H). IR (KBr) ν : 3354, 2953, 1696, 1654, 1558, 1483, 1417, 1363, 1299, 1237, 1201, 1154, 1079, 1035, 943, 872, 844, 755, 704, 581, 499, 441 cm^{-1}. Anal. calcd for $C_{92}H_{129}N_3O_{38}\cdot 10H_2O$: C 53.51 %, H 7.27%, N 2.04%; found: C 53.21%, H 7.17%, N 2.29%. ESI-MS: 1907.87 $[M+Na]^+$.

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