Control of self-aggregation of fullerenes by connection with calix[4]arene: solvent- and guest-effects to particle size†

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A new molecular design of fullerene derivatives exhibiting trigger-responsive self-aggregation in organic solvents has been established. Calix[4]arene was covalently connected with fullerene in order to apply host–guest interaction to the aggregation control. The self-assembly behaviour was studied in organic solvents by UV–vis absorption spectroscopy, dynamic light scattering and transmission electron microscopy. Results show that the bisfullerene formed self-aggregations with a low polydispersity index due to the fullerenes' tendency to aggregate in polar organic solvents. Furthermore, the aggregate sizes can be changed readily by solvent composition and the addition of guest cations. Especially, disaggregation of the bisfullerene was induced by addition of $LiClO_4$ or $NaClO_4$.

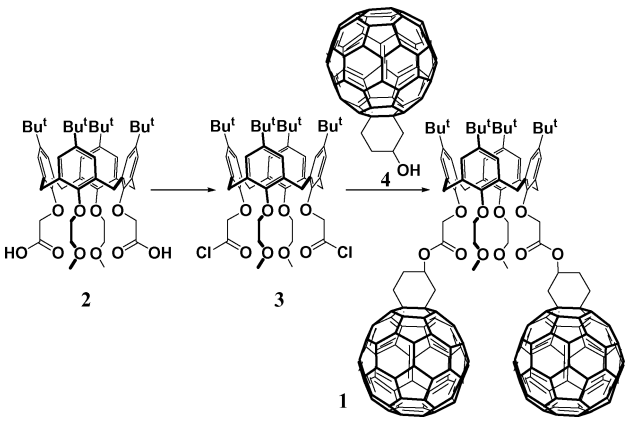
Introduction

Fullerenes and their derivatives have attracted much attention in recent years and have been useful for materials science and biological technology applications.[1] In those applications, controlling the fullerenes' aggregation is an important factor influencing the material properties such as fluorescence, redox, diffusivity (molecular transport), and photochemical reactivity.[2] Therefore, aggregation should control the various C_{60} functions using a trigger. In recent years, many efforts have been made synthetically toward beneficial preparations of C_{60} molecular aggregates. One expected example is dendrimers with peripheral fullerenes.[3] A simple molecular design of well-size-controlled fullerene aggregates can be developed by using dendrimers of adequate size, however, it is difficult to synthetically link all fullerenes with large dendrimers possessing highly dense reactive end-terminals. Several other previous studies have explored the self-aggregation of C_{60} derivatives in an aqueous solution.[4–7] Among these studies, it has been reported that hydrophobic C_{60} moieties aggregate in an aqueous solution by connecting them with hydrophilic groups.[4,5] Recently, unmodified C_{60} and C_{60} derivatives were assembled by formation of supramolecular complexes with DNA or inorganic nanoparticles in water;[6,7] it is expected that the aggregate size is controllable by the size of templates such as DNA and nanoparticles. These previous examples of aggregate size control of unmodified C_{60} and C_{60} derivatives are very limited in organic solvents.[8–10] In this study, we tried to control self-aggregation of fullerenes in organic solvents by using calix[4]arene. It is expected that calix[4]arene is applicable to aggregation control by the host–guest interaction. Herein, we report the preparation of a calix[4]arene-appended bisfullerene (**1**) and its self-assembly behaviour observed by UV–vis absorption spectroscopy, dynamic light scattering and transmission electron microscopy. Furthermore, in the presence of triggers (additives) such as polar solvents or guest ions, the self-aggregation properties of **1** were analysed.

Results and discussion

Chemistry

Compound **3** was prepared by reacting **2**[11] with thionyl chloride in dry pyridine–benzene at the reflux temperature. The preparation of **1** was achieved by adding a dry toluene solution of 1,2-(4′-hydroxycyclohexano)buckminsterfullerene (**4**)[12] to the evaporated reaction mixture of the previous step (Scheme 1). Because **1** has two stereogenic centres arising from **4**, two stereoisomers of **1** are present as *meso* and *racemic* compounds. The *meso* and *racemic* compounds were separable by preparative TLC. The yields of these compounds were 4% and 3%, respectively. The low yields are suspected to be due to the steric hindrance of **4**. It is easy to



Scheme 1 Synthetic routes and compounds used in this work.

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discriminate the NMR signals for *meso*-**1** and *rac*-**1** on the basis of the splitting patterns of the methyl protons in the methoxyethyl groups; in *meso*-**1** with C_s symmetry, the methyl peaks split at 3.63 and 3.49 ppm in a 1 : 1 ratio and in *rac*-**1** with C_2 symmetry, the methyl peak appeared as a singlet at 3.53 ppm. For all of the following experiments, we mention results using only *rac*-**1** because we obtained similar results using *meso*-**1**.

Identification of self-aggregation using UV–vis absorption spectra

First, we examined the solvent effect on the absorption spectra of **1** (50 μM, Fig. 1). Chloroform is known to be a good solvent for both calixarene and C_{60} moieties. Acetonitrile is a poor solvent for C_{60} moieties. Therefore, we predicted that the aggregation of C_{60} moieties themselves gives rise to aggregation of **1** by the presence of acetonitrile.[13] By changing the composition ratio of acetonitrile to chloroform until chloroform–acetonitrile = 1 : 1 (v/v), the absorbance increased over a wide wavelength region concomitant with the acetonitrile ratio. The spectral change was visible as a colour change from faint brown to deep brown, accompanied by turbidity, indicating that the spectral change is attributable mainly to the light scattering by the aggregates of **1**. The absorbance decreased with increasing acetonitrile ratio at a high composition ratio of acetonitrile from chloroform–acetonitrile = 1 : 1 (v/v) to 1 : 9 (v/v), suggesting that the morphologies of the aggregation of **1** changed according to the further increase of the acetonitrile ratio.

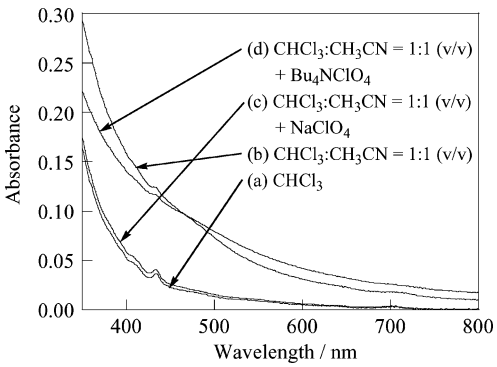
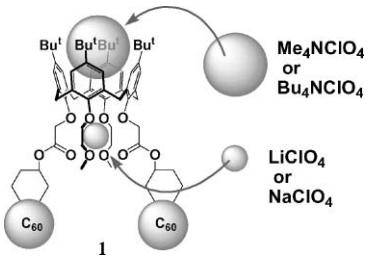


Fig. 1 UV–Vis absorption spectra of **1** (50 μM) (a) in chloroform, (b) in chloroform–acetonitrile = 1 : 1 (v/v), (c) in chloroform–acetonitrile = 1 : 1 (v/v) in the presence of $NaClO_4$ (500 μM) and (d) in chloroform–acetonitrile = 1 : 1 (v/v) in the presence of Bu_4NClO_4 (500 μM); 1 cm cell; 20 °C.

Second, we examined the guest effect on the absorption spectra of **1** (50 μM, Fig. 1). Although the association between **1** and Na^+ is detectable by ^1H NMR spectroscopy through the addition of $NaClO_4$ (500 μM; 10 equivalents to **1**) in a chloroform solution of **1**, the UV–vis absorption spectrum did not change. This result is consistent with previous results, which indicate that alkali metal cations bound to C_{60} derivatives covalently-linked to ionophoric moieties cannot directly affect the absorption spectra of the C_{60} moiety,[14] except for azafulleroid.[15] In contrast, the absorbance of **1** in chloroform–acetonitrile = 1 : 1 (v/v) decreased on addition of $NaClO_4$ (500 μM); the spectrum was similar to that of **1** in a chloroform solution.[16] The deep brown promptly changed to faint brown and the turbidity disappeared. These results show definitively that the disaggregation of **1** occurred by the inclusion of Na^+ in the lower rim of the calixarene moiety, as shown in Scheme 2.[17] On the other hand, when tetrabutylammonium perchlorate (Bu_4NClO_4, 500 μM; 10 equivalents to **1**) as a larger cation was added to a solution of **1** in chloroform–acetonitrile = 1 : 1 (v/v), the absorbance became greater than that before the addition. The results imply that the spectral change was caused by the increase in light scattering as a result of an increase in the aggregate size of **1**. More detailed analyses of the aggregates–disaggregates and the changes in the aggregate size are given below.



Scheme 2 Schematic formation of complexes between **1** and $LiClO_4$, $NaClO_4$, Me_4NClO_4 or Bu_4NClO_4.

Determination of aggregate sizes by dynamic light scattering (DLS) measurements

Aggregate sizes of **1** were estimated by DLS. First of all, the time course of the aggregate size was observed in chloroform–acetonitrile = 1 : 1 (v/v). Fig. 2 shows that the aggregate size changed very slowly and saturated to 321 nm after 12 h. Therefore, we determined all aggregate sizes of **1** in various ratios of chloroform–acetonitrile mixed solvents after 12 h. On the other hand, Fig. 2 illustrates that the polydispersity index increased from under 0.1, which is defined as a monodisperse system, to over 0.1, which is defined as a multidisperse system, until 10 min, then subsequently decreased to 0.05 and then saturated after 12 h. To the best of our knowledge, the polydispersity index has the smallest value in a self-aggregation system of low-molecular-weight molecules in organic solvents. These results indicate that about 50 nm nanoparticles of **1** were generated and then grew up to larger aggregates through further aggregation. The reasons will be discussed below in the section describing the TEM measurements. Fig. 3 shows that the aggregation was not observed between chloroform–acetonitrile = 10 : 0 to 8 : 2 (v/v). Between chloroform–acetonitrile = 7 : 3 to 5 : 5 (v/v), the aggregate size

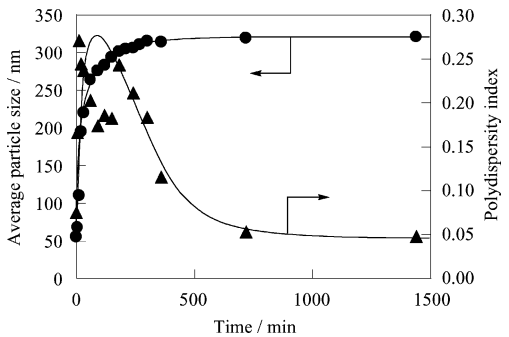


Fig. 2 Time course of the average particle sizes (filled circles) and the polydispersity index (filled triangles) of **1** (50 μM) in chloroform–acetonitrile = 1 : 1 (v/v); 20 °C.

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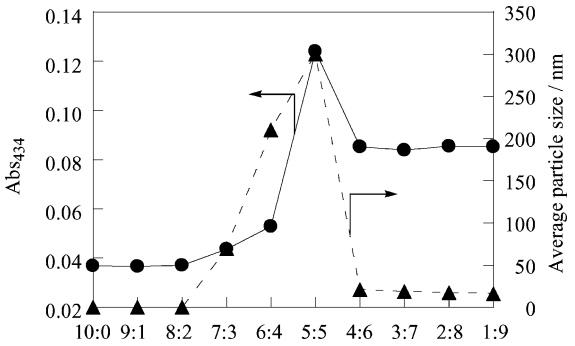


Fig. 3 Plots of absorbance at 434 nm (solid line, filled circles) and average particle size in DLS measurements (dashed line, filled triangles) *versus* chloroform–acetonitrile ratios; [**1**] = 50 μM; 20 °C.

increased concomitant with the composition ratio of acetonitrile. To the contrary, the aggregate size decreased with increasing acetonitrile ratio in the range of chloroform–acetonitrile = 5 : 5 to 1 : 9 (v/v). These size changes agreed well with the absorption spectral changes (Fig. 3) because light scattering increased with the increase in the aggregate size of **1**.

When $LiClO_4$ or $NaClO_4$ was added to a solution of **1** in chloroform–acetonitrile = 1 : 1 (v/v), no scattered light was detected by DLS measurement, indicating that the **1**·Li^+ and **1**·Na^+ complexes did not aggregate in the mixing solvent (Table 1). It is known that calixarene derivatives with ester and ether groups can include Li^+ or Na^+ ions in the ionophoric cavity (Scheme 2).[17] Therefore, we inferred that the disaggregation of **1** was caused by the improvement of the solubility in the polar solvent and the electrostatic repulsion by formation of a **1**·Li^+ or **1**·Na^+ complex (Scheme 3(iii)). On the other hand, the aggregate size increased respectively from 321 nm to 2150 nm and 2370 nm in diameter by the addition of Me_4NClO_4 and Bu_4NClO_4 indicating that the addition of these large ions gave rise to the further aggregation of **1** (Table 1). Since it is known that an ammonium ion can be included in the π-cavity of calixarene by cation–π interactions,[17] it is expected that ammonium ions bridged between the aggregation surface of **1** (Scheme 2). These size changes caused by the addition of cationic guests agreed well with the results of the absorption spectral changes.

Table 1 Average particle sizes (nm) and polydispersity indices of **1** determined by dynamic light scattering (DLS) in chloroform–acetonitrile = 1 : 1 (v/v) in the absence and presence of guest ions; 20 °C

Guest ion	Average particle size/nm	Polydispersity index
—	321	0.05
$LiClO_4$	N. D.[a]	—
$NaClO_4$	N. D.[a]	—
Me_4NClO_4	2150	0.39
Bu_4NClO_4	2370	0.92

[a] N. D. denotes that particle aggregation was not detected.

Morphology of aggregates of 1 by transmission electron microscopy (TEM)

The morphology of **1** was observed using TEM (Fig. 4a–d). Immediately after addition of acetonitrile (chloroform–acetonitrile =

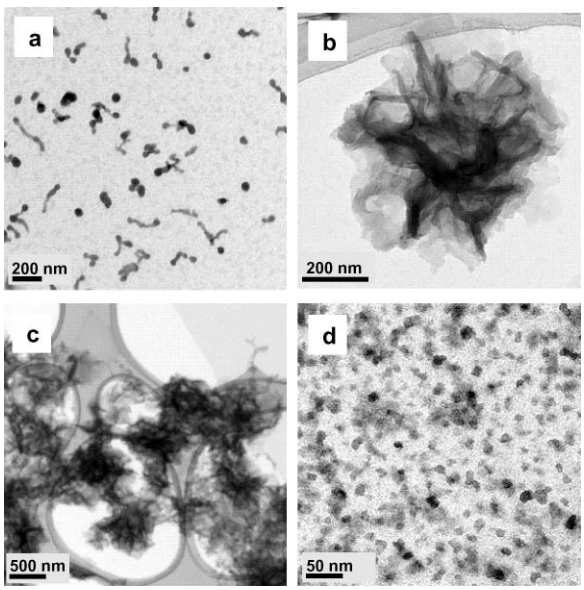
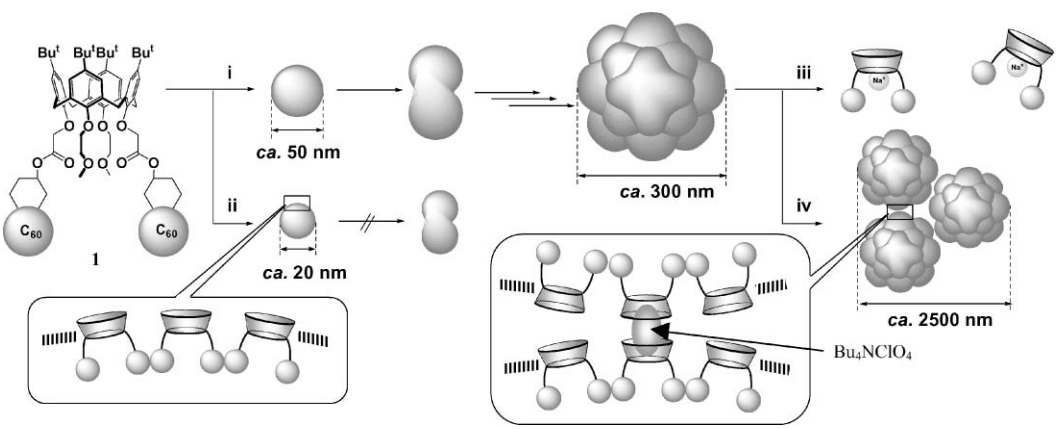


Fig. 4 TEM images of aggregates formed from 50 μM **1** in chloroform–acetonitrile = 1 : 1 (v/v) (a) 10 min and (b) 12 h after the addition of acetonitrile, (c) in chloroform–acetonitrile = 1 : 1 (v/v) in the presence of Bu_4NClO_4 (500 μM) and (d) in chloroform–acetonitrile = 1 : 4 (v/v); using uranyl acetate staining.



Scheme 3 Schematic representation of self-aggregations of **1**. *Conditions*: (i) [**1**] = 50 μM, chloroform–acetonitrile = 1 : 1 (v/v); (ii) [**1**] = 50 μM, chloroform–acetonitrile = 1 : 4 (v/v); (iii) [$NaClO_4$] = 500 μM; (iv) [Bu_4NClO_4] = 500 μM.

1 : 1 (v/v)), we recognized that a part of **1** formed discrete spherical particles with sizes of 30–50 nm (Fig. 4a), which are comparable to the particle sizes determined by DLS measurements (average particle size; 50 nm); a part of the particles commence to aggregate and to fuse (Fig. 4a). The fusion might be considered to occur by loosening interactions among C_{60} moieties caused by the lower stoichiometry of acetonitrile (Scheme 3(i)). Fig. 4b shows TEM images obtained from a solution that was left to stand for 12 h. Since it is clearly not a spherical particle, but a petal-like aggregate, their fusion proceeded further. The size (*ca.* 400 nm) is comparable with that of the particle size determined by DLS measurements (321 nm). Moreover, larger aggregates were observed by the addition of Bu_4NClO_4 (Fig. 4c). Subsequently, it was observed that the petal-like aggregates aggregate further, as shown in Fig. 4b, as suggested by Bu_4NClO_4 bridging between the aggregation surface of **1** (Scheme 3(iv)). On the other hand, by the addition of much acetonitrile (chloroform–acetonitrile = 1 : 4 (v/v)), we can recognize that all **1** formed discrete spherical particles and the particles did not fuse (Fig. 4d). The reason is that tight interactions among C_{60} moieties form as a result of the higher stoichiometry of acetonitrile (Scheme 3(ii)).

Conclusion

This study demonstrated that a calix[4]arene-appended bis-fullerene can form a trigger-sensitive self-aggregation with a high monodispersity in organic solvents. The aggregate size depended on the solvent composition and guest molecules in the calix[4]arene moiety. Disaggregation was induced by the addition of $NaClO_4$. Further aggregation was induced by the addition of Bu_4NClO_4. To the best of our knowledge, this is one of only a few examples of controlled self-aggregation of C_{60}. We hope to continue to strictly control both the size and shape of the self-aggregation by tuning the balance between solvophobic moieties (fullerenes) and solvophilic moieties (calixarenes) and to investigate applications of these aggregations in this laboratory.

Experimental

Chemicals

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(hydroxycarbonylmethoxy)-26,28-bis(methoxyethoxy)calix[4]arene (**2**)[11] and 1,9-(4-hydroxycyclohexano)buckminsterfullerene (**4**)[12] were synthesized according to methods described in previous reports. Lithium perchlorate, sodium perchlorate, tetramethylammonium perchlorate and tetrabutylammonium perchlorate were purchased from Wako Pure Chemical Industries, Ltd.

Preparation

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(3′,4′-buckminsterfullarocyclohexyloxacarbonylmethoxy)-26,28-bis(methoxyethoxy)calix[4]arene (1). Compound **2** (0.14 g, 0.15 mmol) was dissolved in 20 ml of dry benzene; to this solution were added pyridine (31 µl, 0.38 mmol) and thionyl chloride (0.24 ml, 3.1 mmol). The mixture was stirred at the reflux temperature for 12 h. After evaporation to dryness, to the residue (**3**) was added racemic **4** (0.37 g, 0.46 mmol), dry toluene (180 ml) and pyridine (31 µl, 0.38 mmol). The mixture was stirred at the reflux temperature for 48 h. After evaporation to dryness, the residue was purified by column chromatography (silica gel, toluene–ethyl acetate = 4 : 0–4 : 1 (v/v)) and by preparative TLC (silica gel, toluene–ethyl acetate = 40 : 1 (v/v) to give two isomers of **1**; *rac*-**1**, yield 4%; mp (decomp.) >300 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.04 (4H, s, ArH); 6.66 (4H, s, ArH); 6.31–6.27 (2H, m, OC*H*CH_2–C_{60}); 5.24–5.14 (4H, m, OCHCH_2–C_{60}); 4.93–4.79 (4H, m, $ArCH_2Ar$); 4.26–4.12 (8H, m, $OCH_2C{=}O$, CH_2–C_{60}); 3.99–3.74 (12H, m, ArOCH_2CH_2O, $ArOCH_2CH_2O$, CH_2–C_{60}); 3.53 (6H, s, OCH_3); 3.31–3.27 (4H, m, $ArCH_2Ar$); 2.70–2.66 (2H, m, C*H*CH_2–C_{60}); 1.25 (18H, s, Bu^t); 0.98 (18H, s, Bu^t); MS (MALDI-TOF, $CHCl_3$, $NaClO_4$) m/z 2454 $[M + Na]^+$. *meso*-**1**, yield 3%; mp (decomp.) >300 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.04 (4H, s, ArH); 6.66 (4H, s, ArH); 6.31–6.29 (2H, m, OC*H*CH_2–C_{60}); 5.25–5.17 (4H, m, OCHCH_2–C_{60}); 4.90–4.87 (4H, m, $ArCH_2Ar$); 4.25–4.17 (8H, m, $OCH_2C{=}O$, CH_2–C_{60}); 4.01–3.86 (12H, m, ArOCH_2CH_2O, $ArOCH_2CH_2O$, CH_2–C_{60}); 3.63 (3H, s, OCH_3); 3.49 (3H, s, OCH_3); 3.30–3.29 (4H, m, $ArCH_2Ar$); 2.75–2.71 (2H, m, C*H*CH_2–C_{60}); 1.25 (18H, s, Bu^t); 0.98 (18H, s, Bu^t); MS (MALDI-TOF, $CHCl_3$, $NaClO_4$) m/z 2454 $[M + Na]^+$.

Preparation of the solutions containing 1

Compound **1** was dissolved in chloroform. Acetonitrile with and without $LiClO_4$, $NaClO_4$, Me_4NClO_4 or Bu_4NClO_4 was added to this solution. All measurements were carried out at 20 °C.

Characterization of aggregates of 1

UV–Vis spectroscopic measurements were measured on a Shimadzu UV-2550PC spectrophotometer. Dynamic light-scattering measurements (DLS) were carried out using an Otsuka Electronic DLS-7000. Transmission electron microscopy (TEM) investigations were carried out with a JEOL JEM-3100FEF-3500N instrument. The solutions of **1** in chloroform–acetonitrile (1 : 1 or 1 : 4 (v/v)) with or without Bu_4ClO_4 were dropped onto a carbon-deposited 200-mesh copper grid (JEOL Datum Co. Ltd.) and the solvent was then evaporated at room temperature.

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