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Editor's Choice

Photocrosslinking of the Exterior of a Fullerene Bilayer that Prevents Vesicle Aggregation

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An amphiphilic molecule bearing a coumarin moiety attached to a water-soluble fullerene via an alkyl linker forms a water-soluble fullerene bilayer vesicle in water that spontaneously agglomerates and fuses upon standing. The agglomeration–fusion ability of the vesicle was endowed probably by the intervesicular interaction between the coumarin group and the fullerene core, as judged by the lack of such ability for the vesicles bearing only alkyl groups and by complete inhibition by conversion of the coumarin group into a photodimer.

Chemical modification of bilayer vesicles made of lipids or artificial amphiphiles is a standard method for modulating the functions of vesicles.[1–3] A typical example is the structural modification of the interior of the bilayer; for instance, by photocrosslinking.[4,5] We considered that a vesicle made of a water-soluble pentasubstituted amphiphilic fullerene $R_5C_{60}{}^-K^+$ [6–9] provides an interesting new opportunity for modification of the vesicle properties through functionalization of the exterior R groups. We report here the preparation and nanometer-size vesicle formation of a fullerene amphiphile **1** bearing coumarin groups connected via a hexamethylene linker in water, spontaneous agglomeration and fusion of the coumarin-modified fullerene vesicle, and photocrosslinking of the coumarin groups that prevents vesicle agglomeration (Figure 1a). A coumarin-modified vesicle spontaneously agglomerates and fuses upon standing at room temperature in a way similar to that of lipid vesicles (Figure 1b).[10,11] This behavior contrasts with that of the fullerene vesicles covered only by alkyl chains that do not agglomerate at all. The latter vesicles are robust, and their sizes do not change even after a year of being in water, and they can be analyzed without decomposition by electron microscopy in vacuum.[8] Upon photoirradiation of the coumarin vesicle with low-intensity light at 365 nm, the coumarin dimerizes, and the vesicle agglomerization stops (Figure 1c). This effect of exterior photocrosslinking is different from that of the photocrosslinking of the fullerene molecules in the interior of the bilayer under intense UV irradiation, which stabilizes the vesicle instead.[12]

A potassium salt of coumarin-bearing pentasubstituted fullerene **1** was prepared in THF by deprotonation of the protio precursor, which was synthesized from penta(4-sulfanylphenyl)-fullerene (see details in Supporting Information).[13–15] Injection of a THF solution of **1** into water followed by removal of the THF yielded an orange-colored aqueous solution of the bilayer vesicles of **1**, as previously reported for a variety of other $R_5C_{60}{}^-K^+$.[9] Dynamic light scattering (DLS) analysis of the resulting vesicle solution just after preparation showed a unimodal size distribution with an average diameter of 20–80 nm (e.g., Figure 2a red). Atomic force microscopy (AFM) measurement on mica displayed circular objects, the heights of

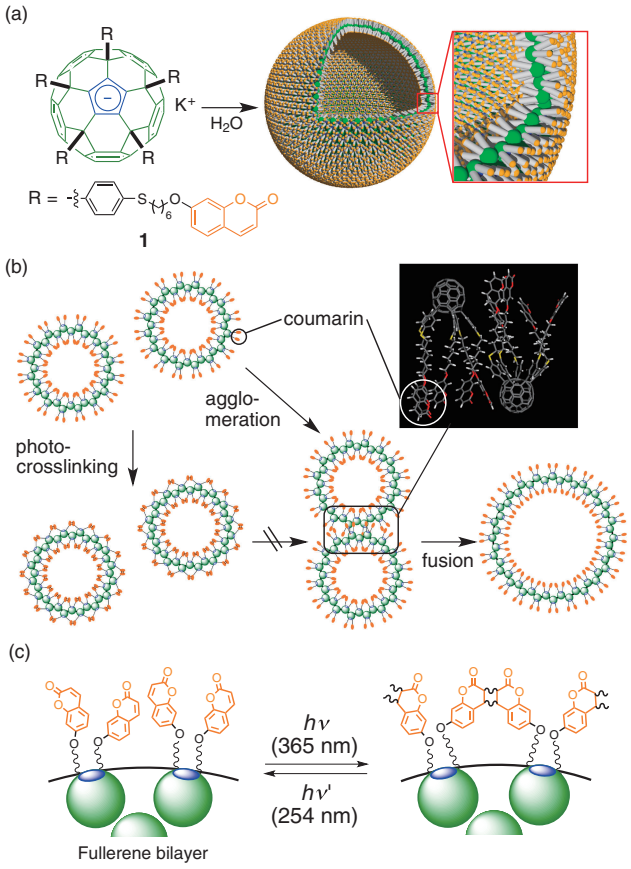

Figure 1. A bilayer vesicle made of a fullerene amphiphile bearing photoresponsive coumarin pendants. (a) Structure and vesicle formation of coumarin–fullerene amphiphile **1**. (b) Agglomeration and fusion of vesicles induced by intervesicular coumarin–fullerene interaction. (Inset) A molecular model of the anionic part of **1**. C, gray; H, white; O, red; S, yellow. (c) Schematic illustration of photodimerization and photocycloreversion of coumarin-appended fullerene vesicles upon UV irradiation.

which ranged from 10 to 40 nm for a sample with the average diameter of 30 nm based on DLS (see Supporting Information). The coumarin-modified fullerene vesicle was found to be less stable than previously reported $R_5C_{60}{}^-K^+$ vesicles covered by alkyl chains, as judged from their propensity to agglomerate and fuse (see below) and from their instability against an added metal ion (Mn(II) ion, necessary for the study of water permeation through the bilayer).[16]

The size of the vesicles made of **1** was found to increase after preparation. Figure 2b shows the increase in the size of the vesicles with an initial diameter of ca. 30 nm. When we left the

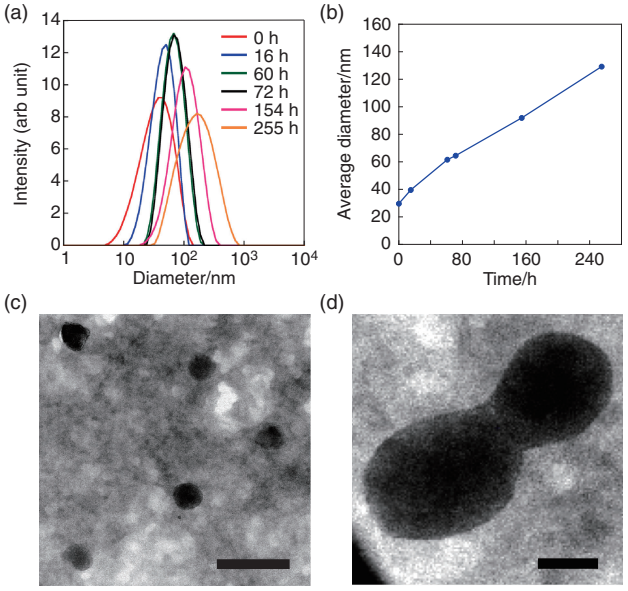

Figure 2. Characterization of bilayer vesicles made of **1**. (a) DLS analysis showing unimodal size distribution of vesicles at various time points after the preparation (H_2O, 25 °C, [**1**] = 0.4 mM). (b) Size change of the vesicles monitored by DLS (25 °C, [**1**] = 0.4 mM). (c, d) STEM images of the vesicles on a carbon film. (c) Dispersed vesicles. Scale bar is 200 nm. (d) Interlinked vesicles. Scale bar is 50 nm.

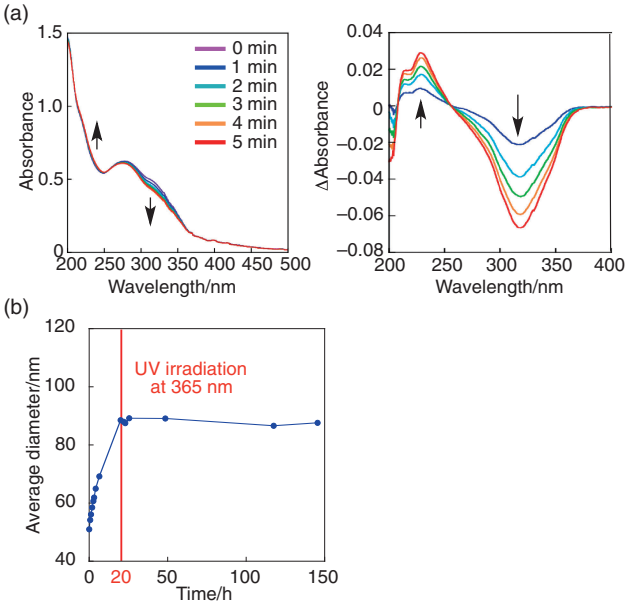

Figure 3. Photodimerization and cycloreversion of coumarin on fullerene vesicles. (a) UV–vis absorption spectral changes of coumarin-appended vesicles formed of amphiphilic fullerene **1** upon irradiation at 365 nm on the left, and the difference spectra on the right ([**1**] = 6 μM, 25 °C, H_2O). (b) Size change of the vesicles monitored by DLS ([**1**] = 0.4 mM) upon UV light irradiation (365 nm for 25 min) at 20 h after vesicle preparation.

vesicle solution to stand at 25 °C without stirring, the diameter gradually increased to 40 nm after 16 h and reached 129 nm after 255 h. A precipitate then began to form. For scanning transmission electron microscopic (STEM) analysis, we then placed, on a thin carbon film, a solution of the vesicles that had a hydrodynamic radius of 70.2 nm (by DLS analysis). We confirmed visually that the size had increased from 30 nm to 79 ± 16 nm (Figure 2c, based on 40 vesicles). Although the vast majority of the vesicles were spherical, we also found dumbbell-shaped objects (Figure 2d),[17] suggesting that the structurally labile coumarin vesicles first agglomerate and then fuse. As flat aromatic molecules similar to coumarin penetrate deep into the fullerene vesicle, because of strong π–π interactions with the fullerene group,[9,18] we consider that the intervesicular coumarin–fullerene interaction (Figure 1b inset) promotes agglomerization and fusion.[19–21]

The polydispersity index (PDI) of the vesicles based on the DLS analysis decreased from 0.25 at 0 h to 0.13 at 16 h, and then started to gradually increase up to 0.26 at 255 h (see Figure 2a and Supporting Information). The decrease in PDI during the initial stage indicates that smaller vesicles preferentially undergo agglomeration and fusion. This phenomenon can be rationalized by considering larger curvature of the smaller vesicles, which facilitates the penetration of coumarin units into the vesicle membranes. The gradual increase in PDI during later stages is consistent with the agglomeration and fusion of the vesicles.

Upon UV light (365 nm, 6 W lamp) irradiation, the absorption peak at 320 nm, due to the coumarin group, decreased, and a new absorption peak at 225 nm, due to the dimer, appeared. The appearance of isosbestic points at 260 and 380 nm (Figure 3a) indicates that the weak irradiation kept the fullerene intact (it only dimerizes under irradiation with a 400 W high-pressure mercury lamp).[22–24] The yield of the photocyclization is calculated to be ca. 20% after 5 min of irradiation from the change in absorption of the coumarin units. The dimerization reaction completely stopped the vesicle agglomeration, as shown in Figure 3b. UV irradiation of 365 nm at 20 h after the preparation of the vesicles (average diameter 90 nm, Figure 3b) stopped the increase in the vesicle diameter.

The photocrosslinking of, and cycloreversion to, the coumarin can be achieved by sequential irradiation at 365 nm and at 254 nm, respectively, as shown by the disappearance and reappearance of the coumarin absorption at 320 nm accompanying isosbestic points (Figure 4a). This process is known to be partially (ca. 20%) irreversible (Figure 4b) because of the formation of isomers of the dimer that do not regenerate the coumarin.[25]

The photocycloreversion of the coumarin dimer does not restore the agglomeration–fusion ability of the coumarin vesicle, as shown in Figure 4c, in spite of the cycloreversion in >80% yield, leaving only ca. 4% of the coumarin units dimerized (i.e., one out of five fullerene molecules in the membrane is crosslinked). This is probably because partial crosslinking of the amphiphile components in the bilayer is sufficient to stabilize the bilayer and to prevent agglomerization and fusion. A similar observation was also made for the photocrosslinking of the fullerene core of fullerene vesicles, where crosslinking of only a small fraction of the fullerene molecules significantly decreased the water permeability of the fullerene bilayer.[12]

In conclusion, we synthesized a fullerene molecule to which five coumarin groups were connected through a hexamethylene linker, and formed a vesicle in water that bears the coumarin

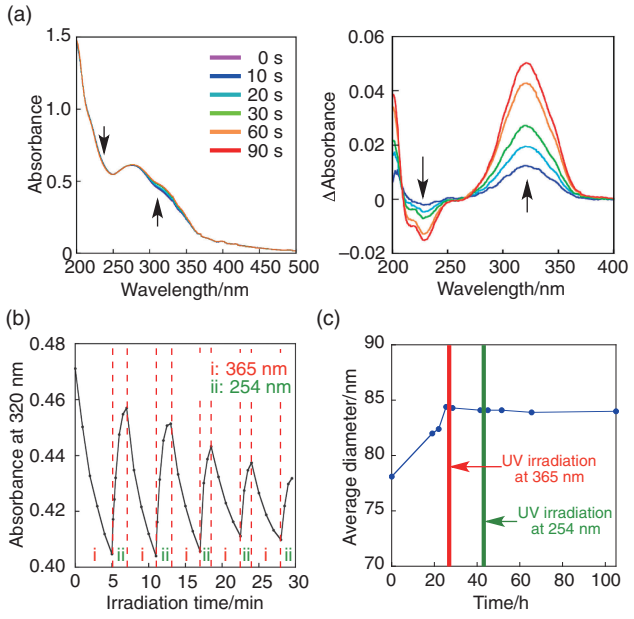

Figure 4. Photocleavage of coumarin dimers on fullerene vesicles. (a) UV–vis absorption spectral changes of the photo-crosslinked vesicles upon irradiation at 254 nm on the left, and the difference spectra on the right ([**1**] = 6 μM, 25 °C, H_2O). (b) Variation of the absorbance of coumarin-appended vesicles formed from amphiphilic fullerene **1** upon alternate irradiation at 365 and 254 nm. (c) Size change of vesicles monitored by DLS ([**1**] = 0.4 mM) upon irradiation of UV light (at 365 nm for 25 min at 26 h and at 254 nm for 8 min at 42 h) after vesicle preparation.

appendage on its exterior surface. Unlike $R_5C_{60}{}^{-}K^{+}$, which bears alkyl chains on the surface, the coumarin vesicle undergoes spontaneous agglomeration and fusion to produce a larger vesicle at room temperature, resulting in the formation of vesicles of >100 nm diameter. We consider that the coumarin vesicle gains its agglomeration–fusion ability because of the ability of the coumarin group to interact intervesicularly with the fullerene group. Our results provide an illustration of the molecular design for programming of the functions of molecular assemblies.[26]

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References and Notes

1 *Vesicles*, ed. by M. Rosoff, Marcel Dekker, New York, **1996**.
2 A. Mueller, D. F. O'Brien, *Chem. Rev.* **2002**, *102*, 727.
3 Y. Wang, H. Xu, X. Zhang, *Adv. Mater.* **2009**, *21*, 2849.
4 W. Meier, *Chem. Soc. Rev.* **2000**, *29*, 295.
5 M. P. Cashion, T. E. Long, *Acc. Chem. Res.* **2009**, *42*, 1016.
6 M. Sawamura, N. Nagahama, M. Toganoh, U. E. Hackler, H. Isobe, E. Nakamura, S.-Q. Zhou, B. Chu, *Chem. Lett.* **2000**, 1098.
7 S. Zhou, C. Burger, B. Chu, M. Sawamura, N. Nagahama, M. Toganoh, U. E. Hackler, H. Isobe, E. Nakamura, *Science* **2001**, *291*, 1944.
8 T. Homma, K. Harano, H. Isobe, E. Nakamura, *J. Am. Chem. Soc.* **2011**, *133*, 6364.
9 K. Harano, R. M. Gorgoll, E. Nakamura, *Chem. Commun.* **2013**, *49*, 7629.
10 A. Shibata, in *Liposomes in Life Science, Experimental Manual*, ed. by H. Terada, T. Yoshimura, Springer, Tokyo, **1992**.
11 Y. Anraku, A. Kishimura, Y. Yamasaki, K. Kataoka, *J. Am. Chem. Soc.* **2013**, *135*, 1423.
12 K. Harano, A. Narita, E. Nakamura, *Chem. Lett.* **2013**, *42*, 1176.
13 H. Isobe, H. Mashima, H. Yorimitsu, E. Nakamura, *Org. Lett.* **2003**, *5*, 4461.
14 H. Isobe, K. Cho, N. Solin, D. B. Werz, P. H. Seeberger, E. Nakamura, *Org. Lett.* **2007**, *9*, 4611.
15 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
16 H. Isobe, T. Homma, E. Nakamura, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14895.
17 B. Jing, X. Chen, Y. Zhao, X. Wang, F. Ma, X. Yue, *J. Mater. Chem.* **2009**, *19*, 2037.
18 L. K. Shrestha, Q. Ji, T. Mori, K. Miyazawa, Y. Yamauchi, J. P. Hill, K. Ariga, *Chem.—Asian J.* **2013**, *8*, 1662.
19 R. Blumenthal, M. J. Clague, S. R. Durell, R. M. Epand, *Chem. Rev.* **2003**, *103*, 53.
20 C. M. Paleos, D. Tsiourvas, Z. Sideratou, *ChemBioChem* **2011**, *12*, 510.
21 M. Ma, D. Bong, *Acc. Chem. Res.* **2013**, *46*, 2988.
22 S. R. Trenor, A. R. Shultz, B. J. Love, T. E. Long, *Chem. Rev.* **2004**, *104*, 3059.
23 J. Motoyanagi, T. Fukushima, N. Ishii, T. Aida, *J. Am. Chem. Soc.* **2006**, *128*, 4220.
24 N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350.
25 Y. Chen, C.-S. Jean, *J. Appl. Polym. Sci.* **1997**, *64*, 1759.
26 H. Nishihara, *Chem. Lett.* **2014**, *43*, 388.

