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Photo-triggered recognition between host and guest compounds in a giant vesicle encapsulating photo-pierceable vesicles

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ABSTRACT

Here, we used centrifugal precipitation to construct a giant vesicle (GV) encapsulating smaller giant vesicles (GV-in-GV) which comprises a photo-resistant outer GV and a photo-pierceable inner GV; the outer GV contained a fluorescent probe (SYBR Green I) in its inner water pool, and the inner GV contained double-stranded DNA (dsDNA) in its inner water pool. The phospholipid membrane of the inner GV was made photo-pierceable by inclusion of *ca.* 15 mol% of a caged phospholipid in its membrane. Immediately after exposure of the GV-in-GVs to UV irradiation, strong fluorescence was detected in the inner water pool of the outer GV, indicating that dsDNA had been released from the inner GV and had complexed with the fluorescent probe. These dynamics can be recognized as a macroscopic representation of the molecular level function of a caged compound.

1. Introduction

Giant vesicles (GVs) with a diameter larger than several μm are easily observed under an optical microscope (Luisi and Walde, 1999; Walde et al., 2010) and so can be used as micro-reaction vessels for the study of enzyme-based processes such as protein expression and DNA replication (Fischer et al., 2002; Monnard et al., 2007; Hosoda et al., 2008; Shoda et al., 2011; Kurihara et al., 2011, 2015; Saha and Chen, 2015; Adamala et al., 2016). In addition, the size and shape of GVs make them potentially useful models of living cells (Kurihara et al., 2011, 2015; Saha and Chen, 2015; Adamala et al., 2016; Janout and Regen, 2009; Fernandez-Trillo et al., 2017). However, controlling the start of biochemical reactions within GVs is difficult as reactions often progress during GV preparation. One way to address this issue would be to prevent interaction of the compounds involved in the reaction by creating a GV encapsulating smaller GV (GV-in-GV) (Fernandez-Trillo et al., 2017; Bolinger et al., 2004; Kisak et al., 2004; York-Duran et al., 2017), which are also called as a vesosome (Fernandez-Trillo et al., 2017; York-Duran et al., 2017), with the walls of the inner GVs being pierceable when exposed to a controllable external stimulus. Previously, Vogel et al. demonstrated release of chemicals from the inner GV of a GV-in-GV by using a heat-labile phospholipid membrane (Bolinger et al., 2004). However, this method cannot be applied broadly to phospholipid GVs containing specific membrane components.

In the present study, we constructed a GV-in-GV in which the phospholipid membrane of the inner GV contained *ca.* 15 mol% of a photo-labile caged phospholipid (2) (Kusumi et al., 1989; Yamagich et al., 1998), which was synthesized from mono-caged dodecanedioic acid (1) and *sn*-glycerophosphocholine (α-GPC)-adsorbed kieselguhr (Ichihara et al., 2005). The GV-in-GV contained two compounds separately, double-stranded DNA (dsDNA) in the inner water pool of the inner GV and the fluorescent probe SYBR Green I in the inner water pool of the outer GV. Upon irradiation with ultraviolet (UV) light, 2 in the phospholipid membrane of the inner GV was converted to a water-soluble phospholipid (3), which produced holes in the membrane through which the dsDNA could pass and form a fluorescent intercalation complex with the SYBR Green I (Fig. 1) (Dragan et al., 2012). Such a GV-in-GV system may provide a method of mixing two chemicals, which are incorporated in nested micro-cavities separately, instantly responding to an external photo-stimulus on demand.

2. Materials and methods

2.1. General

Nitrobenzyl alcohol was recrystallized from an ethanol solution in the dark before use. Dried THF was obtained by distillation with calcium hydride under a nitrogen atmosphere. Dried, alcohol-free $CHCl_3$ was obtained by distillation over P_2O_5 under a nitrogen atmosphere after stirring with conc. H_2SO_4 overnight. Triethylamine was dried on molecular sieves overnight. All other chemicals were used without further purification. Nuclear magnetic resonance (^1H NMR) spectra were obtained by using a Fourier-transform NMR spectrometer (ECS-400S; JEOL, Japan). Scheme of a synthetic route of photo-labile caged

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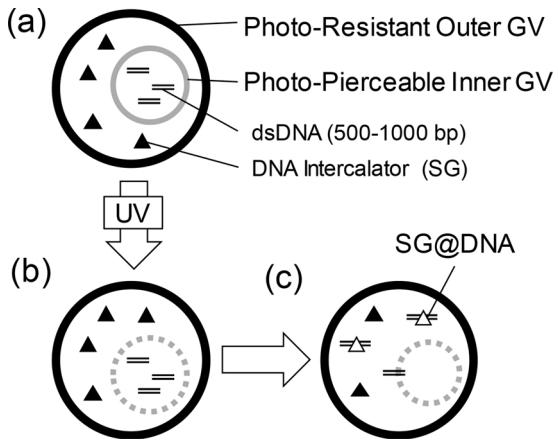

Fig. 1. Schematic illustration of the developed GV-in-GV and its response to UV irradiation. (a) The GV-in-GV comprised two GVs, a photo-pierceable inner GV containing double-stranded DNA (dsDNA) and a photo-resistant outer GV containing the dsDNA intercalator SYBR Green I (SG). (b) Immediately upon UV irradiation, the phospholipid membrane of the inner GV was pierced due to photochemical reaction of a caged phospholipid in the membrane. (c) dsDNA then diffused into the inner water pool of the outer GV, where it formed a fluorescent intercalation complex with SG (SG@DNA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phospholipid 2 is shown in the Supplementary materials (Scheme S1).

2.2. Synthesis of 11-(2-nitrobenzyloxycarbonyl)undecanoic acid (1)

11-(2-Nitrobenzyloxycarbonyl)undecanoic acid was prepared according to the reported procedure (Yamagich et al., 1998). Thionyl chloride (40 mL) was added to dodecanedioic acid (25 g, 0.11 mol), and the mixture was refluxed for 5 h under a nitrogen atmosphere. After cooling the solution to room temperature, excess of thionyl chloride was removed under reduced pressure, and the crude product was distilled *in vacuo* (120 °C, 1 mmHg) to afford decanedioyl dichloride as a colorless liquid (91%). The prepared decanedioyl dichloride (9.1 g 38 mmol) was dissolved in dried THF (100 mL) on an ice bath, and 75 mL of a dried THF solution of 2-nitorobenzyl alcohol (5.2 g 34 mmol) and triethylamine (4.8 mL) was added dropwise for 30 min to the above solution, and the mixture was stirred overnight at room temperature. To decompose the remaining acid chloride, a mixed solution of THF (25 mL), water (25 mL) and triethylamine (4.8 mL) was added dropwise for 1 h at room temperature to the resulting solution, and stirred for 2 h. After removing organic solvents by a rotary evaporator, the residue was dissolved in Et_2O (50 mL), washed with water (3 times) and saturated brine, and the separated organic layer was dried over Na_2SO_4. The crude product was isolated by silica gel column chromatography using $CHCl_3$ as eluent to give 11-(2-nitrobenzyloxycarbonyl)undecanoic acid (1) as white powder (40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) = 8.1–7.5 (m, 4H), 5.5 (s, 2H), 2.4–2.3 (m, 4H), 1.6 (m, 4H), 1.3 (m, 12H).

2.3. Preparation of α-GPC-adsorbed kieselguhr

α-GPC-absorbed kieselguhr was prepared according to the reported procedure (Ichihara et al., 2005). Powdered kieselguhr (Hyflo Super-Cel, Sigma-Aldrich, US) was dried under 140 °C for 1 week before use. Dried 5 mL methanol solution of 300 mg (1.2 mmol) of α-GPC, and 2.3 g of dried powder of kieselguhr was added to a Teflon test tube (F-1118-04, Flon Industry, Japan). The resulted suspension was shaken vigorously by a vortex mixer for several minutes, and methanol was removed by spraying of a dried nitrogen flow. The resulting gruel-like product was dried completely over P_2O_5 in a vacuum desiccator overnight. The α-GPC-adsorbed kieselguhr was obtained as a white cake.

2.4. Synthesis of 1,2-di(10-(2-nitrobenzyl)oxy-10-oxodecanyl)-sn-glycero-3-phosphocholine (2)

1,2-Di(10-(2-nitorobenzyl)oxy-10-oxodecanyl)-*sn*-glycero-3-phosphocholine (2) was synthesized by modifying the reported procedure (Yamagich et al., 1998) using α-GPC-adsorbed kieselguhr (Ichihara et al., 2005) instead of $CdCl_2$ (Bittman, 1993). In a 50 mL Erlenmeyer flask was placed 2.3 g of crashed powder of α-GPC-adsorbed kieselguhr, which contains 270 mg (1.1 mmol) of α-GPC, and 20 mL of dried, alcohol-free $CHCl_3$ was added, and the mixture was stirred for 30 min under a nitrogen atmosphere. To the resulting suspension was added 313 mg (2.5 mmol) of *N*,*N*-dimethyl-4-aminopyridine (DMAP), 1.76 g (4.8 mmol) of 1 and 1.45 g (4.8 mmol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) sequentially, and the mixture was stirred for 1 week at 30 °C under nitrogen atmosphere. $CHCl_3$ (20 mL) was added to the resulting gruel-like mixture, and the extracted solution was filtered. The residue was extracted again with 20 mL of $CHCl_3$ several times, and the combined $CHCl_3$ solution was passed through an ion-exchange column with an H-type ion exchange resin to remove DMAP. The eluted solution was evaporated to afford a crude product, which was separated by gel permeation chromatography using $CHCl_3$ containing 1% of triethylamine to afford 2 as a light-brown oily product (60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.08(d, 2H), 7.63 (t, 2H), 7.57 (d, 2H), 7.48 (t, 2H), 5.49 (s, 4H), 5.20 (m, 1H), 4.4-3.8 (m, 8H), 3.37 (s, 9H), 2.4–2.2(m, 8H), 1.7–1.2 (m, 32H). See the Supplementary material for the ^{1}H NMR spectrum of 2 (Fig. S1).

2.5. Preparation and UV irradiation of photo-pierceable single GVs containing a fluorescent dye

A dried thin film containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (0.4 μmol), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-glycerol sodium salt (POPG) (0.1 μmol), cholesterol (0.1 μmol), and various amounts of the photo-labile caged phospholipid 2 (0, 0.02, 0.05, 0.1, or 0.2 μmol) was prepared on the surface of a glass vial from the $CHCl_3$ solutions of the lipids. The dried thin film was swelled with 0.1 mL PIPES buffer (5 mM, pH 7.5) containing 1 mM of the fluorescent dye pyranine (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) and left to stand for several hours until a vesicular dispersion had formed. The outer water phase of the vesicular dispersion was diluted 20 times with pyranine-free PIPES buffer (5 mM, pH 7.5) (Kusumi et al., 1989); the total concentration of lipids in the resulting dispersion was 0.5 mM.

Samples of the vesicular dispersions were placed in micro-chambers comprising a frame-shaped silicon-rubber spacer (length × width, *ca.* 1 cm × 1 cm; thickness, 0.5 mm; silicone rubber sheet 0.5T; AS ONE, Japan) sandwiched between a pair of thin cover glasses (NEO cover glass, thickness no. 1; Matsunami Glass, Japan). Samples in the micro-chamber were observed under a phase-contrast microscope and a fluorescence microscope (IX71, Olympus, Japan) fitted with an optical filter set (λ_{ex} = 460–490 nm, λ_{em} = 510–550 nm) and a 40 × objective lens.

At the start of each experiment, a phase-contrast microscope was used to select one spherical, hollow GV with a diameter of several tens of micrometers in each vesicular dispersion as the target GV. Phase-contrast and fluorescence microscope images of the target GVs before and after UV irradiation for 5 s (100 W high-pressure mercury lamp; U-LH100HG; Olympus, Japan) were recorded via a CCD camera (exposure time, 300 ms; gain, 1.0; AdvanCam LP2; Advision, Japan) by using the ToupView image capture application (version 3.7; ToupTek, China).

Recorded images were analyzed by using the ImageJ imaging application (National Institutes of Health, USA), and fluorescence intensity was digitized to an arbitrary unit. The net fluorescence intensity emitted by the fluorescent dye within the target GV was calculated as the difference between the intensity at the center of the target GV and that in the surrounding water phase. Residual fluorescence intensity