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Control of the incorporation and release of guest molecules by photodimerization in liposomes

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ABSTRACT

In a drug-delivery system using liposomes, the use of guest molecules bearing hydrophilic moieties results in some leakage from lipid membranes. We suppressed the leakage of coumarins (used as model guest molecules in a drug-delivery system) from lipid membranes by photodimerization at 365 nm. The reason for this phenomenon could be ascribed to an increase in the hydrophobicity of the dimers of the coumarins. The formation of the dimers was detected by ^{1}H NMR, UV–vis absorption, and mass spectra and the leakage percentages of the coumarins were determined by ^{1}H NMR spectra based on the peak intensities. In contrast, when the dimer reverted to a monomer by ultraviolet (254 nm) irradiation, the resulting monomer was released from liposomes.

1. Introduction

In a drug-delivery system, it is very important to control the location of a drug, as well as the timing and amount of drug release in the target tissue (e.g., tumor) [1–5]. Heat, pH, light and enzymes can serve as triggers for controlled release. Liposomes have received increasing levels of attention because of their potential application as drug carriers because poorly water-soluble "guest molecules" (drugs) can be incorporated within lipid membranes [6]. Interest in liposomes as drug carriers is based on their potential to enclose various types of biologic/biomedical materials and to deliver them to diverse cell types [6]. Therefore, the stabilities of lipid membrane-incorporated guest molecules (LMIGs) are very important to increase their bioavailability and circulation in blood. However, the use of guest molecules bearing hydrophilic moieties could result in some leakage from lipid membranes or destabilization of LMIGs by the collapse of liposomes and micelle formation. Schwarzenbach et al. reported a method for determining the liposome–water distribution ratio (log K_{lipw}) of substituted phenols and several other compounds [7]. In contrast, we investigated the relationship between the equilibrium and octanol–water partition coefficient (log P_{ow}) for some small model guest molecules with a π-moiety [8]. Our results showed that, in most of the guest molecules with log $P_{ow} > 1.9$, whole molecules were incorporated into liposomes to form stable LMIGs. However, many of the guest molecules with log $P_{ow} < 1.9$ leaked from the lipid membranes and dissolved in bulk water (Scheme 1a). That is, the threshold for leakage of guest molecules was determined to be ≈1.9. The log P_{ow} value of a guest molecule is usually defined as the ratio of its concentrations in the two phases of a mixture composed of 1-octanol and water [9]. The log P_{ow} values of guest molecules are usually determined experimentally. However, it is also possible to estimate these values using several commercially available computer programs [10]. Therefore, use of log P_{ow} values facilitates prediction of whether some guest molecules leak from lipid membranes. In the present study, we employed coumarins (**1** and **2**; Fig. 1) with relatively low log P_{ow} values (< 1.9) as small guest molecules, and controlled the incorporation and release of these molecules in liposomes by photodimerization (Scheme 1).

2. Materials and Methods

2.1. Materials

Coumarins **1** and **2** were purchased from Tokyo Chemical Industries (Tokyo, Japan). 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was obtained from Funakoshi (Tokyo, Japan).

2.2. Preparation of Liposomes

An appropriate amount of a mixture of DMPC and **1** or **2** ([DMPC]: [**1** or **2**] = 5:1 mol/mol) was dissolved in chloroform. The solvent was evaporated under a gentle stream of nitrogen, followed by a period under vacuum to remove trace solvent. The resulting thin lipid films were hydrated on the wall of the vial above the phase transition temperature with an appropriate amount of water. The hydrated materials were subjected to eight freeze–thaw cycles (−195 and +50 °C) to give unilamellar vesicles, which were extruded 11 times through 0.05-μm

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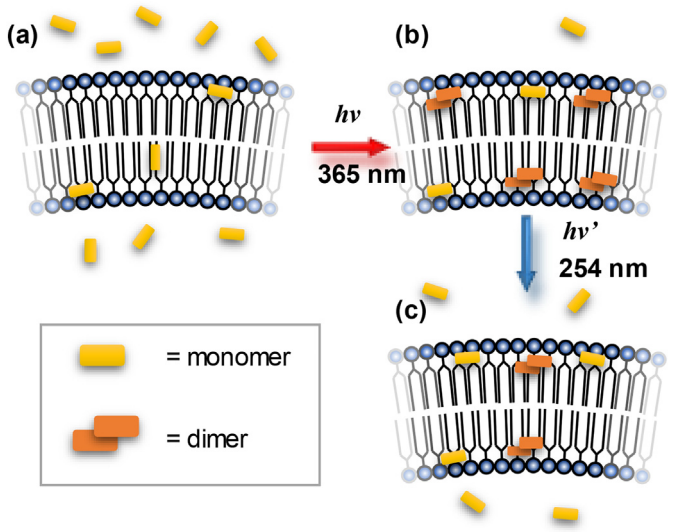

Scheme 1. Guest molecules (yellow) and guest dimers (orange) before (a) and after photoirradiation at 365 nm (b) and 254 nm (c) inside and outside the lipid membrane (schematic).

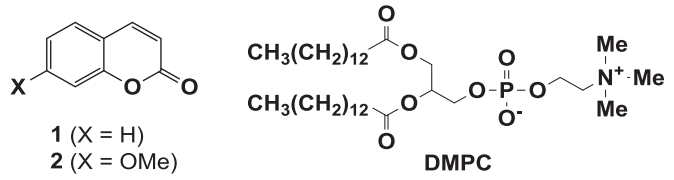

Fig. 1. Structures of compounds.

pores using a LiposoFast™ Mini-extruder (Avestin, Ottawa, ON, USA) above the phase transition temperature. The resulting liposomes were uniform in size with a diameter of ≈90 nm.

2.3. Determination of Leakage Percentages

The leakage percentages of **1** and **2** were determined using ^{1}H NMR spectra of LMI**1** and LMI**2** aqueous solutions (D_2O, [DMPC] = 4.0 mM, [DMPC]:[**1** or **2**] = 5:1 mol/mol). They were based on the peak intensities of **1** and **2** relative to the DMSO peak (0.4 mM), which was added as an internal reference.

2.4. Photoirradiation

Aqueous solutions of LMI**1** and LMI**2** (1.0 ml, [**1** or **2**] = 0.8 mM, [DMPC] = 4.0 mM) were photoirradiated at 365 nm for 12 h or 9 h, respectively, in a 1-cm quartz cell (1.0 W m^{-2}). Furthermore, these solutions were photoirradiated at 254 nm for 12 h and 4 h, respectively, in a 1-cm quartz cell (1.2 W m^{-2}). The photoreactions were monitored by UV–vis absorption and ^{1}H NMR spectra. The formation of dimer **1–1** was confirmed by GC–MS spectra and that of dimer **2–2** by GC–MS and ^{1}H NMR spectra.

2.5. Separation of Dimer 2–2

An aqueous solution of LMI**2** (1.0 ml, [**2**] = 0.8 mM, [DMPC] = 4.0 mM) was photoirradiated at 365 nm for 9 h. The solution was lyophilized and the residue separated by preparative thin-layer chromatography on silica gel ($CHCl_3$, R_f = 0.05). The residue of the fraction was dissolved in $CDCl_3$ and was measured by ^{1}H NMR spectrum to determine containing dimer **2–2**.

2.6. UV–Vis Absorption Spectra

UV–vis spectra were recorded using a UV-2550PC spectrophotometer (Shimadzu, Kyoto, Japan). All experiments were undertaken at 25 °C using a 1-mm cell.

2.6.1. ^{1}H NMR Spectroscopy

^{1}H NMR data were recorded using a Varian 400-MR (400 MHz) spectrometer (Agilent Technologies, Santa Clara, CA, USA).

2.7. Gas Chromatography–Mass Spectrometry (GC–MS)

The GC–MS system used was a JMS-T100GCV AccuTOF GCv 4G (JEOL, Tokoyo, Japan) equipped with a 30 m × 0.25 mm I.D. fused-silica capillary column (J&W; HP-5; film thickness, 0.25 μm). The column temperature was set at 50 °C for 1 min, and then it was programmed to heat from 50 °C to 300 °C at 10 °C/min. The temperature of the injection port and interface was set at 300 °C. Splitless injection mode was used. Helium at a flow rate of 1.5 ml/min was used as a carrier gas. Target analytes underwent chemical ionization at 200 eV and were detected in scan mode. Target ions had a m/z of 293 for dimer **1–1** and 353 for dimer **2–2** as $[M + H]^+$, respectively.

3. Results and Discussion

3.1. Formation of LMIGs by the Premixing Method

Liposomes consisting of dimyristoylphosphatidylcholine (DMPC) were prepared in the presence of coumarin (**1**) or 7-methoxycoumarin (**2**) according to the premixing method [6, 8, 11–14]. Briefly, LMI**1** and LMI**2** were prepared by dissolving DMPC and **1** or **2** in chloroform, followed by evaporation of chloroform under a stream of dry nitrogen gas to give a residue. Multilamellar vesicles (MLVs) were prepared by hydration of the dried lipid films by vortexing. To change from multilamellar to unilamellar vesicles and to obtain a narrow size distribution, the solution was repeatedly frozen and thawed eight times and extruded eleven times with two stacked polycarbonate membranes with a pore size of 50 nm.

The UV–vis absorption spectra of LMI**1** and LMI**2** showed that **1** and **2** were dissolved in water (Fig. 2a, b; black line). However, the results did not indicate whether all of **1** and **2** were incorporated in lipid membranes because hydrophilic **1** and **2** might have leaked from lipid membranes [8].

3.2. Leakage Percentages of Monomer 1 and Dimer 1–1 from Liposomes

We have reported that the proton nuclear magnetic resonance (^{1}H NMR) spectrum of LMI**1** contains several peaks in the range 6.4–8.2 ppm (Figs. 3 and S1) [8]. Because peaks belonging to the guest molecules and lipids in LMIGs should have mostly disappeared due to peak broadening, these peaks were assignable to **1** having leaked from lipid membranes to bulk water (Figs. 3a and S1a) [8]. The leakage percentage of **1** was determined to be 65% for [**1**]/[DMPC] = 20.0 mol % based on the peak intensities of compound **1** relative to DMSO (0.4 mM), which was added as an internal reference (Table 1). The leakage percentage of **1** was scarcely affected by the concentration of **1** ([**1**]/[DMPC] = 5.0–20.0 mol%, Table 1 and Fig. S2). It is well-known that **1** photodimerizes under irradiation at 365 nm to give four regioisomers and stereo-isomers (Scheme 2) [15]. Therefore, we carried out the photodimerization of **1** in a mixture of **1** and liposomes. After photoirradiation at 365 nm for 12 h, the absorption peak at ≈ 275 nm decreased (Fig. 2a, red line). This result suggested that the formation of dimer **1–1** (Scheme 2) shortened a π-conjugation length by changing from a double bond of **1** to the cyclobutane of dimer **1–1**. The latter was detected at a mass-to-charge ratio (m/z) of 293.1 during gas chromatography–mass spectrometry (GC–MS) (Fig. 4). After