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Irradiation-induced fusion between giant vesicles and photoresponsive large unilamellar vesicles containing malachite green derivative

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ABSTRACT

Light-initiated fusion between vesicles has attracted much attention in the research community. In particular, fusion between photoresponsive and non-photoresponsive vesicles has been of much interest in the development of systems for the delivery of therapeutic agents to cells. We have performed fusion between giant vesicles (GVs) and photoresponsive smaller vesicles containing malachite green (MG) derivative, which undergoes ionization to afford a positive charge on the molecule by irradiation. The fusion proceeds as the concentration of GV lipid increases toward equimolarity with the lipid of the smaller vesicle. It is also dependent on the molar percentage of photoionized MG in the lipid of the smaller vesicle. On the other hand, the fusion is hardly affected by the anionic component of the GV. The photoinduced fusion was characterized by two methods, involving the mixing of lipid membranes and of aqueous contents. Fluorescence microscopy revealed that irradiation triggered the fusion of a single GV with the smaller vesicles containing MG.

1. Introduction

Fusion between lipid membranes is one of the most important events in living cells for fertilization, cell fusion, endo- and exo-cytosis, reconstruction of damaged organelles, and cell division [1–3]. Since vesicles have been extensively used as drug nano-carriers, employing vesicle fusion to cell membranes has attracted great interest in the design of systems for the delivery of therapeutic agents to cells. Vesicle fusion to cell membranes allows the direct release of active molecules inside the cytoplasm, and is considered a safe and efficient mechanism for drug delivery. Membrane decoration for vesicle fusion can be achieved by introducing polymers [2,4,5], peptides [6–8], electrostatic charge–charge interaction [9–20], and acid-responsive moieties [21]. Vesicles that function as triggerable drug carriers for drug delivery to desired sites have been developed especially for targeted delivery systems. Among the many current strategies to control drug delivery, the use of light for spatial and temporal control is an attractive approach, which may allow for precise on-demand drug delivery within individual cells. Vesicles can be rendered photoresponsive by using lipids that isomerize [22–27], polymerize [28–31], fragment [32], or induce oxidative reactions [33,34] upon irradiation. Much research on photoresponsive drug delivery to cells has been based on the release of drugs entrapped within vesicles [35–37]. For instance, photoresponsive vesicles incorporating coumarin, which shows dimerization under irradiation, showed photo-triggered release [37]. Coumarin vesicles containing an anti-cancer drug were applied to KB cells (human epidermal carcinoma) and the anti-cancer efficacy was examined. Irradiation did not significantly enhance the inhibition of cancer cell growth. This may be due to endo-cytosis, which is the most common pathway for internalization, wherein the drug remains trapped in the endosome before its cytoplasmic release [38]. Membrane fusion allows the drug molecules to escape from the endo-lysosomal compartments and be released directly into the cytoplasm. However, only a few studies have been conducted on the fusion of photoresponsive vesicles for efficient drug delivery to cells.

We have reported the photoinduced fusion of vesicles incorporating a malachite green (MG, Fig. 1) leuconitrile derivative carrying a long alkyl chain [39]. MG undergoes photoionization to become an amphiphilic compound consisting of a hydrophilic triphenylmethyl cationic head group and a hydrophobic long alkyl chain tail. Under dark conditions, the head group of MG is less polar than it is after irradiation, and MG is thus sufficiently lipophilic to be solubilized in the vesicle membrane. The photoionized charge on MG significantly perturbs the membrane packing and ionized MG carries a

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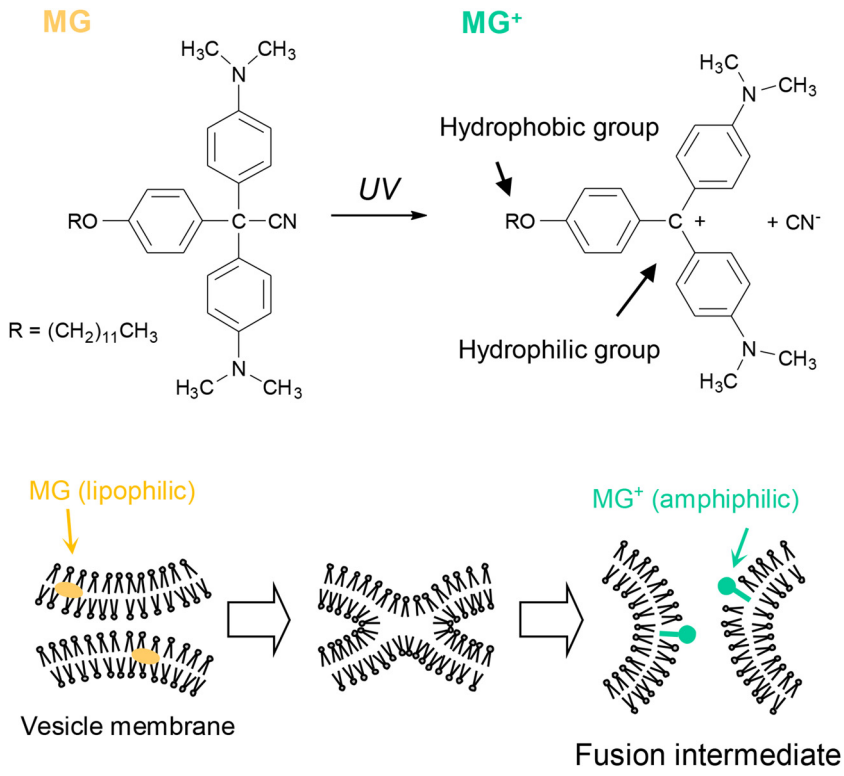

Fig. 1. Photoionization of malachite green (MG) leuconitrile derivative carrying a long alkyl chain induces membrane fusion. Neutral MG is solubilized in the lipid membrane until irradiation. The big head group of ionized MG (MG^+) effectively stabilizes the intermediate of membrane fusion that has been proposed in the popular "stalk-pore" model of vesicle fusion [2,3]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

big hydrophilic head that stabilizes the intermediate of membrane fusion (Fig. 1) [39]. Although fusion between MG vesicles had been observed, questions remained. Do MG vesicles fuse with normal lipid membranes (non-photoresponsive vesicles)? What are the key factors that determine fusion between photoresponsive and non-photoresponsive vesicles? To answer these questions, we used giant vesicles (GVs) in this work. GVs are spherical lamellar structures having diameters greater than one micrometer. They provide a good model for mammalian cells, owing to their comparable diameter, bending energy surface density, and osmotic response [40]. Investigation of the fusion between GVs and MG vesicles was thereby expected to give relevant information on photoresponsive drug delivery using membrane fusion. Furthermore, GVs present the great advantage of being observable by optical microscopy, allowing the investigation of membrane properties by detection of single GVs.

In this work, we report the photoinduced fusion of MG vesicles with GVs consisting of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC). Fluorescence analyses were performed to characterize the fusion events resulting in mixing of the two lipid membranes and the two aqueous contents of the vesicles. We used fluorescence microscopy, which enables the observation of single GVs fused with MG vesicles.

2. Materials and methods

2.1. Materials

MG was synthesized according to literature methods [41]. POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), and *p*-xylene-bis(*N*-pyridinium bromide) (DPX) were purchased from Sigma Aldrich and used as received. 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) was used as received from BioChemika. *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) and *N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine (Rh-PE) were purchased from Invitrogen and used as received. Deionized water was employed in the experiments. All other materials were of analytical grade and were used without further purification.

2.2. Preparation of GVs

GVs were prepared using the gentle hydration of lipid films [42,43]. POPC was dissolved in chloroform and mixed with the desired molar ratio of POPG. Then, 2 mL of the solution was placed in a 12 mm-diameter glass tube and dried using a stream of nitrogen gas at room temperature. The residual solvent was completely removed with a rotary vacuum pump. A small amount (10 μL) of 0.1 M acetate buffer solution at pH 4.0 was added to the lipid film and incubated at 45 °C for 10 min (prehydration). This buffer solution was used to prepare all vesicle samples unless otherwise noted. After prehydration, 2 mL of the acetate buffer was added and the sample was incubated at 37 °C for 2 h. The sample was centrifuged at 12 000*g* for 25 min and the supernatant was collected to remove multilamellar vesicles; this increased the proportion of thin vesicles in the sample, although vesicles with thick membranes were also observed (See Supplementary Material, Fig. S1). The GVs obtained had diameters ranging from 1 μm to 50 μm. The final concentration of POPC was determined for each case by the choline oxidase-DAOS (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) method [44] and the vesicle samples were prepared by diluting the original vesicle dispersion to the desired concentration.

2.3. Preparation of MG vesicle

Appropriate amounts of POPC and MG were dissolved in chloroform. The solvent was evaporated and the residual trace solvent