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Photodimerisation of a styrylpyrazine amphiphile suppresses the release of glucose entrapped in its mixed vesicle with DPPC

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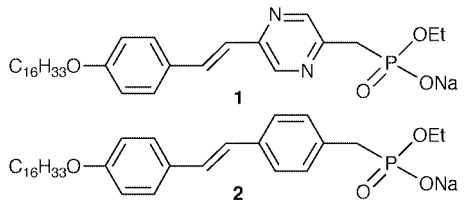
Photolysis of a styrylpyrazine amphiphile in the bilayer of a vesicle made from its mixture with DPPC, with irradiation above 300 nm, effectively suppressed the release of glucose entrapped in the vesicle, while the stilbene analogue induced a sudden perturbation to release the glucose under the same conditions.

Vesicles of lipid bilayers have been extensively studied as an excellent model for a drug-delivery system (DDS) over 20 years.[1] The study of DDSs using vesicles can be generally classified into two main areas: the transport of vesicles targeting a specified site in a living body, and the release control of drugs entrapped in the interior space of vesicles. Concerning the latter case, much effort has been devoted to introducing photochromic molecules such as azobenzene, spiropyran and stilbene into the bilayer of vesicles as a device for changing the permeability of the bilayer membrane in response to light irradiation.[2,3] Most of these studies, however, have been focused on application of intramolecular photochromism, such as *cis–trans* isomerism and a conformational isomerism in the case of spiropyran, to the release control system of substances entrapped in vesicles by light. Although this intramolecular photochromism has been successfully used to regulate the release rate with irradiation at two independent wavelengths, it was impossible to stabilize the bilayer and suppress the release using this strategy.

In our previous paper,[4] we reported that styrylpyrazine amphiphile **1** showed very fast and topochemically-controlled photodimerisation and intermolecular photochromism between



the monomer and its *syn* head-to-head cyclobutane dimer in aqueous dispersion. Our recent study has been directed toward application of this intermolecular photochromism to the photo-regulated release control of substances entrapped in vesicles. In the course of this study, we found that styrylpyrazine amphiphile **1** stabilizes the bilayer of the vesicle made from its mixture with L-α-dipalmitoyl phosphatidylcholine (DPPC) to suppress the release of glucose entrapped in the vesicle with irradiation above 300 nm, while the stilbene analogue **2** induces a sudden perturbation of the bilayer to release the glucose. Here we discuss these interesting observations from the point of view of the difference in molecular stacking modes of the two chromophores in the bilayer of the vesicles.

The mixed vesicles of **1** or **2** with DPPC were prepared by the conventional sonication method.† Permeability properties of the vesicles were studied by the measurement of glucose (%) released from vesicles in the course of storage time at 25 °C, as shown in Fig. 1. The release rate of glucose was decreased by incorporating **1** into the DPPC vesicle compared to that from the DPPC vesicle, whereas the release rate was greatly increased by incorporating **2** into the DPPC vesicle. Upon irradiation of the vesicle of **1** (**1**:DPPC = 1:4 molar ratio, hereafter referred to as vesicle **1**), which was stored for 13 h before irradiation, for 10 min under Ar atmosphere with a 500 W Xenon short-arc lamp through Pyrex glass, the release of glucose from the vesicle **1** was more suppressed than that from the untreated one. The mixtures of **1** with DPPC (**1**:DPPC = 1:3 and 1:2), however, were not able to form vesicles entrapping glucose under the same dispersion conditions. On the other hand, upon irradiation of the vesicle of **2** (**2**:DPPC = 1:6 molar ratio, hereafter referred to as vesicle **2**), which was stored for 17 h before irradiation, for 30 min under the same conditions, the release was initially accelerated and then suppressed. The vesicle of **2** (**2**:DPPC = 1:4 molar ratio) also indicated a similar acceleration effect of the release rate by the photolysis.

In order to explain these interesting results, we investigated the photochemical reactivities of **1** and **2** by irradiation above 300 nm in the bilayer matrix of DPPC vesicles by ^{1}H NMR spectroscopy. The aqueous dispersion of **1** (**1**:DPPC = 1:4) or **2** (**2**:DPPC = 1:6) obtained by the same procedure as referred to above was irradiated under Ar atmosphere with a 500 W Xenon short-arc lamp through Pyrex glass. ^{1}H NMR measurements were conducted in a mixed solvent (CD_3OD–$CDCl_3$ = 2:3) after removing the water *in vacuo*. It is obvious that the overall reaction process of **1** is very different from that of **2**, as shown in Fig. 2. In the case of **1**, the content (%) of the *trans*-monomer decreased rapidly with irradiation to give directly the corresponding *syn* head-to-head cyclobutane dimer, although a trace of the *cis*-isomer was detected. In the case of **2**, however, the content (%) of the *cis*-monomer increased rapidly to the

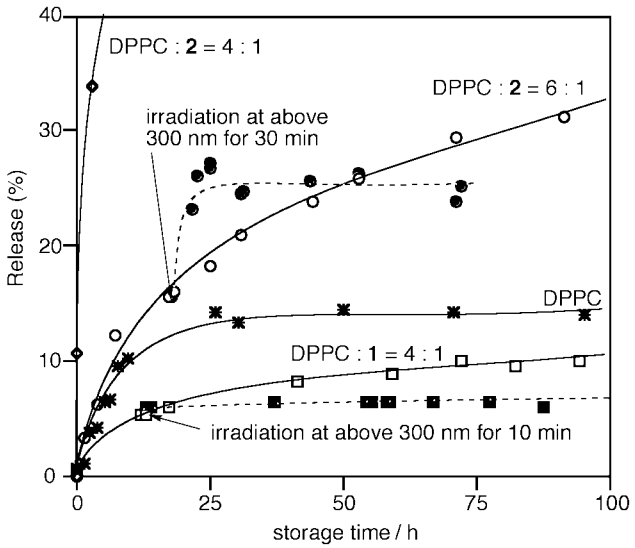


Fig. 1 Release (%) of glucose entrapped inside vesicles made from (※) DPPC, (□) a mixture of DPPC:**1** = 4:1, (■) DPPC:**1** = 4:1 after irradiation above 300 nm for 10 min at time = 13 h, (○) DPPC:**2** = 6:1, (●) DPPC:**2** = 6:1 after irradiation above 300 nm for 30 min at time = 17 h and (◇) DPPC:**2** = 4:1 as a function of storage time at 25 °C.

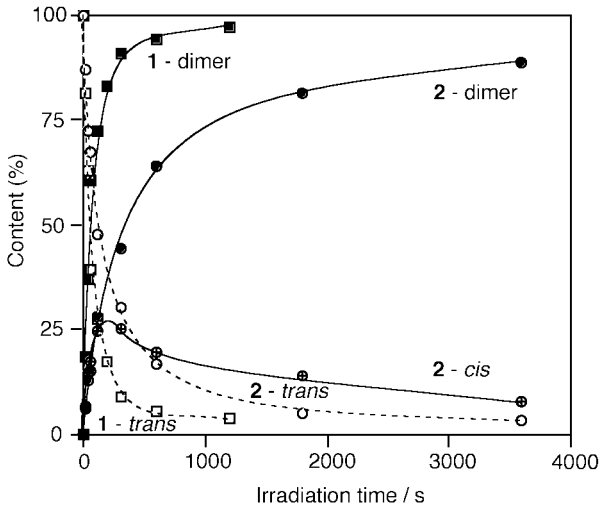


Fig. 2 Comparison of photoreactivities of **1** and **2** incorporated in the bilayer matrix of DPPC with irradiation at above 300 nm. Variation of content (%) of (□) **1**-*trans*, (■) **1**-*syn* head-to-head dimer, (○) **2**-*trans*, (⊕) **2**-*cis* and (●) **2**-*syn* head-to-head dimer as a function of the time of irradiation, where the suspension of **1** or **2** was prepared by dispersion of the mixture (**1**:DPPC = 4 μmol:16 μmol) or (**2**:DPPC = 2.8 μmol:17.2 μmol) in 2 ml of Milli-Q water, respectively.

level of 25% at the initial stage of irradiation with simultaneous formation of the corresponding *syn* head-to-head cyclobutane dimer, and then decreased progressively with the increase of the dimer formation. The reaction behavior of **1** in the bilayer matrix of DPPC was similar to that in the pure aqueous dispersion.[4] This observation suggests that **1** forms aggregates with a 'translation' structure having a strong face-to-face stacking through the multipole-multipole interaction even when **1** is diluted in the bilayer matrix of DPPC and, therefore, the photoisomerism is suppressed by the strong aggregation. On the other hand, the reaction behavior of **2** in the bilayer matrix of DPPC was considerably different from that in the aqueous dispersion.[4] The pure aqueous dispersion of **2** did not form the *cis*-monomer upon irradiation above 300 nm, while 5.5 mol% of 4-hexylstilbene-4′-butyric acid in a dihexadecyl phosphate vesicle gave a photostationary state containing 80% *cis*- and 20% *trans*-isomer upon irradiation at 315 nm.[3] These observations suggest that the intermolecular interaction of **2** is much weaker than that of **1** in the aggregate and, therefore, the fluidity of the bilayer membrane of the vesicle **2** is much higher than that of the vesicle **1**. This speculation is supported by the higher phase transition temperature (T_c) from the gel state to the liquid crystalline state of pure **1** (64 °C) than that of pure **2** (53 °C),[4] and the much lower permeability of the vesicle **1** (1:4) than that of the vesicle **2** (1:4). The weak intermolecular interaction of **2** may be due to the 'pinwheel' unit[5] aggregate having a face-to-edge stacking, as proposed by Whitten *et al.*

It is well known that the photoisomerisation of amphiphilic compounds containing *trans*-azobenzene and *trans*-stilbene to the *cis*-isomers in the bilayer membrane of vesicles increases the permeability of substances entrapped in their vesicles.[2] Therefore, the drastic acceleration of the release from the vesicle **2** at the initial stage of photolysis can be reasonably explained by considering the perturbation of the bilayer membrane induced by formation of the *cis*-isomer, and the suppression of the release from the vesicle **2** at the late stage and from the vesicle **1** may be ascribed to the formation of the corresponding *syn* head-to-head cyclobutane dimers. This was confirmed by investigating the release from the vesicle made from the pure *syn* head-to-head cyclobutane dimer of **1** and DPPC (1:8), the extent of which was smaller than that from the vesicle **1**.

Recently, a 'catastrophic' destruction of the vesicle made from a mixture of a styrylthiophene amphiphile with DPPC through the photodimerisation of the styrylthiophene,[6] inducing the release of substance entrapped within the vesicle, has been reported by Whitten *et al.* They proposed that the aggregation structure of the styrylthiophene amphiphile is a 'pinwheel' tetramer having face-to-edge stacking in the bilayer matrix of DPPC and, therefore, on the occasion of photodimerisation, some additional motion should be necessary to bring adjacent molecules close enough to dimerise. They pointed out the dimerisation, including a structural change, as the main reason for the vesicle disruption.

Considering the release property of the vesicle **2** and Whitten's result,[6] suppression of the release from the vesicle **1** without any perturbation during the dimerisation is unique. A simple topologically-controlled photodimerisation is expected to result in minimal disruption of the vesicle microstructure. Obviously, the vesicle **1** is just such a case. To the best of our knowledge, this is the first case for clean suppression of the release of substances from vesicles by [2 + 2] cycloaddition.

From the viewpoint of using the photochromism between a 1,2-diarylethylene monomer and the corresponding cyclobutane dimer as a new strategy for controlling the release of substances entrapped in vesicles, this clean suppression of the release is noteworthy. Naturally, since the reverse reaction, the cleavage of the cyclobutane dimer to the monomer,[4] is expected to increase the release rate, further study on the reversible release-control of vesicles by using **1** is now in progress in our laboratory.

Notes and references

† The thin layers of the mixtures **1** (**1**:DPPC = 4 μmole:16 μmole), or **2** (**2**:DPPC = 4 μmole:16 μmole, 2.8 μmole:17.2 μmole) with DPPC were prepared on the inside wall of a test tube by dissolving them in 2.5 ml of a MeOH–$CHCl_3$ (1:4, v/v) mixed solvent and evaporating the solvent *in vacuo*. After drying in a vacuum desiccator, 2 ml of an aqueous solution of glucose (300 mM) was added and sonicated for 5 min at 70 °C with a probe-type sonicator. Small unilamellar vesicles containing glucose were separated from the untrapped glucose by gel-filtration chromatography (Sephadex G-50, 1.5 × 18 cm, eluent: 150 mM NaCl aqueous solution). The formation of vesicles was confirmed by a well-established gel-filtration method (ref. 7). The determination of glucose was conducted by the measurement of absorbance at 505 nm of quinone dye produced by the Mutarotase GOD method described by Miwa *et al.* (ref. 8). The amount of glucose released (%) from the vesicles was calculated using eqn. (1),

$$\text{Glucose released (\%)} = A_t/A_{total} \times 100 \quad (1)$$

where A_t is the absorbance of the suspension after a definite period of storage, and A_{total} is the absorbance after addition of an aqueous solution of Triton X-100 (200 g l^{-1}) to the suspension.

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