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Photo- and thermo-responsive assembly of liposomal membranes triggered by a gemini peptide lipid as a molecular switch

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Abstract

A gemini peptide lipid composed of L-histidyl residues, hydrophobic double-chain segments, and an azobenzene spacer unit was synthesized as a photoresponsive molecular switch for assembly of liposomal membranes. Photoisomerization of the azobenzene moiety between the *trans*-form and the corresponding *cis*-form reversibly proceeded in the liquid crystalline state of the liposome formed with phospholipids, but not in the gel state below the phase transition temperature. The gemini peptide lipid with the *cis*-azobenzene spacer recognized Cu^{2+} ions to induce vesicular assembly in the liquid crystalline state of the liposomal membrane. On the other hand, the corresponding *trans*-form, which exhibited little affinity toward Cu^{2+} ions, did not act as a molecular switch for vesicular assembly. Furthermore, photo-switching behavior performed by the gemini peptide lipid was tuned using the thermotropic phase transition of the matrix membrane.

Keywords: Gemini lipid; Molecular switch; Liposome; Photoresponsive; Thermoresponsive

1. Introduction

Lipid bilayer vesicles formed with phospholipids, so-called liposomes, have been used widely in drug and gene delivery systems and as bioreactors [1–4]. Liposomal membranes are also prepared by synthetic lipid molecules, and various biologically important events such as material transport, catalysis, energy conversion, and information processing can be simulated in functionalized liposomal systems as artificial cells [5–10]. Although liposomal membranes have been usually handled as a uni-vesicular state, formation of multi-vesicular assemblies as artificial tissues would be of great importance in light of the superiority of multi-cellular organisms in biological systems over corresponding unicellular states. On those grounds, various approaches for liposomal assembly have been reported to date [11–19]. The common strategy for construction of artificial multi-cellular systems seems to be how to introduce inter-vesicular interactions that are appropriate for assembly of liposomes, but not for further induction of membrane fusion.

We recently developed an assembly system for liposomal membranes using ion-recognizable gemini peptide lipids [20]. A gemini peptide lipid bearing L-histidyl residues, hydrophobic double-chain segments, and a tri(oxyethylene) spacer performed as a molecular switch embedded in liposomal membranes to assemble and disassemble the vesicles through ditopic recognition toward transition metal ions and alkali metal ions, respectively. Our molecular design of the lipids was inspired by a naturally occurring gemini lipid, cardiolipin, which has a unique dimeric lipid structure and interesting biological functions [21,22]. Although much attention has been directed toward physicochemical properties of synthetic gemini surfactants, which consist of two surfactant molecules connected at the level of head group via a spacer group [23–26], few reports describe synthetic gemini lipids composed of two double-chain segments, such as the cardiolipin.

This article reports photo-induced switching behavior of assembly of liposomal membranes equipped with a gemini peptide lipid having L-histidyl residues and an azobenzene spacer unit (**1**) as a photoresponsive molecular switch (Chart 1). Upon UV light irradiation to the aqueous liposomes in the presence of Cu^{2+} ions, the metal-binding affinity of the imidazolyl groups in the gemini peptide lipid changed markedly through photoisomerization of the azobenzene moiety to induce assembly of the liposomes (Fig. 1). Disassembly of liposomal membranes was induced reversibly by visible light irradiation. Furthermore, such photo-switching was repeatable in liquid crystalline state of the membrane. In addition, the present photo-switching behavior of the liposomal assembly was tuned through the thermotropic

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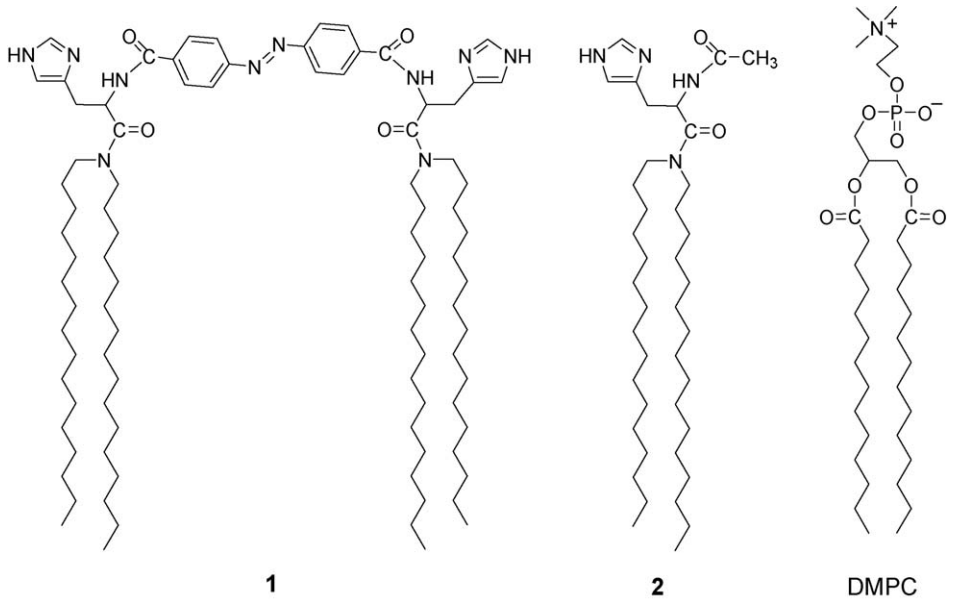

Chart 1. Molecular structures for the gemini peptide lipid **1**,the non-gemini peptide lipid **2**, and DMPC.

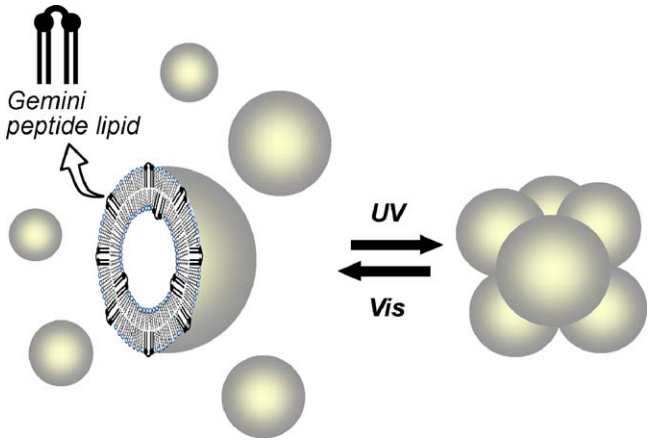

Fig. 1. Assembly control of liposomal membranes using a gemini peptide lipid. Each liposome with gemini peptide lipids is represented as a solid sphere.

phase transition of the matrix membrane. To date, a considerable number of investigations have reported molecular switches that are capable of controlling functions and material properties [27–30]. Among these studies, photochemically driven movement of molecules induced by a molecular switch is an attractive research subject in light of the construction of molecular machines. Although excellent photo-switching systems accompanying linear movements or rotary motions of molecules have been developed [31–34], the present study is the first example of photo-driven movements of molecular assemblies of lipid vesicles, which allow reversible organization of an artificial multi-cellular system.

2. Experimental

Dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine (DMPC and DPPC, respectively; NOF Corp., Tokyo, Japan) were obtained as guaranteed reagents and were used without further purification. Copper(II) chloride from Kanto Chemical Co. Inc., Tokyo, Japan, was dissolved in distilled and deionized water and was standardized using conventional chelatometric titration. A gemini peptide lipid, N^2,$N^{2'}$-azobenzene-4,4′-dioyl-bis(*N*,*N*-dihexadecyl-L-histidinamide) (**1**), was synthesized through condensation of *N*,*N*-dihexadecyl-N^{τ}-tosyl-L-histidinamide [20] with azobenzene-4,4′-dioyl dichloride and followed deprotection of the tosyl groups: Anal. Calcd. for $C_{90}H_{154}N_{10}O_4 \cdot 0.5H_2O$: C, 74.59; H, 10.78; N, 9.66. Found: C, 74.61; H, 11.15; N, 9.32. HR–MS (FAB$^+$): Exact mass calcd. for $C_{90}H_{155}N_{10}O_4$ [M+H]$^+$ 1440.2233, found 1440.2238. Preparation and characterization of a non-gemini peptide lipid, N^2-acetyl-*N*,*N*-dihexadecyl-L-histidinamide (**2**), was reported previously [20].

Liposomes containing a peptide lipid were prepared as follows. Appropriate amounts of phosphatidylcholine and peptide lipid in a molar ratio of 10:1 were dissolved in chloroform. The solvent was evaporated under nitrogen gas flow and the residual trace solvent was removed completely in vacuo. Hydration of the thin film thus obtained on the wall of the vial was performed at 40 °C with an appropriate amount of water. Multi-walled bilayer vesicles were formed upon vortex mixing of the aqueous dispersion. The corresponding small vesicles with a diameter of about 200 nm were prepared using sonication of the dispersed sample with a cup-type sonicator above the phase transition temperature for 5 min.

Photoisomerization of the azobenzene moiety in the gemini peptide lipid was performed through irradiation with a Xe lamp (500 W, SX-UID500X; Ushio Inc.). AUV band-pass filter (D33S; Toshiba Ltd.) and L-42 and Y-43 filters (Asahi Techno Glass Corp.) were used, respectively, for UV and visible light irradiation. Molecular modeling studies for stereoisomers were performed using a software package (Cerius^2 ver. 4.2) [35] that was equipped with the DREIDING force field [36] (ver. 2.21).

The structural error was minimized in bond, angle, torsion, inversion, and van der Waals terms. Then the conformational energy was optimized. The DREIDING parameters given in the program were used without modification.

Electronic absorption spectra were taken on a spectrophotometer (UV-2400; Shimadzu Corp.). Circular dichroism (CD) spectra were recorded on a spectropolarimeter (J-820; Jasco Inc.). The zeta potential was evaluated using an electrophoretic light scattering spectrophotometer (ELS-6000; Otsuka Electronics Co. Ltd.) equipped with a laser Doppler system. Polystyrene 200-nm-diameter beads were employed as a standard. The vesicular solution was mixed with NaCl as an electrolyte and its pH was adjusted by aqueous HCl or NaOH. The vesicles' hydrodynamic diameter was measured using a dynamic light scattering spectrophotometer (DLS-6000; Otsuka Electronics Co. Ltd.) equipped with a He–Ne laser at 633 nm. The time course of light scattering from the sample was analyzed using the Cumulant method at an angle of 90° from the incident light. A microcalorimeter (VP-DSC; MicroCal Software Inc.) was used for differential scanning calorimetry (DSC). Transmission electron micrographs (TEM) were obtained using freeze-fracture-replica techniques with freeze-etching equipment (JFD-9010; JEOL) and an electron microscope (JEM-1011; JEOL).

3. Results and discussion

Regarding the molecular design of a gemini peptide lipid as a photoresponsive molecular switch for liposomal assembly, we gave attention to the specific ion binding behavior of the cardiolipins towards divalent ions such as Ca^{2+} and Mg^{2+} to influence the aggregate morphology of the lipid membranes. To enhance ion recognition, we introduced two L-histidyl residues into the synthetic gemini lipid in place of phosphate groups in the cardiolipin. In addition, the molecular design of the gemini lipid is also based on our previous systematic studies of synthetic peptide lipids, their aggregation behavior and functionalization [8,37,38]. Therefore, the gemini peptide lipids bearing L-histidyl residues, hydrophobic double-chain segments, and a flexible spacer unit have exhibited specific binding ability toward Cu^{2+} ions in phospholipid liposomes to induce the vesicular assembly [20].

The gemini peptide lipid **1** mentioned in this article was designed by introducing an azobenzene moiety as a rigid photoresponsive spacer to switch the metal binding behavior. The space-filling models shown in Fig. 2 represent plausible conformations for the *trans*-isomer and *cis*-isomer in the liposomal membrane. Conformations were minimized by molecular mechanics calculations using Cerius^2 software based on the DREIDING force field. Under thermodynamically stable conditions without photoirradiation, the azobenzene moiety takes *trans*-form (*trans*-**1**); two imidazolyl groups in the gemini lipid are present over 0.6 nm apart from each other (Fig. 2a). Therefore, this species cannot act as a bidentate ligand for a metal ion. Upon UV light irradiation, the azobenzene moiety converts to the corresponding *cis*-form (*cis*-**1**) to be capable of forming a metal chelate that is effective for inducing the assembly of liposomes (Fig. 2b). Since *cis*-**1** reverts to *trans*-**1** by visible light irradiation, the metal binding ability that is related to the vesicular assembly would be changed through the photoisomerization reaction.

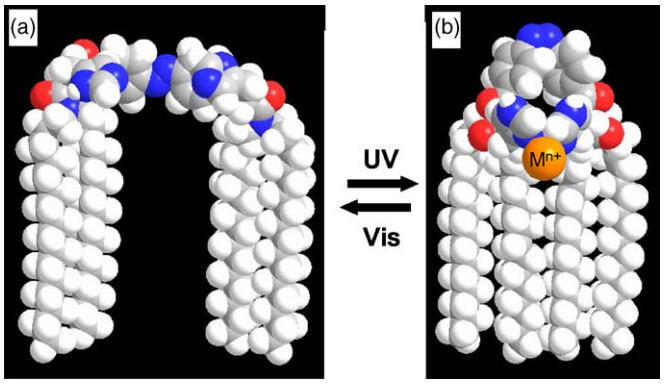

Fig. 2. Molecular models of the gemini peptide lipid **1** evaluated by molecular mechanics calculation using the Cerius^2 software based on DREIDING force field: (a) *trans*-form; (b) *cis*-form bound a metal ion.

Photoisomerization behavior of the gemini peptide lipid embedded in the liposomal membrane was monitored using electronic absorption spectroscopy. Upon UV light irradiation for the DMPC vesicle in the liquid-crystalline state, absorbance characteristic to *trans*-**1** at 333 nm was decreased with a concomitant increase in absorptions at 212 and 440 nm, which are assigned to the corresponding *cis*-isomer, showing isobestic points at 294 and 378 nm (Fig. 3a). Since the present gemini peptide lipid possesses chiral L-histidyl residues that are connected to the azobenzene moiety, the photoisomerization process was also monitored using CD spectroscopy (Fig. 3b). In the liposomal membranes, photoiosomerization of **1** was observed in the liquid-crystalline state, but not in the gel state below the phase transition temperature. In the liquid-crystalline state of the liposomal membrane, thermal isomerization from *cis*-**1** to *trans*-**1** was extremely slow. For example, the half-life monitored by means of electronic absorption spectroscopy was 20 h in the DMPC liposome under conditions shown in Fig. 3. Since all experimental data for *cis*-**1** were taken within 15 min after its photochemical formation, the influence of thermal isomerization is negligible.

In general, metal-ligand interactions are greatly enhanced at the lipid membrane-water interface rather than in aqueous solution [10,39]. The CD spectra of *cis*-**1** embedded in the DMPC vesicle were changed significantly upon addition of Cu^{2+} ions in the liquid crystalline state, reflecting the binding of Cu^{2+} ions to the L-histidyl residues of the gemini peptide lipid (Fig. 4a). The Job's plot analysis in the vesicular system revealed that Cu^{2+} ions bind to the gemini peptide lipid in a ratio of 1:2 (Fig. 4b). Therefore, the binding constant for the 1:2 complex of Cu^{2+} ions with *cis*-**1** in the DMPC vesicle was $5.0 \times 10^9\ M^{-2}$. The value is also represented as $2.5 \times 10^{19}\ M^{-4}$, as apparent binding constant for the 1:4 complex of Cu^{2+} ions with the imidazolyl groups in the lipid. The binding constant of Cu^{2+} ions toward the non-gemini peptide lipid **2** with one imidazolyl group per lipid molecule was $1.5 \times 10^{17}\ M^{-4}$ for the 1:4 complex, which is much weaker than the constants of the corresponding gemini

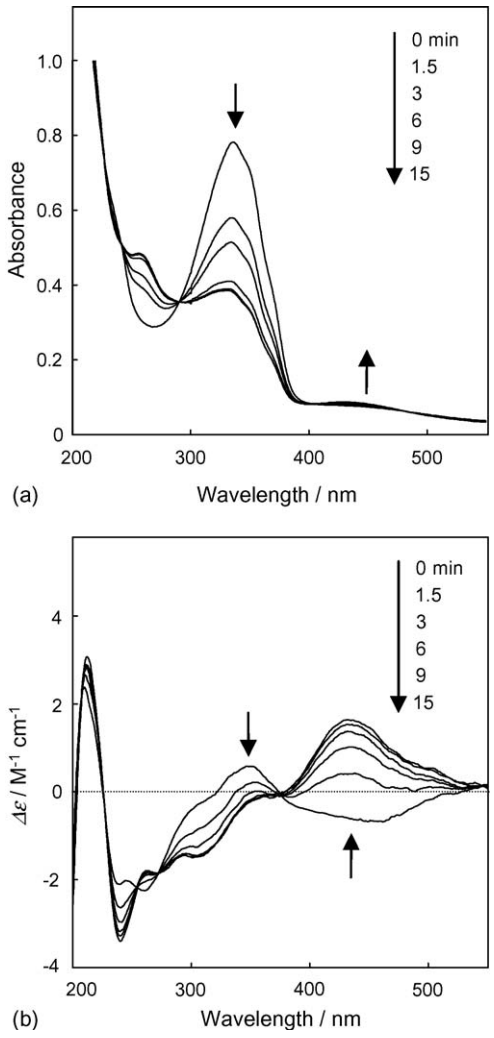

Fig. 3. Photoisomerization behavior of the gemini peptide lipid **1** (50 μM) embedded in the liposome of DMPC (500 μM) at pH 9.0 and 30 °C upon UV irradiation: (a) electronic absorption spectra; (b) CD spectra.

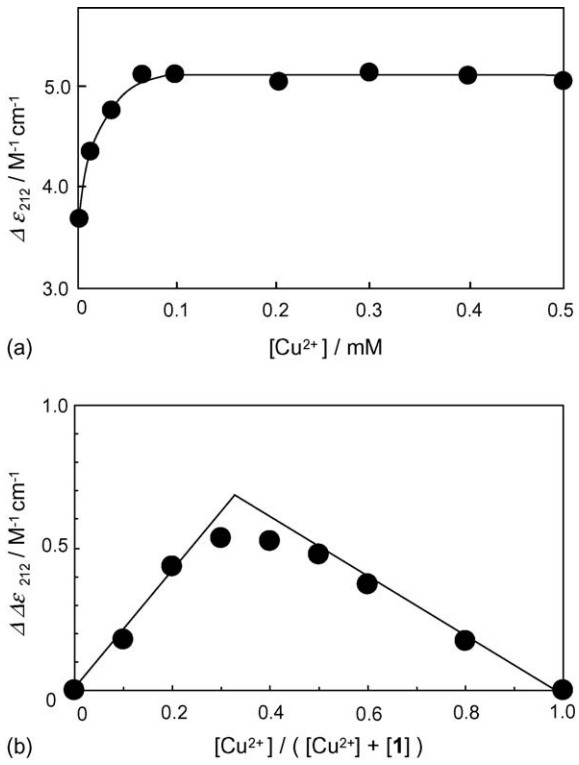

Fig. 4. Cu^{2+}-binding behavior of the gemini peptide lipid **1** in the *cis*-form embedded in the DMPC liposome at pH 9.0 and 30 °C as monitored by CD spectroscopy: (a) Cu^{2+}-dependence of $\Delta\varepsilon$ at 212 nm for **1** (50 μM) in DMPC (500 μM); (b) a Job's plot with a total concentration of **1** and Cu^{2+} being 50 μM.

lipid. The CD spectra of *trans*-**1** embedded in the DMPC vesicle showed no detectable change upon addition of Cu^{2+} ions. Hence, *cis*-**1** recognizes Cu^{2+} ions to form the metal chelate, whereas the corresponding *trans*-isomer has no affinity toward Cu^{2+} ions under the present conditions.

Marked differences in metal binding ability between *cis*-**1** and *trans*-**1** in the liposomal membrane were also clarified by zeta potential measurements. The pH-dependencies of the zeta potential for the DMPC vesicles containing *cis*-**1** in the presence and absence of Cu^{2+} ions are shown in Fig. 5a. In the metal-free system, deprotonation of the imidazolyl groups in the gemini peptide lipid decreased the zeta potential from +40 to −20 mV. On the other hand, a marked increase in the zeta potential was observed in a pH region over 7 in the presence of Cu^{2+} ions. This behavior resembles that for the gemini peptide lipid bearing a tri(oxyethylene) spacer [20], indicating that Cu^{2+} ions bind to the L-histidyl residues of the lipids. In contrast, the zeta potential for the DMPC vesicles containing *trans*-**1** showed similar pH-dependencies in the presence and absence of Cu^{2+} ions (Fig. 5b), supporting the interpretation from the molecular modeling study and the CD spectroscopy that *trans*-**1** embedded in the liposome has little affinity to Cu^{2+} ions.

On these grounds, we evaluated the assembly behavior of the liposomal membranes induced by the gemini peptide lipid **1** as a molecular switch. As shown in Fig. 6, the hydrodynamic diameter (D_{hy}) of the DMPC liposomes containing *cis*-**1** with high metal binding affinity increased from 210 to 500 nm with increased Cu^{2+} concentration. The increased D_{hy} value recovered to the original value after addition of excess amounts (5 mM) of a strong metal chelating agent, ethylenediaminetetraacetate. Therefore, the increase in the hydrodynamic diameter derives from assembly of the liposomes without accompanying membrane fusion. On the other hand, the D_{hy} value of the DMPC liposomes containing *trans*-**1** with little metal binding affinity was independent of Cu^{2+} concentration. The non-gemini peptide lipid **2** embedded in the liposomes was also not effective in the change of hydrodynamic diameter under comparable conditions. Our TEM observation using freeze-fracture-replica techniques supported that the assembly of the liposomal membranes was induced by *cis*-**1**, but not by *trans*-**1**.

To clarify the assembly behavior of the present vesicular system, the hydrodynamic diameter and phase transition temperature (T_m) were measured using changes in the combination of the matrix lipid and the stereoisomer of the gemini peptide lipid in the presence and absence of Cu^{2+} ions (Table 1). Results indicate that the liposomal membrane assembly proceeds in vesicles containing *cis*-**1** in the presence of Cu^{2+} ions above the phase transition temperature of the membrane. Consequently,

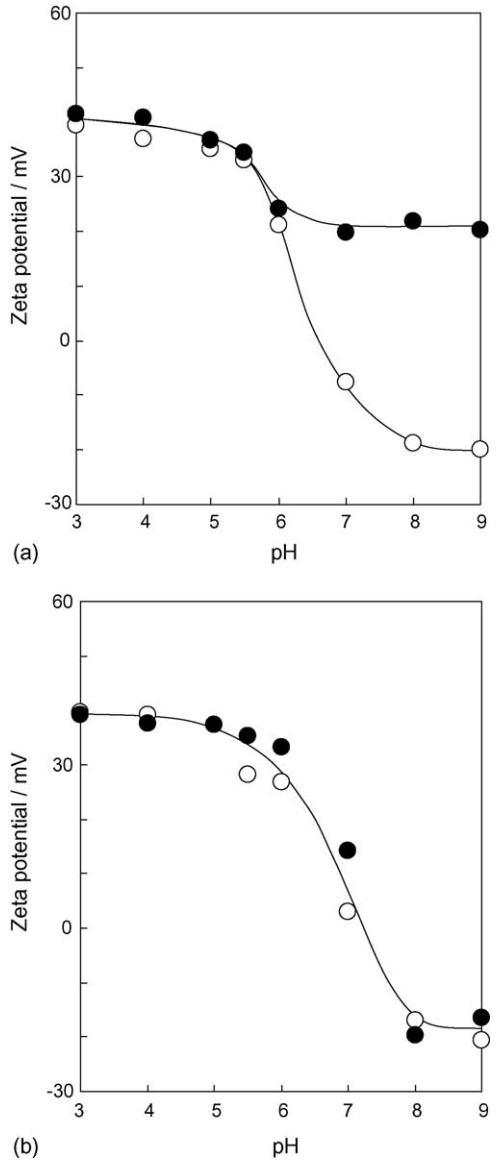

Fig. 5. The pH-dependencies on zeta potentials for the liposomes of DMPC (500 μM) containing the gemini peptide lipid **1** (50 μM) at 30 °C in the presence (●) and absence (◯) of Cu^{2+} (500 μM): (a) the *cis*-**1**; (b) the *trans*-**1**.

Table 1
Effects of matrix lipid on the hydrodynamic diameter of the liposomes induced by the gemini peptide lipid **1** as a molecular switch at pH 9.0 and 30 °C

Entry	Matrix lipid	**1**	Metal ion	T_m (°C)	D_{hy} (nm)
1	DMPC	*trans*-form	free	23.1	210
2	DMPC	*trans*-form	Cu^{2+}	23.0	210
3	DMPC	*cis*-form	free	23.3	210
4	DMPC	*cis*-form	Cu^{2+}	23.9	500
5	DPPC	*trans*-form	free	40.6	200
6	DPPC	*trans*-form	Cu^{2+}	40.7	200
7	DPPC	*cis*-form	free	40.6	200
8	DPPC	*cis*-form	Cu^{2+}	40.7	200

Concentrations in μM: PC, 500; 1, 50; Cu^{2+}, 500.

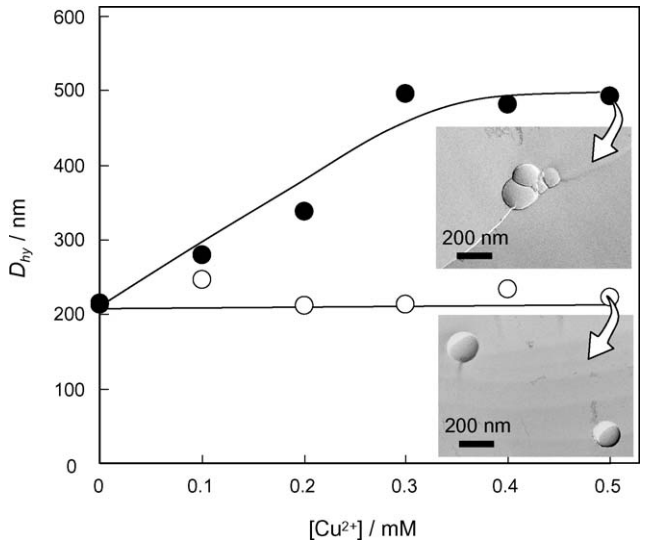

Fig. 6. Effect of Cu^{2+} concentration on the hydrodynamic diameter of the liposomes of DMPC (500 μM) containing the gemini peptide lipid **1** (50 μM) in the *cis*-form (●) or the *trans*-form (◯) at pH 9.0 and 30 °C. Insets show the freeze-fracture-replica TEM images for the DMPC liposomes with **1** at Cu^{2+} (500 μM).

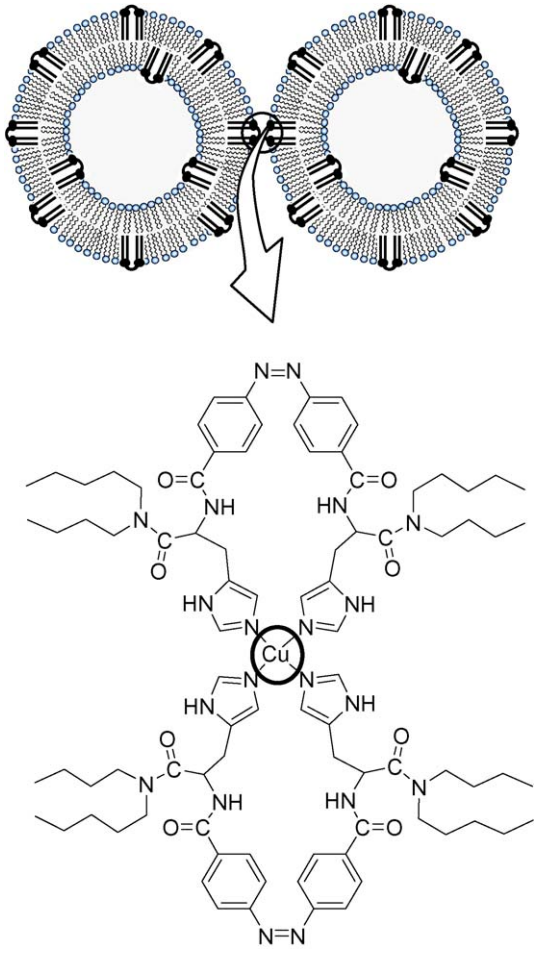

Fig. 7. Molecular junction formed with the gemini peptide lipids for liposomal assembly.

in the liquid crystalline state and the presence of Cu^{2+} ions, the photo-driven switching of vesicular assembly was observed repeatedly, at least more than several times, using *cis-/trans-*photoisomerization of the gemini peptide lipid embedded in the membrane. To maintain the two lipid bilayer surfaces at separation less than about 2 nm in aqueous media, some attractive force that overcomes the hydrated lipid membranes is generally required [40,41]. The 1:2 complex of Cu^{2+} ions with *cis*-**1** shown in Fig. 7 would play a pivotal role as the vesicular junction to stabilize the assembled vesicles.

4. Conclusions

It became apparent that movements of liposomal membranes between the assembly and disassembly states can be controlled reversibly using a gemini peptide lipid as a photoresponsive molecular switch. Movements of the molecular assembly in mesoscopic scale were triggered by photo-driven ion recognition of the molecular switch in nanoscale. In addition, photo-switching behavior was tuned by a thermotropic feature of the matrix membrane. We believe that our results revealed a new function of gemini surfactants and propose a guidepost to design photo-driven molecular machineries. Photo-switching behavior of the liposomal assembly observed as the D_{hy} value changes was repeated with cycles of UV–vis irradiation. It was synchronized with the absorption spectral changes for the *cis/trans-*isomerization of the gemini peptide lipid and zeta-potential changes for the liposomal membrane. Details of the switching behavior are reported elsewhere.

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