Liposomal Sorting onto Substrate through Ion Recognition by Gemini Peptide Lipids

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Selective liposomal assembling in aqueous solution was achieved by ion-recognizable gemini peptide lipids as an inducer. The methodology was extended to liposomal sorting on a substrate covered with a supported bilayer membrane, which was confirmed by means of fluorescence and atomic force microscopy, fluorescence spectroscopy, and quarts crystal microbalance measurements.

In biological systems, molecular recognition processes are utilized to induce selective interaction at the interface of cell membrane surfaces resulting in aggregation, adhesion, and fusion. The development of liposomal systems undergoing the interfacial events provides an unique approach for building multi-liposomal systems as tissue mimetics. It might show the relatively complex physiological functions such as coupling and feedback between multiple reactions and network operations on the multi-liposomal array. On these ground, a variety of approach for controlling the liposomal assembling through the interfacial events such as polymer bridging,[1] DNA conjugation,[2] electrostatic interaction,[3] ligand–receptor interaction,[4] and metal-ion complexation,[5] has been reported.

Recently, we reported gemini peptide lipids for the ion-triggered liposomal assembling through a novel strategy in which ion-specific inter-liposomal linkages were formed by the gemini lipid.[6] In this study, we utilized the interfacial ion recognition to achieve ion-triggered sorting of liposomes encoded with the gemini lipids onto supported lipid bilayer membrane. Surface immobilizations of intact liposomes on the solid substrate have been reported[7] and self-sorting of liposomes onto the patterned supported bilayer was also demonstrated by using the molecular recognition between complementary oligonucleotides displayed on the liposomal surface and the supported bilayer.[8] However, the present liposomal sorting enabled not only sequential separation of tagged liposome from liposomal mixture, also the construction of multi-liposomal array regulated in time and space by external stimuli.

Synthesis of the gemini peptide lipids was reported previously (Chart 1).[6] The liposomes were prepared by sonication of an aqueous dispersion of dimyristoylphosphatidylcholine (DMPC), and a gemini lipid **1** in a 10:1 molar ratio with a cup-type sonicator at 30 W for 6 min. The hydrodynamic diameter (D_{hy}) and polydispersity index of the liposome were evaluated to be 160 nm and 0.2, respectively by means of dynamic light scattering (DLS) measurements. Upon addition of Zn^{2+} ions to the aqueous liposomes, the D_{hy} was increased to 350 nm. On the other hand, replacement of the L-histidyl moieties with L-aspartate residues **2** gave the liposome showing Ca^{2+}-

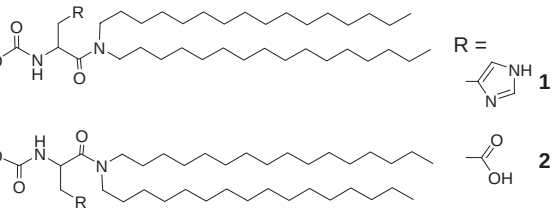

Chart 1.

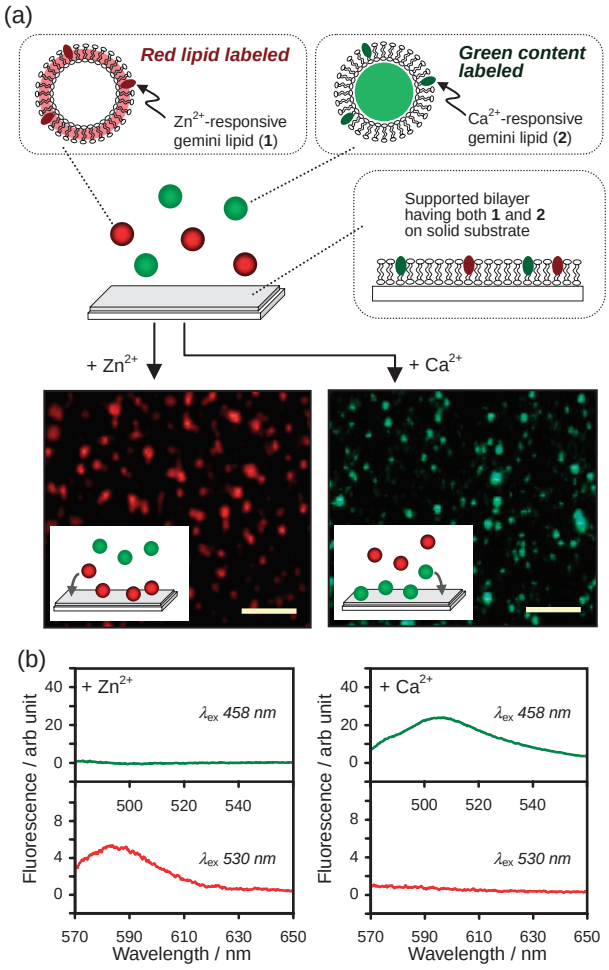

Figure 1. (a) Ion-triggered sorting from aqueous liposomal mixture onto a glass-supported lipid bilayers. Two types of liposomes formed with DMPC (0.5 mmol dm^{-3}), each having one or the other gemini peptide lipid (0.05 mmol dm^{-3}), one is Zn^{2+}-responsive liposome having **1** labeled with Rho-PE (red lipid labeled) and the other is Ca^{2+}-responsive liposome with **2** labeled with pyranine (green content labeled). Both types of liposomes were premixed and incubated with glass-supported lipid bilayer of DMPC containing both **1** and **2**. Subsequently, Zn^{2+} or Ca^{2+} was added and washing resulted in immobilization of corresponding liposomes on the supported bilayer. Bar = 5 μm. (b) Fluorescence emission spectral changes of the substrate upon addition of Zn^{2+} or Ca^{2+} ion: upper, excited at 458 nm; lower, excited at 530 nm.

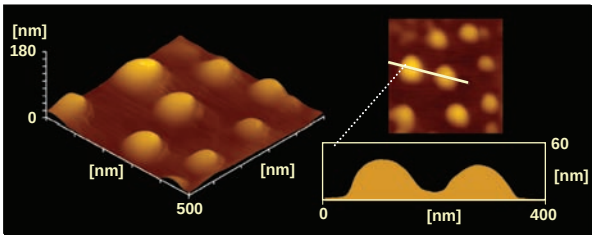

Figure 2. AFM tapping-mode image of immobilized DMPC liposome with gemini lipid onto glass-supported bilayer and the section analysis showing the presence of spherical liposomes.

specific assembling (See Supporting Information).

Based on the ion-selective inter-liposomal assembling, sorting of the liposomal mixture onto the substrate covered with supported bilayer containing gemini peptide lipids was performed (Figure 1). Supported bilayers were first prepared by liposomal fusion to a cationic polymer formed by layer-by-layer assembling technique on a glass substrate (See Supporting Information). The DMPC liposome for the supported bilayer formation was containing both lipid **1** and **2**. Thus, prepared glass substrate was soaked in a mixture of DMPC liposomes in the following way. Two types of DMPC liposomes (0.5 mmol dm^{-3}) having gemini peptide lipid (0.05 mmol dm^{-3}), red lipid labeled liposomes with **1** and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl), ammonium salt (Rho-PE), and green content liposome with **2** and pyranine were premixed and incubated with the glass substrate. Subsequently, Zn^{2+} or Ca^{2+} ions as external triggers were added to the liposomal solution in the presence of the glass substrate, leading to ion-specific immobilization of the corresponding liposomes onto the supported bilayer membrane. After excess unattached liposomes and ions were rinsed away, the immobilized liposomes were visualized by fluorescence microscopic observation of the substrate.

The liposomes having lipid **1** were selectively immobilized onto the substrate in the presence of Zn^{2+} ions (0.5 mmol dm^{-3}), whereas the liposome with **2** was not detected on the supported bilayer (Figures 1a and 1b). In case of Ca^{2+} addition (5 mmol dm^{-3}), the selective immobilization of the liposomes with **2** was observed. Control experiment revealed that liposomes did not associate with the supported lipid bilayer unless appropriate ion species were added to the solution. Furthermore, even in the presence of Zn^{2+} or Ca^{2+} ions, the liposome immobilization was not observed when the supported bilayer or the liposomes did not include the gemini lipids. In addition, replacement of the liposomal labeling did not affect the ion-specificity for liposomal sorting, indicating that the gemini lipids were behaved as ion-specific liposomal sorters (See Supporting Information).

Atomic force microscopic observation of the liposome-immobilized substrate revealed that liposomes were attached onto the supported bilayer keeping their vesicular structures without marked morphological changes such as fusion or fission (Figure 2), since the size of the immobilized liposomes was well correlated with the D_{hy} value in aqueous solution as evaluated by DLS. This sorting process onto the substrate was also evaluated in more extensive area by means of fluorescence spectroscopy (Figure 1b), indicating that the liposomes were immobilized all over the substrate. The amounts of the liposome immobilized onto the substrate under each condition were individually determined by quartz crystal microbalance (QCM) measurements. Table 1 clearly shows that the liposomes are attached onto the substrate covered with supported bilayer only in the presence of corresponding ionic stimuli. In addition, the coverage of the liposome (ca. 40%) calculated by frequency change of QCM caused by liposome immobilization,[9] was well correlated with the results obtained by AFM.

Table 1. Amounts of assembled liposomes onto supported bilayer determined by quartz crystal microbalance at pH 9.0 and 30 °C[a]

Gemini peptide lipid	Ion species	Δm/μg cm^{-2}	Coverage/%
1	Zn^{2+}	4.0	41
1	Ca^{2+}	0.0	0
2	Zn^{2+}	0.0	0
2	Ca^{2+}	3.9	40

[a]Concentrations in mmol dm^{-3}: [DMPC], 0.5: [gemini lipid], 0.05; [Zn^{2+}], 0.5, [Ca^{2+}], 5.

In conclusion, we have demonstrated that the gemini peptide lipids act as liposome sorter, capable of encoding the identity of various liposomes with a certain composition, content, and origin. We believe that the results provide a general basis for the sorting of liposomal membrane triggered by a variety of external stimuli, for example, gemini peptide lipid responsive to pH, temperature, and light can be used for this purpose; this will be reported separately.

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