Lyoprotective Effect of Alkyl Sulfobetaines for Freeze-drying 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine Liposomes

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Abstract: A liposome is a molecular assembly in the form of a vesicle comprised of a phospholipid bilayer. Liposomes can be used as molecular containers in various fields such as pharmaceutical, cosmetic, and food industries. It is difficult to maintain the original structure of liposomes in an aqueous medium. Phospholipids, which are components of liposomes, are susceptible to hydrolysis, which causes disruption of the liposomal structure and dysfunction of the molecular container. In this context, freeze-drying liposomes is a preferable method to improve the shelf life of liposomes. However, when freeze-drying liposomes, a lyoprotective agent is required to preserve their original structure. In this study, we investigate whether alkyl sulfobetaines (SB_{*n*}, *n*: number of carbons in the alkyl chain, *n* = 1–18) can be used as lyoprotectants for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes. The results indicated that the length of the alkyl chain of the SB_{*n*} was an important factor to prevent liposome disruption during the freeze-drying and subsequent rehydration processes. The use of SB_{*n*} with an alkyl chain of intermediate length (*n* = 6–10) could prevent liposome disruption and remarkably reduce the gel-to-liquid crystal phase transition temperature (*T*_m) of the freeze-dried liposomes. This indicates that these SB_{*n*} could intercalate in the dried bilayer and reduce intermolecular interaction between DPPC in the bilayer. The *T*_m reduction of the freeze-dried liposomes should contribute to prevention of the gel-to-liquid phase transition of the liposomes during the rehydration process, which has been known to be a main cause of liposome disruption. We expect that the results from this study will provide an insight into the influence of zwitterionic additives on freeze-dried lipid bilayers and the lyoprotective effect, which should be useful in many biochemical and biomedical fields.

Key words: liposome, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), lyoprotection, freeze-drying, alkyl sulfobetaine

1 Introduction

A liposome is a vesicle composed of phospholipids, whose diameter ranges from several tens of nanometers to several micrometers. Since liposomes can encapsulate a wide variety of molecules, they may act as molecular containers, which can be used in various fields like the pharmaceutical (drug delivery system)^{1–6}, cosmetic^{7, 8} and food industries^{9–11}. In general, liposomes can be obtained as an aqueous dispersion containing vesicular phospholipids. In the aqueous dispersion, phospholipids constituting the liposomes are sensitive to hydrolysis¹². When using phos-

pholipids with an unsaturated acyl group, the lipids are sensitive to oxidation as well¹³. Water and oxygen in the lipid dispersion exacerbate these undesirable reactions¹². Hydrolysis and oxidation trigger disruption of the vesicular structure of the liposomes¹⁴. Such disruptions induce fusion/aggregation of the liposomes, resulting in increment in liposome size. This causes dysfunction of the liposomes as molecular containers. For example, if the liposomes are being used as drug delivery carriers, a change in their size may deprive the liposomes of their drug accumulation function, which relies on their enhanced permeability and

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retention effect. Leakage of encapsulated molecules from the liposomes is also induced by liposome disruption. Thus, the short shelf life of aqueous dispersions of liposomes limits their applicability as molecular containers.

In this context, lyophilization, which is also called freeze-drying, has been proposed as a preferable method to remove water and oxygen contained in the liposome dispersion¹⁵. However, the vesicular structure of liposomes is destabilized during the freeze-drying (FD) and subsequent rehydration (RH) processes. To prevent disruption of the liposomes during the FD/RH processes, the use of additives called “lyoprotectants” are required. Lyoprotectants can prevent the collapse of liposome structure and leakage of entrapped molecules from the liposomes even after FD/RH of the liposomes. To date, it has been demonstrated that some types of lyoprotectants, such as amino acids^{16, 17}, carbohydrates¹⁸ (e.g. glucose¹⁹), trehalose^{20, 21}, arbutine²²) and glycerol²³) could exert a lyoprotective effect. In particular, trehalose could work effectively as a lyoprotectant.

In this study, we propose alkyl sulfobetaines (SB_{*n*}, *n*: number of carbons in the alkyl chain, *n* = 1–18) as a new class of lyoprotectants for liposomes composed of 1,2-dipalmitoyl-*sn*-glycero-phosphocholine (DPPC), which is a typical phospholipid. Molecular structures of SB_{*n*} and DPPC are shown in Fig. 1. The lyoprotective effect derived from trehalose is based on strong binding of trehalose to the zwitterionic headgroups of the phospholipids in the dried state. Although the molecular structure of SB_{*n*} is completely different from that of trehalose, we expect that SB_{*n*} can also act as lyoprotectants. As shown in Fig. 1, the

headgroup charge in SB_{*n*} is reversely arranged with respect to the zwitterionic headgroup of DPPC. In our previous report, we revealed that there was an attractive interaction between sulfobetaine and phosphocholine moieties of 1,2-diacyl glycerides²⁴. Considering that the lyoprotective effect is based on binding of additives to headgroups of liposomes, SB_{*n*} are expected to be lyoprotectants for DPPC liposomes because sulfobetaine can attractively interact with phosphocholine, which is the headgroup of DPPC.

In this study, we examined the applicability of SB_{*n*} as lyoprotective agents for DPPC liposomes. In order to investigate whether DPPC liposomes collapsed during FD/RH, liposome sizes were compared before and after FD/RH. In addition, to examine the integrity of the lipid bilayer of the liposomes after FD/RH, the amount of model solutes in the liposomes after FD/RH was compared to that before FD/RH. The effect of SB_{*n*} that were intercalated in the hydrated or freeze-dried lipid bilayer on the intermolecular interaction between DPPC was examined by differential scanning calorimetry (DSC).

2 Materials and Method

2.1 Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Yuka Sangyo Co. Ltd. (Tokyo, Japan). Alkyl sulfobetaines were purchased from the industries or synthesized by our group (see Supporting information). 5 (6)-Carboxyfluorescein was purchased from Sigma-Aldrich (MO, USA). All reagents were used without further purification.

2.2 Preparation of the liposomes

Liposomes were prepared by a conventional sonication method. Briefly, DPPC (100 μmol) and SB (25 μmol) were added to a round-bottom flask and dissolved in methanol. The lipids/methanol solution was slowly evaporated on a rotary evaporator under reduced pressure to form a uniformly thin lipid film on the bottom of the flask. The dried lipid film was completely hydrated with 5 mL of distilled water at 55°C (above the gel-to-liquid crystal phase transition temperature of DPPC) for 0.5 hours. The hydrated lipid film was vigorously stirred with a probe-type sonicator (UD-200, TOMY, Tokyo, Japan) at 30 W until the appearance of the dispersion changed from milky white to hazy transparent. The resulting dispersion was incubated for 1 h at 55°C, and then filtered (0.22 μm-pore).

2.3 Lyophilization and rehydration of the liposome dispersion

Lyophilization of the liposome dispersion was performed using a freeze dryer (FDU-1200, EYELA, Tokyo, Japan). The prepared liposome dispersion was added to a 5 mL

Fig. 1 Chemical structures of phospholipid and alkyl sulfobetaines (SB_{*n*}, *n*: number of carbons in the alkyl chain, *n* = 1–18) used in this study.

glass vial and frozen in liquid nitrogen. The frozen sample was placed under reduced pressure (~ 13 Pa) at room temperature. The lyophilized lipid cakes were rehydrated in deionized water at 55°C for 1 h.

2.4 Measurement of the size distribution

To determine the size distribution of the liposomes before and after lyophilization, dynamic light scattering (DLS) measurements were carried out using Nicomp 380 (Particle Sizing Systems, CA, USA) equipped with a helium-neon laser (5 mW) operating at a wavelength of 632.8 nm. The scattering light was collected at a 90° angle at 25°C . All sample dispersions were filtered through $0.2\text{-}\mu\text{m}$ syringe filters before analysis.

2.5 Freeze-fractured transmission electron microscopy (FF-TEM)

The liposome dispersion was frozen rapidly in liquid nitrogen and fractured using a cold knife (FR-7000A, Hitachi High-Technologies, Tokyo, Japan). To prepare the liposome replicas, platinum vapor was exposed to the cross section of the frozen sample, and then the cross section was treated with carbon vapor to build up the replica. Liposomes on the replica were washed off with methanol and distilled water, after which the replica was transferred onto a copper grid. The liposome replica was visualized using a transmission electron microscope (H-7650, Hitachi High-Technologies) with an acceleration voltage of 120 kV at -160°C .

2.6 Solute retention study

To investigate the integrity of the lipid bilayer of the liposomes after FD/RH, the amount of solute entrapped in the liposomes after FD/RH was compared to that before the FD/RH. The liposomes were prepared by the same method, except for the hydration buffer. For this solute retention study, we used carboxyl fluorescein (CF) as a model solute, which is typically used for such retention studies. The following method was used for determining the CF retention value. CF was dissolved in TES buffer (10 mM, pH 7.58). The CF solution (10 mM in TES) was used for hydration of the lipid thin layer during liposome preparation. 4 mL of the liposome dispersion was divided into two aliquots (aliquot A, aliquot B, each volume: 2 mL). To remove free SB_n and CF from aliquot A, which had not been encapsulated in the liposomes following their preparation, aliquot A was passed over Sephadex G-50 (GE Healthcare Japan, Hino, Tokyo) packed in a column ($15\text{ mm} \times 113\text{ mm}$), and then lyophilized. In contrast, aliquot B was lyophilized immediately after liposome preparation. Lyophilized aliquots A and B were rehydrated using TES buffer. To release the entrapped CF from the liposomes into the bulk solution, the liposomes were lysed with Triton-X 100 (final concentration = 0.02 wt%). The CF con-

centration in the bulk solution was determined by fluorometry using FP-8000 (JASCO, Tokyo, Japan). The fluorescence intensity at 515 nm (Ex: 490 nm) was measured and converted to concentration using a calibration curve.

2.7 Differential scanning calorimetry (DSC)

DSC measurements were carried out with DSC 1 (Mettler Toledo, Switzerland). Two different types of liposome samples, aqueous dispersions and lyophilized powders, were applied in the measurement. These samples were carefully added to aluminum pans ($40\text{-}\mu\text{L}$ pan for the powders, $100\text{-}\mu\text{L}$ pan for the dispersions) and then sealed with an aluminum lid. The temperature was controlled using a heating-cooling-heating cycle scan program with a scan rate of $2^\circ\text{C}/\text{min}$. Lyophilized liposomes were equilibrated once the temperature exceeded the gel-to-liquid crystal temperature, leading to disruption of their original state soon after freeze-drying²⁵. Thus, the first heating process was adopted as data for lyophilized liposomes. All measurements were conducted under flowing nitrogen gas ($30\text{ mL}/\text{min}$) through a furnace, and empty pans were used as reference.

3 Results and Discussion

3.1 Structural integrity of DPPC liposomes after FD/RH

If DPPC liposomes were freeze-dried without lyoprotectants, the liposomes aggregated and/or fused, which resulted in increment of the liposome sizes. To investigate whether liposomes aggregate and/or fuse during the FD/RH processes, we compare the sizes of the hydrated liposomes before and after the FD/RH processes (Fig. 2). Before the FD/RH processes, the mean size of the hydrated liposomes was 14–169 nm and there is no clear dependency of the alkyl chain length of SB_n on the liposome sizes. After FD/RH processes, the size of the hydrated liposomes that are prepared without SB_n increases from several tens of nanometers to several micrometers. This result indicates that the liposomes aggregate/fuse in the absence of SB_n during the FD/RH processes. In contrast, when adding the sulfobetaines with an alkyl chain of intermediate length, $\text{SB}_{n=6,8,10}$, the increment in the liposome size is remarkably prevented and the size distribution remains almost constant even after the FD/RH processes. Addition of trehalose, which is known as a typical lyoprotectant, the size distribution of the liposomes remains almost constant after FD/RH. In the presence of alkyl sulfobetaines having shorter ($\text{SB}_{n=1,2,4}$) or longer ($\text{SB}_{n=12,14,16,18}$) alkyl chain lengths, the size of the liposomes increases after the FD/RH processes. These results indicate that the length of the alkyl chain in SB_n is an important factor for lyoprotection of the DPPC liposomes. In our preliminary experiments,

Fig. 2 Size distributions of the DPPC liposomes before (black bars) and after (gray bars) FD/RH. SB_n were added at 20 mol% with respect to DPPC.

when using either a cationic or an anionic additive (20 mol% with respect to DPPC, the same amount as the case of SB_n), the DPPC liposomes aggregated and/or fused through the FD/RH processes. In this experiment, alkyltrimethylammonium bromides or sodium alkyl sulfonates were used as cationic or anionic additives, respectively, with the same alkyl chain length as that of SB_n used above. Regardless of the alkyl chain length of these additives, aggregation and/or fusion of the liposomes was observed. These results indicate that the zwitterionic character and proper hydrophobicity (length of alkyl chain) of sulfbetaines may play an important role in prevention of aggregation and/or fusion of liposomes after the FD/RH processes.

For FF-TEM and the solute retention experiments in this study, $SB_{n=10}$ was used as a representative additive. The FF-TEM observations reveal that the use of $SB_{n=10}$ could prevent aggregation and/or fusion of the liposomes after FD/RH (Fig. 3b). In the absence of additives, the liposomes aggregate and/or fuse after FD/RH (Fig. 3a). The results of FF-TEM are consistent with the results of DLS.

Fig. 3 FF-TEM images of liposomes before and after the FD/RH processes. The images in (a) represent liposomes consisting of a single component of DPPC. The images in (b) represent liposomes containing DPPC and 20 mol% of $SB_{n=10}$ with respect to DPPC.

Table 1 Comparison of CF retention values of the liposomes.

Liposome formulation	CF retention (%)
DPPC	<1
DPPC + $SB_{n=10}$	95 ± 10
DPPC + Trehalose	82 ± 13

All values are presented as mean ± standard deviation ($n = 3$).

Leakage of solutes entrapped in liposomes is caused by disruption of the lipid bilayer of the liposomes. Therefore, the amount of solutes entrapped in the liposomes after FD/RH is a good indicator for investigating the integrity of the lipid bilayer during the FD/RH processes. The results of CF retention values are summarized in Table 1. CF retention values are 95 ± 10% and 82 ± 13% in the presence of $SB_{n=10}$ or trehalose, respectively, significantly higher than those found in the absence of additives (<1%). CF retention in the presence of $SB_{n=10}$ is slightly but significantly larger compared with that in the presence of trehalose, an additive known to exert a significant lyoprotective effect^{20, 21}. This result clearly shows that $SB_{n=10}$ also provides significant lyoprotection for DPPC liposomes. Although use of either $SB_{n=6}$ or $SB_{n=8}$ could prevent disruption of the liposome membrane to some extent (CF retention = 57% for $SB_{n=6}$, 75% for $SB_{n=8}$), its efficacy was lower than that of

SB_{n=10}.

3.2 Effect of SB_n on the phase transitions of hydrated DPPC liposomes

In general, the gel-to-liquid crystal phase transition temperature (T_m) of the phospholipid bilayer is strongly related to the intermolecular interaction between the lipid molecules constituting the lipid bilayer^{25, 26}. For instance, if co-existing molecules (e.g. solvent (H₂O), solute, additives) intercalate between lipids in the bilayer, the T_m of the lipid bilayer sometimes decreases. This decrease in the T_m is generally explained by reduction in intermolecular interaction between the lipids in the bilayer, which is caused by an increase in spacing between the lipids²⁷. The T_m of hy-

drated liposomes composed of pure DPPC was $41.7 \pm 0.2^\circ\text{C}$ (Fig. 4a), which is consistent with general value reported previously²⁵. The T_m of the hydrated liposomes shifted in the presence of SB_n, indicating that the interaction between DPPC in the hydrated lipid bilayer was altered by the addition of SB_n.

The addition of alkyl sulfobetaines having a shorter alkyl chain, SB_{n=1,2,4,6}, results in a slight increase in T_m of the hydrated liposomes ($\Delta T_m = +0.4$ to $+0.9^\circ\text{C}$). This increase in the T_m can be explained by a kosmotropic effect, which can be observed when adding saccharides to liposomes²⁸. Kosmotropes are molecules that can stabilize the structure of water molecules in an aqueous solution. It has been known that kosmotropes, such as saccharides, reduce the amount of water molecules on the surface of lipid bilayers owing to local osmotic imbalance²⁹. This partial dehydration at the lipid bilayer surface should increase the T_m by decreasing the spacing between the lipids, which enhances intermolecular interaction between the lipids. At the same time, the amount of SB_{n=1,2,4,6} partitioned in the lipid bilayer should be quite low because of their relatively short alkyl chain. Most of the SB_{n=1,2,4,6} may be existed in the bulk solution.

As shown in Fig. 4a, in the presence of SB_{n=8,10,12,14,16,18}, the T_m of the aqueous dispersion decreases ($\Delta T_m = -4.5$ to -0.2°C). The kosmotropic effect of alkyl sulfobetaines should be overshadowed by the hydrophobic interaction between their alkyl chains in the SB_{n=8,10,12,14,16,18} and the palmitoyl groups in DPPC in the lipid bilayer. The amount of the SB_{n=8,10,12,14,16,18} that partitioned in the lipid bilayer should be much higher than that of the SB_{n=1-6} because of their relatively long alkyl chain. Within this range of alkyl chain length, a descending trend in the T_m is observed until n value reaches 12. SB_{n=8,10,12} intercalated in the lipid bilayer should create "voids" in the hydrophobic region in the lipid bilayer because the alkyl chains of SB_{n=8,10,12} are shorter than the acyl chains of DPPC. These "voids" can contribute to the reduction in the T_m by reducing intermolecular interaction between the lipids in the bilayer, which is also discussed in the next section regarding the freeze-dried bilayers. When using SB_{n=14,16,18}, there is an increasing trend in the T_m . The hydrophobic interaction should contribute to an increase in the intermolecular interaction between the lipids in the bilayer. It can be assumed that these longer alkyl chains in SB_{n=14,16,18} should fill the "voids" in the hydrophobic region in the lipid bilayer, which contribute to the recovery of the intermolecular interaction between the lipids in the lipid bilayer.

Fig. 4 Plots of gel-to-liquid crystal phase transition temperatures (T_m) for the hydrated (a) and freeze-dried (b) DPPC liposomes prepared in the presence of 20 mol% SB_n with respect to DPPC. Data for the hydrated and freeze-dried liposomes were acquired at 2nd or 1st heating process, respectively ($2^\circ\text{C}/\text{min}$). Data are represented as mean $\pm$ standard deviation ($n \geq 2$).

3.3 Effect of SB_n on the phase transitions of freeze-dried DPPC liposomes

It is important to determine the T_m to clarify the extent of intercalation of additives in freeze-dried lipid bilayers. The DSC thermograms for freeze-dried liposomes were

shown in Fig. S4(b). In the absence of SB_n , T_m of the freeze-dried liposome is $96.4 \pm 6.1^\circ\text{C}$ (Fig. 4b), which is consistent with previously published data²⁶⁾. In contrast, in the presence of SB_n regardless of their alkyl chain length, the T_m of the freeze-dried liposomes is lower compared to that in the absence of additives. This result can be explained by the intercalation of SB_n into the freeze-dried lipid bilayer. The trend of reduction in T_m for the freeze-dried liposomes is different from those of the hydrated liposomes. For instance, even when using alkyl sulfobetaines with shorter alkyl chain ($SB_{n=1,2,4,6}$), which increases the T_m of the hydrated liposomes (Fig. 4a), the T_m of the freeze-dried liposomes decreases compared to that without additives. When using $SB_{n=1,2,4,6,8}$, the T_m of the freeze-dried liposomes decreases with increasing n -value. When using $SB_{n=6,8,10}$, the T_m of the freeze-dried liposomes remarkably decreases. Particularly, when using $SB_{n=8}$, the T_m of the freeze-dried liposomes reaches the lowest value ($38.5 \pm 0.4^\circ\text{C}$), which is lower than that of the fully hydrated pure DPPC liposomes ($41.7 \pm 0.2^\circ\text{C}$). Moreover, multiple endothermic peaks were observed when using $SB_{n=6,8,10,12}$ (Fig. S4(b)), which indicates that phase state of the freeze-dried lipid may be different from that without SB_n . The powder x-ray diffraction revealed that crystallinity of freeze-dried DPPC liposomes containing $SB_{n=10}$ was the same with that composed pure DPPC (data not shown). Further examination should be required to reveal details in packing of lipids in the freeze-dried liposome in the presence of SB_n .

The interaction between SB_n and the freeze-dried lipid bilayer can be illustrated by considering the trend of the T_m reduction compared with the alkyl chain length of SB_n (Scheme 1). SB_n with shorter alkyl chain length could create larger “void” spaces in the freeze-dried bilayers owing to their much shorter alkyl chain length compared

to the palmitoyl groups in DPPC (Scheme 1, left panel). However, the amount of SB_n intercalated in the bilayer should be lower because of the low hydrophobicity of the shorter alkyl chain. Thus, the total space of “voids” in the bilayers reduced, which did not strongly influence the reduction of the T_m interaction between $SB_{n=1}$ and phosphocholines of DPPC.

SB_n having alkyl chain of intermediate length should create the largest total space of “voids” in the freeze-dried bilayers (Scheme 1, middle panel), which results in the marked reduction of the T_m . The largest amount of total “void” space should arise from the increased amount of intercalated SB_n in the bilayers and their shorter alkyl chain compared to the palmitoyl groups in the DPPC, which can still create “voids” in the DPPC bilayer.

The use of SB_n with longer alkyl chain led to an increase in the T_m of the freeze-dried liposomes, indicating that the intermolecular interaction of the freeze-dried bilayer is recovered by filling “voids” owing to their longer alkyl chains (Scheme 1, right panel). The amount of SB_n intercalated in the bilayer further increased due to adequate hydrophobicity of their longer alkyl chain.

The changes in T_m that were observed when using trehalose, which is one of the most effective lyoprotectants^{21, 22)}, were similar to those observed with SB_n . It has been reported that the addition of trehalose decreases the T_m of the freeze-dried liposomes ($\sim 25^\circ\text{C}$ for dried DPPC liposomes²⁶⁾). The lyoprotection mechanism of liposome using trehalose has been explained by the water replacement model^{30, 31)}. During the FD process, trehalose can intercalate between lipids in the dried bilayer via hydrogen bonding between headgroups of lipids and hydroxyl groups in trehalose. This resulted in a marked reduction in the T_m of the freeze-dried liposomes. Thus, reduction in the T_m of freeze-dried liposomes by addition of SB_n implies a possi-

Scheme 1 Schematic of “void” creation mechanism in freeze-dried bilayers by intercalation of SB_n . The total “voids” space in the bilayers should be dependent on the amount of intercalated SB_n and the alkyl chain length of the intercalated SB_n .

bility for replacement of water molecules bound to lipid headgroups with SB_n during the FD process. Although water molecules bound to lipid headgroups may be replaced with SB_n, there should be a difference in the underlying mechanism responsible for the T_m reduction between trehalose and SB_n. In the case of SB_n, the T_m reduction of the freeze-dried liposomes should be related to the “void” created in the hydrophobic region in the bilayer, which is dominated by the hydrophobic interaction between short alkyl chains of the intercalated SB_n and palmitoyl groups in the DPPC (Scheme 1, middle panel). In contrast, in the case of trehalose, hydrogen bonding between the hydroxyl groups in the trehalose and the lipid headgroups may be involved in the T_m reduction of freeze-dried liposomes.

It has been reported that liposome disruption is frequently induced during the gel-to-liquid crystal phase transition during the RH process³²⁾. Therefore, limiting the phase transition during the RH process should be important in preventing liposome disruption. It has been shown that when the T_m of the freeze-dried liposomes is lower than the temperature of the RH process, the gel-to-liquid crystal phase transition of the liposomes can be prevented during the RH process. Thus, it can be assumed that the lyoprotective effect in the present study is related to the markedly reduced T_m of the freeze-dried liposomes. Taken together, molecules that are good candidates for lyoprotectants of lipid bilayers should not solubilize the bilayer in the hydrated state, and should strongly interact with the lipid headgroups in the dried state, leading to the T_m reduction of the dried bilayer.

4 Conclusions

The present study demonstrated lyoprotection of DPPC liposomes by using SB_n. The alkyl chain length of SB_n was a crucial factor for the lyoprotective effect. SB_n having an alkyl chain with intermediate length exerted lyoprotective effect. The lyoprotection offered by SB_n may be explained by its prevention of the gel-to-liquid crystal phase transition during the rehydration of freeze-dried liposomes, which can be achieved by a reduction of the phase transition temperature of the freeze-dried liposomes, to a temperature lower than that of the rehydration process. The use of SB_n having an alkyl chain with intermediate length should increase the total “void” space in the hydrocarbon region in the freeze-dried bilayer owing to their appropriate hydrophobicity and shorter alkyl chain compared to the acyl groups of DPPC. The results from this study provide an insight into the mechanism of zwitterionic amphiphiles for preserving lipid bilayers during freeze-drying.

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Supporting Information

Synthesis of alkyl sulfobetaines used in this study and the DSC thermograms.

This material is available free of charge via the Internet at <http://dx.doi.org/jos.66.10.5650/jos.ess.17100>

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