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Pseudo-Interphase of Liposome Promotes 1,3-Dipolar Cycloaddition Reaction of Benzonitrile Oxide and *N*-Ethylmaleimide in Aqueous Solution

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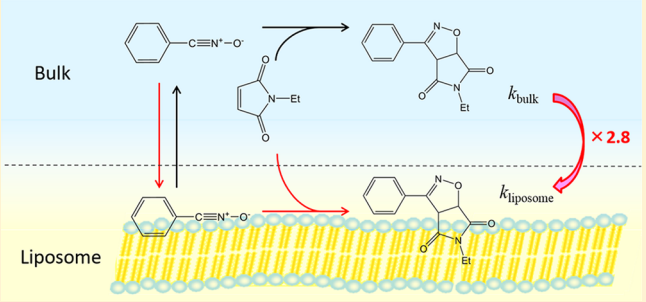
Supporting Information

ABSTRACT: The hydrophobic interior of a liposome membrane was used as a platform for the organic synthesis of hydrophobic compounds in water. The 1,3-dipolar cycloaddition of benzonitrile oxide (BNO) and *N*-ethylmaleimide (EMI) in liposome suspensions was carried out, and an increase in the reaction rate constant was observed depending on the liposome characteristics. While the reaction rate constant in 1,4-dioxane was 1.5 times higher than that in water, the reaction rate constant in an aqueous solution of cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) liposome was 3 times higher than in water. The amount of substrate, BNO, accumulated in the DOTAP liposome was higher than that in 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP), indicating that BNO prefers to be distributed in the liposome membrane in the liquid-disordered phase. The membrane polarity, GP_{340}, as monitored by Laurdan, varied with the presence of BNO, while EMI slightly affected the membrane properties of the liposomes. These results suggest that the pseudo-interphase afforded by the liposome membrane can promote the 1,3-dipolar cycloaddition between BNO and EMI in water.



INTRODUCTION

Water is an ideal solvent, as it is harmless, nonflammable, and stable. Environmentally friendly chemical processes with high atomic efficiency[1] have attracted significant attention, and thus, numerous organic reactions in water have been developed,[2] even though the polarity of water molecules and the insolubility of reactants usually prevent reactions from occurring in the aqueous phase. To overcome such disadvantages, several kinds of catalysts that work at the "interphase" have been reported.[3−5] For example, phase-transfer catalysts (PTC) have been developed as a novel method to catalyze the transfer of reactants across one liquid phase to another immiscible phase.[6−9] The reactants can accumulate in the interphase between two liquid media, where the interphase region can act as a reaction site. The advantage of PTC can be seen in the enantioselectivity of products; nevertheless, they require polar organic solvents and a strong base (or acid) and are not regarded as environment friendly. Organocatalysts, such as L-proline and its derivatives,[10−13] have attracted significant attention from the viewpoint of metal-free and organic solvent-free reaction systems. Several organic syntheses have been reported in water using organocatalysts,[14−18] including Diels−Alder, aldol, and Michael reactions.

Recently, highly organized interfaces, such as the surfaces of self-assembled amphiphilic molecules, have been utilized to achieve successful chemical conversions in water. For example, some micellar aggregates have been reported to promote Diels−Alder reactions.[18−21] Micellar suspensions can provide a hydrophilic−hydrophobic interphase between the bulk solution and hydrophobic interior of the assembly. It has been reported that the pseudo-phase model[22] can be applied to the aldol reaction and Diels−Alder reaction in micellar or emulsion systems.[18,19] Meanwhile, 1,3-dipolar cycloadditions, which are similar to the Diels−Alder reaction, can be influenced by the degree of solvation as well as the frontier molecular orbitals (FMOs) of the reactants.[23,24] In homogeneous solution systems, it is assumed that the relative dielectric constant (ε) and proticity of the reaction media play important roles on the reaction. Although the reaction mechanism might be different in micelle solution, the properties of the hydrophilic−hydrophobic environment around the reactants may also regulate the reactions.

Liposomes, which consist of a lipid bilayer with a 5 nm thick hydrophobic interior, also provide a hydrophobic−hydrophilic interface. Such hydrophobic interiors can be utilized as a nanoreactor. In our previous works, (bio)chemical conversions have been carried out at the surface of liposome membranes.[25−29] Herein, the pseudo-interphase, defined as the surface region of liposome membranes where the hydrophilic aqueous phase and hydrophobic liposome interior are enclosed

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within a nanometer scale, is expected to act as a platform for reactant localization. In addition, the surfaces of the liposome membranes are estimated to be hydrophobic similar to some organic solvents.[30] It is therefore indicated that the reactants localized at the liposome surface could be affected by the surrounding environment, i.e., membrane polarity, etc. Liposome suspensions could be suitable systems for metal-free and organic solvent-free organic syntheses, because the characteristics of the microscopic environment at liposome surfaces (fluidity and polarity among others) are designable; however, previously reported works have not focused on the characterization and design of self-assemblies.

In this study, the 1,3-dipolar cycloaddition[20,31−34] between benzonitrile oxide (BNO) and *N*-ethylmaleimide (EMI) was carried out in the presence of various kinds of liposomes. This is the first report which details the effects of liposome membranes on this reaction. Because the surface of liposome membranes is hydrophilic environment (water rich), the accumulation of reactants at the membrane surface could be regarded as a solvation effect in the aqueous phase. Here, three types of aqueous solutions were used as reaction media: (i) 1,4-dioxane/water binary mixtures (0−100 v/v% 1,4-dioxane), (ii) micellar solutions (cetyltrimethylammonium bromide (CTAB), sodium dodecylsulfate (SDS)), and (iii) liposome suspension systems. The interphase region between hydrophobic medium and hydrophilic medium was defined a pseudo-interphase, and its effects on the 1,3-dipolar cycloaddition are discussed. The microscopic environment of each liposome was characterized using fluorescence probes;[35] membrane polarity was analyzed by 6-lauroyl-2-dimethylaminonaphthalene (Laurdan) and 6-(*p*-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS). As an indicator of water solvation in each medium, the relative dielectric constants (ε') of the liposome suspensions were estimated. The kinetics of the 1,3-dipolar cycloaddition between BNO and EMI were analyzed by pseudo-first-order kinetics. The distribution of each reactant in the liposome membrane was evaluated by ultrafiltration, and the relationship between the adsorption of reactants on the liposome and the dehydration of the membrane surface were then considered. Finally, the reaction kinetics at the pseudo-interphase are discussed, where the possible role of the liposome is to accumulate the reactants and to regulate the reaction in aqueous media.

EXPERIMENTAL METHODS

Materials. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and 1,2-dipalmitoyl-3-trimethylammonium-propane (DPTAP) were purchased from Avanti Polar Lipid (Alabaster, AL, USA). 6-Lauroyl-2-dimethylaminonaphthalene (Laurdan), and 6-(*p*-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Benzaldehyde oxime (BO) and sodium hypochlorite solution (NaClO(aq)) were purchased from Kanto Chemical (Tokyo, Japan). Other chemicals were purchased from Wako Pure Chemical (Osaka, Japan). These chemicals were used without further purification.

Benzonitrile oxide (BNO) was prepared according to the literature.[20] In brief, 21.6 μL of BO were dissolved in 2 mL of the NaClO(aq)/1-propanol solution (50:50). 0.36 g of NaCl was added to this solution to separate it into two phases. After the upper phase was taken and diluted by 1-propanol, 25 mM BNO solution was obtained. The formation of BNO was determined by UV−vis spectroscopy (UV-1800; Shimadzu, Kyoto, Japan) and a high resolution double-focusing magnetic sector mass spectrometer (JMS700; JEOL, Tokyo, Japan), where the conversion of BO was >85%. The prepared BNO solution was stable for at least 3 h, and the experiments were performed soon after the BNO was prepared.

Preparation of Liposomes. A chloroform solution with lipids was dried in a round-bottom flask by evaporation under a vacuum.[35] The obtained lipid thin film was dissolved in chloroform again, and the solvent was evaporated. The lipid thin film was kept under a high vacuum for at least 3 h, and was then hydrated with distilled water at room temperature. The liposome suspension was frozen at −80 °C and thawed at 50 °C to enhance the transformation of small vesicles into larger multilamellar vesicles (MLVs). This freeze−thaw cycles were performed five times. MLVs were used to prepare the LUVs by extruding the MLV suspension 11 times through two layers of polycarbonate membranes with mean pore diameters of 100 nm using an extruding device (Liposofast; Avestin Inc., Ottawa, Canada).

Evaluation of Membrane Polarities. Laurdan is sensitive to the polarity around the molecule itself, and its fluorescence properties enable to evaluate the surface polarity of lipid membranes.[30] The Laurdan emission spectra exhibit a red shift caused by dielectric relaxation. The emission spectra were measured with an excitation wavelength of 340 nm, and the general polarization (GP_{340}), the membrane polarity, was calculated as follows:

$$GP_{340} = (I_{440} - I_{490})/(I_{440} + I_{490})$$

where I_{440} and I_{490} represent the fluorescence intensity of Laurdan at 440 and 490 nm, respectively. To compare the polar environment of the liposome or micelle surface and solvent, the apparent polarity of liposome, ε', was calculated (see Supporting Information Figure S1). The total concentrations of lipid and Laurdan were 100 and 1 μM, respectively.

For measuring membrane polarity by TNS, a TNS/ethanol solution (100 μM) was used instead of a Laurdan/ethanol solution and the rest of the process is the same as that for the measurement using Laurdan.

1,3-Dipolar Cycloaddition of BNO and EMI (Scheme 1). 10 μL of BNO solution (25 mM in 1-propanol) were mixed with 1 mL of sample solution, composed of a dioxane/water mixture, liposome suspension (lipid concentration = 0.25 mM), or micelle solution (lipid conc. = 250 μM). The sample solution was incubated for 30 min at room temperature, and then, 5 μL of EMI solution (200 mM in 1-propanol) were added to initiate the 1,3-dipolar cycloaddition. The total concentrations of BNO and EMI were 250 μM and 1 mM, respectively.

Kinetics Measurement of UV Absorbance. The UV absorbance spectra of sample solution was analyzed from 500 to 200 nm, using a UV spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). The UV absorbance at 278 nm is originated from product[20,31] that is distinguishable from the peak of BNO (258 nm) or EMI (300 nm). The reaction was started in a quartz cuvette, and a time course of the UV absorbance at 278 nm was measured for 15 min at room temperature. The reaction rate constant was analyzed via pseudo-first-order kinetics, because of the excess of EMI (4-fold), which is calculated as follows:

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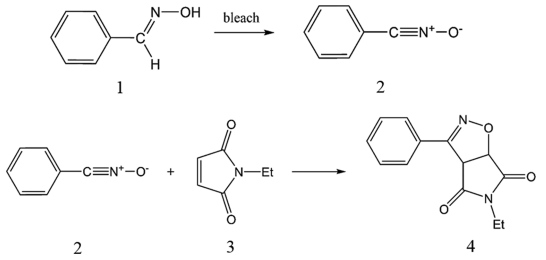
J. Phys. Chem. B 2015, 119, 9772−9779

Scheme 1. 1,3-Dipolar Cycloaddition of Benzonitrile Oxide (BNO) and *N*-Ethylmaleimide (EMI)[a]



[a](1) BO, (2) BNO, (3) EMI, (4) product.

$$-\ln\left(1 - \frac{A_{278}}{A_{278,e}}\right) = kt$$

where A_{278} and $A_{278,e}$ represent the UV absorbance at 278 nm at any moment t in the reaction and that at 15 min (end of the reaction), respectively. k is the reaction rate constant, and t is the time. The concentrations of reactants were 0.25 mM for BNO and 1.0 mM for EMI. The concentration of lipid was 0.25 mM. The relative reaction constant, k_{rel}, was calculated as follows:

$$k_{rel} = k/k_{water}$$

where k_{water} represents the reaction rate constant in water.

Adsorption of Reactants onto Liposome. The adsorption amounts of the reactants were also evaluated by UV spectrophotometer. 10 μL of BNO solution (25 mM in 1-propanol) were mixed with 1 mL of liposome suspension (lipid concentration = 0.25 mM) and incubated for 30 min at room temperature to measure the adsorption of BNO. To measure the adsorption of EMI, 5 μL of *N*-ethylmaleimide solution (200 mM in 1-propanol) were mixed with 1 mL of liposome suspension (lipid concentration = 0.25 mM) and incubated for 30 min at room temperature. After the incubation, the liposome and reactants adsorbed on the membrane were removed with the ultrafiltration unit USY-20 (molecular weight cutoff: 200 000, Advantec Toyo, Ltd., Tokyo, Japan). The adsorption percentages were calculated from the difference in UV absorbance of the solution before and after the filtration:

$$\text{Adsorption percentage} = ((A_{initial} - A_{filtrated})/A_{initial}) \times 100$$

where $A_{initial}$ and $A_{filtrated}$ represent the absorbance of the reactant (BNO or EMI) in the solution before and after ultrafiltration, respectively.

RESULTS AND DISCUSSION

1. Characterization of Liposomes. Pseudo-interphase reactions constitute an important group in chemical reactions in self-assembled systems.[38] Notably, the polarity and proticity of solvents can regulate the partitioning of reactants; the microscopic environment of the liposome surface can also regulate the 1,3-dipolar cycloaddition reaction, as evaluated in this study.[20] In order to investigate the membrane properties of the liposomes, the general polarization (GP_{340}) was estimated using Laurdan. The GP_{340} values varied with the acyl chain length and surface charge of the lipid molecules that comprised the liposome membranes (Table S1). POPC and DOTAP liposomes showed lower GP_{340} values ($GP_{340} < -0.2$), indicating that they were in the liquid-disordered phases.[29] In contrast, DSPC and DPTAP showed higher GP_{340} values at room temperature. Because the phase transition temperatures of DSPC and DPTAP are 55 and 45 °C, respectively, DSPC and DPTAP were in the solid-ordered phases. Because Laurdan peak shifts are dependent on environmental hydrophobicity, GP_{340} values can indicate the hydrophobicity as well as the relative dielectric constants. To investigate the relative dielectric constant, the nonpolarity factor (NF_{340}) was examined; NF_{340} values reveal the degree of nonpolarity of the solvents and were applied to characterize the liposomes. The NF_{340} values were calculated using the ratio of the fluorescence intensities of Laurdan in organic solvents and water (see Experimental Methods and Figure S1). The relative dielectric constant, ε', can be calculated from NF_{340} values, suggesting that the approximate amount of water at the liposome membrane surface can be estimated by analyzing the GP_{340} values (Figure 1). Liposomes in the liquid-disordered phases showed high ε' values ($\varepsilon' = 20-25$), while low ε' values ($\varepsilon' = 3-5$) were observed in liposomes in the solid-ordered phases. CTAB micelles also showed a higher ε' value, implying that the membrane surface of CTAB micelles is similar to that of liposomes in liquid-disordered phases. It is known that the permeability of small molecules across liposome membranes can be influenced by the phase states of the liposomes.[37] Furthermore, it has been reported that the distribution of BNO in positively charged DOTAP liposomes is higher than that in zwitterionic DOPC liposomes.[38] It is therefore expected that the reactants can accumulate at the surface of liposomes, and the reaction can occur at the pseudo-interphase of the liposomes.

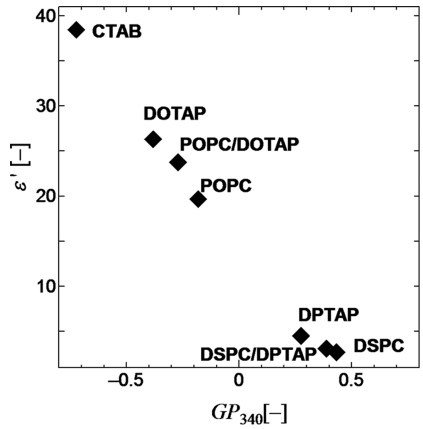


Figure 1. Characteristics of liposome. Membrane polarity (GP_{340}) was measured from the experiment using Laurdan, and relative dielectric constant (ε') was calculated by the fitting equation from Figure S1.

2. Effect of Liposomes on 1,3-Dipolar Cycloaddition. In the 1,3-dipolar cycloaddition of BNO and EMI, the surrounding dielectric environment is a key factor.[34] Notably, the reaction between BNO and EMI was carried out in water (Figure S2). In order to estimate the effects of the reaction media on the 1,3-dipolar cycloaddition between BNO and EMI, the relative dielectric constant ε' and the relative reaction constant, k_{rel}, were plotted (Figure 2). With the 1,4-dioxane/water systems, the k_{rel} values became higher with a decrease in the relative dielectric constant of the solvent. The relative reaction rate constant in 1,4-dioxane ($\varepsilon = 2.2$) was 1.6 times higher than that in water ($\varepsilon = 78$), indicating that the

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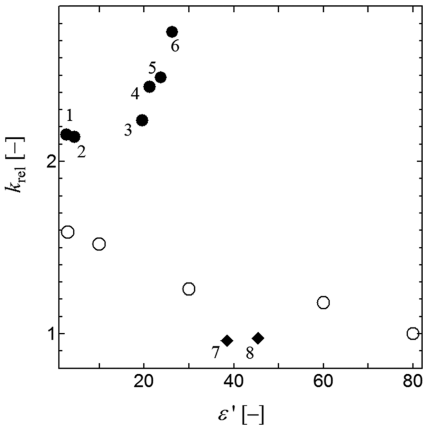

Figure 2. Reaction rate constant in various conditions. The reaction of BNO (0.25 mM) and EMI (1.0 mM) was carried out in water at 25 °C. The reaction was initiated after 30 min incubation of BNO with the medium. For the 1,4-dioxane/water system (open circle), 1,4-dioxane/water was used as the medium to vary the relative dielectric constant. For liposomes (closed circle), liposome suspensions (0.25 mM) were used as the reaction medium, and 5 mM micelle solutions were used for micelles (closed diamond). Each point indicates the following: (1) DSPC, (2) DPTAP, (3) POPG, (4) POPC, (5) POPC/DOTAP, (6) DOTAP, (7) CTAB, (8) SDS. Rate constant values are relative values compared to the reaction conducted in water (ε' = 78).

hydrophobic environment could promote the 1,3-dipolar cycloaddition. BNO is hydrophobic and thus prefers hydrophobic environments.[38] Because 1,4-dioxane is an aprotic solvent, the reaction between BNO and EMI in 1,4-dioxane/water systems would be dependent on the solvation of the reactants. With the micellar suspensions, a nonpromotion effect was observed even though the surface of the micelles was hydrophobic (ε' = 35–45). This result indicated that the reactants could not accumulate on the surface of the micelle aggregates. In the case of the liposomes, the k_{rel} values increased by 2.2–2.8 times as compared to the water solution, depending on the characteristics of the liposomes. Specifically, the reaction was more promoted in liposomes in the liquid-disordered phases (ε' = 20–25) as compared to that in liposomes in the solid-ordered phases. Among the liposomes used in the present study, DOTAP liposome showed the highest promotion effect on the reaction, indicating that the DOTAP liposome could provide a better environment for the reaction. Rispens and co-workers reported that 1-propanol containing 40 M of water was the best medium for the 1,3-dipolar cycloaddition between BNO and EMI.[20] Although the surface charge densities of liposomes or micelles differed (Table S1), no relationship was obtained between the surface charge density and the promotion of the reaction. It is notable that the k_{rel} values were not so high just after the mixing of the reactants in each medium (Figure S3). To find a better environment and to promote this reaction in liposome systems, the condensation of the reactants should be considered.

3. Accumulation of BNO and EMI in Liposome Membranes. The local concentrations of the reactants were estimated based on the ultrafiltration method.[38] The accumulation of the reactants on the liposome membranes could be critical to the enhancement of the 1,3-dipolar cycloaddition reaction between BNO and EMI at the DOTAP liposome membrane. Figure 3 shows the amount of BNO and EMI distributed on each liposome. DOTAP showed a higher adsorption percentage of BNO as compared with POPC (Figure S4). DPTAP, the cationic liposome in the solid-ordered phase, did not significantly promote the distribution of BNO as compared to DOTAP, while the distribution of BNO on DPTAP at 60 °C increased to 34%, when DPTAP was in the liquid-disordered phase. The BNO distribution analysis indicated that BNO was distributed on the DOTAP liposome not only because of the positive charge but also because of the phase state. Only a small amount of BNO was distributed in the CTAB micelle. In contrast, BNO molecules could accumulate in the membrane. DOTAP, the cationic liposome in the liquid-disordered phase, formed a suitable environment for BNO accumulation. In the case of EMI, the distribution ratio was not much higher than that of BNO, and the adsorption percentage values were almost similar within the liposomes. EMI is more

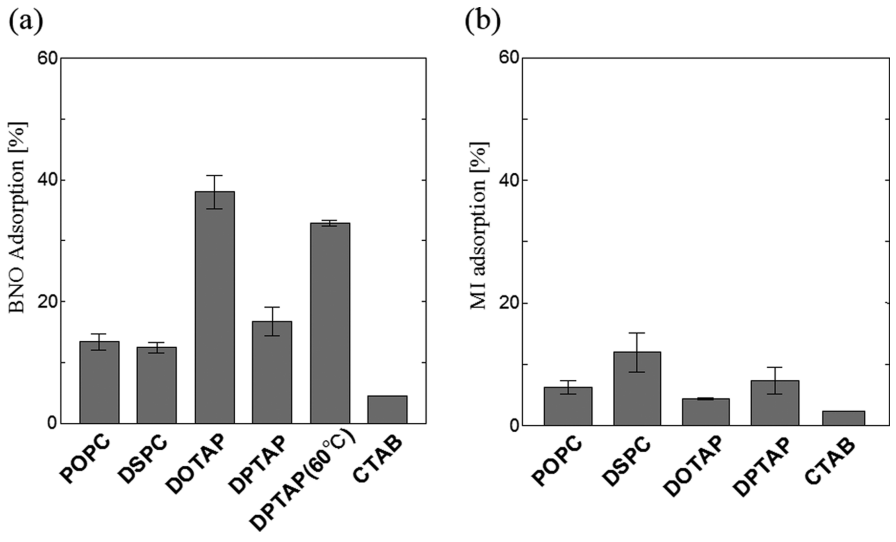

Figure 3. Adsorption Behavior of Substrate Molecules on Liposome Membrane. Each reactant (BNO: 0.25 mM and EMI: 1.0 mM) was incubated with liposome suspension (0.25 mM) in water for 30 min. Adsorption percentage was calculated from the difference of the absorbance before and after filtration (adsorption percentage = $((A_{initial} - A_{filtrated})/A_{initial}) \times 100$). The absorbance was measured at 25 °C unless notified. (a) BNO adsorption, (b) EMI adsorption.

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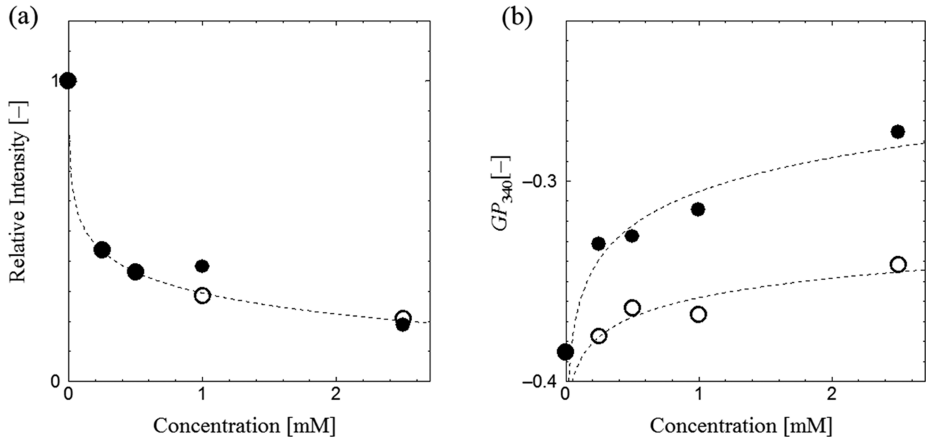

Figure 4. Change in values of fluorescent probes. (a) Intensity of TNS fluorescence was measured. TNS (1.0 μM), DOTAP liposome (100 μM), and optional amount of substrates were incubated for 30 min before measurement. (b) Fluorescent spectrum of Laurdan was obtained. Laurdan (1.0 μM), DOTAP liposome (100 μM), and optional amount of substrates were incubated for 30 min before measurement. GP_{340} values were calculated as written in Experimental Methods section.

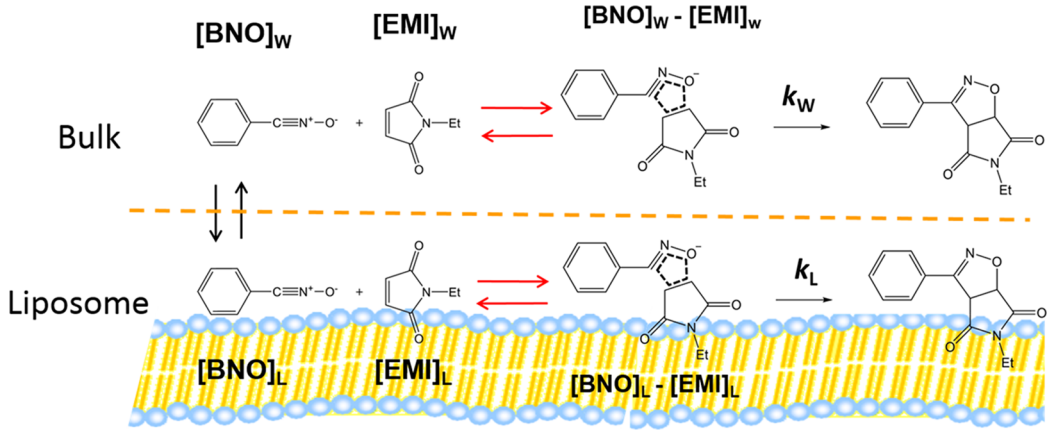

Figure 5. Scheme of the reaction at the interface of liposome membrane.

hydrophilic than BNO: the calculated log *P* values for BNO and EMI were 1.82 and −0.18, respectively.[39] Although the distributed amount of EMI was smaller than that of BNO, it was assumed that EMI preferred to be distributed on the solid-ordered membranes, such as DSPC and DPTAP. In general, 1,3-dipolar cycloadditions are concerted reactions.[40] Thus, both reactants must accumulate at the pseudo-interphase region simultaneously if the reaction occurs around the surface of the liposome membrane. This indicates that strong interactions between reactants and liposome membranes might inhibit the reaction. In the case of the micelle solutions, the mismatched localization of reactants would inhibit the 1,3-dipolar cycloaddition.[20] It is therefore important to estimate the colocalization of BNO and EMI at the surface of the liposomes.

4. Estimation of the Localization of Reactants in Liposome Membranes. The distributions of the reactants in the DOTAP liposome membrane were investigated. Based on the hierarchic binding of the fluorescent probes,[41] the localization behaviors of BNO and EMI were analyzed. TNS and Laurdan exist in the hydrophobic−hydrophilic interface region. After these fluorescent probes were embedded in the liposome membranes, BNO and EMI were added to each sample, and variations in the membrane properties were evaluated (Figure 4). After BNO was added, the fluorescence intensity of TNS decreased and the GP_{340} values increased, indicating that BNO could be localized in the regions where TNS and Laurdan were embedded. It was therefore determined that BNO could be localized at the hydrophobic−hydrophilic interface of the DOTAP liposome. On the other hand, the Laurdan signals were not significantly altered in the presence of EMI. The fluorescence intensity of TNS decreased after addition of EMI, indicating that EMI could be localized at the hydrophilic membrane surface region of the liposomes. From these results, it can be assumed that these two reactants were enclosed at the surface of the DOTAP liposome and the 1,3-dipolar cycloaddition would occur in the hydrophilic region of the membrane (not in the hydrophobic interior region). It is therefore expected that the accumulation of reactants in the DOTAP liposome can promote Diels−Alder reactions and 1,3-dipolar cycloadditions.

The polar environment of the liposome membrane was monitored by analyzing the variations in GP_{340} values before and after BNO distribution (Figure S5). The ΔGP_{340} value of DOTAP was highest among the liposomes used in this study, although the change in the GP_{340} value of DOTAP was not as significant as compared to the phase transition from the liquid-disordered phase to the solid-ordered phase.[29] BNO could be embedded in the hydrophobic−hydrophilic interface region where water molecules bind, and could replace the water molecules, resulting in the dehydration of the membrane

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surface (i.e., an increase in the GP_{340} value). Here, a few percent of water was excluded from the liposome membrane (the relative dielectric constant ε' varied to 25.0 from 26.3; see below). In the presence of BNO, the dehydration degree of DOTAP liposome was most significant (Table S2). Based on the above results, the reactants were distributed in the water-rich environment on the surface of the DOTAP liposome (pseudo-interphase), and the reaction was promoted due to the enclosure of the two reactants.

5. Estimation of the Reaction Rate Constants Occurring at the Liposome Surface. In this study, pseudo-first-order kinetics were applied to calculate the reaction rate constant of BNO and EMI in different media. Since the reaction rate constant of the 1,3-dipolar cycloaddition could depend on the degree of solvation and FMO of the reactants,[19,20,34,42] the relative dielectric constant, ε', is helpful to understand the effects of the reaction media. In a homogeneous solution of 1,4-dioxane/water, a linear relationship between k_{rel} and ε' was approximately obtained, indicating that the solvation of the reactants is critical to promote the reaction. In the case of the micelle solutions, the k_{rel} values were almost the same as those in water. The result shown in Figure 3 reveals that small amounts of BNO (4.5%) and EMI (2.3%) were distributed on the micelle membrane, where a non-promoting effect was observed on the surface of micelle aggregates. In contrast, 2.1-to-2.8-fold higher k_{rel} values were obtained in liposome suspensions. However, the apparent reaction rate constant can be divided into two types of reactions: in bulk water and at the pseudo-interphase of the liposome (Figure 5). In this way, the calculated reaction rate k_{cal} can be written as

$$(\text{reaction rate}) = k[\text{BNO}]_{\text{total}} = k_{\text{W}}[\text{BNO}]_{\text{W}} + k_{\text{L}}[\text{BNO}]_{\text{L}}$$

where k_W is the reaction rate constant for the reaction occurring in the bulk water phase and k_L is the reaction rate for the reaction occurring at the pseudo-interphase of the liposome; $[BNO]_W$ and $[BNO]_L$ are the concentration of BNO in bulk water and at the pseudo-interphase of the liposome, respectively. The calculated values of (k_L/k_W) are shown in Table 1. The reaction rates at the pseudo-interphase of the liposome were much higher than those in water or micellar solutions. The increase in reaction rate in liposome systems might be because of hydrated water at the liposome surface. Because EMI is localized at the surface of the liposome due to its hydrophilicity, the reaction was considered to take place at the surface of the liposome, where hydrated water molecules exist. It has been reported that an increase in the amount of water also increases the reaction rate;[42] therefore, hydrated water molecules at the liposome surface can play an important role in the 1,3-dipolar cycloaddition. On the other hand, the reaction rates decreased in 1,4-dioxane/water systems with increasing amounts of water. This is possibly because of the solvation of BNO in aqueous media; since BNO is a hydrophobic molecule, BNO might not be solvated in the bulk aqueous phase. However, when BNO is at the pseudo-interphase, BNO can be stabilized and solvated by water. Therefore, the solvation of the reactants by water molecules could be critical in forming the activated state and promoting the reaction.

Table 1. Values of the Reaction Rate with Liposomes and Micelle

	ε' [−]	k_{rel} (=k/k_w) [−]	BNO ads. [%]	k_L/k_w [−]
POPC	19.7	2.24	13.4	10.25
DSPC	2.69	2.15	12.4	10.27
DOTAP	26.3	2.75	38.0	5.61
DPTAP	4.52	2.14	16.7	7.83
CTAB	38.5	0.96	4.5	0.11

From Table 1, it can also be seen that the k_L values in DOTAP and DPTAP liposome systems were smaller than those in POPC and DSPC liposomes. This result shows that adsorption of BNO was not directly related to the value of k_L. Rather, it is indicated that the strong electrostatic interaction between BNO and liposomes can inhibit the reaction. A high adsorption of BNO might make BNO more solvated by water molecules, but a high affinity toward the reactants also influences the reaction, possibly because of the restriction imposed on the reactive moiety.

CONCLUSIONS

The environment of the pseudo-interphase of liposomes, as evaluated by Laurdan, was found to be an important factor that can regulate the accumulation of BNO, and its reaction with EMI was improved in the aqueous medium. Liposomes draw both reactants toward the pseudo-interphase and enrich the reactants to promote the reaction. DOTAP liposomes attract more BNO such that more reactants are in a favorable environment for the reaction. The accumulation of BNO at the membrane surface makes DOTAP liposomes unique in regard to the adsorption of the reactants, but strong interactions might not be directly related to the promotion of the reaction. It is assumed that the liposome membrane surface and hydrated water play essential roles in promoting the 1,3-dipolar cycloaddition reaction between BNO and EMI at the pseudo-interphase. It is assumed that hydrated water and "activated" BNO would be localized at the liposome membrane surface. In general, by utilizing the liposome membrane as a designable interface, novel chemical processes can be developed without organic solvents.

ASSOCIATED CONTENT

Supporting Information

Calculation of relative dielectric constants (ε'); Kinetic data of the reaction; Additional data of the reaction rate and absorption behavior. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b03762.

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Notes

The authors declare no competing financial interest.

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