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Switching of Enzymatic Activity through Functional Connection of Molecular Recognition on Lipid Bilayer Membranes

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This article provides an overview of our recent studies on developing functionalized lipid bilayer membranes, on which enzymatic activity was controlled by a molecular switch through connection of molecular recognition. A water-soluble enzyme, such as lactate dehydrogenase, was immobilized noncovalently on the lipid vesicular surface maintaining the catalytic activity. Various types of molecular switches capable of performing as ditopic receptors for an organic signal molecule and a metal ion were prepared and embedded in the lipid membrane. The resulting supramolecular assemblies exhibited the functions of molecular devices triggered by an input signal, which synchronize the enzymatic activity with the recognition of the molecular switch by using a metal ion as a mediator species between the enzyme and the molecular switch. The cationic bilayer membrane formed with the synthetic peptide lipids or the Cerasome-forming lipids is an effective platform for such molecular devices.

Keywords: Molecular device; Lipid bilayer membrane; Enzymatic activity; Artificial receptor; Molecular switch

INTRODUCTION

A lipid bilayer membrane is a powerful and versatile tool in the fields of supramolecular chemistry and medical science. For example, lipid bilayer vesicles formed with phospholipids, the so-called liposomes, have been widely used in drug and gene delivery systems [1–3] and as micro- or nanoscale bioreactors [4]. The discovery of lipid bilayer membranes formed with synthetic molecules [5] has removed one of the limitations of liposome chemistry, and various approaches using lipid membranes, such as structural control of membranes and their domains [6–9], membrane transport [10–12] and catalysis [13–15], have been developed. In order to draw out the best in the lipid membrane characteristics, however, the functional connection of the supramolecular events as observed in biomembrane systems would also be required in artificial systems [16]. A few successful examples of intermolecular communications assembled on lipid bilayer membranes, such as an artificial photosynthetic membrane [17] and an ion-channel-linked biosensing system [18], have been reported.

Recently, our attention has been focused on the functional simulation of a biological signal transduction system by using artificial self-organized assemblies in an aqueous media. From the viewpoint of supramolecular chemistry, the signal transduction appearing on the biomembrane surface [19] can be understood as a naturally occurring supramolecular device, in which the intermolecular communication among the functional biomolecules, such as receptors and enzymes, was efficiently achieved on the lipid bilayer membrane platform. On these grounds we designed an artificial supramolecular device, shown schematically in Fig. 1. The system is basically composed of three supramolecular elements: a bilayer-forming lipid, a molecular switch and an enzyme. The molecular switch was designed so as to tune the enzymatic activity, depending on the molecular recognition of the switch toward an input signal. As the species for tuning, we selected metal ions capable of binding to both the enzyme

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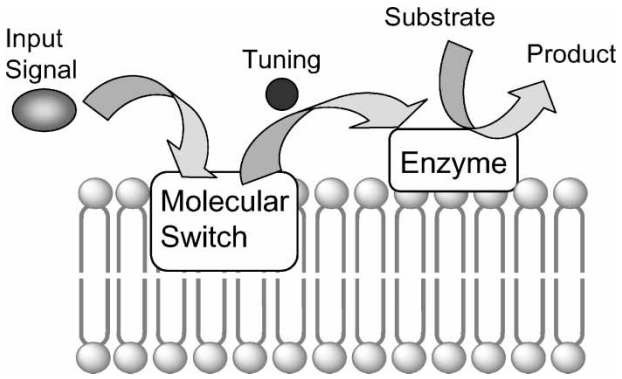

FIGURE 1 Molecular device capable of tuning enzymatic activity through connection of molecular recognition by a molecular switch on a lipid bilayer membrane.

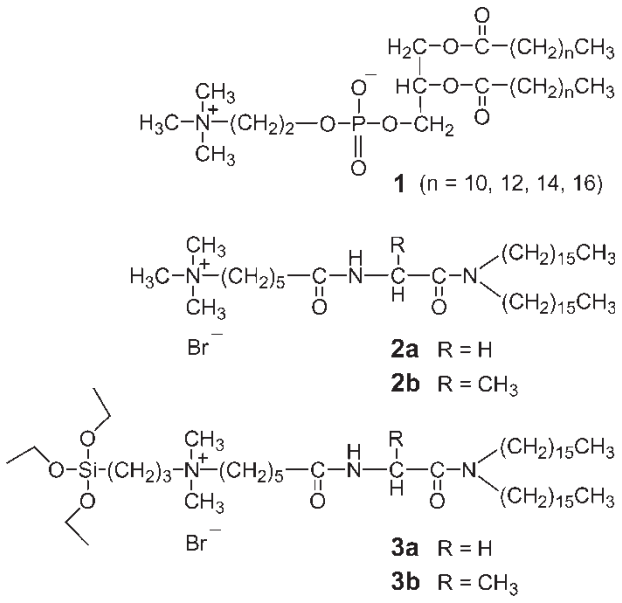

CHART 1

and the molecular switch, and also capable of affecting the catalytic activity upon binding to the enzyme. In addition, it is required that the metal-binding affinity of the molecular switch is changed in the presence and absence of an input signal. In this review article, we describe how to design such supramolecular devices and the consequences for artificial signaling behavior.

LIPID BILAYER MEMBRANES AS PLATFORMS FOR MOLECULAR DEVICES

For the design of molecular devices using lipid bilayer membranes, selection of the bilayer-forming lipid suitable for assembling various supramolecular elements is essential. Phospholipids, such as phosphatidylcholines (**1**; Chart 1), forming liposomal membranes are typical and convenient to use, because the physicochemical properties of the liposomes have been characterized in detail [3]. The liposomes are also available for construction of our supramolecular systems. However, synthetic bilayer vesicles with higher morphological stability are preferable.

The synthetic peptide lipids (**2**) having an amino acid residue inserted between a polar head moiety and a hydrophobic double-chain segment form bilayer membranes with vesicle parameters comparable to the liposomes formed with egg-yolk phosphatidylcholine [20,21]. Formation of a hydrogen-bonded "belt" in the peptide lipid membrane resulted in extremely high morphological stability of the single-walled vesicles. We can fully realize the morphological stability of the peptide lipids as compared with those of the phospholipids and the simple bilayer-forming synthetic lipids such as dialkyldimethylammonium bromide. Owing to the presence of the belt domain, the phase transition from gel to a liquid-crystalline state is mainly governed by the double-chain length of the hydrophobic segment, and not by other moieties in the peptide lipid molecules. Thus, we can readily design the peptide lipids having an appropriate phase transition temperature, 28.0 and 25.5°C for **2a** and **2b**, respectively. It is noteworthy that the phase transition temperature of the bilayer-forming lipids generally depends not only on the double-chain length but also on other parts of the lipid molecules, such as the polar head, and the linker between the head group and hydrophobic chains.

We have recently developed lipid bilayer vesicles having ceramic surfaces (named Cerasomes), as novel organic–inorganic hybrids [22,23]. The Cerasomes were simply obtained upon vortex mixing of an aqueous dispersion of a double-chain lipid bearing a triethoxysilyl head, followed by hydrolysis of the head moiety. On the relatively hydrophobic vesicular surface, condensation among the resulting silanol heads proceeded spontaneously to form a siloxane network, like silica. A drastic increase in the morphological stability of the Cerasomes, as compared with the conventional liposomes and the synthetic lipid vesicles, was confirmed by resistance of the vesicular structure toward micellar-forming single-chain surfactants [24,25]. Such morphological stability allowed the Cerasomes to construct three-dimensional nanoarchitectures of the lipid vesicles on a solid surface as multicellular models [26,27]. In the present work, Cerasome-forming peptide lipids with phase transition temperatures at 25.7 and 23.5°C for **3a** and **3b**, respectively, were used.

ENZYMATIC ACTIVITY ON LIPID BILAYER MEMBRANES

In the light of the components of molecular devices, enzymes are regarded as the amplifiers of chemical signals. We adopted here immobilization of a water-soluble enzyme on the lipid bilayer membrane through noncovalent interactions. For example, NADH-dependent L-lactate dehydrogenase (LDH) effectively binds to the vesicular surface formed with the cationic peptide lipid **2** mainly through electrostatic interactions [28]. The LDH activity was maintained upon formation of the enzyme-lipid hybrid, while an effective molecular assembly could not be constructed with LDH and the cationic hexadecyltrimethylammonium bromide micelle because interaction between the surfactant molecules and LDH caused denaturation of the protein, resulting in the loss of catalytic activity. Detailed analysis, based on Michaelis–Menten-type kinetics for the reduction of pyruvate to L-lactate in the presence and absence of the bilayer vesicles, revealed that LDH is strongly bound to the membrane surface, maintaining the conformation of the active site in aqueous media [29]. Similar behavior was also observed for the enzyme–lipid hybrids by replacing LDH with alcohol dehydrogenase or carbonic anhydrase, or by replacing the peptide lipid with the Cerasome-forming cationic lipid **3**. The present type of protein binding is a very simple but effective method in the successful hybridization of water-soluble proteins with lipid membranes.

Some metal ions act as inhibitors of LDH. We confirmed that Cu(II) and Ag(I) ions were potent inhibitors on a micromolar level for the LDH used here. On the other hand, the inhibitory effects of Zn(II) and Ni(II) ions toward LDH were much smaller than those of the former metal ions at similar concentrations. The inhibition of LDH by Cu(II) ions was reversible and competitive in the presence and absence of the bilayer vesicles, as analyzed by Lineweaver–Burk plots.

MOLECULAR SWITCHES EMBEDDED IN LIPID BILAYER MEMBRANES

The following requirements must be fulfilled for the molecular switch set in the lipid bilayer membrane to exhibit functions of the molecular device shown in Fig. 1: (i) the molecular switch effectively binds in the membrane domain; (ii) the molecular switch acts as a ditopic receptor toward a signal species as an input and a metal ion as a mediator between the enzyme and the molecular switch; (iii) binding affinity of the molecular switch toward a mediator varies as the recognition of an input signal is changed. We adopted the imine bond formation between a primary amine and an activated aldehyde for specific recognition of an input signal by the molecular switch, as the binding process is in equilibrium under physiological conditions. Thus, the hydrophobic molecules shown in Chart 2 were used as the membrane-anchored molecular switches.

The steroid cyclophane **4** performs as a versatile receptor for aromatic guest molecules in the lipid bilayer membranes as well as at the air–water interface and in aqueous solution [30–32]. As for 1-hydroxy-2-naphthaldehyde as an input signal, the steroid cyclophane embedded in the bilayer vesicle of **2a** formed a 1:1 signal–receptor complex with a binding constant of about $10^6\,M^{-1}$ at pH 7 and 30°C [33]. Complexation through the imine bond formation is reversible and greatly enhanced upon

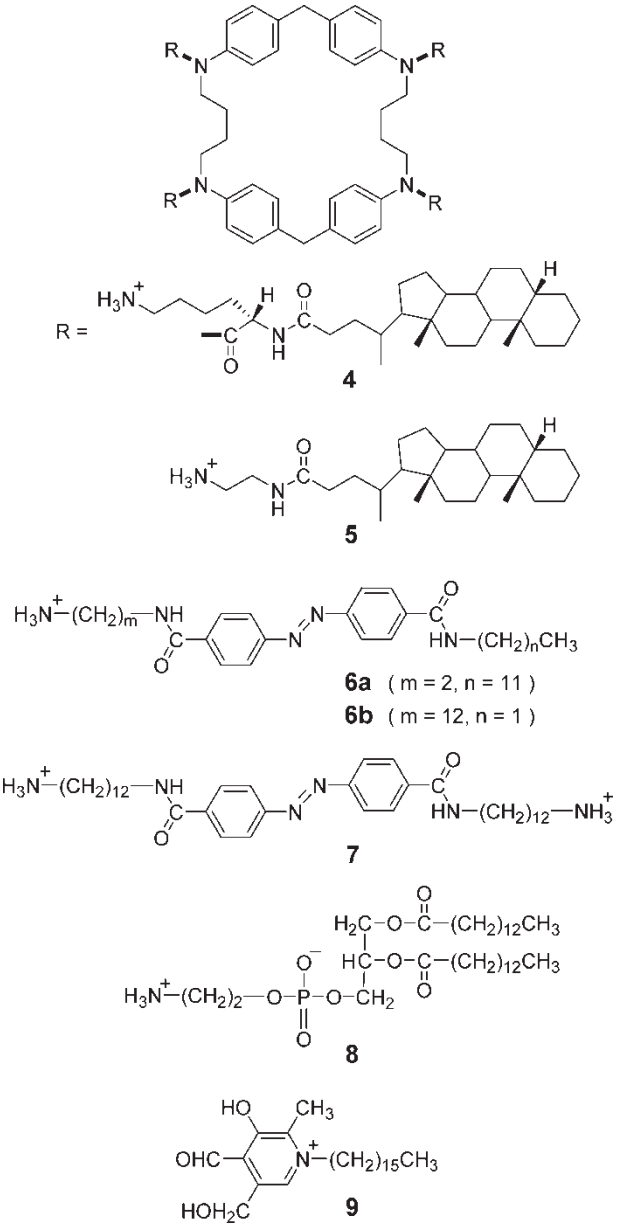

CHART 2

addition of Cu(II) ions to give the corresponding metal complex.

The hydrophobic amine derivatives **5**–**8** also recognize activated aldehydes in the lipid bilayer membrane to form the corresponding imines. The molecular recognition behavior of these molecular switches toward the aldehydes and metal ions is readily explained, as illustrated in Fig. 2 [29]. In the absence of input signals, the molecular switch is homogeneously distributed in the lipid membrane. The binding affinity of an amino group in the head moiety toward transition metal ions is very low. Upon addition of an input signal such as 2-hydroxy-substituted aromatic aldehydes, the imine bond formation between an amino group of the molecular switch and a formyl residue of the signal effectively proceeds on the relatively hydrophobic membrane surface. The resulting signal–switch complex behaves as a strong bidentate ligand for metal ions, such as Cu(II), to form a 2:1 chelate. Thus, the binding affinity of the molecular switch toward metal ions varies drastically as the recognition of an input signal is changed.

As for the hydrophobic molecular switch having an activated aldehyde moiety **9**, various primary amines are used as an input signal, and the metal binding affinity of the molecular switch is drastically changed in the presence and absence of the signal species in a manner analogous to that shown in Fig. 2.

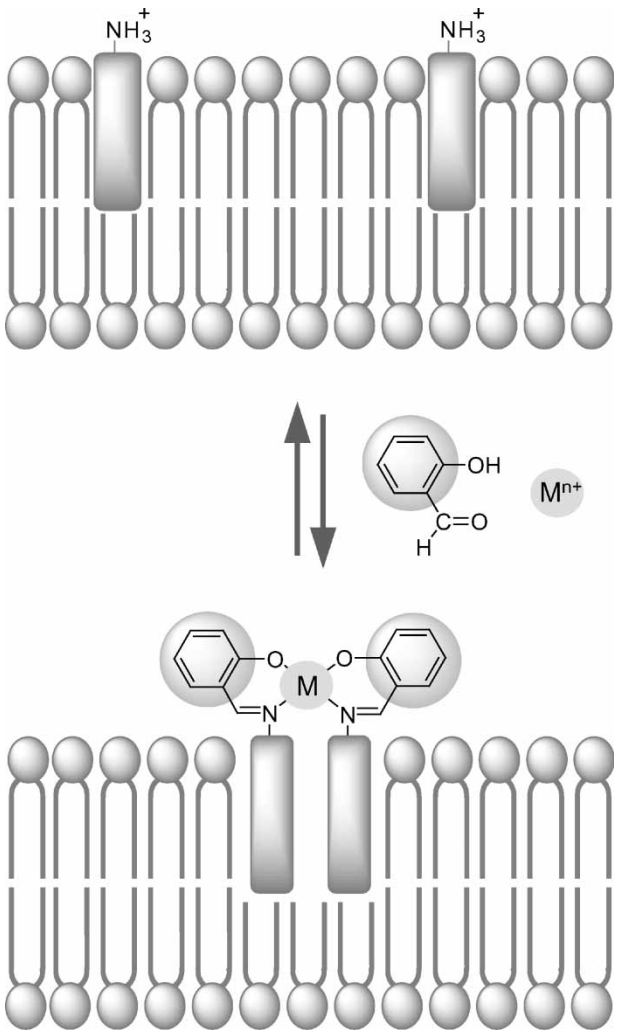

FIGURE 2 On/off behavior of a molecular switch for metal-ion binding triggered by an input signal.

FUNCTIONAL CONNECTION ON LIPID BILAYER MEMBRANES

First, we examined the functional connection between the steroid cyclophane **4** as a molecular switch and LDH as an enzyme on the bilayer vesicle of **2a** by using Cu(II) ion as a mediator [31,33–35]. In the absence of input signals, Cu(II) ions are selectively bound to the active site of LDH and the enzyme is in the inactive state. On addition of 1-hydroxy-2-naphthaldehyde as an input signal, the steroid cyclophane recognizes the signal to form the corresponding imine complex with a large metal binding affinity. Thus, Cu(II) ions bound to LDH are moved to the signal–switch complex and the enzyme turns to be in the active state. The Cu(II) ion is the specific and effective mediator species in the present system, while the catalytic activity of LDH was not switched in the presence of Zn(II) or Ni(II) ions under similar conditions. The switching behavior of the LDH activity is controlled depending on the molecular recognition ability of the steroid cyclophane toward the input signals and stability of the resulting Cu(II) complex. For example, the tendency of the activation of LDH triggered by various input signals is in the following order: 2-hydroxy-1-naphthaldehyde > 1-hydroxy-2-naphthaldehyde > salicylaldehyde > 2-naphthaldehyde. Accordingly, the molecular device depicted in Fig. 1 first became a reality by using the supramolecular assembly composed of the steroid cyclophane **4**, LDH, Cu(II) and the peptide lipid **2a**.

We would like to refer to the correlation of the present molecular device with a biological signal transduction system concerning the G-protein-linked receptor. On the cell membrane surface, the recognition of an external signal by a transmembrane receptor is connected to the catalytic reaction by an enzyme through a G-protein as a signal transmitter between the receptor and the enzyme. Such intermolecular communication is a common mechanism in biological signaling. Thus, our supramolecular system can be regarded as a biomimetic molecular device by replacing the G-protein with a simple metal ion.

Next, we designed another molecular device by using a steroidal receptor **5** with a molecular

structure simpler than the steroid cyclophane. The switching behavior of the enzymatic activity, triggered by recognition of an input signal, was observed in the supramolecular assembly composed of the steroidal receptor **5**, LDH, Cu(II) and the peptide lipid **2a** as a molecular switch, an enzyme, a mediator and a matrix lipid, respectively [29,36]. 1-Hydroxy-2-naphthaldehyde was an effective input signal in the present system. Molecular recognition by the steroidal receptor toward the input signal and the mediator proceeds according to the mechanism as mentioned above (Fig. 2). When the steroidal receptor was replaced by an amine derivative lacking the steroid moiety, or *N*-acetylethylenediamine, the LDH activity was little affected by the input signal under similar conditions. The result indicates that anchoring of the molecular switch into the lipid membrane is essential for the construction of the present type of molecular device. In addition, marked selectivity in input signals was also observed. In order to enhance the response speed of the molecular device, pyridoxal 5′-phosphate with a highly reactive formyl group seems to be the best input signal. As a membrane matrix, the Cerasome prepared from lipid **3** is better than the bilayer vesicle formed with the peptide lipid **2**, as the phase separation behavior of the molecular switch in the matrix membrane would be more enhanced in the former than in the latter [25]. Thus, all-or-none switching was obtained by using pyridoxal 5′-phosphate as an input signal in the supramolecular system composed of the molecular switch **5**, LDH, Cu(II) and the Cerasome-forming lipid **3**, as shown schematically in Fig. 3. As morphologically stable Cerasomes have an advantage in the construction of multicellular models as mentioned above, the present signaling system could be extended to artificial intercellular communication systems.

In order to develop a molecular device capable of reversible and multiple switching of enzymatic activity, we designed artificial receptors responsive to both chemical and photonic signals (Fig. 4) [37,38]. The receptor **6a** comprises an amino group as a chemosignal recognition site, an azobenzene moiety as a photoresponsive moiety, a short connector unit, and a long alkyl chain as a hydrophobic anchor in the membrane. The molecular length of the receptor **6b** is similar to that of **6a** but the former has a longer connector unit between the two kinds of signal recognition parts. The lipid **7** acts as an artificial transmembrane receptor, recognizing chemical signals on both membrane surfaces. The supramolecular assemblies were formed with combinations of the receptor, LDH, Cu(II) and the phospholipid **1** as a molecular switch, an enzyme, a mediator and a membrane matrix lipid, respectively. For 1-hydroxy-2-naphthaldehyde as an input signal, the present systems show signaling behavior

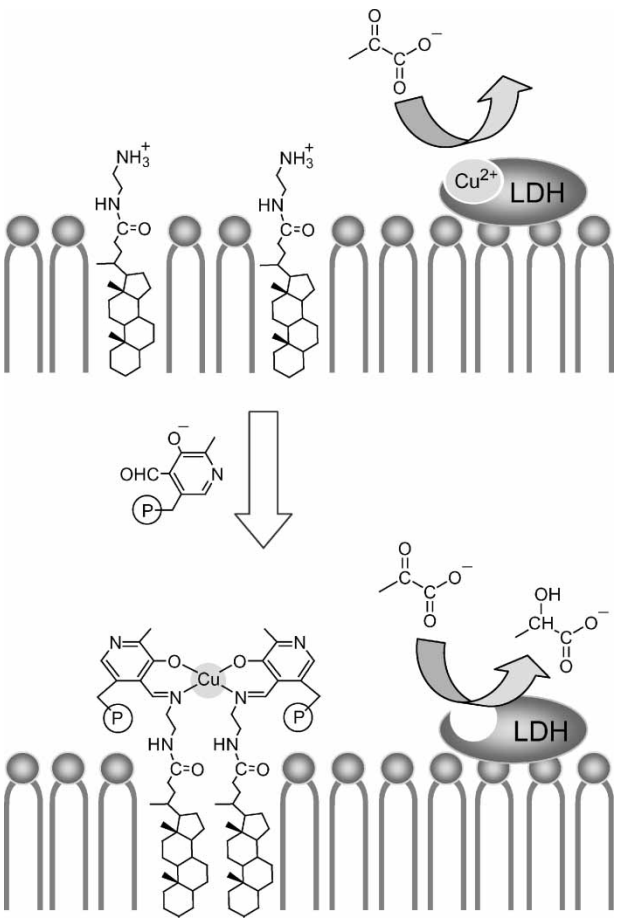

FIGURE 3 Switching of LDH activity triggered by molecular recognition of the molecular switch **5** toward pyridoxal 5′-phosphate as an input signal.

analogous to the hybrid system containing the steroidal receptor **5**. UV irradiation of the azobenzene chromophore of **6a** embedded in the bilayer membrane resulted in photoisomerization of the azobenzene moiety from the *trans* to the *cis* form, and the reverse reaction proceeded upon visible light irradiation. In the lipid bilayer membrane, stability of the Cu(II) chelate of the *cis*-signal–switch complex

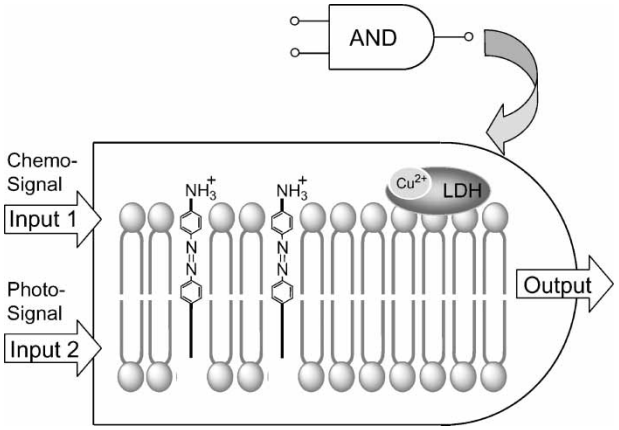

FIGURE 4 AND logic gate constituted with LDH and a molecular switch **6a** immobilized on a lipid bilayer membrane.

is much lower than that of the corresponding *trans*-isomer complex. Thus effective signal transduction behavior was observed only for the *trans*-receptor in the presence of the input chemosignal, but not for other combinations of chemo- and photo-signals. Such behavior can be rationalized by the AND-type logic gate. As the photoisomerization of the azobenzene moiety is a reversible process, we can repeat the switching of the molecular device. It is noteworthy that functions such as the AND logic gate were obtained by using **6a**, with a short connector unit, but not **6b**, which has a longer connector unit between the two kinds of signal recognition parts. This observation reveals that synchronization of responses in the chemosignal recognition site and the photoresponsive part is important in the following design of the molecular switch for constructing the present logic gate.

As one of the common key steps in cell signaling, it is well known that an inositol-containing lipid, or phosphatidylinositol 4,5-bisphosphate, embedded in the cell membrane is converted by the reaction with phospholipase C to inositol 1,4,5-triphosphate and diacylglycerol, which activate the intracellular Ca(II) release and protein kinase C, respectively [19]. Comparing this biological system with the artificial molecular device depicted in Fig. 1, the phospholipid and phospholipase C are regarded as a molecular switch and an input signal, respectively. On these grounds, we designed a bioinspired molecular device by using a phospholipid **8** and a coenzyme, pyridoxal 5′-phosphate, as a molecular switch and an input signal (as shown in Fig. 1), respectively [39,40]. Thus the hybrid vesicle formed with the peptide lipid **2** as a matrix lipid, containing the phospholipid **8**, LDH and Cu(II), effectively performed as the molecular device, switching the enzymatic activity by pyridoxal 5′-phosphate as an input signal. When the phospholipid **8** was replaced by ethanolamine phosphate and ethanolamine, which were metabolic products of **8** catalyzed by phospholipase C and D, respectively, significant switching of the LDH activity was not observed, reflecting poor binding affinities of these hydrophilic amines toward pyridoxal 5′-phosphate. The switching behavior was also tunable by phase transition from gel to the liquid-crystalline state of the bilayer membrane.

The receptor **9** has a highly activated formyl group as a signal recognition site and a long alkyl chain as a hydrophobic anchor in the membrane. The supramolecular assembly was composed of **9**, LDH, Cu(II) and **2** as a molecular switch, an enzyme, a mediator and a membrane-forming lipid, respectively. Among tryptamine, serotonin, taurine, dopamine and histamine, the molecular switch specifically recognized tryptamine by forming an imine bond between the amino group of the signal and the formyl group of the switch. The resulting signal–switch complex strongly bound Cu(II) ions in a 2:1 stoichiometry, in a manner analogous to the steroidal receptor system as mentioned above. Thus the LDH activity inhibited by Cu(II) ions was recovered by a harmonic coupling of the signal recognition behavior of the molecular switch with the enzymatic reaction [41].

CONCLUSIONS

In this article we described our recent approaches to developing molecular devices capable of tuning enzymatic activity through the connection of molecular recognition among multiple supramolecules. Our strategy to construct such molecular devices is based on using the lipid bilayer membrane as a platform for assembling the supramolecular elements, which is inspired by biological signal transduction systems appearing on cell surfaces. The supramolecular systems shown here are capable of performing intermolecular communication between a naturally occurring enzyme and an artificial molecular switch by using a chemical and/or photonic signal. The roles of the lipid bilayer membrane as a platform for these supramolecular systems are not only fixation of the enzyme and the switch within an entropically favorable distance through noncovalent interactions, but also the control of molecular recognition behavior among various chemical species, playing a role on the membrane surface, such as a molecular switch, an enzyme, an input signal species, a mediator, and substrates and products of the enzyme. The present multicomponent system seems to be complex at first sight, but in fact the system is constructed by a combination of simple and clear molecular recognition events. We are now trying to extend the present system toward highly organized supramolecular architectures.

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