Intermolecular Communication on Lipid Bilayer Membrane. Control of Enzymatic Activity Triggered by a Lipid Signal

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(Received November 18, 2003; CL-031116)

Functionalized lipid membrane, on which enzymatic activity was switched by a lipid signal, was constructed by self-assembling of the following components in aqueous media; a cationic peptide lipid as a bilayer-forming matrix, dimyristoylphosphatidylethanolamine as a lipid signal, lactate dehydrogenase as a membrane-binding effector, and Cu^{2+} ion as a mediator between the signal and the effector. Upon addition of pyridoxal 5′-phosphate as a signal activator, specific binding of the activator to the lipid signal induced activation of the enzyme.

Construction of integrated molecular systems being capable of communicating among mutual components is of much importance for design of molecular devices and machines.[1] As the key building blocks for the molecular architectures, biomolecules such as nucleotides[2] and peptides[3] and synthetic molecules such as macrocyclic compounds[4] and molecular assemblies[5] have been widely employed up to the present time. In biological signaling, however, we can realize that various types of organic chemical species also act as signals for information processing.[6] On these grounds, our recent attention has been focused on development of integrated molecular systems controlled by chemical signals on the lipid bilayer membranes in aqueous media.[7–9] The advantage to use the lipid membrane as a scaffold for integration of the functional molecules is that these molecules can be readily self-organized on the membrane surface to perform specific molecular recognition through multiple non-covalent interactions. In this article, we report on an artificial intermolecular communication system (Figure 1), which is inspired by the biological signal transduction triggered by a lipid signal on the biomembrane.

It is well known that an inositol-containing lipid, or phosphatidylinositol 4,5-bisphosphate (PIP_2), in the cell membrane is converted by the reaction with phospholipase C (PLC) to inositol 1,4,5-triphosphate and diacylglycerol, which activate the intracellular Ca^{2+} release and protein kinase C, respectively.[6,10] This process is one of the common key steps in the cell signaling, and PIP_2 and PLC are regarded as a lipid signal and the signal activator for the effectors, respectively.

In our artificial signaling system, a cationic bilayer membrane formed with a peptide lipid having an L-alanine residue[11] (**1**) was employed as the morphologically stable scaffold. NADH-dependent lactate dehydrogenase (LDH) from pig heart (Boehringer Manheim, Germany) was selected as the effector to evaluate the signal transduction efficiency of the present system, since we have clarified that this enzyme is quantitatively bound to the membrane surface keeping its catalytic activity.[8,9] The LDH activity in the reduction of pyruvate to L-lactate was monitored spectrophotometrically by following a consumption rate (v_0) of NADH in the presence of Cu^{2+} ions as the competitive inhibitor of LDH. Dimyristoylphosphatidylethanolamine (**2a**) and a coenzyme pyridoxal 5′-phosphate (**3a**) were chosen as the lipid signal and the signal activator.

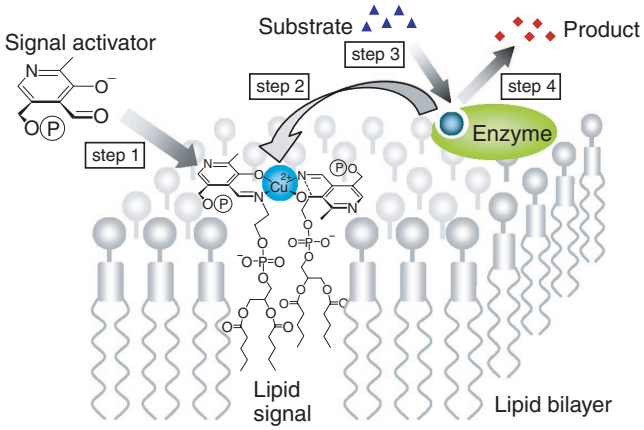
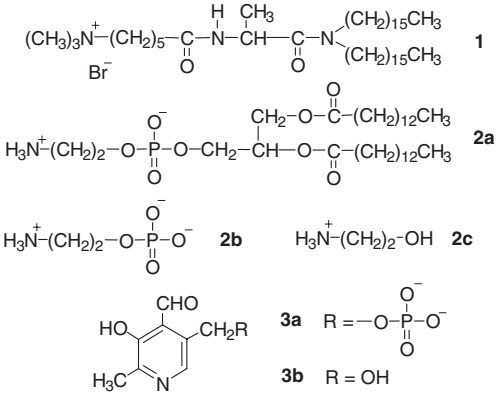


Figure 1. Schematic illustration of functionalized lipid bilayer membrane to control the enzymatic activity by a lipid signal.



Thus, the following signaling behavior would be observed. In the absence of the signal activator, the enzyme is in inactive state, since the binding affinity of Cu^{2+} ions to LDH ($K = 4.5 \times 10^6$ dm^3 mol^{-1})[9] is presumably much higher than that to the polar head of the lipid signal. Upon addition of the signal activator, however, favorable complex formation between the signal and the signal activator proceeds on the hydrophobic membrane surface to provide a binding site for Cu^{2+} ions more suitable than the enzyme (step 1 in Figure 1). Then, Cu^{2+} ion shifts from the enzyme active site to the activated lipid signal (step 2 in Figure 1) and pyruvate is capable of incorporating into the en-

zyme active site (step 3 in Figure 1). As a result, the enzyme turns to be in active state and catalytically amplifies the chemical signal as the reaction product (step 4 in Figure 1). Such crosstalk among molecules was systematically evaluated in the presence and absence of each functional molecule.

The bilayer vesicles with hydrodynamic diameter of 110 nm were prepared by sonication of an aqueous dispersion of **1** with a cup type sonicator at 60 W for 5 min in aqueous 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonate (HEPES) buffer (10 mmol dm^{-3}, pH 7.0) at 40 °C, in the presence and absence of 3 mol % of **2a**. The formation of single-walled bilayer vesicles was confirmed by negative-staining electron microscopy. The LDH activities on the vesicular surface upon addition of the signal species and/or the signal activator in the presence and absence of Cu^{2+} ions were listed in Table 1. In the absence of the signal and the signal activator, the enzymatic activity was suppressed to 8% by binding of Cu^{2+} ions (Entry 1 in Table 1). In the presence of **2a**, the activity was slightly increased, presumably due to weak metal binding ability of the polar head moiety of the lipid signal (Entry 2 in Table 1). When **2a** was replaced by ethanolamine phosphate (**2b**) and ethanolamine (**2c**), which were metabolic products of **2a** catalyzed by phospholipase C and D, respectively, analogous enzymatic activity was observed (Entries 3 and 4 in Table 1). Upon addition of the signal activator (**3a**, **3b**) to the bilayer vesicle lacking the signal, the enzymatic activity did not show significant change (Entries 5 and 6 in Table 1).

In the presence of both **2a** and **3a**, however, the activity recovered to 83% suggesting that the complex of the lipid signal and the signal activator withdrew Cu^{2+} ion from the enzyme active site (Entry 7 in Table 1). We evaluated on the complex formation by means of electronic absorption spectroscopy, since **3a** has unique and sensitive spectrophotometric behavior depending on interaction with amine derivatives.[12] The signal activator **3a** reversibly interacts with the polar head of **2a** to form an imine bond between formyl group of the former and amino moiety of the latter species. The binding behavior was evaluated from the absorption spectral change for the complex formation at 390 nm to give a relatively large binding constant; $K = 3.4 \times 10^5$ dm^3 mol^{-1}. On the other hand, the corresponding K values for **2b** and **2c** with **3a** are 3.7×10^3 and less than 10^2 dm^3 mol^{-1}, respectively, since these signals are hydrophilic and can not effectively form the activated signal species on the membrane surface. In addition, the activated signal formed with **2a** and **3a** has extremely high affinity to Cu^{2+} ions to quantitatively form a 2:1 complex having an absorption maximum at 377 nm under the present conditions, as judging from the Job's plot analysis. As a result, signal specificity in the binding with the signal activator reflected on the signal output, or the enzymatic activity, in the present multi-molecular system (Entries 7–9 in Table 1). The signal transduction efficiency was also tuned by replacement of the signal activator from **3a** to **3b** having smaller binding constant for **2a**; $K = 3.7 \times 10^4$ dm^3 mol^{-1} (Entry 10 in Table 1).

Table 1. LDH activities in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 40.0 °C[a]

Entry	Species / μmol dm^{-3}					v_0[b] /10^{-7} mol dm^{-3} s^{-1}	Activity[c] /%
	[2a]	[2b]	[2c]	[3a]	[3b]		
1	0	0	0	0	0	0.25 (3.15)	8
2	50	0	0	0	0	0.44 (3.17)	14
3	0	50	0	0	0	0.53 (3.11)	17
4	0	0	50	0	0	0.43 (3.09)	14
5	0	0	0	20	0	0.28 (2.83)	10
6	0	0	0	0	20	0.41 (3.13)	13
7	50	0	0	20	0	2.59 (3.12)	83
8	0	50	0	20	0	0.81 (3.12)	26
9	0	0	50	20	0	0.48 (3.18)	15
10	50	0	0	0	20	1.54 (3.20)	48

[a]Concentrations in mmol dm^{-3}: **1**, 1.5; β-NADH, 0.25; pyruvate, 0.5; LDH, 1.4×10^{-6}. [b]Initial velocity for LDH catalyzed reduction of pyruvate to L-lactate was monitored spectrophotometrically by following a consumption rate of NADH as a coenzyme in the presence of Cu^{2+} ions (4 μmol dm^{-3}). The value in the absence of Cu^{2+} ions is in parenthesis. [c]A magnitude of v_0 in the presence of Cu^{2+} ions relative to that of corresponding metal-free system.

In conclusion, we demonstrated here that the control of enzymatic activity on the synthetic bilayer membrane was triggered by specific binding of a signal activator with a lipid signal. The lipid bilayer membrane provides an interface preferable to molecular recognition through multiple non-covalent interactions and connection of its response. To the best of our knowledge, the present system is the first example of artificial intermolecular communication between a lipid as an input signal and an enzyme as an amplifier for the signal output, inspired by the biological phosphatidylinositol signaling on the cell membrane.

This work was financially supported by the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (A), 15205022, 2003.

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Published on the web (Advance View) January 26, 2004; DOI 10.1246/cl.2004.226