Intermolecular Communication on Lipid Bilayer Membrane. Tuning of Enzymatic Activity with Phase Transition of the Matrix Membranes

Wen-Jie Tian,[†] Yoshihiro Sasaki, Sheng-Di Fan,[1] and Jun-ichi Kikuchi*

Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192

[1]Department of Biological Engineering, Dalian Nationalities University, Economical and Technological Development Zone, Dalian 116600, P. R. China

Received October 6, 2004; E-mail: jkikuchi@ms.naist.jp

A molecular device, in which the catalytic activity of lactate dehydrogenase was controlled by a response of ditopic molecular recognition of a lipid signal, was constructed on lipid bilayer membranes. The enzymatic activity was tuned up sensitively through phase transition of the matrix membrane as a platform for the intermolecular communication.

Development of molecular devices is one of the fascinating research targets in supramolecular chemistry.[1,2] As a candidate to be a platform for the molecular devices, lipid bilayer membrane has been widely employed,[3–5] since it is the basic and common structure of the biomembranes on which various supramolecular events, such as materials conversion, transport, and information processing, can be systematically arranged. We have recently developed functionalized lipid bilayer membranes as the molecular devices that are capable of controlling an enzymatic activity by a molecular switch through connection of molecular recognition.[6] In our preliminary communication,[7] we reported that catalytic activity of lactate dehydrogenase (LDH) was switched by specific binding of a signal activator with a lipid signal. The 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.[8]

In this article, we report that the enzymatic activity is tuned up through gel to liquid-crystalline phase transition of the lipid bilayer matrix in aqueous media. The supramolecular system is constituted with a combination of NADH-dependent LDH from pig heart (Boehringer Manheim) as a membrane-binding effector, dimyristoylphosphatidylethanolamine (DMPE) as a lipid signal, Cu^{2+} ion as a mediator between them, and bilayer-forming matrix lipids (Fig. 1). In order to evaluate the phase transition effect on switching of the enzymatic activity, we employed multi-walled mixed bilayer vesicles formed with a cationic peptide lipid (**1**)[9] and a phosphatidylcholine having a different chain length (**2a**, **2b**, or **2c**) as the matrix membranes (Chart 1). The phase transitions from gel to liquid-crystalline state for the bilayer membranes of **1**–**2a**, **1**–**2b**, and **1**–**2c**, in a 1:9 molar ratio, appeared as a single peak at 22.4, 39.5, and 51.5 °C, respectively, as evaluated by differential scanning calorimetry (DSC; VP-DSC, MicroCal, Ltd). Upon addition of 5 mol% of DMPE to the matrix membranes, the phase transition temperatures (T_m) were not so influenced; 24.0, 39.7, and 51.3 °C for **1**–**2a**, **1**–**2b**, and **1**–**2c** system, respectively. We have also confirmed that LDH, bound on a cationic vesicular surface mainly through electrostatic interactions, had no significant effect on the phase transition.[10]

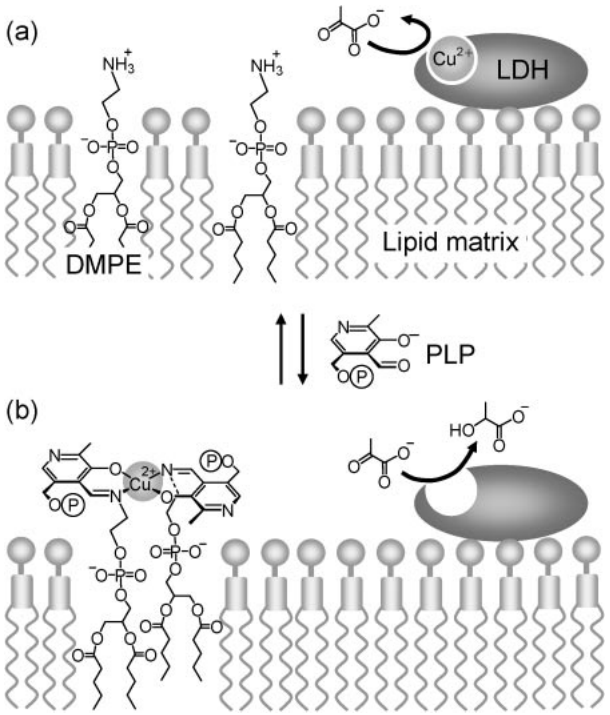
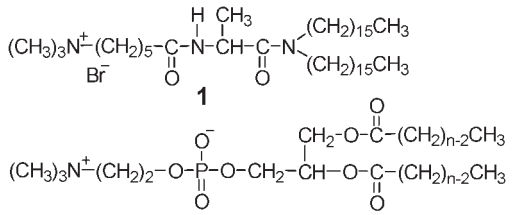


Fig. 1. Schematic illustration for the switching of LDH activity triggered by a lipid signal: a, off-state; b, on-state.



2a (n=14), **2b** (n=16), **2c** (n=18)

Chart 1.

The switching behavior of LDH activity by the lipid signal was evaluated at 35.0 °C in the presence and absence of pyridoxal 5′-phosphate (PLP) as a specific signal activator. The LDH activity in the reduction of pyruvate to L-lactate was monitored spectrophotometrically by following the consumption rate (v_0) of NADH and is represented as the magnitude

† Present address: Department of Biological Engineering, Dalian Nationalities University, Economical and Technological Development Zone, Dalian 116600, P. R. China

Published on the web April 8, 2005; DOI 10.1246/bcsj.78.715

Table 1. LDH Activities on the Lipid Membrane Matrix Containing DMPE as a Lipid Signal in the Presence and Absence of PLP as a Signal Activator[a)]

Entry	Lipid matrix	Lipid signal	Signal activator	v_0[b)]/10^{-7} mol dm^{-3} s^{-1}	Activity[c)] /%
1	**1**–**2a**	DMPE	none	0.24 (2.85)	8
2	**1**–**2a**	DMPE	PLP	1.19 (2.98)	40
3	**1**–**2b**	DMPE	none	0.26 (2.58)	10
4	**1**–**2b**	DMPE	PLP	1.78 (2.63)	68
5	**1**–**2c**	DMPE	none	0.28 (2.55)	11
6	**1**–**2c**	DMPE	PLP	1.84 (2.59)	71

a) In an aqueous HEPES buffer (10 mmol dm^{-3}) at pH 7.0 and 35.0 °C. Concentrations in mmol dm^{-3}: **1**, 0.1; **2**, 0.9; β-NADH, 0.25; pyruvate, 0.5; LDH, 1.4×10^{-6}. b) Initial velocity for LDH catalyzed reduction of pyruvate to L-lactate in the presence of Cu^{2+} ions (4 μmol dm^{-3}). The value in the absence of metal 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.

of v_0 in the presence of Cu^{2+} ions relative to that of the corresponding metal-free system (Table 1). In the absence of a signal activator, the enzymatic activity of the **1**–**2a** system in the liquid crystalline state was suppressed to 8% by binding of Cu^{2+} ions to the enzyme (Entry 1 in Table 1). Upon addition of the signal activator, the activity increased to 40% (Entry 2 in Table 1), since Cu^{2+} ions bound to the enzyme active sites partly shifted to the complex of the lipid signal with the signal activator to form a 2:1 metal chelate complex, as shown in Fig. 1b. Such ditopic molecular recognition behavior of the lipid signal toward the signal activator and the mediator was confirmed spectrophotometrically by monitoring interactions of PLP with DMPE and Cu^{2+} ions.[7]

When we replace the membrane matrix from **1**–**2a** to **1**–**2b**, the LDH activity was switched much more effectively from 10% to 68% at 35.0 °C by an input of the signal activator (Entries 3 and 4 in Table 1). A similar extent of the switching, from 11% to 71%, was observed for the **1**–**2c** system in the gel state (Entries 5 and 6 in Table 1). The results strongly suggest that the switching of the LDH activity is more sensitive in the gel state of membrane matrix rather than in the liquid-crystalline state. Under the similar conditions in the absence of LDH, the spectrophotometric study showed that the concentrations of the 2:1 metal chelate complexes were 2.9 and 4.0 μmol dm^{-3} for the **1**–**2a** system in the liquid-crystalline state and the **1**–**2c** system in the gel state, respectively, at 35.0 °C. Accordingly, the difference in the stability of the 2:1 metal chelate complex above and below the phase transition temperature of the lipid matrix would govern the LDH activity, since the enzymatic activity in the present supramolecular system is indirectly controlled by the Cu^{2+} affinity for the activated lipid signal.

In order to clarify the phase transition effect on tuning of enzymatic activity, we examined the temperature dependence of the v_0 value for LDH bound on the **1**–**2b** membrane surface in the presence and absence of Cu^{2+} ions. In a metal-free system, LDH on the matrix membrane exhibited bell-shaped temperature dependence with maximum activity around 40 °C as in an aqueous solution. The LDH activities in the presence of Cu^{2+} ions relative to those of corresponding metal-free system at various temperatures are shown in Fig. 2, with the DSC thermogram of the matrix.

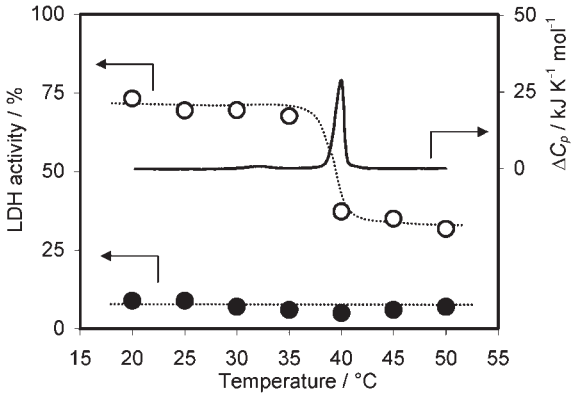


Fig. 2. Temperature dependences of the LDH activities on the **1**–**2b** matrix containing DMPE as a lipid signal in the presence (○) and absence (●) of PLP as a signal activator, and DSC thermogram for the **1**–**2b** matrix containing DMPE: Concentrations in mmol dm^{-3}: **1**, 0.1; **2b**, 0.9; DMPE, 0.05; Cu^{2+}, 0.004; PLP, 0.02; β-NADH, 0.25; pyruvate, 0.5; LDH, 1.4×10^{-6}; in an aqueous HEPES buffer (10 mmol dm^{-3}) at pH 7.0.

In conclusion, we have demonstrated that the enzymatic activity on the surface of lipid bilayer membranes is capable of switching through intermolecular communication between the enzyme and a lipid signal, and is additionally capable of tuning up by phase transition of the lipid matrix. We believe the results provide a useful guidepost for designing artificial molecular devices inspired by biological supramolecular devices in the cells.

This work was financially supported by the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (A), 15205022, 2004.

References

1 J.-M. Lehn, "Supramolecular Chemistry," VCH, Weinheim (1995), Chap. 8.

2 V. Balzani, M. Venturi, and A. Credi, "Molecular Devices and Machines," Wiley-VCH, Weinheim (2003).

3 D. Gust, T. A. Moore, and A. L. Moore, *Acc. Chem. Res.*, **34**, 40 (2001).

4 J. Kikuchi, K. Ariga, and Y. Sasaki, "Advances in Supramolecular Chemistry," ed by G. W. Gokel, Cerberus Press, Inc., South Miami (2001), Vol. 8, p. 131.

5 J.-H. Fuhrhop and T. Wang, *Chem. Rev.*, **104**, 2901 (2004).

6 a) W.-J. Tian, Y. Sasaki, S.-D. Fan, and J. Kikuchi, *Supramol. Chem.*, **17**, 113 (2005). b) W.-J. Tian, Y. Sasaki, A. Ikeda, J. Kikuchi, X.-M. Song, and S.-D. Fan, *Acta Chim. Sin.*, **62**, 1230 (2004). c) Y. Sasaki, M. Yamada, T. Terashima, J.-F. Wang, M. Hashizume, S.-D. Fan, and J. Kikuchi, *Kobunshi Ronbunshu*, **61**, 541 (2004). d) J. Kikuchi, K. Ariga, Y. Sasaki, and K. Ikeda, *J. Mol. Catal. B: Enzym.*, **11**, 977 (2001). e) K. Fukuda, Y. Sasaki, K. Ariga, and J. Kikuchi, *J. Mol. Catal. B: Enzym.*, **11**, 971 (2001). f) J. Kikuchi, K. Ariga, and K. Ikeda, *Chem. Commun.*, **1999**, 547. g) J. Kikuchi, K. Ariga, T. Miyazaki, and K. Ikeda, *Chem. Lett.*, **1999**, 253.

7 W.-J. Tian, Y. Sasaki, A. Ikeda, J. Kikuchi, and S.-D. Fan, *Chem. Lett.*, **33**, 226 (2004).

8 B. Payrastere, K. Missy, S. Giuriato, S. Bodin, M. Plantavid, and M. Gratacap, *Cell Signalling*, **13**, 377 (2001).

9 Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitomi, and Y. Matsuda, *J. Am. Chem. Soc.*, **106**, 3613 (1984).

10 J. Kikuchi, Y. Kamijyo, H. Etoh, and Y. Murakami, *Chem. Lett.*, **1996**, 427.