An Artificial Signal Transduction System. Control of Lactate Dehydrogenase Activity Performed by an Artificial Cell-surface Receptor

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A supramolecular bilayer assembly as an artificial signaling system was constituted in combination with a steroid cyclophane as an artificial receptor, an NADH-dependent lactate dehydrogenase as an effector, and a bilayer-forming peptide lipid. Signal transduction from the receptor to the effector was observed for the present hybrid system by employing two kinds of signaling species, 1-hydroxy-2-naphthaldehyde as an external signal and a Cu(II) ion as a signal transmitter.

Signal transduction based on molecular recognition by artificial receptors is of great interest in the field of supramolecular chemistry.[1] While functional simulation of intracellular receptors in homogeneous solutions has been actively carried out,[2] signal transduction by artificial cell-surface receptors embedded in molecular assemblies has been scarcely studied up to the present time, except for channel-linked receptors.[3] We have recently developed various artificial receptors being capable of recognizing organic signaling ligands in bilayer membranes.[4] A steroid cyclophane (**1**) constituted in combination with three functional components, a cyclophane ring, four bile acid moieties, and four L-lysine residues connecting them, is a receptor prepared along this line.[5] Recent progress in biochemical understanding of G-protein-mediated cell signaling[6] stimulated us to develop a novel supramolecular system by employing the steroid cyclophane as an artificial cell-surface receptor, keeping attention to signal transduction mechanism through G-protein-linked receptor.

Since we can basically understand that G-protein is a signal transmitter operating between a receptor and an enzyme as a specific effector, G-protein could be replaced in an artificial system by a simple signal transmitter which is capable of affecting both receptor and enzyme functions. In addition, the following requirements must be fulfilled by artificial receptors. (1) The receptor is capable of recognizing both an external signal ligand and a signal transmitter species. (2) Binding affinity of the receptor toward a signal transmitter varies as the recognition of a signal ligand is changed. On the other hand, an NADH-dependent lactate dehydrogenase (LDH) catalyzes transformation between pyruvate and lactate and its activity is inhibited by metal ions.[7] On these grounds, we constituted an artificial signaling system in combination with the following supramolecular elements: steroid cyclophane (**1**), cationic peptide lipid (**2**), pig heart LDH (Boehringer Manheim, Germany), 1-hydroxy-2-naphthaldehyde (**3**), and Cu(II) ion as an artificial cell-surface receptor, a bilayer-forming lipid, an effector, an external signal, and a signal transmitter, respectiviey, as schematically shown in Figure 1.

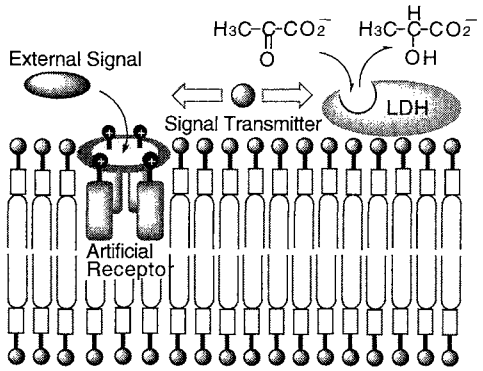

Figure 1. Schematic representation of a signal transduction system mediated by an artificial cell-surface receptor.

Signal transduction behavior was examined in an aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (100 mmol dm^{-3}, pH 7.0) at 30.0 °C. Bilayer vesicles were prepared by sonication of an aqueous dispersion containing **1** and **2** in a 1:40 molar ratio with a bath-type sonicator at 80 W for 60 min. The vesicular size as evaluated by means of dynamic light scattering measurements is 120 nm, and the formation of bilayer vesicles was confirmed by negative-staining electron microscopy. The present vesicles showed a phase transition from gel to liquid crystalline state at 22 °C in differential scanning calorimetry measerements. Upon addition of LDH to the vesicular solution, the enzyme is bound to the cationic membrane surface mainly through electrostatic interactions.[8] The catalytic activity of LDH in the reduction of pyruvate to L-lactate was evaluated spectrophotometrically by following a consumption rate (v_0) of NADH in the presence and absence of the supramolecular elements and the signaling species (Table 1).

First, we examined effects of each component on LDH activity. Inactivation of LDH by Cu(II) ions was highly specific (Table 1, entry 1), since the catalytic activity was not influenced in the presence of Zn(II) or Ni(II) ions under the similar concentration. The inhibition of LDH by Cu(II) ions was reversible and competitive as analyzed by the Lineweaver-

Table 1. LDH activities in an aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0 °C

Entry	Species / μmol dm^{-3}				v_0[b]	Activity[c]
	[1]	[2]	[3]	[Cu^{II}]	μmol dm^{-3} s^{-1}	%
1	0	0	0	8	0.69 (1.20)	58
2	0	0	30	8	0.77 (1.15)	67
3	0	1200	0	8	0.59 (1.45)	41
4	0	1200	30	8	0.25 (1.28)	20
5	30	1200	0	8	0.74 (1.40)	53
6	30	1200	30	8	1.21 (1.36)	89
7	0	1600	0	5	0.05 (1.12)	4
8	40	1600	40	5	1.07 (1.18)	91

[a]Concentrations: pyruvate, 500 or 150 μmol dm^{-3} for entries 1-6 or 7-8, respectively; NADH, 250 μmol dm^{-3}; LDH, 170 μg dm^{-3}. [b]Initial velocity for LDH catalyzed reaction. The value in the absence of Cu(II) ions is in parenthesis. Each value is mean of at least duplicated runs and accurate within ±3%. [c]A magnitude of v_0 in the presence of $Cu(ClO_4)_2$ relative to that of the corresponding metal-free system.

Burk plots; the inhibition constant (K_i) defined as the dissociation constant of LDH–Cu(II) complex being 2.2 μmol dm^{-3} in an aqueous solution. While **3** gave no significant effect on the LDH activity directly, this species behaved as a ligand of Cu(II) ions to somewhat participate in recovering of the catalytic activity (Table 1, entry 2). In the presence of the bilayer vesicles of **2**, the v_0 value was larger than that in lipid-free system (Table 1, entry 3). We can evaluate an amount of LDH bound to the cationic vesicles from the Michaelis constant (K_m); the K_m value in an aqueous solution (35 μmol dm^{-3}) was decreased with an increase in the lipid concentration to reach a constant value (5.6 μmol dm^{-3}) above 500 μmol dm^{-3} of **2**. On the other hand, the maximum initial velocity (V_{max}) was independent on the lipid concentration; 1.6 μmol dm^{-3} s^{-1}. Thus, under the lipid concentrations in Table 1, LDH is quantitatively bound to the membrane surface keeping a conformation of the active site in aqueous media. We can understand that the smaller K_m value observed in the vesicular system relative to that in an aqueous solution are owing to proximity effects between LDH and pyruvate on the cationic membrane surface. We also evaluated the K_i value for LDH–Cu(II) complex in the presence of the bilayer vesicles of **2** to be 0.22 μmol dm^{-3}. Accordingly, the K_m/K_i value, an extent of inactivation of LDH by Cu(II) ions, in the vesicular system was larger than that in an aqueous solution (Table 1, entries 1 and 3). It is noteworthy that an effective molecular assembly can not be constructed with LDH and the cationic hexadecyltrimethylammonium bromide micelle due to a fact that an interaction between the surfactant molecules and LDH causes denaturation of the protein, resulting in loss of the catalytic activity.

Although the activity of LDH bound to the bilayer vesicles of **2** was not so influenced by coexistence of **3**, an addition of Cu(II) ions to this system drastically decreased the enzymic activity (Table 1, entry 4). Such behavior contrastive to that in the absence of **2** is presumably due to inactivation of LDH by the lipid molecules whose stable bilayer structure was perturbed by interaction with **3**–Cu(II) complex. Meanwhile, LDH activity in the hybrid assembly formed with **1** and **2** was comparable to that in the bilayer vesicle of **2** alone (Table 1, entries 5 and 3). Upon addition of Cu(II) ions to these vesicular systems, the enzymic activity in the former was a little larger than that in the latter, reflecting that the lysine residue of **1** acted as a metal binding site.

When we employed **3** as a signal ligand, this species was effectively recognized by **1** embedded in the bilayer vesicle of **2** by forming an imine bond between the formyl group of the ligand and the amino group of the cyclophane; the dissociation constant being about 1 μmol dm^{-3}. The complexation is reversible and much enhanced upon addition of Cu(II) ions due to the formation of the corresponding metal complex as confirmed by electronic absorption spectroscopy. Thus the supramolecular system composed of **1**, **2**, **3**, LDH, and Cu(II) ions as schematically shown in Figure 1 exhibited 89% of the catalytic activity relative to the corresponding system lacking Cu(II) ion as a signal transmitter (Table 1, entry 6). Since LDH activity of the vesicular system in the absence of **1** and **3** was 41% (Table 1, entry 3), such recovery of the enzymic activity would come from an enhanced metal-binding ability of the receptor–ligand complex. By changing concentrations of the individual species, more distinct signaling behavior was observed (Table 1, entries 7 and 8). The results clearly indicate that an artificial signaling system depicted in Figure 1 now turned into reality. The present system shows marked signal selectivity. For example, 89% of LDH activity (Table 1, entry 6) was changed to 93, 67, and 50% by replacement of **3** with 2-hydroxy-3-naphthaldehyde, salicylaldehyde, and 2-naphthaldehyde, respectively.

In conclusion, we demonstrated here the first example of supramolecular assemblies in which an artificial cell-surface receptor transmits an external signal to an enzyme in collaboration with a signal transmitter. Although we employed a bilayer membrane without discrimination between the inner and the outer surfaces at present, more intelligent signaling systems may be developed by further modification of an artificial receptor.

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