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Control of enzymic activity by artificial cell-surface receptors

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Abstract

A novel type of artificial cell-surface receptor capable of affecting enzymic activity was designed by employing steroidal moiety. Bile acid derivatives having an amino group, effectively recognized both the aromatic aldehydes and copper(II) ions in bilayer membrane formed with a synthetic peptide lipid. On the other hand, pig heart L-lactate dehydrogenase (LDH) was bound to the bilayer membrane surface mainly through electrostatic interactions, and the LDH activity was specifically inhibited by copper(II) ions. On these grounds, we constituted artificial signaling systems in combinations, with a steroidal receptor, a bilayer-forming synthetic lipid, and LDH. The present supramolecular vesicles acted as nano-reactors in which the artificial receptor was able to switch on the enzymic activity via accompanying double signal recognition and phase reorganization. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Lactate dehydrogenase; Protein–lipid interaction; Artificial receptor; Bile acid; Signal transduction

1. Introduction

In recent years, much attention has been focused on molecular information networks in cells, especially on signal transduction mediated by cell-surface receptors [1–6]. The signal transduction systems in a typical eukaryotic cell consist of a network of proteins that transform multiple external stimuli into appropriate cellular responses. Molecules that form this network can be placed into ordered biochemical pathways, in which signal propagation occurs through the sequential establishment of molecular interactions in the supramolecular level. Molecular recognition and its response by a cell-surface receptor embedded in lipid bilayer membrane are initial steps of such signal transduction process. On these grounds, biomimetic approaches to develop the artificial signaling systems as molecular devices [7] have been reported by employing synthetic ion channels as receptors in cell membrane models [8–16]. However, signal propagation between a receptor and an effector has been an unsettled problem in artificial systems up to the present time.

We have recently developed an artificial receptor, so-called steroid cyclophane, capable of recognizing organic molecules in aqueous media [17–22]. We found that the steroid cyclophane embedded in bilayer membrane acts as an artificial cell-surface receptor which transmits an external signal to an enzyme, pig heart L-lactate dehydrogenase (LDH), in collaboration with a second messenger signal as schematically shown in Fig. 1 [23,24]. In this article, we are to report that simple bile acid derivatives also

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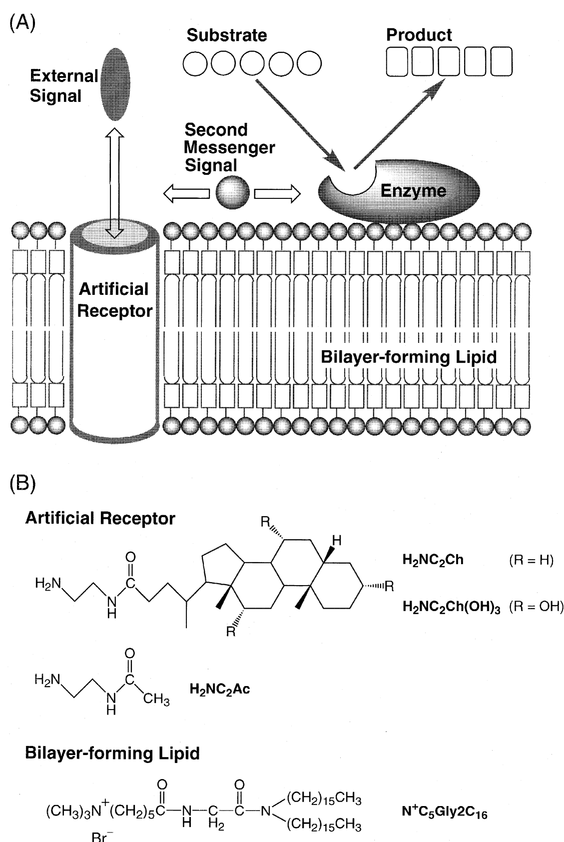

Fig. 1. Schematic representation of control of enzymic acitvity by an artificial cell-surface receptor (A) and structural formula of the artificial receptors and the bilayer-forming lipid (B).

act as artificial cell-surface receptors capable of activating the LDH by performing double signal recognition and phase reorganization in synthetic bilayer membranes [25]. A characteristic of the present signal transduction system is that the molecular recognition ability of the steroidal receptors can be affected by their aggregation in bilayer membrane, while the steroid cyclophane behaves as a cell-surface receptor in the monomeric state.

2. Experimental

2.1. Materials

LDH from pig heart was purchased from Boehringer Mannheim, Germany, as a crystalline suspension in 3.2 mol dm^{-3} ammonium sulfate; the specific activity for pyruvate as the substrate was listed as 550 U mg^{-1} at 25°C. The following compounds were also commercially available as guaranteed reagents and used without further purification: reduced form of β-nicotinamide adenine dinucleotide disodium salt (NADH) (Sigma, USA), sodium pyruvate, 2-naphthol, pyridoxal hydrochloride, 1-hydroxy-2-naphthaldehyde (1H2NA), salicylaldehyde (all from Wako, Japan), 2-naphthaldehyde (Aldrich, USA), copper(II) perchlorate hexahydrate, silver(I) perchlorate, zinc(II) perchlorate (these are from Kanto Chemicals, Japan), and nickel(II) perchlorate hexahydrate (Kishida Chemical, Japan). 2-Hydroxy-3-naphthaldehyde (2H3NA) was prepared according to Ref. [26]: a yellow crystal, mp 98–99°C. Anal. Calcd. for $C_{11}H_8O_2$: C, 76.73; H, 4.68%. Found: C, 76.44; H, 4.69%. A cationic peptide lipid, *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-glycinamide bromide ($N^+C_5Gly2C_{16}$), was prepared according to a procedure similar to that adopted for the synthesis of the peptide lipid having an L-alanine residue [27]: a white solid, final mp 240°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 [6H, t, $J = 6.4$ Hz, $(CH_2)_{15}CH_3$], 1.1–1.9 [62H, m, $CH_2(CH_2)_3CH_2CO$ and $CH_2(CH_2)_{14}CH_3$], 2.33 [2H, t, $J = 6.8$ Hz, $(CH_2)_5CH_2CO$], 3.16 [2H, t, $J = 7.8$ Hz, CH_2-$(CH_2)_{14}CH_3$], 3.30 [2H, t, $J = 7.3$ Hz, CH_2-$(CH_2)_{14}CH_3$], 3.45 [9H, s, $(CH_3)_3N^+$], 3.60 [2H, t, $J = 8.8$ Hz, N^+CH_2], 4.02 [2H, d, $J = 4.4$ Hz, $NHCH_2CO$], 6.93 [1H, br, NH]. Anal. Calcd. for $C_{43}H_{88}BrN_3O_2$: C, 68.04; H, 11.68; N, 5.54%. Found: C, 67.94; H, 11.71; N, 5.49%.

2.2. *N*-(2-aminoethyl)-5β-cholan-24-amide [H_2NC_2CH]

Ethyl chloroformate (56 mg, 0.52 mmol) was added to a mixture of 5β-cholan-24-oic acid (170 mg, 0.47 mmol) and dry triethylamine (53 mg, 0.52 mmol) in dry tetrahydrofuran (15 ml) at room temperature, and the reaction vessel was placed immediately in an ice-bath for 20 min while the mixture was gently stirred. A filtrate of the mixture was added to a dry tetrahydrofuran solution (20 ml) of ethylenediamine (280 mg, 4.7 mmol), and the resulting mixture was stirred for 18 h at room temperature. The solvent

was removed under reduced pressure and the residue was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluant. Evaporation of the solvent under reduced pressure gave a white solid (141 mg, 75%): mp 145–147°C. ^1H NMR ($CDCl_3$) δ 0.64 [3H, s, 18-H (steroid)], 0.91 [3H, s, 19-H (steroid)], 0.92 [3H, d, $J = 7.3$ Hz, 21-H (steroid)], 2.0–2.3 [2H, m, $COCH_2$], 2.84 [2H, m, CH_2CH_2NHCO], 3.31 [2H, m, CH_2CH_2NHCO], 5.92 [1H, br, NHCO]. Anal. Calcd. for $C_{26}H_{46}N_2O \cdot H_2O$: C, 74.23; H, 11.02; N, 6.66%. Found: C, 74.89; H, 11.43; N, 6.65%.

2.3. *N-(2-aminoethyl)-3α,7α,12α-trihydroxy-5β-cholan-24-amide [$H_2NC_2Ch(OH)_3$]*

This was prepared from 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid (0.97 g, 2.4 mmol) and ethylenediamine (1.44 g, 24 mmol) in reference to the method for preparation of H_2NC_2Ch. The crude product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluant. A white solid (0.82 g, 76%): mp 124–126°C. ^1H NMR (CD_3OD) δ 0.71 [3H, s, 18-H (steroid)], 0.92 [3H, s, 19-H (steroid)], 1.03 [3H, d, $J = 6.4$ Hz, 21-H (steroid)], 2.73 [2H, m, CH_2-CH_2NHCO], 3.24 [2H, m, CH_2CH_2NHCO], 3.37 [1H, m, 3-H (steroid)], 3.79 [1H, m, 7-H (steroid)], 3.95 [1H, m, 12-H (steroid)]. Anal. Calcd. for $C_{26}H_{46}N_2O_4 \cdot 0.5H_2O$: C, 67.94; H, 10.31; N, 6.09%. Found: C, 67.67; H, 10.17; N, 5.82%.

2.4. *Measurements*

Melting points were measured with a Yanaco MP-500D apparatus (hot-plate type). Elemental analyses were performed at the Microanalysis Center of Kyushu University. NMR spectra were recorded on a JEOL JNM-EX400 spectrometer. Negatively stained electron micrographs were taken on a JEOL JEM200-FX electron microscope installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University, in a manner described by Murakami et al. [27]. A Seiko Instruments DSC-6100 calorimeter was used for differential scanning calorimetry (DSC). Dynamic light-scattering measurements were carried out with a Photal (Otsuka Electronics) DLS-700 dynamic light-scattering spectrometer. Electronic absorption spectra were taken on a Shimadzu 2400-PC spectrophotometer.

2.5. *Preparation of hybrid assemblies*

Hybrid assemblies employed in this study were generally prepared as follows. Ethanol solutions of the peptide lipid and the steroidal receptor were homogeneously mixed in a vessel. The solvent was evaporated under nitrogen gas flow and completely removed in vacuo to give a thin film. Then, aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonate (HEPES) buffer (100 mmol dm^{-3}, pH 7.0) was added to the vessel. Multi-walled bilayer vesicles were formed upon vortex mixing of the aqueous dispersion. The corresponding single-walled vesicles were prepared by sonication of the dispersion sample with a bath-type sonicator at 80 W for 60 min.

2.6. *Enzyme assay*

LDH activity was measured at 30.0°C using sodium pyruvate as substrate. The assay sample (3 ml) contained 170 μg dm^{-3} LDH, 250 μmol dm^{-3} NADH, and 10–500 μmol dm^{-3} pyruvate in aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0). The reaction was started upon the addition of the stock solution of pyruvate. The catalytic activity of LDH in the reduction of pyruvate to L-lactate was evaluated spectrophotometrically by following a consumption rate of NADH in the presence and absence of the supramolecular elements and the signaling species. For the calculation of concentration, a value of 2580 dm^3 mol^{-1} cm^{-1} was used as a molar extinction coefficient of NADH at 370 nm. The signal transduction efficiency depicted in Fig. 1 was judged from the LDH activity which represents the magnitude of initial velocity for the enzyme reaction in the presence of metal ion as a second messenger signal relative to that of the corresponding metal-free system.

3. Results and discussion

Our strategy to control enzymic activity is to develop a supramolecular system by employing an artificial cell-surface receptor, keeping attention to the signal transduction mechanism through G-protein-linked receptor [3–5]. Thus, we designed an artificial signaling system depicted in Fig. 1. In the present system, an artificial receptor embedded in bilayer membrane can be regarded as the command module for an enzyme as a specific effector. Since we can basically understand that G-protein is a signal transmitter operating between a receptor and an effector, G-protein could be replaced in an artificial system by a simple signal transmitter which is capable of affecting both the receptor and enzyme functions. In addition, the following requirements must be fulfilled by the artificial receptor: (1) The receptor is capable of recognizing both an external signal and a second messenger as the signal transmitter; (2) Binding affinity of the receptor toward a second messenger signal varies as the recognition of an external signal ligand is changed.

We chose here an NADH-dependent LDH as an effector, since the signal transduction efficiency of the present supramolecular system can be evaluated by monitoring the enzymic activity spectrophotometrically. First, we investigated the candidates of the second messenger signals for the enzyme inhibitors [28]. For example, Gordon and Doelle [29] and Steinbüchel and Schlegel [30] reported that some metal ions act as inhibitors of LDH. We confirmed that copper(II) and silver(I) ions were potent inhibitors in a micromolar level for the LDH employed here. On the other hand, inhibitory effects of zinc(II) and nickel(II) ions toward LDH were much smaller than those of the former metal ions under similar concentrations. The inhibition of LDH by copper(II) ions was reversible and competitive as analyzed by the Lineweaver–Burk plots; the inhibition constant (K_i) defined as the dissociation constant of the LDH–Cu(II) complex is 2.2 μmol dm^{-3}. Thus, copper(II) and silver(I) ions seem to be employed as second messenger signals in the present signal transduction system.

For an efficient signal transmission between an artificial receptor and LDH, it should be preferable that both molecules are bound to a bilayer membrane and close to each other. We have previously clarified that synthetic peptide lipids having an amino acid residue interposed between a polar head moiety and a hydrophobic double-chain segment form morphologically stable bilayer membranes in aqueous media [31]. We employed here a cationic peptide lipid bearing a glycine residue, $N^+C_5Gly2C_{16}$. The lipid forms multi-walled bilayer vesicles in an aqueous dispersion state as confirmed by negative-staining electron microscopy. Upon sonication of the dispersion with a bath-type sonicator at 80 W for 60 min, the formation of the single-walled bilayer vesicles with a diameter of ca. 100 nm was observed in the electron micrograph. The vesicular size was consistent with a hydrodynamic diameter (d_{hy}) evaluated by means of dynamic light scattering measurements. Upon addition of LDH to the cationic vesicular solution, the d_{hy} value was increased reflecting the formation of a complex of the vesicle with LDH. On the other hand, the d_{hy} value for the vesicle of the corresponding anionic peptide lipid remained constant without any effect by the presence of LDH [32]. In addition, the phase transition behavior from gel to liquid-crystalline state for the single-walled vesicles was not influenced upon the addition of LDH, as evaluated by means of DSC. Accordingly, LDH is capable of binding to the vesicular surface of $N^+C_5Gly2C_{16}$ mainly through electrostatic interactions.

In order to evaluate an amount of LDH bound to the vesicles, the Michaelis constant (K_m) and the maximum initial velocity (V_{max}) were evaluated in various lipid concentrations. As shown in Fig. 2, the K_m value (35 μmol dm^{-3}) in an aqueous HEPES buffer was decreased with an increase in the lipid concentration to reach a constant value (5.6 μmol dm^{-3}). On the other hand, the V_{max} value was independent on the lipid concentration. Thus, under the lipid concentration above 500 μmol dm^{-3}, 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 values observed in the vesicular system relative to that in the 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 $N^+C_5Gly2C_{16}$ to be 0.22 μmol

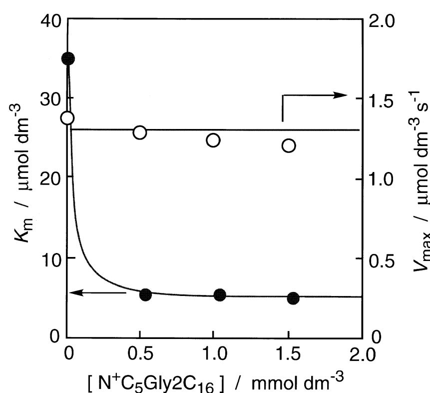

Fig. 2. Correlations of K_m and V_{max} values of LDH with lipid concentration: in aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0°C. Concentrations: NADH, 250 μmol dm^{-3}; LDH, 170 μg dm^{-3}.

dm^{-3}. Accordingly, the K_m/K_i value, an extent of the inactivation of LDH by copper(II) ions in the vesicular system, was larger than that in an aqueous solution. It is noteworthy that an effective molecular assembly cannot be constructed with LDH and cationic micelle of hexadecyltrimethylammonium bromide due to the fact that an interaction between the surfactant molecules and LDH causes denaturation of the protein, resulting in the loss of the catalytic activity [32].

Recently, bile acids have been employed as key building blocks for the design of artificial hosts capable of performing molecular recognition in various physical phases such as the solid state [33,34], and aqueous [22,35] and organic media [36–39]. The molecular rigidity, provided by the steroidal moiety, is of great advantage for the functionalization of such host molecules. While studies on the aggregation behavior of bile acids with phospholipids have been extensively carried out [40–45], there are a few reports on bile acid derivatives performing as supramolecular functional elements in bilayer membranes [20,46]. On these grounds, we employed here bile acid derivatives having an amino group, H_2NC_2Ch and $H_2NC_2Ch(OH)_3$, as artificial cell-surface receptors. In the bilayer membrane, these molecules are capable of reversible binding an aromatic aldehyde as an external signal through the formation of an imine bond between the amino group and the formyl group. Thus, the signal transduction behavior to activate LDH mediated by an artificial receptor as depicted in Fig. 1 would come off under the conditions that the metal binding affinity of the components increases in the following order: the receptor < LDH < the external signal–receptor complex.

Now, we examined the interaction of the artificial receptors with the peptide lipid membrane and its consequence in the signal recognition ability. Fig. 3 shows the DSC thermograms for the multi-walled bilayer membranes of $N^+C_5Gly2C_{16}$. In the absence of the artificial receptor, the lipid aggregate showed a relatively sharp transition with a phase transition temperature (T_m) at 28.0°C (Fig. 3A). Upon the addition of H_2NC_2Ch to $N^+C_5Gly2C_{16}$ in a 1:10 molar ratio, the phase transition peak was broadened and the T_m value was decreased, reflecting that the receptor was homogeneously dispersed in the membrane (Fig. 3B). While 1H2NA as an external signal was quantitatively bound to H_2NC_2Ch embedded in the membrane under the present conditions, as confirmed by electronic absorption spectroscopy (vide infra), there were slight differences in the DSC thermograms in the presence and absence of the signal (Fig. 3C). This implies that the distribution mode of the receptor in the membrane was scarcely

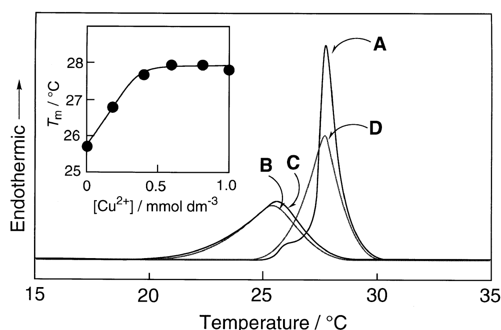

Fig. 3. DSC thermograms for bilayer membranes of peptide lipid dispersed in aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0): (A) $N^+C_5Gly2C_{16}$ (10 mmol dm^{-3}); (B) $N^+C_5Gly2C_{16}$ (10 mmol dm^{-3}) and H_2NC_2Ch (1 mmol dm^{-3}); (C) $N^+C_5Gly2C_{16}$ (10 mmol dm^{-3}), H_2NC_2Ch (1 mmol dm^{-3}), and 1H2NA (1 mmol dm^{-3}); (D) $N^+C_5Gly2C_{16}$ (10 mmol dm^{-3}), H_2NC_2Ch (1 mmol dm^{-3}), 1H2NA (1 mmol dm^{-3}), and copper(II) ions (1 mmol dm^{-3}). Inset shows the correlation between copper(II) concentration and phase transition temperature (T_m) for the hybrid assembly formed with $N^+C_5Gly2C_{16}$ (10 mmol dm^{-3}), H_2NC_2Ch (1 mmol dm^{-3}), 1H2NA (1 mmol dm^{-3}), and copper(II) ions.

perturbed by the signal binding. Upon the addition of copper(II) ions, however, drastic change in the DSC thermogram was observed, reflecting a phase reorganization of the bilayer membrane (Fig. 3D). Judging from the T_m-dependence of the concentration of copper(II) ions, the external signal–receptor complex binds copper(II) ions in a 2:1 molar ratio. Similar thermal behavior was observed when H_2NC_2Ch was replaced by $H_2NC_2Ch(OH)_3$.

The stoichiometry of the signal-binding by the receptor was also confirmed by means of electronic absorption spectroscopy. In aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0°C, 1H2NA showed an absorption maximum at 390 nm. In the presence of the single-walled vesicles formed with $N^+C_5Gly2C_{16}$ and H_2NC_2Ch in a 40:1 molar ratio, 1H2NA binds to the receptor site to form the corresponding Schiff's base having absorption maxima at 420 and 440 nm. Similar spectral behavior was observed when H_2NC_2Ch was replaced by $H_2NC_2Ch(OH)_3$. Dissociation constants for the external signal–receptor complexes were evaluated by spectrophotometric titration to be 1.5 and 2.3 μmol dm^{-3} for H_2NC_2Ch and $H_2NC_2Ch(OH)^3$, respectively. Upon the addition of copper(II) ions, the ternary complex of the receptor, 1H2NA, and copper(II) ions having absorption maxima at 390 and 410 nm was effectively formed. A continuous variation plot for the ternary complex for H_2NC_2Ch was shown in Fig. 4. The result clearly indicates that the external signal–receptor complex binds copper(II) ions in a 2:1 molar ratio. For the ternary complex for $H_2NC_2Ch(OH)_3$, the stoichiometry was also identical. When we replaced the copper(II) ions by other metal ions such as silver(I), zinc(II), and nickel(II) ions, the formation of the corresponding ternary complex was not detected by DSC and electronic absorption spectroscopy.

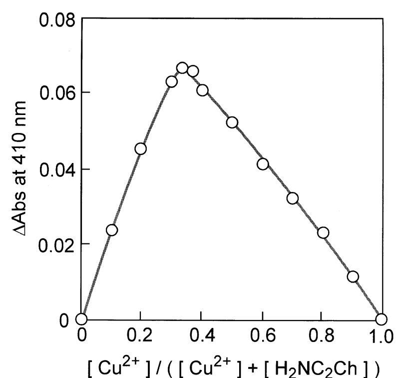

Fig. 4. A continuous variation plot for the complexation of copper(II) ions with Schiff's base derived from H_2NC_2Ch and 1H2NA in aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0°C: total concentration of copper(II) ions and H_2NC_2Ch, 30 μmol dm^{-3}; molar ratio of H_2NC_2Ch–1H2NA–$N^+C_5Gly2C_{16}$ (1:1:40).

Table 1
Effect of supramolecular elements on LDH activity (%)[a]

Entry	Receptor	Lipid	External signal	
			None	1H2NA
1	None	None	37	32
2	None	$N^+C_5Gly2C_{16}$	21	12
3	H_2NC_2Ac	None	52	47
4	H_2NC_2Ac	$N^+C_5Gly2C_{16}$	32	41
5	$H_2NC_2Ch(OH)_3$	$N^+C_5Gly2C_{16}$	15	78
6	H_2NC_2Ch	$N^+C_5Gly2C_{16}$	15	82

[a] In aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0°C. Concentrations: pyruvate, 150 μmol dm^{-3}; NADH, 250 μmol dm^{-3}; LDH, 170 μg dm^{-3}; receptor, 30 μmol dm^{-3}; lipid, 1200 μmol dm^{-3}; external signal, 30 μmol dm^{-3}. The LDH activity represents the magnitude of initial velocity for the enzyme reaction in the presence of copper(II) ions (2 μmol dm^{-3}) relative to that of the corresponding metal-free system. Each value is the mean of at least duplicated runs and accurate within ± 3%.

On these grounds, we constituted an artificial signaling system as schematically shown in Fig. 1 by employing the steroidal receptor, $N^+C_5Gly2C_{16}$ and LDH. The signal transduction efficiency was evaluated from the LDH activity which represents the magnitude of initial velocity for the enzyme reaction in the presence of copper(II) ion as a second messenger signal relative to that of the corresponding metal-free system. The effect of the supramolecular elements on the LDH activity is listed in Table 1. In the presence of 2 μmol dm^{-3} of copper(II) ions, the LDH activity in aqueous HEPES buffer was decreased to 37% under the present conditions (Table 1, entry 1). The catalytic activity of LDH bound to the bilayer vesicle of $N^+C_5Gly2C_{16}$ was somewhat decreased relative to that in the corresponding lipid-free system (Table 1, entry 2), reflecting that the K_i value for the LDH–Cu(II) complex formed on the membrane surface is smaller than that in aqueous solution (vide supra). Upon the addition of 30 μmol

dm^{-3} of 1H2NA, the LDH activity was slightly decreased, suggesting that 1H2NA also acted as an inhibitor. Such inhibitory effect was rather pronounced in the presence of the bilayer membrane. On the other hand, the LDH activity in aqueous solution was increased upon the addition of H_2NC_2Ac as a reference compound of the steroidal receptor (Table 1, entry 3), since this molecule behaves as a weak unidentate ligand for metal ions. Further addition of 1H2NA to this solution does not enhance the LDH activity. Analogous behavior was also observed for H_2NC_2Ac in the presence of the lipid bilayer membrane (Table 1, entry 4). Thus, the signal transduction depicted in Fig. 1 was not successful for the water-soluble H_2NC_2Ac lacking the hydrophobic steroidal moiety. The LDH activity of the hybrid system composed of $H_2NC_2Ch(OH)_3$ and $N^+C_5Gly2C_{16}$ was low in the absence of the external signal (Table 1, entry 5), reflecting the larger metal-binding ability of LDH than the steroidal receptor. Upon the addition of an external signal 1H2NA to this system, however, the LDH activity was drastically increased from 15% to 78% as a consequence of the change in the metal-binding mode. The results clearly indicate that the signal transduction as schematically shown in Fig. 1 occurs with an extremely high efficiency on the membrane surface of the hybrid molecular assembly. When the receptor was replaced by H_2NC_2Ch, the signal transduction efficiency was further increased (Table 1, entry 6). Although the difference in the signal transduction efficiency between $H_2NC_2Ch(OH)_3$ and H_2NC_2Ch was not so large under the conditions employed in Table 1, the difference may be more pronounced by the modification of the reaction conditions such as temperature, since the intermolecular hydrogen bonding interaction among the hydroxyl groups of the former steroidal receptors strongly affects the phase behavior of the hybrid assembly.

The present hybrid system shows marked signal selectivity. As listed in Table 2, efficient signal transduction was observed for the system formed with H_2NC_2Ch, $N^+C_5Gly2C_{16}$ and LDH by employing combinations with an aromatic *o*-hydroxyaldehydes as an external signal and copper(II) ion as a second messenger signal. Among the external signals used here, salisylaldehyde, which would be capable of forming stable chelate species of the external signal–receptor complex with the metal ions in a 2:1 molar ratio, was most effective. Although silver(I) ions also act as potent inhibitors for LDH, the hybrid assembly utilizing silver(I) ion as the second messenger signal did not work as an effective signal transduction system.

Table 2
Signal selectivity of artificial signal transduction system[a]

External signal	Second signal	LDH activity (%)
None	Cu^{2+}	15
2-Naphthol	Cu^{2+}	27
2-Naphthaldehyde	Cu^{2+}	38
Pyridoxal	Cu^{2+}	68
1H2NA	Cu^{2+}	82
2H3NA	Cu^{2+}	92
Salisylaldehyde	Cu^{2+}	96
None	Ag^+	37
1H2NA	Ag^+	26

[a] In aqueous HEPES buffer (100 mmol dm^{-3}, pH 7.0) at 30.0°C. Concentrations: pyruvate, 150 μmol dm^{-3}; NADH, 250 μmol dm^{-3}; LDH, 170 μg dm^{-3}; H_2NC_2Ch, 30 μmol dm^{-3}; $N^+C_5Gly2C_{16}$, 1200 μmol dm^{-3}; external signal, 30 μmol dm^{-3}. The LDH activity represents the magnitude of initial velocity for the enzyme reaction in the presence of the second signal, Cu^{2+} (2 μmol dm^{-3}) or Ag^+ (10 μmol dm^{-3}), relative to that of the corresponding metal-free system. Each value is the mean of at least duplicated runs and accurate within ±3%.

4. Conclusions

We have demonstrated here that the supramolecular vesicles formed in combinations with the steroidal receptor, the cationic peptide lipid, and LDH acted as nano-reactors in which the artificial receptor was able to switch on the enzymic activity via accompanying the double signal recognition and phase reorganization. If we choose an appropriate combination of the external and the second messenger signal in the hybrid vesicular systems, the regulation of the catalytic activities for multi-enzyme complexes would become possible. From this point of view, the present results may provide a useful guide for the design of supramolecular devices that mimic the signal transduction behavior observed in biological systems such as G-protein mediated cell-signaling.

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