Signal transduction mediated by artificial cell-surface receptors: activation of lactate dehydrogenase triggered by molecular recognition and phase reorganization of bile acid derivatives embedded in a synthetic bilayer membrane

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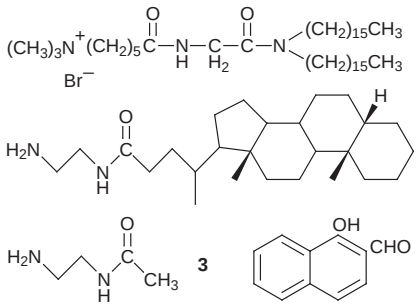
A bile acid derivative, *N*-(2-aminoethyl)-5β-cholan-24-amide, acts as an artificial cell-surface receptor capable of activating lactate dehydrogenase by performing double signal recognition and phase reorganization in a synthetic bilayer membrane.

Recently, bile acids have been employed as a key building block for design of artificial hosts capable of performing molecular recognition in various physical phases such as the solid state,[1] and aqueous[2] and organic media.[3] The molecular rigidity provided by the steroidal moiety is of great advantage for the functionalization of such host molecules. Meanwhile, there are few reports on bile acid derivatives, such as steroidal porphyrins[4] and cyclophanes,[5] each being a supramolecular element in bilayer membranes. We report here on the signal recognition and response of an artificial steroidal receptor, *N*-(2-aminoethyl)-5β-cholan-24-amide **2**, embedded in the bilayer membrane formed with a synthetic peptide lipid, *N*,*N*-dihexadecyl-*N*$^{\alpha}$-[6-(trimethylammonio)hexanoyl]glycinamide bromide **1**.†



1 **2** **3** **4**

In aqueous media **1** formed a morphologically stable bilayer membrane with a gel-to-liquid crystalline phase transition at 26.0 °C (ΔH = 31.0 kJ mol^{-1}) in a manner similar to those reported for the analogous peptide lipids having a different amino acid residue.[6] Bilayer vesicles were prepared by sonication of an aqueous dispersion containing **1** and water-insoluble **2** in a 40 : 1 molar ratio with a bath-type sonicator at 80 W for 60 min in 2-[4-(2-hydroxyethyl)piperazin-1-yl]-ethanesulfonate (HEPES) buffer (100 mmol dm^{-3}, pH 7.0). The formation of single-walled bilayer vesicles was confirmed by negative-staining electron microscopy, and the vesicular size as evaluated by means of dynamic light scattering measurements was 120 nm.

Next we examined the molecular recognition behavior of the receptor **2**. 1-Hydroxy-2-naphthaldehyde **4** was effectively bound to **2** homogeneously dispersed in the bilayer vesicle by forming an imine bond between the formyl group and the amino group, as confirmed by electronic absorption spectroscopy. The binding process was reversible and in equilibrium under the present conditions. The dissociation constant for the Schiff's base was evaluated from the aldehyde-dependent absorbance changes at 440 nm to be 1.5 μmol dm^{-3} at 30.0 °C. The binding of **4** with **2** was much enhanced upon addition of copper(II) ions due to the formation of the corresponding metal chelate having an absorption maximum at 388 nm. The continuous variation method[7] applied to the present bilayer system clearly indicates that the Schiff's base and copper(II) ions form a 2 : 1 chelate accompanying the phase reorganization of the steroidal moieties in the liquid crystalline state of the membrane. Such metal-ion-induced phase reorganization behavior was reflected by an increase in the phase transition temperature and sharpening of the peak for the hybrid membrane in the dispersion states, as evaluated by differential scanning calorimetry. Thus **2** acts as an artificial host being capable of recognizing both **4** and copper(II) ions in the bilayer membrane.

We have also clarified that pig heart lactate dehydrogenase (LDH; Boehringer Manheim, Germany) was bound to the membrane surface of the cationic peptide lipid mainly through electrostatic interactions, and that the LDH activity was specifically inhibited by copper(II) ions.[8] On these grounds, we constituted an artificial signaling system with **1**, **2** and LDH as schematically shown in Fig. 1 and evaluated the signal transduction ability of the steroidal host as an artificial cell-surface receptor. We chose **4** and copper(II) ions as an external signal of the receptor **2** and the second messenger signals between **2** and LDH, respectively. Under the conditions used the metal binding affinity of the components increases in the

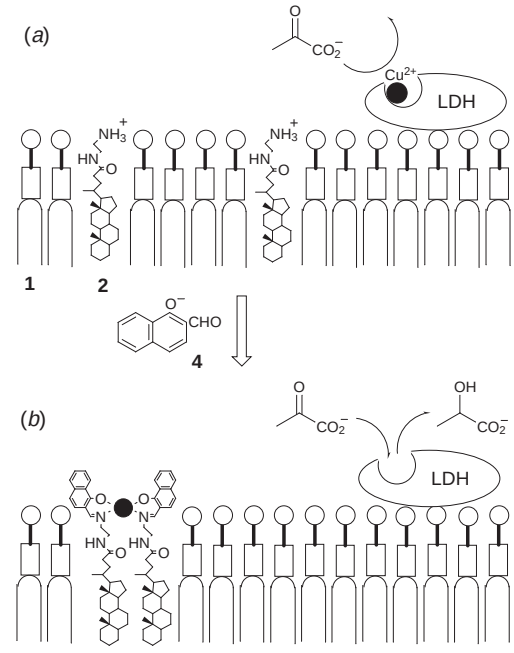


Fig. 1 Schematic illustration of switching of LDH activity mediated by an artificial cell-surface receptor **2**. (*a*) and (*b*) represent the off- and on-states of LDH in the absence and presence of an external signal **4**, respectively.

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

Entry	Species/μmol dm^{-3}				v_0[b]/μmol dm^{-3} s^{-1}	Activity[c] (%)
	1	**2**	**4**	Cu^{2+}		
1	0	0	0	2	0.45 (1.20)	37
2	0	0	30	2	0.37 (1.14)	32
3	1200	0	0	2	0.25 (1.17)	21
4	1200	0	30	2	0.15 (1.18)	13
5	1200	30	0	2	0.17 (1.16)	15
6	1200	30	30	2	1.00 (1.22)	82

[a] Concentrations: pyruvate, (150 μmol dm^{-3}); NADH (250 μmol dm^{-3}); LDH (170 μg dm^{-3}). [b] Initial velocity for LDH catalyzed reaction. The value in the absence of copper(II) ions is in parentheses. Values are accurate within ±3%. [c] Magnitude of v_0 in the presence of $Cu(ClO_4)_2$ relative to that of the corresponding metal-free system.

following order: **2** < LDH < the **2**–**4** complex, giving the signal transduction behaviour shown in Fig. 1. In the absence of an external signal ligand, the catalytic performance of LDH is blocked by coordination of copper(II) ions [Fig 1(*a*)], since the steroidal receptor behaves as a weak unidentate ligand for metal ions. Upon binding of the signal ligand **4** with the receptor **2**, the resulting receptor–ligand complex acts as a strong bidentate ligand to snatch the metal ion from LDH, resulting also in phase reorganization of **2** in the bilayer membrane [Fig. 1(*b*)]. Thus the LDH activity can be indirectly controlled by an external signal in the present artificial receptor system.

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). In the absence of copper(II) ions, the LDH activity was not significantly influenced by the presence of **1**, **2** and **4**. However, inactivation of LDH by copper(II) ions was highly specific, since the catalytic activity was not influenced by the presence of zinc(II) or nickel(II) ions at similar concentrations. The inhibition of LDH by copper(II) ions was reversible and competitive as analyzed by the Lineweaver–Burk plots; the inhibition constant, defined as the dissociation constant of the LDH–copper(II) complex, was 0.22 and 2.2 μmol dm^{-3} in the presence and absence of the bilayer membrane, respectively. Thus the extent of inactivation of LDH by copper(II) ions in the vesicular system was larger than that in aqueous solution (Table 1, entries 1 and 3). Inhibition of the enzymic activity by copper(II) ions was somewhat enhanced upon addition of **4** (Table 1, entry 2), and such behavior was also observed in the presence of the bilayer membrane (Table 1, entry 4). As expected, the LDH activity of the hybrid system depicted in Fig. 1(*a*) was low (Table 1, entry 5), reflecting the larger metal-binding ability of LDH than the steroidal receptor. Upon addition of the signal ligand **4** to this system, however, the LDH activity, defined as the magnitude of the initial velocity in the presence of copper(II) ions relative to that of the corresponding metal-free system, was drastically increased to 82% from 15% (Table 1, entry 6) as a consequence of the change in the metal binding mode, as shown in Fig. 1(*b*). When the receptor **2** was replaced by water-soluble **3** lacking the steroidal moiety in the supramolecular system shown in Fig. 1, the LDH activity increased only a little to 41% from 32% under similar concentration conditions. Accordingly, it is clear that the signal transduction occurrs with extremely high efficiency on the membrane surface of the hybrid molecular assembly formed from the peptide lipid **1**, the steroidal receptor **2** and LDH. The present hydrid system shows marked signal selectivity. For example, only 38% LDH activity was observed by replacement of **4** with 2-naphthaldehyde under the conditions of entry 6 in Table 1.

In conclusion, we have demonstrated here the first example of a supramolecular assembly in which an artificial cell-surface receptor is able to switch on enzymic activity *via* accompanying double signal recognition and phase reorganization. Although the reversibility in the system remains a problem, the present results may provide a useful guide for the design of supramolecular devices that mimic the signal transduction behaviour observed in biological systems such as G-protein mediated cell signaling. [9]

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Notes and references

† All new compounds gave C, H and N elemental analyses within 0.4% of theory, and ^{1}H and ^{13}C NMR spectra consistent with their structures.

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