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PAPER

Switching of the enzymatic activity synchronized with signal recognition by an artificial DNA receptor on a liposomal membrane

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We constructed a supramolecular system on a liposomal membrane that is capable of activating an enzyme *via* DNA hybridization. The design of the system was inspired by natural signal transduction systems, in which enzymes amplify external signals to control signal transduction pathways. The liposomal membrane, providing a platform for the system, was prepared by the self-assembly of an oligonucleotide lipid, a phospholipid and a cationic synthetic lipid. The enzyme was immobilized on the liposomal surface through electrostatic interactions. Selective recognition of DNA signals was achieved by hybridizing the DNA signals with the oligonucleotide lipid embedded in the liposome. The hybridized DNA signal was sent to the enzyme by a copper ion acting as a mediator species. The enzyme then amplified the event by the catalytic reaction to generate the output signal. In addition, our system demonstrated potential for the discrimination of single nucleotide polymorphisms.

Introduction

Cell membranes play a pivotal role as platforms in biological systems. They act as a boundary against the extracellular environment and as a functional unit for the systematic transformation of materials, energy and information. To simulate such biological functions, lipid bilayer vesicles (liposomes), formed with phospholipids or synthetic lipids, have been widely used as cell membrane models.[1] To date, important biological events, such as material transport, catalysis and energy conversion have been successfully simulated in liposomal membranes.[2]

Signal transduction systems, in which signal transmission occurs among biomolecules such as receptors and enzymes, are also integrated into cell membranes. We recently reported the construction of a new class of supramolecular system to mediate information processing, the design of which was inspired by biological signal transduction systems.[3] These systems essentially comprise three molecular components: a synthetic receptor, an enzyme, and a liposomal membrane. The receptor and the enzyme are immobilized on the liposomal membrane. The catalytic activity of the enzyme is activated by an external signal acting on the synthetic receptor, which is mediated by copper ions (Cu^{2+}).[4] In this system, the receptor adjusts the enzymatic activity depending on the molecular recognition of the receptor for the signal.

In this paper, we report the construction of a supramolecular system that senses a specific DNA signal on a liposomal membrane. The system functions through the cooperation of several functional elements, including an oligonucleotide lipid as a receptor with the ability to recognize DNA signals *via* hybridization, and a natural enzyme to amplify the response of the molecular recognition (Fig. 1). The advantage of using DNA sequence-specific hybridization to construct a supramolecular system is well established. For example, DNA hybridization has been used to program biomolecular self-assembly pathways,[5] organize functional nanoparticles,[6] and prepare three-dimensional DNA assemblies such as polyhedra[7] and hydrogels.[8] The present liposomal system uses an oligonucleotide lipid (ONL-**1′**, Table 1 and Fig. 2) as a synthetic receptor for DNA signals (**1–6**), L-lactate

Table 1 Sequences of oligonucleotide lipid (ONL-**1′**)[a], water-soluble single-stranded DNA **1′** (as a control of ONL-**1′**), and DNA signals **1–6**[b]

Name	Sequence
ONL-**1**’	Cholesterol-5′-GAG CGT GGG TAG AGA GAG G-3′
1’	5′-GAG CGT GGG TAG AGA GAG G-3′
1	5′-C CTC TCT CTA CCC ACG CTC-3′
2	5′-A GTA TTC AAC ATT TCC GTG-3′
3	5′-C CTC TCT CTA CCC ACT CTC-3′
4	5′-CAC GGA AAT GTT GAA TAC TCC CTC TCT CTA CCC ACG CTC-3′
5	5′-GAG CGT GGG TAG AGA GAG GGA GTA TTC AAC ATT TCC GTG-3′
6	5′-CAC GGA AAT GTT GAA TAC TCC CTC TCT CTA CCC ACT CTC-3′

[a] The structure of the oligonucleotide lipid is shown in Fig. 2. [b] The single-base mismatches in DNA signals **3** and **6** are underlined.

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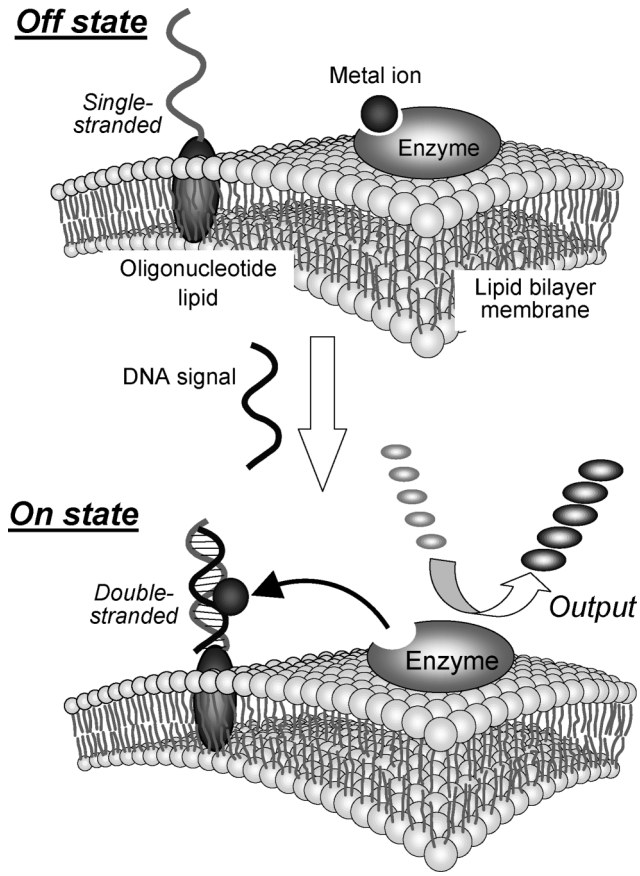

Fig. 1 Schematic representation of a supramolecular system to activate an enzyme *via* DNA hybridization to an oligonucleotide lipid embedded in the liposome.

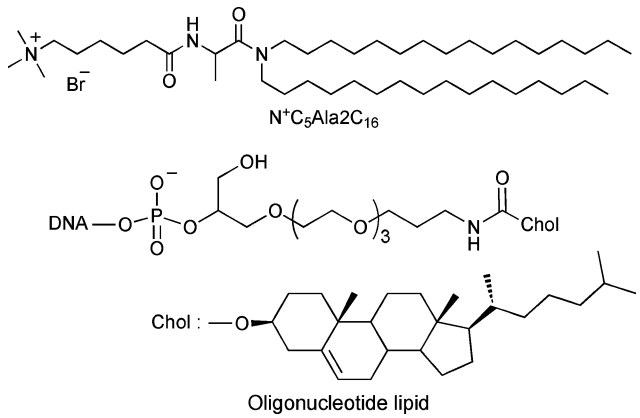

Fig. 2 Chemical structures of $N^+C_5Ala2C_{16}$ and the oligonucleotide lipid.

dehydrogenase (LDH) as the enzyme, Cu^{2+} ions for signal transduction, and a liposomal platform constructed with a cationic peptide lipid ($N^+C_5Ala2C_{16}$) and a phospholipid (DMPC). The Cu^{2+} ion acts as a competitive inhibitor of LDH. Lamanna *et al.* suggested that Cu^{2+} ions have higher affinity with double-stranded DNA than single-stranded DNA from spectroscopic measurements, whereas quantitative data were not provided.[9] More recently, Wang *et al.* determined the binding affinities between Cu^{2+} ions and single- or double-stranded DNA by surface-based electrochemical methods. Thus, the obtained binding constant indicated a more than three-fold higher binding of Cu^{2+} ions to double-stranded DNA compared with the corresponding single-stranded DNA. These results indicate that the binding of Cu^{2+} ions with the DNA receptor varies according to the level of hybridization with a complementary DNA signal.[10] This is one of the most important properties of DNA in the current DNA sensing system. In the absence of a DNA signal with a complementary sequence to the oligonucleotide lipid on the liposomal membrane, the mediator binds to LDH because single-stranded DNAs have lower affinity than does LDH to Cu^{2+}. This results in an enzymatically inactive state (Fig. 1, "off state"). When the receptor detects a DNA signal through hybridization, the resulting double-stranded DNA strongly binds to Cu^{2+}, which switches the enzyme to the active state (Fig. 1, "on state"). Thus, provision of a DNA signal to the liposome containing the oligonucleotide lipid amplifies the signal output through translocation of the mediator between the DNA duplex and the enzyme.

Results and discussion

Design of the supramolecular system

The liposome used in the current system is a spherical and hollow nanoparticle with a shell several nanometres thick. It has been widely used in drug and gene delivery systems, and as a bioreactor.[11] The liposome was formed from the mixture of a cationic peptide lipid, *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$, Fig. 2) and a phospholipid, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC). In the liposomal system used here, the enzyme acts as an amplifier of DNA signals. We immobilized a water-soluble enzyme, LDH, on the liposomal surface through non-covalent, electrostatic interactions.[12] The catalytic activity of LDH was maintained on the liposome; however, an effective molecular assembly could not be constructed with LDH and micelles of a cationic surfactant, cetyltrimethylammonium bromide (CTAB). CTAB micelles denature LDH, resulting in the loss of catalytic activity.[13] Thus, the use of a cationic lipid membrane in this study represents a very easy and simple but effective technique to provide a sophisticated scaffold for the supramolecular assembly of water-soluble enzymes and synthetic receptors.

Several metal ions can inhibit LDH. We previously reported that Cu^{2+}, at micromolar concentrations, is a potent inhibitor of LDH.[13] The inhibition of LDH by Cu^{2+} is reversible and competitive, regardless of the presence of the bilayer vesicles, as analyzed by Lineweaver–Burk plots.[14]

The functional synthetic receptor for use in the current system must fulfil the following requirements: (i) the receptor effectively binds to the liposomal membrane; (ii) the receptor acts as a ditopic molecular recognition site for a specific signal and a metal ion acting as a mediator between the receptor and enzyme; and (iii) the binding affinity of the receptor toward the mediator varies according to the recognition of a signal. With respect to these criteria, an oligonucleotide lipid (Fig. 2), which is capable of changing its binding affinity for metal ions depending on its level of hybridization with complementary single-stranded DNA (ssDNA), was adopted as a synthetic receptor for a DNA signal.

Table 2 Hydrodynamic diameters[a] (D_{hy}) of liposomes

	Species/μmol cm^{-3}						
			DNA signals				
Entry	[Lipid]	[ONL-**1′**]	[**1**]	[**2**]	[**3**]	[**4**]	D_{hy}/ nm (*p.i.*)[b]
1	1000	0	0	0	0	0	124 (0.10)
2	1000	0.50	0	0	0	0	120 (0.08)
3	1000	0.50	0.50	0	0	0	120 (0.12)
4	1000	0.50	0	0.50	0	0	117 (0.15)
5	1000	0.50	0	0	0.50	0	120 (0.16)
6	1000	0.50	0	0	0	0.50	122 (0.13)

[a] The hydrodynamic diameter was determined by dynamic light scattering in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 35 °C. [b] Polydispersity indices (*p.i.*) are in parentheses. [$N^+C_5Ala2C_{16}$] / [DMPC] = 2 : 8.

The base sequences in the oligonucleotide lipid and DNA signals were chosen to prevent the formation of G-quadruplexes, hairpin structures and self-dimerization.[15] In addition, this inhibited unexpected hybridization with non-target DNA. The denaturation temperature (T_m) of fully matched double-stranded DNA (ONL-**1′** with **1**) was estimated to be 51 °C using the Wallace–Itakura formula,[16] indicating that the hybridized double-stranded DNA was stable in the experimental conditions.

Characterization of liposomes modified with an oligonucleotide lipid

The liposomes were prepared by sonicating an aqueous dispersion of $N^+C_5Ala2C_{16}$ and DMPC in a 2 : 8 molar ratio using a cup-type sonicator. The hydrodynamic diameters (D_{hy}) of the liposomes, as determined by dynamic light scattering (DLS) measurements, were 124 nm and 120 nm in the presence and absence of ONL-**1′**, respectively, at pH 7.0 and 35 °C (Table 2). This indicates that incorporation of ONL-**1′** into the liposomal membrane did not affect the size of the liposome. The addition of DNA signals **1**–**4** did not affect the D_{hy} of liposomes embedded with ONL-**1′**. These results suggest that the liposomes exist in an intact form without any aggregation or membrane fusion, even in the presence of a DNA signal with a relatively long sequence. The morphology of the liposome was also examined by cryogenic transmission electron microscopy (cryo-TEM). As shown in Fig. 3, the liposomes were spherical, hollow nanoparticles in the presence and absence of a DNA signal, indicating that neither ONL-**1′** nor the DNA signal induced morphological changes of the liposomes at any of the DNA concentrations tested.

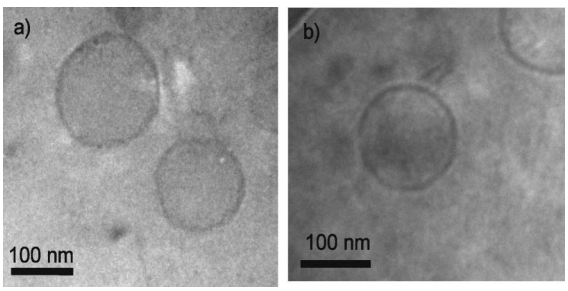

Fig. 3 Cryo-transmission electron microscopy images of a liposome embedded with ONL-**1′** in the presence (b) and absence (a) of DNA signal **1** in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0). Concentration, in μmol dm^{-3}: [$N^+C_5Ala2C_{16}$], 200.

DNA hybridization on the liposomal membrane

Hybridization of DNA signal **1** with ONL-**1′** on the liposome was evaluated by the fluorescence assay using PicoGreen, a fluorescent dye that shows a much higher quantum yield in the presence of double-stranded DNA than ssDNA.[17] Fig. 4 shows the emission spectra of PicoGreen excited at 480 nm in the presence of DNA signals and the liposome. Upon addition of DNA signal **1** to the liposome embedded with ONL-**1′**, the florescence emission was markedly enhanced because of the recognition of double-stranded DNA by PicoGreen (Fig. 4a and b). On the other hand, the non-complementary DNA signal **2** did not greatly enhance fluorescence (Fig. 4c), indicating that DNA signal **1** hybridized with ONL-**1′** on the liposome to form double-stranded DNA.

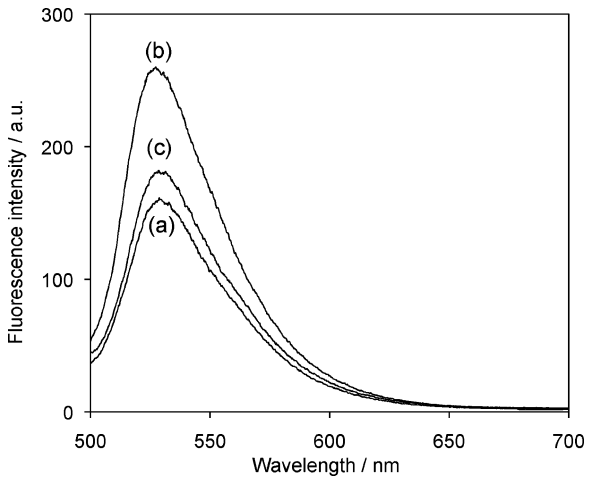

Fig. 4 Fluorescence spectra of PicoGreen on a liposome embedded with ONL-**1′** before (a) and after the addition of DNA signal **1** (b) or **2** (c) in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 35 °C. Concentrations, in μmol dm^{-3}: [$N^+C_5Ala2C_{16}$], 200; [DMPC], 800; [ONL-**1′**], 0.50; [**1**], 0.50; [**2**], 0.50; [PicoGreen], 0.20.

To further confirm hybridization on the liposomal surface, a similar experiment using fluorescence microscopy was conducted in which we replaced the small liposomes with giant liposomes. As shown in Fig. 5, no fluorescence emission from the giant liposome embedded with ONL-**1′** was observed in the absence of DNA signal **1**. When DNA signal **1** was added to the liposome, green fluorescence was observed from PicoGreen that had bound to the double-stranded DNA on the liposomal membrane. Thus, the liposomes modified with ONL-**1′** were shown to recognize the complementary DNA signal through hybridization.

Activation of enzyme by DNA signals

Based on the above results, supramolecular systems composed of ONL-**1′**, Cu^{2+} and LDH were constructed on liposomes formed with $N^+C_5Ala2C_{16}$ and DMPC. In this system, it was expected that the recognition of ssDNA with a complementary sequence would be converted to changes in enzymatic activity. Enzymatic activity was evaluated as a magnitude of the initial velocity in the presence of Cu^{2+} relative to that in the absence of Cu^{2+}. In the

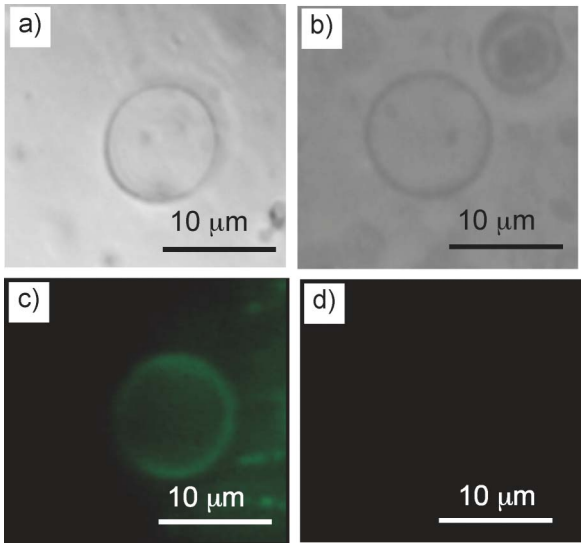

Fig. 5 Phase-contrast microscopic images of giant liposomes embedded with ONL-**1′** in the presence (a) and absence (b) of DNA signal **1** and the corresponding fluorescent images (c and d, respectively) in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0). Concentrations, in μmol dm^{-3}: [N^+C_5Ala2C_{16}], 200; [DMPC], 800; [ONL-**1′**], 0.50; [**1**], 0.50; [PicoGreen], 0.20.

absence of a DNA signal, the activity of LDH (2.8 nmol cm^{-3}) was inhibited by 4.0 μmol cm^{-3} Cu^{2+}, and exhibited 14% catalytic activity relative to the corresponding Cu^{2+}-free system (Table 3, entries 1 and 2). Upon addition of complementary DNA signal **1**, the relative activity of LDH recovered, despite the presence of Cu^{2+} (Table 3, entries 3 and 4), while the LDH activity was not affected by the addition of complementary DNA in the absence of Cu^{2+} (Table 3, entries 1 and 3). This increase in the LDH activity was considered to be due to an enhanced affinity of Cu^{2+} to the double-stranded DNA that formed between DNA signal **1** and ONL-**1′**. To examine the effect of the lipid membrane, ONL-**1′** was replaced with water-soluble DNA, **1′**, because hydrophobic ONL-**1′** is not soluble in aqueous buffer without the liposome (Table 3, entries 5–8).

It was also clearly shown that activation of the enzyme as a result of DNA hybridization was not observed in the absence of the liposome, with relative activities of 82% and 97% in the presence and absence of DNA signal **1**, respectively. This indicates that integration of the artificial receptor and the enzyme on the liposomal scaffold is essential to transfer the detected signal.

The system also exhibited marked signal selectivity (Fig. 6). LDH activity significantly increased to 82% upon the addition of DNA signal **4**, which has an additional 20 mer attached to the 3′ end of DNA signal **1** (39 mer in total), reflecting the enhanced binding affinity of Cu^{2+} to the complex of ONL-**1′** and DNA signal **4**. For non-complementary DNA signals **2** and **5**, the corresponding signal outputs were much smaller than those for complementary DNA signals. This indicates that the present system can detect the DNA signal with a partially matched sequence at its 5′ end. Interestingly, the enzymatic activity, as an output signal, can be applied to detect point mutations (single nucleotide polymorphisms, SNPs), which is consistent with single base mismatches having clearly measurable influences on hybridization processes.[18] The addition of DNA signals **3** or **6**,

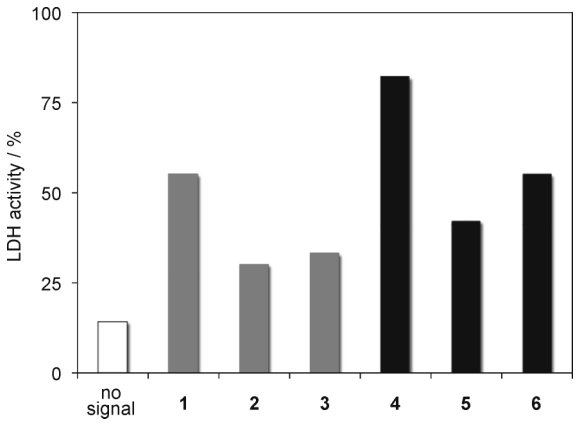

Fig. 6 Activation of LDH by the addition of DNA signals (**1–6**) to a liposome embedded with ONL-**1′** in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 35 °C. Concentrations, in μmol dm^{-3}: [N^+C_5Ala2C_{16}], 200; [DMPC], 800; [DNA signal], 0.50; [ONL -**1**], 0.50; [β-NADH], 0.25; [pyruvate], 0.50; [LDH], 2.8×10^{-6}. The initial velocity of the LDH catalyzed reduction of pyruvate to L-lactate was monitored spectrophotometrically by measuring the consumption of NADH as a coenzyme in the presence of Cu^{2+}. LDH activities were evaluated as the magnitude of v_0 in the presence of Cu^{2+} relative to that of the corresponding Cu^{2+}-free system.

Table 3 LDH activities in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 35 °C[a]

	Species/μmol cm^{-3}						
Entry	[Lipid]	[ONL-**1′**]	[**1′**]	[**1**]	[Cu^{2+}]	v_0 / nmol dm^{-3} s^{-1}[b]	Activity (%)[c]
1	1000	0.50	0	0	0	390	—
2	1000	0.50	0	0	4.0	55	14
3	1000	0.50	0	0.50	0	390	—
4	1000	0.50	0	0.50	4.0	210	55
5	0	0	0.50	0	0	410	—
6	0	0	0.50	0	4.0	340	82
7	0	0	0.50	0.50	0	410	—
8	0	0	0.50	0.50	4.0	400	97

[a] Concentrations, in μmol dm^{-3}: [Lipid] = [N^+C_5Ala2C_{16}] + [DMPC] (2 : 8), 1000; [β-NADH], 0.25; [pyruvate], 0.50; [LDH], 2.8×10^{-6}. [b] The initial velocity of the LDH catalyzed reduction of pyruvate to L-lactate was monitored spectrophotometrically by measuring the consumption of NADH as a coenzyme in the presence of Cu^{2+}. Values are accurate to within ±5%. [c] Activity was defined as the relative ratio of v_0 in the presence of Cu^{2+} ion to that of the corresponding Cu^{2+}-free system.

which contain single base mismatches, decreased LDH activity to 33% and 55%, respectively. These values are similar to those obtained using non-complementary DNA signals. The data clearly show that this system can detect the presence of single base mismatches in the complementary sequences.

We also confirmed that enzymatic activity was dependent on the Cu^{2+} concentration in the presence of complementary or non-complementary DNA signals, as shown in Fig. 7. In the presence of non-complementary DNA signal **5**, the enzymatic activity of LDH on the liposome embedded with ONL-**1′** was markedly decreased by increasing Cu^{2+} concentration, reaching a plateau at 8 μmol dm^{-3}. On the other hand, the magnitude of the decrease in enzymatic activity was significantly reduced in the presence of complementary DNA signal **4**, which caused an upward shift in the concentration of Cu^{2+} required to inhibit LDH activity. This shows that there is a window of Cu^{2+} concentration between 2 and 9 μmol dm^{-3} within which the activation of LDH activity is dependent on DNA hybridization. Further studies are required to better understand the activation/inactivation of enzymes in the presence of a liposome compared with that in a liposome-free system. However, our initial studies have demonstrated effective regulation of enzymatic activity *via* DNA hybridization on liposomal membranes.

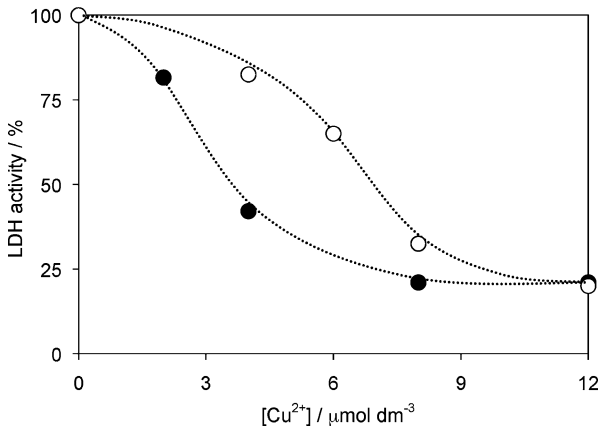

Fig. 7 Effects of Cu^{2+}-concentration on LDH activity on a liposome embedded with ONL-**1′** in the presence of complementary DNA signal **4** (open circle) or non-complementary DNA signal **5** (closed circle) in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) at 35 °C. Concentrations, in μmol dm^{-3}: [$N^+C_5Ala2C_{16}$], 200; [DMPC], 800; [DNA signal], 0.5; [ONL-**1**], 0.50; [β-NADH], 0.25; [pyruvate], 0.50; [LDH], 2.8×10^{-6}. The initial velocity of the LDH catalyzed reduction of pyruvate to L-lactate was monitored spectrophotometrically by measuring the consumption of NADH as a coenzyme in the presence of Cu^{2+}. Activity was defined as the relative ratio of v_0 in the presence of Cu^{2+} ion to that of the corresponding Cu^{2+}-free system.

Conclusions

We created a novel supramolecular system, in which enzymatic activity was activated *via* hybridization of DNA signals to oligonucleotide DNAs on a liposomal membrane, with Cu^{2+} as the mediator. The present system is a unique signal transmission device, in which the molecular recognition of a DNA signal by the receptor is transmitted to the enzyme and is chemically amplified by the catalytic reaction. More importantly, the present system possesses discrimination ability for Cu^{2+}-complexes of single-stranded and double-stranded DNA leading to potential application in SNPs detection. In future experiments, we plan to apply the current system to molecular communication,[19] a new communication paradigm that uses molecules as an information carrier and allows nanomachines (*e.g.*, nanoscale biological or artificially-created devices) to communicate over a short distance.

Experimental

Materials

A cationic peptide lipid, *N*,*N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$) was prepared as described previously.[20] 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was obtained from NOF Corporation, Japan. ONL-**1′** and all ssDNA signals (**1–6** and **1′**) were custom ordered from Nippon EGT, Japan and Eurogentec SA, Belgium, respectively. The following compounds were commercially available and were used without further purification: L-lactate dehydrogenase (LDH) from pig heart (Boehringer Mannheim Gmbh, Germany); β-nicotinamide adenine dinucleotide disodium salt (NADH) (Sigma-Aldrich, USA); sodium pyruvate (Wako Pure Chemical Industries, Japan); copper(II) perchlorate hexahydrate (Kanto Chemical, Japan); PicoGreen (Molecular Probes, USA). Other chemicals used were of analytical grade.

Preparation of liposomes

Liposomes were prepared according to an established protocol[1] as follows. A mixture of lipids containing $N^+C_5Ala2C_{16}$ and DMPC was dissolved in chloroform. The solvent was then evaporated under nitrogen gas flow and the residual trace solvent was completely removed *in vacuo*. Hydration of the resulting thin film on the vial wall was performed at 40 °C with an appropriate amount of pure water or with 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonate (HEPES) buffer (10 mmol dm^{-3}, pH 7.0) to set the concentration of $N^+C_5Ala2C_{16}$ and DMPC to 0.2 and 0.8 mmol dm^{-3}, respectively. Multi-walled bilayer vesicles were formed upon vortex mixing of the aqueous dispersion. Small liposomes were prepared by sonicating the dispersed sample using a cup-type sonicator (Sonifier 250D, Branson, Danbury, CT, USA) above the phase transition temperature for 20 min at 30 W. Before measurements, the liposomes were modified with DNA by incubating the liposome suspension with 0.5 μmol dm^{-3} of ONL-**1′** for at least 1 h to incorporate the hydrophobic cholesteryl group into the bilayer membrane of the liposomes.

Giant liposomes were prepared by gentle hydration of a lipid film. A chloroform solution containing appropriate amounts of the lipid mixture was dried under vacuum for 3 h to obtain a thin film. The film was then hydrated with water at 40 °C for 2 h. The liposome suspension was incubated at 25 °C for 1 h before microscopic observation. The concentrations of $N^+C_5Ala2C_{16}$ and DMPC were set to 0.2 and 0.8 mmol dm^{-3}, respectively. The giant liposomes were modified with ONL-**1′** as described above.

Measurements

Electronic absorption spectra were recorded on a Shimadzu UV-2400 spectrophotometer. Fluorescence spectra were recorded on

a Hitachi F-4500 spectrofluorometer. The D_{hy} of liposomes was measured using a Photal DLS-6000 dynamic light scattering (DLS) spectrophotometer equipped with a He-Ne laser at 633 nm. The time-course of the light scattering from the sample was analyzed by the cumulant method at an angle of 90° from the incident light. Microscopic observation was carried out using an Olympus IX71 fluorescence microscope, and images were acquired using an Olympus DP70 color CCD camera.

TEM observation

The size and morphology of liposomes were visualized by cryo-TEM (JEM-3100FEF, JEOL, Tokyo, Japan) operated at 300 kV. The samples were prepared by depositing one droplet of the liposomal suspension onto carbon-coated copper grids and excess liquid was blotted with filter paper. The remaining liposomal suspension was frozen in liquid ethane cooled to −180 °C. The specimen was mounted onto a Gatan Model 626DH cryo-holder and transferred to the microscope. The liposomes were embedded in a thin layer of vitreous ice and observed at −176 °C at a magnification of ×20000.

Enzyme assay

LDH enzymatic activity in an aqueous HEPES buffer (10 mmol dm^{-3}, pH 7.0) was determined at 30 °C using sodium pyruvate as the substrate. The assay sample solution (1 cm^{-3}) was prepared by mixing LDH (2.8 nmol dm^{-3}) and β-NADH (0.25 mmol dm^{-3}) in the presence and absence of an appropriate concentration of Cu^{2+} using liposomes generated from $N^{+}C_5Ala2C_{16}$ (0.2 mmol dm^{-3}) and DMPC (0.8 mmol dm^{-3}). The reaction was started upon the addition of pyruvate (0.50 mmol dm^{-3}). The catalytic activity of LDH was evaluated spectrophotometrically by measuring the consumption of β-NADH caused by the reduction of pyruvate to L-lactate. The molar extinction coefficient of β-NADH at 340 nm was 6220 $dm^3\ mol^{-1}\ cm^{-1}$.

Acknowledgements

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Notes and references

1 *Liposomes*, ed. V. P. Torchilin and V. Weissig, Oxford Univ. Press, Oxford, 2003; Y. Barenholz, *Curr. Opin. Colloid Interface Sci.*, 2001, **6**, 66; T. Lian and R. J. Y. Ho, *J. Pharm. Sci.*, 2001, **90**, 667; P.-A. Monnard, *J. Membr. Biol.*, 2003, **191**, 87; T. Kunitake, in *Comprehensive Supramolecular Chemistry*, ed. J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle and J. M. Lehn, Pergamon Press, Oxford, 1996, vol. 9, pp. 351; Y. Murakami and J. Kikuchi, in *Bioorganic Chemistry Frontiers*, ed. H. Dugas, Springer-Verlag, Berlin, 1991, vol. 2, pp. 73–113; H. Ringsdorf, B. Schlarb and J. Venzmer, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 113; A. Mueller and D. F. O'Brien, *Chem. Rev.*, 2002, **102**, 727.

2 G. W. Gokel, W. M. Leevy and M. E. Weber, *Chem. Rev.*, 2004, **104**, 2723; K. D. D. Mitchell and T. M. Fyles, in *Encyclopedia of Supramolecular Chemistry*, ed. J. L. Atwood and J. W. Steed, Marcel Dekker, New York, 2004, pp. 742–746; B. D. Smith and T. N. Lambert, *Chem. Commun.*, 2003, 2261; Y. Murakami, J. Kikuchi, Y. Hisaeda and O. Hayashida, *Chem. Rev.*, 1996, **96**, 721; D. Gust, T. A. Moore and A. L. Moore, *Acc. Chem. Res.*, 2001, **34**, 40; J. Kikuchi, Y. Sasaki and K. Ariga, in *Advances in Supramolecular Chemistry*, ed. G. W. Gokel, Cerberus Press, South Miami, vol. 8, 2002, pp. 131–173.

3 *Molecular Biology of the Cell*, ed. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, Garland Science, New York, 2002.

4 J. Kikuchi, K. Ariga and K. Ikeda, *Chem. Commun.*, 1999, 547; J. Kikuchi, K. Ariga, T. Miyazaki and K Ikeda, *Chem. Lett.*, 1999, **28**, 253; K. Fukuda, Y. Sasaki, K Ariga and J. Kikuchi, *J. Mol. Catal. B: Enzym.*, 2001, **11**, 971; J. Kikuchi, K. Ariga, Y. Sasaki and K. Ikeda, *J. Mol. Catal. B: Enzym.*, 2001, **11**, 977; W.-J. Tian, Y. Sasaki, A. Ikeda, J. Kikuchi and S.-D. Fan, *Chem. Lett.*, 2004, **33**, 226; W.-J. Tian, Y. Sasaki, A. Ikeda, J. Kikuchi, X.-M. Song and S.-D. Fan, *Acta Chim. Sinica*, 2004, **62**, 1230; Y. Sasaki, M. Yamada, T. Terashima, J.-F. Wang, M. Hashizume, S.-D. Fan and J. Kikuchi, *Kobunshi Ronbunshu*, 2004, **61**, 541; W.-J. Tian, Y. Sasaki, S.-D. Fan and J. Kikuchi, *Supramol. Chem.*, 2005, **17**, 113; W.-J. Tian, Y. Sasaki, S.-D. Fan and J. Kikuchi, *Bull. Chem. Soc. Jpn.*, 2005, **78**, 715.

5 P. Yin, H. M. Choi, C. R. Calvert and N. A. Pierce, *Nature*, 2008, **451**, 318.

6 D. Nykypanchuk, M. M. Maye, D. Van Der Lelie and O. Gang, *Nature*, 2008, **451**, 549; S. Y. Park, A. K. Lytton-Jean, B. Lee, S. Weigand, G. C. Schatz and C. A. Mirkin, *Nature*, 2008, **451**, 553.

7 Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang and C. Mao, *Nature*, 2008, **452**, 198.

8 E. Cheng, Y. Xing, P. Chen, Y. Yang, Y. Sun, D. Zhou, L. Xu, Q. Fan and D. Liu, *Angew. Chem., Int. Ed.*, 2009, **48**, 7660.

9 J. L. Sagripanti, P. L. Goering and A. Lamanna, *Toxicol. Appl. Pharmacol.*, 1991, **110**, 477.

10 Q. Wang, S. Li, W. Li, F. Gao and K. Jiao, *Bioelectrochemistry*, 2009, **75**, 32.

11 Y. Barenholz, *Curr. Opin. Colloid Interface Sci.*, 2001, **6**, 66; T. Lian and R. Y. J. Ho, *J. Pharm. Sci.*, 2001, **90**, 667; P. A. Monnard, *J. Membr. Biol.*, 2003, **191**, 87.

12 J. Kikuchi, Y. Kamijyo, H. Etoh and Y. Murakami, *Chem. Lett.*, 1996, **25**, 427.

13 Y. Sasaki, Y. Shioyama, W.-J. Tian, J. Kikuchi, S. Hiyama, Y. Moritani and T. Suda, *Biotechnol. Bioeng.*, 2010, **105**, 37.

14 W.-J. Tian, Y. Sasaki, S. D. Fan and J. Kikuchi, *Supramol. Chem.*, 2005, **17**, 113.

15 W. Saenger, in *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, 1984; D. E. Gilbert and J. Feigon, *Curr. Opin. Struct. Biol.*, 1999, **9**, 305; G. Varani, *Annu. Rev. Biophys. Biomol. Struct.*, 1995, **24**, 379.

16 R. B. Wallace, J. Shaffer, R. F. Murphy, J. Bonner, T. Hirose and K. Itakura, *Nucleic Acids Res.*, 1979, **6**, 3543.

17 G. Zimmer, G. Luck, H. Fritzche and H. Tribel, *Biopolymers*, 1971, **10**, 441; H. Richard, J. P. Schereiber and M. Daune, *Biopolymers*, 1973, **12**, 1.

18 J. B. Fiche, J. Fuchs, A. Buhot, R. Calemczuk and T. Livache, *Anal. Chem.*, 2008, **80**, 1049; K. Tawa and W. Knoll, *Nucleic Acids Res.*, 2004, **32**, 2372.

19 M. Mukai, K. Maruo, J. Kikuchi, Y. Sasaki, S. Hiyama, Y. Moritani and T. Suda, *Supramol. Chem.*, 2009, **50**, 541.

20 J. Kikuchi, K. Ariga, Y. Sasaki and K. Ikeda, *J. Mol. Catal. B: Enzym.*, 2001, **11**, 977.

