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Dynamic behavior of a transmembrane molecular switch as an artificial cell-surface receptor

Kentaro Fukuda, Yoshihiro Sasaki, Katsuhiko Ariga, Jun-ichi Kikuchi *

Graduate School of Materials Science, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-0101, Japan

Abstract

Supramolecular functions of a novel synthetic amphiphile with an azobenzene core interposed between two aminoalkyl chains as an artificial transmembrane receptor were investigated. Hybrid vesicular membrane was prepared in combination with the receptor and a bilayer-forming lipid dimyristoylphosphatidylcholine (DMPC). Aggregation behavior of the receptor embedded in the bilayer membrane was evaluated spectrophotometrically by monitoring stacking of the azobenzene moiety. Upon addition of 1-hydroxy-2-naphthaldehyde as an external signal ligand, it was effectively recognized at both ends of the receptor by forming the corresponding Schiff's base, and followed by phase reorganization of the receptor in the membrane. The resulting receptor–ligand complex provided an appropriate binding site for copper(II) ions, which may act as a second messenger signal. Signal transduction behavior of the present supramolecular system was examined by employing NADH-dependent lactate dehydrogenase (LDH) as an effector. We found that the transmembrane receptor is capable of performing as a molecular switch for the enzymatic reaction.

Keywords: Molecular switch; Signal transduction; Lipid bilayer; Azobenzene derivative; Artificial cell-surface receptor

1. Introduction

Signal transduction, or cell communication is simply the mean by which natural cells respond to signals coming from outsides. The purpose of the signal transduction is functional coordination within the cell, between cells or between organs, allowing them to respond to external environment. Many studies have been carried out to understand mechanisms that mediate inter- or intra-cellular signal transduction. Up to the present time, it has been clear that signal transduction is generally accomplished by way of one or more of the following processes: receptor–ligand interaction, ion channeling, second messenger pathway, and transcription of a desired gene product [1]. However, receptor functions remain to be clarified at the molecular level with emphasis on how external signals are recognized, received and transported through the cell membrane to target molecules inside the cells. Thus, the approach to illustrate the principle of signal transduction based on molecular recognition by artificial receptors is one of the most attractive subjects in supramolecular chemistry [2].

So far, we have attempted to simulate and simplify the signal transduction system [3–6], which involves ligand–receptor interaction and G-protein-linked pathway. In this process, binding of an exter-

* Corresponding author. Tel.: +81-743-72-6090; fax: +81-743-72-6099.

E-mail address: jkikuchi@ms.aist-nara.ac.jp (J. Kikuchi).

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nal signal to a receptor embedded in the cell membrane results in activation of the G-protein as a second messenger, which conveys the message to the next effector such as an enzyme in the signaling pathway. Various types of artificial receptors capable of recognizing organic signaling ligands in bilayer membranes have been developed in our research group. For example, a bile acid derivative having an amino group as an artificial cell-surface receptor transmits an external signal to an enzyme in collaboration with a transmitter [6]. The bile acid derivative acts as a host capable of recognizing both aromatic aldehydes and copper(II) ions. On the other hand, lactate dehydrogenase (LDH) binds to the bilayer membrane surface [7,8] and the LDH activity is specifically inhibited by copper(II) ions. Thus, we have successfully simulated signal transduction on the lipid membrane by harmonic constitution of the following components: an artificial receptor, an aromatic aldehyde, a bilayer-forming lipid, copper(II) ions and LDH.

In living cells, however, most signal transduction involves more complex events. For example, cell-surface receptor proteins act as signal transducers. They bind the signaling ligand and convert this extracellular event into one or more intracellular signals that alter the cell function. A kind of G-protein linked receptor responds to light as photoreceptor; rhodopsin receives a photon of light and transduces that to an electrical signal [1]. In order to construct a more organized supramolecular system focused on the transmission of an extra-vesicular signal to the inside of a membrane vesicle and the photo-sensitivity, we have designed and synthesized a novel ampihiphile $Azo(C_{12}N)_2$ as an artificial transmembrane receptor (Fig. 1). This molecule is composed of two amino groups as an external signal binding site and an intracellular regulating site, an azobenzene core as a photosensitive part, and alkyl chains as transmembrane parts. Aggregation behavior and microenvironment of the receptor in membrane can be evaluated spectrophotometrically by monitoring stacking of the azobenzene moiety [9]. In addition, the aggregation of the azobenzene moiety, which affects the affinity towards the signal molecules, can be controlled by irradiation of light, since azobenzene derivatives generally cause reversible conformational changes through photo-induced *cis/trans* isomerization. The reversibility of signaling in cells is another important characteristic for signal transduction. Thus, the isomerization of azobenzene moiety in the artificial receptor may enable to control the signal transduction flow reversibly. In this paper, we report basic behavior of signal transduction ability of the system proposed in Fig. 1. Such an artificial signal transduction system may find novel application in numerous fields, such as medicine, diagnostics, nanotechnology, and artificial intelligence.

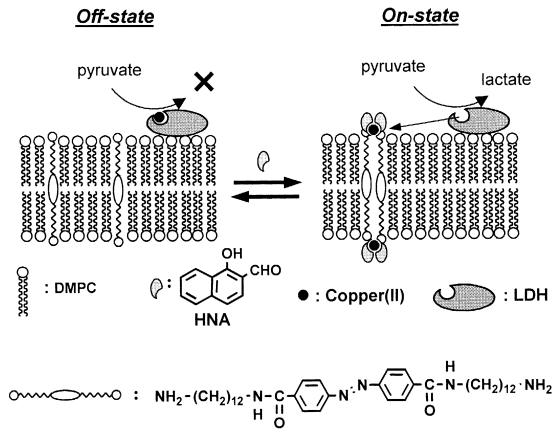

Fig. 1. Schematic representation of dynamic regulation of reversible signaling by an azobenzene-containing artificial cell-surface receptor.

2. Experimental

2.1. Synthesis

An artificial receptor 4,4′-azobis[*N*-(12-aminododecyl)benzamide] [$Azo(C_{12}N)_2$] was synthesized as described below. Reagents and all other chemicals are commercially available and were used without further purification unless otherwise stated. Azobenzene-4,4′-dicarboxylic acid (0.60 g, 2 mmol) prepared according to the literature [10], was transformed to corresponding diacid chloride by refluxing in $SOCl_2$/benzene for 12 h. The product dissolved in chloroform was added dropwise to a chloroform solution of 1,12-diaminododecane (8.0 g, 40 mmol) over 1 h and the solution was stirred for 3 h at room

temperature. The obtained precipitates were collected and dissolved into small amount of trifluoroacetic acid (TFA). The resulting TFA salts were purified by gel permeation chromatography to give the final product Azo($C_{12}N$)$_2$: Yield, 51 mg (3%); mp 276.2–277.5°C. ^{1}H NMR [400 MHz, $CDCl_3/CD_3OD$ (1:1 v/v)] δ 1.34 (32H, m, $N^+(CH_2)_4(CH_2)_8$), 1.66 (8H, m, $N^+CH_2CH_2(CH_2)_8CH_2$), 2.89 (4H, $J = 7.2$ Hz, t, N^+CH_2), 3.42 (4H, $J = 7.2$ Hz, t, $N^+(CH_2)_{11}CH_2$), 8.00 (8H, s, aromatic). Anal. Calcd for $C_{42}H_{64}N_6O_6$: C, 58.45; H, 7.48; N, 9.74%. Found: C, 57.91; H, 7.41; N, 9.53%.

2.2. Preparation of bilayer vesicle containing artificial receptor

Dimyristoylphosphatidylcholine (DMPC) was purchased from The Liposome. Bilayer vesicles were prepared according to established protocols [11,12]. An appropriate amount of Azo($C_{12}N$)$_2$ and DMPC were dissolved in $CHCl_3/CH_3OH$ (1:1 v/v). The solvent was evaporated under nitrogen gas flow and residual trace of solvent was completely removed in vacuo. The thus obtained thin film on the wall of a vial was dispersed in aqueous HEPES buffer (10 mM, pH 7.0) and incubated on a water bath at 40°C for 20 min. The dispersion sample was vortexed for 5 min and then sonicated with a bath-type sonicator at 40–45°C for 1 h to give unilamellar vesicles. The vesicular solution was stored at 40°C.

2.3. Physical measurements

Schiff's base formation and aggregation behavior of Azo($C_{12}N$)$_2$ were evaluated by electronic absorption spectroscopy. The signal transduction behavior was evaluated according to the following protocols. The stock solutions of 1-hydroxy-2-naphthoaldehyde (HNA) and $Cu(ClO_4)_2$ were added to the vesicular solution, and incubated at 40°C for 12 h to equilibrate the solution. Then the stock solutions of reduced form of nicotinamide adenine dinucleotide (NADH, Sigma) and LDH (from pig heart, Boehringer Mannheim) were added to the solution. After 3-min incubation, the reaction was started by addition of the stock solution of sodium pyruvate. The catalytic activity of LDH was monitored following the consumption rate of NADH at 370 nm. The LDH activity was evaluated as magnitude of initial velocity for the reaction in the presence of $Cu(ClO_4)_2$ relative to that of the corresponding metal-free system.

3. Results and discussions

Azobenzene-containing molecules often self-assemble to form supramolecular structures with two- or three-dimensional order accompanying spectral changes upon aggregation of the chromophore. While formation of both H- and J-type aggregate of the azobenzene derivative has been reported [9,13], it seems that the former type of aggregate is generally stable in lipid bilayer membrane [9,14]. First, we examined the aggregation behavior of Azo($C_{12}N$)$_2$ in the DMPC bilayer by means of electronic absorption spectroscopy. The absorption spectra of Azo($C_{12}N$)$_2$ in various media at 40°C are shown in Fig. 2. The prominent absorption band at 331 nm due to π–π transition is characteristic of the monomeric *trans*-form of the azobenzene chromophore in organic solvent (Fig. 2a). In the bilayer membrane Azo($C_{12}N$)$_2$ exhibited the analogous absorption maximum at 334 nm, although a little increase of baseline according to light scattering from the vesicular aggregate was overlapped (Fig. 2b). In contrast, remarkable decrease in the absorbance at

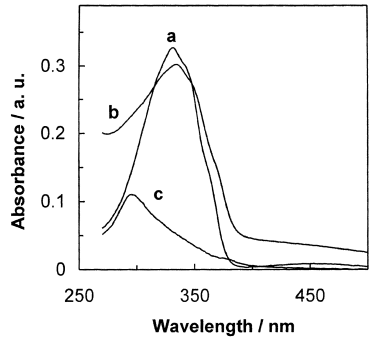

Fig. 2. Absorption spectra of Azo($C_{12}N$)$_2$ (10 μM) in various media at 40°C: (a) in $CHCl_3/CH_3OH$ (1/1 v/v); (b) in 1.0 mM DMPC vesicle dispersion; (c) in 10 mM HEPES buffer (pH 7.0).

334 nm and blue shift of the absorption maximum to 296 nm was observed for Azo($C_{12}N)_2$ dispersed in aqueous HEPES buffer in the absence of DMPC, reflecting the formation of the H-type aggregate [15] (Fig. 2c). Temperature dependence of the absorption spectrum of Azo($C_{12}N)_2$ in the presence of DMPC was also examined in a range of 15–50°C. When the vesicular solution was cooled down, the absorbance at 334 nm was drastically decreased with concomitant increase in absorption at 316 nm in a temperature region between 35°C and 25°C, reflecting formation of the H-type aggregate. The spectral changes were not observed in temperature ranges above 35°C and below 25°C. Since pure DMPC vesicles have a phase transition from gel to lipid-crystalline phase near 23°C [16], the results suggest that the aggregation behavior of Azo($C_{12}N)_2$ in the DMPC vesicle depends on the phase transition of the membrane. Thus, it is clear that the Azo($C_{12}N)_2$ molecules are embedded in the DMPC bilayer in the monomeric state above 35°C.

Next, we examined the molecular recognition behavior of Azo($C_{12}N)_2$ as a receptor at 40°C. HNA as an external signal was effectively bound to the receptor in the DMPC vesicle by forming an imine bond between the formyl group and the animo group, as confirmed by electronic absorption spectroscopy (Fig. 3A). The increase in absorption at 440 nm and the concomitant decrease in absorbance at 340 nm reflect the Schiff's base formation and the followed aggregation of Azo($C_{12}N)_2$ in the bilayer membrane, respectively. Such aggregation would be induced by relaxation of electrostatic repulsion between positively charged amino groups due to the Schiff's base formation of Azo($C_{12}N)_2$ with HNA. On the other hands, dodecyl amine ($C_{12}N$) as a control compound lacking an azobenzene chromophore did not show a change in absorbance at 340 nm upon addition of HNA, while the corresponding Schiff's base with absorption at 440 nm was formed. Time courses of the Schiff's base formation and the aggregation of the azobenzene chromophore are shown in Fig. 3B. The results clearly indicate that the Schiff's base formation induces slow phase reorganization of Azo($C_{12}N)_2$ in the DMPC vesicle as depicted in Fig. 1.

In signal transduction at the cell surface, G-protein acts as a signal transmitter operating between a

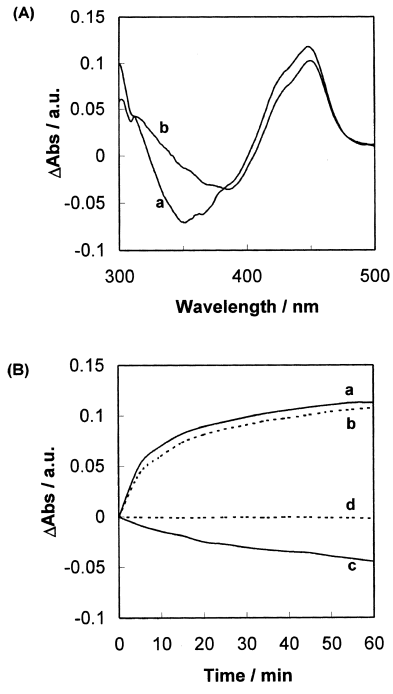

Fig. 3. Spectral changes reflecting Schiff's base formation between amino group and HNA followed by phase reorganization. (A) Differential spectra for Azo($C_{12}N)_2$ (a) and $C_{12}N$ (b). ΔAbs means the difference of absorbance in the presence (Abs_e) and the absence (Abs_0) of HNA ($\Delta Abs = Abs_e - Abs_0$). (B) Time courses of ΔAbs values: (a) for Azo($C_{12}N)_2$ at 440 nm; (b) for $C_{12}N$ at 440 nm; (c) for Azo($C_{12}N)_2$ at 340 nm; (d) for $C_{12}N$ at 340 nm. ΔAbs at 440 nm indicates Schiff's base formation, and ΔAbs at 340 nm reflects degree of H-type aggregation.

receptor and an effector. In order to constitute an artificial signal transduction system mimicking the G-protein mediated cell signaling, the following features are required for an artificial receptor: (1) The receptor embedded in lipid bilayer is capable of recognizing both an external signal ligand and a signal transmitter. (2) The recognition of an external signal ligand induces the change of binding affinity of the receptor toward a signal transmitter. The present Schiff's base of Azo($C_{12}N)_2$ with HNA can bind various metal ions due to formation of the corresponding metal chelates. In practice, the quantitative binding of copper(II) ions to the Schiff's base was also confirmed by the corresponding absorption maximum at 388 nm while metal binding affinity of the unidentate amino group of Azo($C_{12}N)_2$ seems to

be extremely weak in the absence of HNA. Thus, Azo($C_{12}N$)$_2$ can be regarded as an artificial host capable of recognizing both HNA and copper(II) ion on the bilayer membranes and they might act as an external ligand and an effective signal transmitter, respectively. On these grounds, an NADH-dependent LDH, which catalyzes transformation between pyruvate and lactate, was employed as an effector in the present artificial signal transduction system. The LDH activity was effectively inhibited upon addition of copper(II) ions. In addition, it has been already reported that LDH is bound to the membrane surface of the phopholipid vesicles [7,8]. Thus, we now constituted an artificial signal transduction system by employing DMPC, Azo($C_{12}N$)$_2$, LDH, HNA and copper(II) ions as schematically shown in Fig. 1. The catalytic activity of LDH in the reduction of pyruvate to lactate was evaluated spectrophotometrically by following a consumption rate of NADH [17] in the presence and absence of various supramolecular elements. In the absence of copper(II) ions, the LDH activity was not significantly influenced by the presence of receptor, signal molecule, and matrix lipid. When 2 μM of copper(II) ions was added to the solution of LDH in the presence of DMPC vesicle, LDH was completely inhibited by binding of copper(II) ions to the enzyme active site (Table 1). Upon addition of Azo($C_{12}N$)$_2$ or HNA to this system, the enzyme activity was slightly increased because of the amino group of Azo($C_{12}N$)$_2$ or HNA acts as a metal binding site. When we constituted supramolecular system composed of all elements shown in Fig. 1, LDH exhibited the full catalytic activity relative to the corresponding metal-free system. Since reactivation of LDH was not significant in the absence of the receptor or the external signal, such recovery of the enzymatic activity would come from the enhanced metal-binding ability of the receptor-ligand complex. From these results, it is clarified that the signal transduction occurs with extremely high efficiency on the membrane surface of the hybrid molecular assembly depicted in Fig. 1.

Table 1
LDH activity (%) of artificial signal transduction system[a]

Receptor	External signal	
	None	HNA
None	0	7
Azo($C_{12}N$)$_2$	5	100

[a] In aqueous HEPES buffer (10 mM, pH 7.0) at 40°C. Concentrations: Azo($C_{12}N$)$_2$, 10 μM; HNA, 20 μM; Cu(ClO_4)$_2$, 2 μM; LDH, 0.1 mg/ml; pyruvate, 150 μM; NADH, 200 μM; DMPC, 1 mM. The LDH activity represents magnitude of initial velocity for the enzyme reaction in the presence of Cu(ClO_4)$_2$ relative to that of the corresponding metal-free system. Each value is mean of at least duplicated runs and accurate within 3%.

4. Conclusions

We demonstrated here, by the chemical means, the construction of an artificial signal transduction system in which a transmembrane type receptor is able to switch on enzymatic activity through phase reorganization of receptor in the bilayer membrane and double signal transduction mediated by HNA as an external signal and copper(II) ion as a signal transmitter. Shimomura and Kunitake [9] have reported that reversible transformation between an aggregated *trans*-azobenzene derivative and its monomeric *cis*-form could be achieved by *cis/trans* photoisomerization in bilayer membrane. Accordingly, the present novel type of the azobenzene receptor could possibly also control the reversible signaling through mutual transformation between aggregated and monomeric states by photo induced *cis/trans* isomerization of the azobenzene chromophore. Although reversible and transmembrane signaling is an unsettled problem at the present, the results obtained here may provide a useful guidepost for designing more intelligent signaling systems, which are applicable to the development of supramolecular nano-scale devices.

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