Urea Sensitive Field Effect Transistors Based on Dithio Group-Containing Langmuir–Blodgett Membranes

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Synopsis. 2-(Octadecyldithio)pyridine (**1**) and 5-(octadecyldithio)-2-nitrobenzoic acid (**2**) were synthesized in order to prepare Langmuir–Blodgett (LB) membranes which possess reactive dithio groups on the surface for further modification with enzymes. The monolayer membrane, composed of **1** or **2**, was deposited successfully on an ion-sensitive field effect transistor, and the membrane surface was modified with urease covalently by a thiol–disulfide exchange reaction. The LB membrane-based sensor **2** showed a better potentiometric response to urea than that of the membrane-based sensor **1**. The results are discussed on the basis of the reactivity of the dithio groups to urease at the membrane surface.

Recently, much attention has been devoted to the development of biosensors based on Langmuir–Blodgett (LB) membranes, because of the advantages they offer in the form of thinness, uniform thickness, and controlled molecular orientation.[1] We have already reported that a penicillin sensor can be constructed by immobilizing penicillinase through adsorption on the surface of LB membrane deposited on the gate surface of an H^+-ion-sensitive field effect transistor (pH-ISFET).[2,3] Penicillinase, fortunately, can be tightly adsorbed, through electrostatic and/or hydrophobic force of attraction, to the surface of the LB membrane. However, it is not always possible for enzymes to be adsorbed to the LB membrane surface without deactivation. In fact, we have observed poor reproducibility in potentiometric responses of the pH-ISFETs covered with LB membranes adsorbing urease, trypsin, and α-chymotrypsin, presumably on account of the desorption or deactivation of the enzymes. For these reasons, we have developed the reactive LB membranes which can bind enzymes covalently.[4,5] Watanabe et al. also used reactive LB membranes for enzyme sensor preparation.[6]

The present paper reports the preparation of dithio group-containing reactive LB membranes and their application to the ISFET urea sensors.

Experimental

Materials. 1-Octadecanethiol, di-(2-pyridyl)disulfide, and 6,6-dinitro-3,3′-dithiodibenzoic acid were purchased from the Tokyo Kasei Co. Urease (EC 3.5.1.5) was obtained from the Sigma Co. The amphiphile **1** was prepared by a coupling reaction of 1-octadecanethiol (2 g) and di-(2-pyridyl)disulfide (1.6 g) in dichloromethane (20 ml) at room temperature for 16 h. The crude product was washed twice with water and then applied to a silica-gel column chromatograph with dichloromethane as the eluent. It was finally purified by repeated recrystallization from methanol–water. Yield: 1.2 g (44%). Mp 42—43 °C. Found: C, 69.73; H, 10.40; N, 3.47; S, 16.36%. Calcd for $C_{23}H_{41}NS_2$: C, 69.83; H, 10.54; N, 3.54; S, 16.18%. For the synthesis of **2**, a chloroform solution (30 ml) of 1-octadecanethiol (3 g), 6,6′-dinitro-3,3′-dithiodibenzoic acid (4.2 g), and triethylamine (5 ml) was stirred for 5 h. The

amphiphile **2** was purified by column chromatograph and recrystallization. Yield: 2.4 g (47%). Mp 62—63 °C. Found: C, 62.08; H, 8.55; N, 2.90; S, 13.23%. Calcd for $C_{25}H_{41}NO_4S_2$: C, 61.85; H 8.49; N, 2.83; S, 13.20%.

Sensor Fabrication. A benzene solution (0.1 w/v%) of **1** or **2** was spread on a pure water subphase in a Langmuir trough (Kyowa Kaimenkagaku Co.) at 17 °C, and the surface monolayer was compressed at the rate of 0.4 $cm^2 min^{-1}$ up to a surface pressure of 20 $mN\,m^{-1}$ for **1** or 30 $mN\,m^{-1}$ for **2**. Monolayer **1** or **2** was deposited on the gate surface of the pH-ISFET precoated with an octadecanoic acid LB membrane (1, 3, or 5 layers), according to the reported procedure,[3] by dipping the probe vertically through the monolayer at a rate of 0.4 $cm\,min^{-1}$. After the remaining surface monolayer had been removed from the water subphase, the probe was raised. By this kind of treatment, the dithio groups should be located at the uppermost surface of the deposited LB layer. Thus, the surface will be reactive to protein containing free mercapto groups such as urease (Fig. 1). The deposition ratios of **1** and **2** LB membranes were elucidated by the use of glass plates precoated with a 1-layer octadecanoic acid LB membrane, since the small size of the pH-ISFET inhibited precise measurement of the deposition ratio.

The LB membrane-coated pH-ISFET prepared in the above manner, was immersed in a 0.01 mM (1 M=1 mol dm^{-3}) urease solution (100 mM tris(hydroxymethyl)aminomethane–HCl buffer containing 100 mM KCl and 1 mM EDTA, pH 7.2) for 1 h at 23 °C, in order to immobilize urease on the LB membrane surface. The ISFET urea sensors so prepared were rinsed thoroughly with the working buffer before use.

The procedures for the fabrication and the electric property of the bare pH-ISFET used in this study are described elsewhere.[7]

Results and Discussion

Figure 2 shows surface pressure/area isotherms (π–A isotherms) of **1** and **2** spread on a pure water subphase at 17 °C. Both **1** and **2** gave condensed phase monolayers at the air/water interface, with the occupied area of ca. 29 $Å^2$/molecule for **1** and ca. 35 $Å^2$/molecule for **2**. The bulky head group (nitrobenzoic acid) in **2** may be responsible for the larger value of its occupied area. The monolayer **2** gave a higher collapse pressure than
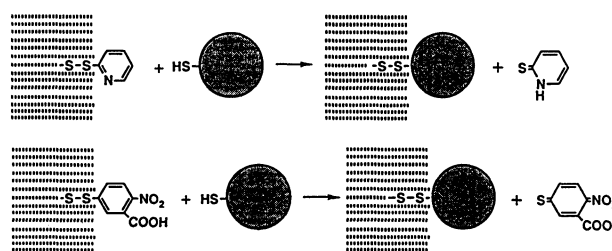

Fig. 1. A schematic illustration of enzyme immobilization on the surface of LB membranes composed of disulfide amphiphiles.
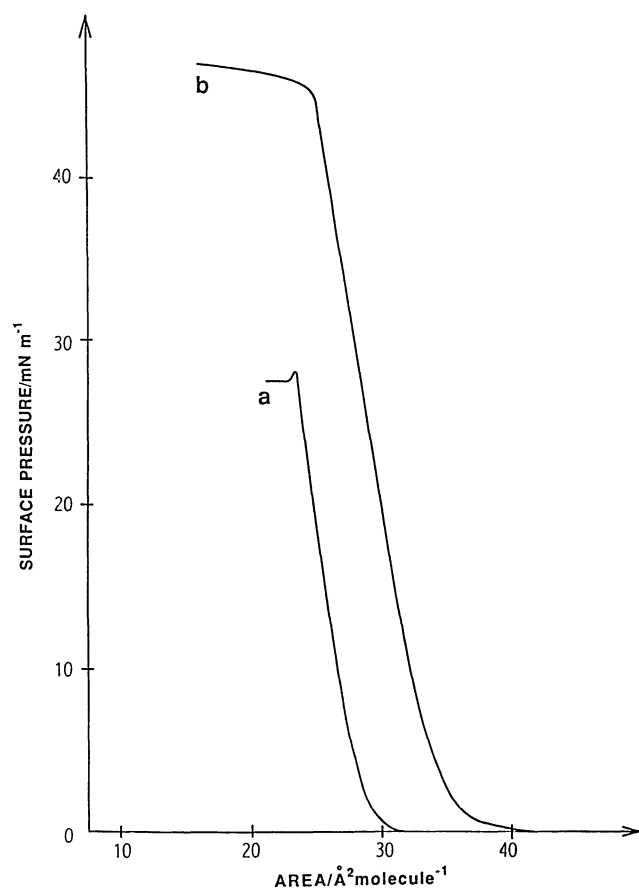
Fig. 2. The π-A isotherms of the **1** (a) and **2** (b) monolayers on pure water at 17 °C.
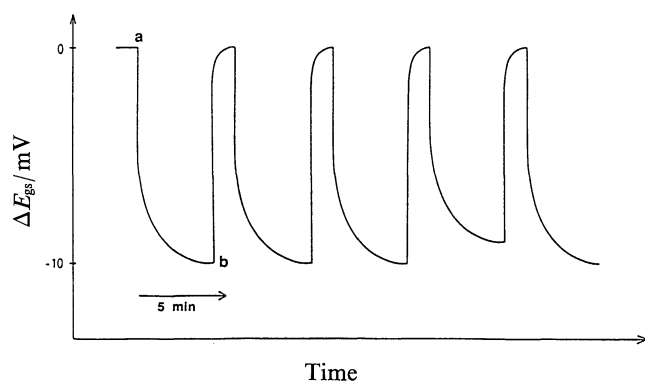

Fig. 3. A typical response curve of the urea sensor prepared with the LB membrane **2**. The prove was immersed in the urea solution (10 mM) at (a) and in the buffer solution at (b). 2 mM KH$_2$PO$_4$ buffer containing 1 mM EDTA (pH 6.0) was used.

monolayers **1** and **2** can be deposited successfully with the dithio groups located at the uppermost surface on the gate of the pH-ISFET precoated with the octadecanoic acid LB membrane.

Figure 3 shows a typical response curve for the ISFET urea sensor prepared with the LB membrane **2** by the use of an ISFET precoated with a 1-layer octadecanoic acid LB membrane. The sensor exhibited a potentiometric response, ΔE_{gs}, to urea, confirming that the urease was immobilized without deactivation and catalyzed the hydrolysis of urea, while changing the pH value at the gate surface of the pH-ISFET as follows:

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow[\text{urease}]{} 2NH_4^+ + HCO_3^-$$
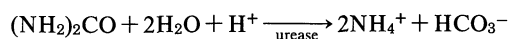
During five successive measurements, the potentiometric response was fairly reproducible, demonstrating clearly the effectiveness of the thiol–disulfide exchange reaction to immobilize urease covalently. However, the sensor response was deteriorated gradually after ten successive measurements.

The ISFET probes precoated with 3- and 5-layer octadecanoic acid LB membranes were also used for urea sensor fabrication. The potentiometric response was suppressed by increasing the thickness of the LB layer. The response of the sensor depended significantly on the buffer capacity of the sample solution. The useful calibration graphs could be obtained in the buffers of lower than 10 mM KH$_2$PO$_4$. These properties of the LB membrane-based sensors are the common case for the potentiometric enzyme sensors reported so far.[1,3,8]

It is interesting to clarify the effects of the different chemical structures of the hydrophilic head groups in **1** and **2** on the potentiometric and enzyme-binding properties of the LB membranes. Firstly, it was checked

monolayer **1**, probably as a result of the stabilizing effect of hydrogen bonding between carboxyl groups in the nitrobenzoic acid moieties.

The deposition ratios of the monolayers **1** and **2** were measured by dipping the glass plate with hydrophobic surface into the pure water subphase through the monolayer at 20 mN m^{-1} for **1** and 30 mN m^{-1} for **2**. The deposition ratios, thus measured, were ca. 1.0 for both the monolayers **1** and **2**. This result suggests that the
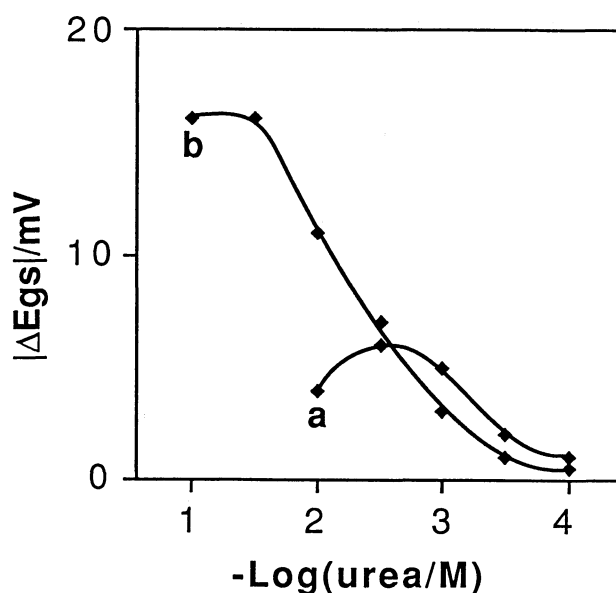

Fig. 4. Typical calibration graphs of the urease-immobilized pH-ISFETs to urea. The sensors were prepared with LB membranes **1** (a) or **2** (b), deposited on the 1-layer octadecanoic acid LB membrane. 2 mM KH$_2$PO$_4$ buffer containing 1 mM EDTA (pH 6.0) was used.

that the LB membrane-coated ISFETs with both **1** and **2** show almost the same pH response with each other (45—50 mV/pH).   Figure 4 shows the typical calibration graphs for the ISFET urea sensors based on LB membranes **1** and **2**.   The potentiometric response of the sensor prepared with LB membrane **2** was higher than that for **1**-based sensor.   The dynamic range of the **2**-based sensor was 0.3—30 mM urea.   This range is comparable to those of the urea sensors based on a urease–albumin crosslinked membrane.[8,9]   The low response of the membrane sensor **1** may stem from the low enzyme loading on the gate surface, which, in turn, probably results from the limited reactivity of LB membrane **1** with mercapto groups in urease.

Thus, we have shown that 5-(octadecyldithio)-2-nitrobenzoic acid is a useful material for the preparation of a reactive LB membrane and it can bind an enzyme covalently to its surface, and so is useful as a biosensor application.

References

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