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Photo-Switched Current through Bilayer Lipid Membrane Containing Spirobenzopyran

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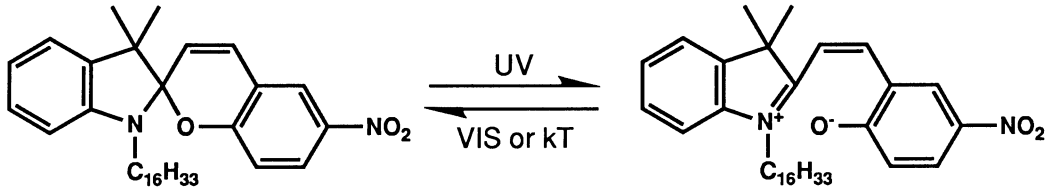
The current through the bilayer lipid membrane (BLM) formed from glyceryl-1-monoolein (GMO) and spirobenzopyran with a long alkyl chain (**1**) increased upon UV light irradiation and decreased upon subsequent irradiation of visible light during the voltage application. This photoinduced increase in the current suggests that defects in BLM are formed as pass ways for ions associated with the conformational change of **1**.

Spirobenzopyran and its derivatives are known to show photochromism (Scheme 1), and they have been embedded in various kinds of membranes such as monolayers,[1] Langmuir-Blodgett multilayers,[2] acetyl cellulose membrane,[3] poly(vinyl chloride) membrane,[4] poly(methacrylic acid) membrane,[5] thin films on glass plate,[6] and other polymer matrices.[7] Photoresponse of the vesicles containing spirobenzopyran,[8] which were spherical bilayer lipid membrane, was also studied by spectroscopic measurements. On the other hand, another representative bilayer lipid membrane, that is, planar bilayer lipid membrane (BLM)[9,10] is suitable for electrochemical measurements. We first report here the photo-switchable current through BLM containing **1** during the voltage application. This photoresponsive behavior of the BLM system may be useful to get insight into structural and functional features of bilayer membranes.



Closed-**1** Opened-**1**

Scheme 1.

Membrane current was measured by using the setup shown in Fig. 1.[11] The cell was separated into two aqueous compartments. A thin Teflon sheet (thickness 25

μm) with a hole (diameter 120 μm) was sandwiched between the compartments. In each compartment a Ag/AgCl electrode with an agar gel bridge was immersed. The electrodes were hidden from the light. They were connected to the voltage-clamp amplifier (EPC7: List Electronic). The computer (Macintosh II: Apple Computer, Inc.) applied the voltage on the membrane and collected the current signal via the amplifier. As BLM has considerably high resistance (10^6-10^8 Ωcm^{-2}),[10] the voltage applied across the membrane was almost the same as that between the electrodes. The light source was 500 W Xenon lamp (UI501C: Ushio Electronic, Inc.) with a glass filter Toshiba UV-D33S (250-390 nm) or Toshiba Y48 (>480 nm). The light was illuminated from the electrically ground side.

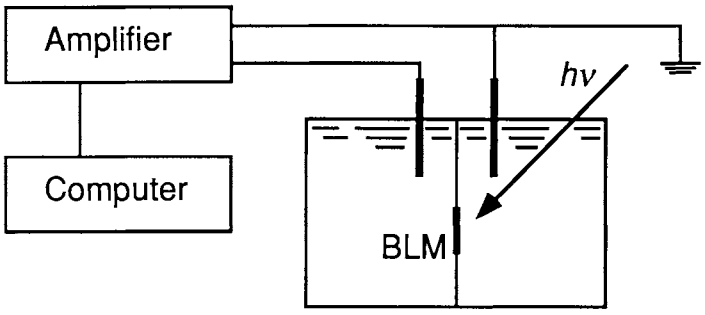


Fig. 1. Diagram of the experimental setup.

BLM was formed by the technique of Montal and Mueller.[10] Before forming BLM, the hole on the Teflon thin film was pretreated with 0.4 μl of 1 vol% hexadecane/hexane solution in each side. Each compartment was filled with aqueous solution (100 mmoldm^{-3} KCl, 0.1 mmol dm^{-3} EDTA, buffered with 2 mmoldm^{-3} 2-(*N*-morpholino)ethanesulfonic acid - KOH: pH 6.2) below the hole and 10 μl of lipid/hexane solution, which contained 1%(w/v) glyceryl-1-monoolein (GMO: Tokyo Kasei Co.) and 0.025%(w/v) **1**, was dispersed. **1** was previously prepared by Dr. Anzai[4] and gifted to us. After the evaporation of hexane, the surface levels of the solution were raised above the hole and then BLM was formed. The formation of BLM was confirmed by the observation of single ion-channel activity of gramicidin under the same conditions as described here. The experiments were performed at 23±1 °C.

A typical photoresponse of the current is shown in Fig. 2. The current increased upon UV light irradiation and decreased upon irradiation of visible light. Irradiation of visible light could not completely return the current to the original level. When BLM was kept in the dark after the UV irradiation, the increase in the current still continued for several tens of seconds and then the current gradually decreased. The spontaneous thermal reaction from opened form to closed one of **1** may be reflected in the gradual decrease in the current.

Fig. 3 shows the voltage dependence of the current. The voltage-current curve after the UV light irradiation was not linear. Although the light was illuminated from one side of the membrane, it was symmetric centering around the origin. It is noteworthy that the shape of the curve is similar to that mediated by ion-carrier such as nonactin.[12] The concave curve in Fig. 3 suggests that the behavior of cations and/or anions which flow through BLM was affected by the surrounding electric field or the structure of the pass way.[13]

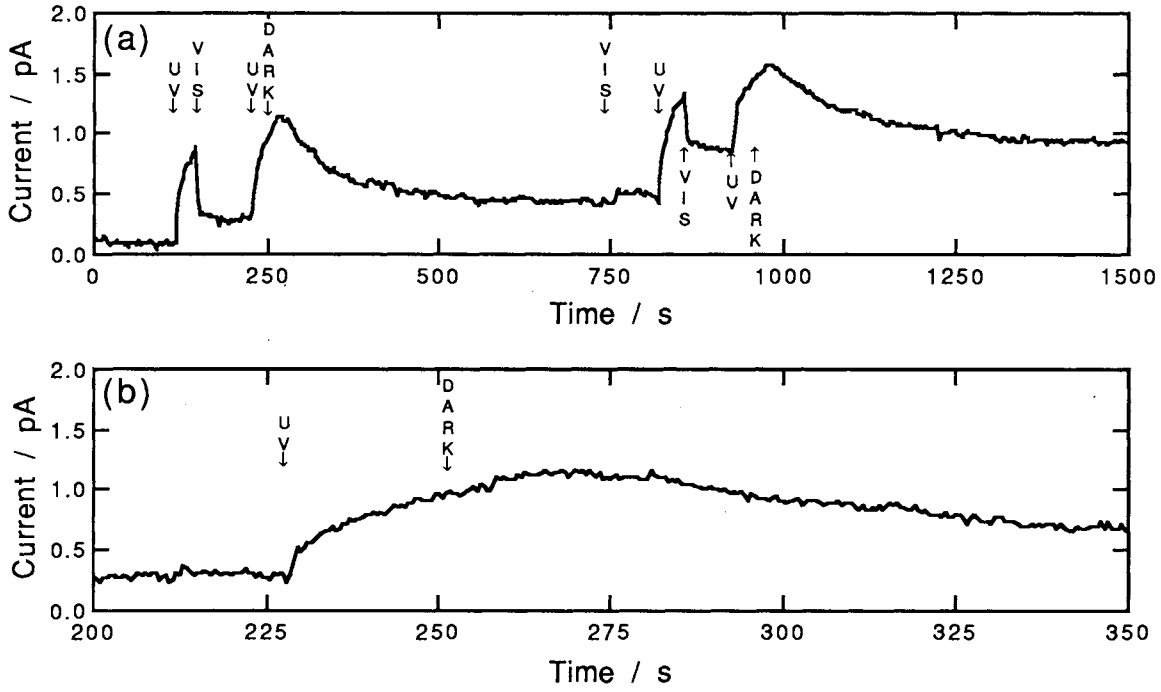


Fig. 2. Time course of the current. During the measurement, 50 mV was continuously applied. BLM had been illuminated by the visible light before the measurement. Part of the chart (a) is expanded and illustrated in (b).

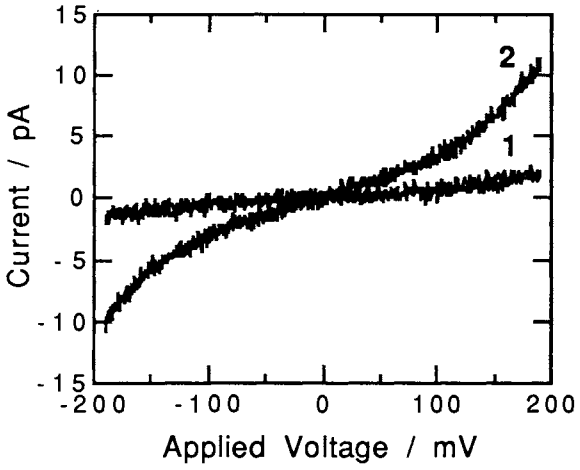


Fig. 3. Changes in the current as a function of the applied voltage before (1) and after (2) the illumination of UV light for 2 min.

A pore with 3.7 Å diameter and 70 Å length in BLM can flow 1 pA at 50 mV,[14] giving fluctuated and discontinuous current.[16] The photoinduced current observed here changed continuously, and therefore the pore formation is unlikely in this case although the magnitude of the current is comparable to that of a typical ion-channel.[15]

A possible explanation for the result is that the energetic barrier for ion migration is lowered by defects formed around opened-**1** in the membrane. The defects may arise from the disordered arrangement of GMO molecules existing near opened-**1**. The ion pair nature or the excess bulkiness of the head moiety of opened-**1** might partly contribute to the formation of the defects. The further increase in the current observed even after BLM was in the dark suggests that the opened form of **1** changes the location from interior to the surface of the membrane so as to make the defects suited for incorporation of ions into the membrane.

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