An Ion Gate Lipid Monolayer Membrane on Gold Electrodes

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Formation of monolayer membranes of a thiol-containing phosphate lipid by the spontaneous assembly onto gold electrodes and their ion gate characteristics are described.

Studies on self-assembled monolayers and bilayers on solid substrates have been the subject of considerable attention.[1-6] Our current interest has concentrated on the fabrication and functionalization of metal electrode surfaces with organized lipid membranes.[6] In this communication we describe the preparation of monolayer membranes of a mercaptan-containing acidic phosphate lipid **1** on gold surfaces *via* chemisorption[1-4] (see Fig. 1) and their ion gate properties examined by an electrochemical method. Characteristics of liposomes and synthetic bilayers of acidic phospholipids in aqueous solution have been investigated extensively in relation to membrane functions such as ion recognition, cell fusion, phase separation and ion transport.[7-10] Recently Umezawa *et al.*[5] have reported the ion responses of dodecylphosphate Langmuir–Blodgett films.

The novel amphiphile **1** was synthesized as follows. Reaction of ω-bromoundecanol and phosphorus oxychloride followed by the addition of H_2O gave bis(ω-bromoundecyl) phosphate, which was treated with mercaptobenzoic acid to obtain the thiobenzoate product. The reduction of the thiobenbzoate with hydrazine gave the final compound, bis(ω-mercaptoundecyl) phosphate **1**.† The experimental procedure for making **1** monolayer electrodes (electrodes *A*, *B* and *C*) is given below.[1-4] A polished gold disk electrode (1.6 mm diameter, Bioanalytical Systems) was immersed in an ethanoic solution of **1** for a given time at ambient temperature. The concentration of **1** and immersing times for making electrode *A*, *B* and *C* are as follows: 1×10^{-3} mol dm^{-3}, 24 h for electrode *A*; 1×10^{-3} mol dm^{-3}, 3 min for electrode *B*; 1×10^{-5} mol dm^{-3}, 3 min for electrode *C*. The modified electrodes were then washed by dipping in ethanol for 2 min and were air-dried.

Fig. 2 shows cyclic voltammograms for the electrode *A* and the bare electrode in the presence of 2 mmol dm^{-3} $Fe(CN)_6^{3-}$. No electrochemistry attributable to $Fe(CN)_6^{3-}$ was observed at the lipid monolayer electrode *A* in the pH range 2.1–10.5 (data at pH 10.5 and pH 2.1 are shown in Fig. 2). This indicates that the monolayer blocks the electrochemical communication of $Fe(CN)_6^{3-}$ with the electrode. Electrode *B* blocked the redox reaction of $Fe(CN)_6^{3-}$ at pH > 6.0, but a small cathodic current was observed at pH < 5.2 (Fig. 3). These are due to the redox reaction of $Fe(CN)_6^{3-}$ because no such current was detected in the absence of $Fe(CN)_6^{3-}$.

The *i–E* curves of the monolayer electrode *C* are shown in Fig. 4. Here, the pH dependence of the voltammograms is more evident than in Fig. 3, *i.e.*, the current is very small at alkaline and neutral pH, while clear redox behaviour was observed in the acidic pH region. This shows that by lowering the pH the monolayer opens a gate allowing $Fe(CN)_6^{3-}$ to penetrate the membrane. At higher pH the gate is closed. The process was reversible with small hysteresis. The apparent pK_a value of the phosphate moiety of **1** monolayer on electrode *C* evaluated from the plot of Δi ($= i_{anodic} - i_{cathodic}$) at 0 V *vs.*

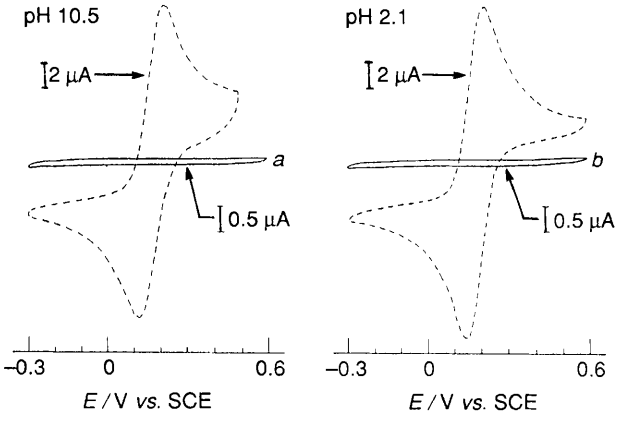
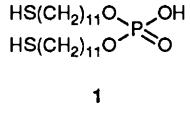


Fig. 2 Cyclic voltammograms of the electrode *A* (trace *a* and *b*) and the bare electrode (dotted lines) at pH 10.5 and 2.1. The solutions were 2 mmol dm^{-3} $K_3Fe(CN)_6$ in 1 mol dm^{-3} KCl. The pH of the electrolyte solution was adjusted with NaOH and HCl. Nitrogen was used for deaerating the solution. Measurement temperature, 25 ± 0.1 °C, scan rate, 100 mV s^{-1}; electrode area, 2.01 mm^2.



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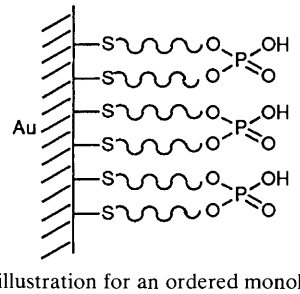

Fig. 1 Schematic illustration for an ordered monolayer membrane of a thiol-containing phosphate lipid *via* chemisorption onto a gold electrode

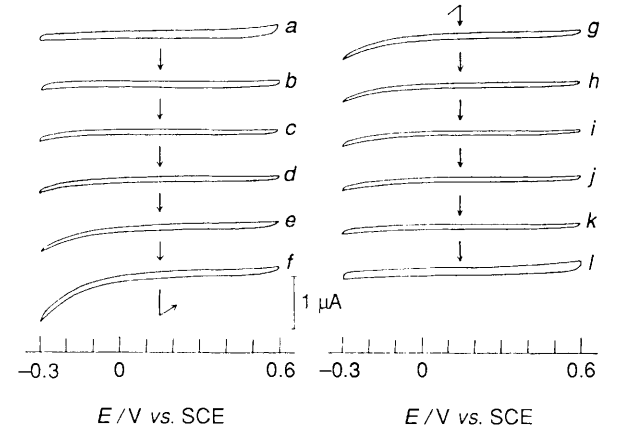

Fig. 3 pH dependence of cyclic voltammograms of the electrode *B*: (*a*) pH 10.5, (*b*) 6.6, (*c*) 5.2, (*d*) 4.1, (*e*) 3.0, (*f*) 2.2, (*g*) 2.9, (*h*) 4.2, (*i*) 5.2, (*j*) 6.0, (*k*) 7.2 and (*l*) 10.4. Other experimental conditions are the same as those in Fig. 2.

† Satisfactory elemental analyses were obtained.

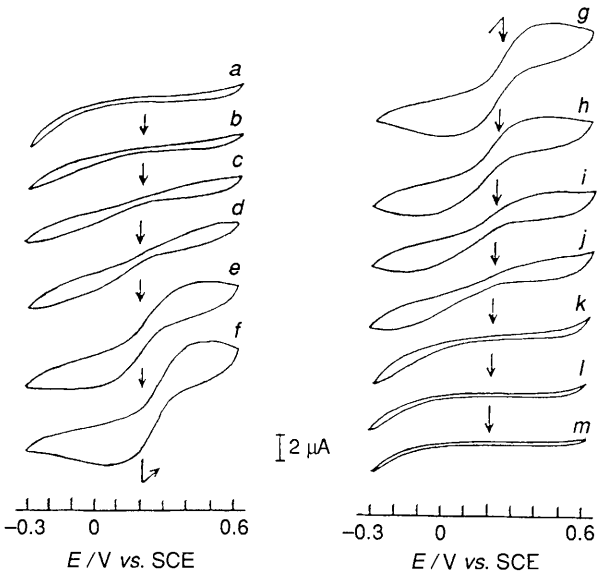

Fig. 4 pH dependence of cyclic voltammograms of the electrode *C*: (*a*) pH 10.4, (*b*) 6.1, (*c*) 5.0, (*d*) 4.0, (*e*) 3.0, (*f*) 2.0, (*g*) 2.5, (*h*) 3.6, (*i*) 4.5, (*j*) 5.4, (*k*) 6.9, (*l*) 8.3 and (*m*) 10.0. Other experimental conditions are the same as those in Fig. 2.

SCE (standard calomel electrode) against pH was about 4, which is larger than values for dialkyl phosphates in aqueous solution (pK_a *ca.* 1.7) and is close to those of phosphatidic acid liposomes.[11] This indicates the existence of intermolecular interaction in the monolayer on electrode *C*.

In conclusion, the ion gate lipid monolayer responsive to pH was formed on the gold electrodes *via* simple chemisorption method.

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