Study of the Ion Channel Behavior of Didodecyldimethylammonium Bromide Formed Bilayer Lipid Membrane Stimulated by PF_6^-

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Bilayer lipid membranes (BLM) formed from didodecyldimethylammonium bromide were made on the freshly exposed surface of a glassy carbon (GC) and were demonstrated by the ac impedance spectroscopy. The ion channels of membrane properties induced by PF_6^- were studied by the cyclic voltammetric methods. Experimental results indicated that the ion channel of BLM was open in the presence of the PF_6^- due to the interaction of PF_6^- with the BLM, while it was switched off in the absence of PF_6^-. Because the ion channel behavior was affected by the concentration of PF_6^-, a sensor for PF_6^- can be developed.

Introduction

Since its inception, the planar bilayer lipid membrane (BLM) has been proven to be a useful model for the biomembrane; it allows charge separation and translocation to be directly measured by electrochemical techniques.[1,2] It has been demonstrated that BLM modified with certain functional materials can be useful in the areas such as membrane reconstitution, light conversion, biosensor, molecular electronic devices, etc.[3,4] However the fragility of conventional planar BLM limits its applications for developing devices of practical use, and as a media for scientists to work on. For the purpose of a better mechanical stability which is crucial for more applications of BLM, recent studies have been introduced dealing with making stable BLM on the solid substrate, which is more convenient for electrochemistry study.[5-7]

The ion channel is among the hot subjects of interest to scientists, because the unique feature of ion channels in biological cell membranes is a selective recognition of substrate followed with amplification of its information by channel switchings: the selective binding of substrates with receptors triggers the opening of an ion-specific channel which allows the permeation of the great amount of ions across the membrane following an electrochemical potential gradient. Some of chemical sensors used in many areas were developed on the basis of the property of ion channel.[8,9] The study of ion channel in membrane aroused scientists' interest.[10,11] Previous work on ion channel has been mainly focused on some substances (special protein, polypeptides or their synthetic analogues) embedded in the lipid membranes.[12-14] The opening and closing of ion channel can be controlled either internally (involving chemical messengers) or externally (lights and electrical potentials). Recently inorganic ions (Ca^{2+}, Mg^{2+}, Ba^{2+}, ClO_4^-) induced lipid channel have received much attention.[10,11,15,16] It seems that the formation of ion channel results from the interfacial reorganization of the lipid assemblies upon the stimulation of these inorganic ions.

In this study, a new inorganic ion (hexafluorophosphate anion) induced ion channel in BLM was investigated (formed by didodecyldimethylammonium bromide on glassy carbon electrode). Hexafluorophosphate anion was firstly demonstrated that it can react with didodecyldimethylammonium bromide formed BLM and can induce ion channel behavior of the BLM. The open-close process of the ion channel is reversible. Furthermore the ion channel current is not only concentration-dependent but also time-dependent.

Experimental

Materials

Dimyristoylphosphatidylcholine (DMPC) was obtained from Sigma (USA). Didodecyldimethylammonium bromide and tris(2,2'-bipyridine)ruthenium(II) chloride were purchased from Acros (Belgium) and used without further purification. Analytical-grade sodium chloride was purchased from Beijing Chemical Reagent Factory (Beijing, China) and sodium hexafluorophosphate from Aldrich (USA). All other reagents were of analytical grade. Pure water was used throughout, obtained by means of a Millipore Q water purification set.

Electrochemical measurements

Cyclic voltammetry and impedance spectroscopy were performed with an Autolab PGSTAT30. Cyclic voltammetry experiments were done in the range of 0.9 to 1.25 V versus Ag/AgCl reference electrode. Impedance spectroscopic experiments were conducted in the frequency range from 10 kHz to 0.1 Hz and with a signal amplitude of 10 mV. All experiments were carried out with a three-electrode system consisting of a Ag/AgCl reference electrode, a platinum coil auxiliary electrode and a glassy carbon (GC) working electrode.

Method for supported bilayer lipid membrane formation

Didodecyldimethylammonium bromide was dissolved in chloroform to give a final concentration of 2.5 mg/mL, which was called BLM forming solution. Prior to supported bilayer lipid membrane formation, a glassy carbon electrode was polished with 1.0, 0.3 and 0.05 μm of alumina slurry, respectively, and then sonicated for 1 min in deionized water and acetone successively. Then the GC electrode was immersed in the 0.1 mol/L NaOH solution, and the potential was held at 1.5 V for 3 min in order to polarize the electrode. After polarizing and washing, the GC electrode was dried under purified nitrogen. Subsequently, the GC electrode was immersed into the forming solution and the electrode was immediately transferred into the 0.1 mol/L KCl solution, where the supported bilayer lipid membrane was formed spontaneously.

Results and discussion

Forming BLM on the GC electrode

The formation of BLM on the GC surface was judged by ac impedance spectroscopy and cyclic votammograms before and after the electrode was coated with BLM.
    Impedance spectroscopy   It has been demonstrated that impedance spectroscopy is an effective method for probing the features of a surface-modified electrode.[17,18] The complex impedance can be presented as the sum of the real, Z_{re} and imaging, Z_{im} components that originated mainly from the resistance and capacitance of the cell, respectively. Fig. 1 shows the result of impedance spectroscopy. Inset of Fig. 1 was a modified Randle's equivalent circuit that was chosen to fit the measured results. R_{sol} is the electrolyte resistance, C_m the lipid membranes capacitance, R_m the lipid membrane resistance, C_{dl} the double-layer capacitance, R_{ct} charge-transfer resistance and Z_w Warburg element. To determine the thickness of the lipid membrane, the equation[19] was given

$$C_m = \varepsilon k / d \qquad (1)$$

where k is dielectric constant ($k = 2.05$),[20] ε permittivity of free space ($\varepsilon = 8.85 \times 10^{-14}$ F· cm^{-1}) and d the thickness of the lipid membranes. Using the equivalent circuit to fit the data of Fig. 1, C_m can be obtained. Thus from Eq. (1) the

thickness of the lipid membrane is 2.13 nm that is well in agreement with that reported for BLM of didodecyldi methylammonium bromide formed on the surface of the GC electrode.[11]
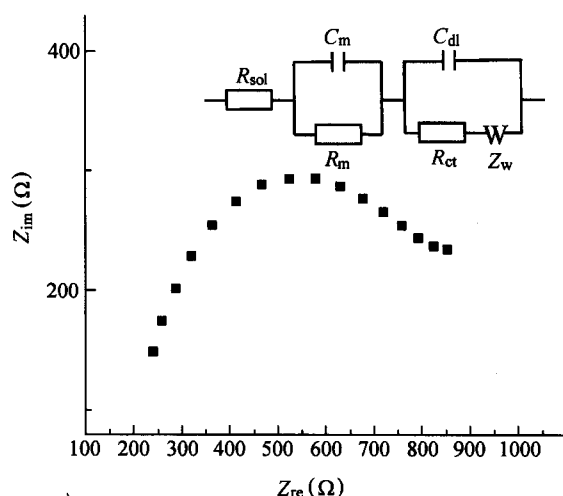

Fig. 1   Polt of complex plane impedance at a modified electrode with supported lipid membrane. Inset: modified Randle's equivalent circuit used to model impedance data in the presence of redox couples. [Ru(bpy)$_3^{2+}$] = 0.5 mmol/L; [Na$^+$] = 10 mmol/L.

Cyclic votammetry   The cyclic voltammograms before and after coating of GC surface with BLM are presented in Fig. 2 (curves a and b). As shown in the figure, before it was coated with BLM, a pair of well-defined reversible waves of [Ru(bpy)$_3$]$^{2+}$ [bpy = tris(2, 2'-bipyridine) ruthenium (II)] couple were obtained. After it was coated, the waves were suppressed, which featured charging and discharging of
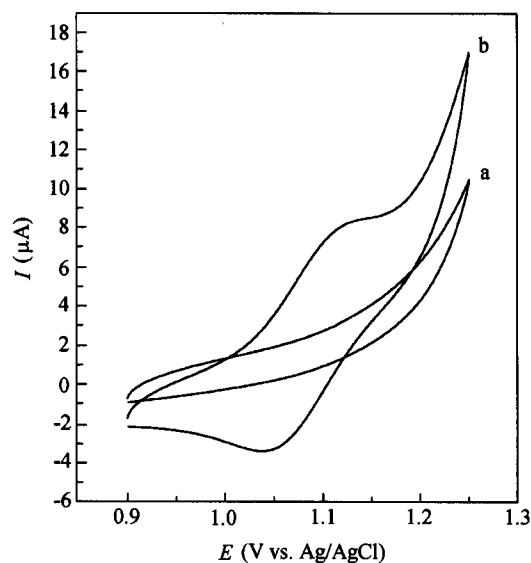

Fig. 2   Cyclic voltammograms of GC electrode. (a) GC electrode coated with lipid membrane; (b) bare GC electrode. [Ru(bpy)$_3^{2+}$] = 0.5 mmol/L; [Na$^+$] = 10 mmol/L. Scan rate is 100 mV/s.

planar capacitance. The BLM blocked [Ru (bpy)$_3$]$^{2+}$ to transfer to the underlying GC electrode. All results indicate that BLM was formed on the GC electrode surface successfully.

Ion channel

Fig. 3 shows the cyclic voltammagrams of [Ru-(bpy)$_3$]$^{2+}$ in the presence of different concentrations of the hexafluorophosphate anion (PF$_6^-$). In the absence of PF$_6^-$, the coated BLM blocked the penetration of ruthenium (II) complex cation through the closed channel toward the underlying electrode. Upon adding PF$_6^-$ the peak currents increased with increasing concentration of the PF$_6^-$ as if the lipid membranes were leaking and the peaks of the marker ion appeared at the same potential value as those observed at the bare GC electrode. It is clear that the PF$_6^-$ anion can stimulate the ion channel of the membrane to be in an open state due to PF$_6^-$ anion-membrane interaction and allowing a great deal of [Ru-(bpy)$_3$]$^{2+}$ to permeate across the membrane. To investigate the influence of [PF$_6^-$] on the ion channel behavior of the membranes, cyclic votammetry measurements with different concentrations of PF$_6^-$ in the electrolyte were taken. Fig. 4 shows the peak current vs. [PF$_6^-$]. The result indicates that the peak current initially increased with the increase of [PF$_6^-$] and reached a plateau demonstrating all the ion channels were open because available site on the BLM was all interacted with PF$_6^-$. The available sites or active sites on the BLM promote the formation of ion channels. The difference in current would reflect the difference in the number of formed ion channels in BLM. The peak current increased with the increase of [PF$_6^-$] and more obvious voltammogram was obtained as shown in Fig. 3. This
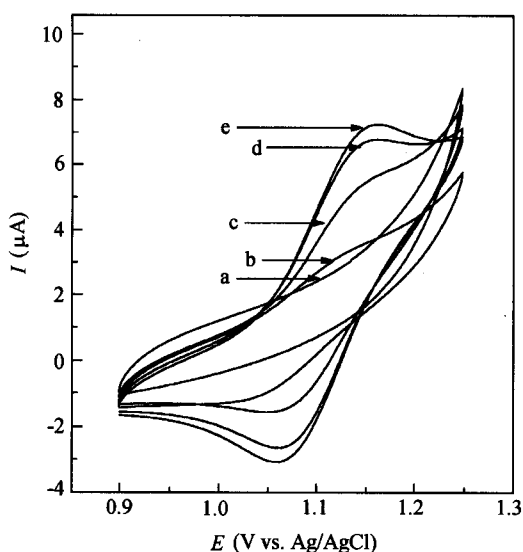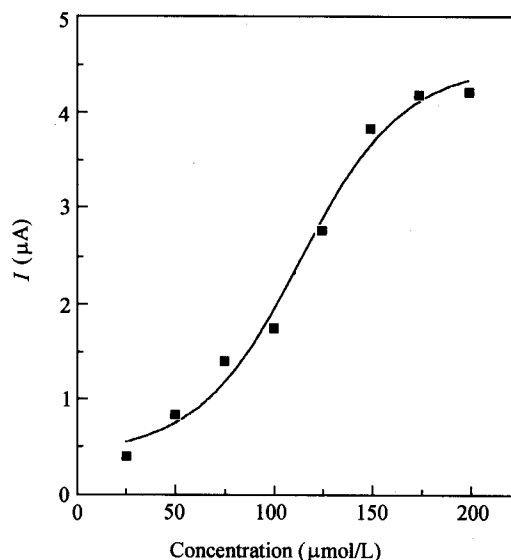

Fig. 4 Dependence of the concentration of PF$_6^-$ ion at the GC electrode coated with lipid membrane on cathodic peak current. [Ru (bpy)$_3^{2+}$] = 0.5 mmol/L; [Na$^+$] = 10 mmol/L. Scan rate is 100 mV/s.

implies that the diffusion of [Ru (bpy)$_3$]$^{2+}$ across the BLM through ion channels is a rate limiting step.

To test whether the interaction of PF$_6^-$ with BLM creates ion channel for the ruthenium(II) complex cation to permeate through the BLM or the BLM desorpts from GC electrode, the electrode was transferred into electrolyte without PF$_6^-$ and the channels were found in a closed state again. The BLM is still on the GC electrode. When the electrode was put into electrolyte with PF$_6^-$, the channels were open again. The reversible open-close process could be repeated many times.

The GC electrode coated with DMPC film could respond reproducibly to PF$_6^-$ ions within 12 h. The responsiveness was lost in a couple of days.

In addition the ion channel behavior is dependent on the time. Fig. 5 shows that the current response of the channel was going as a function of time. The peak current of the ruthenium(II) complex cation increased with time and reached steady state after 5 min.

When the electrode coated with BLM was inserted into solution with C$_2$O$_4^{2-}$, SO$_4^{2-}$, NO$_3^-$, B$_4$O$_7^{2-}$, F$^-$ and Cl$^-$ up to 2 mmol/L but without PF$_6^-$, the cyclic voltammagrams of [Ru(bpy)$_3$]$^{2+}$ appeared as Fig. 3(a). The peak current intensity does not change, i.e., the ion channel can not be opened. These phenomena express channel selectivity.

All the above experiments have shown that the didodecyldimethylammonium bromide formed BLM on a glassy carbon electrode has an ion channel behavior which can be switched on by the stimulus (PF$_6^-$). The channel was opened in the presence of stimulus, otherwise the channel was closed. The interaction of PF$_6^-$ with the BLM may not be due to the electrostatic action nature, because in case in the presence of other ion such as Cl$^-$, SO$_4^{2-}$, C$_2$O$_4^{2-}$, etc. in the so-

Fig. 3 Cyclic voltammograms of 0.5 mmol/L [Ru(bpy)$_3$]$^{2+}$ ions at the GC electrode coated with lipid membrane in different concentration of NaPF$_6$: (a) 0; (b) 0.05; (c) 0.10; (d) 0.15; (e) 0.20 mmol/L. [Na$^+$] = 10 mmol/L. Scan rate is 100 mV/s.

lution without PF_6^- the channel can not be opened. It can be speculated that the PF_6^- anion form an ion-association compound with polar head of the lipid quaternary ammonium cation in the bilayer membrane according to Ref. 10, in which ClO_4^- forms an ion-association compound with lipid quaternary ammonium. The forming of the compound changes the regular alignment of the lipid, resulting in forming the ion channel in the membrane.
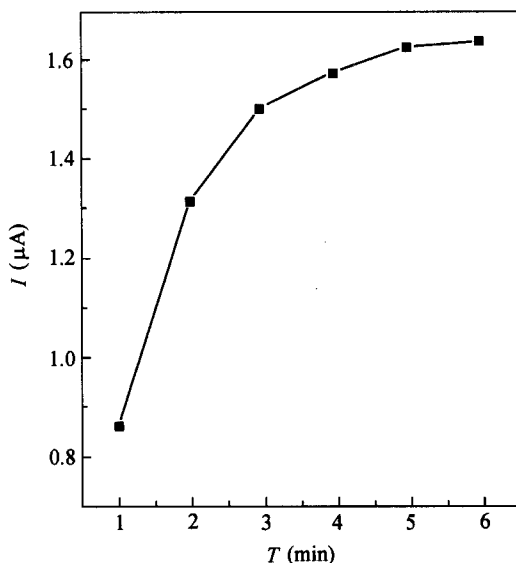

Fig. 5   Dependence of the time on cathodic peak current. [Ru-$(bpy)_3^{2+}$] = 0.5 mmol/L; [Na^+] = 10 mmol/L; [PF_6^-] = 0.20 mmol/L. Scan rate is 100 mV/s.

Conclusion

The didodecyldimethylammonium bromide formed BLM on the surface of a GC electrode was first made to study the interaction with PF_6^-. PF_6^- anion could react with the BLM and stimulate it to show ion channel behavior. The reversible open/close state of the ion channel could be repeated many times. The intensity of the current through the channel increased with the increasing of concentration of PF_6^- and time.

This ion channel behavior could be used to develop a biosensor for PF_6^-.

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