

Ion Channel Behavior of a Supported Bilayer Lipid Membrane Composed of 5,5-Ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane Bromide Modified Glassy Carbon Electrode[†]

GONG, Jing-Ming(龚静鸣) LIN, Xiang-Qin*(林祥钦)

Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China

A synthetic cationic surfactant, 5,5-ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane bromide (DTDB), was used to construct a supported bilayer lipid membrane (s-BLM) coated on an underlying glassy carbon electrode (GCE). Electrochemical impedance spectroscopy (EIS), small-angle X-ray diffraction (SAXD) and cyclic voltammetry were used to characterize the s-BLM. Both EIS and SAXD data indicated that the synthetic lipid exists as a well-oriented bilayer in the membrane. The voltammetric study showed that the lipid membrane can open ion channels in the presence of ClO_4^- stimulant with $\text{Ru}(\text{bpy})_3^{2+}$ as marker ions and give distinct channel currents. The channels can be closed and open up again many times by removing or introducing ClO_4^- anions.

Keywords 5,5-ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane bromide, ion channel behavior, electrochemical impedance spectroscopy, small-angle X-ray diffraction, supported bilayer lipid membrane

Introduction

The ion channel plays an important role in biological activities. For instance, control of ionic flow, conversion of extra-cellular events into intracellular signals, and transfer of information in the nervous system are dependent upon the function of ion channels. The feature of ion channel on biological cell membranes is the selective recognition of substrate (receptor) and the following amplification of the information by channel switching. The interaction between receptor and specific substance triggers an opening of ion-specific channel, which leads to a great amount of ions to permeate across the membrane following an electrochemical potential gradient. Many efforts have been devoted to the research utilizing this principle to fabricate "ion-channel sensors" for detection of various substances. Certainly, detection of several ions and molecules,¹⁻⁴ such as hydrogen ions,⁵ inorganic anions,⁶ nucleotides,^{7,8} and antibodies,^{9,10} have been achieved.

Up to now, studies of ion channel behavior have

aroused great interests, which emphasize the need for establishing an ideal model of bio-membrane. Several kinds of model membranes have been used, including lipid vesicles or liposomes, bilayer lipid membranes (BLM) and Langmuir-Blodgett (LB) layers. Since the reconstituted BLM that separates two solutions was first reported in 1962,¹¹ BLM systems have been employed extensively as an experiment model of bio-membranes. These work has been recently reviewed by Tien *et al.*^{12,13} Studies on the BLM model system have given much insight into the life processes related to biomembranes, and the mimicing of biomembrane processes may find application in the field of electrodes,¹⁴ biosensor devices,¹⁵ biomolecular electronic devices,¹⁶ and solar energy transduction.¹⁷

Though it has been shown that the BLM system formed by conventional methods is very useful, a main deficiency of the BLM is the stability. Because the conventional BLM apparatus can only provide very unstable support, the lipid layer formed was too fragile to be used for practical applications. Recently, it was demonstrated that some BLMs supported on solid substrates, such as metal,¹⁸⁻²¹ Si and polymer,²² glassy carbon (GC),^{23,24} pyrolytic graphite²⁵ and waxed graphite²⁶ have requisite dynamic properties and mechanical stability. The supported BLMs (s-BLMs) become an ideal model for investigating ion channel behavior of bio-membranes. As a typical composition of s-BLM, phospholipid, dialkyldimethyl ammonium bromide surfactants as well as their derivatives,^{22,27-29} have been widely used as the biomembrane-like model. However, to the best of our knowledge, no report has been made on the synthetic cationic lipid with 1,3-dioxane as an s-BLM, whose structure is shown as in Scheme 1.

In our work, an s-BLM of 5,5-ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane bromide was made on GC electrode. It exhibited sensitive permeability changes on ClO_4^- stimulus when $\text{Ru}(\text{bpy})_3^{2+}$ was used as marker ions.

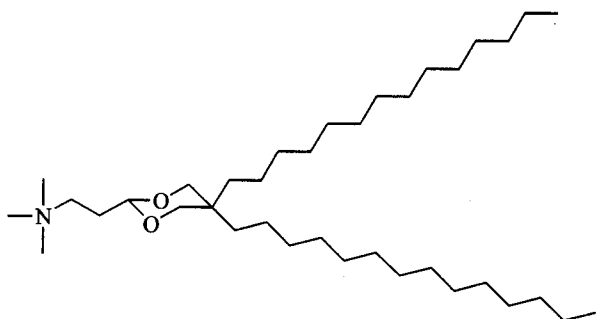
* E-mail: xqlin@ustc.edu.cn; Tel.: 86-551-3606646; Fax: 86-551-3601592

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[†]Dedicated to Professor ZHOU Wei-Shan on the occasion of his 80th birthday.

Scheme 1 Diagram illustrating the chemical structure of the synthetic lipid, 5,5-ditetradecyl-2-(2-triethyl-ammonioethyl)-1,3-dioxane bromide (DTDB)



Experimental

Reagents

5,5-Ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane bromide (DTDB) was synthesized by Lab of Bioorganic Chemistry (USTC, China).³⁰ Tris(2,2'-bipyridine) ruthenium(II), $\text{Ru}(\text{bpy})_3^{2+}$, was purchased from Acros (Belgium) and used without further purification. Analytical-grade sodium perchlorate was purchased from Shanghai Reagent Second Factory (Shanghai, China). Chloroform was analytical grade. Double-distilled water was used throughout. All other chemicals were of the AR quality and used as obtained. High purity nitrogen was used to remove oxygen.

Apparatus and procedures

A computer-controlled electrochemical workstation (CHI 660A, Shanghai, China) was used to perform electrochemical measurements. A three-electrode cell was used, which is composed of a saturated calomel electrode (SCE) as reference electrode, a platinum coil counter electrode, and a working electrode. A glassy carbon electrode (GCE) was used as the basal electrode for fabrication DTDB modified electrode, DTDB/GCE. Surface area of the GCE was determined by cyclic voltammetry (CV) of $\text{K}_3\text{Fe}(\text{CN})_6$ as 0.15 cm^2 with surface roughness of 1.20. Electrochemical impedance spectra (EIS) were measured in the frequency range of 0.1 Hz ~ 100 kHz with a signal amplitude of 10 mV at CHI 660A in a cell with a netted Pt counter electrode of about 2 cm^2 surface area. The measurement was made at the formal potential of $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ in $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ (1:1 mixture) + $0.1 \text{ mol} \cdot \text{L}^{-1}$ KCl solution. A program based on simplex optimization and nonlinear least-square-fit analysis was written for fitting the data.

Sample solutions were purged with N_2 for 10 min to remove oxygen prior to measurement. All electrochemical experiments were carried out at room temperature.

X-Ray diffraction studies were done with a D/Max- γ

A powder diffractometer (Rigaku) using a Cu K α source at 30 kV, 20 mA. Composite film of DTDB for X-ray diffraction was prepared by dispersion and evaporation of the chloroform solution onto a microscopic glass slide in the air.

Preparation of DTDB/GCE

The GCE was abraded to a mirror surface with metallographic SiC sand papers of different grades and then ultrasonicated for 5 min in anhydrous alcohol and $1 \text{ mmol} \cdot \text{L}^{-1}$ nitric acid, successively, to remove any organic and inorganic pollutants and adsorbed micro particles on the surface. Then the GCE was immersed in $0.1 \text{ mol} \cdot \text{L}^{-1}$ NaOH solution at an electrode potential of 1.8 V (vs. SCE) for 2 min for surface anodizing. After rinsed thoroughly with water, the GCE was dried under nitrogen atmosphere and ready for BLM coating.

A $5 \mu\text{L}$ aliquot of the $2 \text{ mg} \cdot \text{mL}^{-1}$ DTDB/chloroform solution ($0.0155 \mu\text{mol}$) was added to the surface of the GCE by using a microsyringe. Allowing the solution to cover the whole range of the GCE surface, then the electrode was immediately immersed into $0.1 \text{ mol} \cdot \text{L}^{-1}$ pH 6.0 phosphate buffer solution (PBS) and kept in the solution for about 0.5 h to allow well assembling of the s-BLM. The obtained DTDB modified s-BLM electrode was labeled as DTDB/GCE.

Results and discussion

Voltammetric behavior of the bare GCE and DTDB/GCE

The DTDB/GCE was characterized by cyclic voltammetry of $\text{K}_3\text{Fe}(\text{CN})_6$, as shown in Fig. 1. It can be seen that $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ redox reaction at the bare GCE before DTDB coating gave a couple of well behaved CV peaks at E_m of 0.232 V with ΔE_p of 65 mV (curve a), however, only a small sigmoid waves were obtained at the electrode

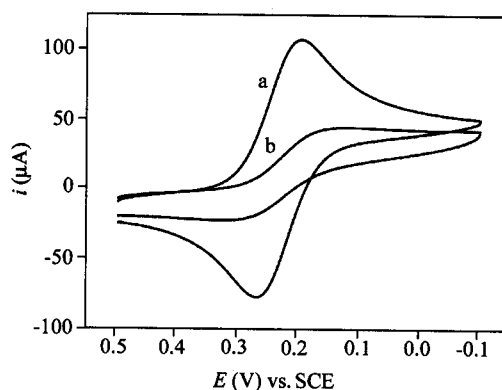


Fig. 1 Cyclic voltammetric responses of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in KCl solutions. $\text{K}_3[\text{Fe}(\text{CN})_6]$: $5 \text{ mmol} \cdot \text{L}^{-1}$, KCl: $1 \text{ mol} \cdot \text{L}^{-1}$, scan rate: 50 mV/s . (a) GCE; (b) DTDB/GCE.

after DTDB coating (curve b). Analysis of the sigmoid waves gave the same E_m value and an $(E_{3/4} - E_{1/4})$ value of 58 mV, indicating a reversible one electron transferred processes of $\text{Fe}(\text{CN})_6^{4-/-3-}$ redox reaction and no significant interaction between the $\text{Fe}(\text{CN})_6^{4-/-3-}$ species and the coated DTDB molecules. It is most likely that the $\text{Fe}(\text{CN})_6^{4-/-3-}$ redox reaction was conducted through pinholes of the modified DTDB film, which may be formed due to disfigurements of the GCE surface. Obviously, $\text{K}_3\text{Fe}(\text{CN})_6$ can be prevented reaching the surface of GCE to a certain degree and a supported lipid membrane had been formed on the surface of GCE successfully.

Electrochemical impedance measurements of DTDB/GCE

EIS is an effective method for probing the features of a surface-modified electrode.^{31,32} The EIS characteristic of the DTDB/GCE in $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ solution is shown in Fig. 2. It can be seen in Fig. 2a that the EIS of the bare GCE is a composition of a semicircle and a straight line with characteristic of a diffusional limiting step of the $\text{Fe}(\text{CN})_6^{4-/-3-}$ processes. Significantly a different spectrum was obtained at the DTDB/GCE, as shown in Fig. 2b. To model this film, a modified Randle's equivalent circuit was used, as shown in Fig. 2b (inset). The total impedance of the system is determined by several parameters: R_{sol} , C_{dl} , capacitance (C_m) and resistance (R_m) of the lipid membrane, charge-transfer resistance (R_{ct}), Warburg impedance Z_w . The fitting procedure showed a good agreement between the theoretical and experimental data. All simulated results are listed in Table 1. From Table 1, it can be seen that R_{sol} is nearly independent of whether the membrane existed or not. Due to the formation of DTDB membrane on the GCE, electron transfer of $\text{K}_3\text{Fe}(\text{CN})_6$ become difficult with R_{ct} greatly increased. A value of $0.39 \mu\text{F}\cdot\text{cm}^{-2}$ for C_m was obtained. The thickness (d) of the lipid membrane can be estimated, according to the equation³³

$$C_m = \frac{\epsilon_0 \kappa}{d} \quad (1)$$

where ϵ_0 is the permittivity of free space ($\epsilon_0 = 8.85 \times 10^{-14} \text{ F}\cdot\text{cm}^{-1}$) and κ , the lipid membranes dielectric constant (estimated as 2.05).³⁴ Under these assumptions, the thickness of the DTDB membrane was estimated as 5.0 nm. This value is in good agreement with the thickness of BLM constructed by DMPC (dimyristoylphosphatidyl choline),³⁵ which also contains hydrophobic C_{14} chains as in DTDB. This value is also in agreement with the result

calculated by Semi-empirical PMS optimization,³⁶ from which a distance of 2.43 nm from the polar head to the tail end of alkyl chains for DTDB monolayer was estimated and thus 4.8 nm for BLM thickness can be expected.

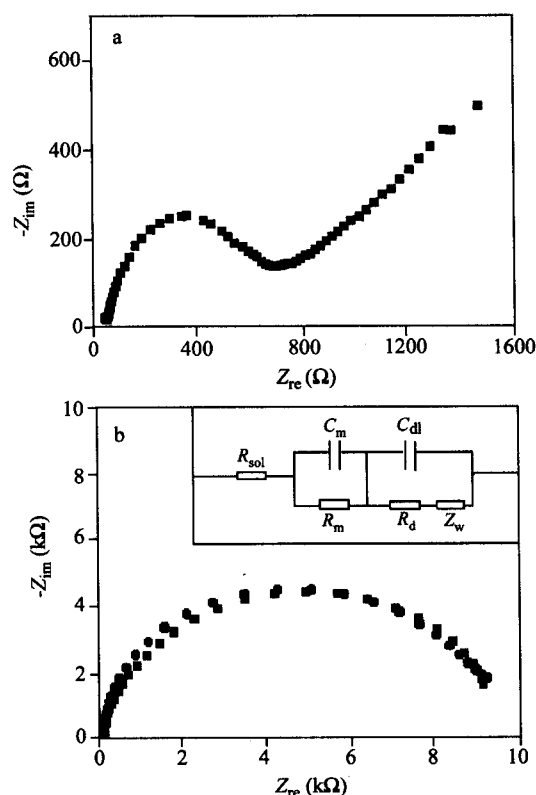


Fig. 2 Electrochemical impedance spectra of bare GCE (a) and DTDB/GCE (b) in $10 \text{ mmol}\cdot\text{L}^{-1} \text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ (1:1 mixture) + $0.1 \text{ mol}\cdot\text{L}^{-1} \text{KCl}$ solution: experimental data (■), fitted data (●). Inset: modified Randle's equivalent circuit used to model impedance data in the presence of redox couples.

Small-angle X-ray diffraction (SAXD) characterization of DTDB

As well known, the reflection angle 2θ of X-ray diffraction can be used to obtain the inter-layer basal spacing of films through Bragg's law and provide useful information about the repeat distance of lamellar structure.³⁷⁻³⁹ According to the result of a small-angle X-ray diffraction (SAXD) experiment, several small angle peaks were observed for different diffraction orders $n = 1, 2$ and 4 in the $1.5^\circ - 10^\circ$ region of 2θ , as shown in Fig. 3. A lamellar D spacing is really close to 5 nm. The SAXD data confirms the creditability for the bilayer structure of DTDB in the s-BLM.

Table 1 Electrochemical impedance spectroscopy fitted results for the bare GCE and the DTDB/GCE

	$C_{\text{dl}} (\mu\text{F}\cdot\text{cm}^{-2})$	$R_{\text{sol}} (\Omega)$	$R_{\text{ct}} (\Omega)$	$C_m (\mu\text{F}\cdot\text{cm}^{-2})$	$R_m (\Omega)$	σ^a
GCE	75.10	16.4	803.1	—	—	532.7
DTDB/GCE	58.84	15.0	8618	0.39	116.0	862.7

^a σ related with Z_w .

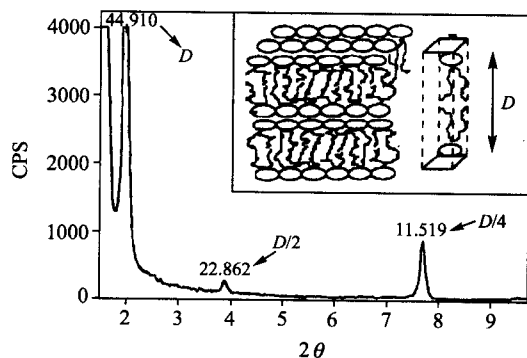


Fig. 3 Small angle X-ray diffraction intensity plotted as a function of scattering angle (2θ) for the film composite of DTDB at room temperature. The inset describes the repeat distance of the structure, D , corresponding to the bilayer membrane thickness.

It is certain from the data shown above that an ideal model of biological membrane on the surface of GCE was successfully constructed.

Ion channel behavior of the s-BLM on DTDB/GCE

Fig. 4 shows the CVs of the DTDB/GCE in $0.5 \text{ mmol} \cdot \text{L}^{-1} \text{ Ru}(\text{bpy})_3^{2+}$ solution. Curve a shows a well behaved redox wave of $\text{Ru}(\text{bpy})_3^{2+}$ containing $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$ at bare GCE. However, the redox reactions were significantly inhibited at the DTDB/GCE (curve b), indicating that the ion channels of the DTDB membrane were almost in a closed state, relatively bulky ruthenium(II) cations can't permeate through the membrane and reach the underlying electrode for electron exchange. Curve c shows the CV of the DTDB/GCE immersed in $\text{Ru}(\text{bpy})_3^{2+}$ solution containing $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KClO}_4$, the distinct CV redox peaks were reformed at potentials almost the same as those observed at GCE. Obviously, the addition of ClO_4^- anions stimulated the ion channel of the DTDB membrane to be in an open state.

In the absence of perchlorate anions, the channels were closed due to a regular alignment of the lipid quaternary ammonium cations on the GCE surface. After introducing ClO_4^- , the channels were opened due to the formation of ion association compounds between ClO_4^- and lipid quaternary ammonium cations in the BLM, which changed the regular alignment of the lipid and created ion channels (gaps) on the membrane. It was demonstrated that the redox current of $\text{Ru}(\text{bpy})_3^{2+}$ was blocked again when the electrode was intentionally transferred into a $\text{Ru}(\text{bpy})_3^{2+}$ solution containing $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$, indicating the ion channels were closed again, as shown in curve d. Certainly, the stage of ion channels can be reversibly open-close-open for many times by exchanging the solutions. The inset in Fig. 4 shows that a reproducible open-close operation was obtained in repeating experiments.

To investigate the influence of ClO_4^- on the charge transfer of $\text{Ru}(\text{bpy})_3^{2+}$ across the BLM, CV measurements

were conducted in solutions containing different concentration of ClO_4^- . Fig. 5 shows the plot of oxidation peak current vs. $[\text{ClO}_4^-]$. It can be seen that the peak current initially increased with $[\text{ClO}_4^-]$ increase, and then reached to a plateau at about $2 \text{ mmol} \cdot \text{L}^{-1}$, which implies all the available sites on the BLM were occupied by ClO_4^- . However, when the DTDB/GCE was immersed into a $\text{Ru}(\text{bpy})_3^{2+}$ solution containing ClO_4^- , a time dependent phenomenon was observed. The CV peak current of $\text{Ru}(\text{bpy})_3^{2+}$ instantly appeared in less than 3 min, then gradually increased with time, and finally stabilized at about 90 min. This is possibly related to a kinetic process of reassembling of membrane structure.

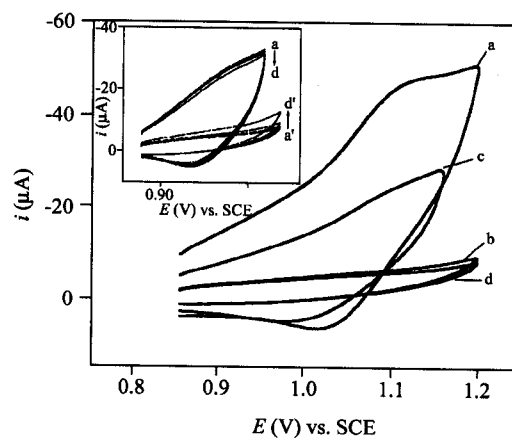


Fig. 4 Cyclic voltammetric response of $0.5 \text{ mmol} \cdot \text{L}^{-1} \text{ Ru}(\text{bpy})_3^{2+}$ at bare GCE (a) and DTDB/GCE in solution of $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$ (b), $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KClO}_4$ (c), $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$ (d). Scan rate, $100 \text{ mV} \cdot \text{s}^{-1}$. Inset: cyclic voltammetric response of $0.5 \text{ mmol} \cdot \text{L}^{-1} \text{ Ru}(\text{bpy})_3^{2+}$ at DTDB/GCE in solution of $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KClO}_4$ (a—d) and $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$ (a'—d'), corresponding to the ion-channel open-close times: the 1st (a, a'), the 3rd (b, b'), the 5th (c, c'), the 8th (d, d').

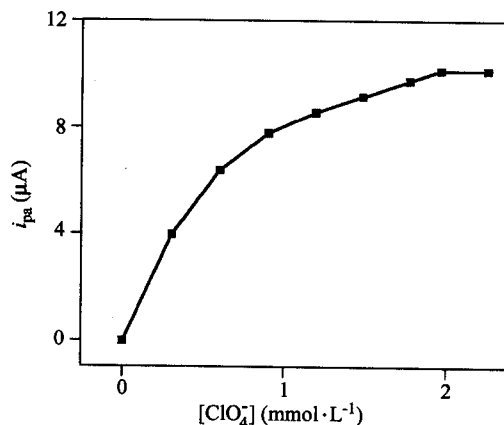


Fig. 5 Oxidation current (i_{pa}) in voltammograms of $0.5 \text{ mmol} \cdot \text{L}^{-1} \text{ Ru}(\text{bpy})_3^{2+}$ at DTDB/GCE in the presence of different concentrations of ClO_4^- in $5 \text{ mmol} \cdot \text{L}^{-1} \text{ KCl}$. Scan rate: $100 \text{ mV} \cdot \text{s}^{-1}$.

The data shown above indicate that the interaction between ClO_4^- and the BLM opens up some kind of channels, which allows the access of $\text{Ru}(\text{bpy})_3^{2+/3+}$ redox couple to the electrode surface. The ion channel behavior of the DTDB/GCE we investigated is similar to the ion channel behavior on the supported lipid membrane composite of didodecyldimethylammonium bromide.^{24,27-29} The function of the ClO_4^- stimulant should interact with the head group of DTDB, and lessen the interaction among the DTDB head groups, which makes the BLM less tightly packed and consequently increases the cation permeability through the membrane.

Conclusions

A new composite s-BLM on GCE using a synthetic cationic surfactant 5,5-ditetradecyl-2-(2-trimethyl-ammonioethyl)-1,3-dioxane bromide (DTDB), DTDB/GCE, was fabricated. The result of EIS and SAXD demonstrated that the supported bilayer lipid membrane has been formed on the surface of GCE. The ion channel behavior of the membrane was studied. DTDB/GCE has reversible ion channel behavior for $\text{Ru}(\text{bpy})_3^{2+}$ marker ion with ClO_4^- stimulant. The channel can open in presence of stimulus, whereas close in absence of stimulus, which can be reversibly repeated many times. Undoubtedly, the s-BLM composite of DTDB, will offer the potential application in ion channel sensor and studies for mimetic bio-membrane. The further work is under progress.

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