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Electrochemical Study of Ion Channel Behavior in Incorporated Poly L-glutamate Bilayer Lipid Membranes

Yuehong Tong,[1] Zhengyan Wu,[1] Xiaojun Han,[1] and Erkang Wang[1,2]

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The lipid layer membranes were fabricated on the glassy carbon electrode (GC) and demonstrated to be bilayer lipid membranes by impedance spectroscopy. The formation of incorporated poly L-glutamate bilayer lipid membrane was achieved. The ion channel behavior of the incorporated poly L-glutamate membrane was determined. When the stimulus calcium cations were added into the electrolyte, the ion channel was opened immediately and exhibited distinct channel current. Otherwise, the ion channel was closed. The cyclic voltammogram at the GC electrode coated with incorporated poly L-glutamate DMPC film response to calcium ion is very fast compared with that at the GC electrode coated only with DMPC film. Ion channel current is not dependent on the time but on the concentration of calcium. The mechanism of the ion channel formation was investigated.

KEY WORDS: Ion channel; bilayer lipid membrane; stimulus; marker ion; poly L-glutamate.

INTRODUCTION

Bilayer lipid membranes (BLM) interposed between two aqueous phases have been successfully applied to the experimental model systems of biomembranes since the 1960s. It has been demonstrated that BLMs modified with certain functional materials can be useful in the areas such as membrane reconstitution, light conversion, biosensor, molecule electronic devices, etc. (Tien, 1988, 1989). But the investigations are primarily impeded by a low stability of artificial planar lipid bilayer systems. This is mainly due to a high sensitivity of such model membranes to mechanical disturbances in the laboratory environment. Consequently, there is strong demand to improve the shortcoming of the BLM. Many methods are proposed to solve the shortcomings of the conventional BLM, such as supporting BLM on solid substrate (Tien and Ottova, 1998; Tien and Salamon, 1989). This kind of supported BLM is highly suitable for further studies and applications, especially in the field of membrane biophysics, cell biology, and biotechnology. Because ion channel in a biological cell membrane is a selective recognition of the substrate to follow amplification of its information by channel switching, the property of an ion channel can be utilized as a model for the construction of devices useful to monitor a large number of biochemical, clinical, environmental, and agricultural interest or for uses in food and pharmaceutical analysis (Nikolelis and Krull, 1993, 1994; Nikolelis and Siontorou, 1995). The investigation of ion channel behavior in supported bilayer lipid membranes on substrates has been a topic of great interest (Wu *et al*., 2000). To open the channel with the stimulus, specific interaction of the stimulus with the membrane assemblies is required to change the membrane permeability for marker ions. This may be implemented by incorporating some appropriate receptors into the membrane.

Increasing interest has been focused on the L-glutamate (L-Glu) because L-Glu is considered to be the principal neurotransmitter that mediates fast excitatory synaptic transmission in the central nervous system of vertebrate. In this study, we have tried to incorporate L-Glu as a calcium receptor into bilayer lipid membrane.

Calcium ion is a very common stimulus which can activate K^+ channel in the plasma membranes of many types of cells (Martymski and Tien, 1991; Tien and Salamon, 1989) and can regulate the permeation of

[1] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China.

[2] To whom correspondence should be addressed; e-mail: ekwang@ns.ciac.jl.cn.

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$Fe(CN)_6^{4-}$ across Langmuir-Blodgett monolayers of 1,3-ditetra-decylglycero-2 phosphocholine on Pt electrodes (Sugawara *et al.*, 1987). The calcium ion interaction with Lecithin (PC)/cholesterol (CH) bilayer lipid membrane on Pt using $Fe(CN)_6^{4-/3-}$ as redox probe has been reported (Jiang *et al.*, 1998). Although calcium ion can induce permeation of $Fe(CN)_6^{4-/3-}$ across LB or BLM supported on Pt electrode, glassy carbon electrode coated incorporated L-Glu DMPC film can give fast response to calcium ion.

This work is aimed at (a) identification of bilayer lipid membrane on the GC and (b) studying the ion channel behavior of bilayer lipid membrane incorporated L-glumatic acid. In the absence of calcium cations, ion channel is in closed state. In the presence of calcium cations, ion channel is open and electrochemical responses toward Ca^{2+} are obtained based on the permeability change. The ion channel current is only concentration-dependent.

EXPERIMENTAL

Materials

Poly L-Glutamate and phosphatidylcholine dimyristoyl (DMPC) were purchased from Sigma Chemical Co. (USA) and used without further purification. Analytical grade potassium ferricyanide was purchased from Bejing Chemical Reagent Factory (Beijing, China). 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. Impedance spectroscopic experiments were conducted in the frequency range 10–0.1 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, platinum coils as an auxiliary electrode, and a GC electrode as a working electrode.

Method for Supported Bilayer Lipid Membrane Formation

Phosphatidylcholine dimyristoyl (DMPC) was dissolved in chloroform to give a final concentration of 2.5 mg mL^{-1}, which was called BLM forming solution. Prior to sBLM formation, a glassy carbon electrode was polished with 1.0, 0.3, and 0.05 μm 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 M KCl solution, and the potential was held at 1.5 V for 3 min in order to polarize the electrode. After polarization, the GC electrode was dried under purified nitrogen. Subsequently, the GC electrode was immersed into the forming solution and then the electrode was immediately transferred into the 0.1 M KCl solution, where the supported bilayer lipid membrane was formed spontaneously.

Formation of a BLM With Incorporated Glutamate

The GC electrode coated with BLM was immersed in a pH = 6 phosphate buffer solution containing poly L-glutamate (2 mg/1 mL^{-1}) for 3 h, so that poly L-Glu existed in glutamate with negative charge (Dalian Light Industry Institute, 1994). Then glutamate incorporated BLM was obtained. This is an important requirement for forming the ion channel because only poly L-Glu anions could form ion association compounds with the calcium.

RESULTS AND DISCUSSION

Cyclic Voltammetric Responses of a Bare GC Electrode and GC Electrode Coated With Lipid Membranes

As described in the introduction, stability of the bilayer membrane is essential for a detailed investigation and application. In addition, evaluation of the membrane formation is another point to be considered. Especially in the case of ion channel in membrane, these two requirements are of crucial importance.

In this study, lipid membranes were fabricated. To evaluate the lipid membranes, cyclic voltammograms were used.

The cyclic voltammograms before and after the coating of GC surface with BLM are presented in Fig. 1(a) and (b). Comparing Fig. 1(b) with Fig. 1(a), the peak currents due to the reversible electrode reaction of a $Fe(CN)_6^{4-/3-}$ system on a bare GC electrode were almost completely suppressed by the lipid layer membranes and peak-to-peak separation between the cathodic and anodic waves of $K_3[Fe(CN)_6]$ were increased. That means the lipid membranes were formed and blocked permeation of the $Fe(CN)_6^{4-/3-}$ (as marker ions) onto the surface of the underlying GC and were successfully formed on

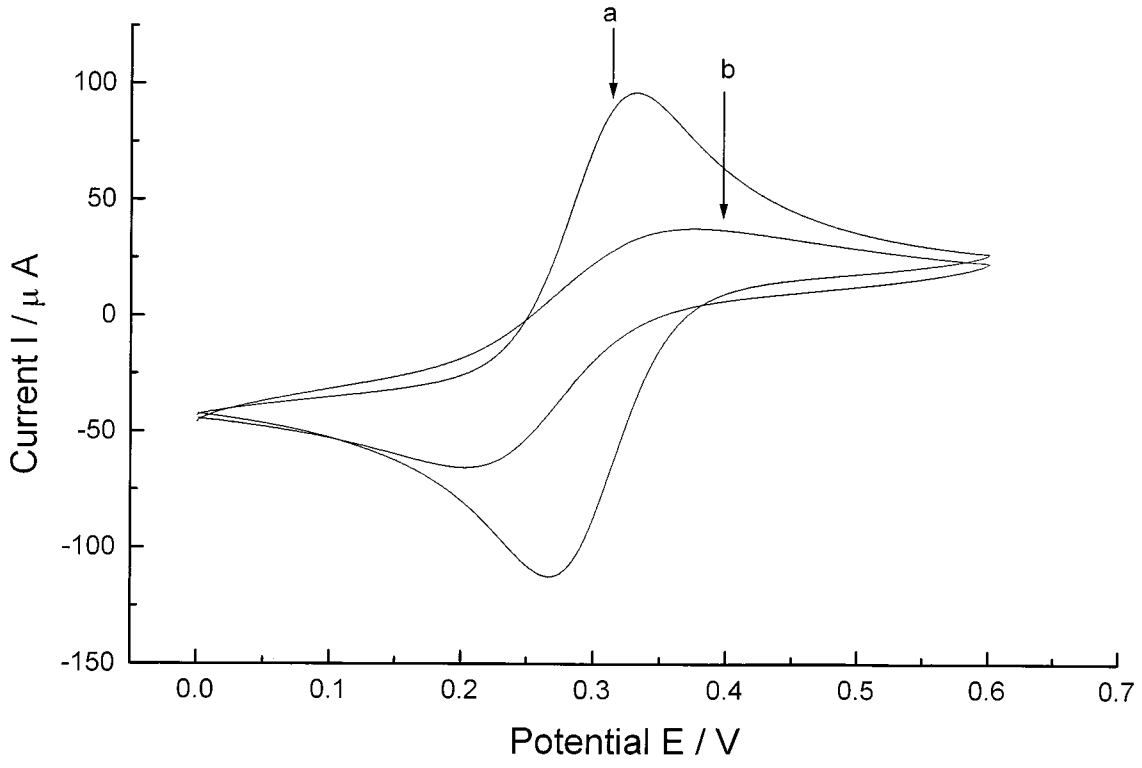

Fig. 1. Cyclic voltammograms of GC electrode (a) bare GC electrode and (b) GC electrode coated with lipid membranes. $[Fe(CN)_6^{3-}] = 5$ mmol L^{-1}; $[K^+] = 1$ mol L^{-1}. Scan rate: 100 mV/s.

the surface of GC. The suppression of CV peaks of the marker ion by coating the DMPC film appeared to be due to a rigid alignment of the phosphate molecules. Relatively bulky ferrocyanide anions are not allowed to permeate through the closed channel toward the underlying electrode.

Impedance Spectroscopy of GC Coated With Lipid Membranes

Bilayer lipid membrane is the ideal model in studying biomembranes (Tien, 1974) and so the method of forming BLM and determination of thickness of lipid membranes are important. To evaluate the thickness of lipid membranes, impedance spectroscopy was widely used in probing the features of a surface-modified electrode (Hall *et al*., 1995; Ren and Pickup, 1997). The complex impedance can be presented as the sum of the real, Z_{re}, and imaginary, Z_{im}, components which originate mainly from the resistance and capacitance of the cell, respectively. Figure 2 is the results of impedance spectroscopy on bare electrode (a) and modified electrode (b) with supported lipid membranes. It can be seen from Fig. 2(a) that the bare GC electrode exhibits an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. With respect to the modified electrode, significant difference in the impedance spectra is observed Fig. 2(b). Inset of Fig. 2(b) was a modified Randle's equivalent circuit that was chosen to fit the measured results. R_s is the electrolyte resistance; C_m the lipid membrane capacitance; R_m the lipid membrane resistance, C_{dl} the double-layer capacitance; R_{ct} charge-transfer resistance and Z_w the Warburg element.

To determine the thickness of the lipid membranes, the capacitance was chosen to show this feature. The double-layer capacitance (C_{dl}) consists of the unmodified GC electrode (C_{GC}) and a capacitance originated from the DMPC membranes (C_m) on the surface of the electrode. The relationship of them can be expressed by Eq. 1 (Plant, 1999; Patolsky *et al*., 1999):

$$1/C_{dl} = 1/C_{GC} + 1/C_m \quad (1)$$

From the Eq. 1, we can get that the value of C_m is 0.40 μF cm^{-2}. With the capacitance value of the lipid membranes and an estimate of its dielectric constant k, $k = 2.05$ (Fettiplace *et al*., 1971), the thickness d of the

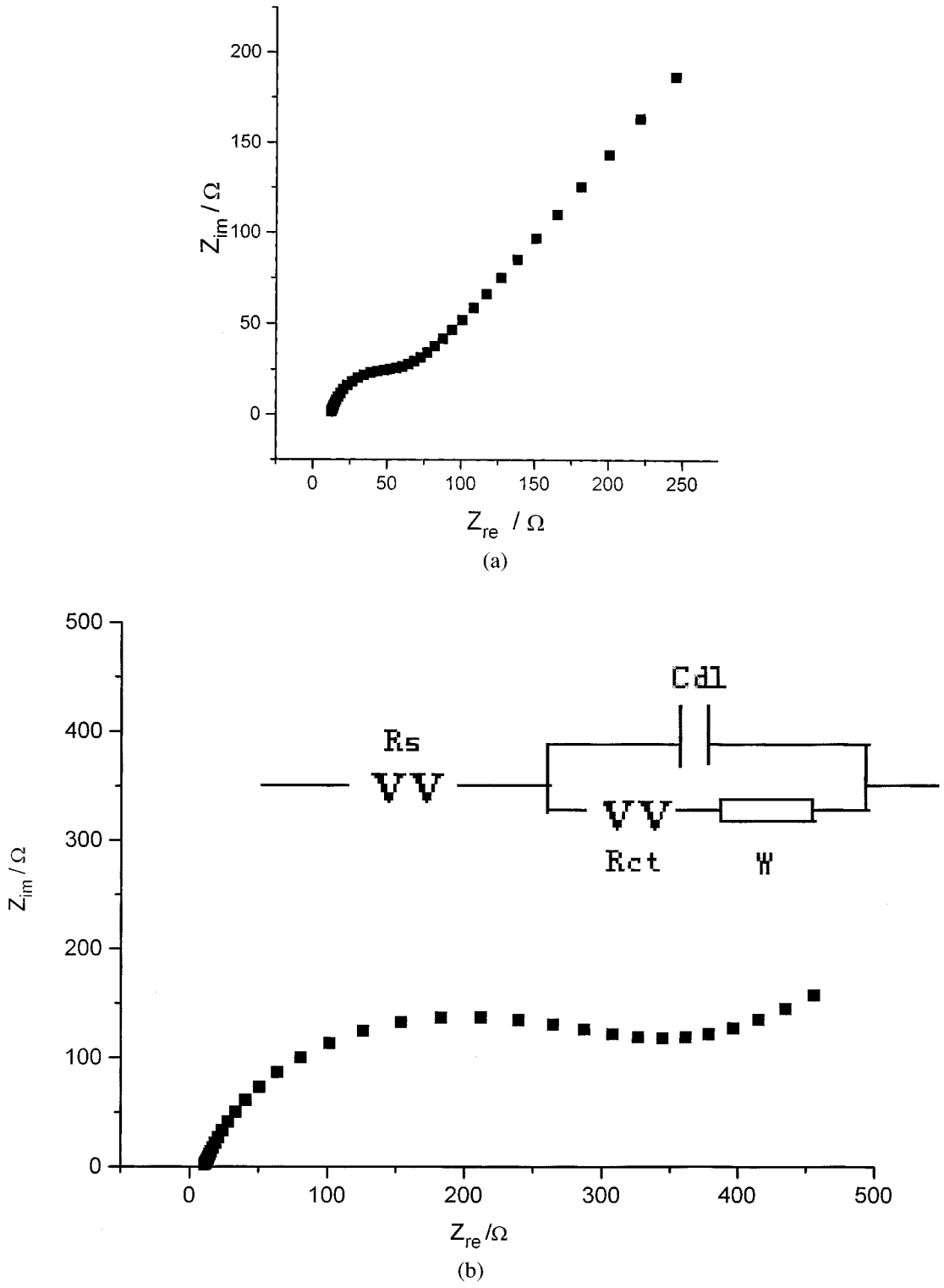

Fig. 2. Complex plane impedance plot (a) at a bare GC electrode; (b) a modified electrode with supported lipid membrane. Inset: modified Randle's equivalent circuit used to model impedance data in the presence of redox couples. $[Fe(CN)_6^{3-}] = [Fe(CN)_6^{4-}] = 5$ mmol L^{-1}; $[K^+] = 1$ mol L^{-1}.

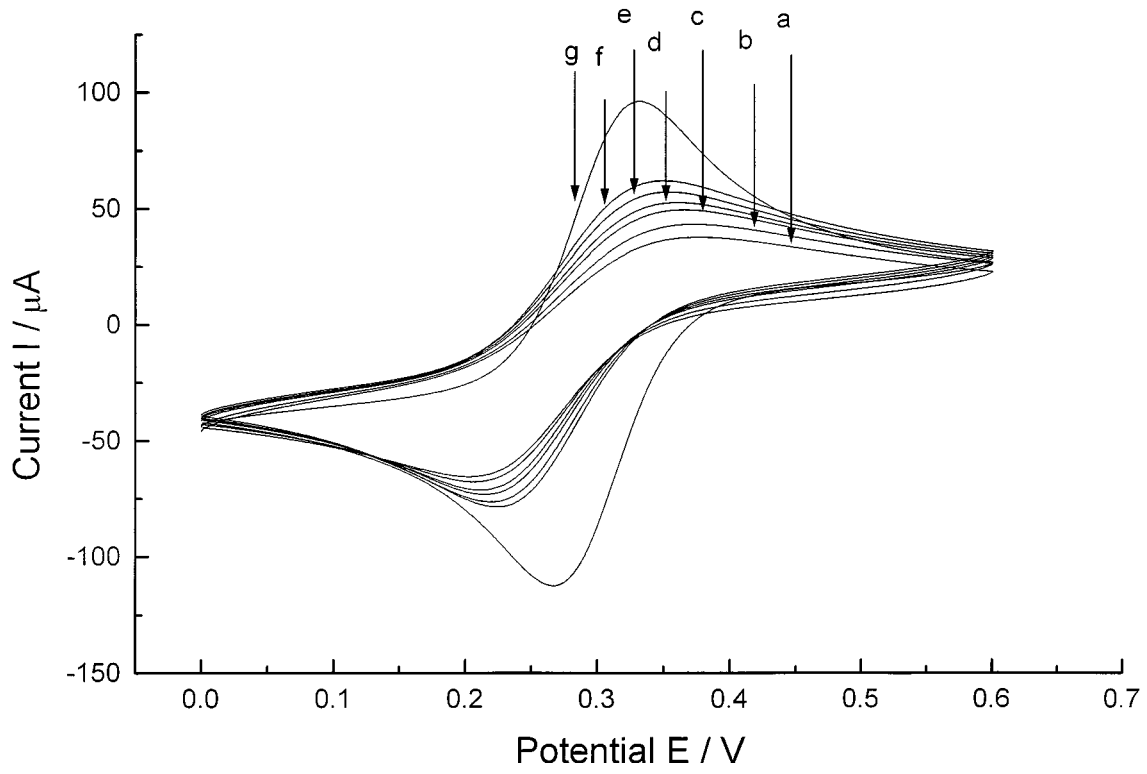

Fig. 3. Cyclic voltammograms of 5 mmol L^{-1} $Fe(CN)_6^{3-}$ ions at a bare GC electrode (g) and the GC electrode coated with lipid membrane incorporated poly L-glutamate in different concentration of Ca^{2+} : (a) 0, (b) 0.05, (c) 0.10, (d) 0.15, (e) 0.20, and (f) 0.30 mmol L^{-1}. $[K^+] = 1$ mol L^{-1}. Scan rate: 100 mV/s.

lipid membranes was estimated according to following equation (Plant, 1999):

$$C_m = \varepsilon k/d \qquad (2)$$

Where ε the permittivity of free space($\varepsilon = 8.85 \times 10^{-14}$ F cm^{-1}). From Eq. 2 the thickness of DMPC lipid membrane was calculated to be 4.5 nm. Because thickness of the single lipid membrane of DMPC should be 2.4 nm (Horber *et al*., 1988; Watt *et al*., 1981), DMPC lipid membrane formed on the GC is the bilayer lipid membrane.

Ion Channel Behavior

One of the important features of ion channel is that stimulus triggered "on/off" switching of the gate function is reversibly made.

Figure 3 shows cyclic voltammetric responses of GC electrode coated with incorporated glutamate DMPC film with different $[Ca^{2+}]$. When Ca^{2+} ions were added to the electrolyte, a distinct CV response from the $Fe(CN)_6^{4-/3-}$ was immediately gained and the intensity of the peak currents did not change with the adding time as if the lipid membranes were "leaking." When the GC electrode only coated with DMPC film was immersed in the same electrolyte with enough amount of Ca^{2+}, the CV response from the $Fe(CN)_6^{4-/3-}$ could be observed after 30 min. That means the poly L-glutamate play an important role to give CV response from the $Fe(CN)_6^{4-/3-}$ in this process. The peak currents increased significantly with increasing concentration of Ca^{2+} ions and the CV peaks of the marker ion appeared at almost the same potential value as those observed at the bare GC electrode. When the electrode coated DMPC film incorporated poly L-glutamate was immersed in electrolyte without Ca^{2+} ions again, the same CV curve as the starting one (Fig. 3a) reappeared. The behavior is fully reversible and the reversible open–close processes can be repeated many times.

The GC electrode coated DMPC film incorporated poly L-glutamate can respond reproducibly to Ca^{2+} ions for 7 h, when stored in 0.1 mol dm^{-3} KCl at room temperature.

Figure 4 shows $[Ca^{2+}]$ dependent changes in the reduction peak current. The current value for the reduction peaks showed almost a linear relationship with $[Ca^{2+}]$

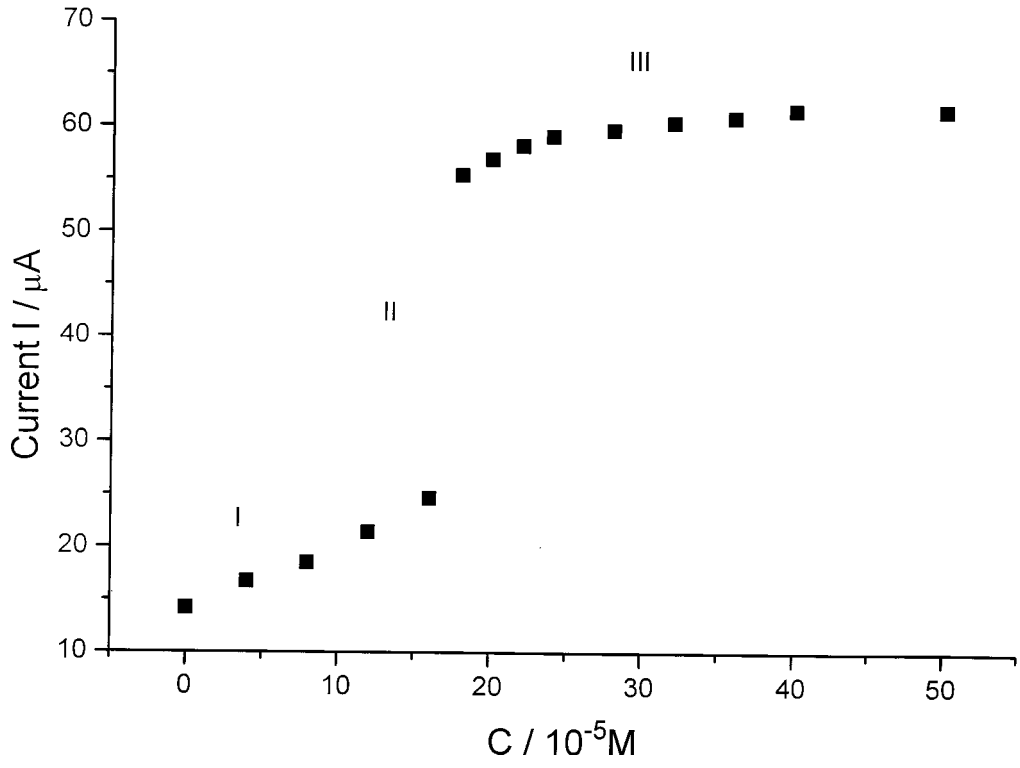

Fig. 4. The plot of anodic peak current on the GC electrode coated with lipid membrane incorporated poly L-glutamate against concentration of Ca^{2+}. $[Fe(CN)_6^{3-}] = 5$ mmol L^{-1}; $[K^+] = 1$ mol L^{-1}.

in the range 1×10^{-5}–1.6×10^{-4} mol dm^{-3} and 1.6×10^{-4}–2.4×10^{-4} mol dm^{-3}. The peak current initially increases with $[Ca^{2+}]$, then increases sharply and finally reaches a plateau. Such ion concentration-dependent changes in CV profiles were not seen for Zn^{2+}, Na^+, and K^+ ions.

These results may be explained as follow: the electrode is first covered with the DMPC film and impedes the redox-active species to electrode. Then glutamate, which forms hydrophilic microdomains, was incorporated into DMPC film. The microdomains containing anionic chains function as "ion channels" for the redox species (Maeda *et al*., 1990). The electrode surface is, however, still covered to some extent with DMPC films which serve as the building blocks. In addition, the polyanion network would reduce the local concentration of the anionic redox couple near the electrode surface, mainly owing to electrostatic repulsion so that the ion channel is in closed state without stimulus. With the addition of Ca^{2+} ionic crosslinking of carboxylate groups should occur, resulting in a contracted conformation of poly L-glutamate chain and a conformational change on the lipid membranes. The relatively "open" feature of the "ion channels" as well as the reduced number of anionic sites in the channel is considered to account for allowing marker ions to permeate across the membrane, leading to the enhancement of the peak currents (Fig. 4). It is apparent that there are three stages in Fig. 4. In range 1×10^{-5}–1.6×10^{-4} mol dm^{-3} and 1.6×10^{-4}–2.4×10^{-4} mol dm^{-3} the intensity of the peak current increased with the concentrations of Ca^{2+} ions. In stage II, the peak currents sharply increased with the concentration of Ca^{2+}. This is attributed to the ion channel gate opened and anionic sites reduced suddenly with marker ion concentration above 1.6×10^{-4} mol dm^{-3}. When all glutamate in the DMPC film totally interacted with Ca^{2+}, which implied all ion channels were all open and no anionic sites existed, peak currents did not increase with stimulus ion concentrations again for stage III.

CONCLUSION

In this paper, bilayer lipid membrane was successfully formed on a GC electrode. The ion channel behavior of the glutamate membrane was investigated and mechanism of stimulated on/off switching of ion channel was discussed. The calcium ion stimulated on/off switching of the gate function was reversibly made and could

repeat many times. The channel was open when stimulus existed. The cyclic voltammogram response to calcium ion was very fast. The peak current increased with the concentration of stimulus. When there was no stimulus, the channel was in closed state. The peak current was affected by the concentration of calcium ion but not by time.

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