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Effects of Mg^{2+} on Supported Bilayer Lipid Membrane on a Glassy Carbon Electrode during Membrane Formation

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The effects of Mg^{2+} on supported bilayer lipid membrane (s-BLM) during membrane formation were investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). There was an increase of the peak-to-peak separation between the cathodic and anodic waves from CV, and an obvious decrease of charge transfer resistance from EIS for the membrane formed in 0.1M $MgCl_2$ compared with that in 0.1M KCl. The higher ionic strength of $MgCl_2$ led to more lipid molecules dropping from the surface of the electrode before they were ready to be in directional arrangement to form s-BLM, so the membrane was not so dense. When reducing the concentration of $MgCl_2$ to 0.03M or 0.05M, the total resistance decreased during the scanning process, which suggested the structure of the lipid membrane changed. The interaction between Mg^{2+} and hydrophilic parts of lipid molecules should be responsible for the phenomena.

Keywords: Supported bilayer lipid membrane, Mg^{2+}; Cyclic voltammetry; electrochemical impedance spectroscopy

1. INTRODUCTION

Mg^{2+} is one of the most abundant metal cations in the intracellular compartment, which plays an important role in controlling ion channel, ATP enzyme activity and metabolism, et al [1-4]. A lot of investigations have been reported on magnesium, which were mainly about the direct interactions between Mg^{2+} and protein [5], ion channel [1]. However, the effect of Mg^{2+} on biomembrane was little reported, especially on the structure and arrangement of membrane lipid [6].

Solid supported bilayer lipid membrane [7-9] is a perfect model of biomembrane, which is widely used to study the transport in cell membrane [10-12], the interaction between some compounds and lipid [13-14], and biosensor [9,15]. The understanding of the process of membrane formation and its electrical properties is the basis of above studies. Many factors, such as preparation, composition and environmental conditions can influence the electrical properties of s-BLM [16-19].

The studies on the interaction between metal cations and biomembrane have attracted considerable interest in recent years. Up to now, a lot of metal ions have been reported on the interaction with biomembrane, such as Ca^{2+} [20], Cu^{2+} [21]. Toyan and his coworkers [22] found that Mg^{2+} also had some effect on the order of membrane lipid, so Mg^{2+} must have influence on the electrical properties of s-BLM during membrane formation, which will be beneficial to the understanding of the role of Mg^{2+} in the cell. In the present paper, the effects of $MgCl_2$ compared with KCl at different concentration on s-BLM formed on a glassy carbon (GC) electrode during membrane formation were investigated by CV and EIS. We found that, compared with KCl, $MgCl_2$ had obvious effects on the electrical properties of s-BLM during membrane formation, and the mechanism was suggested.

2. EXPERIMENTAL PART

2.1. Materials

L-a-phosphatidylcholine (PC) from fresh egg yolk was purchased from Beijing Biological Reagents Plant. The substance was used without further purification. All other reagents were of analytical grade. The water used in all the experiments was ion-exchanged and filtered by a Millipore Milli-Q-system ($R > 18\ M\Omega\ cm^{-1}$).

2.2. Electrochemical measurements

All electrochemical measurements were performed on an electrochemical workstation (CHI650A, CHInstruments, USA). The three-electrode system consisted of s-BLM as the working electrode, a saturated calomel reference electrode (SCE) and a platinum coil auxiliary electrode. All electrochemical experiments were carried out in self-made electrolytic cell and electric isolater at the room temperature ($20 \pm 1^{o}C$).

EIS was performed in the frequency range of 0.1 Hz to 10 kHz. A 5 mV amplitude sine with dc potential 0 V with respect to the open-circuit potential wave was applied between the working and reference electrode. Zview2 program was used for fitting the obtained EIS data.

2.3. Electrode preparation

The methods for the formation of s-BLMs on a GC electrode have been described before [23]. PC was dissolved in chloroform to give a final concentration of 3.0 mg/ml. Prior to s-BLM formation, a 2 mm diameter GC electrode (from Lanlike, China) was polished repeatedly with 1.0, 0.3 and 0.05 μm alumina slurry, followed by successive sonication in deionized water and acetone for 2 min. The electrode was dried in air. Then an 1.5-µl aliquot of the lipid solution was dropped onto the surface of the electrode using a microsyringe, and the electrode was transferred into 0.1 M KCl solution immediately.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of GC electrode and GC electrode coated with lipid membrane

After GC electrode coated with BLM-formation solution had been immersed in 0.1M KCl for about half an hour, it was transferred into 1 mM $[Fe(CN)_6]^{3-}$ with 0.2 M KCl as the supporting electrolyte. Fig. 1 showed the cyclic voltammograms of the bare GC electrode and the modified electrodes. Compared with the bare GC electrode, a decrease existed in the amperometric response of the modified electrodes and an increase in the peak-to-peak separation between the cathodic and anodic waves of $[Fe(CN)_6]^{3-}$. This implied that $[Fe(CN)_6]^{3-}$ was prevented from reaching the surface of the GC electrode by the lipid membrane.

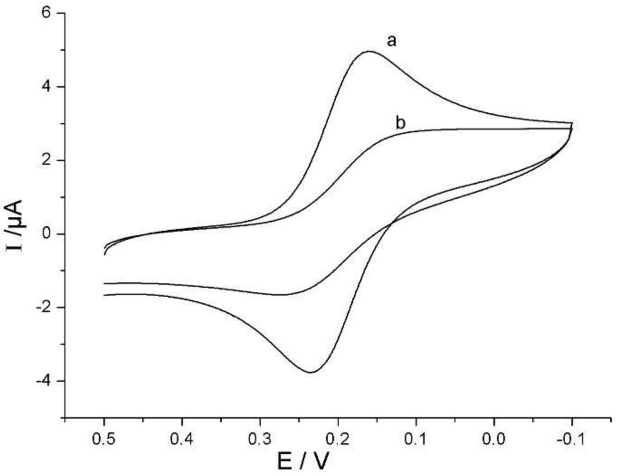

Figure 1. Cyclic voltammograms of (a) bare electrode and (b)glassy carbon electrode supported BLM in 1mM $K_3[Fe(CN)_6]$containing 0.2 M KCl. Scan rate: 50mV s^{-1}.

3.2. EIS measurements of GC electrode supported lipid membrane

EIS is an effective method for probing the features of surface modified electrodes and provides comprehensive information about the dominant electrical process of the system under investigation [24]. Fig. 2 and Fig. 3 showed the impedance spectroscopy as Nyquist plot (-Z"vs Z') of the bare GC electrode and modified electrodes, using 10 mM $[Fe(CN)_6]^{4-/3-}$ as marker ions in 0.1 M KCl at EIS frequency from 0.1 Hz to 10 kHz. It was seen that the Nyquist plot consisting of two parts, one of which was semicircle, another exhibited an almost straight line that was typical characteristic of a diffusional limiting step. With respect to the s-BLM modified GC electrode, the plots showed aberrance semicircle implying a kinetic limiting step.

In order to obtain more detailed information of s-BLMs, a modified Randle's equivalent circuit (inset of Fig.3) was chosen to fit the data. The total impedance was determined by several parameters: (1) electrolyte resistance, Rs; (2) the lipid membrane capacitance, Cm; (3) the lipid membrane resistance, Rm; (4) the constant phase element, CPE; (5) charge transfer resistance, Rct; (6) Warburg element, Zw. The value of Cm of s-BLM was obtained as 0.40 ± 0.02 μF cm^{-2}, which was comparable with the published data [23,25], and Rct of 12.10 ± 0.03 kΩ.

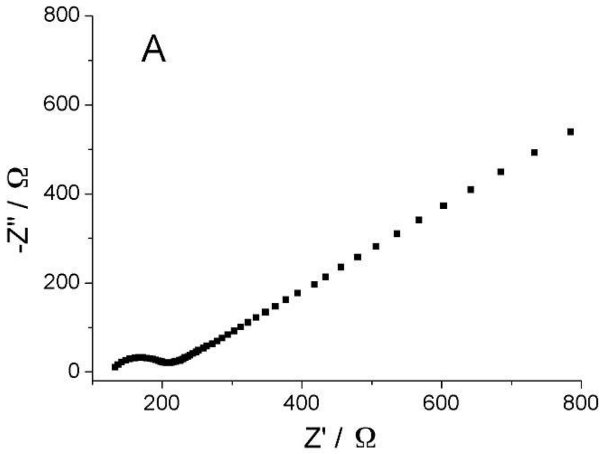

Figure 2. Impedance spectra of bare glassy carbon electrode in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1)mixture containing 0.1M KCl.

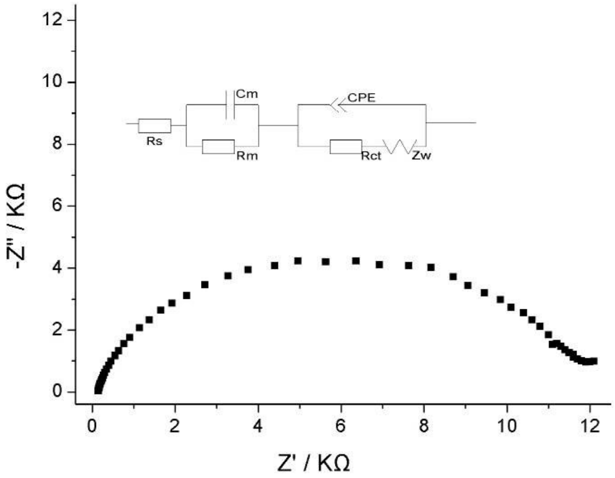

Figure 3. Impedance spectra of glassy carbon supported BLM in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1)mixture containing 0.1M KCl. Inset shows the equivalent circuit used to model impedance data.

3.3. Effects of Mg^{2+} on s-BLM during membrane formation

In the experiments, s-BLMs were formed respectively in KCl and $MgCl_2$ at different concentration. Fig.4 showed cyclic voltammograms of s-BLM formed in 0.01M and 0.1M KCl. We could find that, when we reduced the concentration of KCl to 0.01M, the amperometric response of modified electrode decreased obviously, and the peak-to-peak separation between the cathodic and adnodic waves of $[Fe(CN)_6]^{3-}$ increased, which implied less $[Fe(CN)_6]^{3-}$ could reach the surface of the GC electrode, and the membrane became electrically denser. EIS was also used to investigate the effects, Fig. 5 showed charge transfer resistance increasing obviously and more typical characteristic of kinetic limiting step. This was in agreement with Fig. 4. As we suggested, a lot of factors could influence the electrical properties of s-BLM. Here, we thought the changes of the ionic strength of the electrolyte solutions was the main reason. As we know, during the formation process of s-BLM, the hydrophilic polar groups of lipid molecules form a monolayer on the surface of the GC electrode, which is also hydrophilic. The hydrocarbon parts of the PC are sticking out from the sorbed first monolayer. The second monolayer of PC molecules is therefore formed with an orientation mirror image of the first one. The electrolyte solution induces excess lipid solution drained away, leading to the formation of s-BLM. So the ionic strength of the electrolyte can influence the velocity of s-BLM, and should be proper. In the same time of membrane formation, the lesser ionic strength would make excess chloroform and lipid molecules dropped from the electrode more slowly, so muti-layer lipid membrane was formed instead of bilayer membrane.

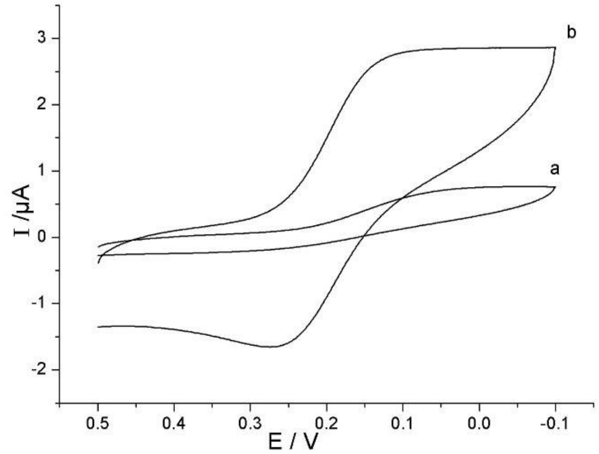

Figure 4. Cyclic voltammograms of supported BLMs formed in 0.01M (a) and 0.1M (b) KCl in 1 mM $K_3[Fe(CN)_6]$ containing 0.2 M KCl. Scan rate: 50mV s^{-1}.

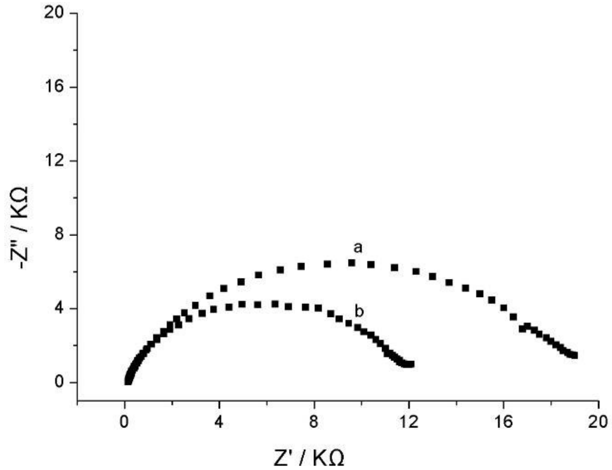

Figure 5. Impedance spectra of glassy carbon supported BLM formed in 0.01M(a) and 0.1M(b) KCl in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1)mixture containing 0.1M KCl.

When the membrane was formed in 0.1M $MgCl_2$, compared with that in KCl at the same concentration, there was an increase of the peak-to-peak separation between the cathodic and anodic waves (see Fig. 6), while the magnitude of the response changed little. Through EIS measurements (Fig. 7), the charge transfer resistance decreased a lot, and the spectra showed a little typical characteristic of diffusion limiting step. This implied the membrane was not so electrically dense any more, and more $[Fe(CN)_6]^{3-}$ ions could reach the surface of the GC electrode. The ionic strength of $MgCl_2$ was higher compared with the same concentration of KCl, which would result in more molecules dropped from the surface of the electrode during membrane formation before they were ready to be in the directional arrangement to form the bilayer lipid membrane, and in the end, the membrane was not so dense. While, we thought that the influence of Mg^{2+} on the structure and order of the lipid membrane by interacting with the hydrophilic parts of PC molecules should also be considered, which needed further experiment to prove.

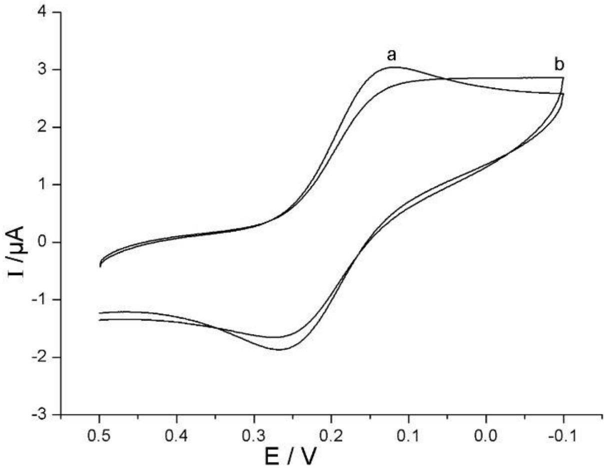

Figure 6. Cyclic voltammograms of supported BLMs formed in 0.1M $MgCl_2$(a) □0.1M KCl(b) in 1 mM $K_3[Fe(CN)_6]$ containing 0.2 M KCl. Scan rate: 50mV s^{-1}.

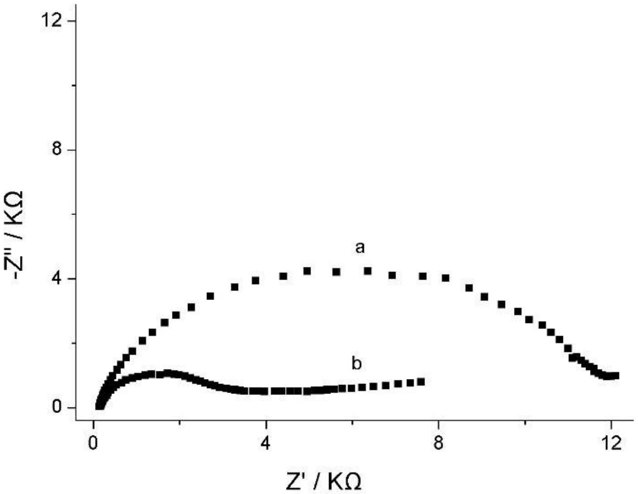

Figure 7. Impedance spectra of glassy carbon supported BLM formed in 0.1M KCl(a) □0.1M $MgCl_2$(b)in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1M KCl.

When we reduced the concentration of $MgCl_2$ to 0.03M, Fig.8 showed the cyclic voltammograms, and we could find that the response decreased obviously and the redox wave was hardly found. EIS (see Fig.9) also showed charge transfer resistance increased. However, the total resistance decreased during the scanning process, which implied the order and structure of the lipid membrane changed, which was different from the membrane formed in KCl or 0.1M $MgCl_2$. we thought this was because Mg^{2+} could entered in the space of lipid molecules during membrane formation. When the modified electrode was transferred into the test solution, Mg^{2+} would gradually moved into the solution due to the weak interaction between Mg^{2+} and lipid molecules and concentration effect. So the arrangement of lipid molecules would be influenced, resulting in the change of charge transfer resistance. While for 0.1M $MgCl_2$, the formed membrane was not dense and the arrangement of lipid molecules was comparatively dispersive, so the effect of Mg^{2+} was not obvious, and for KCl, K^+ could influence s-BLM little.

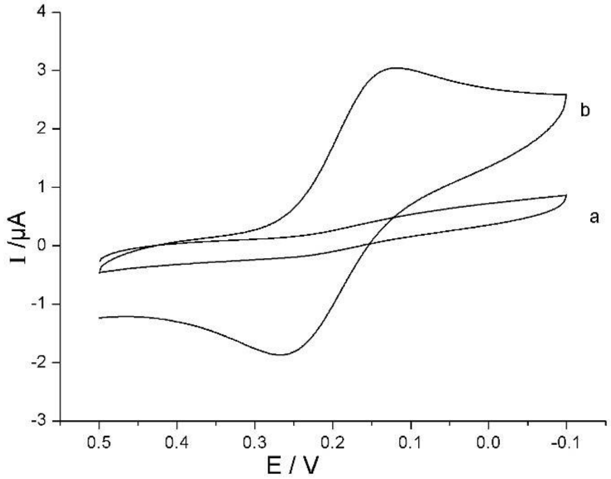

Figure 8. Cyclic voltammograms of supported BLMs formed in 0.03M(a) □0.1M(b) $MgCl_2$ in 1 mM $K_3[Fe(CN)_6]$ containing 0.2 M KCl. Scan rate: 50mV s^{-1}.

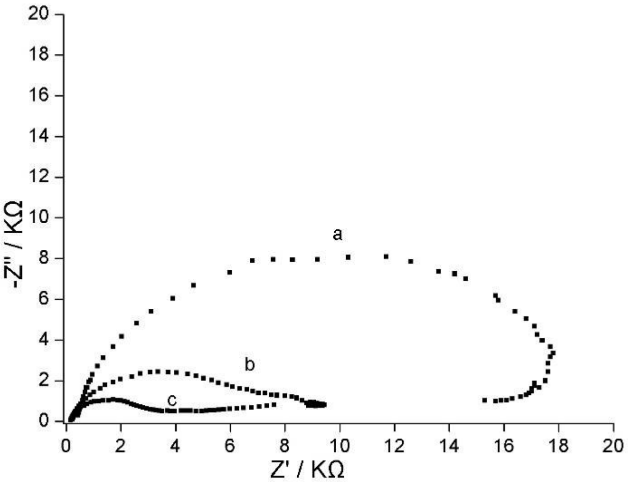

Figure 9. Impedance spectra of glassy carbon supported BLM formed in 0.03M(a), 0.05M(b)and 0.1M(c) $MgCl_2$ in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1M KCl.

4. CONCLUSIONS

The effects of Mg^{2+} on s-BLM on a GC electrode during membrane formation were investigated by CV and EIS. The experiments suggested that, different from KCl, Mg^{2+} had obvious effects on the electrical properties of s-BLM during membrane formation. The changes of ionic strength of the electrolyte solutions and the interaction between Mg^{2+} and the hydrophilic parts of lipid molecules should be responsible for the phenomena. Further experiment was being carried out to understand more details on the mechanism.

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