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Interaction of Quercetin with Supported Bilayer Lipid Membranes on Glassy Carbon Electrode

Xiaoquan Lu [1,], Tianlu Liao [1], Lan Ding [2], Xiuhui Liu [1], Yan Zhang [1], Yina Cheng [1], Jie Du [1]*

[1] College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070, P. R. China
[2] College of Life Science, Northwest Normal University, Lanzhou 730070, China
[*]E-mail: luxq@nwnu.edu.cn

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The interaction of quercetin with a supported bilayer lipid membrane (s-BLM) on a glassy carbon electrode (GCE) was investigated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and scanning electrochemical microscope (SECM). With the presence of quercetin as stimulus and $Fe(CN)_6^{3-/4-}$ as the probe ion, experiments showed that the interaction between quercetin and lipid membranes leaded $Fe(CN)_6^{3-/4-}$ to cross the s-BLM. This phenomenon suggested that some kinds of pores form on the surface of membrane which decreased membrane resistibility to the probing molecules and resulted in the interaction between quercetin and lipid membranes. Based on the investigation, it may be helpful to understand the biological activity of quercetin in vivo.

Keywords: quercetin; bilayer lipid membranes; cyclic voltammetry; impedance spectroscopy; scanning electrochemical microscope

1. INTRODUCTION

The bilayer lipid membrane (BLM) system has been employed extensively as an experimental model of biomembranes, including lipid vesicles, Langmuir–Blodgett monolayer, cast lipid film, bilayer lipid membrane (BLM), supported bilayer lipid membrane (s-BLM), etc [1]. Among these mimetic biomembrane model systems, BLM play an important role in biological activity. However, the major drawback of the BLM system is its instability, which limits its application for mimic experiment. Subsequently, Tien et al. developed a new method of making stable BLM on freshly formed surface of hydrophilic metal such as Pt, Au, Ag, Cu, Ni, stainless steel or glassy carbon, which is abbreviated to s-BLM [2-5]. Nowadays, s-BLM has been wide potential applications in electrochemical biosensors [6, 7] and ion channel behavior [8, 9].

Flavonoids are non-nutritive compounds of plants that have been aroused considerable interest due to their broad pharmacological activity, recently. In fact, flavonoids have been reported to have important medical functions such as antiviral, anti-allergic, anti-platelet, anti-inflammatory and anti-tumor activities [10–13]. The literatures on the electrochemical investigation of flavonoid quercetin are limited. There has not much information on its effect in the transportation of biomembranes so far. The s-BLM is a useful model for understanding the effect of quercetin on biological cell membranes. The studies of interaction between quercetin with s-BLM are important to further understand pharmacology of quercetin. Thus, it is very interesting to study the interaction of drugs with the membrane of the target cell.

In this paper, we described the effect of quercetin on cell membrane model with electrochemical methods. By means of self-assembly of lipid, we made layers of lipid on the surface of a GC electrode. Some electrochemical information was obtained using surface electrochemical method. We found that quercetin interacted with lipid membrane strongly and the action made $Fe(CN)_6^{3-/4-}$ easy reach the surface of GC electrode. Our results showed that quercetin had a biological activity invitro, which enhanced the permeability of the lipid membranes. The aim of the current study was convenient to understand the molecular mechanism of the interaction of quercetin with cell membranes.

2. EXPERIMENTAL PART

2.1. Materials and chemicals

Quercetin was obtained from Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou). Quercetin was dissolved in chloroform and doubly distilled water as stock solution and stock solutions were stored at 4^oC. Phosphatidylcholine (PC) was purchased from Shanghai Biotechnology Company (China). Potassium ferricyanide was purchased from Beijing Chemical Company (China). Acetate salts were purchased from Tianjing Chemical Company (China). The other reagents were of analytical grade and all chemicals were used without further purification.

2.2. Apparatus and procedures

Cyclic voltammetry was performed on a CHI832 electrochemical workstation (CH Instrument Co. Ltd, Austin, USA). Scanning electrochemical microscope (SECM) image experiments were performed on CHI900 electrochemistry station (CH Instruments Co. Ltd, Austin, USA). The SECM tip was a 25-μm diameter Pt UME. Before each experiment, the tip was polished with 0.3-μm alumina and rinsed with pure water. The main quantitative operation was obtained from the feedback mode. AC impedance spectroscopic experiments were carried out with a VMP2 electrochemical workstation (Princeton Applied Research Co. USA) in the frequency range from 200 kHz to 0.1 Hz and with signal amplitude of 10 mV. All experiments were carried out with a three-electrode system, including an Ag/AgCl (KCl-saturated) electrode as reference electrode, a platinum wire served as the counter electrode, a 4mm diameter GC electrode (from Lanlike, China) as the working electrode and were

performed in the presence of a 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1 moll^{-1} KCl as a redox probe at the potential of the system. A Branson 200 Ultrasonic cleaner (USA) was used to clean the working electrode.

Above experiments were carried out at room temperature. The buffer and sample solution were deaerated with purified nitrogen for 10 min for removing oxygen prior to the beginning of a series of experiments.

2.3. Method for supported bilayer lipid membrane formation

The methods for the formation of s-BLMs on a GC electrode have been described [4, 14]. Herein, we made a little modification. Phosphatidylcholine (PC) was dissolved in chloroform to give a final concentration of 20 mg/ml, which was called a BLM-forming solution. A supported bilayer lipid membrane was prepared as follows. Prior to s-BLM formation, a glassy carbon electrode was first polished successively with 0.1, 0.3 and 0.05μm alpha alumina powder and then cleaned ultrasonically in doubly distilled water and acetone for 5 min, respectively. Then, the GC electrode was immersed in 0.1 moll^{-1}KOH 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 electrode was immersed in the BLM-forming solution for 20s and immediately transferred into the 0.1 moll^{-1}KCl solution for 30 min., where the supported bilayer lipid membrane was formed spontaneously.

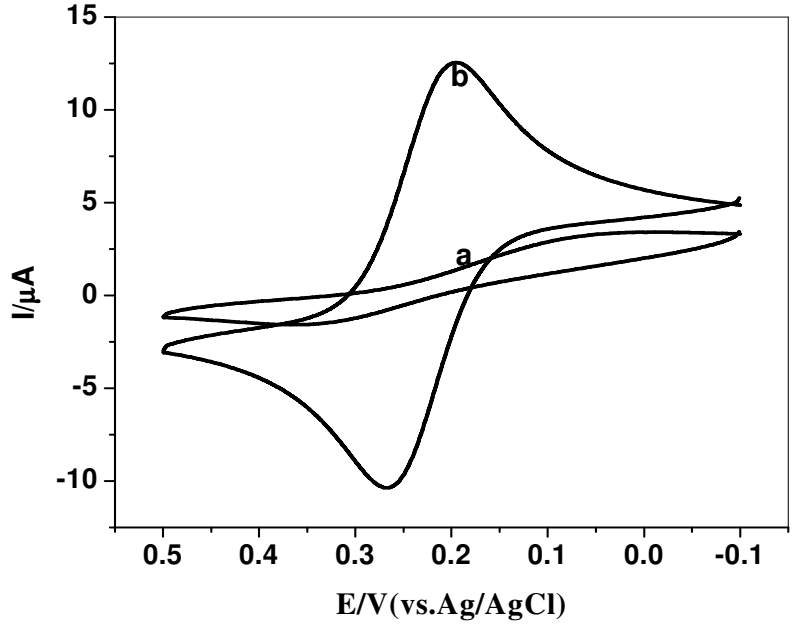

Figure 1. Cyclic voltammetric responses of 1.0mmoll^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1 moll^{-1} KCl on (a) bare GC electrode, (b) s-BLM. Scan rate 50mV s^{-1}.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of the GCE and the GCE supported lipid membranes

The cyclic voltammetry was applied as a convenient and informative electrochemical method for the examination of membrane integrity and $Fe(CN)_6^{3-/4-}$played the role of electroactive species as a probe to assess the ionic permeability of the membrane. Fig.1 shows the cyclic voltammetric response of probe ion on the bare GCE (Fig. 1a) and the GCE coated with supported bilayer lipid membranes (Fig.1b) in 1.0 $mmoll^{-1}$ $Fe(CN)_6^{3-/4-}$ solution. Fig.1a shows the redox process of $Fe(CN)_6^{3-/4-}$ on the bare GCE. It was observed that the peak separation ΔE_{pac} was 71 mV at scan rate $50mVs^{-1}$. The data for GCE is consistent with a reversible electron transfer model. Comparing Fig.1b with Fig.1a, we could find a noticeable decrease in the current response of the electrode and an increase in the peak-to-peak separation between the cathodic and anodic waves of $Fe(CN)_6^{3-/4-}$. It means that $Fe(CN)_6^{3-/4-}$ was prevented from reaching the surface of GC electrode. Hence, it is clear that the supported lipid membranes had been formed on the surface of GC electrode successfully.

3.2. Impedance measurement of the GC electrode and the GC electrode supported lipid membranes

AC impedance spectroscopy has become an effective method for probing the features of the surface-modified electrodes and often applied as a complementary technique to cyclic voltammetry. Fig.2 shows the impedance spectroscopy as Nyquist plots of (a) the bare GCE and (b) the GCE modified with a lipid membrane by using $1.0mmoll^{-1}$ $Fe(CN)_6^{3-/4-}$as probe ions at their formal potential. Fig. 2a illustrates the results of impedance spectroscopy of a bare electrode. It can be seen that the bare GC electrode exhibits almost straight line that is the characteristic of a diffusional limiting step of the electrochemical process. However, with the presence of the bilayer lipid membrane, a significant difference in the impedance spectra is observed (Fig.2b). The semicircle located near the origin is probed by higher frequencies, which means that the dynamics of electron transfer in higher frequency range could be observed and the current due to voltage excitation is under kinetic control [15]. The obvious semicircle of modified electrode indicates the s-BLM hindered electron transfer of marker ions.

For the sake of giving more detail information about the impedance property of the membrane, we chose a modified Randle's equivalent circuit [4](inset of Fig.2) to fit the measured results. The total characteristic of impedance was determined by several parameters, where R_{sol} is the electrolyte resistance, R_m the lipid membrane resistance, C_m the lipid membrane capacitance, C_{dl} the double-layer capacitance, R_{ct} charge-transfer resistance, and Z_w the Warburg element. The value of C_m of s-BLM was determined directly from the impedance spectroscopy using Randle's equivalent circuit as 4.0×10^{-7} Fcm^{-2}.

There is a relationship between the capacitance value and the thickness of the bilayer lipid membrane. Considering the bilayer lipid membrane as a plate condenser, the thickness of lipid membrane is estimated according to the following equation: $C_m=\varepsilon_0\varepsilon/d$, Where d is the thickness of s-BLM, ε_0 is the relative dielectric permittivity of the lipid ($\varepsilon_0=8.85\times10^{-14}$ Fcm^{-1}) and ε is the dielectric

constant of the lipid (ε =2.05) [16]. From the equation, we could get the result that the thickness of PC lipid membrane coated on the GCE is about 4.53 nm. It is very close to the value of 4–10 nm, which is the thickness of the bilayer of PC [17]. It is clear that the s-BLM have been formed on the surface of GC electrode.3.3. The interaction of quercetin with s-BLM

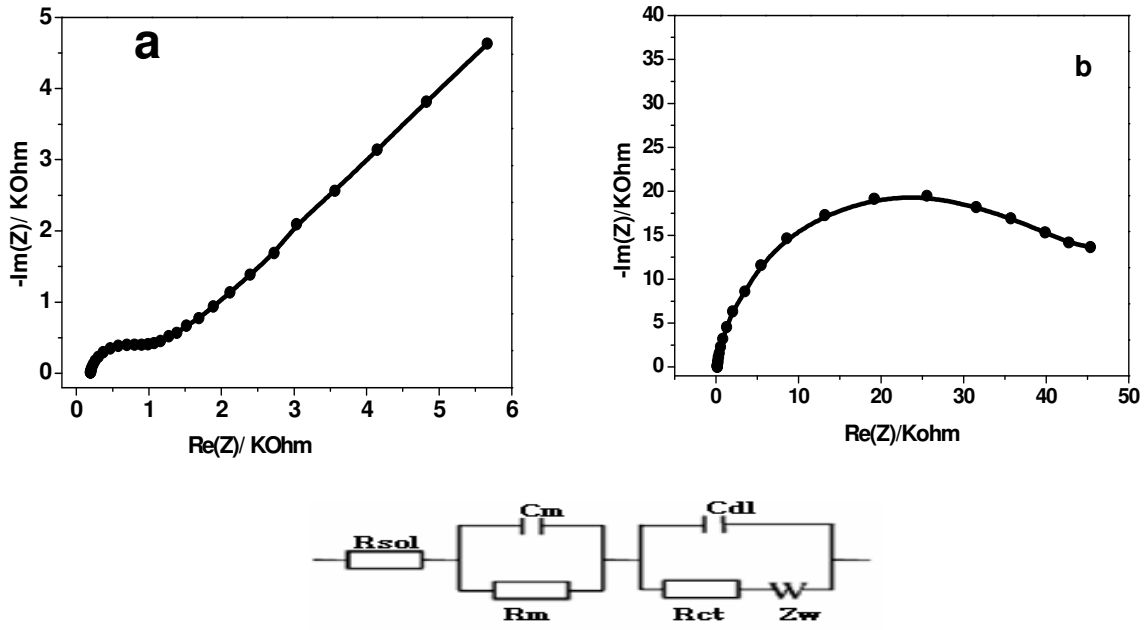

Figure 2. Ac impedance spectroscopy of (a) a bare GCE and (b) s-BLM in 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1moll^{-1} KCl as the supporting electrolyte. Frequency range: 200 kHz-0.1Hz. Inset: Randles sequivalent circuit.

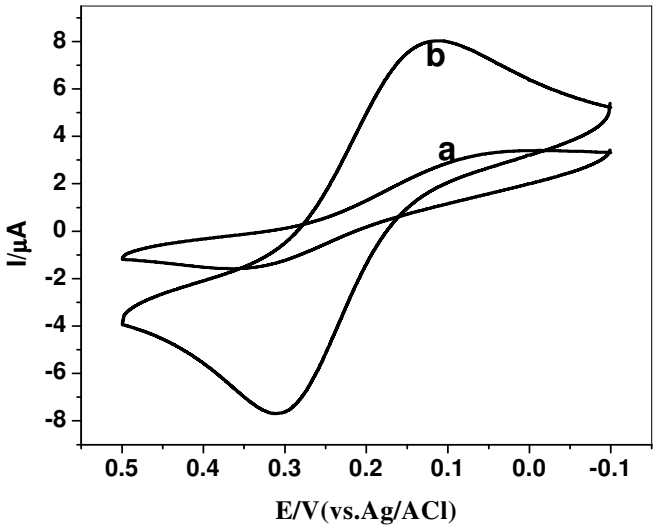

Figure 3. Cyclic voltammetric responses of (a)s-BLM and (b) s-BLM after interacting with 4.0mmoll^{-1} quercetin for 5 min. in 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$ solution with 0.1 mmoll^{-1} KCl as supporting electrolyte. Scan rate 50 mVs^{-1}.

3.3.1. Cyclic voltammetric responses of the interaction between quercetin and s-BLM

After the BLM was formed on the surface of GC electrode, the electrode was immersed in 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$ solution. Cyclic voltammetric (CV) response of the electrode was recorded as Fig.3a. When we put the electrode into the solution containing 4.0 mmoll^{-1} quercetin for 10min, then took out, immersed it in 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$ solution. Its CV response was recorded as Fig.3b. A distinct CV response from the $Fe(CN)_6^{3-/4-}$ complex ion was gained as if the lipid membranes were 'leaking'. It means that the effective electrode area was increased. It can be known that quercetin induce some active sites on the BLM and $Fe(CN)_6^{3-/4-}$ can diffuse across the s-BLM at the active sites, which we call pores. The reasonable explanations are the quercetin is not simply adsorbed on the surface of s-BLM, it interacts with s-BLM and produces some pores. The pores do not have selectivity so that $Fe(CN)_6^{3-/4-}$ can reach the surface of electrode and produce CV response.

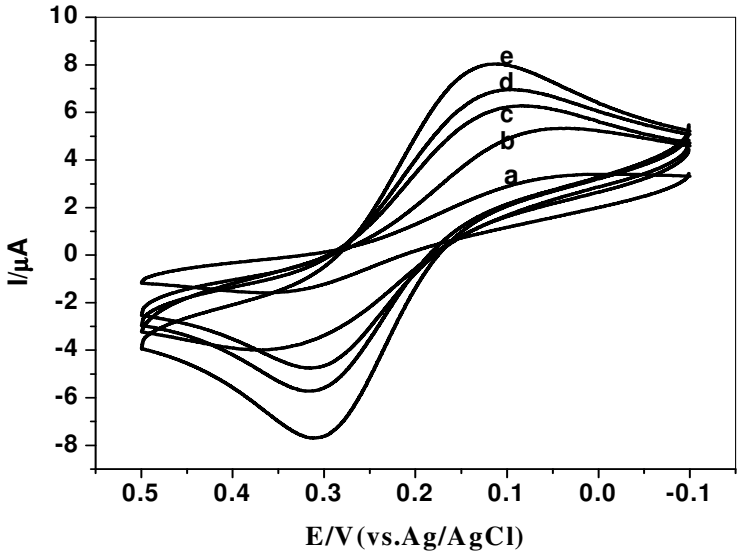

Figure 4. Cyclic voltammetric response of 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$, at GC electrode supported bilayer lipid membrane after interaction with different concentration of quercetin for 5 min: (a) 0.0 mmol l^{-1}, (b) 1.0 mmol l^{-1}, (c) 2.0 mmol l^{-1}, (d) 3.0 mmol l^{-1}, (e) 4.0 mmol l^{-1}. Scan rate 50 mVs^{-1}.

With the presence of quercetin as stimulus and $Fe(CN)_6^{3-/4-}$ as the marker ion, Cyclic voltammetric response of 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$, at GC electrode supported bilayer lipid membrane after interaction with different concentration of quercetin for 10 min. The intensity of the peak current increased with the concentration of quercetin (Fig.4).This can be attributed to higher concentration of quercetin producing more pores, which causes more $Fe(CN)_6^{3-/4-}$ reach the surface of the electrode.

Furthermore, the pore behavior is time-dependent. Fig.5 shows the current response of the channel as a function of time. The peak current of $Fe(CN)_6^{3-/4-}$ increased with time and reached steady state after 30 min. Subsequently, We took out the electrode and then immersed it in the solution

containing 0.1 moll^{-1} KCl and found the pore behavior was kept stable more than 12h. It was concluded that the bond between quercetin and Phosphatidylcholine was firm.

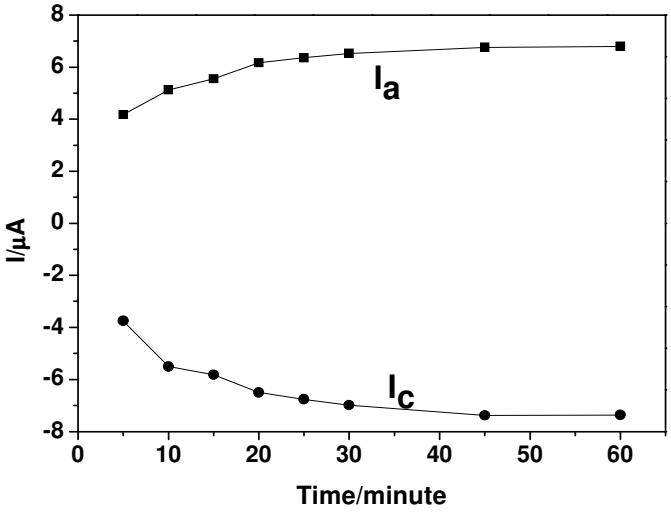

Figure 5. Cathodic and anodic peak currents of 1.0 mmoll^{-1} $Fe(CN)_6^{3-/4-}$, at GC electrode supported bilayer lipid membranes as a function of time after interaction with 3.0 mmoll^{-1} quercetin, Scan rate 50mVs^{-1}.

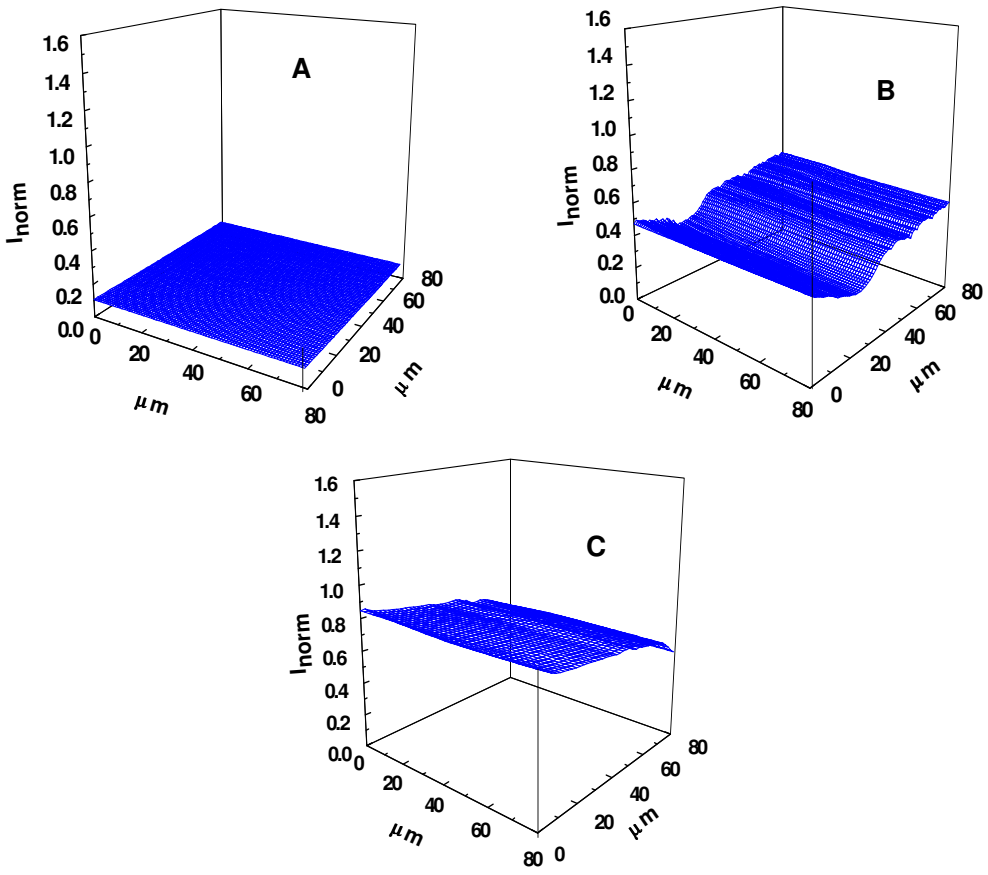

Figure 6. A-C: SECM image of after s-BLM interacted with 1.0 mmol l^{-1} quercetin for different time (A: 0 min, B: 15 min C: 30 min). The solution is 1.0 mmol l^{-1} $K_3Fe(CN)_6$ and 0.1 mol l^{-1} KCl.

3.3.2. Study of interaction of quercetin with s-BLM by Scanning electrochemical microscope

Scanning electrochemical microscope (SECM) is a powerful method for imagine the surface on a molecular scale and has been extensively used to study the various structures of biomembrane system [18-20]. The interaction of quercetin with s-BLM was further demonstrated by SECM. Here, the modified GCE was acted as substrate, and a 25-μm-diameter Pt UME was immersed into a 1.0 $mmoll^{-1}$ $K_3Fe(CN)_6$ solution forming the SECM tip. The main qualitative operation was obtained from the feedback detection mode. All values of tip current (i_T) was divided by the steady state current ($i_{T,\infty}$) to show as the normalized current. The interaction was imaged through monitoring the normalized current change. Fig.6 A-C were a series of images obtained by SECM on the GC electrode supported bilayer lipid membrane after interaction with 4.0 $mmoll^{-1}$quercetin for different time (A: 0 min, B: 15 min, C: 30min).The corresponding approach curves were shown in Fig.7. By comparing the current intensities of these images, it is clear that the SECM feedback current of the s-BLM after interacting with 4.0$mmoll^{-1}$ quercetin is greater than that of the BLM covered substrate. From Fig. 6 A, we can see that the GCE modified with BLM is smooth and flat relatively. The formation of membrane prevented $Fe(CN)_6^{3-/4-}$ redox couple from reaching the surface of the electrode to a great extent. The same s-BLM used in experiments to interact with quercetin and the image was shown in Fig.6 B, C. With increasing of interaction time, defects increased. It was clear that the image changed markedly with a sharp increase in the current after interacted with quercetin for 30 min and there were some bumps and collapses shown in the image. The experimental results showed that quercetin caused the formation of defects and micropores on the surface of BLM, which caused the promotion of the redox couples reaching the surface of electrode. Obviously, the result was consistent with the electrochemical results.

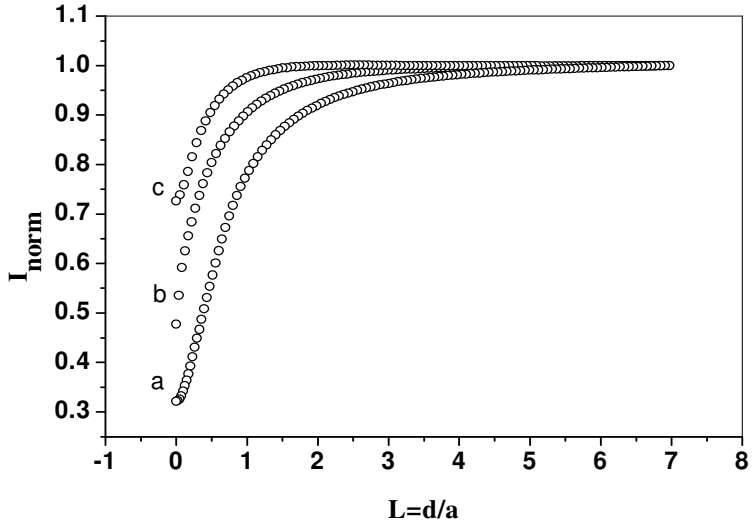

Figure 7. Probe approach curves with respect to the corresponding the modified electrode of A-C images in Fig.6.

4. CONCLUSIONS

From all the above experiments, we can concluded that the interaction of quercetin with the s-BLM produces pores, which allow the redox couple reach to the surface of the electrode. As the membrane is a lipid bilayer with hydrophilic groups on the surface and hydrophobic hydrocarbon chains inside. The change of dipolar direction may affect the orientation of hydrophobic chains with respect to the surface of electrode. We presumed that the interaction may be quercetin with the head of PC，which lessens the interaction among PC head groups. The resulting molecular arrangement changes loose, even produces some channels, Probe ions $Fe(CN)_6^{3-/4-}$can diffuse and reach the surface of the electrode surface.The results provide new insight into rational drug design and would lead us to understand the biological activity of quercetin in vivo for the further development of a stabilized lipid film biosensor for quercetin.

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