J Appl Electrochem (2009) 39:1163–1167
DOI 10.1007/s10800-009-9778-5

ORIGINAL PAPER

Electrochemical sensing of the ion-channel formation of OmpF

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Received: 8 April 2008 / Accepted: 23 December 2008 / Published online: 14 January 2009
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Abstract The ion-channel formation of outer membrane protein F (OmpF) can be achieved in phosphatidylcholine bilayer membrane modified on a pyrolytic graphite electrode, which makes it possible to sense the porin functionality conveniently with an electrochemical technique through the change in the redox peaks currents of an electroactive marker. The effect of ionic strength, pH on the ion permeability of OmpF and the effect of the protein concentration on the ion-channel formation have been thus examined. This study may provide a simple and rapid way to probe other similar biological processes within natural cellular membranes.

Keywords OmpF · Porin · Phosphatidylcholine · Ion-channel sensor

1 Introduction

Gram-negative bacteria are protected by an outer membrane which acts as a permeability barrier against external harsh environments [1]. Many crucial cellular activities, such as solute uptake, protein translocation and signal transduction, are fulfilled by the embedded proteins in this membranous structure present outside the cytoplasmic membrane and the peptidoglycan layer [2]. The outer membrane contains a large amount of porins which form passive water-filled channels for the diffusion of hydrophilic molecules (<600 Da) without much selectivity [2].

Outer membrane protein F (OmpF) is the first intrinsic membrane protein to be crystallized [3] and has been well characterized in terms of structure and ion-channel activity [4]. OmpF has a trimeric assembly of monomeric 16-stranded β-barrels, which are connected by amino acid sequences referred to as loops and turns [5]. Relatively, OmpF has long loops on the extracellular side and short turns on the intracellular side, where the L3 loop folds back into the pore and forms the so-called constriction zone [6]. Each of these three identical and functionally independent subunits contains its own hydrophilic pore with poor selectivity and ion specificity [7]. The pore-forming properties of OmpF have been extensively studied, where the permeability for nutrients and antibiotics is especially important for biotechnological and medical applications [8]. OmpF is believed to be slightly cation selective ($Li^+ < Na^+ < K^+$) and is permeable for hydrophilic molecules up to 600 Da [9]. It is suggested by many attempts that the ion permeability is significantly regulated in response to external conditions such as pH [10], ionic strength [11] and membrane potential [12].

As is well known, the ion-channel is an important biological system in cellular activities. The unique features of analyte-triggered membrane permeability switching and selective substrate binding have attracted much attention since Umezawa and co-workers first introduced the principle of mimetic ion-channel sensors [13]. Based on the employment of simple-composition structures to mimic biological cellular membranes, much interest has been given to the study of analyte-stimulated ion-channel behavior [14–17]. However, to the best of our knowledge,

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there is still no report on directly sensing ion-channel formation of a protein and studying the related biological processes. Therefore, electrochemical sensing the ion-channel formation of such proteins would be promising and provide a convenient way to study their reactions responding to external stimulus.

Purified porins are demonstrated to be able to reconstitute into lipid bilayers and form ion-permeable pores. Efforts have been made to help study the ion-channel properties of OmpF tethered to metal surfaces [18, 19] or assembled in nanostructures [8] both supported with lipid bilayer membranes (BLM). BLM and lipid vesicles have been proven to be ideal systems to investigate the function of channel-forming peptides or proteins, providing a friendly environment needed for the maintainance of the active state of integral proteins [20, 21]. In this work OmpF is successfully reconstituted in phosphatidylcholine (PC) membrane modified on a pyrolytic graphite (PG) electrode for the first time. Its ion-channel formation can then be sensitively monitored by an electrochemical method. The commonly used electroactive probe $Fe(CN)_6^{3-/4-}$ can be employed to indicate the ion-channel permeability through the solid BLM. The influence of ionic strength, pH and protein concentration on the ion-channel formation of OmpF in this system has also been examined. This study has presented a convenient electrochemical system to investigate the ion-channel formation of OmpF, as well as the effectors that regulate the interaction between OmpF and lipid membranes. Taking advantage of simplicity and rapidness, this electrochemical method may be applicable and extended to monitor other basic interactions within biological membranes.

2 Materials and methods

2.1 Materials

OmpF was isolated and purified from *Escherichia coli* as previously described [22]. PC was purchased from Sigma and used without further purification. Other chemicals were of analytical reagent grade. Water was purified with a Milli-Q purification system (Barnstead, MA) to a specific resistance >18 MΩ cm^{-1} and was used to prepare all solutions. PC vesicle dispersion was prepared by ultrasonicating 1 mM PC suspension for at least 2 h until it became clear.

2.2 Electrode preparation and electrochemical measurements

The PG electrode was prepared by putting a PG rod (geometric area, 5.45 mm^2) into a glass tube (∅5 mm) and fixing it by epoxy resin. Electrical contact was made by fixing a copper wire to the rod with the help of Wood alloy. The PG electrode surface was pretreated by sanding on rough and fine abrasive papers, followed by polishing to mirror smoothness with an alumina (particle size of about 0.05 μm)/water slurry on silk. Finally, the electrode was thoroughly washed through ultrasonicating in both ethanol and doubly distilled water for about 5 min prior to modification. BLM formation on PG electrode was based on the interaction between the hydrophilic polarized electrode surface and the amphipathic lipid [17]. A mixture containing 10 μL OmpF and 10 μL PC vesicle was placed onto the PG electrode surface. The surface was dried overnight at room temperature. The OmpF-embedded BLM modified electrode was gently rinsed with pure water before use.

Cyclic voltammetric measurements were performed using a CHI 660C electrochemical analyzer (CH Instruments) and a three-electrode system. A one-compartment glass cell consisting of a modified PG working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode was used for the measurements; the cell had a working volume of 5 mL. All experiments were carried out in 0.1 M PBS solution containing 1 mM Fe $(CN)_6^{3-/4-}$ (1:1), where 20 mM KCl was employed as supporting electrolyte.

3 Results and discussion

Cyclic voltammetry can be used to observe the ion-channel formation of OmpF embedded in BLM. The redox couple, $Fe(CN)_6^{3-/4-}$, gives a pair of well-defined peaks at a bare PG electrode (data not shown). Assembled BLM on the PG electrode surface forms a solid barrier that blocks access of this redox couple to the electrode surface, resulting in inhibited electron transfer between $Fe(CN)_6^{3-/4-}$ and the substrate electrode, as is displayed by the dashed line in Fig. 1a. The solid line in Fig. 1a shows the cyclic voltammogram (CV) obtained at the OmpF-embedded BLM modified electrode. Obviously, an enhanced response of $Fe(CN)_6^{3-/4-}$ can be observed, compared with the wave obtained at BLM-alone modified electrode. Therefore, it is reasonable to believe that OmpF has been reconstituted into the lipid bilayers and has formed ion-permeable pores in the solid lipid membrane for the redox probe to reach the surface of the electrode. An OmpF-alone modified electrode has also been tested in this system as comparison. The peak current of $Fe(CN)_6^{3-/4-}$ obtained at the OmpF-alone modified electrode is even lower than that obtained at the BLM-alone coated electrode (Fig. 2), indicating that the accumulation of OmpF will more strongly inhibit the electron transfer between the redox probe and the electrode. This result demonstrates that OmpF itself cannot

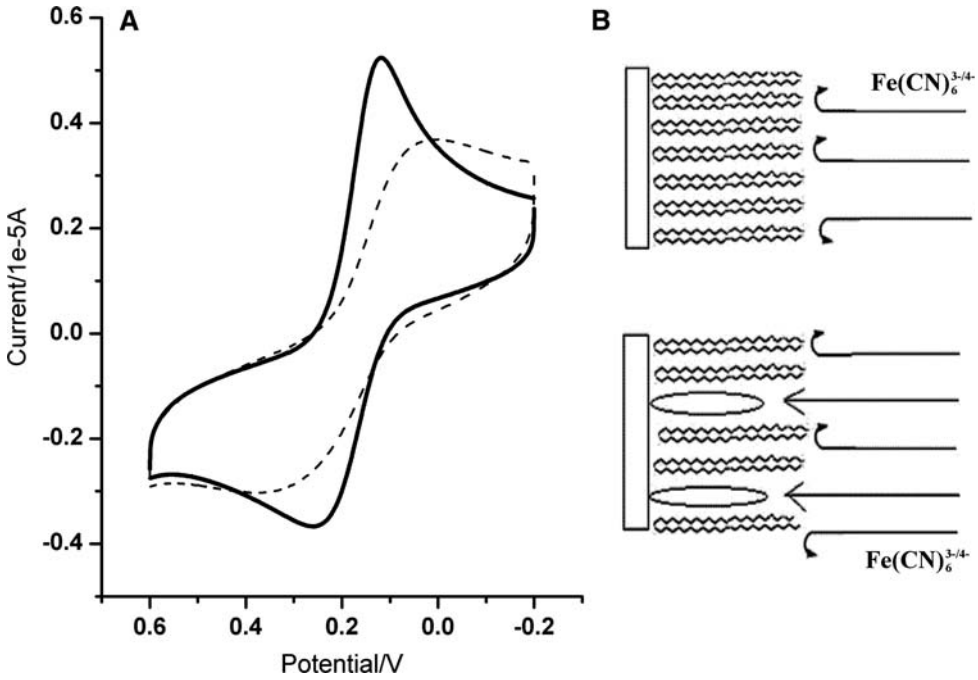

Fig. 1 **a** CVs of 1 mM $Fe(CN)_6^{3-/4-}$ in PBS buffer solution (0.1 M, pH 7.0, containing 0.02 M KCl as supporting electrolyte) obtained at OmpF-embedded BLM modified electrode (solid line) and BLM-alone modified electrode (dashed line). Scan rate: 100 mV s^{-1}. **b** Illustration of electrochemical sensing the ion-channel formation of OmpF in BLM modified on a PG electrode surface

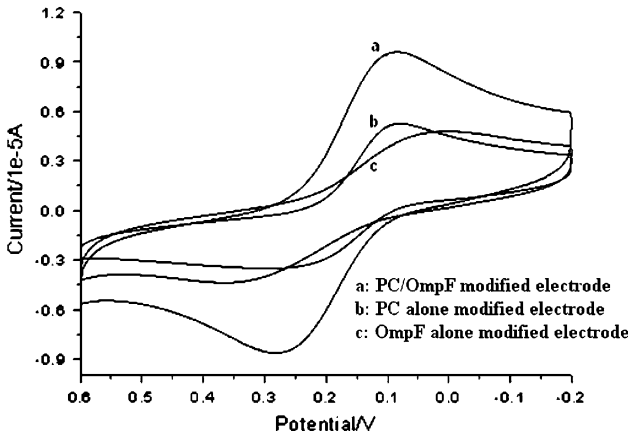

Fig. 2 CVs of 1 mM $Fe(CN)_6^{3-/4-}$ in PBS buffer solution (0.1 M, pH 7.0, containing 0.02 M KCl as supporting electrolyte) obtained at (a) OmpF-embedded BLM modified electrode, (b) BLM-alone modified electrode and (c) OmpF alone modified electrode. Scan rate: 100 mV s^{-1}

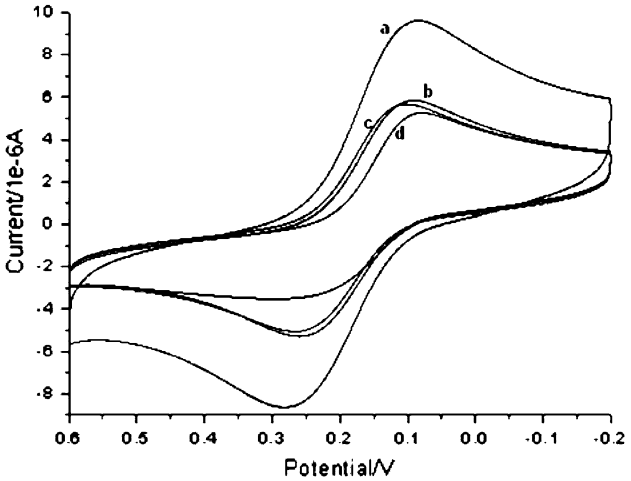

Fig. 3 CVs of 1 mM $Fe(CN)_6^{3-/4-}$ obtained at OmpF-embedded BLM modified electrode prepared with different volume ratio between PC and OmpF. (a) 1:1, (b) 1:1.5, (c) 1:0.5. Curve d shows the case of BLM-alone modified electrode. Others same as in Fig. 1

form ion-channels on the electrode surface without the support of BLM,which is consistent with the natural porin functionality of OmpF in cellular membranes. The mechanism of electrochemical sensing the ion-channel formation of OmpF supported with BLM on a PG electrode surface has been illustrated in Fig. 1b.

The stability of the OmpF-embedded BLM modified electrode and BLM-alone modified electrode have been examined. Both can present a satisfactory result. On the one hand, the modified electrodes can be used in the test buffer for as long as one week. On the other hand, several hundred cycles of scanning of the electrodes can give the same voltammogram. These results also reveal that the stability of the developed ion-channels in BLM on the electrode surface can be also satisfactory.

Different volume ratios between PC vesicle and OmpF were tested for modification of the electrode surface, where the volume ratios were 1:0.5, 1:1, 1:1.5, respectively. Experimental results reveal that the peak currents of the redox probe is the highest when PC vesicle and OmpF is co-modified as 1:1 by volume (Fig. 3). So, a smaller amount of OmpF ($V_{PC}/V_{OmpF} = 1:0.5$) makes less channels for the probe to cross through, while an excessive amount of OmpF ($V_{PC}/V_{OmpF} = 1:1.5$) partially inhibits the electron transfer

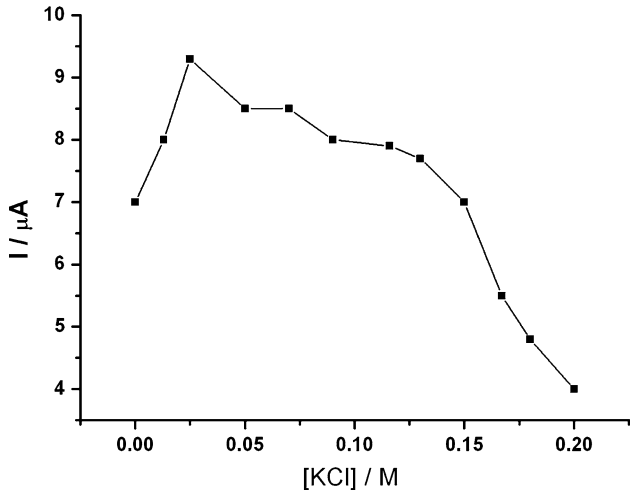

Fig. 4 Plot for the dependence of the anodic peak current of $Fe(CN)_6^{3-/4-}$ obtained at the OmpF-embedded BLM modified electrode on the concentration of KCl in the test buffer solution. Others same as in Fig. 1

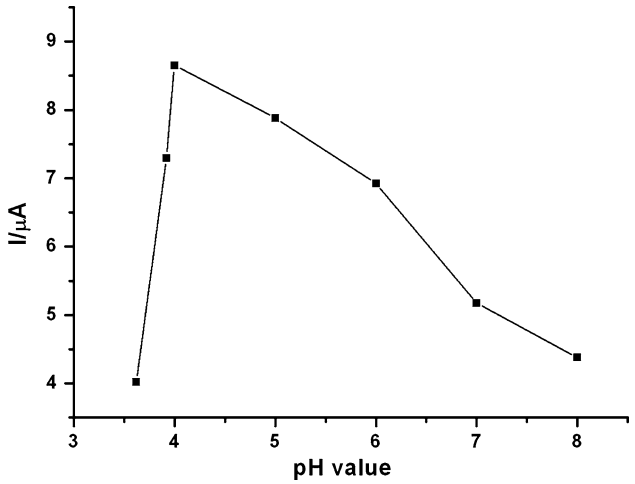

Fig. 5 Plot for the dependence of the anodic peak current of $Fe(CN)_6^{3-/4-}$ obtained at the OmpF-embedded BLM modified electrode on the pH value of the test buffer solution. Others same as in Fig. 1

between the redox probe and the electrode. This result has also further confirmed the important role of BLM in the ion-channel formation of OmpF.

The effect of external ionic strength on the ion-channel behavior of OmpF in BLM has been revealed by changing the concentration of KCl in the test buffer solution. Figure 4 shows the relationship between the electrochemical response of $Fe(CN)_6^{3-/4-}$ obtained at the OmpF-embedded BLM modified electrode and the concentration of KCl in the test buffer solution. The redox peak current of $Fe(CN)_6^{3-/4-}$ increases with the introduction of KCl into the system at lower concentration levels. However, as the KCl concentration increases, the redox peak current of $Fe(CN)_6^{3-/4-}$ gradually declines. When the concentration of KCl is higher than 0.1 M, the redox wave of $Fe(CN)_6^{3-/4-}$ begins to descend rapidly. The result obtained by this proposed electrochemical sensing technique is in good agreement with reports that lower ion-strength helps open the ion-channels formed by OmpF in BLM, while at higher ion-strength, the ion-channel will be shut for self-protection against the harsh environment.

The ion-channel behavior of OmpF is reported to be pH dependent. The pH in the periplasm has been found to not only affect the voltage gating, but also influence the porin channel size and cooperativity of OmpF [10]. We have examined this issue. Figure 5 displays the relationship between pH and the electrochemical response of $Fe(CN)_6^{3-/4-}$ obtained at the OmpF-embedded BLM modified electrode for 0.1 M phosphate buffer solution at different pH values. At mildly acidic pH (typically at pH 4), the OmpF-embedded BLM exhibits enhanced electron transfer activity between the redox probe and the electrode, indicative of better ion-channel behavior. However, when the acidity of the test buffer becomes lower than pH 4, the peak current of the redox probe sharply decreases, indicating strongly reduced ion permeability. On the other hand, the redox peak current of $Fe(CN)_6^{3-/4-}$ also decreases gradually as the pH value becomes higher than 4.0, suggesting that alkaline conditions also result in an inhibition to the ion-channel behavior of OmpF in BLM.

4 Conclusions

Electrochemical technique has been successfully employed in this work to sense the ion-channel formation of OmpF reconstituted in BLM. Based on this electrochemical sensing method, the appropriate amount ratio between OmpF and PC vesicle may induce high ion permeability through the BLM modified on the solid surface. It has been also demonstrated that appropriate ion-strength and mildly acidic conditions benefit the formation of OmpF in BLM. High ion-strength, alkaline conditions, as well as extremely acidic pH, inhibit the ion-channel formation of OmpF, reflecting the self-protection ability of natural cellular membranes against severe external conditions. This study may provide a simple and convenient way to sense other similar biological processes in the future.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant No. 90406005, 20575028), the Program for New Century Excellent Talents in University and the Chinese Ministry of Education (NCET-04-0452).

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