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Characterization and property of DNA incorporated bilayer lipid membranes

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Abstract

Calf-thymus DNA-incorporated bilayer lipid membranes supported on a glassy carbon (GC) electrode was prepared by making layers of phosphatidylcholine dimyristoyl (DMPC) on GC electrode. DNA in the BLM was characterized by cyclic voltammetry, IR and AFM, and lipid layers formed on the GC electrode were demonstrated to be a bilayer lipid membrane by electrochemical impedance experiment. In IR and AFM experiments the findings indicated that DNA was incorporated into BLM. The ion channel of bilayer lipid membranes incorporated was studied. The result showed that the ion channel was opened in the presence of the stimulus quinacrine. In the absence of quinacrine the channel was switched. The process can repeat itself many times. The impedance spectroscopy measurements demonstrate that the stimulus quinacrine opens the channel for permeation of marker ion. The mechanism of forming an ion channel was investigated.

Keywords: Ion channel; Bilayer lipid membrane; DNA; Quinacrine

1. Introduction

During four decades of studies, bilayer lipid membranes (BLM) have demonstrated their usefulness for analytical purposes and biomembrane modeling. It has been shown that BLM modified with suitable species can be ion-selective and photosensitive. They can be employed in drug and toxic testing, in immunological and cancer research [1–3]. Because the BLM formed by the conventional method has usually poor mechanical strength and is very fragile, this is of limited use for protracted studies and for practical applications. In order to improve the BLM shortcoming, BLM supported on the solid substrates is developed [4–6] and is highly suitable for further studies and applications, especially in the field of membrane biophysics, cell biology, biotechnology and ion channel behavior of a membrane [7].

The studies of ion channel behavior of a membrane has aroused the interest of more scientists since the unique feature of ion channels in biological cell membrane is a selective recognition of substrates and subsequent amplification of its information by channel switchings [8]. The chan-

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nel is opened reversibly by a stimulant (analyte)—membrane interaction and great deal of marker ions are allowed to permeate across the membrane.

A growing interest has recently arisen in the development of a DNA sensor which is expected eventually to replace conventional methods in gene diagnoses using nucleic acid hybridization techniques for genetic or pathogenic diseases [9,10]. DNA biosensors being presently developed are based largely on piezoelectric, electrochemical, or optical transducers [11–14]. Because of their unique capability for the separation and purification of genes as well as their potential application for molecular recognition, these kinds of DNA biosensors will be of practical value in both molecular biology and modern biomedical engineering.

The goal of our work is to investigate the use of lipid membranes either for the modification of carbon electrodes for improved incorporation of DNA, or as transducers to monitor directly electrochemical responses to a DNA-binding drug on the basis of the redox couple-mediated 'ion-channel' mechanism. The work herein reports the characterization of ds DNA in bilayer lipid membrane formed on glassy carbon using IR, AFM, CV, etc. The lipid membrane supported on the GC was demonstrated to be a bilayer lipid membrane by electrochemical impedance experiments. The mechanism of an ion channel in a ds DNA bilayer lipid membrane was discussed. With the stimulus present, the channel is opened, and a large amount of marker ions are allowed to permeate through the membrane, which are immediately detected electrochemically at an underlying electrode. With elimination of the stimulus, the channel is again closed reversibly.

2. Materials and methods

2.1. Materials

Phosphatidylcholine dimyristoyl (DMPC) was purchased from Sigma Chemical Co. (USA) and used without further purification. Analytical-grade potassium ferricyanide was purchased from Beijing Chemical Reagent Factory (Beijing, China). All other reagents were of analytical grade. Ultrapure water was prepared with a Quartz distillatory below boiling point and then purified with a Milli-Q ultrapure water system.

2.2. Electrochemical measurements

Cyclic voltammetry and impedance spectroscopy were performed with an Autolab PGSTAT30 (ECO CHEMIE BV, The Netherlands). Impedance spectroscopic experiments were conducted in the frequency range from 10 kHz to 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.

2.3. AFM

2.3.1. Substrates

The mica was freshly cleaved.

2.3.2. Sample preparation

Dimyristoylphosphatidylcholine (DMPC) was dissolved in chloroform to give a final concentration of 2.5 mg ml^{-1}, which was called BLM forming solution. The aliquot droplet of forming solution was applied to cover a piece of freshly cleaved mica. The other process is identical to the method mentioned in Section 2.5.

Surface images were acquired in tapping mode under ambient conditions (Nanoscope IIIa; Digital instruments, Inc.). Si cantilevers having integral tip (spring constant, 0.58 N/m) were used. All images, which were obtained reproducibly over at least four spots on the sample surfaces, were recorded by oscillating the cantilever slightly below its resonance frequency (typically 200–300 kHz) and raster scanning across the surface.

2.4. Fourier-transformed infrared spectroscopy (FTIR)

IR spectra at a resolution of 4 cm^{-1} were recorded on a Nicolet 520 FTIR spectrometer (Nicolet Company, USA) interfaced with a Compaq 2000 computer and continuously purged with dry nitrogen. One hundred and twenty scans were signal averaged. The sample was spread on a

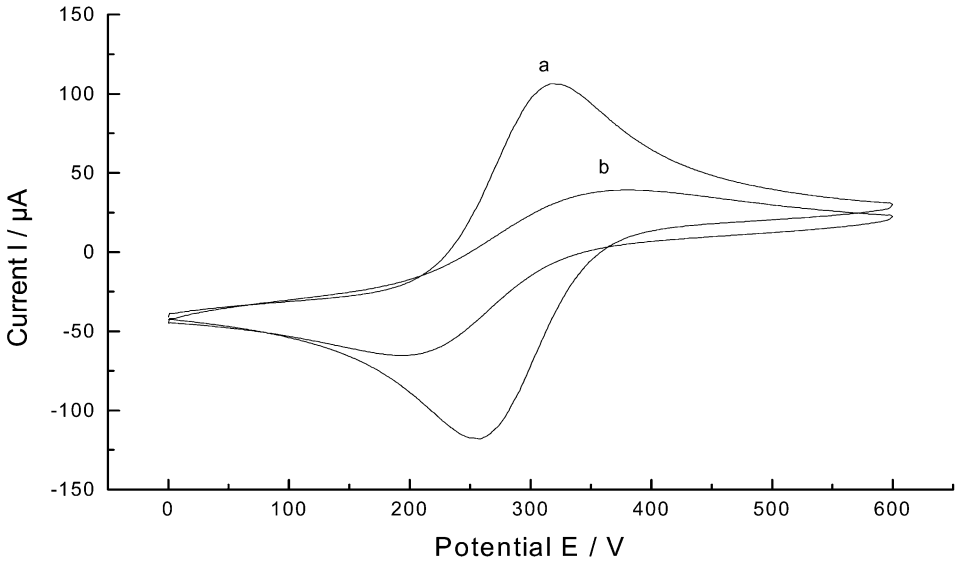

Fig. 1. Cyclic voltammograms of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ at a bare glassy carbon electrode (a) and a glassy carbon electrode modified by a bilayer lipid membrane with incorporated DNA (b). $[K_4Fe(CN)_6]=[K_3Fe(CN)_6]=5$ mM, [KCl] = 1 M, 100 mV/s.

glassy carbon plate. The method is identical to that in Section 2.5. The background was air and subtracted by Nicolet software; the resultant spectra was not subjected to any smoothing process.

2.5. 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 alumna slurry, respectively, and then sonicated for 1 min in deionized water and acetone successively. Then the GC electrode was immersed in 0.1 M KCl solution, and the potential was held at 1.5 V for 3 min in order to polarize the electrode. The surface of the electrode polarized is hydrophilic. After polarization, the GC electrode was dried under purified nitrogen. Subsequently, the GC electrode was immersed into the forming solution for 1 min and the electrode was immediately transferred into the 0.1 M KCl solution, where the supported bilayer lipid membrane was formed spontaneously.

2.6. Formation of a BLM with incorporated DNA

The GC electrode coated with BLM was immersed in a solution containing ds DNA (2.5 mg ml^{-1}) for 1 h. Then, ds DNA incorporated BLM was obtained.

3. Results

The formation of a BLM on the GC surface was judged by a.c. impedance spectroscopy and cyclic voltammograms before and after the electrode was coated with BLM.

Cyclic voltammetric (CV) measurements of ferrocyanide ions as a marker ion with a glassy carbon (GC) electrode and electrode coated with phosphatidylcholine dimyristoyl film are shown in Fig. 1. The reversible CV peaks of a $Fe(CN)_6^{4-/3-}$ system at an uncoated GC electrode (Fig. 1a) are almost completely suppressed by a lipid membrane on the surface of the GC electrode (Fig. 1b).

The impedance spectroscopy is an effective method for probing the features of a surface-modified electrode [15,16]. The complex impedance can be presented as the sum of the real, Z_{re}

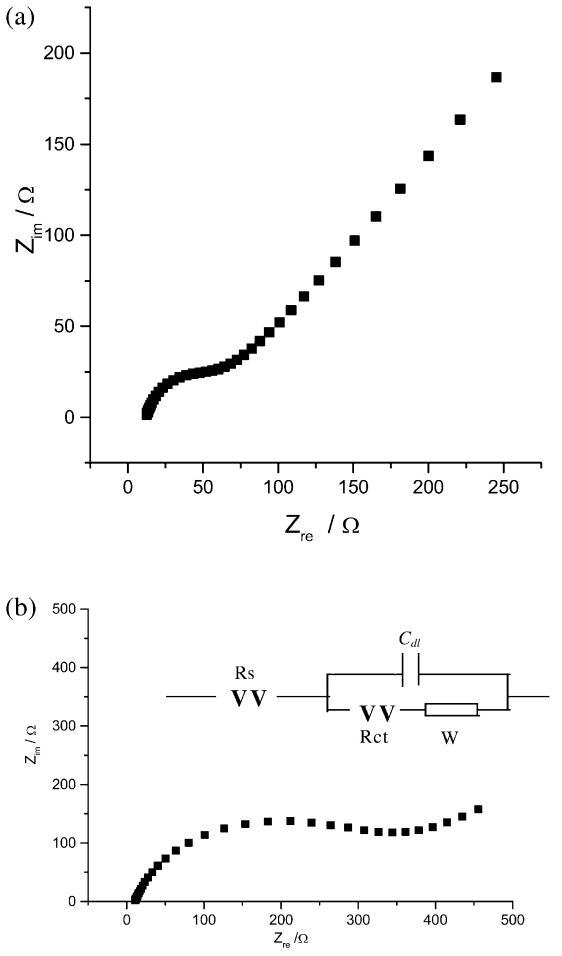

Fig. 2. Complex plane impedance plot (a) at a bare GC electrode; (b) a modified electrode with supported lipid membrane. Inset: modified Randle's equivalent circuit used to model impedance data in the presence of redox couples. $[Fe(CN)_6^{3-}] = 5$ mmol l^{-1}; $[K^+] = 1$ mol l^{-1}.

and Z_{im} components that originated mainly from the resistance and capacitance of the cell, respectively. Fig. 2 is the result of impedance spectroscopy. Inset of Fig. 2b 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 membranes capacitance, R_m the lipid membranes 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 membrane, the equation [17] was given

$$C_m = \varepsilon k / d \quad (1)$$

Where k is the dielectric constant, ε permittivity of free space and d the thickness of the lipid membranes. From Eq. (1) the thickness of the lipid membrane was calculated to be 4.5 nm.

An electrode modified by DNA incorporated BLM behaves in a particular way.

To assess whether the DNA was incorporated into BLM, we conducted characterization of DNA in the BLM with AFM and IR.

Fig. 3a shows DMPC films formed on new cleaved mica. Supported films made of lipids are planar and very smooth. The cross-sectional plots of Fig. 3c show the height of the islands along the line. The thickness of the film (from Fig. 3c) was measured to be approximately 5 nm. The same DMPC/BLM film as in Fig. 3a was used in experiments to incorporate ds DNA (Fig. 3b). From Fig. 3b, the image with DNA shows elongated bumps, while the image of DMPC film without DNA shows a flat surface. We conclude that DNA was incorporated into the DMPC film.

To further evaluate whether the DNA was incorporated into BLM, we analyzed the data from FTIR. Fig. 4 shows the FTIR spectra of BLM incorporated with DNA and BLM. As one can see from Fig. 4 (top line) the IR spectrum of the incorporated DNA BLM has a distinct 1728 cm^{-1} band, and intensive doublet of 1097 and 1060 cm^{-1} bands in the 1250–950 cm^{-1} regions compared to that of the DMPC/BLM.

In order to open the channel with the stimulus, some 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. So ds DNA was incorporated into BLM for this purpose.

Cyclic voltammetric responses of the DMPC film coated DNA to different concentrations of quinacrine are shown in Fig. 5. The CV peaks of $Fe(CN)_6^{4-/3-}$ ions as marker ion appear by adding electroinactive quinacrine ions and the peak current increases with increasing concentration of quinacrine ions. The reversibility of the electrode reaction of ferrocyanide ions is also improved by adding higher concentration of quinacrine ions.

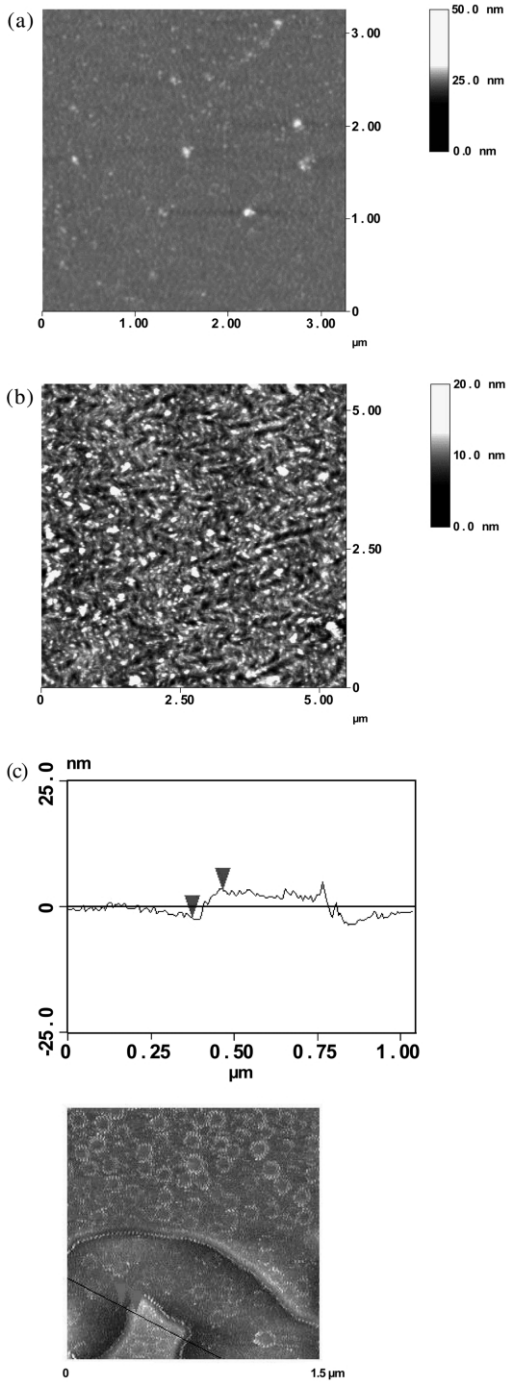

Fig. 3. (a) AFM image of the DMPC films on mica. (b) AFM image of DNA incorporated DMPC films on mica. (c) Cross-sectional plots showing the height of the islands along line.

When the electrode was transferred into an electrolyte without quinacrine, the CV peaks of marker ions disappear again. This process can be repeated many times and the electrode is quite stable. The responsiveness is lost 3 days later.

Fig. 6 shows anodic peak current vs. quinacrine. The plot can be divided into four stages. In stages I and III, the peak currents increase sharply with increasing concentration of the quinacrine. When the channel was in a closed state, very few stimuli can open the gate, leading to the apparent current change for stage I, then the current gradually increases with increasing quinacrine (stage II). When ion channels were opened to some extent, adding a small amount of stimulus can easily open the gate and remarkably reduce the negative charge, leading to the currents increasing sharply (stage III). When all the DNA in the DMPC film totally interacted with quinacrine, this implied that all ion channels were open due to interaction between quinacrine and DNA. Thus, no anionic sites existed, and peak currents did not increase with stimulus ion concentrations again for stage IV. From Fig. 6, it is observed that the current value for the reduction peaks showed almost a linear relationship with [quinacrine] in the range 2×10^{-7}–4×10^{-6} mol dm^{-3} and 4×10^{-6}–6×10^{-6} mol dm^{-3}.

The important features of these results are as follows: (i) a quinacrine ion is chemically recognized by the incorporated DNA/DMPC film and this information is quantitatively transferred into an electrochemical signal corresponding to the amount of permeated marker ions ($Fe(CN)_6^{4-/3-}$ ions), and (ii) the quinacrine ion stimulated 'on/off' switching of the gate function is reversibly made. As we saw when DNA, which was regarded as quinacrine ion receptors, was incorporated into the DMPC film, channel opening by quinacrine ions was clearly observed as the permeation of $Fe(CN)_6^{4-}$ ions. Obviously, the bilayer lipid membrane without incorporation of DNA exhibited no change in cyclic voltammograms with quinacrine ion stimulus.

Fig. 7 shows the a.c. impedance spectroscopy measurements in solutions with different concentrations of quinacrine ion. From Fig. 7 it can be seen that with increasing concentration of quina-

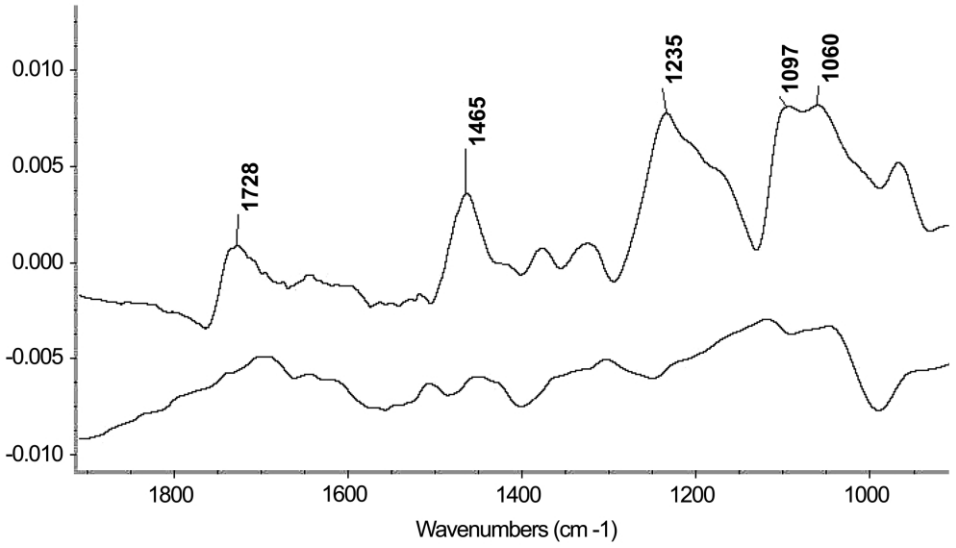

Fig. 4. FTIR spectra of BLM incorporated with DNA (top) and BLM only (bottom). Background: atmosphere. Average scan number: 120. Resolution: 4 cm^{-1}.

crine, spectroscopic measurements gradually decrease.

4. Discussion

In the present study, lipid membranes were fabricated. In order to evaluate the lipid membranes, cyclic voltammograms and a.c. impedance spectroscopy were used.

From the CV result, the suppression of the peak current of the marker ion by coating lipid membrane appears to be due to a rigid alignment of the phosphate molecules which function as a 'closed' channel. Relatively bulky ferrocyanide

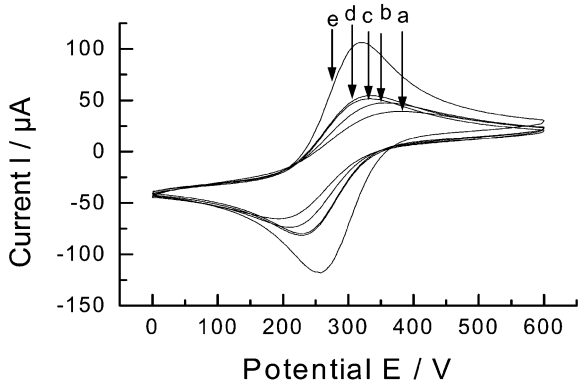

Fig. 5. Cyclic voltammograms of $Fe(CN)_6^{4-}$/ $Fe(CN)_6^{3-}$ at a bare glassy carbon electrode (e) and the glassy carbon electrode modified by a bilayer lipid membrane with incorporated DNA in different concentrations of the quinacrine. (a) 0; (b) 4; (c) 6; and (d) 7×10^{-6} M; experimental conditions are the same as those in Fig. 1.

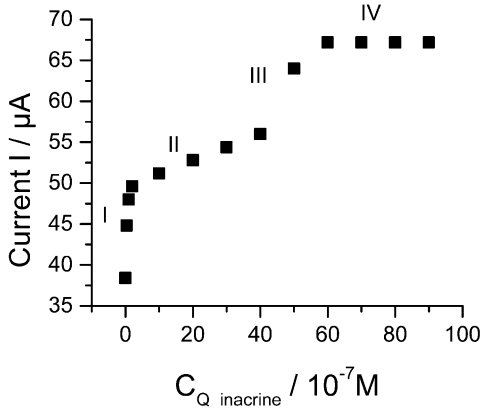

Fig. 6. Plot for anodic peak current dependence on the concentration of quinacrine at the glassy carbon electrode modified by a bilayer lipid membrane with incorporated DNA; experimental conditions are the same as those in Fig. 1.

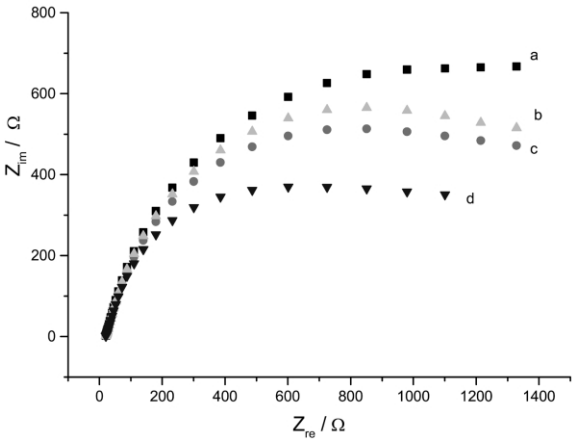

Fig. 7. AC impedance spectroscopy of the glassy carbon electrode modified by a bilayer lipid membrane with incorporated DNA with different [quinacrine]. (a) 0; (b) 2; (c) 4; (d) 7×10^{-6} M. Experimental conditions are the same as those in Fig. 2.

anions are not allowed to permeate through the closed channel towards the underlying electrode.

We measure the thickness of the lipid membrane approximately 5 nm from the a.c. impedance and AFM experiments. It could be concluded that the membranes of phosphatidylcholine dimyristoyl formed on the surface of the GC electrode were bilayer membranes, because the thickness of the DMPC monolayer should be approximately 2.4 nm [18,19].

We observed that the DNA was incorporated into BLM from the AFM. The image with DNA shows elongated bumps. The fact that DNA incorporated DMPC film might be explained by the idea that DNA molecules are able to diffuse onto the lipid bilayer and hydrophobically interact with the lipid, which will play a key role in stabilizing the electrostatically formed primary complexes of the lipids with DNA [20].

Further IR studies have also shown DNA incorporated in BLM. At high relative humidity (r.h.> 75%), DNA molecules are regular double helices and have a 1712 cm^{-1} absorption band of C=O stretching vibrations of stacked base pairs. The 1712 cm^{-1} band disappears when the rear double helix is destroyed, for example when the DNA film is dried [21,22]. Another IR spectral criterion of the DNA double helical structure is the presence of two intensive narrow bands in the sugar-phosphate backbone vibration range 1250–950 cm^{-1}. One of them, 1088 cm^{-1}, is attributed to PO_2^- symmetric stretching vibrations (u_s) and the other is 1052 cm^{-1}, attributed to the complex mixed O_5–C_4–C_5–O_4 vibrations [21,23]. From Fig. 4 (bottom line), DMPC does not show marked IR absorption in the range 1250–950 cm^{-1} and in the double bond range above 1700 cm^{-1}. This circumstance enables us to use the spectral criterion of the double helical state mentioned above to analyze the DNA conformation and existence in the DMPC/BLM. The adsorption bands shift to the lower wavelengths possibly owing to hydrophobic interactions between lipids and DNA [20], making the energy lower and DNA being decomposed to form single strands [24]; 1235 cm^{-1} is assigned to the antisymmetric stretching vibration mode of PO_2^-. This indicates that DNA molecules are incorporated into BLM.

Incorporated DNA DMPC film blocks $Fe(CN)_6^{3-}$ ions from permeating to the underlying electrode due to not only the rigid alignment of the phosphate molecules which functions as a ‘closed’ channel, but also the electrostatic repulsion between the polyanionic DNA and the anionic redox couple ions. On adding quinacrine, the peak current of $Fe(CN)_6^{3-}$ reappeared. Neither the bare electrode nor coated DMPC electrode showed any response to quinacrine. The ion channels were formed due to binding of the cationic quinacrine to the polyanionic ions (DNA), not only changing the molecular configuration of membrane but also reducing the negative charge on the electrode surface, resulting in the enhancement of the current intensity. Such concentration-dependent changes in CV profiles on incorporated DNA/DMPC film electrode were not seen for Na^+ and K^+. This can be attributed to the specific and strong interaction between the DNA double helix and quinacrine [25].

Previous studies have indicated that the BLM incorporated DNA behaves like an ion channel. The a.c. impedance results give further demonstration for this conclusion. The sunken semicircle is characteristic of porous electrodes [26], and with the increasing concentration the impedance

decreases; the spectroscopy changes from that featuring a dielectric electrode to that featuring a porous electrode. The impedance spectroscopy experiments on the other hand, have proven that incorporation of channel receptors such as dsDNA in the DMPC BLM, interacts selectively with analytes and open channels for permeation of marker ions.

In conclusion, the electrode-coated, BLM incorporated dsDNA was obtained by preparing the forming DMPC on the GC electrode surfaces, then incorporating dsDNA into BLM. The film of DMPC formed on the GC electrode was demonstrated to be DMPC bilayer membranes by impedance spectroscopy. The dsDNA was characterized by CV, FTIR and AFM, which indicates that DNA was in BLM. The method used to prepare this type of electrode is reliable and satisfactory. The functional membrane expresses an ion channel behavior and the formation of ion channel was demonstrated by electrochemical impedance spectroscopy. The behavior was investigated. The channel was formed and open in the presence of stimulus; the intensity of the channel current increases with the concentration of stimulus. In the absence of stimulus, the channel was in a closed state; the reversible open–close processes could be repeated many times. Thus, this type of DNA-modified electrode will find application in electrochemical biosensors for gene detection and investigation of interactions of DNA with other molecules, etc. The mechanism of forming an ion channel is discussed.

Acknowledgments

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References

[1] H.T. Tien, Bilayer Lipid Membranes (BLM): Theory and Practice, Marcel Dekker, New York, 1974, pp. 1–68.
[2] H.T. Tien, Membrane photobiophysics and photochemistry, Prog. Surf. Sci. 30 (1989) 1–199.
[3] C.G. Siontorou, V.G. Andreou, D.P. Nilcolelis, U.J. Krull, Detection of DNA hybridization using self-assembled bilayer lipid membranes (BLMs), Electroanalysis 12 (2000) 747–751.
[4] A. Wardak, H.T. Tien, Cyclic voltammetry studies of bilayers on solid substrates, Bioelectrochem. Bioenerg. 24 (1990) 1–11.
[5] H.T. Tien, A.L. Ottova, Supported planar lipid bilayer (s-BLMs) as electrochemical biosensors, Electrochim. Acta 43 (1998) 3578–3610.
[6] E. Sackman, Supported membranes: scientific and practical applications, Science 271 (1996) 43–48.
[7] Z. Wu, J. Tang, Z. Cheng, X. Yang, E. Wang, Ion channel behavior of supported bilayer lipid membranes on a glassy carbon electrode, Anal. Chem. 72 (2000) 6030–6033.
[8] M. Sugawwara, K. Kojima, H. Sazawa, Y. Umezawa, Ion-channel sensors, Anal. Chem. 59 (1987) 2842–2846.
[9] J. Wang, M. Jiang, Dendritic nucleic acid probes for DNA biosensors, J. Am. Chem. Soc. 120 (1998) 8281–8282.
[10] K. Peterlinz, R. Georfiadis, T. Herne, M. Tarlov, Observation of hybridization and dehybridization of thiol-tethered DNA using two-color surface plasmon resonance spectroscopy, J. Am. Chem. Soc. 119 (1997) 3401–3402.
[11] Y. Okahata, Y. Matsunobu, K. Ijiro, M. Mukae, A. Murakami, K. Makino, Hybridization of nucleic acids immobilized on a quartz crystal microbalance, J. Am. Chem. Soc. 114 (1992) 8229–8230.
[12] S. Yamaguchi, T. Shimomura, T. Tatsuma, N. Ouama, Adsorption, immobilization, and hybridization of DNA studied by the use of quartz crystal oscillators, Anal. Chem. 65 (1993) 1925–1927.
[13] C.F. Mandenius, A. Chollet, M.M. Lenburg, I. Lundstrom, Optical surface methods for detection of nucleic acid binding, Anal. Lett. 22 (1989) 2961–2973.
[14] R.P.E. Cordes, G.A. Technitz, Acridine orange sensitization and DNA inhibition effects on the cyclic voltammetry of naphthoquinoses using bare and DNA modified gold electrodes, Electroanalysis 12 (2000) 351–355.
[15] E.A.H. Hall, N.G. Skinner, C. Jung, S. Szunerits, Investigating polymers and conducting metals as transduction mediators or immobilization matrices, Electroanalysis 7 (1995) 830–837.
[16] X. Ren, P.G. Pickup, An impedance study of electron transport and electron transfer in composite polypyrrole + polystyrenesulphonate films, J. Electroanal. Chem. 420 (1997) 251–257.
[17] A.L. Plant, Supported hybrid bilayer membranes as rugged all membrane mimics, Langmuir 15 (1999) 5128–7135.
[18] J.K.H. Horber, C.A. Lang, T.W. Hansch, W.M. Heckl, H. Mohwald, Scanning tunneling microscopy of lipid films and embedded biomolecules, Chem. Phys. Lett. 145 (1988) 151–158.

[19] A. Watt, K. Harlos, D. Marsh, Charge-induced tilt in order-phase phosphatidylglycerol bilayers evidence from X-ray diffraction, Biochim. Biophys. Acta 645 (1981) 91–96.

[20] C.H. Spink, J.B. Chaires, Thermodynamics of the binding of a cationic lipid to DNA, J. Am. Chem. Soc. 119 (1997) 10920–10928.

[21] M. Tsuboi, Application of infrared spectroscopy to structure studies of nucleic acids, Appl. Spectrosc. Rev. 3 (1969) 45–90.

[22] J.F. Neault, H.A.T. Riahi, DNA–chlorophyll in interaction, J. Phys. Chem. B 102 (1998) 1610–1614.

[23] M. Ghomi, R. Lettelier, J. Liquier, E. Taillandier, Interpretation of DNA vibrational spectra by normal coordinate analysis, Int. J. Biochem. 22 (1990) 691–699.

[24] Z. Wang, D. Liu, S. Dong, Study on adsorption and oxidation of calf thymus DNA at glassy carbon electrode, Electroanalysis 12 (2000) 1419–1421.

[25] W.D. Wilson, I.G. Lopp, Analysis of cooperativity and ion effects in the interaction of quinacrine with DNA, Biopolymers 18 (1979) 3025–3041.

[26] M. Blank, I. Vodyanou, Biomembrane Electrochemistry, Advances in Chemistry Series, 235, American Chemical Society, Washington, DC, 1994, pp. 491–512.