Ion Channel Behavior of Amphotericin B in Sterol-Free and Cholesterol- or Ergosterol-Containing Supported Phosphatidylcholine Bilayer Model Membranes Investigated by Electrochemistry and Spectroscopy

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ABSTRACT Amphotericin B (AmB) is a popular drug frequently applied in the treatment of systemic fungal infections. In the presence of ruthenium (II) as the maker ion, the behavior of AmB to form ion channels in sterol-free and cholesterol- or ergosterol-containing supported phosphatidylcholine bilayer model membranes were studied by cyclic votammetry, AC impedance spectroscopy, and UV/visible absorbance spectroscopy. Different concentrations of AmB ranging from a molecularly dispersed to a highly aggregated state of the drug were investigated. In a fixed cholesterol or ergosterol content (5 mol %) in glassy carbon electrode-supported model membranes, our results showed that no matter what form of AmB, monomeric or aggregated, AmB could form ion channels in supported ergosterol-containing phosphatidylcholine bilayer model membranes. However, AmB could not form ion channels in its monomeric form in sterol-free and cholesterol-containing supported model membranes. On the one hand, when AmB is present as an aggregated state, it can form ion channels in cholesterol-containing supported model membranes; on the other hand, only when AmB is present as a relatively highly aggregated state can it form ion channels in sterol-free supported phosphatidylcholine bilayer model membranes. The results showed that the state of AmB played an important role in forming ion channels in sterol-free and cholesterol-containing supported phosphatidylcholine bilayer model membranes.

INTRODUCTION

Amphotericin B (AmB) is one of the main polyene antibiotics widely used to treat deep-seated fungal infections (Brajtburg et al., 1990). Unfortunately, the classical formulation of AmB, Fungizone, has negative side effects (e.g., nephrotoxicity) that seriously impair its efficacy. The mechanism of biological action of AmB is most probably directly related to the ability of the drug to form hydrophilic pores in the hydrophobic membrane core, where it increases the permeability of the cells to ions and small molecules (Fujii et al., 1997; Kruijff et al., 1974; Marin et al., 1991). It has been proposed in the 1970s that the interaction between membrane sterols and AmB is responsible for the selectivity of the drug. It has therefore been assumed that the selective toxicity of AmB for fungi results from its capacity to bind more strongly to ergosterol, the principal fungal sterol, than to cholesterol, the principal sterol of mammalian cells. However, the detailed molecular mechanisms of the interaction of AmB with the membrane, as well as the formation of a transmembrane pore structure, are still imperfectly understood. The evidence for channel formation without sterols challenges the idea that the most widely accepted and oldest AmB channel models have included sterols as staves in a barrel-type structure (Croquin et al., 1983; Hartsel et al., 1988; Ruckwardt et al., 1998). However, when studies are made with model membranes, the situation is not simple, and contradictory results are also found in the literature (Bolard et al., 1991; Butyan and McPhie, 1996; Fujii et al., 1997; Ockman, 1974). The molecular structures of the components used in the study are shown in Fig. 1. AmB (Fig. 1 *A*) has quite a special structure, with one hydrophobic side containing seven conjugated double bonds and the other side, hydrophilic, containing several polar substituents. Cholesterol (Fig. 1 *B*) and ergosterol (Fig. 1 *C*) are very similar molecules, the main difference in the two structures resulting from the presence in ergosterol of an additional methyl group and a double bond on the side chain and the presence of an additional double bond on the steroid nucleus.

Supported lipid bilayers and lipid vesicles are useful model systems to study basic interaction mechanisms that are responsible for the structure and function of biological membranes. The simple composition of an artificial bilayer in contrast to the complex mixture of lipids and proteins in biological membranes facilitates a detailed examination of a single membrane function, e.g., ion transport.

In this paper, to shed some light on the interactions between AmB, phospholipids, and the sterols, we have performed electrochemistry and spectroscopy studies on the ion channel behaviors of AmB on glassy carbon electrode (GCE)-supported pure phosphatidylcholine bilayer model membranes and GCE-supported cholesterol- or ergosterol-containing supported phosphatidylcholine bilayer model membranes. We found that AmB could form ion channels in supported bilayer lipid membranes and that the states of AmB controlled the ion channel activities. The current study was undertaken in an attempt to better understand the

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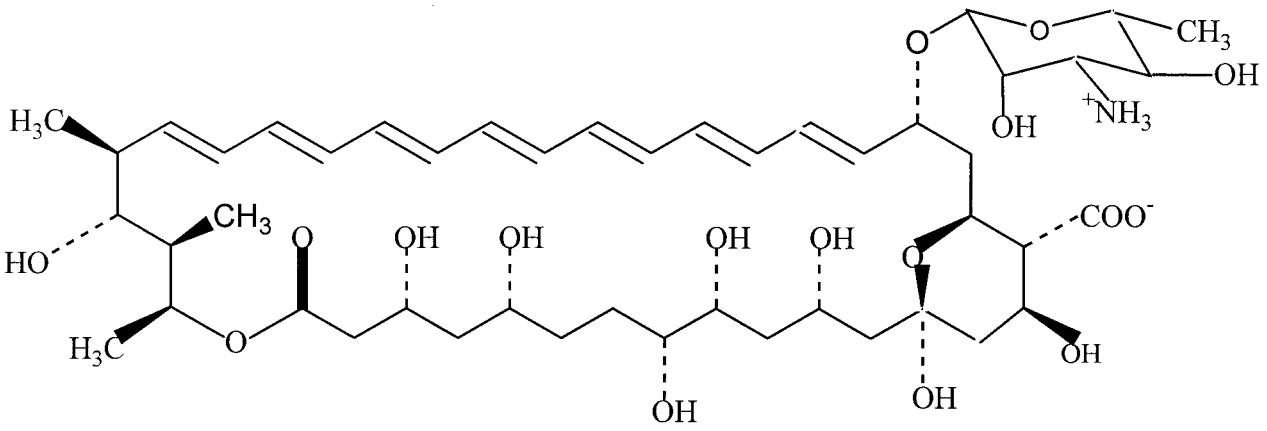
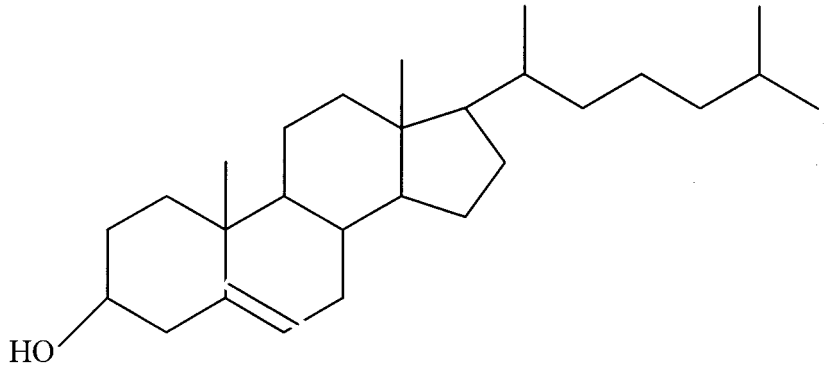
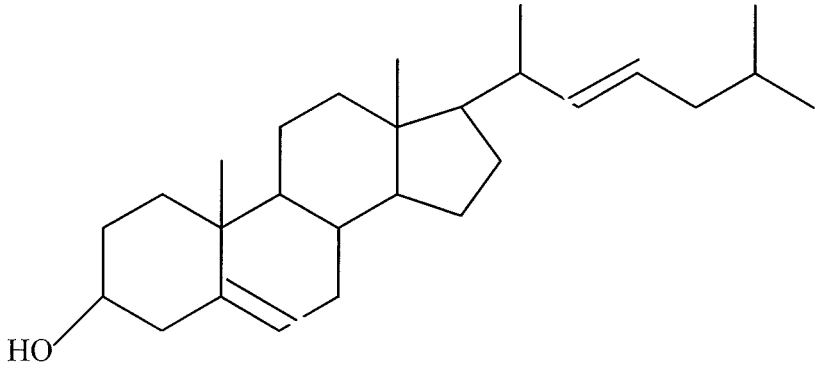


AmB



cholesterol



ergosterol

FIGURE 1 The primary structure of AmB, cholesterol, and ergosterol.

molecular mechanism of the formation of AmB ion channels in supported bilayer lipid model membranes in the presence or absence of sterol.

MATERIALS AND METHODS

Reagents

AmB, ergosterol, cholesterol, and dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma Chemical Co. (St. Louis, MO). Ergosterol and cholesterol were twice recrystallized from ethanol. Tris(2,2′-bipyridine)ruthenium (II) was obtained from Acros (Belgium).

On the basis of DMPC, cholesterol, and ergosterol stock solutions in chloroform, the final mixtures of lipids containing 0% and 5% (mol/mol) sterol were prepared. Similarly, the solutions corresponding to a series of concentrations of AmB were prepared from stock solutions of AmB first dissolved in pure dimethyl sulfoxide and then diluted with water. The final concentration of dimethyl sulfoxide did not exceed 0.1% (v/v).

Other chemicals were of the highest quality possible, and all chemicals were used without further purification. Pure water obtained by means of a Millipore Q water purification setup was used throughout.

Apparatus

All cyclic voltammetric experiments were carried out with a Cypress Systems (Lawrence) model CS-1087 connected to a PC for control and data storage. The apparatus used for electrochemical impedance measurement was composed of an Autolab with potentiostat/galvanostat PGSTA30 and frequency response analysis system software (FRA, Eco Chemie, Utrecht, The Netherlands), to provide fully computer-controlled electrochemical impedance spectroscopy. Impedance measurements were performed in the frequency range from 0.1 to 10,000 Hz with a signal amplitude of 5 mV. A standard three-electrode cell was used for the electrochemical experiment. A GCE was used as the substrate electrode, and the counter-electrode was a platinum wire. All potentials reported in the paper are referenced to a Ag/AgCl (KCl-saturated) electrode. The buffers were purged with purified nitrogen (N_2) for 20 min before a series of experiments. A nitrogen environment was kept over solutions in the cell for exclusion of oxygen.

Absorbance spectra were obtained for samples containing different concentrations of AmB in 10 mM phosphate buffer, pH 7.18. The monomeric or aggregated state for AmB was judged by the following approximations. 1) At low AmB concentration (below 10^{-6} M), the absorption spectrum is similar to the one observed in natural conjugated polyenes (heptaenes): it presents a vibrational structure, and the main band is located at ~408 nm. 2) At 10^{-6} M, a totally new spectrum is observed, and the absorption maximum at 340 nm is detected. All spectra were recorded at room temperature on a Cary 500 Scan UV-vis-NIR spectrophotometer (Varian).

Method for supported bilayer lipid membrane formation

Before supported bilayer lipid membrane formation, a GCE 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 GCE was immersed in the 0.1 M NaOH solution, and the potential was held at 1500 mV for 3 min to polarize the electrode. After the electrode was polarized, it was dried under purified nitrogen. Subsequently, a 5-μl aliquot of the lipid solution was dropped onto the surface of the electrode by a microsyringe, and the electrode was immediately transferred into the phosphate buffer. Thirty minutes after transformation of the lipid-coated GCE into the buffer solution, a bilayer lipid membrane was formed on the substrate. Our technique for forming a lipid bilayer membrane on GCE is based on the interaction between the hydrophilic polarized GCE surface and amphipathic lipid, as described previously (Han and Wang, 2001; Wu et al., 2000).

RESULTS

Absorption spectroscopy of AmB

At low concentration (2×10^{-7} M), three defined peaks are observed at 408, 385, and 365 nm, while a band that looks wide but minor is observed at 340 nm. As the concentration of AmB increases to 1×10^{-6} M, the spectrum is progressively modified as shown in Fig. 2. When the AmB concentration was increased, all the peaks enhanced their intensity and the 340-nm peak increased in a drastic way without a shift in their position. The 340-nm peak is regarded as characteristic of aggregates in which the polyene chromophores are stacked so as to interact electronically (Bolard, 1991; Fujii et al., 1997; Saka and Mita, 1998; Tancrède et al., 1990) On the other hand, a 408-nm peak is regarded as characteristic of monomers (Barwicz et al., 1998; Bolard, 1986; Chapados et al., 1994; Madden et al., 1990). The absorption spectra change with AmB concentration is due to a displacement of the equilibrium between monomer and aggregate. Fig. 2 shows a monotone increase of the absorbance at 340 nm when the AmB concentration was increased, which suggests the formation of aggregates.

Formation and characterization of supported bilayer lipid membrane

The formation of supported bilayer lipid membrane on the GCE surface was judged by capacitance and cyclic voltammogams before and after the electrode was coated with bilayer lipid membrane. Fig. 3 showed the cyclic voltammograms of the bare GCE (Fig. 3 *a*) and the lipid-bilayer-coated GCE (Fig. 3 *b*) in the presence of $Fe(CN)_6^{3-}$. Comparing Fig. 3 *b* with Fig. 3 *a*, we find that before GCE was coated with bilayer lipid membrane, a pair of well defined reversible waves of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ was obtained. After it was coated, there were no waves observed, but a cyclic voltammogram was obtained of only the capacitor's charging and discharging, which featured a solid substrate-supported bilayer lipid membrane electrode. This result shows that the bilayer lipid membrane self-assembled on GCE dramatically slows down the electron transfer between the solution and the GCE, which is consistent with previous results (Gao et al., 2000; Tien and Ottova, 1998; Umezawa et al., 1998).

Impedance spectroscopy is an effective method for probing the features of a surface-modified electrode. Fig. 4 illustrates the results of impedance spectroscopy on a bare electrode (Fig. 4 *a*) and a modified electrode (Fig. 4 *b*) with supported lipid membranes in the presence of 1 mM $Fe(CN)_6^{3-/4-}$, which were measured at the formal potential

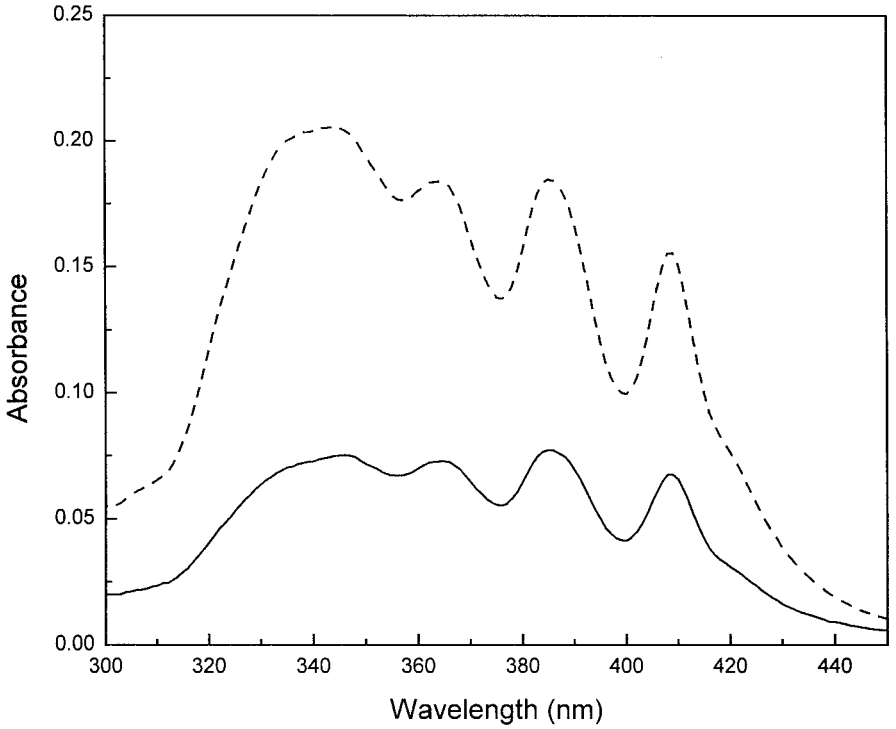


FIGURE 2 Absorption spectra of AmB at 2×10^{-7} M (——) and 1×10^{-6} M (— — —), in 0.1 M KCl, 10 mM phosphate buffer at pH 7.18. The temperature was 25°C.

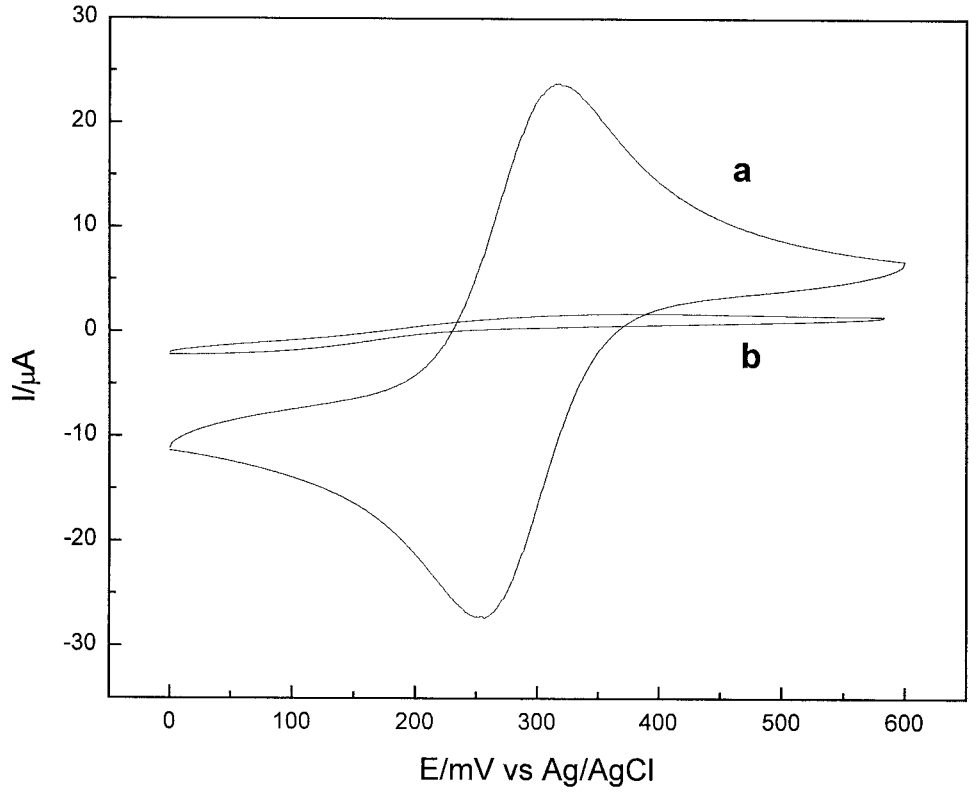


FIGURE 3 Cyclic voltammograms of GCE in 1 mM $K_3[Fe(CN)_6]$ solution containing 0.1 M KCl, 10 mM phosphate buffer at pH 7.18. (*a*) Bare GCE; (*b*) GCE coated with lipid membrane. The scan rate was 50 mV s^{-1}.

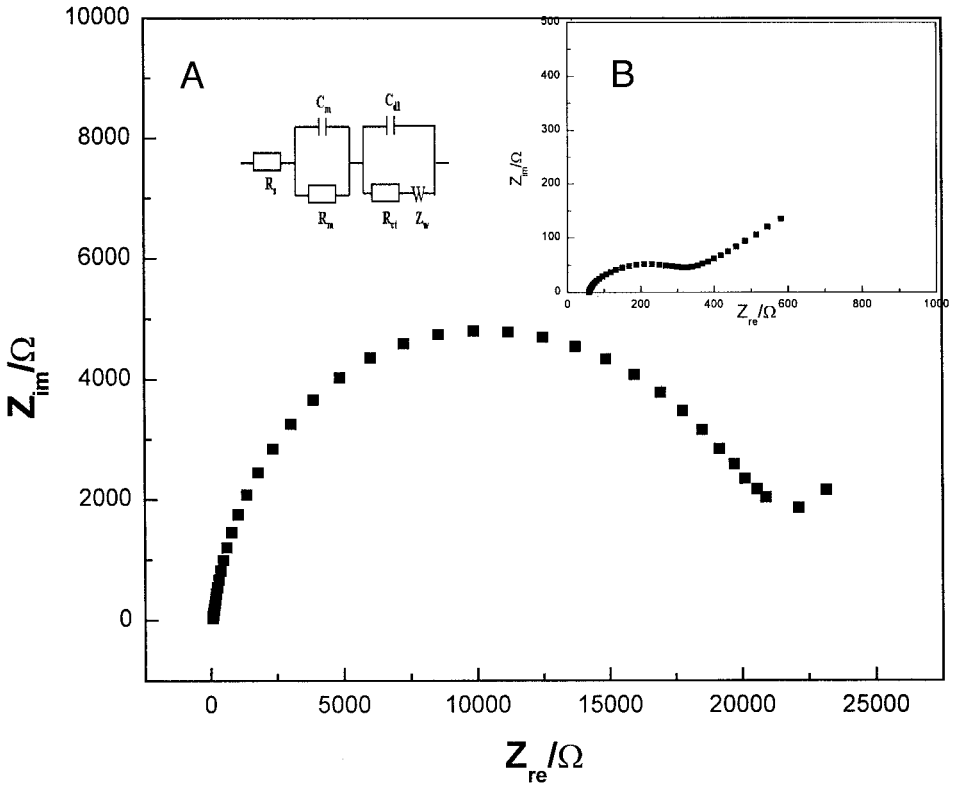


FIGURE 4 AC impedance spectroscopy in 1 mM $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1M KCl, 10 mM phosphate buffer, pH 7.18. (A) Bare GCE; (B) Modified electrode with supported lipid membrane. Frequency range was 0.1–10,000 Hz. (*Inset*) Modified Randle's equivalent circuit used to model impedance data in the presence of redox couples: R_s, electrolyte resistance; C_m, lipid membrane capacitance; R_m, lipid membrane resistance; C_{dl}, double-layer capacitance; R_{ct}, charge-transfer resistance; Z_w, Warburg element.

of $Fe(CN)_6^{3-/4-}$. The impedance plots of supported bilayer lipid membranes are characterized by a single semicircle in the high-frequency domain followed by a Warburg-like mass transfer impedance in the low-frequency portion. These results indicate that the equivalent circuit of the GCE-supported bilayer lipid membrane system could be represented by a membrane bulk impedance in series with an impedance consisting of the charge transfer resistance, the double-layer capacitance, and intramembrane mass transfer impedance (Plant et al., 1994). It could be seen that the presence of the bilayer lipid membrane caused the charge transfer resistance to increase and the double-layer capacitance to decrease compared with that of the bare GCE, further proving the formation of a bilayer lipid membrane. It has been known that defects in the lipid bilayer could provide locations where ions may be able to approach more closely to the electrode surface and to accumulate. The presence of a single semicircle in the high-frequency domains following a Warburg-like mass transfer impedance in the low-frequency portion indicated that the GCE-supported bilayer lipid membrane was not sufficiently defect-free to entirely eliminate direct electron transfer between redox species and the bilayer lipid membrane-modified GCE.

Ion channel formation by AmB in the supported bilayer lipid membranes

The formation of ion-permeable channels by AmB was monitored by measuring the responses of ruthenium (II) complex cations as the marker ions on the GCE. Six typical concentrations of AmB were used: 10^{-7} M, 2×10^{-7} M, 1×10^{-6} M, 5×10^{-6} M, 2×10^{-5} M, and 5×10^{-5} M. From the absorbance spectroscopy of AmB we conclude that they are in the form of monomers at the first two concentrations and in the form of aggregates at the last four concentrations. In each case, the supported bilayer model membrane systems are the following: GCE-supported pure DMPC (S-DMPC), GCE-supported DMPC plus cholesterol 5% (S-CHOL), and GCE-supported DMPC plus ergosterol 5% (S-ERGO). After the bilayer lipid membrane was formed on the surface of GCE, it was immersed in buffer solution containing 0.5 mM Tris(2,2′-bipyridine)ruthenium (II). Fig. 5 shows the cyclic voltammograms in solutions with different concentrations of AmB upon S-DMPC, S-CHOL, and S-ERGO. At concentrations of 10^{-7} M and 2×10^{-7} M for S-DMPC and S-CHOL, no obvious characteristic of redox peaks of ruthenium (II) complex appeared on the cyclic voltammograms (Fig. 5, *A* and *B*, curves *a* and *b*). The suppression of CV peaks of the ruthenium (II) complex appeared to be due to the closed channels of the lipid membrane. However, for S-ERGO, the enhanced amperometric response of ruthenium (II) complex was found gradually (Fig. 5, *A* and *B*, curve *c*). AmB molecules can open some kind of channel for the ruthenium (II) complex to cross the lipid membrane to reach the surface of the electrode. When the concentration of AmB was increased to 1×10^{-6} M, the amperometric response of ruthenium (II) complex was also found in S-CHOL (Fig. 5 *C*, curve *b*). It meant

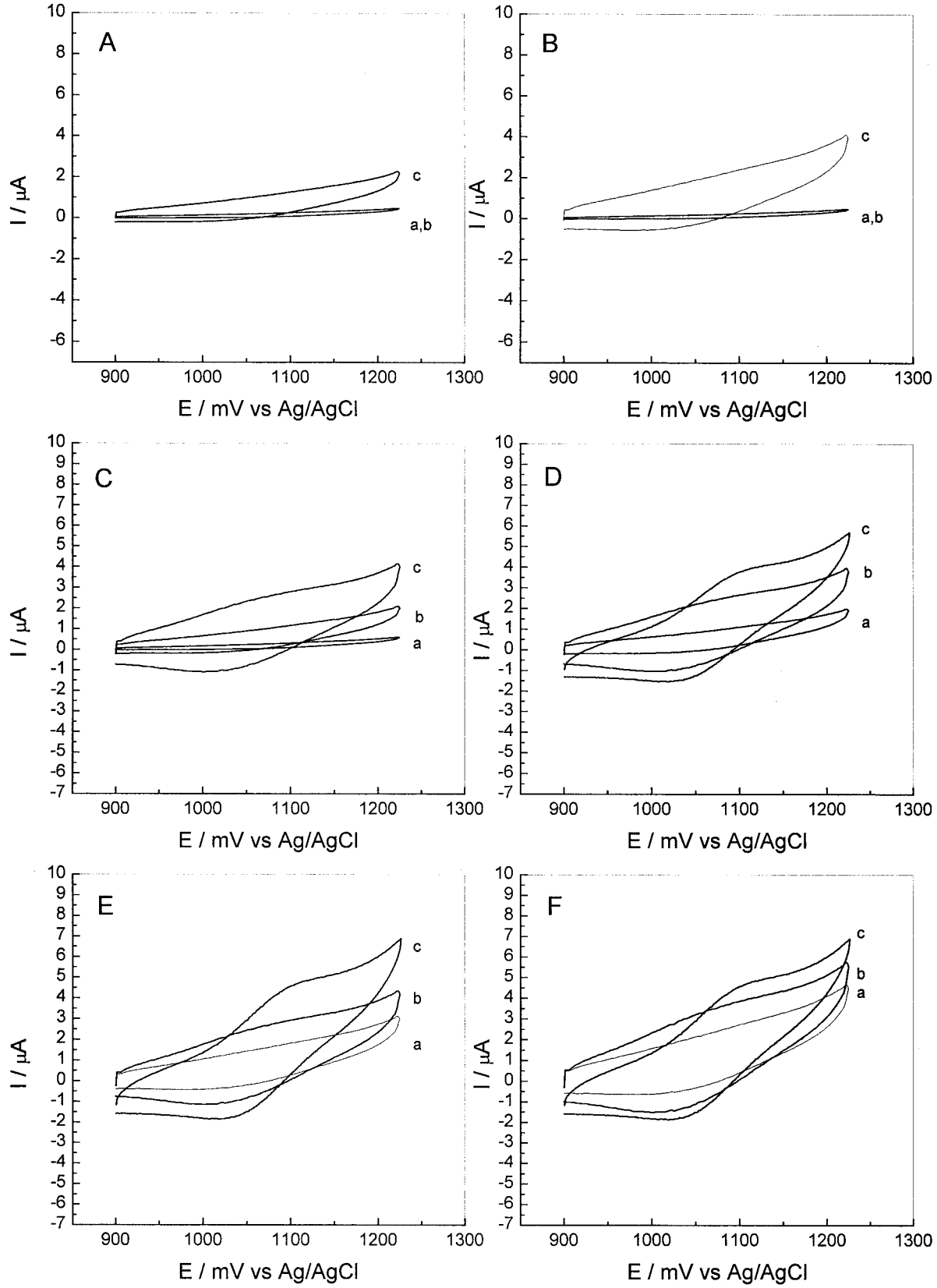


FIGURE 5 Cyclic voltammetric responses of 0.5 mM ruthenium (II) complex cation containing 0.1 M KCl, 10 mM phosphate buffer, pH 7.18 at S-DMPC (*curve a*), S-CHOL (*curve b*), and S-ERGO (*curve c*) in different concentrations of AmB: (*A*) 10^{-7} M; (*B*) 2×10^{-7} M; (*C*) 1×10^{-6} M; (*D*) 5×10^{-6} M; (*E*) 2×10^{-5} M; (*F*) 5×10^{-5} M. The scan rate was 50 mV s^{-1}.

that AmB could form ion channels in S-CHOL at this concentration. As for S-DMPC, no cyclic voltammetric response of the ruthenium (II) complex appeared yet (Fig. 5 *C*, curve *a*). As for S-ERGO, the enhanced amperometric response of the ruthenium (II) complex was obtained again (Fig. 5 *C*, curve *c*). At a concentration of 5×10^{-6} M, the suppression of CV peaks of the ruthenium (II) complex became dramatically higher (Fig. 5 *D*, curve *a* vs. Fig. 5 *A–C*, curve *a*), indicating that AmB began to form ion channels in S-CHOL. At the same time, the enhanced amperometric response of the ruthenium (II) complex was obtained again in S-CHOL and S-ERGO (Fig. 5 *D*, curve *b* and *c*). However, when the concentration of AmB reached 2×10^{-5} M, a maximal amperometric response of the ruthenium (II) complex was found in S-ERGO (Fig. 5 *E*, curve *c*). When the concentration of AmB was increased to 5×10^{-5} M, increased current responses of ruthenium (II) complex were also found in S-DMPC and S-CHOL From the result, we can see that AmB can form ion channels in S-ERGO at the lowest concentration among the three different supported model membranes. However, AmB can form ion channels in S-ERGO at the highest concentration. In other words, AmB can form ion channels most easily in S-ERGO and with the most difficulty in S-DMPC among the three membrane systems investigated. However, the potential for AmB to form ion channels depends on the different constituents of the supported model membranes and the nature of the component (cholesterol or ergosterol)

Fig. 6 shows the AC impedance spectroscopy measurements in solutions with different concentrations of AmB upon S-DMPC, S-CHOL, and S-ERGO. When AmB interacted with S-DMPC at the concentrations 10^{-7}, 2×10^{-7}, and 1×10^{-6} M (Fig. 6, *A–C*, curve *a*), there were no changes on AC impedance spectroscopy, which was almost the same as in Fig. 4 *B*. It showed that AmB could not form ion channels sufficiently at low concentrations. However, the spectroscopy began to sink at a concentration of 5×10^{-6} M (Fig. 6 *D*, curve *a*). It showed that AmB could begin to form ion channels in S-DMPC at this threshold concentration. Moreover, with increasing concentration above the threshold (Fig. 6, *E* and *F*, curve *a*), the spectroscopy sank gradually and the channel activity for AmB in S-DMPC increased dramatically. Similarly, there was yet a different threshold concentration for AmB showing channel activity when AmB interacted with S-CHOL (Fig. 6 *C*, curve *b*). Below the threshold, AmB did not show channel activity in S-CHOL (Fig. 6, *A* and *B*, curve *b*). On the contrary, channel activity of AmB increased with the concentration of AmB above the threshold (Fig. 6, *D–F*, curve *b*). When AmB interacted with S-ERGO at concentration ranges from 10^{-7} to 2×10^{-5} M (Fig. 6, *A–E*, curve *a*), a considerable decrease was observed in the charge-transfer resistance compared with Fig. 4 *B*. The spectroscopy changed from that featuring a dielectric electrode to that featuring a porous electrode, implying that pores had been formed in S-ERGO because of the presence of drug molecules. With the increase in concentration of AmB, the spectroscopy gradually sank. The sunken semicircle was characteristic of porous electrodes (Blank and Vodyanoy, 1994), and with the increase in concentration of AmB, the impedance decreased. However, when the concentration of AmB reached 5×10^{-5} M (Fig. 6 *F*, curve *a*), the spectroscopy did not change any more, showing that channel activity in S-ERGO had already reached the maximum. We can see that the results obtained from AC impedance spectroscopy were consistent with the results obtained from the cyclic votammetry.

DISCUSSION

The binding of AmB to biological and model membranes has been studied for a long time. Its mechanism of action is thought to involve interaction with sterols, leading to pore formation and increased permeability and, ultimately, to membrane disruption and cell lysis. The AmB pore was proposed to consist of antibiotic molecules intercalated with sterol molecule (Finkelstein and Holz, 1973). The higher affinity for fungal ergosterol over mammalian cholesterol has been invoked as the explanation for the antibiotic's selectivity for the fungal membrane, because the equilibrium between monomers and aggregates appears to play a key role in drug activity. In more recent years, work has aimed at understanding the membrane effects of the antibiotic in terms of its aggregation state and of the differences in its affinities for cholesterol and ergosterol as a function of the aggregation state. Bolard et al. (1991) found that the binding of the antibiotic to membranes in monomeric or in aggregated form depends on the lipid composition, and toxic effects were ascribed to the aggregated state. Despite ambiguities associated with much of the reported data regarding the structure of the AmB channel and its relevance to the mechanism of action, it thus seems likely that AmB, depending upon the specific conditions, possesses several distinct mechanisms of channel activity.

The solid supported bilayer lipid membrane has the advantage of ease and reproducibility of preparation, long-term stability, and the possibility to use an electrically conductive support. At low AmB concentration, a rigid molecule of AmB interacts with the lipid alkyl chains by van der Waals force (Aracava et al., 1981) and acts like other similar structure-modifying agents, such as cholesterol or membrane-spanning polar carotenoids. Hydrophobic interactions of alkyl chains with AmB are most probably responsible for the formation of certain fractions of the lipid. Furthermore, the AmB molecules were immersed in the sea of lipid and were separated by lipid molecules. They could not contact each other and remain in their monomeric form. Thus, AmB could act only as a helper for stabilizing the supported bilayer lipid membrane, and the redox probe could not cross the membrane to give an electrochemistry response at the surface of the GCE. However, this is not the

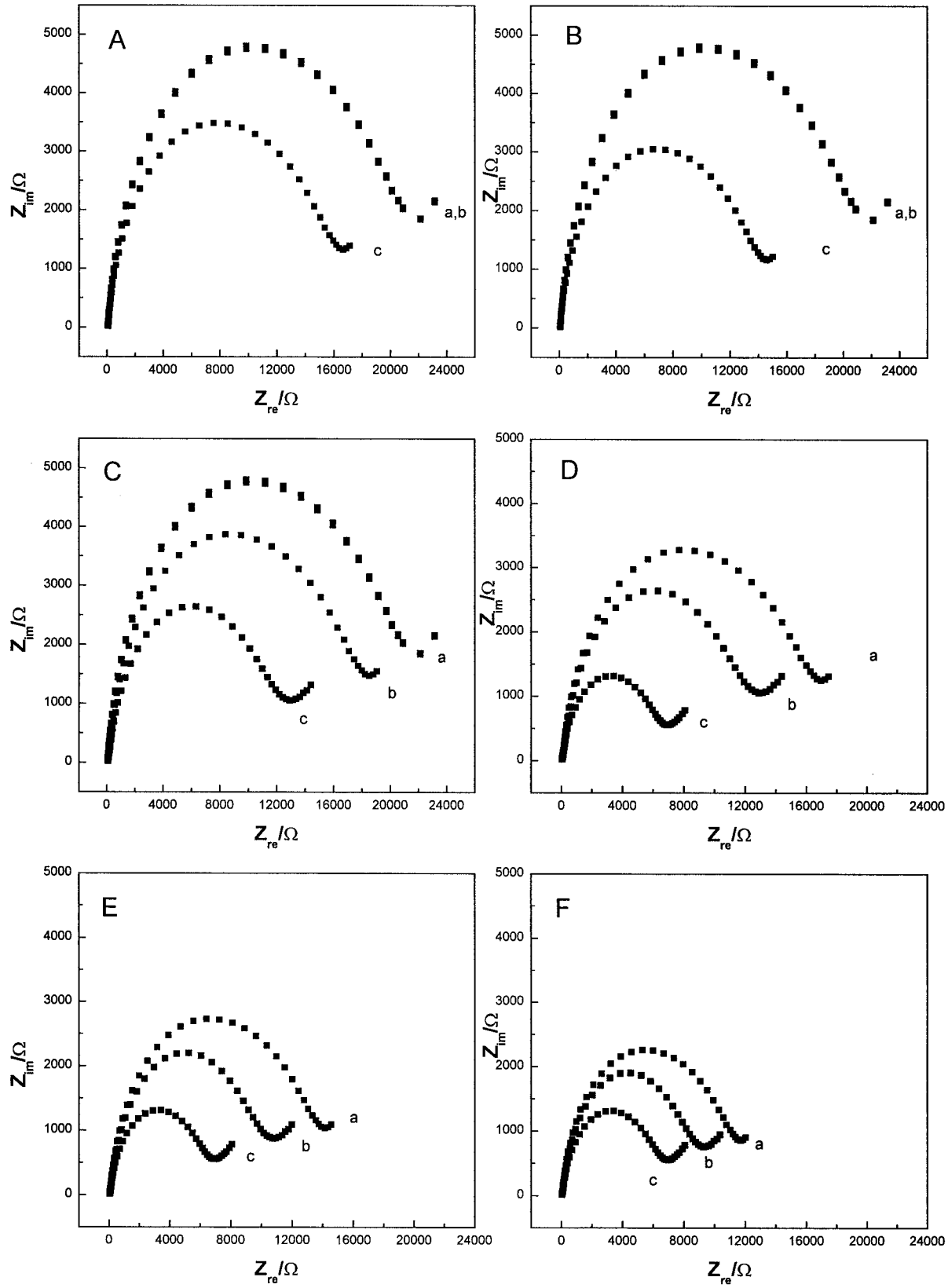


FIGURE 6 AC impedance spectroscopy of S-DMPC (*curve a*), S-CHOL (*curve b*) and S-ERGO (*curve c*) in the presence of 0.5 mM ruthenium (II) complex cation containing 0.1 M KCl, 10 mM phosphate buffer, pH 7.18 with different concentrations of AmB: (*A*) 10^{-7} M; (*B*) 2×10^{-7} M; (*C*) 1×10^{-6} M; (*D*) 5×10^{-6} M; (*E*) 2×10^{-5} M; (*F*) 5×10^{-5} M. The frequency range was 0. 1–10,000 Hz, and the equivalent circuit used is the same as the one used in Fig. 3.

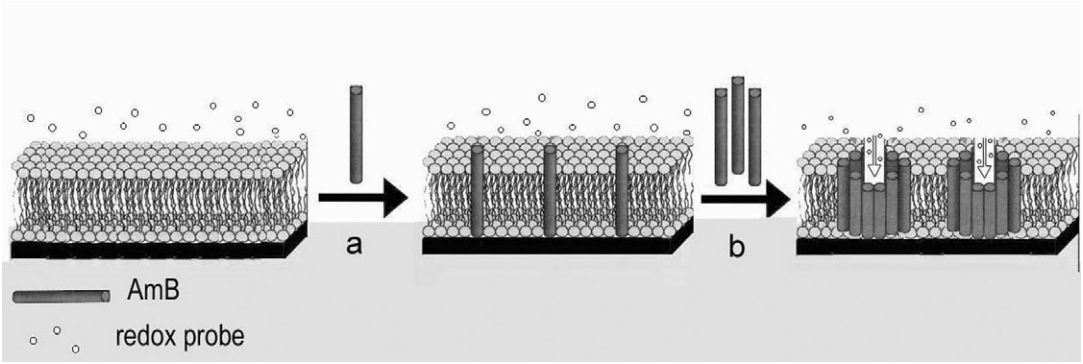


FIGURE 7 The process of AmB forming ion channels in the GCE-supported sterol-free supported phosphatidylcholine bilayer model membrane. (*a*) At low concentration, AmB molecules were isolated by lipid molecules and could not form ion channels for the redox probe to pass. (*b*) When performing at a relatively highly aggregated state, AmB molecules aggregated each other to form ion channels.

case when ergosterol was present in the supported bilayer model membrane.

It has been postulated that preferential destruction of fungal cells by AmB is due partly to its higher affinity for the ergosterol-rich fungal membranes than for the cholesterol-containing membranes typically found in mammalian cells (Archer and Gale, 1975). In addition, AmB has a higher binding affinity constant for ergosterol than for cholesterol (Readio and Bittman, 1982), so AmB could interact with ergosterol contained in S-ERGO and lead to formation of ion channels even at such a low concentration. It has been widely reported that AmB can form self-associated complexes in water when the AmB concentration increases (Bolard et al., 1980; Hemenger et al., 1983; Mazerski et al., 1982). In aqueous solution, AmB has been found to exist as a mixture of various species: monomers and soluble as well as insoluble aggregates, whose concentration depends on factors such as total antibiotic concentration, method of preparation, and even the concentration of the stock solution (Legrand et al., 1992). However, there is a discrepancy in the critical concentration value of AmB at which the transition from the monomeric to the aggregated state of AmB begins. In other words, below the critical value, AmB exists in the form of the monomeric state. With the amount of AmB increased to the value above the critical value, they aggregated one another in solution. (From the absorption spectroscopy of AmB in our experiment, the drug molecules had aggregated at the concentration of 1×10^{-6} M.) For S-ERGO, the increasing current responses of the redox probe were found, and the ion channel behavior of AmB was greatly promoted, which coincided with results obtained from other studies that aggregates indeed represented the active form of AmB (Barwicz and Tancrède, 1997; Bolard et al., 1991; Gruda and Dussault, 1988). At the same time, the ion channel behavior of AmB was also found in S-CHOL. It had been reported that AmB should be in a self-associated form to induce K^+ permeation in cholesterol-containing liposomes, and any form of the antibiotic (monomeric or aggregated) induced K^+ leakage from ergosterol-containing membrane models (Bolard et al., 1991; Legrand et al., 1992; Yu et al., 1998).

Gruda and Dussault have proposed that ergosterol interacts slowly with monomeric AmB, but dimeric AmB allows complexation with ergosterol strikingly; it is considered that cholesterol does not interact with monomeric or dimeric AmB but reacts with AmB in a self-associated form (Gruda and Dussault, 1988). However, no current responses of the redox probe could be found in S-DMPC, indicating that there was not enough AmB in solution to form ion channels in S-DMPC. Actually, several AmB aggregate structures have been proposed, such as stacked dimers, in-plane dimers, and single or double helicoidal aggregates. Mazerski et al. (1999) have proposed a multi-step model of polyene antibiotic self-association. For this model, in aqueous solution, the polyene antibiotic can exist in many different species with a molecular weight in the range of a thousand (monomer) to a few million (colloid micelles). With a further increase in concentration, AmB tended toward the highly aggregated state in solution, and the ion channel behavior of AmB was also found in S-DMPC.

We propose three conceivable mechanisms by which AmB may affect the permeability of the pure DMPC bilayer. The first is to form hydrophilic pores of molecular dimensions for the redox probe in the hydrophobic membrane surrounding, which would lead to formation of the ion channel in the supported bilayer lipid membrane. The redox probe could reach the electrode along the channel formed by AmB, easily corresponding to the electrochemical response obtained. Second, because of the mismatch between AmB aggregation and the lipid bilayer, the preexisting defects could enlarge in or on the bilayer, which would allow the redox probe to get to the surface of the GCE along the defects. Finally, there could be a combination of the above mechanisms. Furthermore, AmB, because of both its rigidity and its position within the membrane structures, the chains of DMPC are aliphatic. As a consequence, these

chains interact more, through van der Waals interactions, with AmB than the aliphatic chains of the lipid interact with themselves. So the aliphatic chains of the lipid are rigidified upon their interaction with the drug. This is consistent with the idea that AmB can form aggregates or pores within the bilayer in the absence of sterols (Fournier et al., 1998).

Much work done recently led to the conclusion that sterols do promote, but are not necessary to produce, highly selective cationic AmB channels (Wolf and Hartsel, 1995). This has led to the proposal that AmB solution self-association is necessary for activity against sterol-free membranes but that ergosterol-containing membranes are also sensitive to monomeric AmB and may form stable channel-forming aggregate structures with this sterol at much lower AmB concentrations (Bolard et al., 1991; Cotero et al., 1998; Lambing et al., 1993). Fournier et al. (1998) studied the structure effects of AmB on pure and ergosterol- or cholesterol-containing dipalmitoylphosphatidylcholine bilayers by differential scanning calorimetry. They showed that AmB interacted strongly with the aliphatic chains of the lipid, consistent with the idea that AmB could form pores in a lipid matrix. The exact molecular architecture of an AmB channel is unknown. Incorporation of AmB into the hydrophobic core of the membrane results in the formation of molecular aggregates, which probably take the form of hydrophilic pores composed of six to nine molecules (Baginski et al., 1997; Gagos et al., 2001; Kruijff et al., 1974; Marin et al., 1991). AmB can readily form sterol-free channels under special circumstances: in the presence of osmotic stress (Lambing et al., 1993) or with sonicated small unilamellar vesicles (Fujii et al., 1997; Hartsel et al., 1988). Transition of AmB from a monomeric state to an aggregated state is the key factor in forming ion channels. One of the processes where AmB formed ion channels in the sterol-free GCE-supported bilayer lipid membrane (defect-free) was predicted (Fig. 8.) It involved the initial accumulation of AmB at the membrane surface or inside the membrane, and then it was assumed that several molecules associated with the membrane to form a pore. Whether this aggregation of molecules began before insertion or in the membrane after insertion is unknown. As yet, it is also unknown how many monomers were required to form a pore. The AmB molecules were thought to align around a central channel, with the hydrophobic faces toward the lipid bilayer and the hydrophilic faces toward the pore center. However, after the ion channel of AmB formed in pure lipid-supported model membrane, it is hard to avoid the conclusion that some of the unstable channels are affected by the high concentration of AmB still left in solution, which would lead to the emulsification processes where extremely aggregated AmB/lipid mixed micelles might form and cause the electrode to be depleted of lipid to some extent.

Although much work has been directed toward characterizing pore formation by AmB, it has been difficult to obtain a definitive answer because of the complex interactions of the drug with itself and with the lipid and sterols.

CONCLUSION

In the present paper, the behavior of AmB in forming ion channels in supported bilayer lipid membrane was studied comparatively between sterol-free and cholesterol- or ergosterol-containing supported phosphatidylcholine bilayer model membranes by electrochemical and spectroscopic methods. The state transition of AmB played the key role for channel formation in supported bilayer model membranes. The results showed that AmB could form ion channels in S-ERGO no matter what the state of AmB; AmB could form ion channels in S-CHOL only in the state of aggregation for AmB; and AmB could form ion channels in S-DMPC with the most difficulty among the three membrane systems studied and only in the state of extremely high aggregation for AmB.

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