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EFFECT OF AMPHOTERICIN B ON CHOLESTEROL-CONTAINING LIPOSOMES OF EGG PHOSPHATIDYLCHOLINE AND DIDOCOSENOYL PHOSPHATIDYLCHOLINE

A REFINEMENT OF THE MODEL FOR THE FORMATION OF PORES BY AMPHOTERICIN B IN MEMBRANES

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Summary

(1) Binding and K^+-permeability measurements were performed on egg and 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes with or without cholesterol.

(2) Amphotericin B binds specifically to cholesterol in both types of liposome despite the difference in bilayer thickness.

(3) Addition of amphotericin B to one side of the cholesterol-containing egg phosphatidylcholine bilayers induces a fast K^+ efflux from the outermost compartment of the liposomes. In contrast, the total K^+ content of sonicated unilamellar cholesterol-containing egg phosphatidylcholine vesicles is released by amphotericin B.

(4) Amphotericin B addition to one side of the cholesterol-containing 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes does not cause a change in K^+ permeability. The presence of amphotericin B on both sides of the bilayer, however, induces an increase in K^+ permeability.

(5) A model is proposed which accounts for the effect of bilayer thickness on the amphotericin B-induced permeability changes in membranes.

Introduction

Amphotericin B is one of the most studied polyene antibiotics (for a recent review see ref. 1). Its complete stereochemical structure is known (Fig. 1). A variety of biochemical and physicochemical studies on different types of model and biological membranes have led several groups to propose a detailed molecular mechanism for its antibiotic action [2—4]. The basic characteristic of these

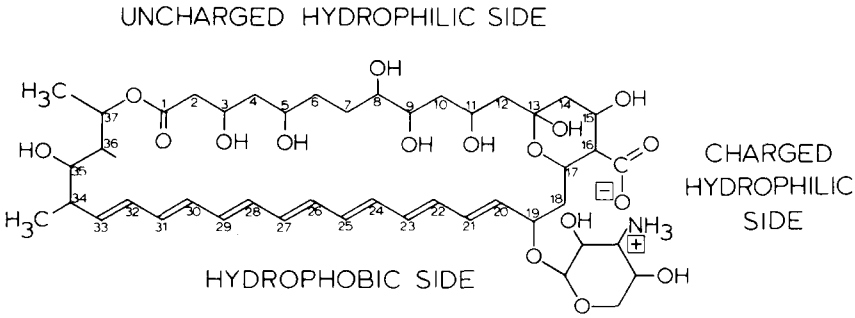

Fig. 1. Structure of amphotericin B [5].

models is that upon interaction with sterol-containing membranes (in both monolayers) complexes are formed of about 8 amphotericin B molecules and a similar number of sterol molecules. In these complexes the molecules are arranged in a circular manner so that the charged hydrophilic side of amphotericin B is at the membrane-water interface, while the ring system is oriented perpendicular to the membrane and the uncharged hydrophilic side is pointing inward such that it is shielded from the hydrophobic core of the membrane. This complex forms a half pore in which an aqueous channel of 8 Å diameter is present (see Fig. 5 in ref. 4). Two such half pores located opposite each other in the membrane would form a conducting pore through which small molecules and ions, such as K^+, leave the cell until it dies. The presence of sterols in the membrane is a prerequisite for the amphotericin B-induced permeability changes in natural membranes and egg phosphatidylcholine liposomes [1].

An intriguing feature of amphotericin B not explained by the proposed models is the difference in membrane sidedness of the amphotericin B action between black lipid membranes on the one hand and liposomal and biomembranes on the other. With black lipid membranes amphotericin B has to be added to both sides of the bilayer [6,7], whereas for the other membranes addition to one side only is sufficient to produce the characteristic permeability changes [4]. An important difference between the two types of membrane is the bilayer thickness, the black lipid membrane being thicker than the liposomal bilayer [8]. We therefore thought it of interest to study the amphotericin B-induced K^+ permeability changes in liposomes prepared from two unsaturated phosphatidylcholines differing by four carbon atoms in chain length e.g., egg phosphatidylcholine (containing mainly oleic acid: $18 : 1_c$) and didocosanoyl phosphatidylcholine ($22 : 1_c/22 : 1_c$-phosphatidylcholine). It will be shown that the character of the amphotericin B-induced K^+ leak is very different for the two phosphatidylcholines, leading to the proposal that for egg phosphatidylcholine liposomes and biological membranes the half pore is sufficient to act as a conducting pore.

Experimental

Materials

Amphotericin B was supplied by the Squibb Institute for Medical Research,

New Brunswick, N.J. The antibiotic was dissolved in dimethyl sulfoxide or dimethyl formamide at a concentration of 1 mg/ml and was stored in the dark at −15°C for a maximum of three days. Cholesterol was obtained from Fluka (Buchs, Switzerland). Egg phosphatidylcholine and phosphatidic acid derived from it were obtained as described before [9]. 1,2-Didocosenoyl-*sn*-glycero-3-phosphocholine (22 : 1_c/22 : 1_c-phosphatidylcholine, double bond $\triangle$13) was synthesized as described elsewhere [10]. All other chemicals were of analytical grade.

Preparation of liposomes

(a) Liposomes and vesicles for K^+-efflux experiments. A chloroform solution containing 40 μmol egg phosphatidylcholine and 1.6 μmol phosphatidic acid with or without 7.75 μmol cholesterol (15.7 mol %) was dried down with a rotary evaporator. The dry lipid film was dispersed at 20°C in 1.0 ml 150 mM KCl/10 mM Tris · HCl, pH 7.5, buffer (KCl buffer) by agitating on a vortex mixer for 1 min. Lipid vesicles were prepared by ultrasonication [11]. To remove the untrapped K^+ the liposomes or vesicles were dialyzed three times for 30 min against 200 ml 100 mM $CaCl_2$/10 mM Tris · HCl, pH 7.5, buffer ($CaCl_2$ buffer). The dialysis was performed at 0°C in the case of egg phosphatidylcholine liposomes and 17°C in the case of 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes. This was done in order to avoid possible complications due to the lipid phase transition which occurs at 10°C for the latter lipid (van Dijck, P.W.M., personal communication).

(b) Liposomes for K^+-efflux experiments with enclosed amphotericin B. 25 and 50 μg amphotericin B was added to the chloroform solution containing egg phosphatidylcholine and 22 : 1_c/22 : 1_c-phosphatidylcholine, respectively, whereafter the solvent was evaporated and the liposomes were prepared as described under (*a*). The liposomes were first dialyzed in the dark three times (each time 30 min) against 200 ml KCl buffer to remove untrapped amphotericin B, and three times against the $CaCl_2$ buffer to remove the K^+ outside the liposomes. The presence of amphotericin B outside the dialyzed liposomes was tested with control experiments in the following way: liposomes were made in and dialyzed against the $CaCl_2$ buffer, aliquots of KCl-enclosed liposomes prepared from egg phosphatidylcholine and cholesterol as described under (*a*) were added, and the amount of K^+ released was used to calculate the amphotericin B concentration, using the K^+ leaks induced by known amounts of amphotericin B as a reference (detection limit 0.1 μg amphotericin B/ml).

(c) 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes for K^+-influx experiments. The lipid film of 22 : 1_c/22 : 1_c-phosphatidylcholine with or without either 15.7 mol % cholesterol or 50 μg amphotericin B was dispersed in 1 ml of the $CaCl_2$ buffer at 17°C, as described above, and was dialyzed at 17°C in the dark: (1) three times each time for 30 min against 200 ml KCl buffer with or without 5 μg amphotericin B/ml; (2) three times for 30 min against KCl buffer without amphotericin B and (3) three times for 30 min against the $CaCl_2$ buffer, whereafter the K^+ content of these liposomes was measured.

(d) Liposomes for binding experiments. Liposomes were prepared as described under (*a*) except that the dialysis step was omitted. The 22 : 1_c/22 : 1_c-phosphatidylcholine-containing liposomes were prepared in 250 mM

glucose/25 mM NaCl/10 mM Tris · HCl, pH 7.5, buffer. The density of this buffer is higher than the density of the KCl buffer which facilitates the pelleting of the liposomes by centrifugation (see below).

Binding experiments

100 μl egg phosphatidylcholine liposomes was added to 10 ml of $CaCl_2$ buffer or 50 μl 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes was added to 5 ml of 150 mM LiCl/10 mM Tris · HCl, pH 7.5, buffer. (Due to the low density of this buffer the 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes containing glucose can be centrifuged.) Subsequently, 5 μg amphotericin B was added to the egg phosphatidylcholine liposomes and 2 μg to the 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes and the mixtures were incubated for 30 min. Similar aliquots of amphotericin B were incubated without liposomes for control. The liposomes were pelleted by centrifugation for 15 min at 12000 × *g*. All manipulations with the egg phosphatidylcholine liposomes were carried out at 0°C and with the 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes at 17°C. The pellets were dissolved in 1.0 ml chloroform/methanol (1 : 1, v/v) and the amount of amphotericin B was measured spectrophotometrically at 408 nm [12].

Analytical methods

K^+ influx or efflux from the liposomes was measured with a potassium specific glass electrode at 21°C as described before [9]. The amphotericin B-induced K^+ efflux was always corrected for small passive K^+ leaks. The ultraviolet spectrum of amphotericin B which is sensitive to chemical degradation and complex formation with cholesterol [12,13] was routinely measured with a Perkin-Elmer two wavelength double-beam spectrophotometer Model 356. Phospholipid phosphorus was measured after perchloric acid destruction of the lipids by the Fiske-Subba Row procedure [14].

Results

Effect of amphotericin B on egg phosphatidylcholine liposomes and vesicles

Table I shows that amphotericin B specifically binds to cholesterol in egg phosphatidylcholine liposomes in agreement with previous spectroscopic and calorimetric studies [12]. In the absence of cholesterol no significant binding to the liposomes occurs. The relative large fraction of the added amphotericin B recovered as a pellet in the absence of liposomes reflects the formation of amphotericin B aggregates in the buffer (see ref. 1).

The effect of the amphotericin B · cholesterol complex formation on the K^+ permeability of the egg phosphatidylcholine bilayer is shown in Fig. 2. Low concentrations of amphotericin B release about 35% of the liposomal K^+ content from unsonicated liposomes (Fig. 2A). Higher amounts of amphotericin B (up to 2 μg/ml) only increase the rate of the K^+ efflux but do not increase the final extent of the K^+ release. In the absence of cholesterol similar amounts of amphotericin B only induce a very small K^+ leak (Fig. 2A).

To test whether the multibilayer character of the liposomes is responsible for the incomplete K^+ release induced by the amphotericin B · cholesterol complex

TABLE I

BINDING OF AMPHOTERICIN B TO EGG- AND 22 : 1_c/22 : 1_c-PHOSPHATIDYLCHOLINE LIPOSOMES CONTAINING NO OR 15.7 mol % CHOLESTEROL

For experimental details see Experimental.

	% of added amphotericin B recovered in pellet (mean ± S.D.)			Amount of amphotericin B bound to cholesterol in the liposomes (nmol amphotericin B/ μmol cholesterol)
	No liposomes	Liposomes —cholesterol	Liposomes +cholesterol	
Egg phosphatidylcholine	30.3 ± 1.2	30.0 ± 1.2	33.2 ± 1.2	0.2
22 : 1_c/22 : 1_c Phosphatidylcholine	22.0 ± 2.5 *	22.0 ± 2.5	30.0 ± 2.5	0.4

* The lower amount of amphotericin B must reflect the smaller aggregate size of amphotericin B in the LiCl buffer as compared to the $CaCl_2$ buffer.

the experiment was repeated with small single bilayer vesicles (Fig. 2B). With these vesicles the K^+ leak is also greatly enhanced by the amphotericin B · cholesterol complex and in this case all the K^+ is released from the vesicles. This difference is most easily interpreted in the sense that amphotericin B cannot get across the various bilayers of the liposome and can excert its action on the outermost bilayer, thus releasing the K^+ only from the outermost aqueous compartment in the liposome. Theoretically there are two possibilities to explain this: firstly, after the amphotericin B addition to the liposomes half pores are formed in both monolayers of the outermost bilayer, thus the charged hydrophilic sides of amphotericin B are present in the polar headgroup region of both monolayers. Half pores located opposite each other in the bilayer form conducting pores through which K^+ can leave the outermost aqueous compartment (as was proposed originally in ref. 4). Secondly, amphotericin B addition leads to the formation of half pores in the outer bilayer in which the charged

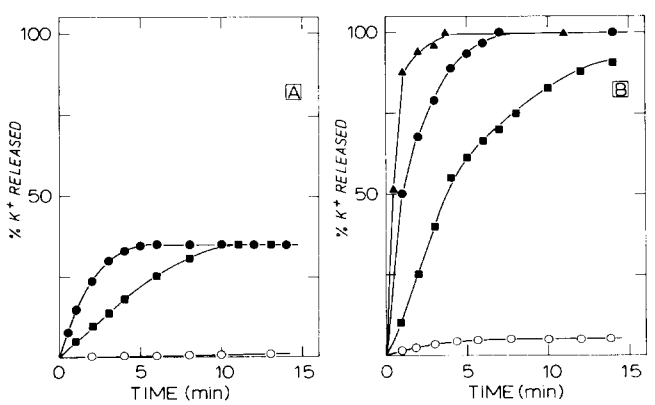

Fig. 2. Effect of amphotericin B on the K^+ leak from egg phosphatidylcholine liposomes (A) or sonicated vesicles (B) with or without 15.7 mol % cholesterol. (A) ○———○, no cholesterol, 0.8 μg amphotericin B/ml; ■———■, with cholesterol, 0.4 μg amphotericin B/ml; ●———●, 0.8 μg amphotericin B/ml. (B) ○———○, no cholesterol, 0.71 μg amphotericin B/ml; ■———■, with cholesterol, 0.14 μg amphotericin B/ml; ●———●, 0.71 μg amphotericin B/ml ▲———▲, 17.8 μg amphotericin B/ml.

hydrophilic sides of all amphotericin B molecules are localized in the polar headgroup region of the outer monolayer only. These half pores would already be long enough to span the bilayer and to cause the K^+ leak. There are several arguments against the first and in favor of the second alternative. In the first mechanism amphotericin B molecules must undergo a very rapid transbilayer movement. Because amphotericin B resembles more or less a phospholipid molecule, as it has roughly the same size and is also amphiphatic in character, and since the transbilayer movement of phospholipids in general is extremely slow (for review see ref. 15) this seems highly unlikely. For example, after the addition of lysophosphatidylcholine to phosphatidylcholine vesicles the lyso compound is readily incorporated into the outer monolayer of the vesicle but even 45 h later it still cannot be detected in the inner monolayer [16]. The first mechanism also requires that amphotericin B cannot go from the inner monolayer of the outer bilayer to the outermost aqueous compartment of the liposome and thereby to the next bilayers, since in that case more K^+ would leak out of the liposomes. That amphotericin B can rapidly exchange between the aqueous phase and the bilayer is borne out by the results presented in Fig. 2B. At an amphotericin B concentration of 0.14 μg/ml almost all K^+ is release from the vesicles in about 15 min. It can be calculated from the size of the vesicle [11] that an average of 3 amphotericin B molecules are present per vesicle. A second addition of a similar amount of vesicles (thus now only 1.5 amphotericin B molecules are present per vesicle) resulted again in a nearly complete release of the K^+ content of the vesicles in 15 min. Since one half pore requires about 8 and one double half pore about 16 amphotericin B molecules [4] it follows that amphotericin B can exchange rapidly between the various vesicles like the mobile carrier valinomycin [17]. Furthermore, the closely related polyene antibiotic nystatine is completely removed from erythrocytes within seconds after resuspending the cells in nystatin-free medium [18], which also shows that these polyene antibiotics can readily exchange between the aqueous phase and the membrane. With the second alternative the total release of all the K^+ from the vesicles at the low amphotericin B concentration can be envisaged as the rapid association-dissociation of the halfpore and a fast exchange of amphotericin B between the vesicles.

To distinguish further between the two possible mechanisms an experiment was performed, as outlined in Table II, in which amphotericin B was incorporated in all layers of the liposome by dispersing a mixed lipid-amphotericin B film in the KCl buffer (method (*b*), Experimental). The untrapped amphotericin B was first removed by dialysis against KCl buffer whereafter the KCl was replaced by $CaCl_2$. As shown in Table II, in the case of the double half pore mechanism it is expected that after dialysis the liposomes contain no amphotericin B and have a K^+ content which is similar for liposomes with or without cholesterol. In the case of the half pore mechanism most amphotericin B is still associated with the liposomes but the K^+ content must be greatly reduced in the presence of cholesterol and less reduced in the absence of cholesterol. Experimentally it was found that in the absence and also in the presence of cholesterol 60% of the initial amphotericin B was still associated with the liposomes after dialysis. The ultraviolet spectrum of amphotericin B showed the characteristic shift in the 3/1 ratio indicative of the amphotericin B · choles-

TABLE II

THEORETICALLY POSSIBLE MECHANISMS OF AMPHOTERICIN B ACTION ON 15.7 mol % CHOLESTEROL CONTAINING EGG PHOSPHATIDYLCHOLINE LIPOSOMES

A, Amphotericin B; A · C, amphotericin B · cholesterol complex; the inside compartment represents the total liposome without the outermost bilayer.

	Double half pore mechanism			Half pore mechanism		
	Inside	Outermost bilayer	Outside	Inside	Outermost bilayer	Outside
Expected situation after preparation of liposomes	K^+ A	A · C/A · C	K^+ A	K^+ A	A · C A · C	K^+ A
Exptected situation after dialysis against KCl	K^+		K^+	K^+ A	A · C	K^+
Expected situation after dialysis against $CaCl_2$	K^+			A	A · C	

terol complex formation [12]. No amphotericin B could be detected outside the liposomes assayed as described in Experimental. The K^+ content was 271 nmol K^+/μmol phosphatidylcholine for liposomes without cholesterol and 60 nmol K^+/μmol phosphatidylcholine for liposomes with 15.7 mol % cholesterol (normal K^+ content for both liposomes is 750 nmol K^+/μmol phosphatidylcholine). The addition of up to 5 μg amphotericin B/ml to the liposomes did not cause any K^+ leak from the liposomes (see Fig. 2A). These results again strongly support the second alternative in that (a) the halfpore is already sufficient to cause the K^+ efflux and (b) that amphotericin B cannot get across the lipid bilayer.

Effect of amphotericin B on 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes

Binding experiments were performed in order to compare the amphotericin B-cholesterol interaction in the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer with the interaction in the egg phosphatidylcholine bilayer; the results are presented in Table I. Amphotericin B only binds to the 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes in the presence of cholesterol. The affinity of amphotericin B for cholesterol in the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer is even higher than that for the egg phosphatidylcholine bilayer, demonstrating that the longer fatty acid chains do not interfere with the amphotericin B · cholesterol complex formation. The amphotericin B-induced K^+ leaks from the 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes are summarized in Fig. 3. Antibiotic concentrations as high as 10 μg/ml do not cause any detectable K^+ leak from these liposomes both in the absence and presence of cholesterol, which is in strong contrast with the effect of amphotericin B on the egg phosphatidylcholine liposomes (compare Fig. 2). This demonstrates that the membrane thickness of the bilayer is a very important parameter in the ampho-

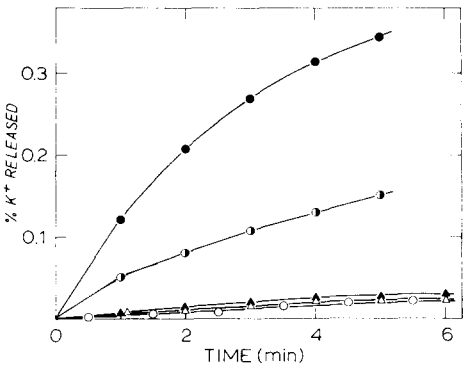

Fig. 3. Effect of amphotericin B on the K^+ permeability of 22 : 1_c/22 : 1_c-phosphatidylcholine liposomes with or without 15.7 mol % cholesterol. ○———○, 10 μg amphotericin B/ml added to cholesterol-free liposomes containing no amphotericin B inside; ▲———▲, 10 μg amphotericin B/ml added to cholesterol-containing liposomes with no amphotericin B inside; △———△, 10 μg amphotericin B/ml, added to cholesterol-free liposomes with amphotericin B inside. ◑———◑, ●———●, 4 and 9 μg amphotericin B/ml, respectively, added to cholesterol-containing liposomes with amphotericin B inside. The error in the determination of the % K^+ released is estimated to be maximally 10%.

tericin B-induced K^+ leak. Apparently the half pores formed in the outer monolayer do not span the bilayer. To test whether for these bilayers the simultaneous presence of amphotericin B on both sides of the bilayer would facilitate the K^+ efflux (as is the case with the black lipid membranes) liposomes were prepared with enclosed K^+ and amphotericin B, and these were subsequently dialyzed against KCl and $CaCl_2$ buffers as described under method (*b*) in Experimental. The liposomes were found to contain 80% of the original amphotericin B, which, based on its ultraviolet spectrum (measured as described in ref. 12), showed an interaction with cholesterol similar to that in egg phosphatidylcholine bilayers. No amphotericin B could be detected outside the liposomes. Both liposomes with and without 15.7 mol % cholesterol contained 500 nmol K^+/μmol phosphatidylcholine. This shows that amphotericin B cannot cross the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer and also that half-pores present in the inner monolayer of the outer bilayer do not permit the efflux of K^+ (compare with Table II). Addition of amphotericin B to these

TABLE III

EFFECT OF THE SIDENESS OF AMPHOTERICIN B ADDITION ON THE K^+ INFLUX AT 17°C IN 22 : 1_c/22 : 1_c-PHOSPHATIDYLCHOLINE LIPOSOMES CONTAINING NO OR 15.7 mol % OF CHOLESTEROL

For experimental details see Experimental.

Cholesterol presence in liposomes	Amphotericin B enclosed in liposomes	Amphotericin B presence outside the liposomes	K^+ influx (nmol K^+/h per μmol phosphatidylcholine)
+	—	—	23
+	—	+	48
+	+	+	193
—	+	+	60

liposomes induces a small but significant K^+ leak which was dependent upon the amount of amphotericin B added and the presence of cholesterol in the bilayer (Fig. 3). It can be concluded that the presence of amphotericin B · cholesterol complexes on both sides of the bilayer of 22 : 1_c/22 : 1_c phosphatidylcholine liposomes induces a K^+ efflux. To further substantiate this finding the K^+ influx in these liposomes was studied under conditions where amphotericin B was present on one and on both sides of the bilayer (Table III). The maximal influx again is observed when (1) cholesterol is present in the bilayer and (2) amphotericin B is present on both sides of the bilayer.

Discussion

The addition of amphotericin B to one side of a sterol-containing egg phosphatidylcholine bilayer (Fig. 2) or a biological membrane (refs. 9 and 18 and papers cited in ref. 19) leads to the release of enclosed K^+. Furthermore, amphotericin B can exchange readily between the bilayers and the aqueous medium, but cannot get across the bilayer. Fig. 4A illustrates the model we propose for the amphotericin B action on these membranes to account for these observations. It visualizes a cross-section through the circular arrangement of 8 amphotericin B molecules forming a half pore (see also Fig. 5 in ref. 4). Cholesterol molecules are thought to be present near the hydrophobic side of the amphotericin B molecules [4]. New features of this model are: (1) the charged groups of all the amphotericin B molecules are localized on the side of the bilayer to which the antibiotic was added, and (2) the half pore is sufficient to cause the change in K^+ permeability. The length of the amphotericin B molecule (21 Å) is shorter than the hydrophobic core of the cholesterol-containing egg phosphatidylcholine bilayer (35 Å, [20]). We propose that meniscus formation around the half pore is possible due to the flexibility of the phospholipid acyl chains. This would minimise the contact between the hydrophobic core of the bilayer and the aqueous phase. The half pore must be a highly dynamical structure with a short life time. The half pore can possibly shuttle up and down in the bilayer to some extent which could provide some mobile carrier characteristics for the half pore. The $C_{35}OH$ group on the amphotericin B molecules are thought to be important in the anchoring of the half pore in the interphase between the inner monolayer and the aqueous phase. A detailed description of the possible mode of formation of the half pore in the bilayer is given in ref. 4.

Addition of amphotericin B to one side of the bilayer is insufficient to cause a permeability change in the cholesterol-containing 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer despite the formation of amphotericin B · cholesterol complexes in the bilayer. The presence of amphotericin B on both sides of the bilayer induces a change in K^+ permeability. This closely resembles the effect of amphotericin B on black lipid membranes [6,7]. Both model membranes have a thicker hydrophobic core than the egg phosphatidylcholine bilayer. No direct X-ray measurements are available for the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer but from the data on egg phosphatidylcholine and the average length of a methylene group in an acyl chain in the liquid crystalline state [21] a thickness of the hydrophobic part of 45 Å can be calculated. For egg phosphatidyl-

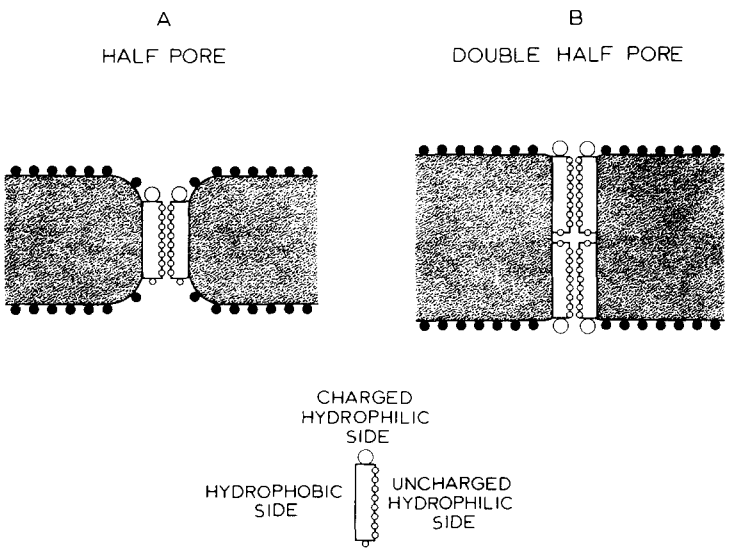

Fig. 4. Model of the half pore in the egg phosphatidylcholine bilayer (A) and the double half pore in the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer (B). The size of the amphotericin B molecule, the bilayer thickness and the average distance between the polar headgroup of the phosphatidylcholine molecules (black spheres) are on scale. The shaded part represents the hydrophobic core of the bilayer. The small open circles on the amphotericin B molecule represents the various oxygen functions on the non-charged hydrophilic side of amphotericin B and the $C_{35}OH$ group. The large open circle on the amphotericin B molecule represents the mycosamine and carboxyl group.

choline-decane films, which are commonly used as black lipid membranes, a value of 48.6 Å was reported [8]. Apparently, the length of the half pore is now insufficient to span the bilayer. The presence of half pores in both monolayers (which can only be achieved by the addition of amphotericin B to both sides of the membrane) can give rise to double half pores (conducting pores) as is shown in Fig. 4B (see also ref. 4). The much less efficient K^+ permeation through the double half pores of the 22 : 1_c/22 : 1_c-phosphatidylcholine bilayer as compared to that through the egg phosphatidylcholine bilayer containing only half pores must reflect the low probability of the two half pores to be located opposite to each other in the bilayer. Such a double half pore could possibly be stabilized by the interaction between the $C_{35}OH$ group of amphotericin B molecules in opposite located half pores [4].

Sterol specificity is generally accepted to be of major importance for the membrane specificity of the polyene antibiotics [1,19]. The bilayer thickness, which is dependent on the lipid composition of the membrane and thus greatly varies between different biological membranes could also be an important factor determining the susceptibility of the sterol-containing membrane, towards the pore-forming polyene antibiotics.

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