Biochimica et Biophysica Acta, 339 (1974) 57–70

BBA 76569

POLYENE ANTIBIOTIC–STEROL INTERACTIONS IN MEMBRANES OF *ACHOLEPLASMA LAIDLAWII* CELLS AND LECITHIN LIPOSOMES

III. MOLECULAR STRUCTURE OF THE POLYENE ANTIBIOTIC–CHOLESTEROL COMPLEXES

B. DE KRUIJFF and R. A. DEMEL

Biochemical Laboratory, State University Utrecht, Vondellaan 26, Utrecht (The Netherlands)

(Received September 18th, 1973)

SUMMARY

1. Based on the analysis of the complexes of cholesterol and the polyene antibiotics filipin, amphotericin B, nystatin, etruscomycin and pimaricin, which can be built with space-filling models, and from data on the polyene antibiotic–cholesterol interaction, mechanisms of the polyene antibiotic induced permeability changes in membranes are proposed.

2. The amphotericin B–cholesterol complex is visualised as a circular arrangement of 8 amphotericin B molecules interdigitated by 8 cholesterol molecules. The outside of this complex is hydrophobic, the inside is hydrophilic due to the presence of the hydroxyl groups of the amphotericin B molecules. Two such complexes (half pores) will generate a pore which traverses the membrane. The hydrophilic channel of such a pore has a diameter of about 8 Å.

3. The nystatin–cholesterol and etruscomycin–cholesterol complexes also are visualised as pores. However, the pimaricin–cholesterol complex cannot form a conducting pore because the length of the half pores is considerably less than the length of half the thickness of the lipid core.

4. The filipin–cholesterol complex is visualised as an aggregate of 150–250 Å in diameter oriented in the hydrophobic core of the membrane. This aggregate might be composed of two regular arrays of stacked filipin molecules such that the exterior of this aggregate is hydrophobic due to the presence of the double bonds of filipin to which equal amounts of cholesterol are complexed. The presence of this aggregate causes membrane fragmentation.

INTRODUCTION

The structures of filipin [1], etruscomycin [2], pimaricin [3], nystatin [4] and amphotericin B [5] are given in Fig. 1. It was demonstrated that for the interaction of the polyene antibiotics with cholesterol, leading to complexes in the membrane, an intact ring system, a conjugated double bond system and the hydroxyl groups of the polyene

TABLE I

STOICHIOMETRY OF THE POLYENE ANTIBIOTIC-CHOLESTEROL INTERACTION OBTAINED FROM VARIOUS STUDIES

Polyene antibiotic used	mole cholesterol/mole polyene antibiotic				
	Ultraviolet spectroscopy on liposomal and free cholesterol [8, 11]	Fluorescence spectroscopy on free cholesterol [12]	Differential scanning calorimetry on liposomal cholesterol [11]*	Permeability studies on cholesterol containing *A. laidlawii* cells [9]	Binding to cholesterol containing *A. laidlawii* membranes [10]
Filipin	1.5	0.80–0.95	1.2	0.7	1.2
Amphotericin B	—	—	3.9	3.3	0.7
Nystatin	—	—	1.2	1.6	—
Etruscomycin	—	—	0.6	0.3	1.6
Pimaricin	—	—	1.7	—	1.5

* Data are corrected for the amount of cholesterol in the liposome not available for the polyene antibiotic [9].

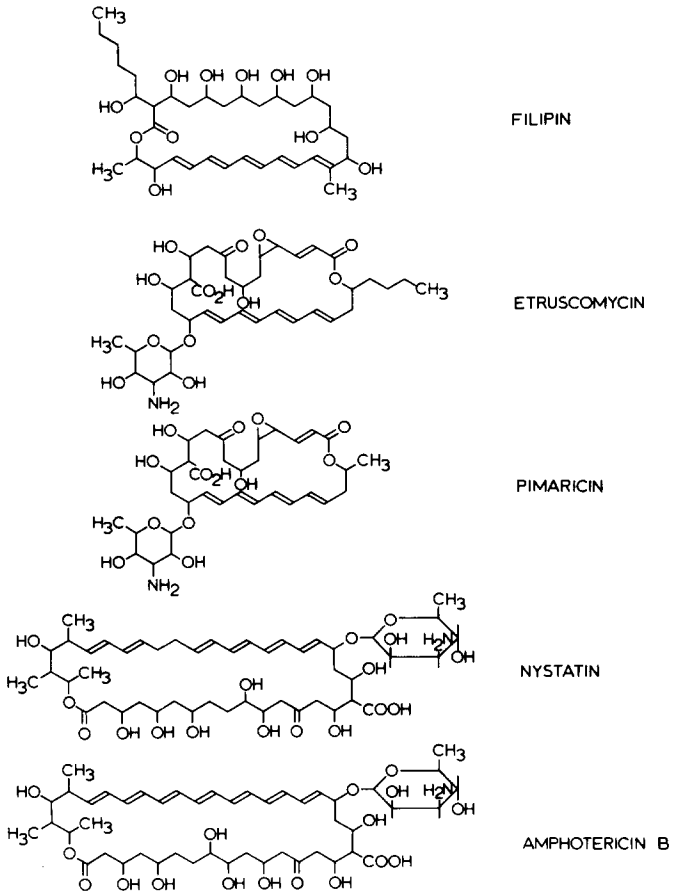


Fig. 1. Structures proposed for the different polyene antibiotics.

are prerequisites [5–7]. Complexes of the above mentioned polyene antibiotics with cholesterol have some characteristics in common. (a) The interaction within these complexes is primarily hydrophobic [8–10]. (b) The number of cholesterol molecules per mole of polyene antibiotics in the complex is of the same order. Table I summarizes the stoichiometry of the polyene antibiotic–cholesterol interaction measured in various systems with different techniques. For filipin, which has been most extensively studied, the data from the literature indicate that the interaction with cholesterol is equimolar; for the other polyene antibiotics the data are more scattered, but it is obvious that only a small number of cholesterol molecules interact with one molecule of antibiotic. (c) All polyene antibiotics can complex in a membrane only with sterols which have a 3β-OH group, a planar ring system and a hydrophobic side chain at C_{17} [8, 9, 11, 13].

Despite these common features of the various polyene antibiotic–cholesterol complexes there are remarkable differences in the effect of these complexes upon membrane permeability. The filipin–cholesterol complex, which can be visualized as an aggregate of 150–250 Å in diameter lying within the hydrophobic core of the mem-

brane [14, 15], fragments the membrane and causes a release of all cytoplasmic components [9]. Amphotericin B–cholesterol, nystatin–cholesterol and probably also the etruscomycin–cholesterol complexes function as aqueous channels traversing the membrane [9, 7, 16]. Pimaricin–cholesterol complexes do not affect the membrane permeability of *A. laidlawii* cells and egg lecithin liposomes [9, 10]. In order to understand these differences in mode of action of the various polyene antibiotics from the differences in chemical structure we investigated in this paper the features of complexes which can be built from space-filling models of the polyene antibiotics and cholesterol. From these models and the available experimental data a mechanism of the polyene antibiotic induced permeability changes in membranes is proposed.

Amphotericin B–cholesterol complex

The space-filling models of amphotericin B, cholesterol and distearoyl lecithin show several interesting features (Fig. 2). In the amphotericin B molecule the hydrophilic groups, namely 7 hydroxyl groups, the keto group, the carboxyl group and the ester linkage are aligned along one side and directed to the outside of the ring-shaped molecule. However, the other side of the ring system is hydrophobic and rigid due to the conjugated double bond system. The length of the double bond system equals the length of a cholesterol molecule. Since the polar mycosamine group and the carboxyl residue are located at one side of the molecule, amphotericin B also has amphipathic properties. The only hydroxyl group of the ring system not aligned with the other hydroxyl groups at the far end of the molecule opposed to the charged groups, is surrounded by three methyl groups (see also Fig. 4B). The length of the amphotericin B molecule is about the length of the lecithin molecule as measured from the charged phosphate group to the terminal methyl group. The cross section of the amphotericin B molecule is nearly rectangular, while the cross section through the ring system of the

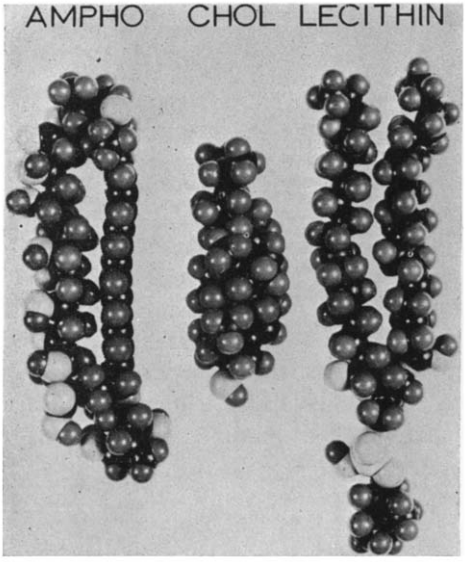


Fig. 2. Space-filling models of amphotericin B, cholesterol and distearoyl lecithin.

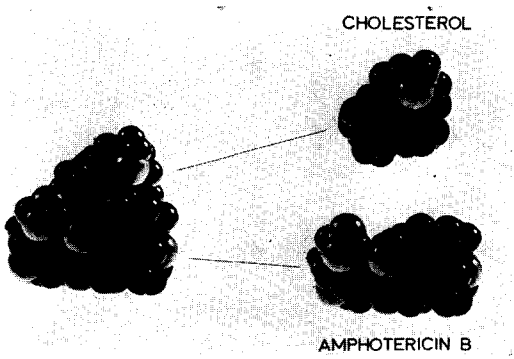
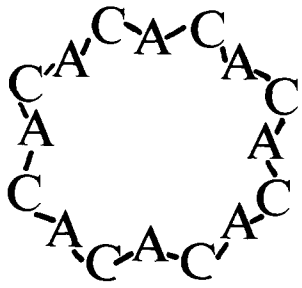
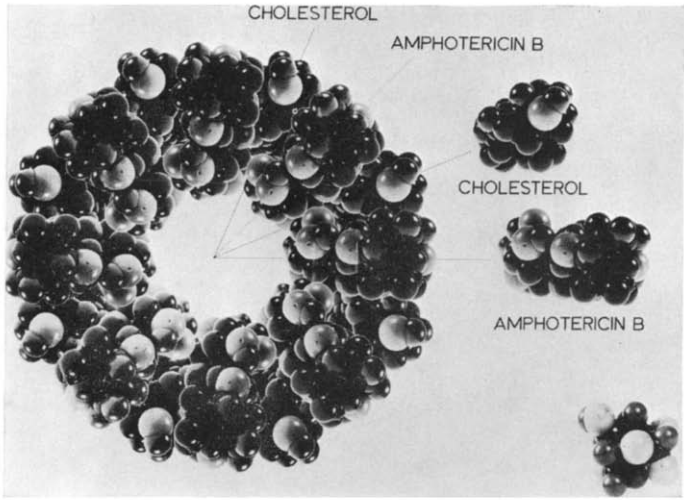


Fig. 3. Space-filling models of amphotericin B (A), cholesterol(C) and the A–C complex. The view is along the long axes of the molecules and is facing the mycosamine group of A and the hydroxyl group of C (top view).

cholesterol molecule is more conical (Figs 3 and 4A). These characteristics of the molecules make it possible to form an amphotericin B (A)–cholesterol (C) complex in which the cholesterol molecule fits partially in the ring of the amphotericin B molecule (Fig. 3). In this A–C complex cholesterol is in close contact with the double bond system only; there is no direct contact between the hydrophilic groups of amphotericin B and cholesterol. Since the cholesterol molecule can be present on both sides of the double bond system of the amphotericin B molecule, two almost equivalent complexes A–C and C–A can be visualised (Fig. 3 only shows one possibility). The radial angle of the segmental A–C complex is about 23 ° (Fig. 3). This complex can be extended to the formation of an A–C–A–C complex with a radial angle between two amphotericin B molecules of 45°. A further extension of the complex leads to a circular arrangement of 8 A–C units forming a $(-A-C-)_8$ complex.



The inside of the $(-A-C-)_8$ complex is hydrophilic in character due to the hydroxyl groups of the amphotericin B molecules whereas the outside is hydrophobic in nature due to the conjugated double bond systems of the amphotericin B molecules and the interdigitating cholesterol molecules. A photographic representation of a molecular model of this last complex is given in Figs 4A and 4B. In Fig. 4A the view is along the conjugated double bond system and faces the charged groups of amphotericin B and the hydroxyl group of cholesterol. In Fig. 4B the view is along the conjugated double bond system and faces the end of the amphotericin B and the tail of the cholesterol



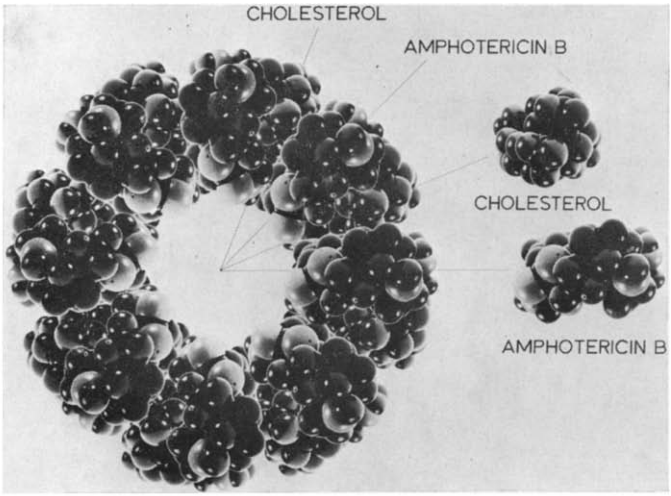


Fig. 4. Space-filling model of the $(-A-C-)_8$ complex (half pore). The view is along the long axes of the molecules. A. Top view half pore. At the right bottom part of the figure a space-filling model of the glucose molecule is included. B. End view half pore.

molecule. The hydroxyl group of cholesterol and the charged groups of amphotericin B are located on the same side of the complex. These residues are thought to be of critical importance for the orientation of the complex in a lipid bilayer. The charged groups will be coplanar with the polar residues of the phospholipid molecules. The apolar backbones of amphotericin B and cholesterol are oriented parallel to the fatty acid chains of the phospholipid. The total length of the amphotericin B molecule equals the length of the fatty acid and glycerol moiety of the phospholipid molecules, so that the $(-A-C-)_8$ unit arrangement can form a half pore through the lipid bilayer. Two such half pores on either side of the lipid bilayer are necessary for a complete conducting pore (Fig. 5). The hydroxyl groups of amphotericin B which were not

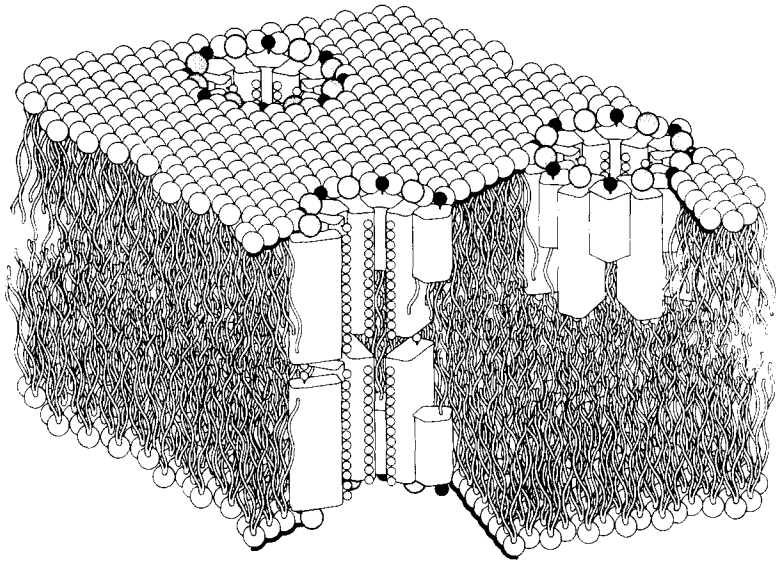


Fig. 5. Schematic representation of the pores formed by amphotericin B and cholesterol in a lipid bilayer. The figure shows in a cross section the interior of a conducting pore formed by two half pores. Furthermore the hydrophobic outside of a half pore and the entrance of another pore in the membrane is visible.

lined up in the pore interior may be involved in anchoring the two half pores thus stabilizing the conducting channel.

For the formation of the half pores in membranes we propose the following mechanism. Throughout the membrane A–C complexes are formed with two possible orientations. In one case the complex will be oriented more or less parallel to the plane of the membrane so that the hydrophilic site of the amphotericin B molecule is at the membrane/water interface. Cholesterol will also be oriented parallel to the membrane. A–C complexes in this orientation will probably not tend to aggregate. The other possibility is that the A–C complex is oriented perpendicular to the plane of the bilayer. In this case cholesterol has its normal perpendicular orientation but now the hydrophilic site of amphotericin B in the complex is in the hydrophobic core of the membrane leading to a rather unfavourable situation. However, the hydrophilic side of a second A–C complex in this orientation can associate with the hydrophilic site of the first A–C complex to form a larger complex A–C–A–C which is also perpendicularly oriented to the membrane. This orientation of the A–C–A–C complex is already energetically preferred because the hydrophilic sides of the two A–C complexes partially shield each other from the hydrophobic core of the membrane. Due to a cooperative effect the perpendicular orientation of the $(-A-C-)_8$ complex (half pore) which would eventually result, is highly stabilized since now all the hydrophilic sites of the amphotericin B molecules are directed inside the pore whereas all hydrophobic groups are at the outside. The diameter of the hydrophilic channel in the pore is 8 Å.

The molecular model of the amphotericin B-cholesterol pore we are proposing is in agreement with many experimental data. (a) Amphotericin B interacts with

cholesterol in such a way that the sterol is withdrawn from its interaction with other lipids [10, 11]. The hydrophobic interaction of the rigid double bond system of amphotericin B with the rigid steroid nucleus of cholesterol must be more favourable than the interaction between the rigid double bond system of amphotericin B or the rigid steroid nucleus of cholesterol with a mobile alkyl chain of a lipid. This might provide an explanation for the absolute sterol requirement of the polyene antibiotics. (b) The interaction between cholesterol and amphotericin B in the model proposed is hydrophobic in nature [8–10]. (c) From studies on the stoichiometry of the amphotericin B–cholesterol interaction it has become apparent that a small number (0.7–3.9) of cholesterol molecules are interacting with one molecule amphotericin B (Table I). In the present model we suggest that equal amounts of cholesterol and amphotericin B are needed to form a pore. The cholesterol molecules in this model interdigitate with the amphotericin B molecules. However, at the outer surface of the pore the conjugated double bond systems of the amphotericin B molecules are capable of interaction with additional cholesterol molecules, in which case the cholesterol: amphotericin B ratio would exceed unity. (d) The induced membrane permeability changes are strongly dependent upon the amphotericin B concentration. This indicates that 5–10 [7] or about 6 [9] molecules of amphotericin B are required to form a conducting pore. (e) With black-lipid membranes it was demonstrated that addition of amphotericin B to one side of a cholesterol containing membrane often did not influence the membrane resistance while addition of amphotericin B to both sides markedly decreased the membrane resistance. From these studies Cass et al. [7] suggested that half pores have to be formed at each side of the lipid bilayer to make the formation of a complete pore possible. In some cases, however, they also observed that addition of amphotericin B to one side of the membrane was sufficient to produce a strongly decreased resistance. In the black-lipid membrane studies of Van Zutphen et al. [17] it was found that addition of amphotericin B to one side of a cholesterol containing film decreased the resistance to a significant extent. Amphotericin B added to the other side of the membrane did not further decrease the resistance. In liposomes and various cells [9, 10, 18–20] amphotericin B can increase the membrane permeability when added to only one side of the membrane. This might indicate that in liposomes and biological membranes the amphotericin B–cholesterol half pore apparently can form on both sides of the membrane. We suggest the following mechanism: Formation of A–C in the outer most layer of the membrane gives an expansion of the lipid molecules around the complex, since cholesterol is removed from its interaction with these molecules. Dissociation of A–C gives a condensation of the lipid molecules because of the lipid–cholesterol interaction. Such a lateral compressibility that is induced by the A–C complex formation would permit an increase in translocation rate of A or A–C analogous to that described by Linden et al. [21] and Papahadjopoulos et al. [22]. These authors observed that the translocation of various compounds is enhanced through membranes which have both gel and liquid crystalline lipids present. In black-lipid membranes the presence of decane might interfere with this translocation, so that the antibiotics have to be added to both sides of these membranes to produce a conducting channel. (f) From permeability studies it was concluded that the glucose molecule did not pass [7, 16] or passed rather slowly [9] through the amphotericin B–cholesterol pores. It was suggested that the diameter of the aqueous channel in the pore must be very similar to the radius of the glucose molecule (Fig. 4A). (g) The fact

that an intact ring system of amphotericin B is required for its action is also inherent to our model. Hydrolysis of the lactone bond results in loss of activity [7]. The hydrophilic sites of the hydrolysed amphotericin B molecules will be at the membrane/water interface and it will become nearly impossible to orient them within the lipid core, even after interaction of the conjugated double bond system with cholesterol. Thus, no pore can be formed with such a modified polyene. In our model the intact ring system is needed to pull the hydrophilic site of amphotericin B into the hydrophobic interior of the membrane.

Nystatin–cholesterol complex

The structure of nystatin is closely related to that of amphotericin B (Fig. 1). The differences consist of a change in the position of the hydroxyl groups at the hydrophilic site of the molecule and an interruption of the conjugated double bond system. In amphotericin B seven double bonds are conjugated whereas in nystatin the double bond system is separated into two and four double bonds by two methylene groups. The interruption of the double bond systems allows a bending of this otherwise rigid region. The effects of nystatin and amphotericin B upon both model and biological membranes are also very similar [7, 9, 16, 18, 19]. From the permeability studies described in the preceeding paper we concluded that the pore formed by the nystatin–cholesterol complex is somewhat smaller than that formed by the amphotericin B–cholesterol complex [9]. We propose a model for the nystatin–cholesterol complex which is basically the same as for amphotericin B. The somewhat smaller pore size could be explained by the bending of the hydrophobic backbone of the nystatin molecule.

Etruscomycin–cholesterol complex

There are several important differences between the chemical structure of etruscomycin and amphotericin B (Fig. 1). The ring size of etruscomycin (26 atoms) is smaller than the ring size of amphotericin B (38 atoms). Etruscomycin contains 4 conjugated double bonds compared to 7 in amphotericin B. The hydrophilic side of the ring system of etruscomycin contains 3 hydroxyl, 1 keto, 1 carboxyl and 1 epoxide group, making it smaller than the hydrophilic side of amphotericin B. Furthermore, a hydrophobic tail of 4 carbon atoms is attached to the ring system of etruscomycin. This hydrophobic tail must play an important role in the permeability changes induced by etruscomycin because pimaricin which does not affect the membrane permeability [9] has a structure which is identical to etruscomycin with the exception of the hydrophobic tail. The orientation of the charged groups, e.g. the carboxyl group and the amino sugar residue, is identical for etruscomycin and amphotericin B.

Analogous to amphotericin B and nystatin, half pores can be constructed from etruscomycin and cholesterol. The total length of this half pore including the hydrophobic tail is similar to the length of the amphotericin B half pore and the permeability changes induced by etruscomycin in cholesterol containing *A. laidlawii* cells are similar to the permeability changes induced by amphotericin B and nystatin [9]. However, the temperature dependence of the etruscomycin induced K^+ leak was much stronger than those of the amphotericin B and nystatin induced K^+ leaks. At room temperature etruscomycin did not affect the membrane permeability but at 0 °C a strong K^+ release from the cells was observed [10]. The amphotericin B and

nystatin induced K^+ leaks was also increased at lower temperatures but this effect was much less pronounced [10]. This indicates that hydrophobic forces are more important for the formation of the etruscomycin–cholesterol complex than for the formation of the amphotericin B-cholesterol and nystatin–cholesterol complex.

We suggest that, in contrast to amphotericin B and nystatin, the two half pores of etruscomycin opposing each other in the membrane are stabilized by hydrophobic interactions between the alkyl chains at the end of the two half pores.

Pimaricin–cholesterol complex

As indicated above the pimaricin molecule differs from the etruscomycin molecule only in the absence of the hydrophobic tail. The pimaricin–cholesterol complex did not affect the membrane permeability of *A. laidlawii* cells and egg lecithin liposomes [9]. We suggest that the pimaricin–cholesterol complex is also a half pore, but because the length of this structure is less than half the thickness of the membrane no conducting pores can be formed.

Filipin–cholesterol complex

The most obvious features in the chemical structure of filipin, when compared with the other polyene antibiotics are the absence of the charged carboxyl and mycosamine groups (Fig. 1). Furthermore, one hydroxyl group is close to the conjugated double bond system and an alkyl chain is present which lies in one line with the other hydroxyl groups. These properties make the formation of a filipin–cholesterol complex with the configuration of a pore and an orientation perpendicular to the plane of the bilayer unlikely. Furthermore, the hydrophobic side chain of the filipin molecule would interrupt an aqueous channel and make the formation of a conducting pore impossible. It should be noted that the four carbon hydrophobic side chain of

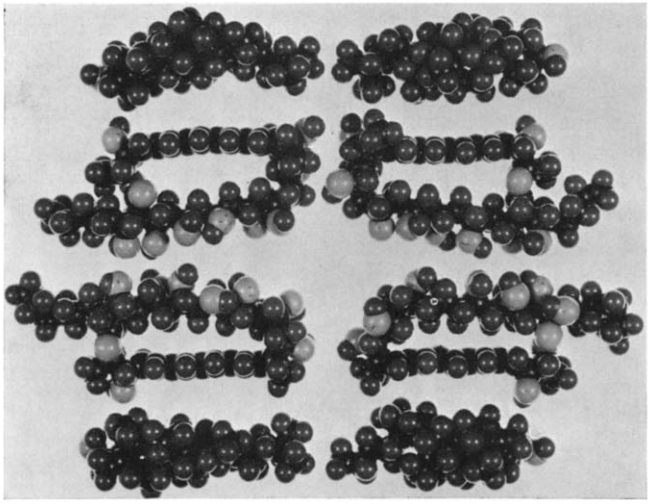


Fig. 6. Space-filling models of four filipin (F) and four cholesterol (C) molecules forming a $(F–C)_4$ complex which can be extended in two dimensions in a plane perpendicular to the complex and parallel to the long axes of the molecules as shown in Fig. 7.

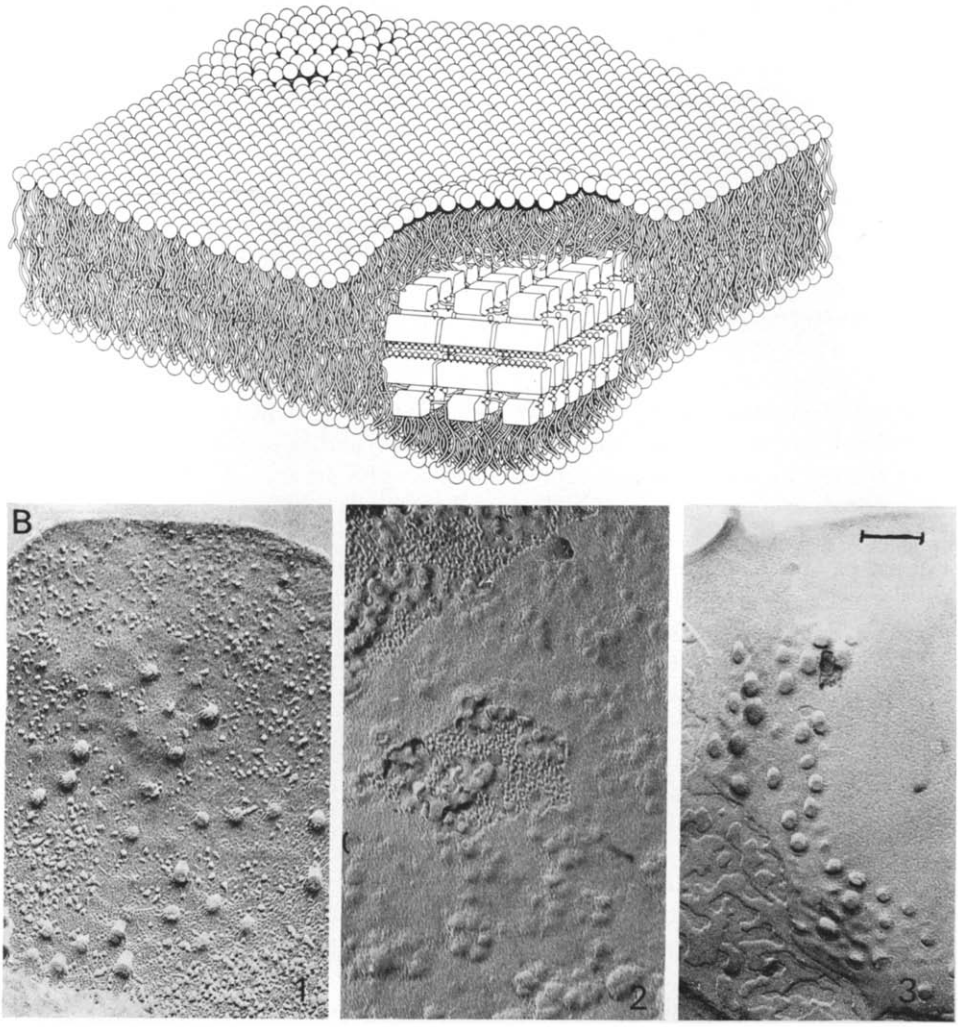


Fig. 7. A. Schematic representation of the $(F–C)_n$ complex in a lipid bilayer. The figure shows a cross section through a part of a $(F–C)_n$ complex lying in the bilayer and the projection on the lipid bilayer of an underlying $(F–C)_n$ complex. B. Freeze etch electron micrograph of the $(F–C)_n$ complex. (1) Complexes in the outer fracture face of the *A. laidlawii* cell membrane [14]. (2) Etch face of the *A. laidlawii* cell membrane showing the projections of underlying complexes [14]. (3) Complexes in fracture face of cholesterol–egg lecithin liposomes [14]. The electron micrographs were generously supplied by Drs A. J. Verkleij from our laboratory.

etruscomycin is aligned with the hydrophobic conjugated double bond system so that the formation of an aqueous channel is not prevented.

A unique property of filipin well illustrated with space-filling models, is the packing of molecules in a parallel array. This conformation is possible due to the absence of the bulky mycosamine residue. The plane of the filipin molecules is oriented perpendicular to the array and the arrays then can be combined in parallel to form an extensive aggregate (Figs 6 and 7A). One side of this planar aggregate is hydrophobic

by the presence of the double bond systems while the opposite side is hydrophilic by the presence of the hydroxyl groups. The hydrophilic side of one planar aggregate can associate with the hydrophilic side of a second planar aggregate to form a double layer aggregate. The exterior of this aggregate would then be composed of the conjugated double bond systems of all the filipin molecules. At this surface cholesterol molecules could interact in a hydrophobic and parallel manner (Figs 6 and 7A). The possibility also exists for hydrogen bonding between the hydroxyl group of cholesterol and the hydroxyl group of filipin which is located close to the double bond system. This filipin–cholesterol aggregate prefers not only a location in the hydrophobic core of the membrane but one that is oriented parallel to the plane of the membrane. In such a structure the stoichiometry is one molecule of cholesterol per molecule of filipin (cf. Table I).

Filipin–cholesterol aggregation leads not to pore formation but to disruption of the cell membrane. We suggest the following mechanism. One cholesterol molecule (C) interacts hydrophobically with the double bond system of one filipin molecule (F). There are, analogous to the A–C complex, two possibilities for the orientation of this F–C complex in the membrane. The hydroxyl groups of filipin can be oriented at the membrane/water interface, with the double bond systems of filipin and cholesterol oriented parallel to the plane of the bilayer. The other possibility is that the F–C complex is oriented perpendicularly to the membrane. The hydrophilic side of filipin is now in the hydrophobic region of the membrane and can interact with the hydrophilic side of a second F–C complex to form F–C–F–C in which the hydrophilic sides are now (partially) shielded from the hydrophobic interior of the membrane. As this aggregation proceeds the outside of the $(F–C)_n$ complex will become increasingly hydrophobic. This $(F–C)_n$ complex is not necessarily circular and indeed may form a regular array of parallel stacked F–C complexes (see above). The absence of charged groups would enable movement of $(F–C)_n$ within the plane of the bilayer. Several $(F–C)_n$ complexes could aggregate in the hydrophobic core of the membrane. The $(F–C)_n$ complexes can finally be visualised by electron microscopy as shown in Fig. 7. At the edges of these aggregates there is a strong curvature of the lipid layers in the membrane. We suggest that at these places the observed fragmentation of the membrane occurs.

There is considerable experimental support for the molecular structure and the mechanism of formation of the proposed filipin–cholesterol complex. (a) The filipin–cholesterol interaction is stronger than the (phospho)lipid–cholesterol interaction since cholesterol is withdrawn from its interaction with polar lipids as shown by differential scanning calorimetry [8, 10, 11]. (b) The filipin–cholesterol interaction is mainly hydrophobic as revealed by spectroscopic and permeability studies [8, 10]. (c) A possible hydrogen bonding between filipin and cholesterol was suggested from the monolayer studies of Demel et al. [6]. (d) Freeze-etch electron microscopy showed [14, 15] that the filipin–cholesterol complex was an aggregate of 150–250 Å in diameter lying in the hydrophobic core of the membrane (see also Fig. 7B). At the etched face of the membrane, projections of about 50 Å in height were visible (cf. [14] and Fig. 7B). (e) In the preceding paper we suggested from the temperature dependence of the interaction and binding of filipin with cholesterol in *A. laidlawii* membranes that initially primary filipin–cholesterol complexes were formed all over the membrane [10]. Aggregation of these complexes results in the formation of electron microscopi-

cally and spectroscopically detectable filipin-cholesterol complexes [9, 10]. (f) The effect of cholesterol upon the ultraviolet spectrum of the various polyene antibiotics is maximal for filipin although the length of the double bond system is intermediate between the length of the double bond systems of amphotericin B and pimaricin [8, 11]. In the models of the polyene antibiotic–cholesterol complexes we presented in Figs 5 and 7, the conjugated double bonds of adjacent filipin molecules are in close contact with each other whereas in the complexes of the other polyene antibiotics the cholesterol molecules are interdigitating the conjugated double bond systems. We suggest that the cholesterol induced change in the ultraviolet spectrum of the polyene antibiotic is a result of the orientation of the polyene antibiotic–cholesterol complex such that the chromophores of adjacent molecules are influencing each other. This effect would therefore be maximal for filipin because the chromophores are at the smallest distance from each other in the filipin–cholesterol complex. This suggestion is supported by the finding that the ultraviolet spectrum of a concentrated filipin ($1.0 \cdot 10^{-4}$ M) solution in water resembles the ultraviolet spectrum of a dilute (10^{-6} M) aqueous dispersion of the filipin–cholesterol complex [8]. It is likely that concentrated filipin solutions in water contain micelles or aggregates [8] of filipin, the interior of which will be hydrophobic by the presence of the conjugated double bonds. Apparently the chromophores of adjacent filipin molecules in these micelles or aggregates are influencing each other in an identical way as in the filipin–cholesterol complex. (g) Cholesterol containing *A. laidlawii* cells suspended in isotonic NaCl are completely fragmented by filipin; even after prolonged high speed centrifugation no pellet is recovered [9]. This fragmentation is much less pronounced in isotonic $CaCl_2$. There is a decrease in absorbance of the cell suspension but large membrane fragments can easily be recovered by centrifugation (see the electronmicrographs in the preceding publication [9]). A similar phenomenon was observed with rat erythrocytes (de Kruyff, B., unpublished observations). Under conditions identical to those used for the *A. laidlawii* cells no pellet could be recovered from filipin treated suspended in isotonic NaCl whereas in $CaCl_2$ large membrane fragments could be isolated. (i) From a comparison of the rate constants of the formation of the filipin–cholesterol aggregate and the induced K^+ leak it was suggested that the formation of the complexes precedes the membrane fragmentation [10].

NOTE ADDED IN PROOF (Received January 16th, 1974)

Recently, Finkelstein and Holz [23] proposed structeres for the amphotericin B and nystatin formed pores which are basically similar to the structures proposed in this paper.

ACKNOWLEDGEMENTS

We are indebted to Mr H. Harmsen of the U.N.F.I. for the photography of the molecular mcdels and to Mr N. Van Galen for the drawing of the figures. The authors wish to thank Professor Dr L. L. M. Van Deenen for his helpful discussions during the course of this investigation.

REFERENCES

1 Bergy, M. E. and Eble, T. E. (1968) Biochemistry 7, 653–659
2 Gandiano, G., Bravo, P., Quilico, A., Golding, B. T. and Rickards, R. W. (1966) Tetrahedron Lett. 30, 3567–3571
3 Golding, G. T., Rickards, R. W., Meyer, W. E., Patrick, J. B. and Barber, M. (1966) Tetrahedron Lett. 30, 3551–3557
4 Borowski, E., Zielinsky, J., Falkowski, L., Ziminsky, T., Golik, J., Kolodziejczyk, P., Jereczek, E. and Golulewicz, M. (1971) Tetrahedron Lett. 685–692
5 Borowski, E., Zielinsky, J., Ziminsky, T., Falkowski, L., Kolodziejczyk, P., Golik, J. and Jereczek, E. (1970) Tetrahedron Lett. 3909–3913
6 Demel, R. A., Crombag, F. J. L., Van Deenen, L. L. M. and Kinsky, S. C. (1968) Biochim. Biophys. Acta 150, 1–14
7 Cass, A., Finkelstein, A. and Krespi, V. (1970) J. Gen. Physiol. 55, 100–124
8 Norman, A. W., Demel, R. A., De Kruyff, B. and Van Deenen, L. L. M. (1972) J. Biol. Chem. 247, 1918–1929
9 De Kruyff, B., Gerritsen, W. J., Oerlemans, A., Demel, R. A. and Van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 339, 30–43
10 De Kruyff, B., Gerritsen, W. J., Oerlemans, A., Van Dijck, P. W. M., Demel, R. A. and Van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 339, 44–56
11 Norman, A. W., Demel, R. A., De Kruyff, B., Geurts Van Kessel, W. S. M. and Van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 290, 1–14
12 Schroeder, F., Holland, J. F. and Bieber, L. L. (1972) Biochemistry 11, 3105–3111
13 Bittman, R. and Fischhoff, S. A. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3795–3799
14 Verkleij, A. J., De Kruyff, B., Gerritsen, W. F., Demel, R. A., Van Deenen, L. L. M. and Ververgaert, P. H. J. (1973) Biochim. Biophys. Acta 291, 577–581
15 Tillack, T. W. and Kinsky, S. C. (1973) Biochim. Biophys. Acta 323, 43–54
16 Dennis, V. W., Stead, N. W. and Andreoli, T. E. (1970) J. Gen. Physiol. 55, 100–124
17 Van Zutphen, H., Demel, R. A., Norman, A. W. and Van Deenen, L. L. M. (1971) Biochim. Biophys. Acta 241, 310–330
18 Kinsky, S. C. (1970) Annu. Rev. Pharmacol. 10, 119–142
19 Kinsky, S. C. (1967) in Antibiotics (Gottlieb, D. and Shaw, P. D., eds.), Vol. I, pp. 122–141, Springer Verlag, Heidelberg
20 Kinsky, S. C., Haxby, J., Kinsky, C. B., Demel, R. A. and Van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 152, 174–185
21 Linden, L. D., Wright, K. L., McConnel, H. M., Fox, C. F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271–2275
22 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348
23 Finkelstein, A. and Holz, R. (1973) Membranes (Eiserman, ed.), Vol. 2, pp. 377–407, M. Dekker Inc., New York