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# Conformational Analysis of Amphotericin B — Cholesterol Channel Complex

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**Abstract.** A molecular model of ionic channel formed by flexible molecules of amphotericin B and cholesterol is proposed. Complexes with axial symmetry from 5 to 11 were simulated. In contrast to the model of the channel formed from rigid molecules, flexible molecules form a tightly packed structure consolidated by both dispersive forces and intermolecular hydrogen bonds. Contributions of a lactone ring, polar heads, cholesterol and lipid environments to the global energy of the complex formation are discussed. Among the complexes capable of ionic transport, that of axial symmetry eight is preferable. Two types of complexes, differing by the number of intramolecular hydrogen bonds, are shown to be possible.

**Key words:** Amphotericin B — Cholesterol — Ionic channel structure — Conformational analysis

## Introduction

The polyene antibiotic amphotericin B is known to induce ionic transport in biological membranes [3, 20]. It was also shown to form ionic channels in black lipid membranes [7, 8]. The presence of sterol molecules in the membrane is necessary for channel formation. The channels are permeable to univalent ions and small organic compounds [2]. Qualitative models of the channel, based on its transport properties and structural peculiarities of the amphotericin B molecule, were proposed by Andreoli [1], De Kruijff and Demel [6] and Finkelstein and Holz [9]. However, these models are too simplistic to allow simulation of the channel transport properties at the molecular level.

The chemical structure of the amphotericin B molecule was determined by Borowski et al. [5]. The spatial structure of its iodinated derivative was solved by Ganis et al. [10]. Conformational analysis of a free amphotericin molecule or its complexes is hampered due to the presence of a macrolide lactone ring in the molecule. This problems was resolved by Rinnert and Maigret [23]. The stable structure found in a vacuum approximation was shown to be similar to that in the crystalline state. NMR stereochemical data obtained by Sowinski et al. [26] have shown the identity of the antibiotic conformation in solution and in the crystal. Based on these findings, some models of the ionic channel made of rigid molecules of the antibiotic and sterol were proposed by Khutorsky et al. [16, 18, 19]. Attempts to conduct a simulation of electric properties of the channel based on the models made from the rigid molecules have been published [4, 17].

There is no reason to expect large energetic hindrances in a conformational mobility of the amphotericin B lactone ring. The rigid frame of the ring does not allow tight packing of the channel structure; while one may suppose that only a tightly packed structure of the ionic channel ensures stability of its basic properties such as ionic selectivity and permeability.

The problem of amphotericin B molecule flexibility was resolved by Mazerski and Borowski [21] using a molecular dynamics method. In contrast to Rinnert and Maigret [23], they demonstrated that a variety of conformational states of the amphotericin B molecule with significantly different values of dihedral angles are possible.

A molecular conformation is largely determined by its environment. This general statement is supported by the results of amphotericin B molecule simulations by Mazerski and Borowski [21]. The significant differences in the conformations of the molecule in a vacuum and in an aqueous environment were shown.

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The amphiphilic molecule of the antibiotic, being in a channel structure, lies on the border of an aqueous solution and a membrane. The polar part of the molecule, apparently, faces the water, while the lactone ring should pierce the lipid membrane, i.e., is in a hydrophobic environment. To simulate the amphotericin B molecule in this position, its two moieties were studied separately.

Meddeb et al. [22] conducted a comprehensive conformational analysis of polar head conformations in a vacuum or in the presence of 'hydration water' molecules. Possible stable conformations of the hydrated polar parts of the amphotericin B molecule and its derivative amphotericin methyl ester were compared.

We confirmed the conclusion of Maserski and Borowski [21] on the lactone ring flexibility by the method of conformational analysis for the amphotericin B molecule with the truncated polar head [25]. Seven types of stable conformations, differing by their patterns of intramolecular hydrogen bonds and the lactone ring twisting, were obtained by the global energy minimization. The intrinsic energies of the molecule in these conformations were shown to be similar.

The importance of the channel environment for the amphotericin channel was demonstrated by Golovanov and Tsigankova [11]. It was shown, that the complex made from the rigid molecules taken in the conformation corresponding to the crystal structure should be unstable in the lipid bilayer. This conclusion is based on a comparative analysis of the interaction of basic components of the antibiotic lactone ring with either a lipid molecule or a neighboring antibiotic molecule in the channel structure. The former is energetically preferable.

Thus the problem of amphotericin channel simulation is still open to debate. The approach developed by us in [25] was used in this study to simulate the structure of channel complexes made of flexible molecules of amphotericin B and cholesterol.

## The Problem Setup

An ionic channel is supposed to be formed by an oligomeric equimolar complex of the amphotericin B and cholesterol molecules. Complexes with the axial symmetry from 5 to 11 were simulated.

Two variants of the antibiotic structures were used: the intramembrane fragment and the complete molecule. We assume that large fragments of molecules make additive contributions to the global energy of a molecular complex. In the vacuum approximation used, the energy increment of the intramembrane fragments is evaluated more correctly than interactions of the whole molecules in the interface. Interactions of polar and especially charged groups of molecules facing aqueous solution seem to be overestimated in the vacuum approximation.

An attempt at a quantitative evaluation of the global energy of the channel structure made from the intramembrane fragments is presented in this paper, while only qualitative conclusions for the channels from intact amphotericin B molecules may be obtained.

In computer experiments with intramembrane fragments of the channel, a curtailed amphotericin B molecule (cAMB, truncation is shown in Fig. 1) with removed amino sugar, carboxyl group and adjacent hydroxyl radical was used.

The curtailed molecule of amphotericin B was also used to estimate the possibility of channel formation in the lipid environment. To simulate the hydrophobic moiety of the lipid molecule, we used two saturated aliphatic chains of $C_{17}H_{36}$ in a tight contact, optimized in a preliminary simulation of its flexible chains.

To demonstrate the possible role of the polar groups in the complex structure, we simulated the channel complex made of total amphotericin molecules. The carboxyl and amino groups of the molecule were assumed to be charged. The simulation was performed in the vacuum approximation, assuming 0.5 elementary charge for the amino and carboxyl groups to simulate partially the screening effect of a water solution.

## Materials and Methods

The original software 'Diana2' and a detailed protocol of the computer experiment are described in our previous paper [25]. This interactive program can simulate molecular interactions for multiple applications including conformational analysis of flexible cyclic molecules. It also can be used for solution of axially symmetrical problems. The program includes a description of molecular subjects, molecular editor, force field for energy evaluations, programs for conformational analysis and service programs. The basic features of the computational method are presented below.

### Molecules

The initial coordinates of the heavy atoms in the amphotericin B and cholesterol molecules were taken from X-ray data of Ganis et al. [10] and Shich et al. [24]. The bond distances and the values of valence angles were regarded as invariable. In the curtailed molecule, the truncated fragments were replaced by hydrogen atoms.

The dihedral angles of the amphotericin B molecule marked in Fig. 1 were taken to be the intrinsic degrees of freedom (33 variables for the curtailed amphotericin B and 40 for the whole molecule). Flexible molecules were regarded as a sum of rigid fragments connected by torsion bonds. Taking into account the data from the molecular dynamics study [21], we assumed the heterocyclic mycosamin and hemiketal rings to be rigid.

In a cholesterol molecule, the dihedral angle of the hydroxyl group and five of those in its aliphatic tail chain were taken as six more variables.

### Energy Calculations

The global energy of a complex is the sum of intramolecular and intermolecular energies for all molecules in the complex. The global

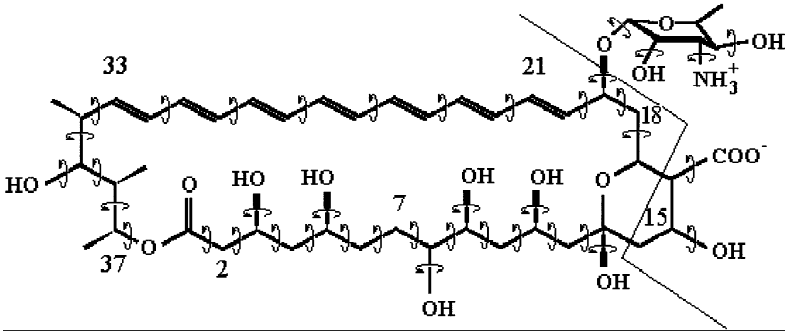


**Fig. 1.** Structural formula of amphotericin B. Dihedral angles rotated in conformational analysis are shown. Polar fragments are truncated to provide a curtailed amphotericin B molecule. Removed atoms are separated from right. Carbon atoms of the lactone ring are numbered.

energy of a large molecular complex is evaluated as the sum of interaction energies $E_{ij}$ for pairs of adjacent molecules making the complex. Stable conformations of molecular complexes were found by the global energy minimization.

The global energy was estimated using an empirical force field proposed by Golovanov et al. [12]. This force field has been given preference as it includes explicitly the interaction energy for double bonds, characteristic of both the amphotericin B and sterol molecules. This simple method was shown to reproduce well a wide range of experimental data and *ab initio* calculations on molecular interactions of a large set of small molecules [12]. Peculiar features of this approach are as follows:

(i) The hydrocarbon fragments $CH_3$, $CH_2$ and CH are considered as unit pseudoatoms;

(ii) to describe the effects of lone pairs and π-electrons, a molecule structure is supplemented with negatively charged pseudoatoms *Q;*

(iii) the energy of atom-atom interactions is described by the sum of electrostatic energy and Lennard-Jones potentials;

(iv) the energy of hydrogen bond formation is taken into consideration;

(v) the intrinsic energy of a molecule also includes the torsion potentials, describing the barriers of the inner rotation.

Due to axial symmetry of the channel structure, the interaction of only two neighboring monomers (each monomer consisting of one amphotericin B molecule or a pair of amphotericin B and cholesterol molecules) was taken into account in computations of the global energy for channel complexes.

The global energy per monomer was calculated as

$$E_{ch} = E_{i,i+1} + E_{int} - E_{int0} \quad (1)$$

where $E_{i,i+1}$, $E_{int}$ and $E_{int0}$ are the energy of interaction of $A_i$ *and* $A_{i+1}$ monomers, the intrinsic energies of the monomer in the channel complex and in the free state respectively.

For a bimolecular complex, changes in the intramolecular energy during the bimolecular complex formation were also taken into account.

## Selection of Initial Conformations

Preliminary simulation of possible molecule conformations and arrangement of the molecules in a channel structure were done by hand using space-filling molecular models of CPK type, manufactured by Tartu University (Estonia). General physical principles were used for a preliminary selection of possible conformations. Then the conformations obtained were transferred to computer files using molecular editor of 'Diana2' software.

The initial conformations 1 and 6 of single curtailed amphotericin B molecule (cAMB) optimized in the computer experiment [25] were chosen to simulate the ionic channel structure. The lactone ring in conformation 1 is very close to that in the crystal [10]. In conformation 6, characterized by presence of hydrogen bond between hydroxyl groups at C5 and C8 atoms (possibility of this conformation was shown first by Maserski and Borowski [21]), the lactone ring conformation differs strongly from that in the crystal.

In conformation 6 of the curtailed molecule all the intramolecular hydrogen bonds were preserved and corresponding conformation of the molecule from [25] were taken as a start point without modification.

The conformation 1 obtained in [25] was modified to realize formation of the net intermolecular hydrogen bonds in the channel structure. In the crystal, the hydroxyl groups of the lactone ring face the polyene chain of the antibiotic, while to form the net of the hydrogen bonds, the OH groups ought to have access from two sides. To provide such access, the chain of OH groups was shifted out of the polyene chain. The conformation obtained was taken as an initial one for this computer experiment.

In experiments with whole amphotericin B molecules, the initial conformation of the intramembrane fragment and its position in the channel complex were taken from the data obtained by optimization of the channel structure made from cAMB1. Then an initial conformation of the polar head was chosen to achieve maximal proximity of oppositely charged groups in two neighboring amphotericin B molecules.

## Protocol of Computer Experiment

The computer experiment consisted of several successive optimization procedures. In contrast to Rinnert and Maigret [23], the value of the lactone ring closing accuracy was taken large enough at first (0.5 – 1 Å) to provide more possibilities for conformational changes of the complex. For the next optimizations, the closing accuracy was successively diminished.

The optimization procedure also has several steps. A final conformation obtained in a preliminary step is taken as an initial one for the next step. In the first step, the molecules were considered rigid. Ten variables were allowed at this step: the distance to the channel axis, three Euler angles for amphotericin B molecule as well as Cartesian coordinates and Euler angles for cholesterol molecule (as it was taken by Khutorsky [16]).

A procedure for the minimization of the complex global energy was conducted by the method of varying polyhedron (method of Nelder and Mead in [14]). In the beginning of the first minimization, the initial polyhedron was created by the method of successive coordinate descent. For the next minimizations, it was created by random deviation of the variable set from optimal position ('shaking' of the complex under study).

After the adjustment of the rigid molecules, the intramolecular

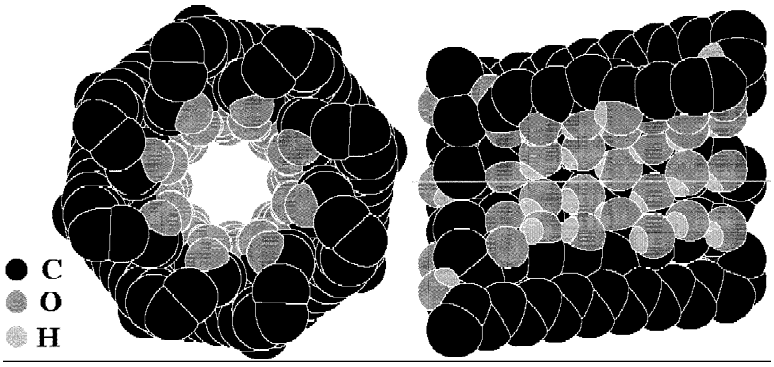


**Fig. 2.** Spatial structure of the channel complex, formed by eight curtailed amphotericin B molecules in conformation 6 (cAMB6), corresponding to the minimum of the global energy of the complex. Left, view along the channel. Right, half of the channel complex is shown parallel to its axis to present the channel lining with hydroxyl groups.

degrees of freedom were added. For the closing of the lactone ring in the flexible amphotericin B molecule, the approach proposed by Rinnert and Maigret [23] was used. Conformations with the closed lactone ring only were considered during the minimization procedure. If a conformation with the open ring was proposed during minimization, an additional procedure was used to replace this conformation by the nearest closed conformation of the molecule.

The energy minimization of the complex from the flexible molecules was repeated until, in spite of significant 'shaking' of the complex after each minimization, the minimal values of the global energy retained constant level.

The final conformation obtained during this optimization procedure was used for the next optimization with a lower value of the ring closure accuracy. The optimization procedures were repeated until the optimized conformation with the ring closing accuracy of 0.1 – 0.2 Å was obtained.

After the computer experiment completion, the resulting conformation was modified to search for other stable structures differing qualitatively by the pattern of hydrogen bonds, relative position of molecules, and complex symmetry etc. The results of the computer experiments with these modified initial conformations were compared with the preliminary one. The conformations with the lowest minimum of global energy are presented below.

### Simulation of Lipid Environment

To evaluate the probability of the channel formation in the lipid environment, the interaction energy of two neighboring channel-forming molecules was compared with that of one channel-forming molecule and a lipid molecule.

The channel complex $A_n$ is formed by oligomerization of $n$ amphotericin B molecules $A$ released from their lipid environments with concomitant formation of lipid dimers $L_2$:

$$nLAL \Rightarrow A_n + nL_2 \quad \text{(R1)}$$

A similar reaction is proposed for the channel complex formed from amphotericin B and cholesterol C molecules:

$$nLACL \Rightarrow (AC)_n + nL_2 \quad \text{(R2)}$$

The channel complex formation is energetically advantageous if these reactions are exothermic. The global energy differences $\Delta E$ are equal respectively:

$$\Delta E = n^*(E_{AA} + E_{LL} - 2\,E_{AL}) \quad (2),$$
$$\Delta E = n^*(E_{ACAC} + E_{LL} - E_{AL} - E_{CL}) \quad (3),$$

where subscripts $A$, $L$ and $C$ refer to amphotericin B, lipid and cholesterol respectively, $E_{AA}$ and $E_{ACAC}$ are energies of monomer interactions in a channel calculated by Eq. (1); other values are energies of bimolecular interactions (Table 2).

### Size of The Inner Pore

To evaluate the position of an atom in the channel structure, we compared the distance from this atom to the channel axis $R_i$ with the minimal value equal to the radius of a ring formed by $n$ such atoms in tight contact ($R_{min}$, minimal ring radius for symmetry $n$). In a symmetrical problem a distance $d_{ii}$ between equivalent atoms is proportional to $R_i$:

$$d_{ii} = 2\,R_i \sin(\alpha/2), \quad (4)$$

where

$$\alpha = 2\,\pi/n. \quad (4a)$$

Thus,

$$R_{min} = r_{vdw}/\sin(\pi/n) \quad (5)$$

where $r_{vdw}$ is the van der Waals radius of the atom.

A channel is supposed to be permeable to an ion if the clearance between the channel axis and atoms lining the inner pore is larger than the van der Waals radius of this ion. Amphotericin B channels were experimentally shown [8] to be permeable to halide ions including iodide (2.3 Å radius). Taking into account van der Waals radius of an oxygen atom (1.5 Å) and methyl group (2.0 Å) accepted in our calculations, the distances of corresponding heavy atoms to the channel axis should be more than 3.8 and 4.3 Å respectively. From relation (5) it follows that the minimal number of monomers in an axial symmetric channel lined with hydroxyl and methyl groups and permeable for halide ions should be equal to eight.

## Results and Discussion

### Channels Containing Eight cAMB

Computer optimization of the channel structure made of eight clipped amphotericin molecules starting from configuration 6 (cAMB6) resulted in the structure shown in Fig. 2. The antibiotic molecules are tightly packed. All

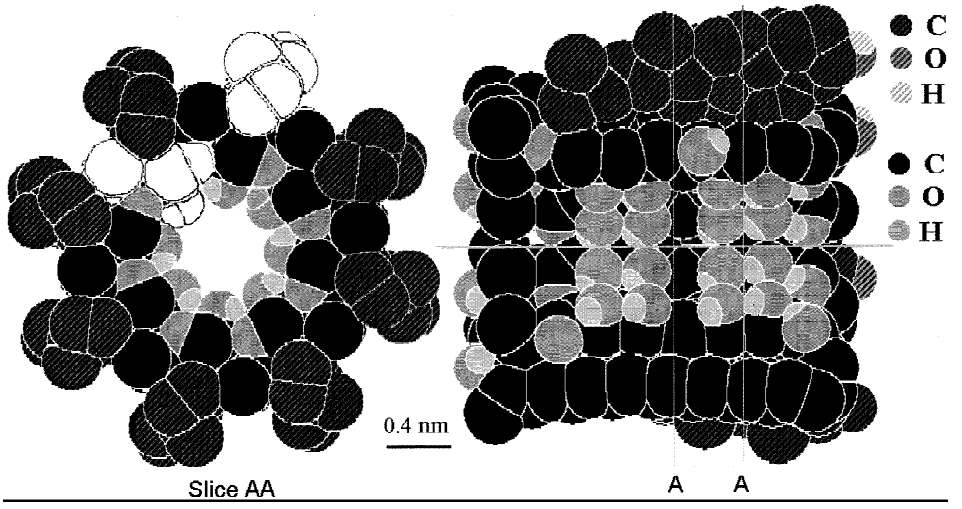


**Fig. 3.** Computer-optimized structure of the channel complex from eight curtailed amphotericin B molecules in conformation 1 (cAMB1) and eight cholesterol molecules. The atoms belonging to cholesterol molecules are hatched. Left, cross-section of the channel; atoms of one cAMB1 and one cholesterol molecules are bleached to show contributions of molecules to the channel structure. Right, half of the channel is shown parallel to its axis to present the channel lining with hydroxyl groups.

intramolecular hydrogen bonds of the amphotericin molecule in the channel structure are preserved. The lactone ring is twisted and tilted to the channel axis: the angle between the polyene chain and the channel axis is 24° and the angle between the polyol chain and the axis is 18°.

For the channel made of curtailed molecules in configuration 1 (cAMB1) the structure obtained is more ordered (Fig. 3). The tilts of the two chains are 5° and 2° respectively. Four hydroxyl groups of the antibiotic polyol chain HO-3, HO-5, HO-9 and HO-11 (numbers refer to the corresponding carbon atoms of the lactone ring in Fig. 1) take part in the formation of four rings lining the channel walls. The formation of both intra- and intermolecular hydrogen bonds occurred. The C-O bonds of these hydroxyl groups deviate from the channel radius-vector direction by not more than 7°.

The principal difference between the two structures is the distance from oxygen atoms O-8 to the channel axis in the middle part of the channel complex (Fig. 4). In cAMB6, these atoms are included in the lining net of hydrogen bonds while, in the case of cAMB1, there is an annular groove in this place (compare the channel linings in the right parts of Figs. 2 and 3). The former type of structure is energetically preferable. The latter structure with higher global energy is nonetheless presented here because we suppose that, in the presence of water molecules inside the channel cavity, those two types of complexes will have values comparable to their global energies. Indeed, in contrast to the first type of structure, the groove in the middle of the second type structure may bind at least four water molecules.

Addition of cholesterol molecules to both cAMB1 and cAMB6 channel complexes does not change the channel configuration significantly. Figure 3 demonstrates how a sterol molecule fills the space between two neighboring molecules of the antibiotic. Corresponding changes of the global energy are presented in Table 1.

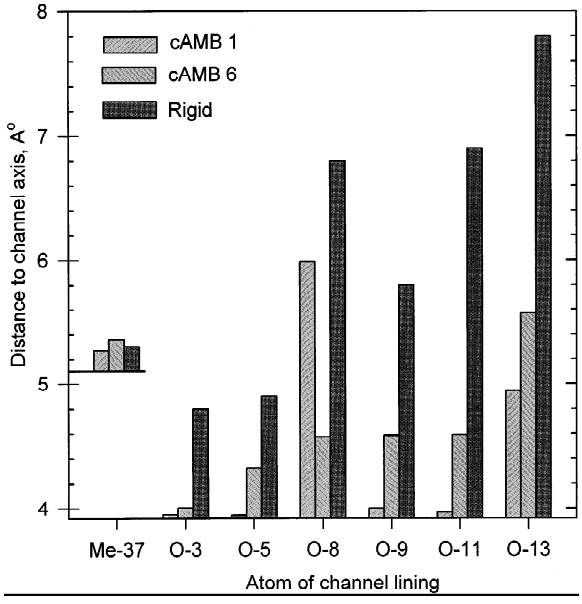


**Fig. 4.** Distances from the atoms lining the channel cavity to the channel axis. Numerals at the atom symbols correspond to those of bound carbon atoms in the amphotericin B lactone ring shown in Fig. 1. *cAMB1*, channel optimized from eight curtailed amphotericin molecules taken in conformation 1 and eight cholesterol molecules; *cAMB6*, the same, but the initial conformation was 6; *Rigid*, data of Khutorsky from [16]. Background values (3.92 Å for oxygen and 5.10 Å for methyl groups) are equal to the minimal ring radii for symmetry eight (*see* Materials and Methods).

**Table 1.** Global energies (in kcal/mol) per monomer in simulated channel complexes of axial symmetry *n* from curtailed (cAMB1, cAMB6) or total amphotericin B molecules with (1:1) or without cholesterol

| *n* | cAMB1 | cAMB1 + chol | cAMB6 | cAMB6 + chol | AMB | AMB + chol |
|---|---|---|---|---|---|---|
| 5 | −17.9 | −19.4 | −32.5 | −39.0 | −39.8 | −49.9 |
| 6 | −20.6 | −22.3 | −31.4 | −37.6 | −44.4 | −49.1 |
| 7 | −22.6 | −25.3 | −29.7 | −33.6 | −45.1 | −55.2 |
| 8 | −22.3 | −25.9 | −28.1 | −31.0 | −42.3 | −53.2 |
| 9 | −22.1 | −24.9 | −27.1 | −29.5 | −38.2 | −47.6 |
| 10 | −21.1 | −21.7 | −25.1 | −25.0 | −37.9 | −43.7 |
| 11 | −19.1 | −19.8 | −24.3 | −24.3 | −36.7 | −39.4 |

Note: Differences between total energy per monomer and energies of free monomer are presented.

Replacement of the truncated fragments of the amphotericin B molecule by methyl groups instead of hydrogen atoms was not followed by remarkable changes in the complex conformation, i.e., truncated fragments do not induce steric hindrances in the complex formation.

To compare the structures cAMB1 and cAMB6 channels with that of a channel made of rigid molecules obtained in [16], we evaluated the distances from the polyol chain oxygen atoms to the channel axis. Figure 4 shows that the methyl groups Me37 are positioned at about minimal radius $R_{\min}$ from the channel axis for the three structures compared. The positions of oxygen atoms lining the channel cavity are different. Four oxygen atoms in cAMB1 are in tight contact with similar atoms of the neighbor molecules ($R_i \cong R_{\min}$), while two others are more distant from the channel axis: O8 is inside the groove in the middle part of the complex and O13 is at the channel entrance. In cAMB6, only O3 atoms are in tight contact, while the other four are posed ~0.5 Å farther.

Thus, intramembrane fragments of the antibiotic molecules may form channel complexes stabilized by both dispersive forces and hydrogen bonds.

## Different Axial Symmetry

Figure 5 and Table 1 show the results of computer experiments on optimization of the channel complexes with different axial symmetry. The structures obtained have conformations similar to those shown above for the octamer complexes.

The distances from the cavity lining atoms to the channel axis in two types of complexes (cAMB1 and cAMB6) are shown in Fig. 5. The background level 3.8 Å corresponds to the assumed minimal distance of the oxygen atoms to the axis of the permeable channel (*see* Materials and Methods). The inclined lines in Fig. 5 correspond to minimal ring radii of methyl groups and oxygen atoms for different symmetries.

Table 1 shows the values of the channel global energy per monomer for the cAMB1 and cAMB6 complexes with and without cholesterol. The energy dependence on the channel symmetry for the two types of complexes have different patterns. The cAMB1 complexes reveal an energy minimum that is better pronounced in the presence of cholesterol molecules, while the energy of cAMB6 complexes rises with the number of monomers in the channel.

In the cAMB1 type of channel, four hydroxyl groups lining the inner pore are in tight contact and form intermolecular hydrogen bonds for the complexes with symmetry from 5 to 9 (as shown in Fig. 3). The structures with larger symmetry numbers may not form similar hydrogen bonds because of steric hindrances induced by lactone ring interactions. Indeed, distances of the lining OH groups to the channel axis become larger than for the model of contacting atoms (straight line in Fig. 5). As a consequence of nonoptimal distance between hydroxyl groups involved in an intermolecular hydrogen bond, the global energy increases (Fig. 6).

Among the complexes of cAMB6 type, pentamere may form five optimal hydrogen bonds between neighbor molecules only. As steric hindrances induced by lactone ring interactions are more pronounced for this type of complex, the global energy per monomer increases with symmetry number (Table 1).

The cholesterol molecules fill the space between two polyene chains of the neighbor amphotericin B molecules. The optimal size of the space fitting to cholesterol molecule corresponds to the symmetry number of eight (Fig. 3).

Possible deviations of the radius from its equilibrium value were assessed taking into account that real molecular complexes are dynamic systems. The computer assay shows that changes in the channel radii by 0.1 – 0.15 Å resulted in the increase of the global energy per monomer to about 1 kT (~0.6 kcal/mol).

## Simulation of Lipid Environment

The values of interaction energies for pairs of flexible molecules taking part in the channel formation in the lipid environment are shown in Table 2. Changes in intramolecular energy during formation of the pair complex were negligible for the 'lipid' molecule; they did not exceed 1 – 2 kcal/mol for amphotericin B: 'lipid' pair and cholesterol: 'lipid' pair. At the same time, the molecule interaction energy was much higher due to the flexibility of the amphotericin B and cholesterol molecules.

Taking the energy values for monomer interactions in the channel complex with different symmetry $E_{AA}$ and

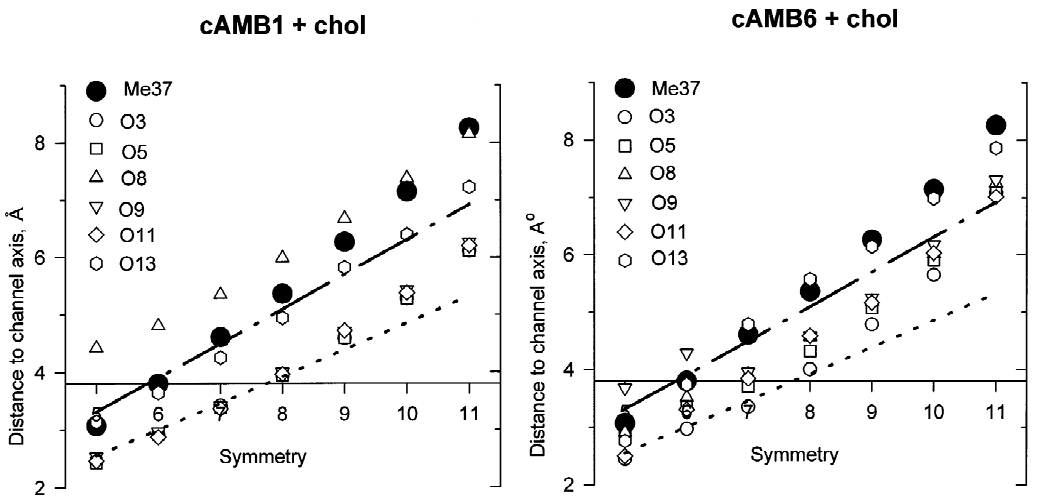


**Fig. 5.** Distances from the lining atoms to the channel axis at different symmetries of the channel complexes. Atom numbering as in Fig. 4. Background value of 3.8 Å is assumed to be the low limit of the cavity radius. Straight inclined lines correspond to minimal ring radii for oxygen and methyl groups (*see* Materials and Methods). Left, channel structures, optimized from cAMB1 and cholesterol; right, from cAMB6 and cholesterol.

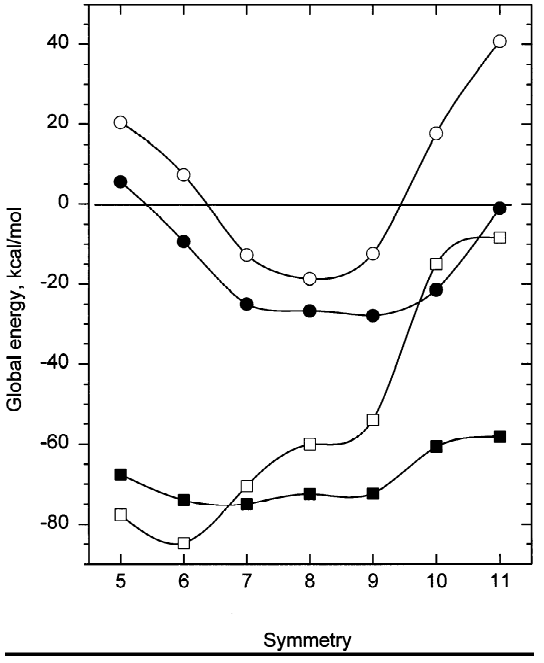


**Fig. 6.** Global energies of the channel from cAMB1 (circles) or cAMB6 (squares) with (empty symbols) or without (filled symbols) cholesterol molecules obtained in a computer experiment for different axial symmetry numbers.

$E_{ACAC}$ from Table 1, relationships (2) and (3) were used to evaluate global energy differences of channel formations in the lipid environment. The values obtained are presented in Fig. 6. The structures with the positive energy difference are unfavorable.

The complexes of composition $n \times n$ made of cAMB1 and cholesterol molecules are stable in a lipid environment for $n$ from 7 to 9. The same complexes made of cAMB6 and cholesterol are stable in the lipid environment for all $n$ studied and the hexamer is energetically preferable.

Figure 6 shows that among the permeable channels ($n \geqslant 8$) octamer complexes are preferable for both structure types. According to experimental data reported by Ermishkin et al. [8], only one conducting state of the amphotericin B channel was registered. The data presented above show that the octamer complex best fits the experimental data. Formation of smaller complexes in configuration cAMB6 is preferable, but their presence is not detectable by electrical measurements.

**Table 2.** Total formation energies of pair molecular complexes

| Interacting molecules | Abbreviation | Total energy[a], kcal/mol |
|---|---|---|
| cAMB[b] − cholesterol | $E_{AC}$ | −29 |
| cAMB − 'lipid'[c] | $E_{AL}$ | −27.5 |
| 'Lipid' − 'lipid' | $E_{LL}$ | −36 |
| 'Lipid' − cholesterol | $E_{CL}$ | −32 |

[a] Differences between total complex energy and energies of free molecules are presented; [b]curtailed amphotericin B; [c]hydrophobic moiety of the lipid molecule, simulated by two tightly packed $C_{17}H_{36}$ molecules.

## Channels Made of Complete Amphotericin Molecules

Conformational analysis of intact amphotericin B molecule shows that its stable conformation in the vacuum

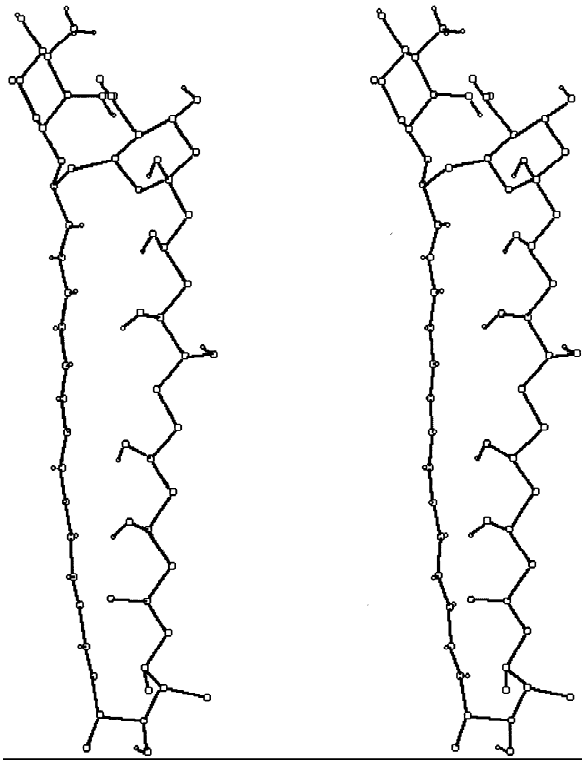

**Fig. 7.** Stereo view of free amphotericin B molecule in the optimized conformation.

(Fig. 7) is far from that in the crystal (*cf.* Fig. 4 in [23]). In the conformation obtained, the charged groups of the molecule are brought to 2.8 Å distance from each other. Simultaneously, the lactone ring of the amphotericin B molecule is twisted significantly.

As a result of strong interaction between charged amino and carboxyl groups of the molecule, optimization of the intact amphotericin B molecule structure led to a maximal contact between these groups. Due to the lactone ring flexibility and changes in torsion angles C16-C17-C18-C19 and C17-C18-C19-C20, the distance between these groups is smaller than that obtained by Meddeb et al. (conformation C1 in [22]) by at least 0.3 Å.

The values of global energy per monomer for the resulting channel complexes made of whole antibiotic molecules with and without cholesterol for different symmetry numbers are included in Table 1. The complex structure with axial symmetry equal to seven has the minimal value of global energy per monomer.

The structure resulting from the computer experiment for the channel with the symmetry of eight is shown in Fig. 8. At the channel entrance, $NH_3^+$ and $COO^-$ groups are seen to form an alternating ring of charged groups reinforcing the structure (Fig. 8, left). The van der Waals profile of the channel is featured by two opposite antibiotic molecules in the right part of Fig. 8. The profile is heterogeneous. It has a wider part (vestibule) of about 5 Å long at the channel entrance and a narrow part corresponding to the intramembrane fragment of the channel.

Finally, we checked the suggestion of Finkelstein and Holtz [9] and De Kruijff and Demel [6] on a possible role of hydroxyl groups HO-35, posed in the tail part of amphotericin B molecule, in the assembly of a double-length channel. Our simulations show that these hydroxyl groups can form a ring of hydrogen bonds between the two halves of the channel. The double-length channel from the side of the lipid environment is shown in Fig. 9. The hydrogen bond ring in the complex center is seen to be partially masked by the tail methyl groups.

Figure 9 also shows the pecularities of the cholesterol molecule packing in the channel structure. These molecules are hidden in the right half of the double-length complex and hatched in the left one. The position of the cholesterol molecule is determined by the groove between the two polyene chains of the adjacent amphotericin B molecules on one side (cross-section of the channel formed from the whole amphotericin B molecules is the same as in Fig. 3) and by the formation of the hydrogen bond with a hydroxyl group of amino sugar on the other. One may find this hydroxyl group in Fig. 1 between the amino group and oxygen O-19 binding the amino sugar and the lactone ring.

The experimental data of Herve et al. [13] have shown the involvement of $NH_3^+$ group of the amino sugar to interaction with sterol molecule. A hypothetical chain of hydrogen bonds between the 3β-OH group of the sterol and the $NH_3^+$ group of amino sugar through an intermediary water molecule was suggested (Fig. 4 in [13]). Our data agree with this hypothesis if it is assumed that the above-mentioned OH group of the amino sugar plays the role of the intermediary water molecule.

Interactions of the adjacent charged groups ($NH_3^+$ and $COO^-$) make the largest contributions to the global energy of the simulated complex. Undoubtedly, this contribution is overestimated in the vacuum approximation. Indeed, both water molecules and electrolyte ions of the channel complex environment strongly decrease this electrostatic interaction. As an ultimate approximation of the environment effect, the complex formation was simulated when these charges were decreased from $|e|/2$ to zero value. The resulting conformation obtained after optimization is the same as for the charged molecules, in spite of significant perturbations induced by 'shaking' of the molecular complex during the computer experiment. Specifically, the distance between these charged groups increased only from 2.8 to 3.0 Å. At the same time, the value of the global energy was strongly reduced.

Thus, results of computer experiments show the possibility of tight interaction between the alternating charged groups of two amphotericin B molecules. This

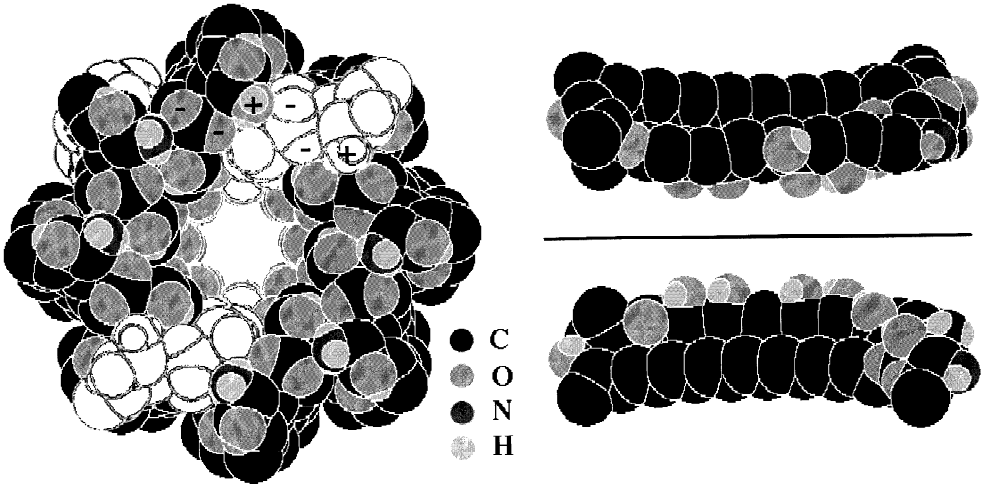


**Fig. 8.** Computer-optimized spatial structure of the channel complex made from eight total amphotericin B and cholesterol molecules. Left: the view along the channel axis on the side of polar groups; two of amphotericin B and one cholesterol molecules are bleached to show contributions of molecules to the channel structure; charged atoms (carboxyl and amino groups) of two neighboring amphotericin B molecules are marked. Right, side view of the amphotericin B molecules bleached in the left part of the figure.

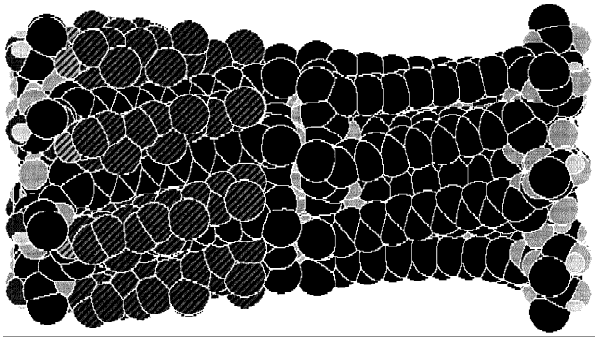

**Fig. 9.** Hydrophobic side of a double-length channel complex from total amphotericin B and cholesterol molecules (8 × 8) optimized in a computer experiment. Cholesterol molecules are hatched in the left and hidden in the right parts of the channel.

interaction decreases global energy of the complex and provides complex stabilization. This result corresponds to the experimental data of Kasumov et al. [15] and Herve et al. [13] which showed that modification of the charged groups results in the decrease of channel stability.

Interactions of the hydrophobic moieties of both lipid and sterol molecules are similar. Table 2 shows that energies of amphotericin B molecule interaction with both cholesterol and 'lipid' molecules are similar. A hydrophobic groove between amphotericin B molecules (Fig. 3) may be filled with either 'lipid' or cholesterol molecules. The principal difference in interaction of amphotericin B molecule with these two molecules may be found in the region of polar heads. As shown in Fig. 9, the amino sugar groups form a ring at the channel entrance with an outer radius larger than the intramembrane fragment of the complex (right part of the complex in Fig. 9 where cholesterol molecules are hidden). Thus, a sterol molecule may fill the hydrophobic pocket between the two polyene chains of the neighboring amphotericin B molecules and the amino sugar fragment of the amphotericin molecule (as it is shown in left part of Fig. 9). The hydrogen bond formation between the sterol hydroxyl group and the OH group of the amino sugar in the hydrophobic surroundings fixes the sterol position.

Although computer simulation of the channel complex of amphotericin B and intact lipid molecules was not performed, it can be expected that a lipid molecule incorporated into the channel complex instead of cholesterol would force apart the charged groups of the neighboring amphotericin B molecules with its polar head. Thus, by disturbing the interaction of the polar heads of the neighboring molecules, the intact lipid molecules would weaken the complex.

## Conclusion

The symmetric complex of flexible molecules of amphotericin B and cholesterol forms a tightly packed structure that is fastened by the set of hydrogen bonds between the neighboring amphotericin B molecules.

Opposite charged groups of the neighboring amphotericin B molecules may contact each other and form a ring of alternating charges that strengthen the channel complex.

A cholesterol molecule may fit tightly into the hydrophobic pocket between the polyene chains of the two neighboring amphotericin B molecules and the amino

sugar group of the antibiotic. The 3β-OH group of cholesterol, the hydroxyl group of the amino sugar and its amino group may form a chain of hydrogen bonds fixing the position of cholesterol molecule.

The preferable number of molecules in the channel structure is eight.

A computer simulation of the channels formed from different sterols and analogues of amphotericin B molecules is proposed. The structures obtained in this study will be used in a new round of simulations of the amphotericin B channels filled with water molecules and permeable inorganic ions in order to explain the electrical properties of these channels at the molecular level.

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