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# Molecular modeling of amphotericin B–ergosterol primary complex in water II

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ABSTRACT

The work presented is a part of our continual study on the behavior of the polyene macrolide antibiotic amphotericin B (AmB) complexes with sterols on the molecular level. In contrast to the previously researched AmB–ergosterol binary complex, the AmB–ergosterol–AmB aggregates simulated of 2:1 stoichiometry retain significantly higher stability and relatively rigid, "sandwich" geometry. Van der Waals forces with a considerable share of the electrostatic interactions are responsible for such behavior. System of the intermolecular hydrogen bonds also seems to be of notable importance for the complex's structure preservation. The most energetically favored geometries match fairly close the geometric criteria and the network of interactions postulated in the contemporary hypothetical and computational models of antibiotic–sterol complexes. On the basis of works previously published and the present study novel hypotheses on the AmB selectivity towards sterols varying in chemical structure and on the possible mechanisms of channel structure formation were presented.



## 1. Introduction

Despite its high toxicity polyene macrolide antibiotic amphotericin B (AmB; Fig. 1) still remains a drug of choice in the treatment of systemic fungal and yeast infections — for review see [1]. The mode of action of amphotericin B relies on its differential interaction with cell membrane sterols — ergosterol (Fig. 2) and cholesterol (for review see [1,2]). Regardless of extensive studies led by many researchers the detailed mechanism of action of the antibiotic remains unknown. Thus, rational development of novel AmB derivatives is, at least, hampered.

It was indirectly found on the basis of experimental data that antifungal activity of AmB relates to creation of specific channels in membranes (see [1,2]); however, different modes of action are also taken into consideration — see [3] for review. Hypothetical models of such channel complexes emerged rapidly after determination of amphotericin B structure [4]. The most comprehensive one was created by deKruijff and Demel in the early 1970s [5]. According to this model the channel structure is circular and consists of 8 AmB molecules interdigitated by 8 sterol molecules. Because of functional groups exposition, the complex is hydrophilic inside, contrary to the hydrophobic outside. Two such complexes coupled can transverse the membrane and create a water pore. Unfortunately, in spite of the experimental data, the exact mechanism of the channel formation remains unknown till now.

Still, there are quite strong experimental indicators supporting hypotheses on sequential formation of the AmB–sterol channel in model membranes [6,7]. The first stage of such a process appears to be an aggregation of several molecules into so called primary complexes. However, there is only indirect evidence for this phenomenon, which is clearly followed by yet unknown geometry and even stoichiometry of primary complex(es).

It had been found on the basis of UV and CD experiments that various AmB–sterol complexes are present not only in lipid bilayers, but also in water and hydroalcoholic media [8,9]. Structures of the latter ones remain unknown as well. Both species, i.e. those formed in the bilayer and in the solution, could have analogous structures as their CD spectra are highly similar. In our opinion, potential correspondence of the complexes' geometries in various media makes it possible to extrapolate the behavior of our relatively simple model to primary complex(es) present in membranes.

In spite of the experimental data all the proposals on the shape of primary complexes are based on theoretical considerations or computer calculations. All these models presuppose that van der Waals interactions of the AmB chromophore and the lipophilic part of sterols preserve such complexes, although Coulombic forces may also play a significant role in the proper placement of complex constituents. In the earliest proposal put forward by Herve et al. the sterol hydroxyl group is bound to the charged fragments of the antibiotic molecule [10]. Mazerski et al. [8] postulate that the primary complex could be a part or the complete structure of the de Kruijff's channel [5]. They specify three types of interactions responsible for the complex existence: binding forces between hydrophobic parts of the molecules, stabilizing ones between charged groups of adjacent AmB molecules, and orienting forces between the sterol hydroxyl group and an

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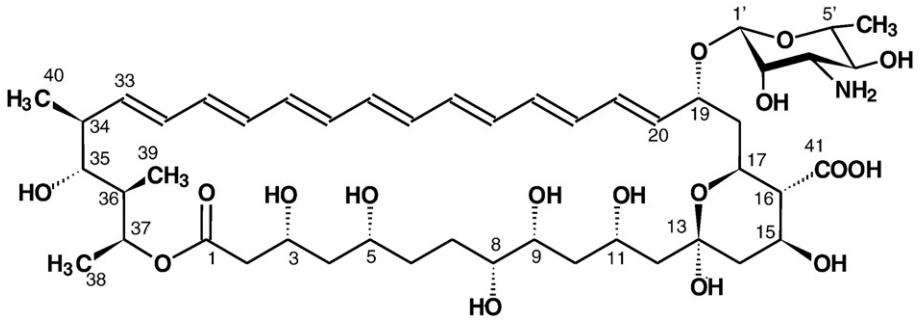

**Fig. 1.** Structure of amphotericin B.

unidentified polar fragment of the antibiotic molecule. The 2:1 stoichiometry of the primary complex suggested by Mazerski finds indirect support in results of biophysical experiments on AmB aggregation with ergosterol in hydroalcoholic media [11].

So far, computational approach to the problem was first applied by Langlet et al. with the use of a somehow simplified *ab initio* method. The authors calculated energies and obtained possible structures of the antibiotic–sterol complex (1:1 stoichiometry). However, constrains needed to keep the method numerically effective almost completely "froze" the system and reduced the study to a kind of rigid conformational analysis of limited area of phase space [12]. In addition, raw energy calculations of 2:1 complexes were there presented. The next and yet the last computational study was completed by us [13]. With the use of the molecular dynamics simulation we modeled the 1:1 AmB–ergosterol complex. In brief, we found incompatibility or only partial compatibility of the complex properties to the previous hypotheses on the geometric criteria and the network of interactions of primary complex and channel. The system simulated presented a dynamic and relatively variable nature.

Implicit evidence, based on experimental and model studies cited below, indicates that AmB–sterol complexes of higher stoichiometries can exist and may be more stable as compared to the 1:1 complex. Antibiotic and sterol molecules consist of hydrophilic and hydrophobic domains, AmB is amphoteric in addition, thus there is no doubt that the molecules should highly tend to complex. UV, CD and NMR studies on antibiotic self-association and complexing with sterols in membranes and water or hydroalcoholic media show existence of various species besides AmB–AmB or antibiotic–sterol complexes of 1:1 stoichiometry [7,8,14]. Theoretical works on the AmB dimer behavior [15–17], energy calculations of AmB higher aggregates [17,18] and the above mentioned raw energy calculations of 2:1 complexes [12] also indicate possible stability of such more or less organized species.

Taking into account the above results, we decided to simulate molecular dynamics of a system that consists of two antibiotic molecules and an ergosterol molecule in water.

## 2. Methods

The starting geometry was based on the structure acquired from our colleagues [19] and rebuilt to obtain the complex with ergosterol.

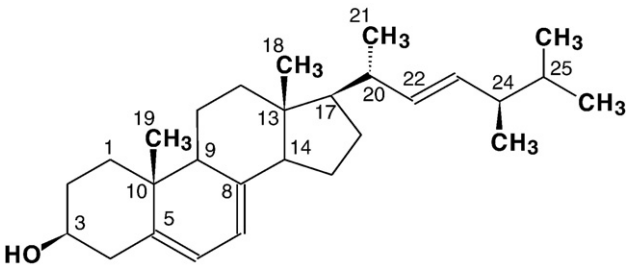

**Fig. 2.** Structure of ergosterol.

The united atom approach was employed except for hydroxyl hydrogen atoms. Standard atomic charge densities included in the GROMOS force field were used.

Minimizations and dynamics simulations were done using the GROMOS 96 molecular modeling package [20]. The integration of the classical equations of motion was done with a 2-fs time step with all bond lengths constrained within a $10^{-4}$ relative to the reference lengths with the use of the SHAKE method [21]. The *leapfrog* integration scheme was employed during all the simulations. The energy function included terms describing bonds, bond angles, dihedrals, improper dihedrals, van der Waals, and electrostatic interactions. No explicit hydrogen bond term was employed in this function. A rectangular periodic boundary was used. All the computations were carried out for molecules in water with a dielectric constant equal to 1, as required when using the standard GROMOS force field [20]. The Coulomb and van der Waals interactions were neglected when the distance between interacting atoms was greater than 1 nm (i.e. the cut-off value was less than half of the minimal vector of the periodic element as a result of periodic boundary treatment in GROMOS).

Energy minimization was performed for the system first. The next step was a 20-ps pre-simulation to relax the system and to remove the strains which eventually appeared due to the initialization procedure. At the beginning of this step, atomic velocities were adjusted according to the Maxwell–Boltzmann distribution at 300 K with periodic scaling after each 0.1 ps if the temperature deviated from the desired value of 300 K by more than 5 K. The list of non-bonded neighbors was updated every 10 MD steps. Following the relaxation period, the simulation was continued for additional 200 ps. The temperature was kept constant at 300 K by coupling the kinetic

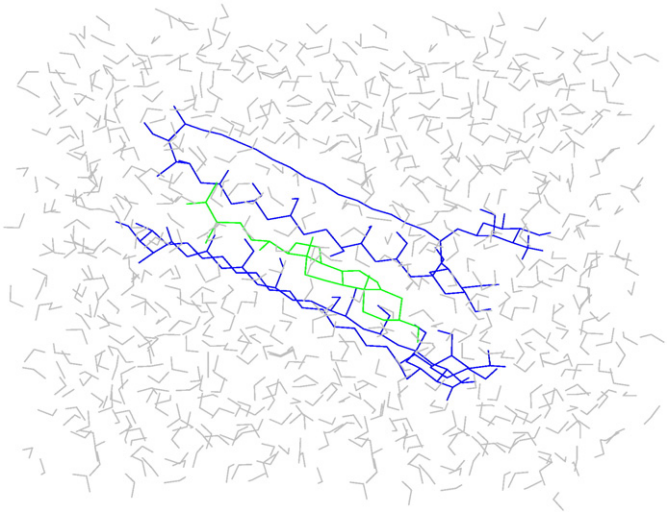

**Fig. 3.** Complex geometry after energy minimization (sterol molecule green, AmB blue, water light grey).

**Table 1**
Run averaged values of intermolecular interactions energies.

| Symbol of run | Van der Waals interactions energy [kJ/mol] | | | | Electrostatic interactions energy [kJ/mol] | | | | Total value of energy |
|---|---|---|---|---|---|---|---|---|---|
| | AmB1–AmB2 | AmB1–sterol | AmB2–sterol | VdW.subtotal | AmB1–AmB2 | AmB1–sterol | AmB2–sterol | Estat.subtotal | |
| I | −147±18.6 | −56±11.1 | −86±12.8 | −289±25.2 | −82±25.1 | 1±6.3 | −1±3.3 | −83±26.1 | −372±36.3 |
| II | −138±15.0 | −21±19.0 | −62±16.4 | −221±29.2 | −37±21.5 | −6±11.1 | 0±0.9 | −43±24.2 | −264±38.0 |
| III | −112±17.6 | −32±11.5 | −80±14.8 | −224±25.7 | −49±27.7 | −12±13.4 | −1±1.8 | −61±30.8 | −285±40.1 |
| IV | −141±22.3 | −68±10.6 | −93±9.3 | −303±26.4 | −44±21.3 | −16±11.6 | −2±2.2 | −62±24.3 | −365±35.9 |
| V | −123±19.7 | −53±13.7 | −92±13.5 | −268±27.6 | −49±23.9 | −19±12.0 | 0±2.9 | −68±26.8 | −336±38.5 |
| VI | −123±17.4 | −53±9.3 | −92±8.8 | −268±21.6 | −43±18.1 | −5±11.6 | −1±1.5 | −49±21.5 | −318±30.5 |
| Average | −131±18.4 | −47±12.5 | −84±12.6 | −262±26.0 | −51±22.9 | −10±11.0 | −1±2.1 | −61±25.6 | −323±36.6 |

energy of the system to a heat bath with a relaxation time of 100 fs. The pressure was kept at 0.06102 (1 bar) by diagonal ($X$, $Y$, $Z$), anisotropic position scaling with a relaxation time of 500 fs during the main dynamic runs.

The calculations included six runs described above, i.e. 1200 ps of the main simulation time in total.

## 3. Results

The parameters of the system (Fig. 3) were the following:

- the initial dimensions of the periodic element: $X=2.12$ nm, $Y=2.93$ nm, $Z=3.91$ nm;
- the number of water molecules generated: $n=700$;
- the total number of atoms: $i=2286$.

The molecules were kept in complex together by van der Waals forces similarly to the case of the binary complex we simulated previously [13], but electrostatic interactions added a significant, 20÷30% contribution of the total intermolecular energy this time (Table 1).

Van der Waals interactions between AmB molecules were stronger than interactions between AmB and the ergosterol. In all probability, it is an effect of the bigger size of the antibiotic molecule as compared to the sterol which favors the larger contact surface in the AmB–AmB set (Table 1/Fig. 4b). With regard to their variability, the values of AmB1–AmB2 vdW interactions fit one another and the average quite well (Table 1). The same can be stated about the angle values between the long axes and the mean planes of the antibiotic molecules (Table 2). Relatively greater variability of the values of electrostatic forces attracting the antibiotic molecules is caused by existence of AmB–AmB water bridges, not considered as direct intermolecular interactions, and directional properties of hydrogen bonds included in the electrostatic part of the GROMOS forcefield equation. The Coulombic forces between the antibiotic molecules covered up to 40% of the AmB–AmB total interaction energy and, as point-to-point interactions, had fundamental importance for the proper mutual placement of the complex components (Table 1/Fig. 4a). The hydrogen bonds linking the adjacent molecules and attraction of the charged groups contributed these interactions. AmB–ergosterol interactions were an important part of the forces keeping the complex together, but only one of the antibiotic molecules (AmB1) took part in the electrostatic interactions (Table 1/Fig. 4a). On the above basis one can presume preferable bonding of the sterol's 3βOH group to polar fragments of AmB1. Van der Waals energy value of the ergosterol–AmB2 interactions was higher than the interaction level of the sterol and AmB1 during all dynamic runs (Table 1/Fig. 4b), probably because of differences in mutual orientation of the mean planes of the molecules (Table 2/Fig. 5). The dihedral angle between the ergosterol and AmB1

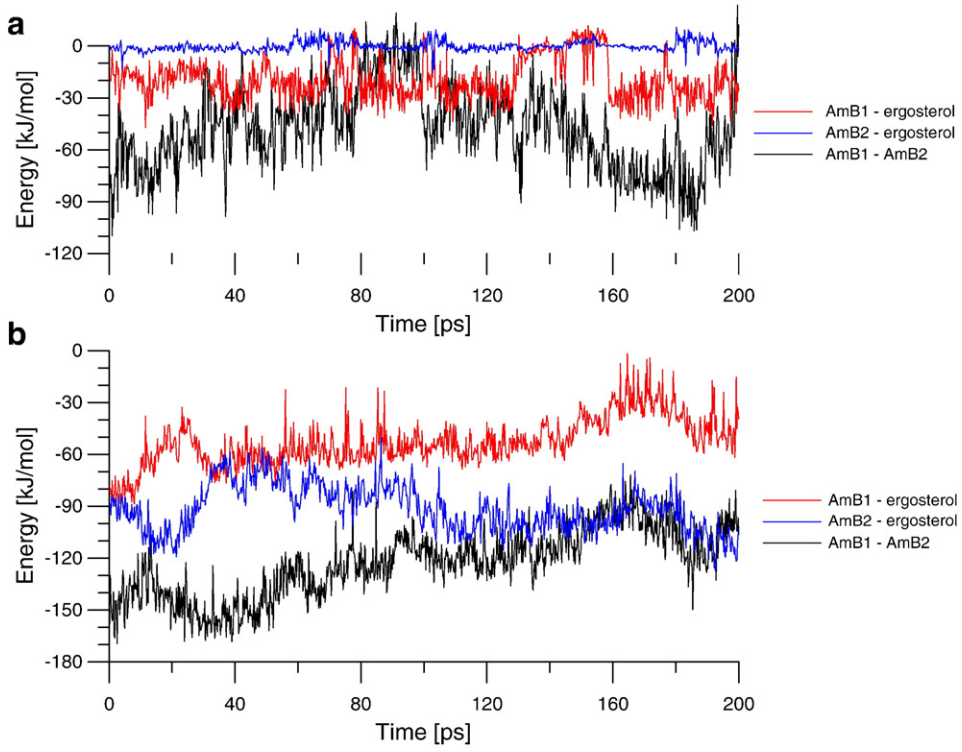


**Fig. 4.** Typical histories of intermolecular energy value changes of electrostatic interactions (a) and van der Waals interactions (b) versus simulation time (run V).

**Table 2**
Run averaged values of angles between mean planes and long axes of AmB and sterol molecules.

| Symbol of run | Angle between long axes [deg] | | | Angle between mean planes [deg] | | |
|---|---|---|---|---|---|---|
| | AmB1–sterol | AmB2–sterol | AmB1–AmB2 | AmB1–sterol | AmB2–sterol | AmB1–AmB2 |
| I | 8 ± 5.0 | 7 ± 3.6 | 4 ± 2.3 | 150 ± 16.9 | 60 ± 16.4 | 92 ± 14.5 |
| II | 35 ± 18.3 | 27 ± 15.6 | 12 ± 4.8 | 72 ± 49.9 | 139 ± 19.7 | 119 ± 44.5 |
| III | 24 ± 9.0 | 16 ± 9.1 | 12 ± 4.0 | 153 ± 14.9 | 101 ± 24.2 | 64 ± 20.7 |
| IV | 8 ± 4.8 | 8 ± 3.9 | 7 ± 4.4 | 145 ± 26.2 | 96 ± 40.4 | 51 ± 20.7 |
| V | 17 ± 6.1 | 9 ± 5.2 | 14 ± 8.5 | 138 ± 20.1 | 54 ± 20.3 | 87 ± 13.9 |
| VI | 14 ± 7.0 | 13 ± 6.1 | 9 ± 4.5 | 157 ± 13.4 | 125 ± 16.1 | 69 ± 9.9 |
| Average | 18 ± 8.4 | 13 ± 7.3 | 10 ± 4.8 | 136 ± 23.6 | 96 ± 22.9 | 80 ± 20.7 |

took higher values as compared to the sterol–AmB2 one. It can be an indicator of smaller intermolecular distances favorable for stronger vdW interaction of AmB2 and the ergosterol.

The AmB–ergosterol–AmB system was quite stable from a geometric point of view, as compared to the binary one, presumably because of existence of the network of hydrogen bonds and dispersive interactions which additionally "keep the complex constituents together". The distance between the mean planes of the sterol and antibiotic approximated 0.45 nm. Shape variability of the molecules was not as intensive as in the binary complex, certainly because of higher rigidity of the 2:1 system. The long axes of the molecules persisted nearly parallel to one another during simulations (Table 2/Fig. 5). The angle made by the macrolide rings of the antibiotic molecules took a value of 80 ± 20°. Variability of the values of antibiotic–ergosterol interactions as well as of the angles describing mutual orientation of sterol toward AmB molecules results, in all probability, from the limited rotation of the ergosterol molecule along its long axis (Fig. 5). Lack of stabilizing factors other than the hydrogen bond made by the sterol's 3βOH is the most likely reason for its relatively high "wobbling ability". The distance between the $^{+}NH_3$ and $COO^{-}$ groups of each individual AmB molecule, and between the proximate charged groups of both molecules fluctuated around 0.6 nm, thus it can be presupposed that inter- and intramolecular electrostatic attraction is well balanced (Fig. 6).

Intermolecular hydrogen bonds were of great importance for the mutual orientation of the complex constituents. The interaction of the "equatorial" hydroxyl group 8OH of AmB2 with the polyole system of the AmB1 molecule, which lasted for 40 ÷ 90% of the runs time, played the key role. Its intensity is undoubtedly caused by the proper position of the $8OH_{(AmB2)}$ group towards the nearby polar fragments of the interaction partner. Groups $9OH_{(AmB1)}$ to $8OH_{(AmB2)}$ and, placed the most frequently in the vicinity of the mycosamine ring — $15OH_{(AmB2)}$ to $2'OH_{(AmB1)}$, created quite stable hydrogen bonds as well (Table 3/Fig. 7). In our opinion, these hydrogen bonds (with minor supplement of bonds created by other groups) together with vdW forces ensure relevant stabilization of the complex. In addition, one cannot exclude the replacement of these bonds by water bridges.

Very distinctive was the behavior of the sterol's 3βOH hydroxyl group which during some runs very frequently created hydrogen bonds only with the polar fragments of the aminosugar moiety of the AmB1 molecule (Table 4). This group interacted often with the 2′OH hydroxyl (Fig. 8) and also with oxygen atom of the glycoside bond (19O) and the mycosamine ring (1′O).

## 4. Discussion

The exact stoichiometry of the primary complex(es) has not been established experimentally until now, so it is unknown whether the complex consists of two (sterol molecule and antibiotic molecule) or more components. In all probability there are more than one species having various properties and dynamics [8]. It was postulated that the systems which consist of more than two molecules can be more stable as compared to the binary complexes [12].

The results of our study sustain the above presuppositions and demonstrate that the 2:1 complex retains rigid, quite accurately defined geometry of "sandwich" type kept together by the intermolecular H-bonding and dispersive interactions. This leads to stability of the 2:1 complex, especially as compared to the binary complex characterized by shorter-lived hydrogen bonds created solely by the sterol's hydroxyl group and polar fragments of antibiotic, as well as weaker dispersive interactions [13]. According to the study by Gruda and Dussault [11], binary complexes in polar media might be only transient species. As denoted in the contemporary hypothetical and calculational models, van der Waals interactions are mainly responsible for the above mentioned stability; however, Coulomb forces share significant contribution as well. The mutual orientation of the complex components relies on these forces and, in addition, on the presence of the intermolecular hydrogen bonds. The AmB–AmB interactions have a key importance for cohesion of the complex in water and, probably, of aggregates and the channel structure in membrane. On the other hand, the presence of the sterol stabilizes these systems by increasing the level of the intermolecular dispersive interactions.

Mazerski's hypothetical model of the 2:1 primary complex implies considerable influence of the electrostatic interactions of the charged groups of adjacent AmB molecules on the mutual orientation of the complex components [8]. This suggestion is supported by our results. In the vicinity of the polar channel side exposed to water environment the $^{+}NH_3$ and $COO^{-}$ groups equally distant from one another, as in the primary complexes studied, are to create a multipole of altered positive and negative charges. Such structure was found by Khutorsky [22], Silberstein [23] and Baginski, who moreover observed creation of hydrogen bonds involving these groups [19]. Unlike Khutorsky, we postulate that the intermolecular AmB–AmB hydrogen bonds

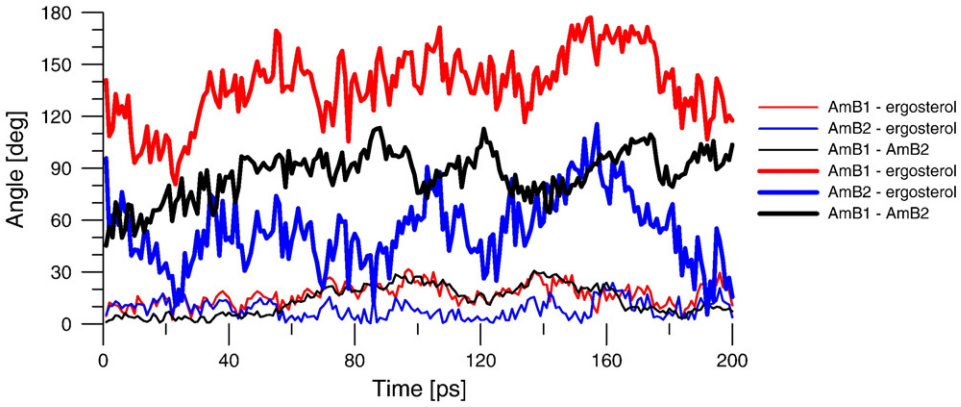


**Fig. 5.** Trajectory of angles describing mutual orientation of the molecules complexed (of run V as an example) — between mean planes (thick lines) and long axes (thin lines).

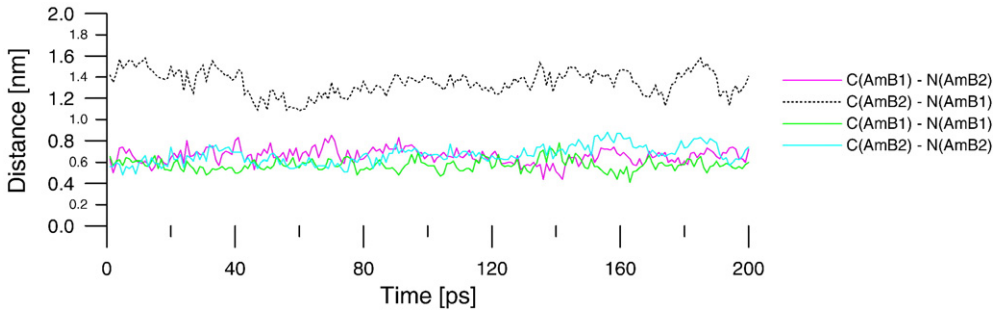


**Fig. 6.** Trajectory of AmBs' $^{+}NH_3$ to $COO^{-}$ intra- and intermolecular distances (run V).

definitely can exist and are of importance for the mutual orientation of antibiotic molecules (particularly 9OH–8OH and 15OH–2′OH). The absence of these bonds in Khutorsky's model can be interpreted as the cumulated effect of steric hindrance of the neighboring molecules coupled with freezing of the AmB's intramolecular degrees of freedom. Presence of such a set of bonds, intermolecularly connected the polyol systems of antibiotic molecules, was confirmed by Baginski and Silberstein [19,23].

As indicated by the hypothetical models, the proper orientation of sterol molecules towards antibiotic originates from a network of hydrogen bonds connecting the sterol hydroxyl group and the charged group(s) of the antibiotic directly or with participation of a water molecule. These claims were partially verified on the basis of a conformational analysis of AmB [24–27] and the primary complex [12], which demonstrated that due to steric reasons there is low probability of existence of such chelate binding sterol's 3βOH with $^{+}NH_3$ and $COO^{-}$, even by a water bridge. With respect to work of Langlet et al. [12], but also with caution emerging from the limitations of the method used there, the only possible interaction could be the water bridge between 3βOH of the sterol and AmB's $COO^{-}$ without participation of ammonium cation. Our results do not support the above suggestion, nonetheless point to another functional group of AmB as a partner for such interaction. Silberstein's conformational analysis of the channel demonstrated the presence of the 3βOH–2′OH hydrogen bond [23]. During the simulations of our system orientation of the sterol molecule resulted from relatively intensive H-bonding of 3βOH and polar fragments of the antibiotic molecule (up to 80% of the simulation time). Similar interactions stabilized to some extent the binary complex; nevertheless the sterol had almost complete conformational freedom there [13]. As presupposed in the hypothetical [5], confirmed in the computational models of other authors [19,22,23] and in our study, the additional stabilizing factors were van der Waals intermolecular forces between AmB and a sterol. Their positioning properties for the sterol molecule arise from its presence in the groove between chromophore systems of AmBs.

Finally, in our opinion, greater stability of the sandwich complex, in relation to the binary one, is based on higher levels of intermolecular vdW interactions facilitated by a better defined system of the intermolecular hydrogen bonds. These are “internal” foundations of the sandwich system stability; however, partial coverage of the molecules caused by their mutual adjacency may be an “external” source of the stability. Similarly to a single AmB molecule and binary complex, the sandwich complex remains of dual hydrophilic and hydrophobic nature; although, in all probability, the enlargement of the system itself leads to the increase of the dispersive interactions with water environment, additionally improving the stability of the 2:1 complex.

**Table 3**
Intermolecular hydrogen bonds connected AmB1 and AmB2 during run V.

| Donor → acceptor (AmB1 → AmB2) | Time of persistence [% of run time] | Donor → acceptor (AmB2 → AmB1) | Time of persistence [% of run time] |
|---|---|---|---|
| 9OH → 8O | 44.5 | 8OH → 9O | 41.5 |
| 2′OH → 15O | 55.5 | 15OH → 2′O | 11.5 |
| 11OH → 9O | 13.5 | 5OH → 5O | 10.0 |
| 13OH → 11O | 10.0 | 15OH → 41O | 8.5 |
| 3OH → 3O | 7.5 | 3OH → 5O | 6.0 |
| | | 3OH → 1O | 5.5 |
| 11OH → 11O | 5.0 | 11OH → 11O | 5.0 |
| | | 8OH → 11O | 5.0 |

## 5. Conclusions

Experimental data allow speculations about potential influence of some structural factors on AmB–sterol complexing ability and selectivity. The currently available data are the following:

- presence of the heptaene chromophore in the antibiotic is indispensable to ensure the van der Waals interaction with sterols — for review see [3];
- essential is also presence of the protonable amino group at the mycosamine moiety [28]; however, the position of this group can be shifted, e.g. by N-amino acylation [29,30]; the degree of the proton affinity of the amino group does not influence the selectivity of the interaction with sterols [31];
- there is no evidence that other functional groups of AmB play an essential role in its activity; however, esterification or amidation of the carboxyl group slightly improves selective affinity of the antibiotic in regard to ergosterol as compared to cholesterol containing membranes [31];
- 3β-hyroxyl in a sterol molecule is crucial for the polyene–sterol complex formation — for review see [3];
- the number and position of the double bonds in the sterols' ring system do not essentially influence the AmB–sterol interactions [10].

Of particular interest are the structural features of the sterols' side chain. Various types of branching in this moiety seem to influence the affinity of these compounds to AmB only to a minor extent. However, the appearance of the double bond in the side chain, as in the ergosterol molecule, essentially influences the affinity to AmB. The

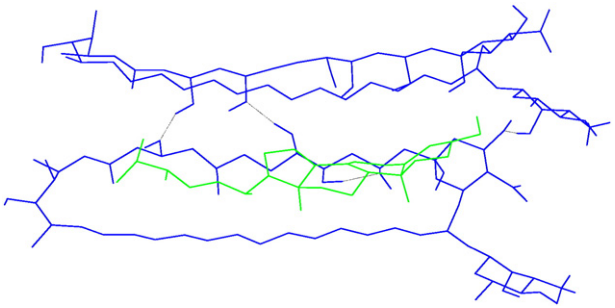

**Fig. 7.** Typical intermolecular AmB–AmB hydrogen bonds system (H-bonds as dotted lines, 3OH(1) → 3O(2), 8OH(2) → 5O(1), 2′OH(1) → 15O(2) — run III).

**Table 4**
Intermolecular hydrogen bonds connected sterol and AmB1 (bonds with participation of AmB's 2′OH are underlined).

| Symbol of run | Donor → acceptor | Time of persistence [% of run time] |
|---|---|---|
| I | 3βOH → 1′O | 14.0 |
| II | 3βOH → 2′O | 21.0 |
| III | 3βOH → 2′O | 33.0 |
| | 3βOH → 1′O | 22.0 |
| | 3βOH → 19O | 9.0 |
| IV | 2′OH → 3βO | 46.0 |
| | 3βOH → 2′O | 32.7 |
| V | 3βOH → 2′O | 67.5 |
| | 3βOH → 1′O | 12.0 |
| VI | 3βOH → 2′O | 23.0 |

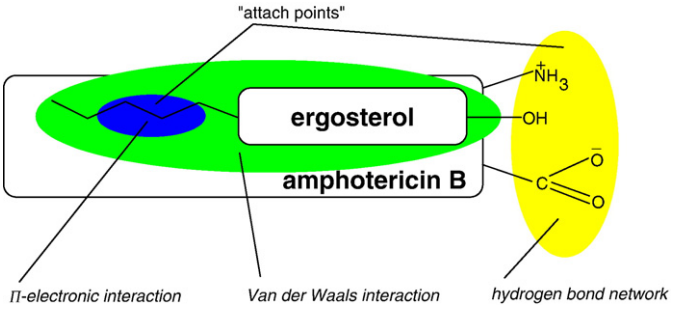


**Fig. 9.** Schematic diagram of AmB–ergosterol interactions.

antibiotic preferentially binds to the membranes that contain a sterol with the double bond in this moiety [10]. This structural factor seems to constitute the major and, moreover, the only so far identified molecular feature of sterols which correlates with the differential affinity of AmB to its membrane sterol targets.

There is no evidence that other than the above mentioned functional groups play an important role in the phenomenon of the antibiotic–sterol affinity and selectivity.

In the light of the above presented facts the following hypothesis on the molecular nature of AmB differential affinity to sterols could be put forward. There is no doubt that the major binding force in the AmB–sterol complex comes from the van der Waals interactions. Moreover, in the case of rod-shaped molecules the extent of the van der Waals interactions strongly depends on their relative orientation and reaches the maximum when both molecules are coplanar and parallel. Van der Waals interactions themselves could not play the role of orienting forces in such a case. Thus, some additional point-to-point interactions are needed to stabilize the correct orientation of the complex constituents. In the case of such molecules at least two points of interaction properly distant from each other are necessary. The hydrogen bond network in which the polar groups of adjacent molecules (polar groups of antibiotic, 3β-OH group of sterol) are directly or indirectly involved may be the first "attach point". A second point ought to be present in the sterol side chain. The presence of the C=C double bond locally increases the electron density in the ergosterol's side chain [32]. Sufficiently high electron density is also ensured near the polyene chromophore of the AmB [33]. Thus, the π–π electronic interactions between these parts of the complex constituents possibly are to be the second orienting "bond" (Fig. 9). This situation does not occur in the case of cholesterol and in consequence the stability of the AmB–cholesterol complex is lower (Fig. 10).

Results of experimental study on correlation between the aggregation state of the antibiotic and the affinity of AmB to ergosterol as compared to cholesterol in model membranes are the indirect proof which supports our hypothesis [34]. The authors of the paper established that amphotericin B affinity to cholesterol depends on the concentration of the AmB dimer. Such relation has no place in the case of ergosterol, for which the total AmB concentration counts. On the basis of our hypothesis these findings can be explained as a lack of the AmB–cholesterol complex stability prior to creation of the 2:1 "sandwich" structure. Such instability does not occur in the case of ergosterol complexes, as ergosterol holds the second, hardening "attach point".

Alternatively, it was shown that ergosterol does not react (or reacts extremely slowly) with monomeric amphotericin B in aqueous solutions of propanol [11]. Such a different behavior may result from the relatively high steric freedom of molecules in solution followed by possible complex instability there, as compared to quite stable AmB–sterol complexes in a rather packed surrounding of phosphatidylcholine. On the other hand, at high concentrations of the AmB dimer, the reaction is immediate and the concentration of amphotericin B complexed with ergosterol is twice as high as the amount of the added sterol. Explanation of this phenomenon based on our computational studies of AmB–ergosterol's 2:1 and 1:1 complexes implies enough stability of the "sandwich" structure even in the water–propanol system. Unfortunately, according to our knowledge, equivalent data on AmB–cholesterol interactions in hydroalcoholic media do not exist.

The method applied during our research adequately describes geometric and dynamic features of the systems studied, yet does not reveal the molecular nature of differential affinity of the antibiotic towards various sterols (unpublished data on AmB–cholesterol complexes molecular dynamics). According to our knowledge, there is no currently available force field based on classical approximation which contains factor describing π–electronic interactions, in our opinion the major reason for the greater AmB–ergosterol affinity. Examination of intramolecular interactions of π–electronic multimolecular systems is inconvenient even with the use of semiempirical quantum chemistry methods. On the other hand, the only valid *ab initio* approach is numerically expensive in the case of more sophisticated systems. Regardless of the entire obstacle one can consider the complex geometries obtained as a good starting point for the determination of the AmB–sterol interaction energies with the support of theoretical quantum chemistry methods.

As the detailed experimental data on the molecular nature and dynamics of creation of the water channel is not available, the below

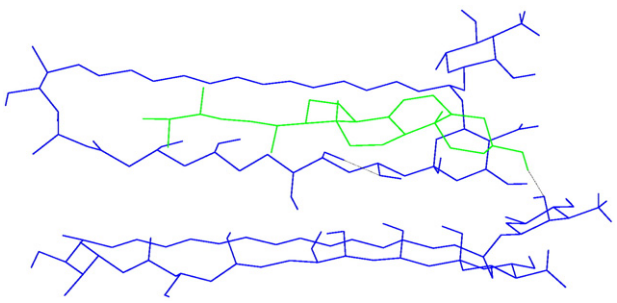

**Fig. 8.** Sterol–AmB hydrogen bonding (3βOH → 2′O — run V).

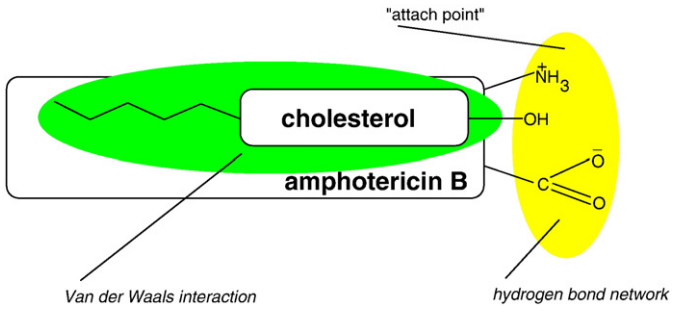


**Fig. 10.** Schematic diagram of AmB–cholesterol interactions.

proposed theoretical model of such process should be only treated as a primer for further discussion. The geometric analogies of hypothetical [8] and computational models of the 2:1 primary complex as compared to hypothetical and computational models of the water channel [5,19,22,23,35,36] might be a basis for anticipation of a possible mechanism of the channel creation. With regard to the high stability of the AmB dimer [15] superior over the stability of the AmB–sterol binary complex [13] and relatively the greatest stability of the AmB–sterol–AmB complex one can presuppose that the cholesterol molecule should join the previously complexed AmB dimer of the "head to head" type. The above events might occur concurrently for ergosterol, as its ability to complex with AmB in the membrane environment does not depend on the AmB dimer concentration [34]. The AmB–sterol–AmB complexes could then unite into a pre-channel structure of 2:1 stoichiometry, and finally the "missing" sterol molecules might fit into free spaces. These two events could happen simultaneously as well. The hypothesis presented concerns a situation where the process is controlled thermodynamically. In model membranes, considering the kinetics of the process and adequate excess of sterol, the first stage of the channel composition could be creation of the binary AmB–sterol complex. Treating the phenomenon from another perspective, one might postulate existence, among other species, of systems with channel-like geometry in membraneless apolar environments.

The above forethoughts on primary/channel complexes' structure and formation process cannot be currently compared directly with experimental results, thus any biophysical studies providing information on AmB's self-organization, as well as on the complexes' properties on the molecular level would be very helpful.

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