ELSEVIER

Biophysical Chemistry 95 (2002) 125–133

Biophysical Chemistry

www.elsevier.com/locate/bpc

Molecular modelling of amphotericin B–ergosterol primary complex in water

Mariusz Baran[a,b], Jan Mazerski[b,*]

[a]*Laboratory of Medical Informatics, Medical University of Gdansk, ul. Debinki 1, PL-80211 Gdansk, Poland*
[b]*Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Technical University of Gdansk, ul. Narutowicza 11/12, PL-80952 Gdansk, Poland*

Received 17 August 2001; received in revised form 30 November 2001; accepted 7 December 2001

Abstract

The properties of amphotericin B–ergosterol primary complex have been studied with the use of the molecular dynamics simulation. Possible geometries of the complex were tested first in order to find the structures with the most favourable values of the intermolecular interactions energy. The molecules studied possessed a tendency to fit each other's shapes, which favours intermolecular van der Waals interactions. The main simulations were performed for the best structures found. Presence of hydrogen bonds between the sterol hydroxyl group and polar fragments of mycosamine (most frequently 2′OH) was coupled with a relatively high level of the intermolecular energy values. The structures obtained are hardly comparable to the hypothetical and 'computational' models of the antibiotic–sterol complex. The geometries found are not suitable to assemble the presupposed structure of the water channel, however, the existence of the complex in the shape anticipated is not in contradiction to the results of biophysical experiments on the complexation in water and in hydroalcoholic media.

Keywords: Amphotericin B; Ergosterol; Primary complex; Computer simulation; Molecular dynamics; GROMOS

1. Introduction

Amphotericin B (Fig. 1) is a potent agent effective against a wide spectrum of invasive fungi and yeasts causing systemic infections [1–5]. It belongs to the group of polyene macrolide antibiotics [6–8]. The activity of these antibiotics is based on their interaction with cell membrane sterols [1,9]. In spite of extensive studies led by many authors, the exact mechanism of action of the antibiotics on the molecular level has not yet been recognised. Because of that, reasonable designing of more potent derivatives of amphotericin B (AmB) remains difficult.

Experimental data indicate that action of AmB leads to the creation of channel-like structures in biological membranes [10–15]. In spite of lack of the structural data on the water channel, molecular models of the AmB–sterol 'channel' complex appeared rapidly after an X-ray determination of

*Corresponding author. Tel.: +48-58-347-11-16; fax: +48-58-347-26-94.
E-mail address: janmaz@altis.chem.pg.gda.pl (J. Mazerski).

0301-4622/02/$ - see front matter
PII: S0301-4622(01)00252-6

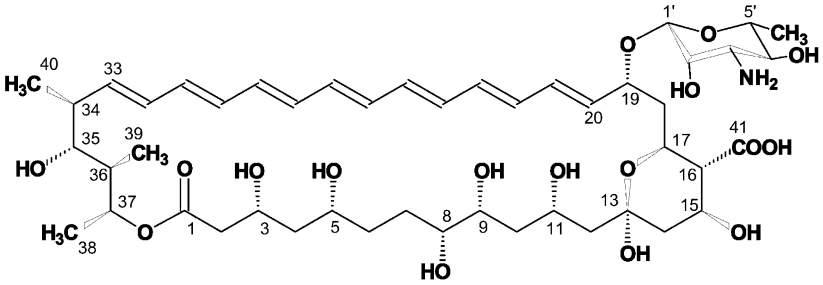

Fig. 1. Amphotericin B.

the structure of AmB *N*-iodoacethyl derivative [16,17]. The most comprehensive hypothesis was published by DeKruijff and Demel in the early 1970s [18]. According to this model, the channel consists of eight AmB molecules arranged circularly and interdigitated by eight sterol molecules. The outside of this complex is hydrophobic; the inside is hydrophilic due to the presence of the hydroxyl groups of the AmB molecules. Two such complexes generate a pore which transverses the membrane.

The process of formation of the channel structure in the membrane probably consists of several stages [19]. The first of them is, presumably, complexing of AmB and sterol molecules into the so-called primary complex(es) [20]. Unfortunately, there are only indirect experimental indications of the existence of the primary complex [21]. Its geometry has not yet been defined. Some hypotheses on the shape of the primary complex arose taking into account the experiments performed and the models of the water channel published. All of them presuppose that van der Waals interaction between the AmB chromophore and a flat, lipophilic sterol molecule is very important for the stability of the complex. Then, the interaction of the sterol 3βOH group and polar fragments of the antibiotic should be responsible for the proper placement of the complex constituents. In the model proposed by Herve et al. [22] the sterol hydroxyl group is bound with the charged groups of AmB, possibly by water bridges. The authors of another hypothesis presume that the primary complex could be a fragment or the complete structure of the water channel [23]. They postulate that three different kinds of forces are responsible for the stability of the complex: binding forces between hydrophobic parts of the antibiotic and sterol molecules; stabilising forces between the amino group and carboxyl group of adjacent AmB molecules; orienting forces between the sterol 3βOH group and an unidentified place in the antibiotic molecule. The authors of the models draw attention to the agreement of the structures proposed with the principles of water channel designed by DeKruijff, in order that the channel structure could be easy assembled from the geometries of the primary complex.

Biophysical experiments proved that AmB–sterol complexes exist not only in lipidic bilayers. UV and CD data indicate that some complexes are also present in water and in hydroalcoholic media [23,24], but their geometry is not known, either. High similarity between the CD spectra of the complex(es) present in solution and of the corresponding complexes formed in the phospholipid bilayer can be observed. This strongly suggests that both species might also have similar geometries. During our research we focused our attention on the primary complexes formed in solution. We hope that the results of the study performed on this relatively simple model system could facilitate the understanding of the processes observed in the membranes.

Some teams applied in their research computational chemistry to override the acute lack of the structural data on the primary complex. In 1994 Langlet et al. [25] published a work concerning the geometry of the AmB–sterol complex. The studies were carried out with the use of the partial

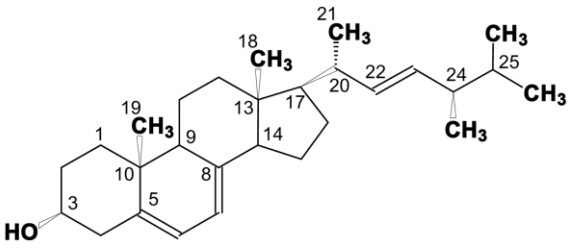

Fig. 2. Ergosterol.

Sum of Interactions Between Fragments computed Ab initio (SIBFA) method. The authors obtained the possible structure and intermolecular energies of the complex studied. However, approximations and simplifications necessary on the level of complexity of the method used require particular caution during a discussion of the results. All bond lengths, bond angle values, and almost all dihedrals of the molecules studied remained frozen. Additionally, water environment was mimicked by a few water molecules arbitrarily located close to the places of hydration.

Molecular dynamics seems to be a more accurate tool in order to simulate a system of such a kind. It is known that AmB, if present in water in low concentration, exists in aqueous medium in monomeric form [26]. Thus, a system in the shape of a rectangular box with periodic conditions, containing one molecule of amphotericin B and one ergosterol molecule (Fig. 2), and filled up with nearly 460 water molecules, has been considered a model of a primary complex. The results of dynamic simulations of this system with the use of the GROMOS 96 force field are described in this paper.

2. Methods

The geometries derived from the X-ray data on *N*-iodoacethyl AmB [17] and ergosterol monohydrate [27] crystals were used as a starting point of our calculations. Hydroxyl hydrogen atoms were added in their standard geometry to the X-ray structure. The unified atom approach was employed for other hydrogen atoms. Standard atomic charge densities included in the GROMOS force field were used during all the calculations.

Minimisations and dynamics simulations were done using the GROMOS 96 molecular modelling package [28]. The integration of the classical equations of motion was done with a 2-fs time step with all bond lengths constrained within 10^{-4} relative to the reference lengths with the use of the SHAKE method [29]. 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 the studied molecules surrounded by water molecules with the use of the dielectric constant equal to 1, as required when using the standard GROMOS force field [28]. 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 minimisation was performed for the system first. The next step was a 20-ps presimulation to relax the system and to remove the strains which eventually appeared due to the initialisation 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°. The list of non-bonded neighbours was updated every 10 MD steps. Following the relaxation period, the simulation was continued for an additional 200 ps. The temperature was kept constant at 300 K by coupling the kinetic energy of the system to a heat bath with a relaxation time of 100 fs. The pressure was kept at 100 kPa by diagonal (*X*, *Y*, *Z*), anisotropic position scaling with a relaxation time of 500 fs during the main dynamic runs.

The main dynamics simulation included six runs described above, i.e. totally 1200 ps of the simulation time.

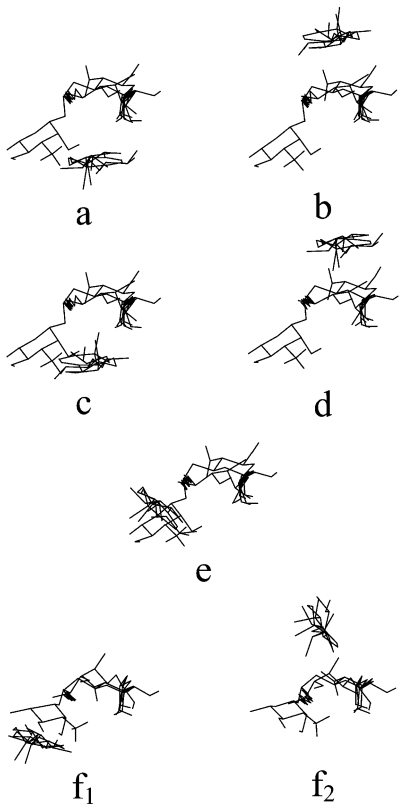

Fig. 3. Configurations of the AmB–ergosterol complex.

3. Exploration of phase space

The authors of the study described above applied a consistent approach during the search for an optimal mutual orientation of the components of the complex [25]. They systematically rotated a sterol molecule and moved it parallel along the long axis of AmB. A similar procedure was used to prepare starting geometries for our calculations; however, the systems obtained were placed next in a water environment. We paid attention to obtaining a maximal contact surface between the sterol and antibiotic molecules. In effect, we gained an abundant set of 20 structures with different placements of ergosterol in relation to AmB (Fig. 3).

The geometries generated can be divided into six groups according to the orientation of the molecules in relation to each other. The relative placement of the molecules considered in the groups are as follows:

a the sterol is under the mean plane of the macrolide ring and its methyl groups point toward the antibiotic;
b the sterol is over the mean plane and its methyl groups point toward AmB too;
c the sterol is under the mean plane, but its methyl groups point toward the opposite direction;
d the sterol is over the mean plane of the macrolide and the methyl groups point to the direction opposite AmB;
e the mean planes of the sterol and the polyene chain are parallel; and
f the orientation of the molecules was chosen on the basis of the water channel structure.

Geometries *1, *2, … of groups (a)÷(e) differ by the relative position of their components — the ergosterol was moved parallel along the long axes of the molecules. In the case of the f1 geometry the sterol is placed under aglicone, and in the f2 geometry — over aglicone.

The parameters of the systems studied are the following:

- the initial dimensions of the periodic element: $X=3.56$, $Y=2.16$, $Z=2.03$;
- the number of water molecules generated: $n=460\pm6$; and
- the total number of atoms: $i=1488\pm18$.

Every system was preliminarily relaxed shortly for 20 ps after the optimisation of the structure to find possible geometries of the complex. This procedure was followed by selection of the systems for further main simulations. The van der Waals interaction seems to be responsible for the stability of the complexes; electrostatic attraction, however, could play some role, too. The energy of the antibiotic–sterol interactions possessed the most favourable values in the case of systems a1, a2, a3 and c1. The electrostatic interaction reached 10÷15% of the total energy in the systems a1, a2, a3 and b2, probably due to intermolecular hydrogen bonding. The value of the energy varied quite intensively during some simulations, but in most cases one could observe its rise after dropping. In 18 dynamic runs the ergosterol molecule remained

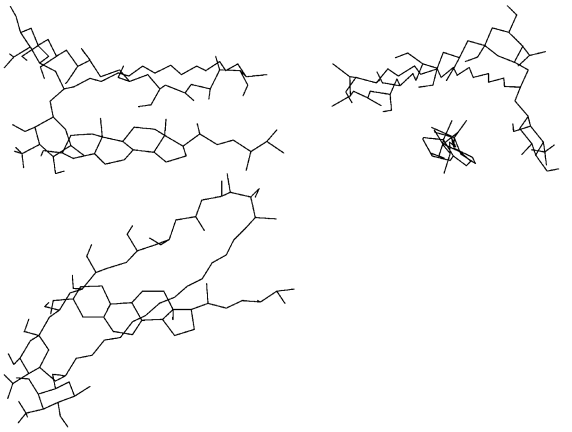

Fig. 4. Fitting of the AmB molecule to ergosterol — run b2.

in close proximity to AmB. The complex appeared to be decomposed only in two relaxation runs.

During the relaxation runs the complexes displayed a dynamic and relatively variable in time nature, but the mutability of their structure was limited, i.e. the final configuration of 'head-to-head' as well as 'tail-to-tail' geometries appeared to be proximate to the initial ones. However, the type of starting geometry only in a limited way affects the mean intermolecular energy level.

Optimal contact surface is a condition of the most favourable van der Waals interaction. A review of temporary snapshots of the geometry of the complex confirmed the tendency of its components to fit their shapes with each other. Both the molecules complexed demonstrated such behaviour (Figs. 4 and 5). However, all the alterations of the shape of the molecules studied were limited by stiffness of the polyene system and the steroid nucleus.

Hydrogen bonds could stabilise the geometry of the complex. Interaction between the 3βOH hydroxyl group of the ergosterol molecule and the 2′OH group of mycosamine moiety was found in several systems studied. Hydrogen bonds engaging the sterol and other polar fragments of the antibiotic lasted for a shorter time.

4. Main simulations

As the exploration of the phase space of the complex described above showed, the 1:1 primary complex could exist in several configurations characterised by similar energy of interaction. Thus, more extensive exploration of the phase space was necessary to determine that its the most probable configuration. To solve this problem we performed the main dynamics simulations with the use of a few different configurations of the complex that were distant in the configurational space. Moreover, several independent simulation runs that differed by the distribution of atom velocities were done for each starting configuration. In our opinion, such a method of exploration of the phase space, followed by statistical interpretation of the trajectories obtained, can be very effective.

Two configurations from among the ones obtained after the relaxation runs were selected for this procedure. The first (a2) was characterised by the best intermolecular energy. The second one was considered as a fragment of the channel (f1). The parameters of the systems were equivalent to the preliminary ones. Three dynamic runs lasting 200 ps were performed for both starting configurations. It is remarkable that, irrespectively of a starting geometry and an atomic velocity distribution, similar final structures of the complex were obtained. It may suggest that the region of the phase space that represents typical behaviour of such a complex was found.

Van der Waals interactions remained on a similar, relatively high level. In the case of some runs, antibiotic polar fragments quite intensively attracted electrostatically the hydroxyl group of ergosterol for $20 \div 50\%$ of the simulation time. A

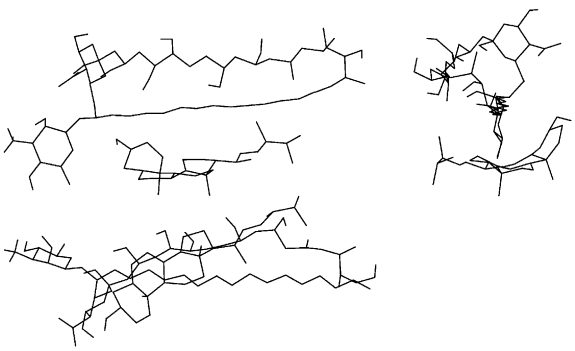

Fig. 5. Fitting of the ergosterol molecule to AmB — run e1.

correlation between favourable intermolecular electrostatic interactions, van der Waals interactions and hydrogen bonds existence was observed (Fig. 6). The rises and drops of the energy values were coupled with the creation and breaking of the hydrogen bonds. That coupling likely originates from the presence of hydrogen bonds which can stabilise relative alignment and extent of the intermolecular surface of the components of the complex.

The molecules studied are 'locally' flexible. In spite of that, their overall shape remains approximately planar and elongated. It allows describing the relative position of the sterol towards the antibiotic with the use of angles between the mean planes and long axes of the molecules. Table 1 presents values of these angles averaged over the simulation time. Fluctuations of the values of the angles remained on a relatively low level. Fig. 7 presents usual mutability of the angle parameters over the period of the simulation distinguished by stable relative orientation of the components of the complex. The ergosterol molecule lies near the aminosugar moiety under the mean plane of the antibiotic in that case. The sterol methyl groups point to the direction opposite AmB. The long axes and mean planes of the molecules are approx-

Table 1
Averaged values of angles describing placement of ergosterol toward the AmB molecule

Symbol of run	Angle between long axes of the molecules (°)	Angle between mean planes of the molecules (°)
a2.1	66±28	138±34
a2.2	25±19	157±16
a2.3	29±18	135±26
f1.1	28±16	150±15
f1.2	16±7	161±12
f1.3	27±17	118±40

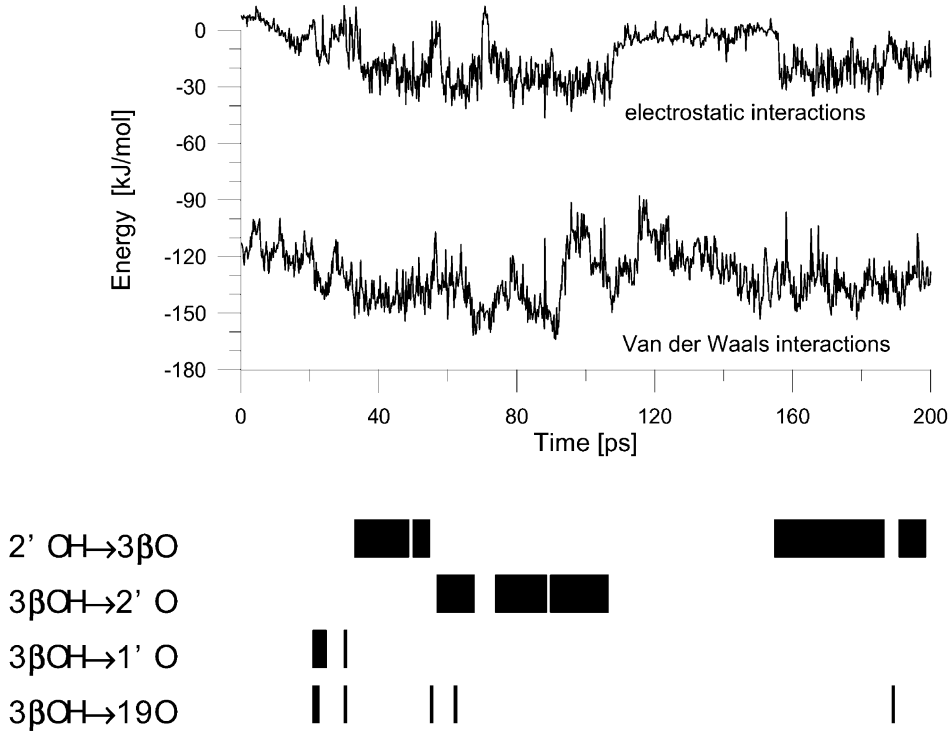

Fig. 6. Correlations between favourable intermolecular electrostatic interactions, van der Waals interactions and hydrogen bonds existence — run a2.2.

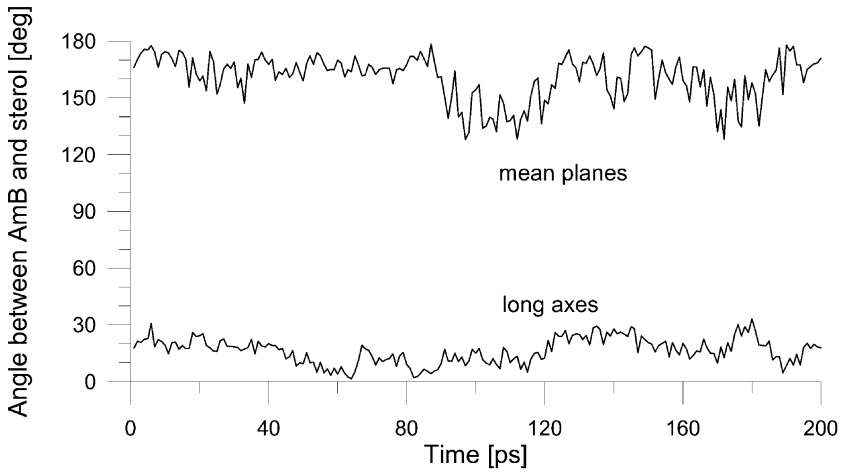

Fig. 7. Trajectory of angles describing placement of ergosterol toward the AmB molecule — run f1.2.

imately parallel. The distance between the carbon backbones of the molecules is equal to 0.4÷0.5 nm. In the figure illustrating the topic discussed (Fig. 8) one can observe that the sterol molecule occupies the space between the polyene chain and the polyol fragment of the antibiotic, and the 3βOH hydroxyl group is placed near the mycosamine.

Such placement of the molecules facilitates interaction between polar groups. Hydrogen bonds existed for up to 52.5% of the period of the runs. They connected the polar sites of the sterol and aminosugar — especially the 2′OH group as well as oxygen atoms 1′O and 19O. Water bridges frequently replaced hydrogen bonds. Polar fragments of AmB and ergosterol 3βOH were therefore connected directly or indirectly for 26÷100% of the main simulation time (Table 2).

5. Discussion

It should be stressed that geometries characterised by the highest level of intermolecular interactions obtained during our calculations are hardly comparable to the current, 'binary' model of the primary complex [22]. They are in contradiction to the results obtained by Langlet et al. [25], too. The presupposed structure of the water channel

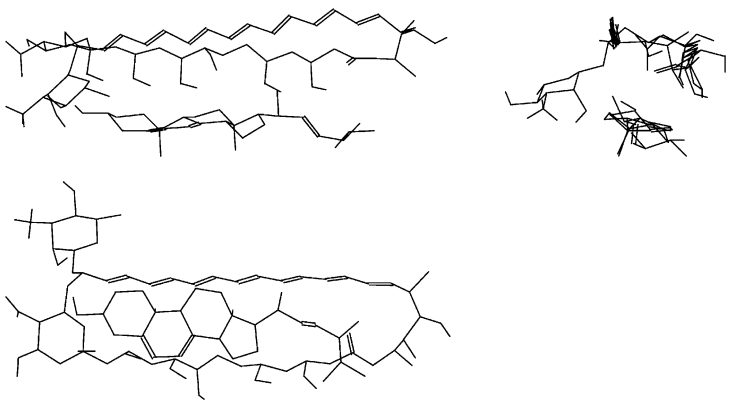

Fig. 8. Snapshot of the AmB–ergosterol complex — the 60th ps of run f1.2.

Table 2
Intermolecular hydrogen bonds and water bridges

Symbol of run	Donor/ acceptor	Time of existence (% of run time)	
		Hydrogen bond	Water bridge
a2.2	2′O	52.5	18
	19O		11
	13O		7
	41O		7
	1′O		5
a2.3	2′O	21.5	16
f1.1	19O		12.5
	2′O		11.5
	15O		9.5
	1′O		9.5
	13O		6.5
f1.2	2′O	21	22
	19O	15	
	13O		5.5
f1.3	2′O		12
	1′O		8.5
	8O		5.5

[18] cannot be created using our geometries, either. There are a few possible reasons for these disagreements.

In all probability, the most important is the simplification applied by the creators of the hypothetical [22] and 'computational' [25] models of the primary complex. In our opinion, they treated the components of the complex too statically — partially or completely neglecting the lability of the interacting molecules that allows them to fit in each other and/or omitting the role of the surrounding medium.

A lack of information on a stoichiometry of primary complex(es) could be pointed out as an explanation of the incompatibility between the geometries calculated and the model postulated by Mazerski et al. [23]. Up to now it remains unknown whether the complex consists of one sterol and one AmB molecule or of more than two molecules. It is highly probable that there can be more species of complexes with different stoichiometries and other properties in the media taken into account [23]. Presumably, the complexes that consist of more than two molecules should be more stable than the binary ones [23,30].

The difference between the geometries of the complex determined by us and the hypothetical channel structure [18] might be explained by the different surroundings in which they are present. Phospholipid molecules are placed perpendicular to the bilayer plane in a lipidic membrane. Their side chains surround the hydrophobic exterior of the water channel and limit a possible range of mutability of the channel components more than the water environment. Moreover, the components are 'stiffed' sterically by the adjacent antibiotic and sterol molecules.

The studies on dynamics of binary systems did not bring the ultimate determination of the geometry of the primary complex that is suitable to assemble the postulated channel structure. Results of biophysical experiments, however, do not negate the existence of a complex in the shape anticipated by us. The effects of our calculations strongly suggest the ability of the ergosterol as well as the antibiotic molecule to adapt quite well with their conformation to their co-partner(s) in interaction, and, consequently, a dynamic and relatively variable in time nature of the complex itself.

Computer simulations of systems consisting of more than two molecules will be the next step of our research. Such systems could be more suitable as a theoretical model of the primary complex.

Acknowledgments

This work was partially supported by the Faculty of Chemistry of the Technical University of Gdansk and by the Medical University of Gdansk. The main simulations were performed in the Academic Computer Center in Gdansk (TASK).

References

[1] G. Medoff, J. Brajtburg, G.S. Kobayashi, J. Bolard, Antifungal agents useful in therapy of systemic fungal infections, Annu. Rev. Pharmacol. Toxicol. 23 (1983) 303–330.

[2] H.A. Gallis, R.H. Drew, W.W. Pickard, Amphotericin B: 30 years of clinical experience, Rev. Infect. Dis. 12 (1990) 308–329.

[3] S. Demarie, R. Janknegt, I.A.J.M. Bakkerwoudenberg, Clinical use of liposomal and lipid-complexed amphotericin B, J. Antimicrob. Chemother. 33 (1994) 907–916.
[4] G.A. Sarosi, S.F. Davies, Therapy for fungal infections, Mayo Clin. Proc. 69 (1994) 1111–1117.
[5] A.C.A.P. Leenders, S. Demarie, The use of lipid formulations of amphotericin B for systemic fungal infections, Leukemia 10 (1996) 1570–1575.
[6] J. Kotler-Brajtburg, G. Medoff, G.S. Kobayashi, et al., Classification of polyene antibiotics according to chemical structure and bilogical effects, Antimicrob. Agents Chemother. 15 (1979) 716–722.
[7] S. Omura, H. Tanaka, Macrolide antibiotics, in: S. Omura (Ed.), Production, Structure, and Antifungal Activity of Polyene Macrolides, Academic Press, Inc, Orlando, 1984, p. 351.
[8] S. Inouye, M. Sezaki, Current antifungal antibiotics, J. Syn. Org. Chem. Jpn. 51 (1993) 73–95.
[9] E.F. Gale, Macrolide antibiotics, in: S. Omura (Ed.), Mode of Action and Resistance Mechanisms of Polyene Macrolides, Academic Press, Inc, Orlando, 1984, p. 425.
[10] K.M. Kasumov, S.D. Karakozov, Effect of amphotericin B added to one side of a membrane, Biofizika 30 (1985) 281–284.
[11] B.E. Cohen, Concentration- and time-dependence of amphotericin B induced permeability changes across ergosterol-containing liposomes, Biochim. Biophys. Acta 857 (1986) 117–122.
[12] J. Bolard, P. Legrand, F. Heitz, B. Cybulska, One-side action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium, Biochemistry 30 (1991) 5707–5715.
[13] D.E. Mickus, D.G. Levitt, S.D. Rychnovsky, Enantiomeric cholesterol as a probe of ion-channel structure, J. Am. Chem. Soc. 114 (1992) 359–360.
[14] S.C. Hartsel, S.K. Benz, W. Ayenew, J. Bolard, Na^+, K^+ and Cl^- selectivity of the permeability pathways induced through sterol-containing membrane vesicles by amphotericin B and other polyene antibiotics, Eur. Biophys. J. 23 (1994) 125–132.
[15] S.F. Hsu, R.R. Burnette, Characterization of the effects of amphotericin B on ion channels in MDCK cells using the patch-clamp technique, Biochim. Biophys. Acta 1329 (1997) 26–38.
[16] W. Mechlinski, C.P. Schaffner, P. Ganis, G. Avitabile, Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B, Tetrahedron Lett. 44 (1970) 3873–3876.
[17] P. Ganis, G. Avitabile, W. Mechlinski, C.P. Schaffner, Polyene macrolide antibiotic amphotericin B. Crystal structure of the *N*-iodoacetyl derivative, J. Am. Chem. Soc. 93 (1971) 4560–4564.
[18] B. De Kruijff, R.A. Demel, Polyene antibiotic-sterol interactions in membranes of acholeplasma laidlawii cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic–cholesterol complexes, Biochim. Biophys. Acta 339 (1974) 57–70.
[19] B.E. Cohen, A sequential mechanism for the formation of aqueous channels by amphotericin B in liposomes. The effect of sterols and phospholipid composition, Biochim. Biophys. Acta 1108 (1992) 49–58.
[20] S.C. Hartsel, C. Hatch, W. Ayenew, How does amphotericin B work?: studies on model membrane systems, J. Liposome Res. 3 (1993) 377–408.
[21] A. Vertut-Croquin, J. Bolard, M. Chabbert, C.M. Gary-Bobo, Differences in the interaction of the polyene antibiotic amphotericin B with cholesterol- or ergosterol-containing phospholipid vesicles. A circular dichroism and permeability study, Biochemistry 22 (1983) 2939–2944.
[22] M. Herve, J.C. Debouzy, E. Borowski, B. Cybulska, C.M. Gary-Bobo, The role of the carboxyl and amino groups of polyene macrolides in their interactions with sterols and their selective toxicity. A 31P-NMR study, Biochim. Biophys. Acta 980 (1989) 261–272.
[23] J. Mazerski, J. Bolard, E. Borowski, Effect of modifications of ionizable groups of amphotericin B on its ability to form complexes with sterols in hydroalcoholic media, Biochim. Biophys. Acta 1236 (1995) 170–176.
[24] I. Gruda, J. Bolard, On the existence of an amphotericin B–sterol complex in lipid vesicles and in propanol–water systems, Biochem. Cell Biol. 65 (1987) 234–238.
[25] J. Langlet, J. Berges, J. Caillet, J.P. Demaret, Theoretical study of the complexation of amphotericin B with sterols, Biochim. Biophys. Acta 1191 (1994) 79–93.
[26] J. Bolard, M. Seigneurt, G. Boudet, Interactions between phospholipid bilayer membranes and the polyene antibiotic amphotericin B, Biochim. Biophys. Acta 599 (1980) 280–293.
[27] S.E. Hull, M.M. Woolfson, The crystal structure of ergosterol monohydrate, Acta Crystallogr. B 32 (1976) 2370–2373.
[28] W.F. Gunsteren, S.R. Billeter, A.A. Eising et al., Biomolecular Simulation: The Gromos 96 Manual and User Guide, vdf Hochschulverlag AG an der ETH Zurich and BIOMOS b.v., Zurich, Groningen, 1996.
[29] J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of *n*-alkanes, J. Comput. Phys. 23 (1977) 327–341.
[30] J. Caillet, J. Berges, J. Langlet, Theoretical study of the self-association of amphotericin B, Biochim. Biophys. Acta 1240 (1995) 179–195.