THE CHEMICAL RECORD

Interaction of Amphotericin B and Its Selected Derivatives With Membranes: Molecular Modeling Studies

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Received 23 August 2006; Revised 18 October 2006; Accepted 13 September 2006

ABSTRACT: Amphotericin B (AmB) is a well-known antifungal antibiotic that has been used in the clinic for about five decades. Despite its chemotherapeutic importance, AmB is quite toxic and many efforts have been made to improve its pharmacological properties, e.g., by chemical modifications. The lipid membrane is a molecular target for AmB, however, due to heterogeneity of its components, the molecular mechanism of AmB action is still unclear. The lack of this knowledge hinders rational designing of new and less toxic AmB derivatives. Our review is a critical presentation of the current understanding of AmB molecular mechanism of action at the membrane level. Except the experimental approach, the extensive overview of molecular modeling studies, performed mostly in our lab, is presented. The results of interactions between AmB or some of its derivatives and lipid model membranes are discussed. In our studies, different biomembrane models and different associate states of the antibiotic were included. Presented molecular modeling approach is especially valuable with regard to a new paradigm of the structure of lipid membrane containing liquid-ordered domains. Hopefully, all these complementary experimental/computational approaches are going to reach the point at which a new hypothesis about molecular mechanism of AmB activity and selectivity will be put forward. © 2007 The Japan Chemical Journal Forum and Wiley Periodicals, Inc. Chem Rec 6: 320–332; 2006: Published online in Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/tcr.20096

Key words: amphotericin B; polyene macrolide; molecular modeling; lipid membrane; sterols

Introduction

Amphotericin B (AmB) belongs to a group of polyene macrolide antibiotics comprising of more than 200 compounds.[1–3] AmB (Fig. 1) was discovered in the fifties of the previous century and very soon it was introduced as an antifungal drug to combat systemic fungal infections. Since then, the antibiotic has been used extensively in the clinic and even now it is regarded as a golden standard and a life-saving drug in the treatment of severe fungal infections.[4–6] Figure 2 presents an overview of AmB history. Without exaggeration, one may say that it is probably one of the oldest antibiotics still being used in medical practice as the same active agent. There are two reasons of this privileged position of AmB among antifungal agents: (i) unique chemotherapeutic properties and (ii) lack of better alternative drugs. Unique properties of AmB as chemotherapeutic include its high activity, wide antifungal spectrum, fungicidal and not only fungistatic action, and reluctance to produce fungal resistance. Altogether, these properties make AmB so special and therefore, many other antifungal agents introduced through the years could not overtake

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Contract grant sponsor: Ministry of Education and Science, Poland; contract grant number 3 P05F 01225

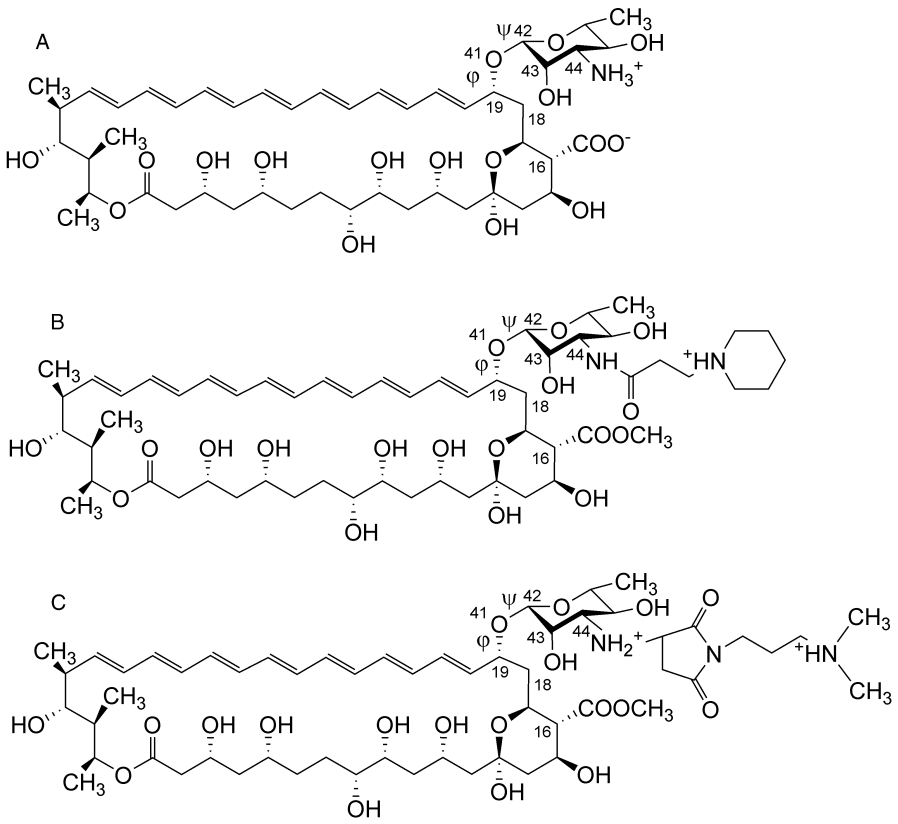

Fig. 1. Chemical structures of (A) amphotericin B (AmB), (B) PAmE, and (C) SAmE. Presented φ and ψ torsion (dihedral) angles are defined by the following atoms: φ = C18-C19-C41-C42, ψ = C19-C41-C42-C43. Please note that the parent AmB molecule is a zwitterion and both derivatives (SAmE and PAmE) are methyl esters and bear only positive charges. The so-called "polar head" of AmB contains the sugar moiety and polar part of the aglycon, comprising the carboxyl group.

the position of AmB. However, the situation is far from being ideal because AmB, despite its high activity, is also highly toxic (i.e., it exhibits poor selectivity between fungi and humans). Additionally, due to its physical and chemical properties, the antibiotic has to be administered intravenously, which is very painful for patients. The lack of AmB selectivity causes many side effects and therefore, continuous efforts have been made to improve the antibiotic.[7,8] One way to do this is to make

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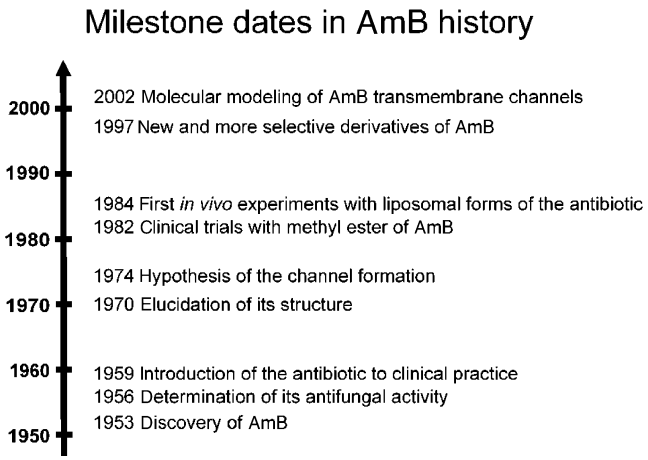

Fig. 2. Selected most important facts concerning amphotericin B (AmB) history.

liposome or lipid formulations of the antibiotic which are known to be less toxic delivery forms.[9,10] These new formulations are somewhat less harmful to patients, but due to the high cost of their preparation they are not available for more extensive use. Another approach is focused on obtaining new and less toxic chemical derivatives of AmB.[8,11] At this point it is worth mentioning that, despite so many years that have passed from the AmB discovery, only the very original molecule of the antibiotic solubilized with sodium deoxycholate (commercial name Fungizone) is used in clinics. Although, there were efforts to introduce methyl ester of AmB as a second generation of the drug, but it appeared that this new derivative was neurotoxic.[12] Searching for new derivatives of a parent drug molecule is usually very fruitful and is a typical way to introduce second or even third generation of the parent drug. However, in the case of AmB, this way is hindered due to the lack of knowledge concerning the molecular mechanism of AmB action. Nevertheless, studies on the mechanism of action of AmB and its new derivatives have been continued in many laboratories.[7,8,13,14] New derivatives were synthesized in our laboratory which are considerably less toxic than AmB, which shows that it is possible to reduce toxicity of the parent molecule by chemical modifications.[15–17] Other derivatives of AmB were obtained by other groups specifically to study the mechanism of action of this antibiotic.[18–25] All these new derivatives are currently being regarded and studied only as model compounds, and further studies are necessary to design and develop an efficient new drug.[16,26–28]

AmB is a membrane-active compound, and this means that components of cellular membrane constitute molecular targets for the antibiotic. Although this review focuses on AmB, some preliminary studies carried out in our laboratory, which concern AmB derivatives (Fig. 1), will also be presented. The molecular mechanism of AmB action has been extensively

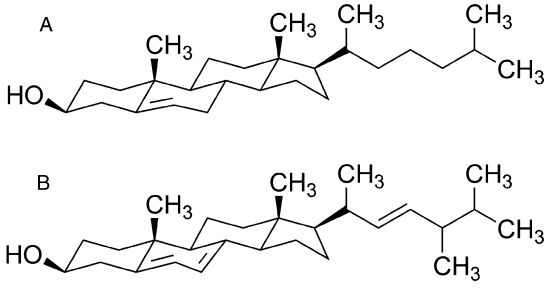

Fig. 3. Chemical structures of (A) cholesterol and (B) ergosterol molecules.

studied by many groups using many different methodologies. In this paper, we will present a comprehensive overview of different studies on this mechanism, but the main effort will be directed toward the presentation of molecular modeling studies which are quite new and promising directions in this field. We believe that by such a multidisciplinary approach, we will be able to finally make a breakthrough in the understanding of the AmB mechanism of action. In consequence, this new knowledge may help to develop a less toxic antifungal drug based on the parent AmB molecule.

Current View of AmB Mechanism of Action

Hundreds of papers have been published about the AmB mode of action. Many often contradicting and inconsistent data were collected through the years concerning the AmB membrane activity. Studies were performed on different mammalian and fungal cells as well as on cell membrane models (lipid monolayers, planar bilayers, and liposomes)[13,14,29] using many experimental techniques. Nevertheless, the molecular mechanism of AmB action is still not clear. There are several hypotheses but there is no consistent theory covering all aspects of this mechanism. Only some fragmentary data are well established, and based on these elements the following working hypothesis can be presented.

AmB is well known as an antifungal agent but it also exhibits antivirus, antiprotozoal, and antiprion activity.[3,7,30–32] The antifungal action of AmB is related directly to the membrane activity of the antibiotic molecules. According to the experimental data reviewed in literature, the molecules of the antibiotic form ionic transmembrane channels.[13,14,29,33] These channels alter/disturb membrane permeability, causing leakage of cell components (mainly potassium ions), which in consequence leads to cell death. It is accepted that higher activity of AmB toward fungal vs. mammalian cells is due to the differences in sterol content.[34,35] Mammalian cell membranes contain cholesterol and fungal cell membranes ergosterol (Fig. 3). The permeability caused by AmB is higher in ergosterol-containing membranes.[36] However, only slightly higher activity of AmB against fungal vs. mammalian membranes is

responsible for the toxicity of the antibiotic. The toxic effects of AmB may also be linked with oxidative damage caused to cells by the antibiotic present in membrane and additionally by interaction of AmB with blood plasma proteins.[7,37,38]

The molecular mechanism of AmB membrane activity and especially the details concerning the antibiotic transport into the plasma membrane, channel formation, and a structure of the pore are only hypothesized.[13,14,33–35,39,40] AmB is an amphiphilic and amphoteric molecule and therefore, tends to associate in distinct polar (aqueous media) and nonpolar (membrane interior) environment. In an aqueous solution outside the cell, the AmB molecules aggregate at a concentration as low as 10^{-6} M.[41,42] On the other hand, inside the membrane the AmB molecules form channels.[39,40] In aqueous media, the nonpolar parts of AmB (Fig. 1) are hidden from the water, and in the membrane they are exposed to lipids. The association state of the antibiotic, lipid composition, and physical state of the membrane are therefore important for AmB transport into the membrane.[13,14,29,43,44]

Depending on the concentration of the antibiotic, monomers, dimers, or soluble oligomers of AmB reach the membrane surface and subsequently have to enter the membrane. The behavior of AmB molecules at the membrane surface is still a puzzle and only recently some models have been postulated based on the studies of lipid model membranes about AmB. The presence of the helical aggregates was proposed by Milhaud et al.[45,46] The surface-adsorbed monomeric forms of the antibiotic (AmB and nystatin, another polyene macrolide antibiotic, which is structurally very similar to AmB) were also observed.[47,48] Different dimers of AmB molecules were recorded by Gruszecki et al.[49] On the other hand, higher order aggregates were observed by atomic force microscopy on the surface of mica-supported membranes.[46] The stability of these aggregates was associated with sterol content and the hollow-centered structures were observed when ergosterol was present in the lipid bilayer.[46] Moreover, the importance of the interactions between the polar head of AmB and the phospholipid polar heads present at the membrane surface, was pointed out by other groups.[50–52] Both AmB and most phospholipids (bearing a choline or ethylamine group) are zwitterionic molecules and may strongly interact with each other through electrostatic forces. Actually, the AmB–phospholipid interactions through their polar heads might be considered a driving force either for the formation of the supramolecular complexes at the membrane surface or for the transport of the antibiotic molecules into the lipid bilayer.

Studies on the behavior of AmB molecules inside the membrane are probably even more difficult than at the membrane surface, due to the fact that plasma membrane is a very heterogeneous medium. For these studies, lipid model membranes in a form of monolayers, planar bilayers, and liposomes, were used.[13,14] These model membranes may contain different phospholipids and sterols and may exist in a gel- and/or liquid-crystalline state. Concerning chemotherapeutic aspects, the most interesting was to trace the AmB–sterol interactions. The early ^{2}H-NMR studies indicated that there are some kind of AmB–cholesterol interactions inside the liposome membrane and that sterol molecule acts as a "buffer" separating the antibiotic molecule from interaction with phospholipids.[53] Nevertheless, the question still remains if sterol is a necessary building component of the channel or it indirectly influences channel formation by modifying properties of the membrane.[54–58] There is only one example that shows that sterol may be directly involved in AmB channel formation. Studies about the enantiomeric form of cholesterol indicated that AmB permeability of membranes containing cholesterol and *ent*-cholesterol is different from what may suggest that sterol molecules interact in a specific way with the AmB molecules in the channel.[59] On the contrary, direct interactions between AmB and dimyristoyl-phosphatidylcholine (DMPC) lipids were observed which led to the ordering of lipid acyl chains.[60] Only some data from the work of Coutinho et al., obtained for nystatin, support an idea that the antibiotic–ergosterol complexes are formed in the lipid bilayer.[48] It was also pointed out that AmB dimers exist in the membrane.[61] Such dimers were also previously considered as building blocks for the channels.[29]

Permeabilization of the membrane by AmB is regarded as a dominant chemotherapeutic action of the antibiotic and therefore, majority of studies carried out about model membranes were devoted to reveal the mechanism of AmB pore formation. Based on the very early permeability studies, a model of AmB channel in the membrane was proposed.[39,40] According to this model, the channel contains eight AmB molecules and the same number of sterol molecules (Fig. 4). The internal diameter of the channel lumen was estimated to be equal to 0.8 nm. Sterol molecules are indispensable elements of the channel in this model. On the contrary, there are data indicating that AmB channels can also form in membranes lacking sterols.[54,55] However, these data are challenged as being not relevant to AmB concentration reached at physiological conditions.[48] Another problem concerning the structure of the AmB channel was its transmembrane length. It seems that the channel proposed by De Kruijff and Demel called single-length channel (SLC), is able to span only part of the lipid bilayer because it is too short.[40] Therefore, this model was extended and the new model assumed that two SLCs can form a so-called double-length channel (DLC).[62,63] However, it has to be stressed that a DLC is expected to form when the antibiotic is added to both sides of the lipid bilayer and this situation does not correspond to the physiological conditions in which AmB is delivered to cells only from one side, i.e., extracellular. Definitely, membrane permeability caused by AmB depends on the length of acyl chains of phospholipids and for

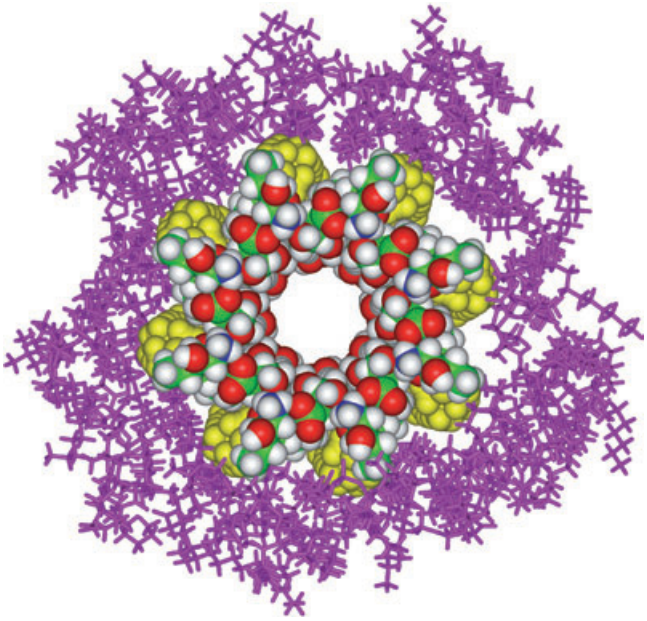

Fig. 4. Model of amphotericin B (AmB)-sterol channel; sterol (yellow balls), AmB (*CPK* balls), phospholipids (magenta sticks). The view presents entrance to the channel from the side containing polar heads of the antibiotic molecules.

the longer ones it is unlikely that SLC can span the whole membrane. On the other hand, one can assume that phospholipids surrounding the channel somehow adopt a proper position in order to enable SLC to span the whole lipid bilayer.[35] This latter behavior of phospholipids with different acyl chain lengths, added to membrane as minor constituents, was observed experimentally in the studies of liposome permeability by AmB.[64] Recently, the formation of the AmB SLCs in DMPC membrane as dominant forms was also confirmed by ^{13}C-NMR studies.[65] One may assume that the formation of DLC would be more difficult than that of SLC and the stability of DLC would be lower than that of SLC (mainly because two leaflets of the lipid bilayer are to some extent independent). Taking into account the available data and this assumption, one has to admit that a model of SLC is more relevant to physiological conditions and looks more convincing. The molecular structure of AmB SLC and especially its size and stoichiometry were based on permeability studies.[39,40,66] In this model, eight AmB molecules are arranged in a form of the pore sticking their hydroxyl groups toward the center of the pore (Fig. 4). The structure of this channel has never been confirmed by experimental methods. However, many theoretical studies support and use this model.[67–74] Moreover, the conformational analysis of AmB membrane channel performed by Silberstein indicates that the stoichiometry proposed originally in the early seventies (i.e., eight AmB molecules per channel) is preferable.[72] Similar results were obtained by Baginski[75] in the tests before the preparation of the initial model of the channel to the molecular dynamics (MD) simulations.[71] In these tests, very different models were taken into account, built from different than the eight AmB molecules as well as taking different orientations of the antibiotic (not only head-to-head but also head-to-tail—similar to the AmB dimer structure observed in the solution).[76] Generally, these studies show that the channel comprising eight AmB molecules (Fig. 4) is the most compact one, and at the same time such structure assures a proper diameter of the pore to be around 0.8 nm. The proposed model of the channel (SLC) is also supported by computational chemistry studies (thermodynamic and electrostatic calculations)[68,70,73] which show that through such a channel both potassium and chloride ions can pass freely, as it was observed experimentally.[77]

The most mysterious and still unknown element of the AmB molecular mechanism of action is the way by which the antibiotic molecules enter the lipid membrane. Currently, only very speculative mechanisms are considered: (i) a "one-step mechanism" and (ii) a "sequential mechanism."[14] In the "one-step mechanism," a certain critical number of AmB molecules have to embed in the membrane surface and then such an aggregate would enter the membrane interior. In this case, the channel could form after reorganization of the AmB molecules inside the membrane. This mechanism can be indirectly supported by the recent work of Silva et al. where the authors showed that the average number of nystatin molecules per lipid vesicle has to reach about 100, in order to see a transient membrane permeability.[57] In the "sequential mechanism," it is assumed that dimers or monomers of AmB can enter the membrane sequentially. Dimers of AmB are more likely to do this because they can hide their polar parts from the lipid environment. Being inside the membrane, AmB dimers would diffuse in order to find other AmB associates and to form the channel. In both mechanisms, sterol could play an active role helping to reorient AmB molecules and further form the channel. However, this active role of sterol in the AmB channel formation or stability is still not clear and will be further discussed in our review.

Concerning molecular mechanism of action of the AmB derivatives, generally, it is expected to be similar to the parent AmB. However, different selective toxicities of these new compounds have been observed which may suggest that this mechanism could be different in ergosterol- and cholesterol-containing membranes. For instance, N-methyl-N-D-fructosyl amphotericin B methyl ester (MFAME) is much less toxic than AmB,[15] and the reduced toxicity of MFAME is partly attributed to the lower concentration of water-soluble oligomers of this derivative.[26] This observation is in agreement with that of a previous study, that the monomeric form of AmB can better differentiate between membranes containing cholesterol and ergosterol.[43] As it was stated in the Introduction, the different AmB derivatives were specially designed

to probe the AmB mechanism of action. A series of such derivatives were synthesized by Murata's group.[19] They obtained covalently linked conjugates containing AmB-AmB, AmB-sterol or AmB-phospholipid molecules.[18,20,78,79] These conjugates are important with regard to the type of interactions expected in the channel as it was shown that these homo/hetero-covalent dimers reproduce respective interactions in the complex exhibiting hemolytic activity.[19] Interestingly, AmB-ergosterol conjugate is more efficient in channel formation than AmB-cholesterol conjugate, which may imply the direct interaction between AmB and ergosterol in the membrane.[18] Recently, it was also shown by solid-state NMR that AmB derivative, containing a linker between amino and carboxyl AmB groups, is able to form large aggregates especially in ergosterol- and not cholesterol-containing DMPC lipid bilayers.[24] This observation points out that only ergosterol plays a direct role in the AmB aggregate/channel formation.[24]

Lipid Membranes as Targets for AmB

The permeabilization action of AmB appeared to be a primary chemotherapeutic activity of this antibiotic and therefore, cell membrane is regarded as a target for the AmB molecules. At this point, it is worth underlining that it is not a typical situation in chemotherapy that a membrane, which is a heterogeneous and multicomponent structure, is a target for the drug. The situation is even more complex, because AmB acts as a supramolecular complex (i.e., a channel) and not as a single molecule as many proteins do. This means that AmB-membrane system is far from a typical drug-target tandem (a classical key-lock system) where the drug is a single molecule (e.g., inhibitor) and the target is also a single macromolecule (e.g., enzyme). Due to this complication, the mechanism of AmB action at the membrane level is still not elucidated.

Eukaryotic cell membranes consist of different arrays of glycerophospholipids and sphingolipids that vary in head group and acyl chain composition. Additionally, these biomembranes contain sterols (cholesterol in mammalian cells and ergosterol in fungal cells). A classical view of cell membranes as fluid-mosaic model of Singer and Nicolson[80] was recently extended by the discovery of membrane microdomains (e.g., lipid rafts).[81] Lipid rafts are conceived as so-called liquid-ordered (*lo*) functional lipid microdomains, rich in sphingolipids and cholesterol.[82–84] The phase diagram for the mixture of cholesterol and phospholipids indicates that the lipid bilayer can exist in gel and in liquid-ordered phase which is unique for cholesterol and higher sterols.[85–87]

Concerning the so-called sterol hypothesis which states that AmB is fully active, i.e., exhibits functional permeability action, only in biomembranes which contain sterols, one has to assume that a direct sterol–AmB interaction is presumably important for the antibiotic mechanism of action. However, the structural role which sterol plays in membrane may also be important for AmB membrane activity and this role of sterol can be regarded as an indirect effect. The influence of sterols on membrane physicochemical properties, especially the effect of cholesterol, has been studied for decades and has been summarized in some recent reviews.[86–88]

In order to understand the molecular mechanism of AmB selective action, i.e., higher activity against ergosterol- than cholesterol-containing membranes, the difference between these two types of membranes should be elucidated. Unfortunately, contrary to extensive studies on cholesterol, physicochemical properties of membranes containing ergosterol are less known.[86,89] However, the situation has greatly improved because general interest in studies of different membrane properties steadily increases.[90] For instance, it was found that lipid rafts are not only present in cholesterol-containing membranes but are likely to exist also in membranes containing ergosterol and sphingolipids.[91,92] Moreover, it was found that the formation of these rafts (ordered lipid domains) is promoted more strongly by ergosterol compared to cholesterol.[93] Recently, it was also shown (phase diagram) that ergosterol, similarly to cholesterol, can promote the separation of liquid-crystalline dipalmitoyl-phosphatidylcholine (DPPC) bilayer into coexisting *lo* and liquid-disordered (*ld*) phases.[94] Several reports demonstrated that the ability of ergosterol to order saturated lipid acyl chains (DMPC and DPPC) is substantially higher than that of cholesterol.[95–98] The situation is reverse in the case of unsaturated lipid acyl chains of palmitoyl-oleoyl-phosphatidylcholine.[89,95,98] Performed micropipette aspiration experiments also showed that elasticity and phase behavior of DPPC membrane are modulated differently by ergosterol and cholesterol.[96,99] Moreover, studies of anisotropic motions of both sterols in DPPC membrane revealed that the cholesterol molecule is more prone than ergosterol to cross the bilayer midplane.[100]

The structural differences between both sterols are responsible for the differences between cholesterol- and ergosterol-containing membranes. However, despite many previous efforts, the relationship between the chemical structure of different sterols and their influence on the lipid bilayer has not yet been precisely determined. Our previous studies of the molecular properties of both sterols pointed out to only some differences which can be crucial for sterol–lipid or sterol–AmB interactions.[101,102] Conformational analysis performed for both sterols revealed that ergosterol molecule is more flat than cholesterol due to the presence of less flexible side chain.[101] On the other hand, studies of the distribution of molecular electrostatic potential for both sterols showed that ergosterol, a molecule containing two additional carbon–carbon double bonds (one in ring B and one in the side chain, Fig. 3), generates extensive negative potential next to the ring B and in the

side chain.[102] A similar negative potential was not observed for the cholesterol molecule. In consequence, these different electrostatic patterns for cholesterol and ergosterol molecule may lead to differences in intermolecular interactions between sterols and lipids, as well as sterols and AmB.

Due to their biological importance, lipid membranes were a subject of extensive studies by different methods (e.g., solid-state NMR, fluorescence techniques, differential scanning calorimetry, etc.). However, using these methods to study complex and heterogeneous lipid systems, it is possible to obtain data corresponding solely to their macroscopic physical properties. The only approach that gives molecular/atomic resolution in studies of biomembranes is the so-called computational chemistry or molecular modeling approach. The successful applications of molecular modeling methods to study membranes were described in many recent reviews.[103–106] Continuous progress of computer technology has enabled to carry out longer simulations, and hence it has become possible to study mixed membranes containing either two types of lipids or lipid and sterol. For the scope of this review, it is worth recalling several studies of lipid model membranes with sterols.[107–116] It has to be also pointed out, that there are only very few studies of membranes containing ergosterol.[117,118] The models of membranes containing not only phospholipids but also sterols are the most suited to study AmB–membrane interactions by molecular modeling methods. One of such models has been used by our group (Fig. 5)[118] but other models containing only pure phospholipids (DMPC), have also been applied as the reference systems.

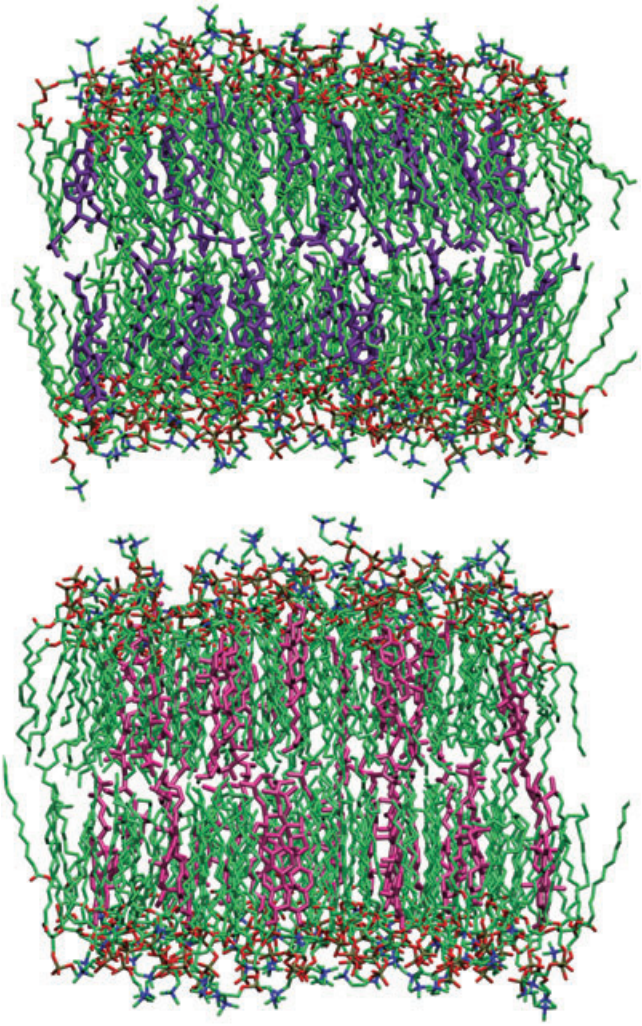

Fig. 5. The final molecular dynamics (MD) frames of the hydrated lipid bilayer models containing 128 dimyristoyl-phosphatidylcholine molecules (green) and 42 cholesterol (violet; top panel) or ergosterol (pink; bottom panel) molecules—the structures come from the MD simulation carried out by Czub and Baginski.[118] For the sake of clarity, water molecules were omitted.

The Antibiotic–Membrane Interactions

In order to study the interactions between AmB or its derivatives with membranes by molecular modeling methods, it was necessary to build and/or select the appropriate model systems.[14,119] These systems had to include an adequate model of lipid membrane, a position (e.g., vertical or horizontal) of the antibiotic molecule(s), and a structure of possible supramolecular complexes formed by the antibiotic molecules. Concerning a model of lipid membranes, three different models have been used: (i) a simple model of membrane, simulated as a low dielectric slab,[14,68,73,102] (ii) a model of lipid bilayer, containing only phospholipids (DMPC),[51,120,121] and (iii) a mixed lipid bilayer, containing DMPCs and sterols (cholesterol or ergosterol).[71,73,74,122] Concerning the position of antibiotic molecules and the stoichiometry of possible complexes, monomers,[51,102,121–123] dimers,[120] and channels[14,68,71,73,74] have been studied. Most of the studies included only AmB but some preliminary studies were also performed for AmB derivatives.[121,123] As for the methodology, different approaches have been applied, including MD, methods based on solution of the Poisson–Boltzmann equations, the semiempirical quantum chemistry methods, and Monte Carlo methods. To make our presentation more concise, the overview of interactions between the antibiotic and lipid model membranes is described next separately for monomers and higher antibiotic associates.

Monomers of the Antibiotic

The studies on the antibiotic monomers interacting with lipid membrane were undertaken in order to find out how the antibiotic molecules interact with membrane components at the membrane surface and inside the lipid bilayer. Another puzzling problem was to solve whether a single AmB molecule is able to enter the lipid bilayer. It was also interesting to find

out if there are substantial differences between AmB and its derivatives concerning membrane interaction.

The very early studies of AmB monomers, with a simple model of membrane simulated as a low dielectric slab, were performed 10 years ago.[102] In this work, the molecular electrostatic potential of AmB was calculated at the molecular surfaces. Our study revealed that both in water and in lipid environment, the antibiotic molecule preserves an amphiphilic/amphipatic character, which is more pronounced in nonpolar membrane. For this reason, AmB molecules have to form different supramolecular hetero- or homo-complexes inside the membrane, in order to hide the polar parts of their molecule.

The studies of AmB and its derivatives interacting with more elaborated lipid membranes containing explicit lipid molecules (DMPC) were performed using MD methods.[51,121,122] Only homogeneous membranes containing DMPCs were used, but this approach should be regarded as the first step in studies of AmB–membrane interactions. On the other hand, the simulations of systems containing only lipids (not sterols) could be compared to many experimental studies that were performed with such membranes. Despite simplicity of the models, our studies provided several important observations which were possible to record only due to the application of molecular modeling methods. First, it was found that AmB molecules present at the surface of the membrane (both horizontal and vertical position was simulated) interact very strongly with the polar heads of phospholipids. Having analyzed the MD trajectories, it was even possible to record lateral diffusion of AmB–lipid complexes.[51,121] It was also revealed that AmB molecules can change position at the surface of lipid bilayer from horizontal to vertical.[51] This observation may suggest that AmB molecules are prone to form a monolayer at the membrane surface. Similar behavior of AmB was shown in experimental studies where such monolayers were observed at the air–water interface.[124] Concerning the behavior of AmB inside the lipid bilayer, it was found that AmB molecules exhibit an ordering effect of lipid acyl chains (Fig. 6).[51,120] However, strong interactions between AmB and the surrounding nonpolar parts of lipid molecules have not been observed. Only the polar head of AmB was able to interact with the polar heads of lipids in a more direct way so the formation of AmB–lipid complexes could be postulated. One of the most interesting properties monitored within the MD simulations were torsional angles φ, ψ (see definition of these angles in Fig. 1), defining mutual position of the aglycon, and the sugar moiety of AmB (including its derivatives). Previous studies showed that it is possible to distinguish two main conformers of the semiflexible AmB molecule: an "open" and a "closed" one.[125,126] When AmB takes the "closed" conformation, the carbonyl and the amino groups of the molecule are close together and they may form an intramolecular hydrogen

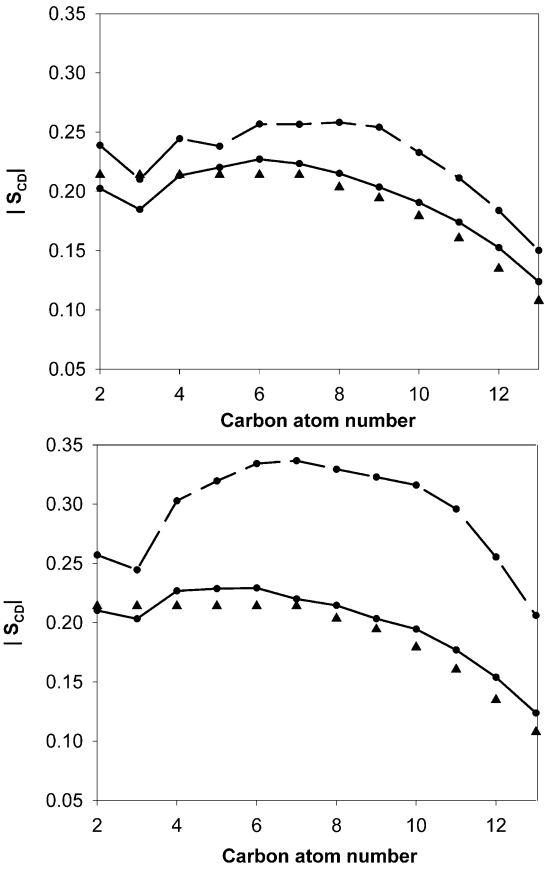

Fig. 6. The deuterium order parameter profiles calculated for the dimyristoylphosphatidylcholine (DMPC) acyl chains from the simulation of the lipid bilayer systems containing amphotericin B (AmB) monomer (top) and dimer (bottom). The profiles averaged over all DMPC molecules in the systems are presented as solid lines with circles and the profiles obtained for six and eight DMPC molecules situated near AmB monomer and dimer, respectively, are shown as a dashed line with circles (data published only as the conference communication).[120] Additionally, experimental values of the $|S_{CD}|$ for the pure DMPC bilayer are presented (triangles).[128] The order parameter $|S_{CD}|$ was calculated according to the formula (for details see ref. 122):

$$|S_{CD}| = \left\langle \frac{3}{2}\cos^2\beta - \frac{1}{2} \right\rangle$$

where β is the angle between the C—D or (C—H) bond vector (in DMPC acyl chains) and the bilayer normal).

bond through the water molecule. In the case of an "open" conformation, both mentioned groups may form only intermolecular hydrogen bonds either with other antibiotic molecules (as it is in the channel)[71,74] or with polar groups of surrounding lipids[51,120]. The distribution of φ, ψ angles for AmB and its derivatives, when they are present at the membrane surface, is given in Figure 7. The φ, ψ data for AmB molecules inside the membrane and AmB channels are also presented. Analyzing the data in Figure 7, it is interesting to note that the distribution of these angles for SAmE and PAmE

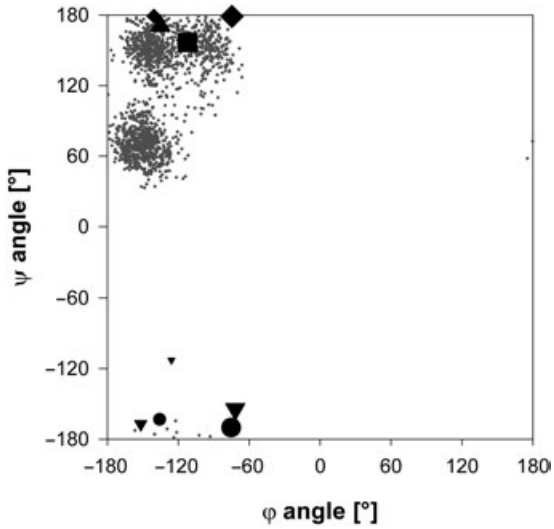

Fig. 7. Ramachandran map of the φ and ψ torsion angles for the antibiotic (Fig. 1), obtained for the amphotericin B (AmB) monomer embedded in the pure dimyristoyl-phosphatidylcholine bilayer (gray dots). Additional symbols show the average values of the φ and ψ torsion angles for: (i) the AmB conformers observed in the aqueous phase at the membrane surface (diamonds),[121] (ii) the SAmE conformers observed in the aqueous phase at the membrane surface (triangles),[121] (iii) the PAmE conformers observed in the aqueous phase at the membrane surface (squares),[121] (iv) the AmB conformers observed in the transmembrane channel structure AmB–cholesterol (circles), and AmB–ergosterol (inverted triangles).[74] The size of each symbol is proportional to the population of this particular conformation.

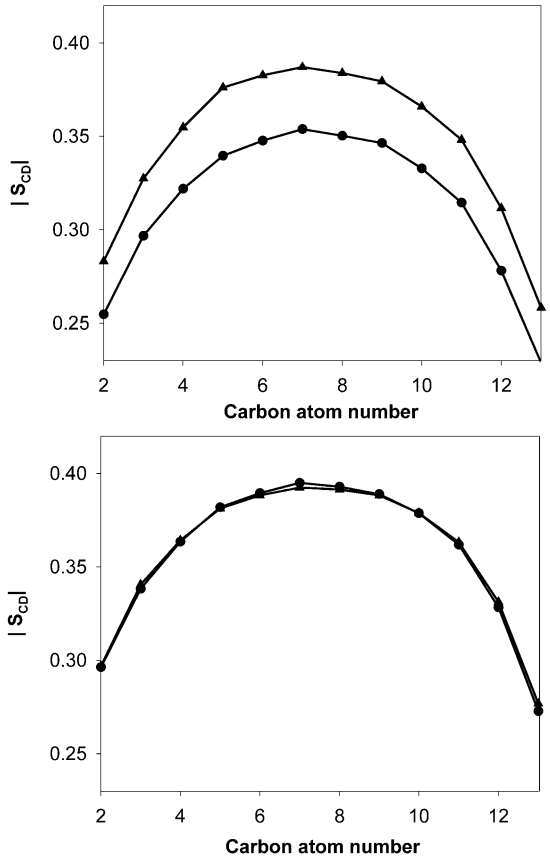

Fig. 8. Profiles of the deuterium order parameter calculated for the dimyristoyl-phosphatidylcholine acyl chains in the lipid bilayer systems containing ~25 mol % of cholesterol (top) or ergosterol (bottom).[122] In addition to the order parameter values calculated for amphotericin B-containing systems (triangles), we present profiles obtained in similar systems without antibiotic molecules (circles).[118]

is very similar to that present in AmB channels ("open" conformers), which may suggest that studied derivatives are more ready to form intermolecular complexes (e.g., channels).

The MD simulations of AmB and its derivatives were also carried out with lipid bilayers containing sterols.[122,123] Such systems are the most relevant to the physiological conditions. Only monomers of the antibiotic were considered so far but higher order associates of AmB are currently a subject of study in our group. Performed calculations revealed that sterols modulate interactions of AmB molecules with lipids and that this modulation is different for ergosterol and cholesterol. First of all, it was found that AmB molecules are able to order lipid acyl chains but it was observed only in cholesterol-containing membranes (Fig. 8).[122] The addition of AmB to ergosterol-containing membranes did not further increase the ordering effect. The position of AmB molecules inside the lipid bilayer was also different in cholesterol- and ergosterol-containing membranes. AmB in the latter membranes was more tilted with regard to the bilayer normal axis. Some differences between these two systems were also recorded concerning dynamic properties of AmB (e.g., lateral diffusion, wobbling motions).[122] In general, after analyzing the interaction of AmB molecules with membranes containing sterols, it was concluded that AmB exhibits higher affinity toward the sterol-containing *lo* phases, and, therefore, may be accumulated in ordered membrane domains.[122] It was also stated that because the partition coefficient between the *ld* and *lo* phases appears to be greater in the case of ergosterol- than cholesterol-containing membranes, this effect may be the possible origin of the AmB selectivity.

Concerning the behavior of SAmE and PAmE inside the membranes containing sterols, some differences were encountered (a full set of data will be published elsewhere).[123] The esterification-induced shift of the AmB's carboxyl group causes the displacement of the whole macrolide part of the molecule toward the center of the membrane. The tilt angle of the studied molecules is different in pure DMPC- and sterol-rich lipid bilayers and some differences are also observed between cholesterol- and ergosterol-containing membranes. Chemical modifications of the AmB ionizable groups cause the significant change in the pattern of interactions (i.e., hydrogen bonds) with neighboring phospholipid molecules. Moreover, the chemical modifications of AmB structure cause the

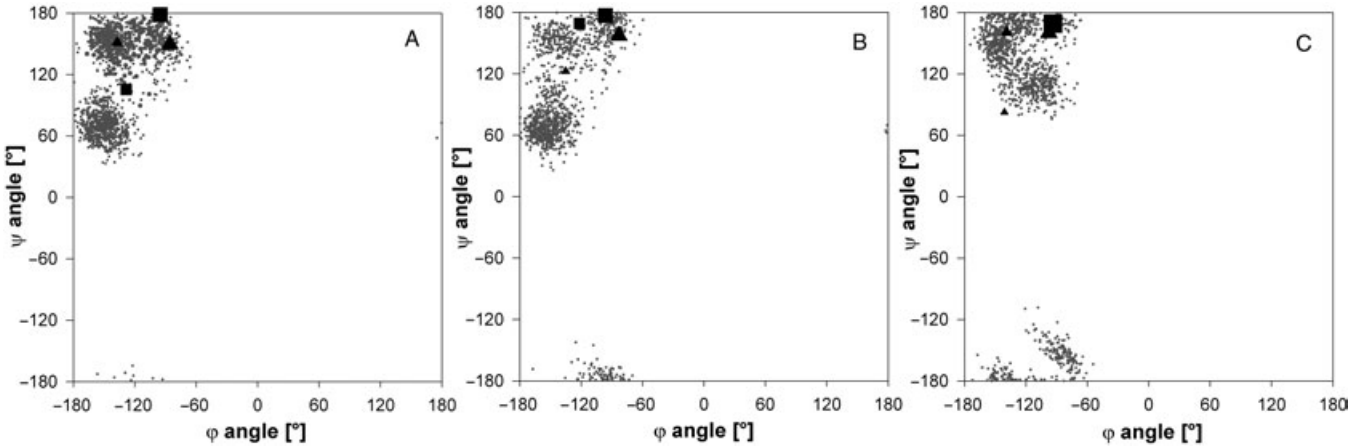

Fig. 9. Ramachandran map of the φ and ψ torsion angles for the antibiotic (gray dots), obtained for the amphotericin B monomer embedded in the pure dimyristoyl-phosphatidylcholine (DMPC) bilayer (A), DMPC-cholesterol bilayer (B), and DMPC-ergosterol bilayer (C).[123] Additional symbols show the average values of the φ and ψ angles for SAmE (triangles) and PAmE (squares) conformers observed inside of three different membranes.[123] The size of each symbol is proportional to the population of this particular conformation.

reasonable accentuation of the difference in the range of the allowed arrangements of molecules in both sterol-containing membranes. Thus, one could propose that while the induction of the vertical orientation enables the activity of polyene macrolides in both sterol-containing membranes, the differentiation of this activity in the case of the derivatives may result from the varied amplitude of the wobbling motion and position of the derivative molecule in the membrane.

It is also worth mentioning that the distribution of φ, ψ angles for AmB and its derivatives inside the membranes containing sterols is slightly different than for sterol-free membranes (Fig. 9). Moreover, a comparison of the data in Figures 7 and 9 indicates that AmB derivatives prefer to take more "open" conformation compared to the parent molecule, which apparently is a dominant one in the channel structure. This observation may also be related to increased drug selectivity because one may assume that such derivatives will be more prone to form the channel.

Complexes of the Antibiotic

The simplest complex of AmB is a dimer (Fig. 10). This system was studied because it had been postulated earlier, that dimers of AmB can enter the membrane and are the simplest building blocks of the channel. Head-to-head dimers of AmB molecules immersed in the lipid bilayer (DMPC lipids) were studied by MD methods.[120] As it is seen in Figure 10, AmB molecules hide polar parts (containing hydroxyl groups) inside the dimer. This behavior of AmB dimer agrees with previous calculations of molecular electrostatic potential for AmB and can be regarded as a typical behavior of amphiphilic molecules in a nonpolar environment.[102] Analyzing the interactions

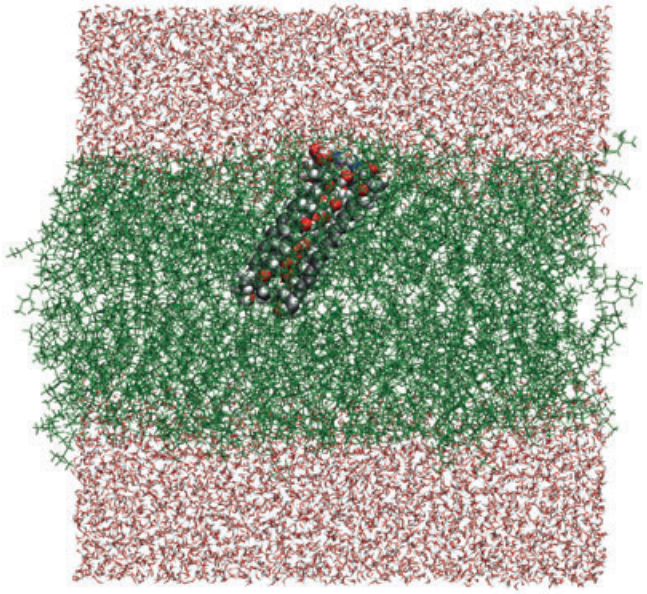

Fig. 10. The molecular dynamics snapshot of AmB dimer (*CPK* model) immersed in the pure dimyristoyl-phosphatidylcholine bilayer (green).[120] Water molecules (red) are also present in the figure to show the interface lipid–water contact. Please note the characteristic tilt of the AmB complex with regard to bilayer normal axis and the fact that hydroxyl groups are hidden inside the complex.

between AmB and DMPC molecules, it was found that monomers of AmB order the acyl lipid chains less effectively than dimers of AmB do (Fig. 6).[120] The ordering effect stems from the fact that AmB molecules are quite rigid and elongated. It appears that the head-to-head dimer of AmB, containing two such rigid molecules, exhibits a superadditive effect. This observation could be discussed with regard to the

mechanism of channel formation. Very recently, it was proposed that nystatin channels form at the boundaries of lipid domains.[127] Lipid domains are more ordered than other parts of the membrane and therefore, dimers of AmB, ordering lipid acyl chains to higher degree than monomers, would be ready to anchor at lipid domain boundary and subsequently form the channel.

The AmB channels, as the most relevant aspect of chemotherapeutic activity of the antibiotic, were studied using different model membranes and methodology. A simple model membrane, as a low dielectric slab, was used to study the thermodynamic profile of ion passage through the channel[68,73] and to calculate the molecular electrostatic potential at the "mouth" of the channel.[14] However, ambiguous results were obtained due to the complexity of the system. Nevertheless, it was observed that potassium or sodium cations can pass freely through the channel along the concentration gradient, with no potential barriers or wells, and potential well was recorded only at the channel "mouth" region. This negative potential well or rather ion "trap" is different for channels containing cholesterol (exhibiting deeper well) and ergosterol (exhibiting shallow well).[14] Such difference may indicate that the former channels are less efficient in cation passage.

The most detailed studies of AmB channels immersed in the lipid bilayer (DMPC molecules) were performed using MD methods.[71,74] Models of SLC channels containing eight molecules of the antibiotic and eight cholesterol or ergosterol molecules were used (Fig. 4). The extensive analysis of MD trajectories revealed that there are many different interactions (chains of hydrogen bonds) between functional groups of adjacent AmB molecules. It was also found that the AmB polar heads can interact with the polar heads of DMPC molecules and that this is very complementary interaction (both molecules are zwitterions). The most meaningful data were obtained in comparative studies of AmB-cholesterol and AmB-ergosterol channel.[74] It was observed that the latter channel is more stable and has a higher internal diameter. This fact may explain, at least to some extent, the higher permeabilization efficacy of AmB exhibited in ergosterol- compared to cholesterol-containing membranes.

Conclusions and Outlook

The studies of interactions between AmB or its derivatives and lipid model membranes performed in our group by molecular modeling methods have shown that this approach is equally valuable as the experimental one. Nowadays, different computational chemistry and molecular modeling techniques can be regarded as equivalent tools to study lipid membranes and membrane–ligand interactions. In the case of AmB, this approach provided a deep insight into the molecular level of the AmB mechanism of action at the membrane level. Obtained atomic details in these studies are beyond experimental possibilities. Nevertheless, molecular modeling should be regarded only as a complementary approach to experimental studies. After analyzing different recent data, it seems that such parallel experimental/computational approach can bring a breakthrough in studies of molecular AmB mechanism of action.[14,23,119] The most essential element of this mechanism is the interaction of the antibiotic molecules with the lipid membrane. These systems, presented as models, can be simulated explicitly by different molecular modeling techniques and may contain different types of lipid and/or sterol molecules. Currently, systematic studies of such systems are carried out and part of the available data were presented in this review. This approach can be especially valuable in light of a new paradigm concerning membrane structure, i.e., existence of liquid-ordered domains in the membranes. These domains are sterol rich. At the same time, sterol is required for AmB membrane activity. On the other hand, sterols and AmB molecules order the lipid chains in a similar way. This means that both molecules may exhibit similar affinities toward *lo* lipid domains. Studies of these domains and their interactions with AmB and its derivatives may become fruitful direction and could be a breakthrough step in the understanding of the mechanism of AmB channel formation and its stability. Thus, there is a hope that joint experimental and computational efforts will result in a new hypothesis concerning AmB molecular mechanism of action, including its selectivity.

The authors would like to thank Prof. Edward Borowski for his continuous support in our studies of polyene macrolides. The authors also thank the TASK Computational Center (Gdansk, Poland) for granting CPU time.

REFERENCES

[1] Omura, S.; Tanaka, H. Macrolide Antibiotics; Academic Press, Inc.: Orlando, FL, 1984, Chapter 9.
[2] Zotchev, S. B. Curr Med Chem 2003, 10, 211.
[3] Aparicio, J. F.; Mendes, M. V.; Anton, N.; Recio, E.; Martin, J. F. Curr Med Chem 2004, 11, 1645.
[4] Gallis, H. A.; Drew, R. H.; Pickard, W. W. Rev Infect Dis 1990, 12, 308.
[5] Meis, J. F. G. M.; Verweij, P. E. Drugs 2001, 61, 13.
[6] Herbrecht, R.; Nivoix, Y.; Fohrer, C.; Natarajan-Ame, S.; Letscher-Bru, V. J Antimicrob Chemother 2005, 56, 39.
[7] Hartsel, S.; Bolard, J. Trends Pharmacol Sci 1996, 17, 445.
[8] Borowski, E. Farmaco 2000, 55, 206.
[9] Hillery, A. M. Adv Drug Deliv Rev 1997, 24, 345.
[10] Antoniadou, A.; Dupont, B. J Mycologie Medicale 2005, 15, 230.
[11] Schaffner, C. P. Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents., L.R. Prous Science Publishers, S.A.: Barcelona, 1987, Chapter 7.

[12] Ellis, W. G.; Sobel, R. A.; Nielson, S. L. J Infect Dis 1982, 146, 125.
[13] Bolard, J. Biochim Biophys Acta 1986, 864, 257.
[14] Baginski, M.; Cybulska, B.; Gruszecki, W. I. Advances in Planar Lipid Bilayers and Liposomes; Elsevier: Amsterdam, 2006, Chapter 9.
[15] Grzybowska, J.; Sowinski, P.; Gumieniak, J.; Zieniawa, T.; Borowski, E. J Antibiot 1997, 50, 709.
[16] Hac-Wydro, K.; Dynarowicz-Latka, P.; Grzybowska, J.; Borowski, E. J Colloid Interface Sci 2005, 287, 476.
[17] Slisz, M.; Cybulska, B.; Mazerski, J.; Grzybowska, J.; Borowski, E. J Antibiot 2004, 57, 669.
[18] Matsumori, N.; Eiraku, N.; Matsuoka, S.; Oishi, T.; Murata, M.; Aoki, T.; Ide, T. Chem Biol 2004, 11, 673.
[19] Matsumori, N.; Masuda, R.; Murata, M. Chem Biodivers 2004, 1, 346.
[20] Yamaji, N.; Matsumori, N.; Matsuoka, S.; Oishi, T.; Murata, M. Org Lett 2002, 4, 2087.
[21] Matsumori, N.; Sawada, Y.; Murata, M. J Am Chem Soc 2005, 127, 10667.
[22] Ibragimova, V.; Alieva, I.; Kasumov, K.; Khutorsky, V. Biochim Biophys Acta-Biomembr 2006, 1758, 29.
[23] Matsumori, N.; Oishi, T.; Murata, M. J Syn Org Chem Japan 2006, 64, 502.
[24] Matsumori, N.; Sawada, Y.; Murata, M. J Am Chem Soc 2006, 128, 11977.
[25] Paquet, V.; Carreira, E. M. Org Lett 2006, 8, 1807.
[26] Cybulska, B.; Gadomska, I.; Mazerski, J.; Grzybowska, J.; Borowski, E.; Cheron, M.; Bolard, J. Acta Biochim Pol 2000, 47, 121.
[27] Szlinder-Richert, J.; Mazerski, J.; Cybulska, B.; Grzybowska, J.; Borowski, E. Biochim Biophys Acta-Gen Subjects 2001, 1528, 15.
[28] Czerwinski, A.; Konig, W. A.; Zieniawa, T.; Sowinski, P.; Sinnwell, V.; Milewski, S.; Borowski, E. J Antibiot 1991, 44, 979.
[29] Hartsel, S. C.; Hatch, C.; Ayenew, W. J Liposome Res 1993, 3, 377.
[30] Warnock, D. W. J Antimicrob Chemother 1991, 28 Suppl B, 27.
[31] Ramos, H.; Valdivieso, E.; Gamargo, M.; Dagger, F.; Cohen, B. E. J Membrane Biol 1996, 152, 65.
[32] Adjou, K. T.; Deslys, J. P.; Demaimay, R.; Dormont, D. Trends Microbiol 1997, 5, 27.
[33] Kerridge, D. Adv Microb Physiol 1986, 27, 1.
[34] Medoff, G.; Brajtburg, J.; Kobayashi, G. S.; Bolard, J. Annu Rev Pharmacol Toxicol 1983, 23, 303.
[35] Gale, E. F. Macrolide Antibiotics; Academic Press, Inc.: Orlando, FL, 1984, Chapter 11.
[36] Teerlink, T.; De Kruijff, B.; Demel, R. A. Biochim Biophys Acta 1980, 599, 484.
[37] Brajtburg, J.; Elberg, S.; Bolard, J.; Kobayashi, G. S.; Levy, R. A.; Ostlund, R. E. J.; Schlessinger, D.; Medoff, G. J Infect Dis 1984, 149, 986.
[38] Barwicz, J.; Gruda, I. Recent Res Dev Antimicrob Agents Chemother 1997, 2, 105.
[39] Andreoli, T. E. Kidney Int 1973, 4, 337.
[40] De Kruijff, B.; Demel, R. A. Biochim Biophys Acta 1974, 339, 57.
[41] Mazerski, J.; Bolard, J.; Borowski, E. Biochim Biophys Acta 1982, 719, 11.
[42] Barwicz, J.; Gruszecki, W. I.; Gruda, I. J Colloid Interface Sci 1993, 158, 71.
[43] Bolard, J.; Legrand, P.; Heitz, F.; Cybulska, B. Biochemistry 1991, 30, 5707.
[44] Barwicz, J.; Tancrede, P. Chem Phys Lipids 1997, 85, 145.
[45] Milhaud, J.; Michels, B. Chem Phys Lipids 1999, 101, 223.
[46] Milhaud, J.; Ponsinet, V.; Takashi, M.; Michels, B. Biochim Biophys Acta-Biomembr 2002, 1558, 95.
[47] Lopes, S.; Castanho, M. A. R. B. J Phys Chem B 2002, 106, 7278.
[48] Coutinho, A.; Silva, L.; Fedorov, A.; Prieto, M. Biophys J 2004, 87, 3264.
[49] Gruszecki, W. I.; Gagos, M.; Herec, M. J Photochem Photobiol B Biol 2003, 69, 49.
[50] Minones, J.; Minones, J.; Conde, O.; Patino, J. M. R.; Dynarowicz-Latka, P. Langmuir 2002, 18, 2817.
[51] Sternal, K.; Czub, J.; Baginski, M. J Mol Model 2004, 10, 223.
[52] Gabrielska, J.; Gagos, M.; Gubernator, J.; Gruszecki, W. I. FEBS Lett 2006, 580, 2677.
[53] Dufourc, E. J.; Smith, J. C. P.; Jarrell, H. C. Biochim Biophys Acta 1984, 776, 317.
[54] Cotero, B. V.; Rebolledo-Antunez, S.; Ortega-Blake, I. Biochim Biophys Acta- Biomembr 1998, 1375, 43.
[55] Venegas, B.; Gonzalez-Damian, J.; Celis, H.; Ortega-Blake, I. Biophys J 2003, 85, 2323.
[56] Zumbuehl, A.; Stano, P.; Heer, D.; Walde, P.; Carreira, E. M. Org Lett 2004, 6, 3683.
[57] Silva, L.; Coutinho, A.; Fedorov, A.; Prieto, M. Biochim Biophys Acta-Biomembr 2006, 1758, 452.
[58] Silva, L.; Coutinho, A.; Fedorov, A.; Prieto, M. Biophys J 2006, 90, 3625.
[59] Mickus, D. E.; Levitt, D. G.; Rychnovsky, S. D. J Am Chem Soc 1992, 114, 359.
[60] Dufourc, E. J.; Smith, J. C. P.; Jarrell, H. C. Biochim Biophys Acta 1984, 778, 435.
[61] Gruszecki, W. I.; Gagos, M.; Herec, M.; Kernen, P. Cell Mol Biol Lett 2003, 8, 161.
[62] Marty, A.; Finkelstein, A. J Gen Physiol 1975, 65, 515.
[63] Van-Hoogevest, P.; De Kruijff, B. Biochim Biophys Acta 1978, 511, 397.
[64] Matsuoka, S.; Murata, M. Biochim Biophys Acta-Biomembr 2003, 1617, 109.
[65] Matsuoka, S.; Ikeuchi, H.; Matsumori, N.; Murata, M. Biochemistry 2005, 44, 704.
[66] De Kruijff, B.; Gerritsen, W. J.; Oerlemans, A.; Demel, R. A.; Van-Deenen, L. L. M. Biochim Biophys Acta 1974, 339, 30.
[67] Khutorsky, V. E. Biochim Biophys Acta 1992, 1108, 123.
[68] Bonilla-Marin, M.; Moreno-Bello, M.; Ortega-Blake, I. Biochim Biophys Acta 1991, 1061, 65.
[69] Golovanov, I. B.; Tsygankova, I. G. Mem Cell Biol 1995, 9, 335.
[70] Khutorsky, V. Biophys J 1996, 71, 2984.

[71] Baginski, M.; Resat, H.; McCammon, J. A. Mol Pharmacol 1997, 52, 560.
[72] Silberstein, A. J Membr Biol 1998, 162, 117.
[73] Resat, H.; Baginski, M. Eur Biophys J Biophys Lett 2002, 31, 294.
[74] Baginski, M.; Resat, H.; Borowski, E. Biochim Biophys Acta-Biomembr 2002, 1567, 63.
[75] Baginski, M. Unpublished results. The studies were performed during preparation of models of AmB channels for molecular dynamics simulations (ref. 71). The channels containing six, eight or ten AmB molecules were considered. Mutual positions of antibiotic and sterol molecules were also varied. Only the model containing eight AmB and sterol molecules, as the most compact, was subsequently studied by molecular dynamics and published (ref. 71).
[76] Balakrishnan, A. R.; Easwaran, K. R. K. Biochim Biophys Acta 1993, 1148, 269.
[77] Hartsel, S. C.; Benz, S. K.; Ayenew, W.; Bolard, J. Eur Biophys J 1994, 23, 125.
[78] Matsumori, N.; Yamaji, N.; Matsuoka, S.; Oishi, T.; Murata, M. J Am Chem Soc 2002, 124, 4180.
[79] Matsuoka, S.; Matsumori, N.; Murata, M. Org Biomol Chem 2003, 1, 3882.
[80] Singer, S. J.; Nicolson, G. L. Science 1972, 175, 720.
[81] Simons, K.; Ikonen, E. Nature 1997, 387, 569.
[82] Munro, S. Cell 2003, 115, 377.
[83] Edidin, M. Annu Rev Biophys Biomol Struct 2003, 32, 257.
[84] Simons, K.; Vaz, W. L. C. Annu Rev Biophys Biomol Struct 2004, 33, 269.
[85] Vist, M. R.; Davis, J. H. Biochemistry 1990, 29, 451.
[86] Mouritsen, O. G.; Zuckermann, M. J. Lipids 2004, 39, 1101.
[87] Mcmullen, T. P. W.; Lewis, R. N. A. H.; Mcelhaney, R. N. Curr Opin Colloid Interface Sci 2004, 8, 459.
[88] Ohvo-Rekila, H.; Ramstedt, B.; Leppimaki, P.; Slotte, J. P. Prog Lipid Res 2002, 41, 66.
[89] Henriksen, J.; Rowat, A. C.; Brief, E.; Hsueh, Y. W.; Thewalt, J. L.; Zuckermann, M. J.; Ipsen, J. H. Biophys J 2006, 90, 1639.
[90] Hilgemann, D. W. Annu Rev Physiol 2003, 65, 697.
[91] Rietveld, A.; Neutz, S.; Simons, K.; Eaton, S. J Biol Chem 1999, 274, 12049.
[92] Bagnat, M.; Keranen, S.; Shevchenko, A.; Shevchenko, A.; Simons, K. Proc Natl Acad Sci USA 2000, 97, 3254.
[93] Xu, X. L.; Bittman, R.; Duportail, G.; Heissler, D.; Vilcheze, C.; London, E. J Biol Chem 2001, 276, 33540.
[94] Hsueh, Y. W.; Gilbert, K.; Trandum, C.; Zuckermann, M.; Thewalt, J. Biophys J 2005, 88, 1799.
[95] Urbina, J. A.; Pekerar, S.; Hong-Biao, L.; Patterson, J.; Montez, B.; Oldfield, E. Biochim Biophys Acta-Biomembr 1995, 1238, 163.
[96] Endress, E.; Bayerl, S.; Prechtel, K.; Maier, C.; Merkel, R.; Bayerl, T. M. Langmuir 2002, 18, 3293.
[97] Bernsdorff, C.; Winter, R. J Phys Chem B 2003, 107, 10658.
[98] Arora, A.; Raghuraman, H.; Chattopadhyay, A. Biochem Biophys Res Commun 2004, 318, 920.
[99] Tierney, K. J.; Block, D. E.; Longo, M. L. Biophys J 2005, 89, 2481.
[100] Endress, E.; Heller, H.; Casalta, H.; Brown, M. F.; Bayerl, T. M. Biochemistry 2002, 41, 13078.
[101] Baginski, M.; Tempczyk, A.; Borowski, E. Eur Biophys J 1989, 17, 159.
[102] Baginski, M.; Borowski, E. Theochem J Mol Struct 1997, 389, 139.
[103] Forrest, L. R.; Sansom, M. S. P. Curr Opin Struct Biol 2000, 10, 174.
[104] Feller, S. E. Curr Opin Colloid Interface Sci 2000, 5, 217.
[105] Scott, H. L. Curr Opin Struct Biol 2002, 12, 495.
[106] Saiz, L.; Bandyopadhyay, D.; Klein, M. L. Biosci Rep 2003, 22, 151.
[107] Robinson, A. J.; Richards, W. G.; Thomas, P. J.; Hann, M. M. Biophys J 1995, 68, 164.
[108] Gabdoulline, R. R.; Vanderkooi, G.; Zheng, C. J Phys Chem 1996, 100, 15942.
[109] Tu, K. C.; Klein, M. L.; Tobias, D. J. Biophys J 1998, 75, 2147.
[110] Smondyrev, A. M.; Berkowitz, M. L. Biophys J 1999, 77, 2075.
[111] Rog, T.; Pasenkiewicz-Gierula, M. FEBS Lett 2001, 502, 68.
[112] Rog, T.; Pasenkiewicz-Gierula, M. Biophys J 2001, 81, 2190.
[113] Chiu, S. W.; Jakobsson, E.; Mashl, R. J.; Scott, H. L. Biophys J 2002, 83, 1842.
[114] Rog, T.; Pasenkiewicz-Gierula, M. Biophys J 2003, 84, 1818.
[115] Hofsass, C.; Lindahl, E.; Edholm, O. Biophys J 2003, 84, 2192.
[116] Pandit, S. A.; Bostick, D.; Berkowitz, M. L. Biophys J 2004, 86, 1345.
[117] Smondyrev, A. M.; Berkowitz, M. L. Biophys J 2001, 80, 1649.
[118] Czub, J.; Baginski, M. Biophys J 2006, 90, 2368.
[119] Baginski, M.; Sternal, K.; Czub, J.; Borowski, E. Acta Biochim Pol 2005, 52, 655.
[120] Czub, J.; Baginski, M. Biophys J 2004, 86[1], 2163-Pos.
[121] Sternal, K. Doctoral Thesis, Gdansk University of Technology, 2005.
[122] Czub, J.; Baginski, M. J Phys Chem B 2006, 110, 16743.
[123] Czub, J.; Baginski, M. Molecular Dynamics as a Tool to Study Drug-Membrane Interactions; ISQBP President's Meeting, Strasbourg, France, June 2006, Book of abstracts.
[124] Seoane, J. R.; Vila Romeu, N.; Minones, J.; Conde, O.; Dynarowicz-Latka, P.; Casas, M. Prog Colloid Polym Sci 1997, 105, 173.
[125] Berges, J.; Caillet, J.; Langlet, J.; Gresh, N.; Herve, M.; Gary-Bobo, C. M. Stud Phys Theoret Chem 1990, 71, 253.
[126] Baginski, M.; Gariboldi, P.; Bruni, P.; Borowski, E. Biophys Chem 1997, 65, 91.
[127] Helrich, C. S.; Schmucker, J. A.; Woodbury, D. J. Biophys J 2006, 91, 1116.
[128] Petrache, H. I.; Dodd, S. W.; Brown, M. F. Biophys J 2000, 79, 3172.