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Biochimica et Biophysica Acta 1768 (2007) 2616–2626

Biochimica et Biophysica Acta BBA

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Interactions of amphotericin B derivatives with lipid membranes—A molecular dynamics study

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Received 6 March 2007; received in revised form 15 June 2007; accepted 15 June 2007
Available online 23 June 2007

Abstract

Amphotericin B (AmB) is a well-known polyene macrolide antibiotic used to treat systemic fungal infections. AmB targets more efficiently fungal than animal membranes. However, there are only minor differences in the mode of action of AmB against both types of membranes, which is a source of AmB toxicity. In this work, we analyzed interactions of two low toxic derivatives of AmB (SAmE and PAmE), synthesized in our laboratory, with lipid membranes. Molecular dynamics simulations of the lipid bilayers containing ergosterol (fungal cells) or cholesterol (animal cells) and the studied antibiotic molecules were performed to compare the structural and dynamic properties of AmB derivatives and the parent drug inside the membrane. A number of differences was found for AmB and its derivatives' behavior in cholesterol- and ergosterol-containing membranes. We found that PAmE and SAmE can penetrate deeper into the hydrophobic region of the membrane compared to AmB. Modification of the amino and carboxyl group of AmB also resulted in the conformational transition within the antibiotic's polar head. Wobbling dynamics differentiation, depending on the sterol present, was discovered for the AmB derivatives. These differences may be interpreted as molecular factors responsible for the improved selectivity observed macroscopically for the studied AmB derivatives.

Keywords: Amphotericin B; Molecular dynamics; Molecular modeling; Lipid membranes; Cholesterol; Ergosterol

1. Introduction

Polyene macrolide antibiotic amphotericin B (AmB) is a well-known antifungal drug which has been used in clinical practice for over 45 years [1–3]. Due to its unique chemotherapeutic properties (e.g., very high activity, broad antifungal spectrum, fungicidal action, and ability to overcome multidrug resistance) as well as the lack of better alternatives, AmB has become a golden standard in the treatment of severe systemic fungal infections. Unfortunately, as a result of poor selectivity toward fungal vs. mammalian cells, AmB is one of the most toxic drugs used clinically [4,5]. To resolve the problem of AmB toxicity, two general approaches were undertaken, namely: (i) search for lipid formulations of the drug lowering its toxicity [6] and (ii) design of new, possibly less-toxic AmB derivatives [7]. New AmB formulations, introduced within last two decades, only partially limited toxic side effects and were found too expensive for a broad clinical application. Therefore, chemical modification of the parent drug still seems to be the most promising approach which, at the same time, repeatedly proved successful in the history of chemotherapy.

A rational design of new, less-toxic AmB derivatives is a hard task not only due to problems with chemical modifications of this complex molecule [8] but mainly due to inadequate knowledge concerning AmB molecular mechanism of action and especially the molecular nature of the antibiotic selective toxicity (fungi vs. mammals) [9–11]. It is widely accepted that AmB molecules interact with cell membranes and form transmembrane ionic channels which lead to release of K^+ ions and small inorganic molecules from the cytoplasm. Eventually, this release directly or indirectly is responsible for the lethal effect to the cell of AmB [9,12]. According to the most popular, so-called sterol hypothesis the presence of sterols in the cell membrane is required for chemotherapeutically relevant manifestation of the AmB channel-forming activity. Chemotherapeutic application of the drug is based on its higher activity against membranes containing ergosterol (principal fungal sterol) than cholesterol

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0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.06.017

(mammalian sterol) [13,14]. However, differential effect of AmB on both types of cell membranes is relatively small, and therefore AmB is characterized by low selective toxicity. Unfortunately, molecular details of the selectivity mechanism are still not clear.

Many experimental studies of AmB-induced membrane effects were undertaken throughout the years [10,11,15] which showed that more selective AmB derivatives exhibit: (i) higher affinity to ergosterol than to cholesterol [16], (ii) lower ability to form self-associated species in water solution [17–19], and (iii) a different interaction pattern with membrane phospholipids [20]. On the other hand, studies performed on different cell models show that the partition coefficient between a cell membrane and the aqueous phase is comparable for AmB and its derivatives [21]. Since none of the experimental findings alone seems to sufficiently explain the increased of selective toxicity of some AmB derivatives, the mechanism of the drug selectivity might be either multi-factorial or experimental models employed so far were inadequate to examine this mechanism at the molecular level. It is possible that the effect of improved drug selectivity results from essentially different interactions of more selective derivatives with membrane components inside lipid bilayers, as compared to the parent drug. In order to study such AmB-lipid interactions at the molecular level, appropriate tools for such microscopic studies are necessary. Recently, computational chemistry methods, offering a direct insight into the microscopic nature of the observed membrane phenomena, were employed as complementary to experimental studies [22–24]. However, these methods have not as yet been applied to study interactions between AmB derivatives and lipid membrane. Two such derivatives were obtained in our laboratory, namely N-(1-piperidinepropionyl)-amphotericin B methyl ester (PAmE) and *N*-(*N*′-3-dimethylaminopropylsuccinimido)-amphotericin B methyl ester (SAmE) (Fig. 1) [25,26]. Experimental studies revealed that these new compounds exhibit a markedly higher selective toxicity than the parent antibiotic [21,25,27]. In the current study, we clarify the molecular bases of the improved selectivity of new AmB derivatives.

To investigate various molecular aspects of the polyene mechanism of action, different molecular modeling studies were carried out in our group within last few years [28–31]. In particular, using molecular dynamics technique, we analyzed and validated various structural and dynamic properties of lipid bilayers containing either cholesterol or ergosterol [32]. These bilayer systems were then used as models to characterize the interactions of AmB with its cellular targets [31]. In the current work, using the same models and analogous molecular dynamics simulation protocols as for the parent antibiotic, we directly compared the behavior of the very highly toxic drug (AmB) and its two more selective derivatives (PAmE and SAmE) inside the lipid membranes. Analysis of our results enabled us to discuss the observed differences in the context of improved selectivity of the studied AmB derivatives.

In a broader sense, our study, to the best of our knowledge, is the first example where detailed molecular dynamics simulation of the series of membrane-active molecules, which interact with lipid membranes containing sterols, were performed to characterize the mode of ligand–membrane interaction. Thus, our results also show that molecular dynamics simulations can be used not only to reproduce complex biological systems but also may help in rational drug design and predict interactions of membrane-targeted molecules within lipid membrane system.

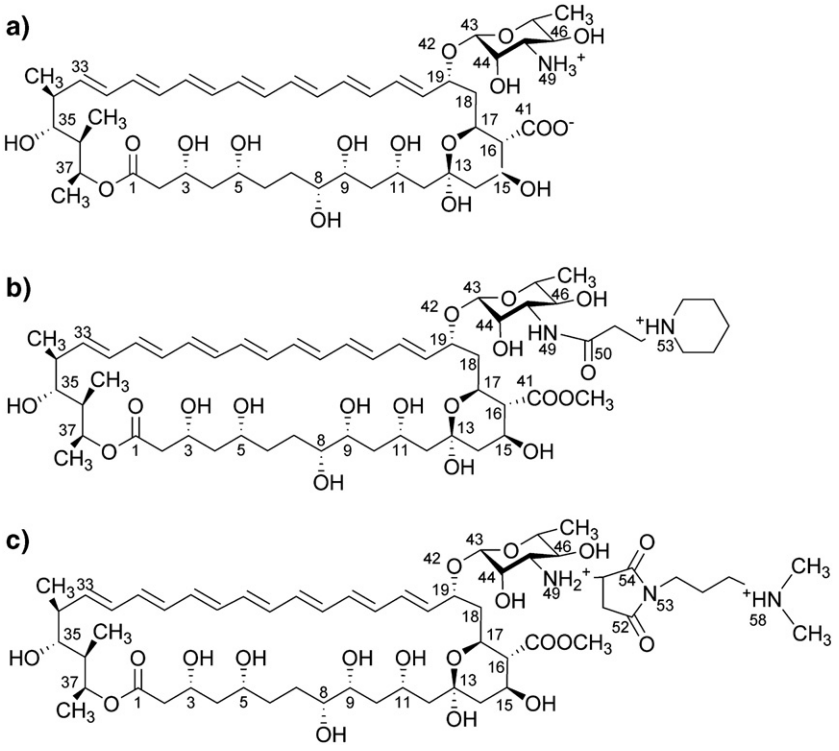

Fig. 1. Structures of AmB (a) and its two semisynthetic derivatives PAmE (b) and SAmE (c).

2. Methods

Three MD simulations were carried out for both PAmE and for SAmE molecules embedded in: (i) pure DMPC bilayer (DMPC/PAmE and DMPC/SAmE systems), (ii) DMPC bilayer containing ~25 mol% of cholesterol (Chol) (DMPC/Chol/PAmE and DMPC/Chol/SAmE systems), and (iii) DMPC bilayer containing ~25 mol% of ergosterol (Erg) (DMPC/Erg/PAmE and DMPC/Erg/SAmE systems). The starting structures of the lipid bilayer systems, containing monomeric forms of AmB derivatives, were prepared using final MD frames obtained in our previous simulations of AmB molecules placed inside sterol-containing membranes [31]. Models with AmB derivatives were constructed by simple replacement of AmB molecules in the previously studied system [31] by modified AmB molecules. The atom coordinates of PAmE and SAmE molecules were taken from a formerly performed conformational analysis [33]. Both studied AmB derivatives bear the net positive charge (Fig. 1), therefore, the proper number of chloride ions were randomly placed in the water lamella to neutralize the systems. The starting structures were composed of 128 DMPC molecules and, in the case of the sterol-doped systems, contained 42 Chol or Erg molecules. Each system contained also 4 isolated antibiotic molecules in each bilayer leaflet. All the bilayers were fully hydrated with about 40 waters per one DMPC molecule. The prepared models were then energy minimized to remove possible bad van der Waals contacts (positions of the antibiotic atoms were kept fixed during this optimization). The obtained structures were subsequently subjected to MD simulation. The final snapshots of the six simulated systems are included in the supplementary material (Fig. A). The molecular images were prepared using the VMD program [34].

All energy minimization procedures and MD simulations were carried out using the GROMACS package [35]. To maintain consistency with our previous works, the topology and force field parameters for DMPC molecules were based on the united-atom parameter set for DPPC [36]. The force-field parameterization of Chol and Erg molecules was also the same as in our previous studies [31,32]. The force field parameters, except atomic charges, for the antibiotic molecules were generated using PRODGR server (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/). Similarly to AmB, the sets of atom charges for both AmB derivatives were obtained by iterative fitting them to the molecular electrostatic potential calculated at the semi-empirical level of theory [31]. The united atom approach was also applied to antibiotic, sterol and DMPC molecules. The same parameterization procedure was previously used for AmB and it was proved to be successful in reproducing many experimental details concerning the behavior of an antibiotic molecule within a lipid bilayer [31,37].

Periodic boundary conditions were used in all our MD simulations. Electrostatic interactions were described using the smooth Particle Mesh Ewald summation method (SPME) with real space cut-off of 0.9 nm, and fast Fourier transform grid spacing of approximately 0.1 nm [38]. Van der Waals interactions within 0.9 nm were evaluated in every MD step and interactions between 0.9 and 1.2 nm were determined every 10 steps. The update of the neighbor list for short-range non-bonded interactions was done every 10 MD steps. All covalent bonds in the system were constrained to their reference values with the LINCS algorithm for phospholipid, sterol, and antibiotic molecules and with the SETTLE scheme for water molecules [39,40]. Equations of motion were integrated using the leap-frog algorithm with a 2-fs time step. The temperature and pressure were maintained around 300 K and 1 bar, respectively. The Berendsen weak coupling method with time constants of 0.1 (for temperature) and 1.0 ps (for pressure) was applied for this purpose. All three dimensions of a rectangular simulation box were allowed to fluctuate independently.

All systems were simulated for 40 ns and the atom coordinates were stored every 2 ps. The last 30 ns of each MD run were regarded as a productive simulation and taken for calculating averages. The total energy, its components (the kinetic and the potential energy), as well as dimensions of the simulation box were monitored during the simulation (see Supplementary Material, Fig. B) and, similarly to the simulations of AmB monomers inside the lipid membrane, the time assigned as equilibration (10 ns) appeared to be sufficient to obtain convergence of the studied properties.

3. Results and discussion

Analysis of the generated MD trajectories was focused on the behavior of the antibiotic molecules, in their monomeric forms, embedded inside DMPC bilayers of different sterol composition (sterol-free and doped with ~25 mol% of Chol or Erg).

3.1. Location and orientation of the antibiotic molecules

The electron density profiles (EDPs) across the bilayer, calculated for the Erg-containing systems, are presented in Fig. 2. They show the preferred location of the key functional groups of the antibiotic within the polar region of the membrane (EDPs calculated for the pure and the Chol-containing membranes are very similar and are included only in the Supplementary Material, Fig. C and Fig. D). The results obtained for the two examined semisynthetic derivatives of AmB and for AmB itself show a significant change of the relative position of the carboxyl ($-COO^-$) and the amino group ($-NH_3^+$) within the bilayer. In AmB, free $-COO^-$ group, is strongly exposed to the water phase [31] but after esterification (in both studied derivatives) shifts noticeably toward the hydrophobic core of the bilayer. This effect is more pronounced

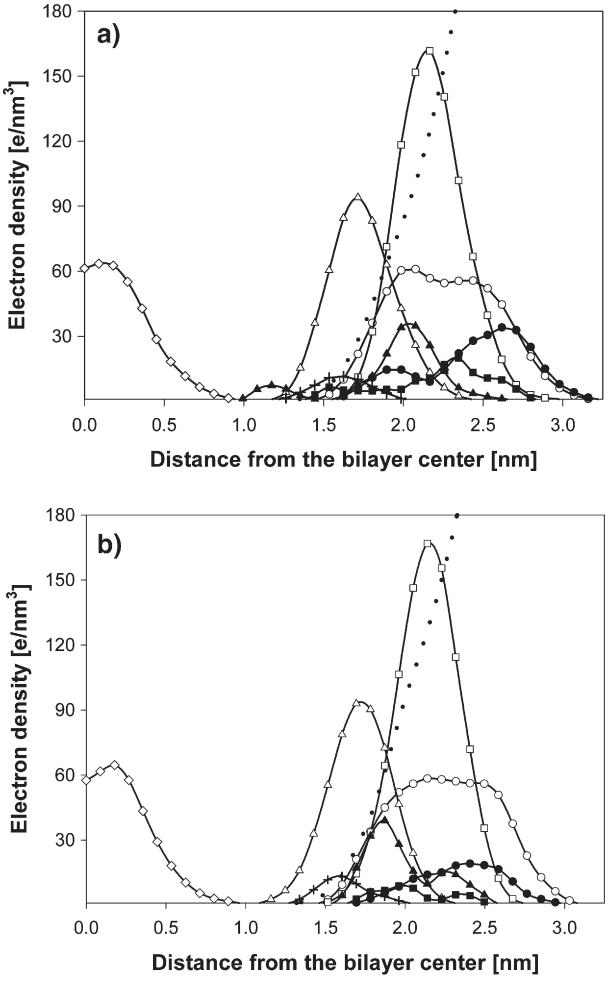

Fig. 2. Electron densities of selected groups across the bilayer for DMPC/Erg/PAmE (a) and DMPC/Erg/SAmE (b) systems: trimethylammonium groups (○), phosphate groups (□), carbonyl groups (△), DMPC terminal methyl groups (◇), sterol hydroxyl groups (+), ester group of PAmE or SAmE (▲), amino group (N-49) of PAmE or SAmE (■), $-NH(CH_2)_5^+$ of PAmE or $-NH(CH_3)_2^+$ of SAmE (●). Electron density of water is shown as the dotted line. To facilitate the analysis of the presented data, the EDPs for antibiotic groups were arbitrarily multiplied by a factor of five.

for SAmE than for PAmE. On the contrary, amino group of AmB at atom N49 (acylated in PAmE and alkylated in SAmE) is still able to form a hydrogen bond with phosphate groups (PO_4^-) of DMPC and remains at approximately the same level in the membrane as it was observed for AmB [31]. This level corresponds to the average location of lipid PO_4^- groups (see also Supplementary Material, Table A). It is also worth noting that additional tertiary amino groups (corresponding to nitrogen atom N58 in SAmE and N53 in PAmE), which are the most distant from the membrane center, are also able to interact with PO_4^- groups. Distributions of their electron density (Fig. 2) partially overlap with PO_4^- peaks suggesting a potential interaction (e.g., hydrogen bonds) between these amino groups and the DMPC PO_4^- groups. A comparison of EDPs calculated for the Chol and Erg-containing systems (see Fig. 2 and Supplementary Material, Fig. D) indicates that both, the presence and type of sterol do not substantially influence the preferred position of the antibiotic functional groups at the membrane interface. Similar sterol-independent behavior was previously observed in the case of AmB [31]. This can also be concluded from the data presented in Table A (Supplementary Material) which show average distances of the selected atoms from the bilayer midplane. Based on these data one can also conclude that esterification of AmB induces a shift of the COO^- group and, consequently, leads to the displacement of the whole macrolide ring towards the center of the membrane. Therefore, penetration of the other leaflet of the bilayer by both AmB derivatives is much stronger than that observed for the parent antibiotic (see, for example, position of the O-35 atom in Supplementary Material, Table A). This penetration depends on the membrane thickness and decreases in the following order: pure DMPC >> DMPC/Chol > DMPC/Erg [32]. The considerably deeper "immersion" of SamE in the bilayer, as compared to PAmE, was recorded in each simulated system. Considering the tendency of the positively charged piperidine group ($-NH(CH_2)_5^+$) of PAmE, and the dimethylamino group ($-NH(CH_3)_2^+$) of SAmE to interact with lipid PO_4^- groups, the above-mentioned effect can be attributed to the fact that the substituent on the nitrogen atom N49 has larger effective length in SAmE, compared to the one present in PAmE. The longer substituent of SAmE allows the macrolide part of the molecule to penetrate more deeply into the hydrophobic region of the bilayer. Analysis of the standard deviations of the average distances from the bilayer center (Supplementary Materials, Table A) indicates that the transverse mobility of the antibiotic molecules reduces with increasing molecular order of the membrane. The highest amplitude values were observed in the pure DMPC (disordered bilayer) and the lowest in the Erg-containing systems (highly ordered bilayer) [32]. The above findings can be discussed in terms of the channel formation mechanism. It seems that penetration of the other leaflet of the bilayer by the AmB derivatives may create some difficulties in the channel formation process. It is assumed that in thick membranes (e.g., sterol-rich liquid-ordered membrane domains) the so-called double length channels (DLC) containing two interacting single length channels (SLC) are likely to form. Thus, the AmB derivatives that penetrate the whole membrane more strongly are presumably less prone to form DLC channels in membranes that are not thick enough. Since Erg was shown to induce a larger increase of the DMPC membrane thickness than Chol, the DLC channels can be more easily formed in the bilayer containing the former type of sterol [32]. Thus ionophoric activity of the studied AmB derivatives should be better expressed in Erg- than Chol-containing membranes (differentiating effect of the membrane type).

Based on both, the experimental [41] and simulation [31] results, it was postulated that the average orientation of the longest principal axis of the rigid macrolide ring is essential for the ability of the polyene antibiotic to permeabilize various types of lipid membranes. In particular, it was found that the presence of sterols (Chol or Erg) in the bilayer forces more perpendicular orientation of AmB with respect to the surface. This orientation, in turn, may favor the aggregation of the antibiotic molecules into stable channel structures [31]. To test this possibility, we calculated distributions of the macrolide ring tilt angle (precise definition is given in the caption of Fig. 3) for both AmB derivatives and compared them with the distribution for the parent antibiotic. It is evident that in the presence of sterols, the average orientation of the macrolide ring axis of both derivatives alters substantially and similarly to AmB becomes approximately parallel to the membrane normal [31]. In addition, the range of possible orientations of the antibiotic molecules becomes reduced in sterol-containing bilayers. It can be assumed that the observed changes arise from the increased order and tight packing of the phospholipid acyl chains in membranes which contain sterols. Interestingly, the tilt angle distributions obtained for SAmE have a significantly higher dispersion. This finding correlates with the deeper location of this derivative within the membrane. As a result of this localization, the interactions between the SAmE macrolide ring and the polar part of the bilayer are relatively weak. Additionally, the whole aglycone moiety, as being closer to the membrane center, is surrounded by the more disordered lipid environment. The above effects presumably result in the increased amplitudes of the macrolide wobbling motion (motion corresponding to changes of the angle between the longest principal axis of the macrolide ring and the bilayer normal). If one compares the tilt angle of AmB and its derivatives (Fig. 3), it is evident that chemical modifications of the AmB structure lead to noticeable increase of the difference in the range of allowed arrangements in both sterol-containing membranes and the distributions are significantly narrower in the systems containing Erg. Thus, one could propose that while induction of a vertical orientation of the antibiotic is essential for its activity in both sterol-containing membranes, differentiation of this activity (i.e., increased selectivity observed for AmB derivatives), may at least in part, result from the varied amplitude of the wobbling motion. Much stronger decrease of fluctuations of the antibiotic molecules in the Erg-containing bilayer could possibly favor self-association of these molecules and, in consequence, formation of the so-called barrel-stave channel, particularly in this type of membrane. The differentiation of the preferred orientation and wobbling dynamics of the macrolactone part of both studied AmB derivatives may result from a deeper immersion of the antibiotics in the bilayer. Since molecular properties of the Chol- and Erg-containing membranes are particularly different in the hydrocarbon region of the bilayer

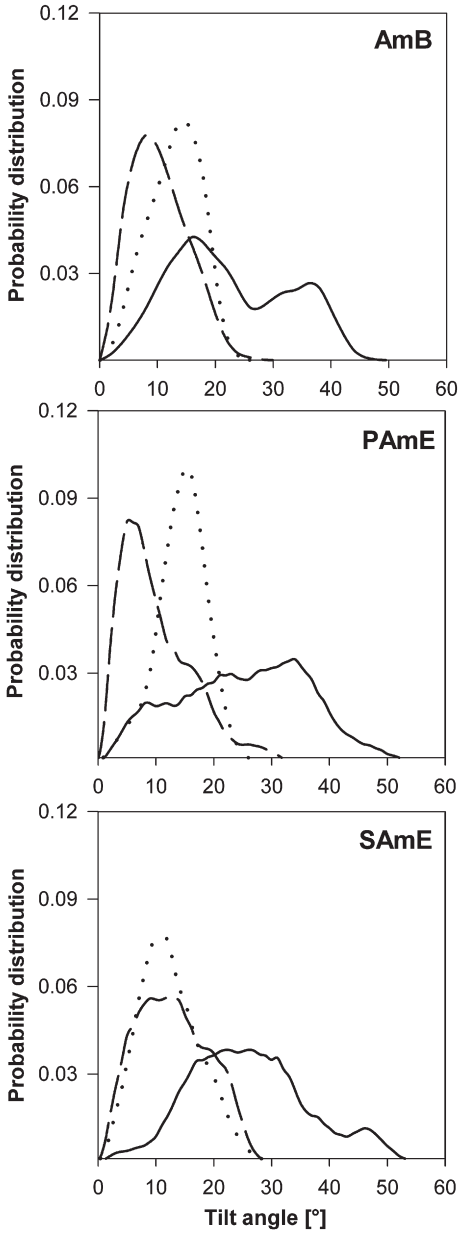

Fig. 3. Distributions of the antibiotic tilt angles (angle between the longest principal axis of the macrolide moiety and the normal to the bilayer plane) calculated for AmB, PAmE and SAmE molecules in the pure DMPC (solid line) and in the DMPC bilayers containing Chol (dashed line) and Erg (dotted line). The distributions for AmB were taken from [31].

[32], a deeper penetration of SAmE and PAmE molecules to the membrane may lead to their different behavior in both types of membranes, compared to AmB.

The orientation of the macrolide ring plane with respect to the membrane surface is another geometric parameter which characterize the arrangement of the antibiotic molecules within the lipid bilayer. The angle distributions between the vector perpendicular to the macrolide plane and the bilayer normal (an angle value of 90° means that the plane is oriented perpendicularly to the membrane surface), obtained for both selective AmB derivatives and for the parent antibiotic are presented in Fig. 4. It was shown in Fig. 3 that in the sterol-containing systems both AmB and its derivatives are positioned nearly vertically. This means that in a liquid-ordered membrane phase there is no preferred orientation of the macrolide ring with regard to the water/bilayer interface. For this reason, in Fig. 4 we present data only for the pure DMPC bilayer, which can be regarded as a model of the lipid bilayer in a liquid-disordered phase. A similar difference in the orientation of the macrolide ring plane is visible between the parent antibiotic and both derivatives. If we take into account that the mutual arrangement of the mycosamine ring and the aglycon part does not change too much (see further in the text and Fig. 6), the above-mentioned difference appears to be due to the altered electrostatic properties of the antibiotic's polar head caused by the chemical modifications. In the case of AmB, the observed inclination of the macrolide B face (see molecular drawing in Fig. 4) toward the bilayer center (orientation angles larger than 90°) stems from the fact that the dipole of the polar head ($-COO^- \rightarrow -NH_3^+$) tends to be oriented inwardly. This, in turn, results from the previously mentioned exposure of the $-COO^-$ group to the water phase together with the involvement of the $-NH_3^+$ group in the direct interaction with PO_4^- groups of DMPC. The above tendency can be also interpreted as the electrostatic "matching" of the antibiotic's polar head dipole to a component of the membrane dipole potential, generated by DMPC headgroups. On the contrary, a shift of the esterified $-COO^-$ group towards the center of the bilayer, together with simultaneous direct interaction between the modified $-NH_3^+$ group and PO_4^- groups, observed for PAmE and SAmE, causes such a change of the macrolide plane orientation that the B face becomes directed toward the membrane surface. Certainly, this rearrangement can greatly influence the mode of interaction between antibiotic molecules and other membrane components. In particular, it can have direct effect on the possibility or efficiency of the polyene:sterol complexes formation (so-called primary complexes).

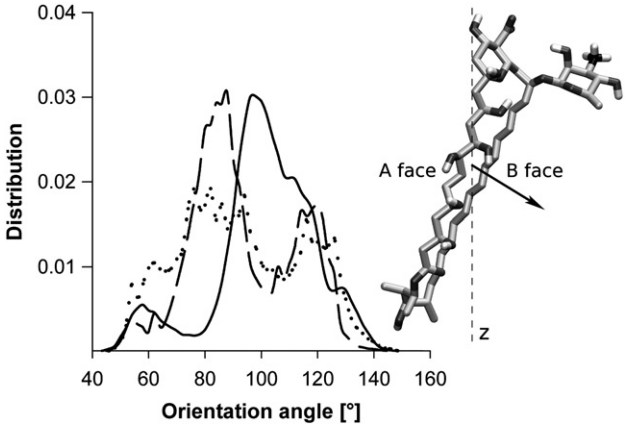

Fig. 4. Distributions of the angle between the vector perpendicular to the macrolide plane and the bilayer normal calculated for AmB (solid lane), PAmE (dashed line) and SAmE (dotted line) in the pure DMPC bilayer. The macrolide plane was calculated as the plane defined by C13, C19 and C35 atoms. In addition, the molecular drawing is presented showing schematically that the analyzed vector points out of the B face of the macrolide ring. Data for AmB were taken from the simulation reported previously [31].

3.2. Ordering of lipid molecules

It was previously found that AmB molecules, when inserted into the membrane, have the ability to increase the conformational order of the acyl chains of phospholipid molecules [31]. To examine if the chemical modifications of the AmB polar head affect the order-inducing effect, the deuterium order parameter, S_{CD}, was calculated as a function of the carbon atom number in the DMPC acyl chains (Fig. 5). The deuterium order parameter S_{CD} was calculated as described previously [31]. Fig. 5 also shows the S_{CD} profiles calculated previously for corresponding antibiotic-free systems [32]. It can be seen that all studied AmB derivatives, similarly to the parent compound, increase the order of the acyl chains which is manifested by the increase of $|S_{CD}|$ values. This means that the ordering and condensing action of AmB have to be a direct consequence of the rigid structure and smooth molecular surface of the macrolide ring [31]. Similar ordering and condensing properties of AmB, PAmE and SAmE may also indicate that both derivatives, similarly to AmB, have a tendency to create the antibiotic-enriched clusters inside the lipid bilayer. Formation of such domains, observed experimentally for AmB [42], was previously postulated to proceed in a way analogous to the separation of liquid-ordered lipid phases in the presence of sterols, such as Chol or Erg [31,43]. It can be also seen in Fig. 5 that antibiotic molecules inserted into the Chol-containing membrane induce further ordering of the hydrocarbon chains, whereas no such induction is visible in the highly ordered membrane with Erg. The same effect was previously observed for AmB [31]. We proposed that full expression of the polyene channel-forming activity may need the sufficient rigidity/order of the membrane environment (for more arguments see Czub and Baginski [31]). Thus, channel-forming activity in the Chol-containing bilayer would require higher concentration of the antibiotic molecules than in Erg-containing membranes and it explains why the same amount of polyene molecules is more effective in the Erg-containing membranes, as compared to the Chol-containing ones. This conjecture could be also interpreted in terms of antibiotic selectivity, and is further supported by our current results indicating that Chol-doped membrane containing 4.5 mol% of PAmE or SAmE is still less ordered than the Erg-containing equivalent with the same amount of the antibiotic.

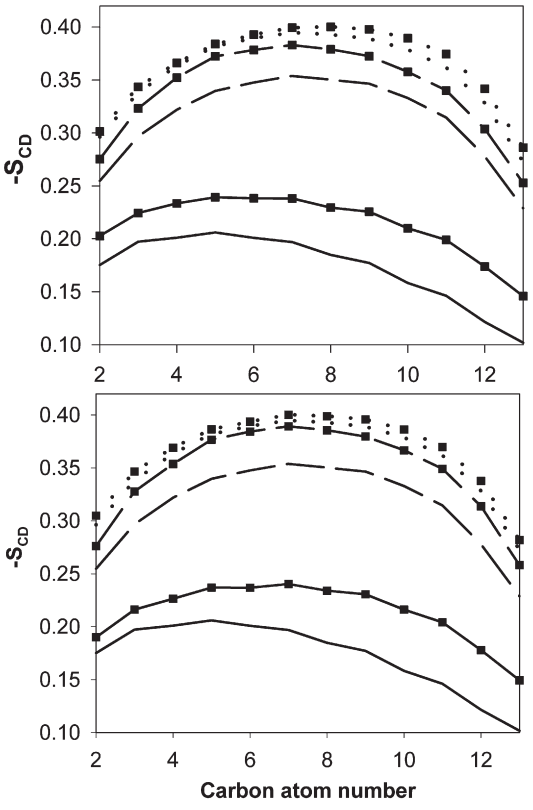

Fig. 5. Profiles of the deuterium order parameter calculated for the DMPC acyl chains in the pure DMPC bilayer (solid lines), and in the DMPC containing Chol (dashed line) and Erg (dotted line). In addition to the order parameter values calculated for antibiotic-containing systems (■), we present profiles obtained in similar systems without antibiotic molecules (no symbol) [32].

3.3. Intermolecular interactions of antibiotic molecules

Since intermolecular interactions play an important role in the mechanism of the polyene-induced permeabilization of a lipid bilayer, we examined in which way the introduced substituents affect the ability of the antibiotic to interact with other molecules. Table 1 shows the time-averaged number of hydrogen bonds between individual functional groups of antibiotic molecules and accepting sites of DMPC (as the DMPC molecule can essentially participate in hydrogen bonds only as an acceptor). The numbers were calculated using the geometric criterion for presence of a hydrogen bond (the distance between donor, D, and acceptor, A, shorter than 0.35 nm; the angle between the vectors D→A and D→H smaller than 60°). As one can see, the positively charged amino groups of the antibiotics are engaged in the interaction with DMPC much more strongly than any other functional groups. These amino groups form stable (often bifurcated) hydrogen bonds mainly with oppositely charged PO_4^- groups. Thus, the stability of these bonds is additionally increased by favorable electrostatic interaction. The covalent modification of the amino group of AmB leads to a considerable weakening of this interaction. Obviously, the decrease is more pronounced in the case of acylation (PAmE), which removes the positive charge from the amino group. As one may expect, the additional charged amino groups of both derivatives ($(-NH(CH_2)_5^+$ in PAmE and $-NH(CH_3)_2^+$ in SAmE) may, in some way, counteract this effect since they can also form hydrogen bonds with PO_4^- groups. However, the participation of the more spatially hindered $-NH(CH_2)_5^+$ group of PAmE in this interaction is much smaller than the one observed for the SAmE's $-NH(CH_3)_2^+$ group. If we consider that the presence of the positively charged amino group in the polar head structure was previously recognized as necessary for preserving the ionophoric activity of AmB derivatives [44], it can be proposed that the direct and stable interaction (hydrogen bonds and/or ion pairs) of the antibiotic polar head with PO_4^- groups of phospholipids may play an important role in the mechanism of membrane permeabilization. For example, this interaction could ensure the proper positioning of the antibiotic polar head at the water/membrane interface. Interestingly, it seems that the ability of new amino groups to form hydrogen bonds with

Table 1
The average number of hydrogen bonds formed between antibiotic and DMPC molecules

AmB group	DMPC group			PAmE group	DMPC group			SAmE group	DMPC group		
	PO_4^-	*sn*-1 C=O	*sn*-2 C=O		PO_4^-	*sn*-1 C=O	*sn*-2 C=O		PO_4^-	*sn*-1 C=O	*sn*-2 C=O
DMPC/AmB system [a]				*DMPC/PAmE system*				*DMPC/SAmE system*			
NH_3^+	0.99	0.07	0.24	$NH(CH_2)_5^+$	0.34	0.08	0.21	$NH(CH_3)_2^+$	0.63	0.04	0.13
OH-46	0.11	0.04	0.11	NH-49	0.17	0.03	0.07	NH_2^+	0.93	0.07	0.20
OH-44	0.10	0.04	0.03	OH-46	0.14	0.08	0.02	OH-46	0.29	0.02	0
OH-15	0.07	0.04	0.04	OH-44	0.04	0.04	0.01	OH-44	0.06	0.02	0.04
				OH-15	0.11	0.01	0.02	OH-15	0.03	0.04	0.01
DMPC/Chol/AmB system [a]				*DMPC/Chol/PAmE system*				*DMPC/Chol/SAmE system*			
NH_3^+	0.96	0.04	0.16	$NH(CH_2)_5^+$	0.26	0.02	0.08	$NH(CH_3)_2^+$	0.40	0.03	0.05
OH-46	0.14	0.02	0.06	NH-49	0.28	0.00	0.02	NH_2^+	0.65	0.00	0.23
OH-44	0.10	0.04	0.03	OH-46	0.12	0.00	0.01	OH-46	0.15	0.00	0.02
OH-15	0.07	0.04	0.04	OH-44	0.12	0.04	0.04	OH-44	0.13	0.02	0.02
				OH-15	0.00	0.01	0.02	OH-15	0.05	0.05	0.06
DMPC/Erg/AmB system [a]				*DMPC/Erg/PAmE system*				*DMPC/Erg/SAmE system*			
NH_3^+	0.98	0.19	0.27	$NH(CH_2)_5^+$	0.52	0.01	0	$NH(CH_3)_2^+$	0.51	0.01	0
OH-46	0.11	0.03	0.06	NH-49	0.23	0.16	0	NH_2^+	0.78	0.13	0.09
OH-44	0.07	0.09	0.06	OH-46	0.15	0.02	0	OH-46	0.15	0.06	0
OH-15	0.05	0.03	0.02	OH-44	0.07	0.07	0.01	OH-44	0.02	0.04	0.04
				OH-15	0	0	0	OH-15	0	0.02	0.06

[a] The values for AmB are taken from ref. [31].

lipid PO_4^- groups is relatively more pronounced in Erg- as compared to Chol-containing membranes (Table 1). The values in Table 1 show also that the pattern of hydrogen bonds formed between the antibiotic molecule and DMPC is significantly different for both selective derivatives. As an example, PAmE makes, on average, fewer hydrogen bonds with DMPC than the parent antibiotic, whereas SAmE makes noticeably more of them. Based on the observation of specific interaction between the $-NH_3^+$ and PO_4^- groups, it was previously suggested that formation of AmB:DMPC complexes of 1:1 stoichiometry can occur in lipid membranes [45,46]. Inspection of the currently obtained trajectories shows that SAmE is able to interact at the same time with two different DMPC molecules by its both charged amino groups. However, as it follows from the comparison of hydrogen bond autocorrelation functions (data not shown), hydrogen bonds created by the SAmE's amino groups generally have a shorter lifetime compared to bonds formed by the AmB amino group. The character of interactions between PAmE and DMPC is much different. Amino and amide functional groups of PAmE form rather weak and unstable hydrogen bonds with DMPC and, therefore, it seems that PAmE is not able to form specific complexes with phospholipids. On the other hand, the participation of hydroxyl groups in the formation of hydrogen bonds is similar and rather weak for both AmB derivatives. OH-46 is the hydroxyl group most strongly engaged in the interaction with DMPC both in the case of studied derivatives as well as for the parent antibiotic. Due to their immersion in the hydrocarbon membrane core, the polyol hydroxyls make hardly any hydrogen bonds with phospholipids (especially, it is the case for both derivatives, which are shown to penetrate deeper into the hydrocarbon region). In general, it could be stated that the different mode of interaction with adjacent phospholipid molecules, exhibited by the polar heads of both studied derivatives with improved selectivity, suggests that this interaction mode may be of secondary importance for the selective toxicity.

The hydration of the polyene molecules embedded in lipid membranes of different sterol composition was also studied. As a measure of the hydration level, the number of hydrogen bonds formed between the antibiotic and water molecules was used. It was found that PAmE derivative makes on average 1.51±0.44, 2.61±0.36 and 2.42±0.37 hydrogen bonds with water in the pure DMPC, DMPC/CHOL and DMPC/ERG systems, respectively. The corresponding values obtained for SAmE are 2.83±0.33, 3.62±0.43 and 3.24±0.46. Therefore, from these data we conclude, that SAmE, as the molecule with the net positive charge of +2, is solvated more strongly than PAmE. For both AmB derivatives, the presence of sterols in the membrane causes a significant increase of the hydration level. This fact can be ascribed to a partial shift of the whole antibiotic molecule toward the water phase, observed in the sterol-containing membranes. Interestingly, both PAmE and SAmE form more hydrogen bonds with water in the membrane containing Chol than in the one with Erg. This effect may in turn arise from differences in water penetration into both sterol-containing membranes namely, water penetration is greater in the case of the less densely packed bilayer doped with Chol. Similar trend in hydration in the three studied lipid environments was previously found for the parent antibiotic (6.04±0.42, 6.31±0.50 and 6.27±0.47, in the same order as above) [31]. However, AmB is hydrated much efficiently than its both derivatives. This result could be expected if we consider the previously mentioned deeper location of both AmB derivatives in a lipid bilayer as well as the type of the performed structural modifications (e.g., esterification leads to a considerable decrease of the polarity).

Previous studies of AmB derivatives [19,26,27] showed that certain chemical modifications of the AmB's ionized groups can result in significant improvement of the selective toxicity of the antibiotic. On the other hand, our simulations allowed us to propose that hydration of these groups has an effect on the overall behavior of the antibiotic molecules inside a lipid bilayer e.g., on the preferred localization within the membrane [31]. Thus, we studied the degree of hydration of the introduced and modified functional groups to find out if there is any correlation between this property and the selectivity of action of a particular derivative. To this end, three-dimensional radial distribution functions (RDF) of hydrogen and oxygen atoms coming from water molecules around central atoms of given functional groups were calculated. The resulting plots (see Supplementary Material, Fig. E) indicate that esterification of AmB's $-COO^-$ group leads to strong desolvation of this group and, in consequence, the above-mentioned shift of the whole macrolide toward the bilayer center. This effect supports our earlier assumptions that the hydrated $-COO^-$ group plays the role of a specific anchor of the AmB molecule at the membrane interface [31]. On the contrary, the obtained RDFs of water atoms around the N-49 nitrogen atom are quite similar for AmB derivatives and the parent antibiotic, implying similar and rather weak interaction of the corresponding amino groups with water molecules. The low hydration of these groups can be ascribed to their strong involvement in the direct interaction with neighboring DMPC PO_4^- groups (in the case of AmB and SAmE). In the case of PAmE this effect can also be a consequence of the charge removal after acylation of the amino group. Additional positively charged amino groups ($-NH(CH_2)_5^+$ in PAmE and $-NH(CH_3)_2^+$ in SAmE), that were shown above to form hydrogen bonds with DMPC, are also rather weakly hydrated.

The formation of the AmB:sterol primary complexes was demonstrated in solution and is also assumed by some authors to occur within lipid membranes. At the same time, the selective toxicity is linked with a type of sterol. Thus, it was postulated that higher selectivity of certain AmB derivatives (e.g., PAmE and SAmE) is due to their different affinity to both sterols, which most likely originate from the modification of the antibiotic amino group [44]. However, in contradiction to earlier assumptions, recent studies suggest that participation of the AmB's $-NH_3^+$ group in the stabilization of the antibiotic–sterol complex is highly improbable due to the steric and geometric conditions [28,31,47]. This conclusion is further supported by the present study showing that the AmB's $-NH_3^+$ and sterol 3β-OH groups are located relatively far from each other along the axis perpendicular to the membrane surface (see Supplementary Material, Table A). These data imply that sterol molecules form more readily hydrogen bonds with the antibiotic's hydroxyl groups, such as OH-44 or OH-13 than with the $-NH_3^+$ group. Unfortunately, the limitations of the method employed made it impossible to directly observe the possible formation of the primary complexes. The calculations intended to compare the stability and geometry of primary complexes preformed by AmB and its selective derivatives are currently in progress and will be published in due time.

3.4. Intramolecular properties of the antibiotic molecules

It was also proposed that chemical modifications of the AmB's polar head may result in the change of the mutual orientation of mycosamine moiety and lactone ring [48]. Such rearrangement would alter the overall shape of the molecule, in particular, changing the position of OH-44 with respect to the macrolide part. Furthermore, it may be presumed that this transition will change the relative affinity of the antibiotic to Erg and Chol. To verify the hypothesis of a conformational change of the polar head of the antibiotic molecule in the environment of a lipid membrane, we present the comparison of the two-dimensional maps of the φ (C18–C19–O42–C43) and ψ (C19–O42–C43–C44) dihedral angles obtained for AmB and both its derivatives (Fig. 6). The introduction of substituents at the $-NH_3^+$ and $-COO^-$ groups of AmB significantly influences the conformation of the glycosidic linkage but in all cases the rotation around the C19–O42 and O42–C43 bonds is, to a large degree, hindered and only a limited range of φ and ψ values is allowed. Three main conformers were found, namely A ($\varphi \approx -80°$, $\psi \approx 150°$), B ($\varphi \approx -140°$, $\psi \approx 150°$) and C ($\varphi \approx -150°$, $\psi \approx 80°$). The first two were also observed previously for AmB in the hypothetical channel structure [28,49] and in the water phase near the surface of the membrane [30]. This means that the orientation of the mycosamine moiety is quite independent of the environment. However, inverted population of the A and B minima is the most visible difference in the conformational behavior between polar heads of AmB and its more selective derivatives. The tendency of both derivatives to preferentially adopt the A conformation corresponds to an approximately parallel arrangement of the mycosamine and macrolide planes (Fig. 7). The aminosugar moiety in the A conformation is situated along the longest principal axis of the macrolide and the axial OH-44 is positioned in such a way that it could easily participate in the formation of the primary complex with a sterol molecule. It can be assumed that in such a complex, the 3β-OH group of the sterol molecule makes a hydrogen bond with one of the AmB's polar head hydroxyls while the steroid nucleus and the AmB's heptaene chromophore interact by van der Waals forces. It was suggested by some authors that AmB is able to form stable primary complexes only with more rigid Erg molecules whereas it does not interact in this way with Chol [17,47]. In this light, the observed changes in the polar-head geometry, possibly favoring the complex formation, should also increase the selectivity of the antibiotic. On the other hand, the discussed conformational transition can also influence the ability of the antibiotic monomers to associate in the functional channel structure. It was previously found that the AmB channel is stabilized by the ring of intermolecular hydrogen bonds localized in the polar-head region of the structure of the pore [28,49]. Formation of this hydrogen bond ring is especially possible when the antibiotic molecules adopt the B conformation, that is much less likely in the case of both more selective derivatives. The C conformation, being noticeably less frequent for both derivatives than for the parent antibiotic, also appears to promote intermolecular interactions. The above results indicate that the introduced chemical modifications should probably

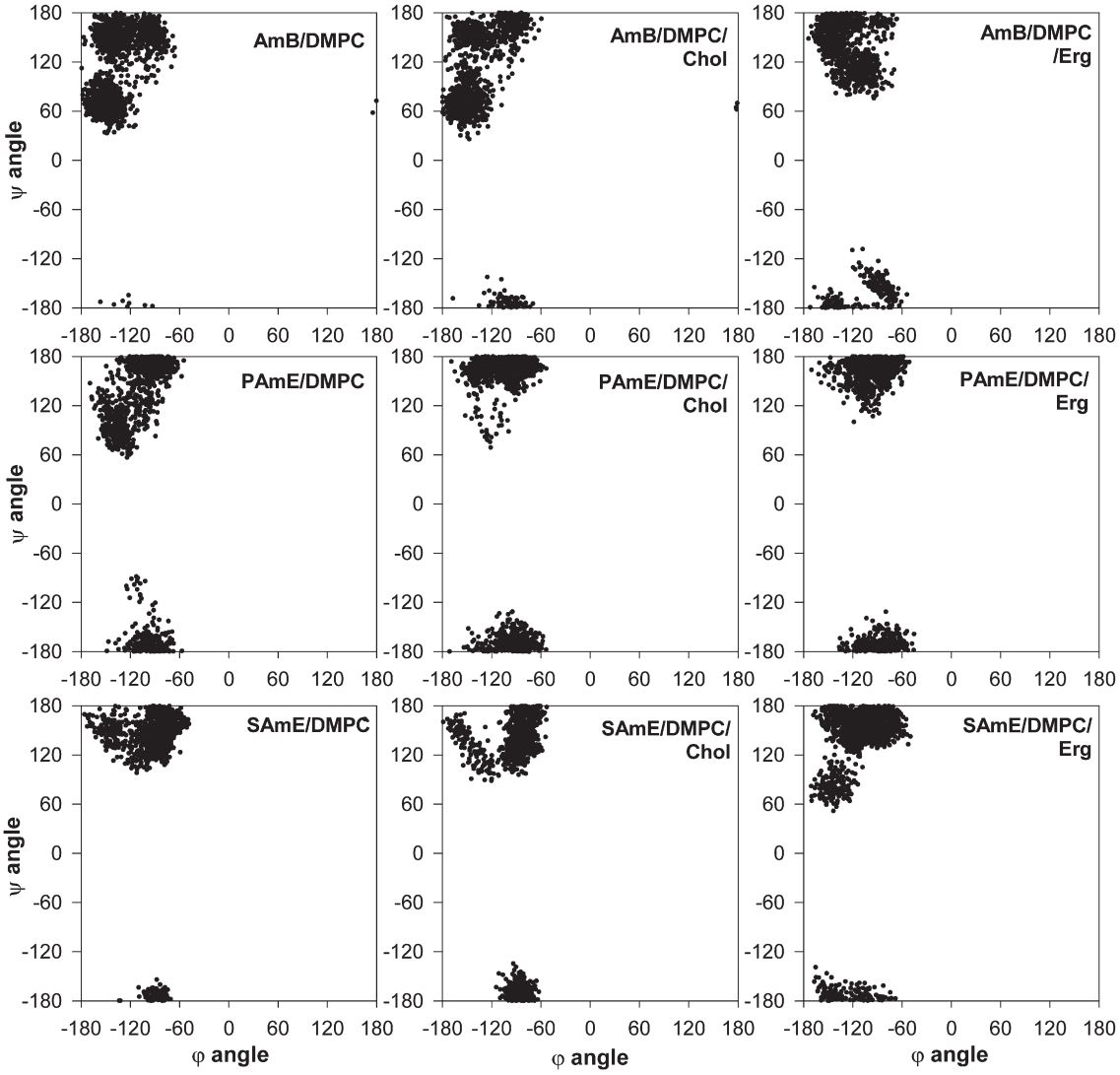

Fig. 6. Distributions of the φ (C18–C19–O42–C43) and ψ (C19–O42–C43–C44) dihedral angles obtained for AmB (top panel), PAmE (middle panel) and SAmE (bottom panel) in the three different membrane systems. Data for AmB come from the simulations reported in ref. [31].

lower the stabilization of the channel, and increase its sensitivity to the lipid environment (e.g., the conformational order and rigidity). Since Erg, as compared to Chol, shows a higher ability to increase the order of the phospholipid acyl chains, one could argue that the above finding supports the hypothesis of an indirect sterol role in the mechanism of polyenes' action. Obviously, the character of structural modifications namely esterification of $-COO^-$ and substitutions at $-NH_3^+$ should also act in the same direction and block, to some extent, possibilities of the channel structure stabilization.

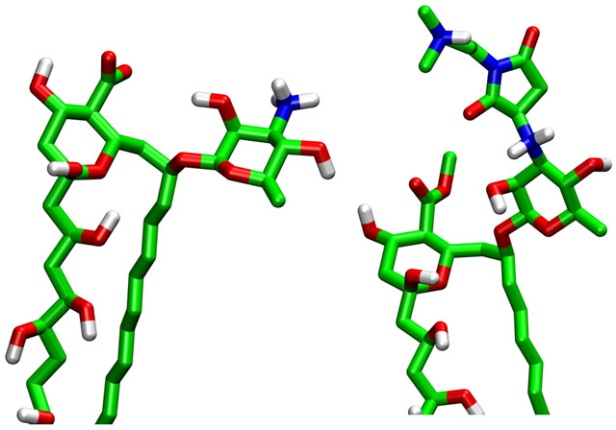

Fig. 7. Typical conformations of the AmB (left) and SAmE (right) polar heads.

3.5. Conclusions

To the best of our knowledge, the present study is the first attempt to examine the mechanism of selective action of polyene macrolide antibiotics toward fungal and mammalian membranes directly at the molecular level. To investigate these phenomena, AmB derivatives (SAmE and PAmE) with significantly improved selectivity (AmB itself is of very poor selectivity) and molecular modeling methods, which are able to tackle the problem at the molecular level, were used. Molecular dynamics simulations of systems containing model membranes with

incorporated molecules of the chosen antibiotics were carried out to study our ligand–membrane systems. Analysis of simulation output data allowed us to identify some essential differences in the behavior of AmB and its two derivatives inside lipid bilayers with different sterol content. The observed differences were then interpreted in the framework of a membrane permeabilization mechanism as possible reasons of improved selectivity of the derivatives.

In particular, it was found that, due to chemical modifications of the amino (introduction of a substituent with an extra amino group) and the carboxyl (esterification) groups of AmB, both derivatives tend to penetrate more deeply into the other leaflet of the bilayer than the parent drug. Since properties of Chol- and Erg-containing membranes differ mainly in the hydrophobic interior [32], a deeper location of the derivatives in the membrane results in a significant differentiation of their wobbling dynamics in both types of the bilayer. A stronger restriction imposed on wobbling motions in more ordered Erg-containing membranes may, in turn, help the antibiotic molecules to self-associate within this type of liquid-ordered phases. We also observed that both AmB derivatives, similarly to the parent compound, exert a condensing and ordering effect on a lipid bilayer. However, it seems that higher concentration of the antibiotic is necessary to obtain a similar degree of ordering in the Chol-containing membrane, as compared to the one with Erg. If we assume that an adequately high level of conformational order is essential for channel formation, the above result can also be recognized as important for the mechanism of selectivity. Furthermore, we found that chemical modifications of the AmB's polar head (particularly, introduction of substituents at the amino group) cause a shift of the conformational equilibrium of this moiety toward the conformation in which shape of the molecule enables to form the primary complex with sterol. According to some authors, such conformations should additionally increase the activity of the antibiotic in the Erg-containing membranes without modifying it in the Chol-containing ones [47]. It was also confirmed that the introduced new amino groups (corresponding to nitrogen atom N58 in SAmE and N53 in PAmE) in both derivatives are able to form hydrogen bonds with phospholipids and to take over the role of the parent amino group in AmB. Interestingly, this ability is relatively more pronounced in the Erg-containing bilayer, as compared to the Chol-containing one.

In conclusion, our calculations revealed particular changes in the molecular properties of the studied AmB derivatives, compared to the poorly-selective parent antibiotic, inside lipid membranes. Moreover, some of these structural and dynamic effects exhibited by AmB derivatives clearly results from different behavior of studied antibiotics in Chol- and Erg-containing membranes. Thus, the observed differences can be interpreted as a possible molecular basis of the improved selectivity of our AmB derivatives. Current findings will be helpful in further studies which will include self-associated antibiotic species (e.g., channel structures) and antibiotic–sterol binary complexes in the membrane. All these efforts together (both experimental and simulations) will be necessary to confirm the present hypotheses concerning the molecular basis of higher selectivity of some AmB derivatives.

Acknowledgments

This research was supported by the Ministry of Education and Science (grant no. 3 P05F 012 25) and Gdansk University of Technology (Poland). The authors would like to thank Dr. A. Skladanowski for critical reading of the manuscript. The authors also thank the TASK Computational Center (Gdansk, Poland) for granting CPU time.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2007.06.017.

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