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

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ABSTRACT

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

1. Introduction

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

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

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

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

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

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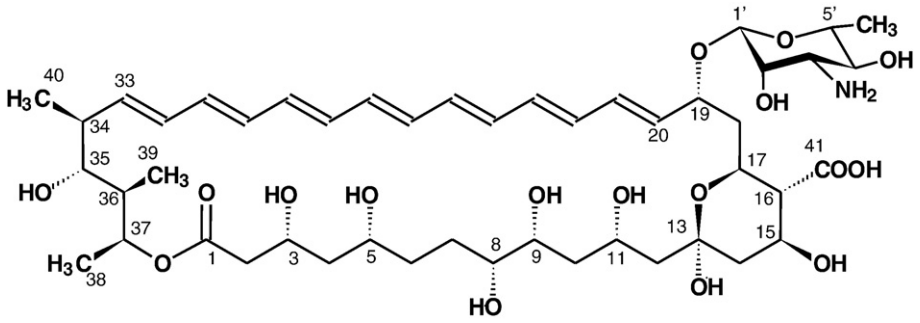

Fig. 1. Structure of amphotericin B.

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

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

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

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

2. Methods

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

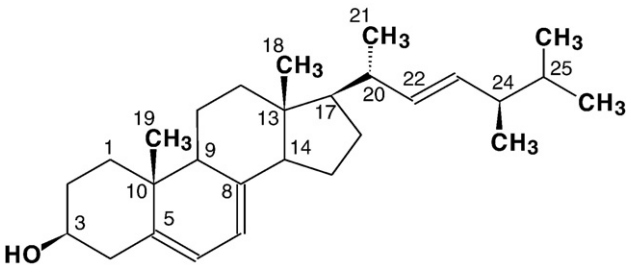

Fig. 2. Structure of ergosterol.

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

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

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

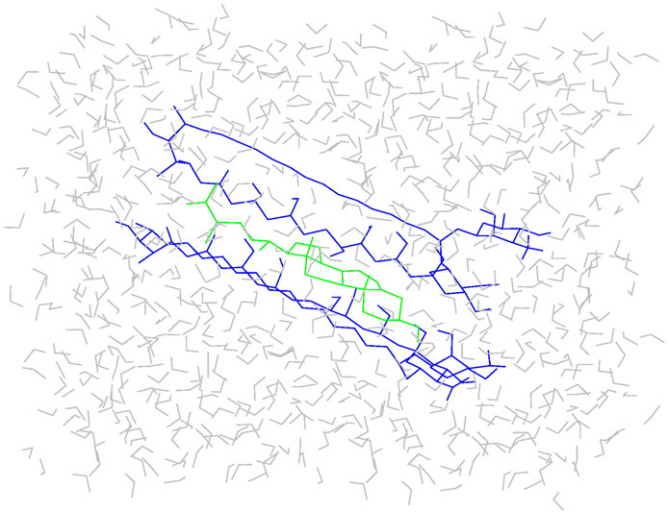

Fig. 3. Complex geometry after energy minimization (sterol molecule green, AmB blue, water light grey).