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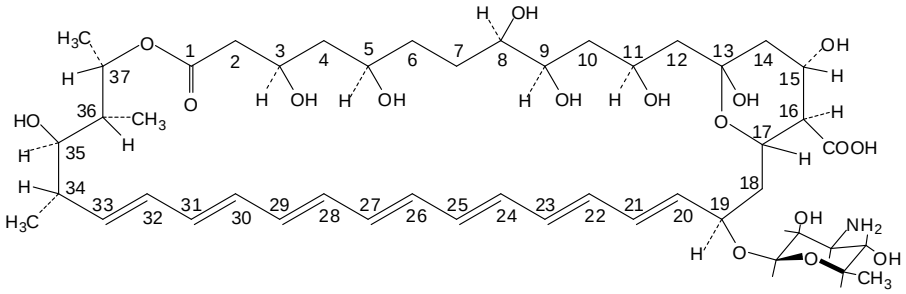
MODELS OF AMPHOTERICIN MEMBRANE CHANNEL BASED ON CONCERTED =C-H…O INTERMOLECULAR INTERACTIONS

Lisnyak Yu. V.

SI "I.Mechnikov Institute of Microbiology and Immunology of National Academy of Medical Sciences of Ukraine", Kharkiv

Amphotericin B, polyene macrolide antibiotic (fig. 1), is well known for several decades as membrane-acting agent that is widely used in medicine to treat advanced fungal infections [1].



Amphotericin B

Fig. 1. Chemical structure of amphotericin B molecule.

It has been shown that molecular mechanism of this antibiotic functioning is closely related to its ability to form hydrophilic channels in hydrophobic environment of cellular membrane that causes leakage of ions and other small molecules from the cell. Membrane sterols such as cholesterol and ergosterol also participate in formation of membrane channels.

Experimental molecular structure of polyene-sterol membrane channels (PSMC) has not been obtained yet and despite of the fact that PSMC were investigated in a number of experimental and theoretical studies [2-7] the structural specificity and characteristics of PSMC still remain subjects of discussion.

Amphotericin B forms two types of channels: single-length channel (SLC) and double-length channel (DLC) that are structurally very similar but differ in length by a factor 2. SLC and DLC span monolayer and bilayer phospholipid membrane, correspondingly. It is assumed that SLC is formed in much the same way as staves of a barrel form a cylinder (so called "barrel stave model") (fig. 2a). The "barrel" is built from "staves" consisting of antibiotic and incorporated sterol molecules, with long axes being nearly parallel and oriented perpendicularly to the membrane surface (Fig. 2b).

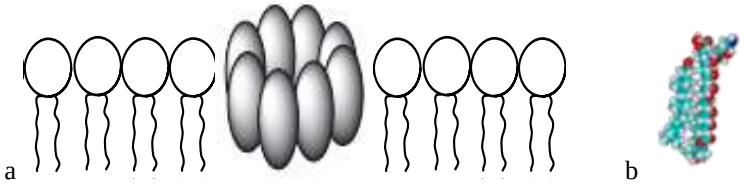


Fig. 2. Single-length channel (SLC): SLC involved in phospholipid monolayer (a) and its structural unit, "stave" (b).

It is assumed that the polar hydroxyl groups of antibiotics are turned to interior of the channel (and form its pore). The external surface of the channel turned toward the lipid phase is formed by hydrophobic conjugated double-bond chains of the antibiotic and by sterol molecules. The polar head of amphotericin B molecule containing the amino sugar portion and carboxyl group (fig. 1) is exposed to outer-membrane space (fig. 3). The channel structure is stabilized mainly by intermolecular hydrogen bonds between hydroxyls at C9, C8 and C5 (fig. 1), electrostatic interactions between amino and carboxyl groups and hydrophobic interactions between non-polar portions

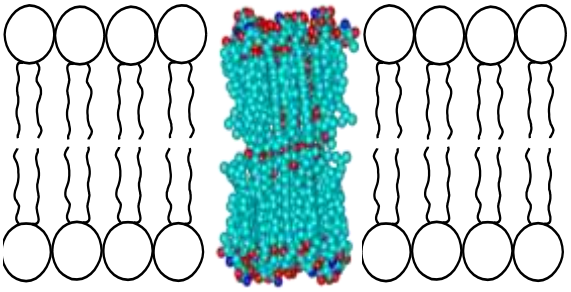


Fig. 3. Double-length channel (DLC).

of adjacent amphotericin molecules. DLC is formed by two barrels bound end to end (fig. 3).
Different channel molarities have been reported, with the number of asymmetrical units ranging from 4 to 12 but the most spread is barrel stave model consisting of 8 amphotericin molecules with amphotericin:sterol molar ratio 1:1.

` Recently, new, alternative, barrel stave model of polyene membrane channel was proposed for another polyene macrolide antibiotic, chainin [8]. The model mimics the intermolecular association mode of polyene macrolides in crystal structures (like that of amphotericin [10], fig. 4) and is based on an idea of concerted weak =C-H…O interactions between unsaturated double bonds and hydroxyl OH groups of adjacent polyene molecules as an important factor stabilizing molecular assembly of polyene channel (fig. 5). Being conceptually attractive from biophysical point of view, the model requires further investigations for its applicability to others polyene macrolide systems. Including into consideration different molecular architectures of membrane channels could be helpful in understanding the known multiplicity of modes of polyene macrolide antibiotics action on membranes. The purpose of our study was to investigate the possibility for amphotericin B to form channel aggregates with concerted weak =C-H…O intermolecular interactions.

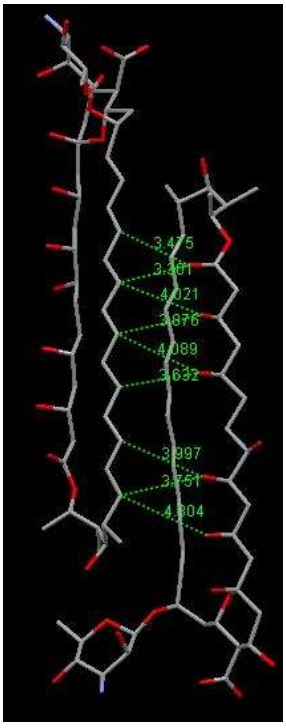


Fig 4. Intermolecular association of two amphotericin B molecules in antiparallel mutual orientation via =C-H···O interactions in the crystal structure [10]. C···O distances (in Å) are presented by green.

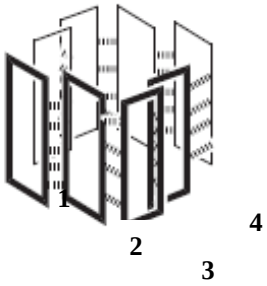


Fig 5 Intermolecular association mode of eight polyene macrolides (rectangular boxes) via concerted weak =C-H…O interactions (dashed lines) Molecules 1 and 2 as well as 3 and 4 are arranged in parallel mutual orientations, correspondingly; molecules 2 and 3 are mutually arranged in perpendicular orientation (figure adopted from [8]).

Methods

Structure of amphotericin B molecule was extracted from X-ray data for N-iodoacetyl-amphotericin B tetrahydrofuran solvate monohydrate [9-10] from Cambridge Crystallographic Data Centre and optimized until energy RMS gradient < 0.001 kcal/(mol·A). Eight or six parallel molecules of amphotericin B were circularly and symmetrically arranged nearby to be involved in intermolecular =C-H…O interactions and obtained molecular aggregates were optimized by block-diagonal Newton-Raphson method. Calculations were performed in vacuo using the molecular modeling system HyperChem™ [11] with MM+ force field and PM3 charges (ε=1.5). Channels pore diameters were measured as the distances between centers of corresponding atoms minus the sum of their van der Waals radii.

Results and discussion

To study the possibility for amphotericin to form membrane channel via intermolecular interactions mimicing the association mode of polyene macrolides in crystal structures we have built two single-length channel models in vacuo: amphotericin B octamer and hexamer.
Barrel-stave channel assembly of eight amphotericin B units (octamer) is shown in fig. 6. Pore diameter in the narrowest part of the channel is too large in this case: 13.5 Å versus 7.0 Å for “classic” barrel stave model. The model does not reproduce the concerted =C-H…O interactions within the whole structure. These interactions were observed only for pairs of amphotericin B molecules interacting in a parallel manner, in contrast to ones interacting in a perpendicular manner (see Fig. 5). This fact possibly reflects the limitations of force field approach and the necessity to use combined QM/MM calculations for such systems. In this channel model, hydroxyl groups of amphotericin B are poorly exposed for hydrophillic interactions in the pore.

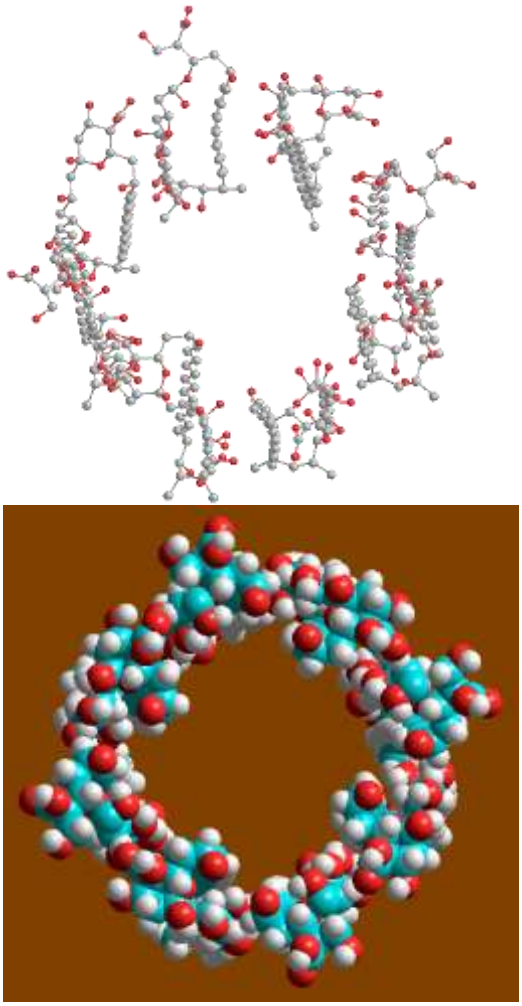


Figure 6 Barrel-stave assembly of eight amphotericin B units (hydrogen atoms and sugar fragments are omitted for clarity).

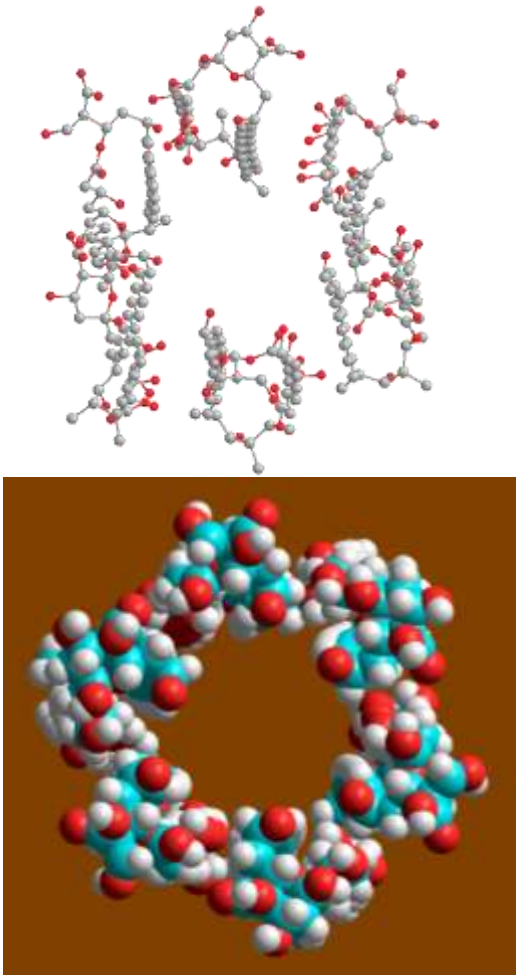


Fig. 7. Barrel-stave assembly of six amphotericin B units (hydrogen atoms and sugar fragments are omitted for clarity).

Barrel-stave assembly of six amphotericin B units (hexamer) is shown in fig. 7. The model rather well reproduces the concerted =C-H…O interactions between amphotericin molecules within the whole assembly. Hydroxyl groups of amphotericin molecules are much better exposed for hydrophillic interactions in the pore. Pore diameter in the narrowest part of the channel equals 8.8 Å being within experimental estimates. Solvent accessible surface of the channel is shown in Fig. 8.

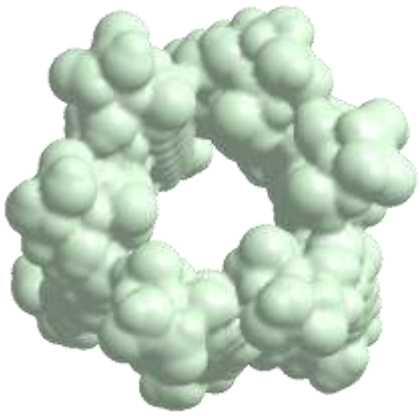


Fig. 8. Solvent accessible surface of the hexamer amphotericin B channel

Conclusions

Thus, to study the possibility for amphotericin to form membrane channel via intermolecular interactions mimicing the association mode of polyene macrolides in crystal structures there have been built two simple single-length channel models in vacuo: amphotericin B octamer and hexamer. In the frame of our force field approach, hexamer model better reproduced characteristic features of a channel with concerted weak =C-H…O intermolecular interactions: association mode, H-bonding pattern along with assembly and accessibility of polyene hydroxyl groups for hydrophyllic interactions in the pore.

The proposed in vacuo models can be incorporated into phospholipid bilayer of cell membrane to serve as starting structures for further comprehensive molecular dynamics simulations of these systems in the presence of aqueous environment.

Involving different molecular models for supramolecular assemblies of membrane channels into analysis of their structural peculiarities could be helpful for understanding the known multiplicity of modes of action of polyene macrolide antibiotics on membranes as well as the general molecular mechanism of channel's permeability.

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To study the possibility for amphotericin to form membrane channel via intermolecular interactions mimicing the association mode of polyene macrolides in crystal structures we have built two channel models in vacuo: amphotericin B octamer and hexamer. The octamer model does not reproduce the concerted weak =C-H…O intermolecular interactions within the whole aggregate structure and has too large pore diameter. For hexamer model, the pore diameter is within experimental estimates. The hexamer model rather well reproduces characteristic features of a channel with such concerted weak interactions: association mode, H-bonding pattern within the whole assembly and accessibility of polyene hydroxyl groups for hydrophyllic interactions in the pore.

МОДЕЛИ АМФОТЕРИЦИНОВОГО МЕМБРАННОГО КАНАЛА, ОСНОВАННЫЕ НА СОГЛАСОВАННЫХ МЕЖМОЛЕКУЛЯРНЫХ =C-H…o ВЗАИМОДЕЙСТВИЯХ

Лисняк Ю. В.

Для исследования возможности формирования амфотерициновых мембранных каналов посредством межмолекулярных взаимодействий, имитирующих способ ассоциации полиеновых макролидов в кристаллических структурах, мы построили две модели мембранной поры в вакууме: октамер и гексамер амфотерицина В. Модель октамера не воспроизводит согласованные слабые межмолекулярные =C-H…O взаимодействия в пределах всей структуры агрегата и имеет довольно большой диаметр поры. В модели гексамера диаметр поры находится в пределах экспериментальных оценок. Модель гексамера хорошо воспроизводит характерные особенности канала с такими согласованными слабыми =C-H…O взаимодействиями: способ ассоциации, картину Н-сязывания в пределах всего агрегата и доступность гидроксильных групп полиенов для гидрофильных взаимодействий в поре.

МОДЕЛІ АМФОТЕРИЦИНОВОГО МЕМБРАННОГО КАНАЛУ, ЯКІ БАЗУЮТЬСЯ НА УЗГОДЖЕНИХ МІЖМОЛЕКУЛЯРНИХ =C-H…o ВЗАЄМОДІЯХ

Лісняк Ю. В.

Для дослідження можливості формування амфотерицинових мембранних каналів завдяки міжмолекулярним взаємодіям, які імітують спосіб асоціації полієнових макролідів у кристалічних структурах, ми побудували дві моделі мембранної пори в вакуумі: октамер і гексамер амфотеріцину В. Модель октамеру не відтворює узгоджені слабкі міжмолекулярні =C-H…O взаємодії в межах всієї структури агрегату та має доволі великий діаметр пори. У моделі гексамеру діаметр пори знаходиться в межах експериментальних оцінок. Модель гексамеру добре відтворює характерні особливості каналу з такими узгодженими слабкими міжмолекулярними =C-H…O взаємодіями: спосіб ассоціації, картину H-зв’язування в межах усього агрегату и доступність гідроксильных групп полієнів для гідрофільних взаємодій в порі.