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Structures of amphotericin B-cholesterol complex

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The structures of amphotericin B-cholesterol complex that forms a channel in a lipid membrane were analysed by molecular mechanics calculations. The symmetric complex consisting of eight rigid antibiotic and cholesterol molecules was considered. The presence of a continuous set of low-energy states of the complex with different values of the channel diameter was shown. These states are characterized by significant tilt of the amphotericin planes to the radial axis of the channel and by strong interaction between the charged ammonium and carboxyl groups of the antibiotic. Changes of the channel diameter may result in changes in pore permeability.

Introduction

The antifungal polyene antibiotic amphotericin B (Fig. 1) forms pores in membranes that are permeable to ions, water and non-electrolytes. It is assumed that the pore is formed by one [1–3] or two complexes [4–7], represented in space-filling models as a cylinder consisting of eight antibiotic and eight incorporated sterol molecules [7]. The channel has conducting and non-conducting states [8,9] with frequent transitions occurring between them [8].

It is natural to assume that the transitions between these states are linked with structural rearrangement of the pore. Therefore, it is essential to calculate low-energy structures of amphotericin-sterol complex.

The main goal of the present study is the detailed analysis of energy surface of the amphotericin-cholesterol complex, in order to ascertain possible structural rearrangement of the pore.

Methods

The computations on the complex were carried out constrained to C-8 rotational symmetry.

The relative position and orientation of eight amphotericin and cholesterol molecules are described in terms of ten variables defining their distances from the axis of the complex, the distance between them along the symmetry axis, the Euler angles fixing their orientations, and the radial angle between them (Fig. 2). The internal degrees of freedom of the antibiotic and cholesterol molecules were fixed. The cholesterol side chain was in the '*trans*' conformation.

This approximation is justified by noting that amphotericin B [10] and the carbocyclic nucleus of

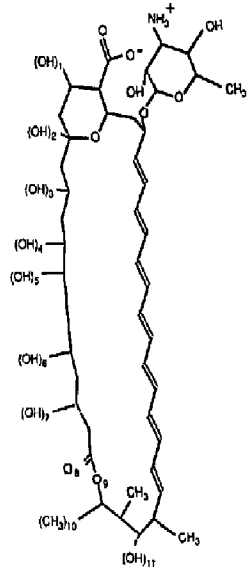

Fig. 1. Stucture of amphotericin B. The channel atoms numbers are given.

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cholesterol [11] are rather rigid, and each possesses just one main conformation [10,11]. Only the side chain of cholesterol is flexible [11], but it would be expected that the interaction between the '*trans*' conjugated double bonds chain of amhpotericin and cholesterol with the '*trans*' comformation of side chain would be the strongest, as in such a case the contact between both molecules is optimal [7,11].

The energy of the intermolecular interaction as a function of the positional and orientational variables introduced was calculated using the Lifson empirical force field [12] that includes electrostatic and Lennard-Jones potentials. Apparently it is very difficult to calculate correctly the interaction between charged ammonium and carboxyl groups of the antibiotic occurring on the membrane/water interface. The charges of these groups were considered as variable parameters. Three values of charges (1.0, 0.75 and 0.50) were used in the calculations.

For the calculations we used the conformations of amphotericin B [13] and cholesterol [14] from the crystallographic data, after preliminary refinement by the energy minimization. The interaction of nearest asymmetrical units of a regular complex concentrating the bulk of the complex energy was considered.

The search of low-energy structures was carried out in two steps. At first a regular complex consisting only of the antibiotic molecules was analyzed. Then the cholesterol molecules were incorporated into the optimal structures of this complex and the energy of the whole complex was minimized. The position of the minimum in the complex energy surface extending in the radial direction (see below) was determined by varying the distance d_A of amphotericin B from the channel axis (Fig. 2) over a range of 10 Å in increment 0.1 Å. The energy was minimized with respect to the others nine variables. A 10 Å range of the radial variable was covered by the computations.

The computations were carried out on a the personal IRIS graphics workstation. The visualization of

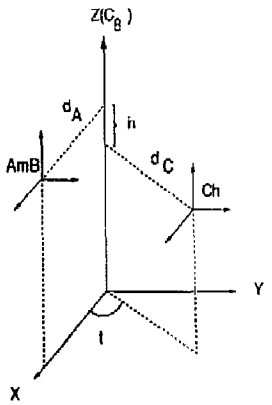

Fig. 2. Diagram of the relative position of amphotericin B (AmB) and cholesterol (Ch) in the asymmetrical unit of the complex. d_A and d_C are distances from the symmetry axis (Z) to AmB and Ch. h is the distance between them along the Z axis, and t is a radial angle between the molecules.

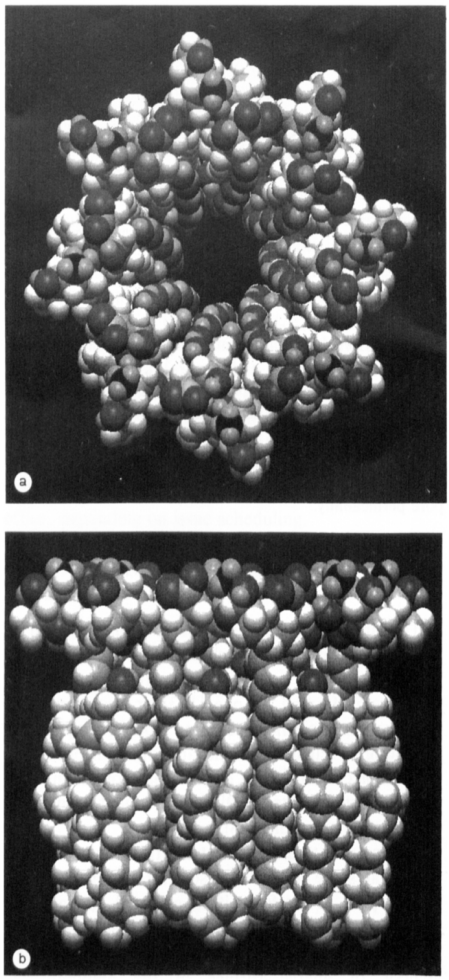

Fig. 3. Computer representation of the amphotericin B-cholesterol complex by CPK models (white CH hydrogens, light grey carbons, medium grey OH and NH hydrogens, dark grey oxygens, and black nitrogens). (A) The view is along the symmetry axis (Z axis) from the charged end of the complex. (B) The view is perpendicular the symmetry axis.

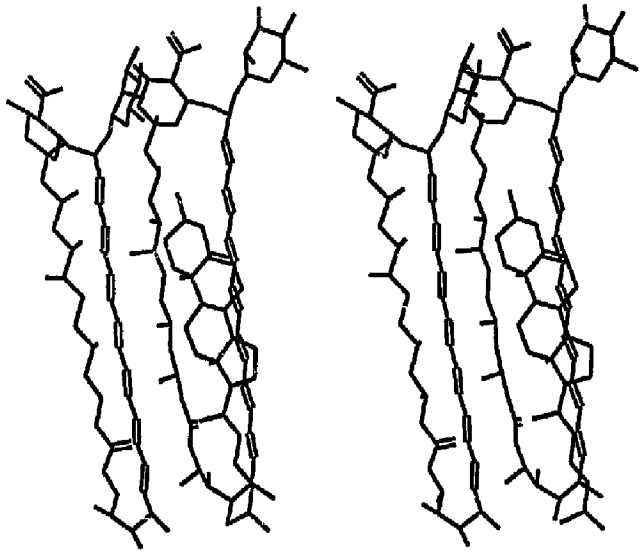

Fig. 4. Stereo view of two adjacent amphotericin B and incorporated cholesterol molecules.

the complex and its components was performed with the program MacroModel [15].

Results and Discussion

Characterization of the most stable complexes

Fig. 3 gives the representations of the structural organization of the amphotericin B channel. The channel is formed by ten hydroxyl and one methyl groups (Fig. 1) of the assembled antibiotic molecules (Fig. 3A). The external surface of the complex is formed by hydrophobic chains of amphotericin and cholesterol molecules (Fig. 3B). The water-exposed part of the complex contains charged ammonium and carboxyl groups. Two ammonium and carboxyl groups of adjacent molecules strongly interact (Fig. 3A).

The shape of the channel is not regular but contains narrow and wide areas. Table I presents the distances of atoms lying on the channel surface from the symmetry axis. The channel is characterized by wide entrances and a narrow center at the two oxygens O-6 and O-7. The distances between all pair of equivalent oxygens on adjacent antibiotic molecules are greater than the sums of their van der Waals radii, and thus there are no hydrogen bonds between them. The intramolecular hydrogen bonds we got found are probably an artifact of having done the calculations in vacuum. In reality the channel hydroxyl groups are probably hydrogen bonded to water molecules, contained in the channel. It is interesting to note that the hydroxyl hydrogens are approximately the same distance from the channel axis as the oxygens they are bonded to (Table I). This is because the adjacent C-O bonds are pointing towards the channel axis. It is known that the charge magnitude of hydroxyl oxygen is twice that of hydrogen, which implies that the pore has a cation rather than an anion affinity. This finding contradicts the popular opinion that the anion selectivity of amphotericin B pore is due to the hydrogens pointing towards the channel axis [16]. The cation selectivity is

TABLE I

Geometrical characteristics (Å) of the most stable complex

Atom [a]	R [b]	D [c]	Z [d]	Hydrogens (OH)		Carbons (OC)	
				R	Z	R	Z
O-1	10.0	7.7	24.2	10.3	25.0	9.6	23.3
O-2	7.8	6.0	21.1	7.9	22.1	8.7	20.9
O-3	6.9	5.3	18.6	7.1	19.4	7.9	18.4
O-4	5.8	4.4	16.2	5.7	17.0	7.1	16.0
O-5	6.8	5.2	14.9	5.9	15.3	7.2	14.7
O-6	4.9	3.7	10.9	4.6	11.7	6.3	10.9
O-7	4.8	3.7	8.3	4.3	9.0	6.1	8.3
O-8	5.6	4.3	5.7			6.3	5.8
O-9	6.8	5.2	4.7				
C-10 (CH_3)	5.3	4.2	2.9				
O-11	7.1	5.4	0.5	7.0	0.0	8.1	1.5
C(CO_2^-)	11.5	8.8	25.0				
N	13.5	10.4	24.0				

[a] See Fig. 1.
[b] Distances of the atoms from the symmetry axis.
[c] Distances between equivalent atoms.
[d] Axial co-ordinates of the atoms.

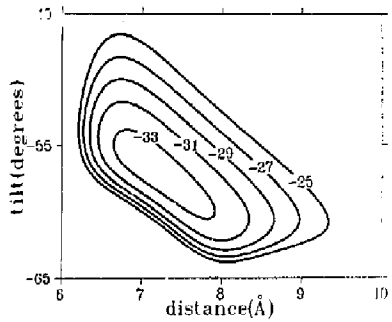

Fig. 5. Potential-energy (kcal/mol) map of the complex consisting only of the amphotericin B molecules. Abscissa is a distance (Å) of O_1 (see Fig. 1) from the symmetry axis, ordinate is a tilt (degrees) of the molecule plane to the radial axis.

observed when the antibiotic is added to one side of the membranes [1–3].

The stabilization of the complex is caused mostly by electrostatic interactions of ammonium and carboxyl groups of adjacent antibiotic molecules. The 4.6 Å distance between C and N of adjacent groups occurs as a result of a significant tilt of amphotericin planes in relation to the radial axis of the channel (Fig. 3A). The remaining distances between charged groups are much larger (Table I) and do not essentially influence these interactions. The charges used for the charged groups do not noticeably influence the structural variables and only cause changes in the interaction energy.

The structure of the hydrophobic part of the complex also has some interesting features. The cholesterol molecules are partly incorporated into the conjugated double bonds groove of amphotericin molecules (Fig. 4). The plane of cholesterol is in contact with hydrophobic edge of one of the antibiotic molecules, and the plane of adjacent antibiotic molecule is in contact with the edge of cholesterol. This type of contact between flat molecules is like that of a benzene dimer [17].

Low-energy structures of the complex

Analysis of the energy surface of the amphotericin-cholesterol complex shows the existence of valley-like region of low-energy values. It is interesting to point out that the complex consisting of only the antibiotic molecules exhibits this same feature. The contours on the complex potential-energy map extend in the radial direction (Fig. 5). The incorporation of cholesterol between amphotericin B molecules does not change this pattern.

Thus, the calculations show the presence of a continuous set of complex low-energy states. The variation in the positional parameters in this low-energy region are represented in the Table II for different charges of the NH_3^+ and COO^- groups. The structural characteristics of the pore appear to be essentially independent of the charges used. Even without knowing the exact charges, we can have reasonable confidence that these results are physically realistic. The main differences in the structures, corresponding to the opposite edges of the valley bottom, are observed near the end of the channel where the charged groups are located. The maximum difference equals about 1 Å for oxygen O-1. It gradually decreases toward the narrow part of the channel and equals 0.7 Å for oxygens O-6. At the opposite end of the channel, the difference is smaller and equals 0.1 Å. After correcting for the van der Waals radii, it is possible to conclude that, within the energy range considered the diameter of the narrow part of the channel can vary from 6.5 to 8 Å. The optimal channel diameter is about 7 Å. The weak observed permeability of the pore for molecules with a diameter of about 8 Å [18,19] and a monotonic increase of the permeability of the pore for molecules with decreasing size [18] are in good agreement with the results obtained.

TABLE II

Minimum and maximum distances (Å) of the channel atoms from the symmetry axis for low-energy structures belonging to a 2 kcal / mol energy range

Atom [a]	O-1	O-2	O-3	O-4	O-5	O-6	O-7	O-8	O-9	C-10 (CH_3)	O-11
|charge| [b] = 1.0											
Min	9.5	7.4	6.6	5.5	6.3	4.6	4.5	5.6	6.7	5.2	7.1
Max	10.6	8.3	7.3	6.2	7.2	5.2	5.2	5.8	7.2	5.6	7.2
|charge| = 0.75											
Min	9.5	7.5	6.6	5.5	6.3	4.6	4.5	5.6	6.6	5.2	7.1
Max	10.6	8.3	7.3	6.2	7.2	5.2	5.1	5.7	7.1	5.6	7.1
|charge| = 0.50											
Min	9.4	7.4	6.6	5.5	6.2	4.7	4.5	5.7	6.7	5.2	7.2
Max	10.7	8.3	7.3	6.2	7.2	5.2	5.1	5.7	7.0	5.5	7.1

[a] See Fig. 1.
[b] Charges of ammonium and carboxyl groups.

The presence of complex similar energies yet with different pore diameters can explain the transition of the channel from the open to the closed state, without postulating overall rearrangement such as a change in the number of molecules in the pore or the relative position of the pores. The decrease in conductivity may be the result of the shrinking of the pore, sharply reducing the permeability of the channel because of the large value of ion hydration energy in the relatively narrow channel.

Conclusions

There is a continuous set of low-energy structures of amphotericin B-cholesterol complex with different values of the channel diameter. These states are characterized by a significant tilt of amphotericin planes to relative the radial axis of the channel and a strong interaction between ammonium and carboxyl charged groups. A change in the channel diameter may result in transitions between the conducting and non-conducting states of the pore.

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