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A CONFORMATIONAL ANALYSIS STUDY OF THE INTERACTION OF AMIODARONE AND CHOLESTEROL WITH LYSOPHOSPHATIDYLCHOLINE

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Abstract—The spatial configuration of amiodarone (in both its protonated and neutral forms) and a hydroxylated analog was studied using conformational analysis in a simulated membrane–water environment. The three compounds and cholesterol were studied as isolated molecules and in interaction with lysophosphatidylcholine. The association of the molecules with lysophosphatidylcholine was further characterized by incorporation in a phosphatidylcholine matrix. Calculation of the mean interaction energy, the surface charge density and the hydrophilic and hydrophobic mean molecular areas showed that the protonated form of amiodarone, and to a lesser extent cholesterol form a stable association with lysophosphatidylcholine. This association was further stabilized when incorporated into a phosphatidylcholine matrix so that the mean interaction energy increased to −96.1 kJ/mol (i.e. 60% higher than the mean lipid–lipid energy of interaction). Lysophosphatidylcholine was shown to possess a cone-shaped structure whilst amiodarone was shown to be in the form of an inverted cone. This association of the two cones forms a stable cylindrical structure.

Amiodarone is now generally accepted as arguably the most effective antiarrhythmic drug currently available for the prevention of post-infarction life-threatening arrhythmias [1]. Structurally, it is a highly hydrophobic cationic amphiphilic agent [2–4] which has a pronounced interaction with the lipid component of the biological membrane [5–7]. Amiodarone lowers the temperature of the liquid to crystalline phase transition of the membrane and like cholesterol either increases or decreases lipid mobility in the gel or liquid–crystalline phase. In the gel state, lipid mobility depends on the concentration of amiodarone, its degree of ionization and the length of the lipid acyl chains. In the liquid–crystalline state, the concentration-dependent decreased lipid mobility is essentially due to hydrophobic interactions. Previously, we have shown that amiodarone increases the lipid order parameter to the same extent as cholesterol and at the same molar concentration [6]. We suggested that amiodarone is a rigid molecule deeply buried in the hydrocarbon core of the lipid and that amiodarone–lipid interactions are mainly hydrophobic.

Lysophosphatidylcholine, being a micellar lipid, acts as a natural modulator of biological membranes [8, 9]. Under certain conditions, the effects of lysophosphatidylcholine are thought to be deleterious as exemplified by cell lysis which ultimately causes cell death [9] or by acting as a "trigger" mechanism in the induction of life-threatening arrhythmias during myocardial ischaemia [10]. The effects of lysophosphatidylcholine can be counteracted by the addition of cholesterol [11–13]. More recently it has been shown that lysophosphatidylcholine and cholesterol interact in a molar ratio of 1:1 and form a stable bilayer [12, 14]. A recent study [15], extending the previous observations, showed that lamellar structures can be formed over a wide range of concentrations and that lysophosphatidylcholine distribution between the inside and the outside of the vesicle is highly sensitive to the cholesterol content. These observations led to the suggestion that one of the functions of cholesterol is to buffer the stability of the bilayer structure through a specific lysophosphatidylcholine–cholesterol complex [14].

The similar effects of amiodarone and cholesterol on lipid dynamics described above are suggestive of a similar pattern of interaction with the lipid membrane. This would be in agreement with the previously suggested "membrane-stabilizing" effect of cationic amphiphilic drugs [17] thus, offering a potential explanation for some of their cardiac activities [16, 17]. Because of the similarities between amiodarone and cholesterol, we have carried out a study using conformational analysis of the association of amiodarone (in both its protonated and neutral forms) and cholesterol with lysophosphatidylcholine.

MATERIALS AND METHODS

The conformational analysis procedure was based on the technique developed by Brasseur and Ruysschaert [18]. The total conformational energy of the molecule at the air–water interface was empirically calculated as the sum of all contributions resulting from local interactions, i.e. Van der Waal's energy, torsional potential, electrostatic interactions and transfer energy. Electrostatic energy was calculated as a function of the dielectric constant. The values of dielectric constant (ε) at the interface, determinated experimentally and theoretically, vary from 10 to 40 [18]. In this study in order to simulate the interface,

the dielectric constant of the hydrophobic and hydrophilic media were taken as 3 and 30, respectively. Between these two media, the dielectric constant was assumed to increase linearly. The transfer of energy for distinct moieties of the molecule has been determined experimentally by numerous authors, and summarized by Tanford [19]. The values used for the valance angles, bond lengths and atomic charges are those currently used in conformational analysis studies [20] and are similar to the values more recently used in computer modelling [21–23].

In the calculation procedure, changes of 60° each were first imposed on each of n torsional angles, yielding 6^n conformers. The internal energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy; such a selection was based on one statistical weight, Boltzmann relationship, associated with the individual configurations.

A simplex minimization procedure [24] was used to reduce further the total internal energy of selected conformations and the molecule was orientated at the air–water interface taking into account the positions of the hydrophobic and hydrophilic centres [25].

The procedure (the Hypermatrix Method) used to surround one molecule A with other molecules B is a modification of the sequential method used to surround one drug with lipid molecules [26]. This method is based on a non-relaxed strategy in which the molecular structure of all compounds are fixed through the hypermatrix procedure. In essence this consists of fixing the position and orientation of molecule A after orientation at the air/water interface. A second molecule B was then orientated at the interface and allowed to move along the x-axis in steps of 0.05 nm. For each position, the second molecule was rotated in steps of 30° around its long axis z' and around the first molecule. l is the number of positions along the x-axis, m the number of rotations of the second molecule around the first one and n is the number of rotations of the molecule itself. For each set of values of l, m and n, the intermolecular energy of interaction was calculated as the sum of the London–Van der Waals' energy of interactions (E^{vdw}), the electrostatic interaction (E^{cb}) and the transfer energy of atoms or groups of atoms from a hydrophobic to a hydrophilic phase (E^{tr}). Then, the second molecule was allowed to move in steps of 0.05 nm along the z'-axis perpendicular to the interface and the position of the z'-axis was varied in steps of 5° with respect to the z-axis, such that the lowest interaction energy state could be obtained for each set of values l, m and n.

The energy values together with the co-ordinates associated to each set of l, m and n, were stored in a hypermatrix and classified according to decreasing values of the interaction energy. The position of the third molecule C was defined as the first energetically favorable orientation stored in the hypermatrix, taking into account the sterical and energetic constraints imposed by the presence of the second molecule. Thus, orientations are disregarded where overlap of atomic co-ordinates of two molecules occurs and where the interaction energy between the two molecules was positive. In order to minimize further the conformational energy, the position of the second and third molecules were then alternatively modified in steps according to the energy classification of the hypermatrix. For the fourth molecule, the same process was repeated but this time the positions of the three surrounding molecules were modified alternatively in order to find the lowest energy state. In this calculation, the interaction energy between all monomers in the aggregate were considered and reduced to a minimum till the lowest energy state of the entire aggregate is reached. We limited this approach to the number of molecules sufficient to surround one central molecule. The configuration of the final mixed monolayer was projected onto the interface plane (X,Y) and the areas occupied per molecule were estimated. The mean interaction energy between one drug surrounded by lipids was equal to the sum of lipid–drug and lipid–lipid interaction energies divided by the number of surrounding lipids [26, 27].

The isoelectrostatic energy contour map showing attractive and repulsive regions was calculated taking as reference a plane located in the centre of the molecule [28]. The electrostatic potential field around a molecule was calculated as follows: a plane was defined in the center of the molecule perpendicular to the interface; a punctual charge (negative charge for P-Am and N-Am, positive charge for lyso PC) was then displaced by steps of 0.01 nm in all directions; the electrostatic energy was calculated in each position taking into account a dielectric constant equal to 10 and all discrete charges of the molecule. Interval of 1 kcal/mol (4.18 kJ/mol) was imposed. Contours with isoelectrostatic energy of 1 kcal/mol were then obtained.

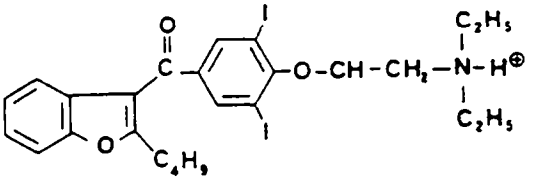
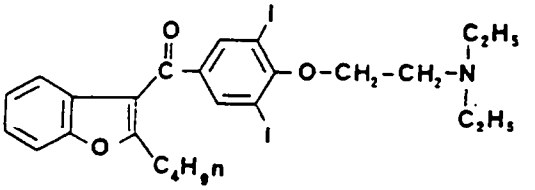
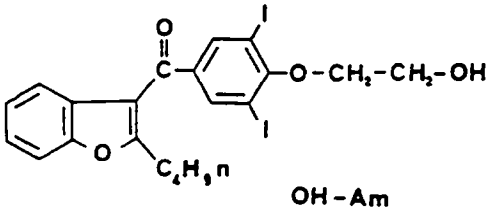
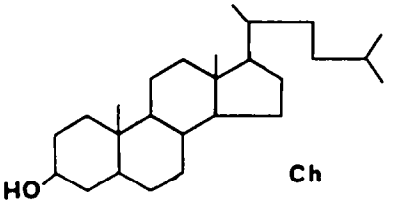
All calculations were performed using an Olivetti XP5 with a 80387 processor. The software used was PC-TAMMO+ (Theoretical Analysis of Molecular Membrane Organization) and PC-MSA+ (Molecular Structure Analysis) procedures [18]. Graphs were drawn with the PC-MGM+ (Molecular Graphics Manipulation) program.

RESULTS

Systematic analysis on the torsional angles and conformation at the air–water interface have been performed previously for amiodarone [4], cholesterol [29], lyso PC (1-palmitoyl-3-phosphatidylcholine) [30] and DPPC (1,2-dipalmitoyl-3-phosphatidylcholine) [26]. However, in the case of amiodarone, two analogues with modifications of the hydrophilic content of the lateral chain were also calculated: the neutral form and the hydroxyl derivative (Table 1). Their conformation at the air–water interface lies only in minor modifications at the level of the lateral hydrophilic chain. This confirms the mobility of the side chain and the major importance of the rotational angles around the carbonyl function [4]. The critical importance of these angles has been noted previously in the study of compounds chemically related to amiodarone but lacking any side chain [31].

The mean energies of interaction, computed by the Hypermatrix Method between amiodarone, its analogues and cholesterol and the various lipids are

Table 1. Chemical structure of amiodarone, analogues of amiodarone and cholesterol and mean interaction energy (E) between these compounds and lyso PC, DPPC and lyso PC incorporated in DPPC

Chemical structure	Interaction energy (E: kJ/mol)		
	Lyso PC	DPPC	Lyso PC-DPPC
 P-Am	−53.1	−73.6	−83.6
 N-Am	−42.2	−53.9	−96.1
 OH-Am	−36.4	−56.4	−72.3
 Ch	−33.5	−58.9	−62.7

P-Am, protonated amiodarone; N-Am, neutral amiodarone; OH-Am, hydroxylated analog of amiodarone; Ch, cholesterol; Lyso PC, 1-palmitoyl-3-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-phosphatidylcholine.

shown in Table 1. When interacting with lyso PC, the protonated form of amiodarone (P-Am) has the highest interaction energy. This value is of the same order (of magnitude) as the interaction energy between lyso PC and DPPC (−53.9 kJ/mol) [18] and DPPC–DPPC molecules (−54.3 kJ/mol) [32]. The interaction energies between amiodarone or analogues are very weak, being smaller than −11.9 kJ/mol. The interaction energy between cholesterol molecules is equal to −13.3 kJ/mol.

These results indicate that amiodarone, analogues and cholesterol interact preferentially with lipid rather than with themselves. The two compounds bearing a hydroxyl group (OH-Am and cholesterol) have the same interaction energy with lyso PC. The neutral form of amiodarone (N-Am) has an intermediate interaction energy.

Figure 1 illustrates the electrostatic field around P-Am, N-Am and lyso PC. The electrostatic environment field of P-Am shows one minimum associate to

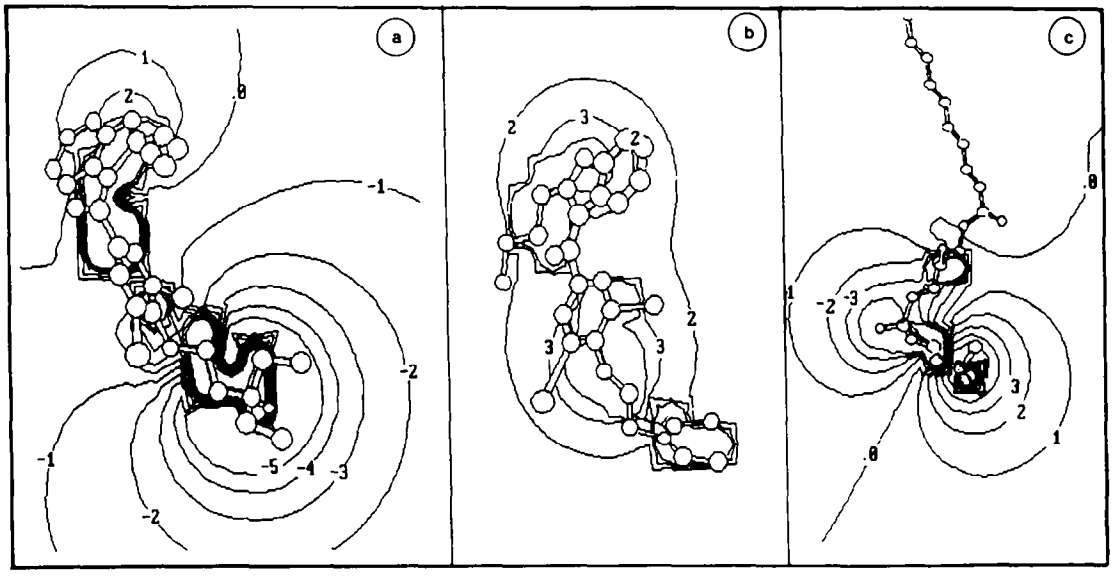


Fig. 1. Electrostatic field contour to contour representation [expressed in kcal/mol (1 kcal/mol = 4.18 kJ/mol)] of P-Am (a), N-Am (b) and lyso PC (c). The dielectric constant is equal to 10. The virtual charge moved by steps of 0.01 nm is negative for P-Am and N-Am and is positive for lyso PC.

the protonated moiety of the molecule and a repulsive region around the benzofuran moiety. The electrostatic environment field of N-Am indicates only a repulsive region uniformly developed around this molecule. For the lyso PC, calculated in this case with a positive charge, a minimum appears in the vicinity of the phosphate, and a repulsive region around the ionized amino group. Moreover, in the hydrocarbon region, the electrostatic environment field is equal to 0. This analysis indicates that a fit in the electrostatic interactions between P-Am and lyso PC possibly exists.

The structure of the complexes between lyso PC and amiodarone are depicted in Figs 2 and 3. One of the iodine atoms of P-Am interacts directly with the glycerol backbone of lyso PC; the proton of P-Am being located in the close vicinity of the phosphate of lyso PC. The acyl chain of lyso PC interacts with the benzofuran (Figs 2A and 3). The interaction of lyso PC with N-Am as compared to P-AM is totally different. An interaction is observed only between the polar head of lyso PC and the neutral amino residue of amiodarone (Fig. 2B). In contrast, cholesterol interacts mainly with the acyl chain of lyso PC (Fig. 2C).

The compounds were further studied by the calculation of another physico-chemical parameter characterizing their behaviour at the membrane interface: the area occupied per molecule. This corresponds to the projection on the plane of the interface between hydrophobic and hydrophilic phases of the mean molecular area occupied by either the hydrophilic of the hydrophobic moiety of a molecule. The values obtained for P-Am, N-Am, cholesterol and lyso PC isolated or in association are compiled in Table 2. In the case of lyso PC, the conformation of the molecule corresponds to a cone [30] due to the large difference in the area of the hydrophilic and hydrophobic moieties. This results in a micellar organization. P-Am, N-Am and cholesterol display no particular characteristics based on the molecular area of the isolated molecule. When interacting with lyso PC, it is important to note for P-Am and N-Am that the hydrophilic and hydrophobic surface areas are of the same order of magnitude as the isolated structures. This suggests some reduction in size of the 1/1 complex. The area occupied by the hydrophobic moieties in the complex corresponding approximately to the acyl chain for lyso PC and the benzofuran for P-Am, is equal to 0.61 nm^2 and the area occupied by the hydrophilic moieties is equal to 0.58 nm^2. These two values indicate that the P-Am–lyso PC complex can produce a bilayer organization. Thus, the micellar organization seen in the case of lyso PC can be modified. In the case of P-Am, the association could produce a stable bilayer. This could also be the case for cholesterol. By contrast, the association between N-Am and lyso PC shows no tendency to promote a stable structure.

Although the amiodarone– or cholesterol–lyso PC mean interaction energy is of the same order of magnitude as the DPPC–DPPC mean interaction energy, the former association displays some structural characteristics (e.g. complementarity in conformation and interactions) which deserve further investigation. Therefore the lyso PC-drug complexes were incorporated into a DPPC matrix. The interaction energies between DPPC and a 1/1 complex consisting of lyso PC and cholesterol or the various analogs of amiodarone are listed in Table 1. In comparison to the 1/1 complex with lyso PC, the interaction energy of the corresponding mixed matrix increases by 60 to 130%. In all cases, the interaction energy is higher than the DPPC–DPPC interaction

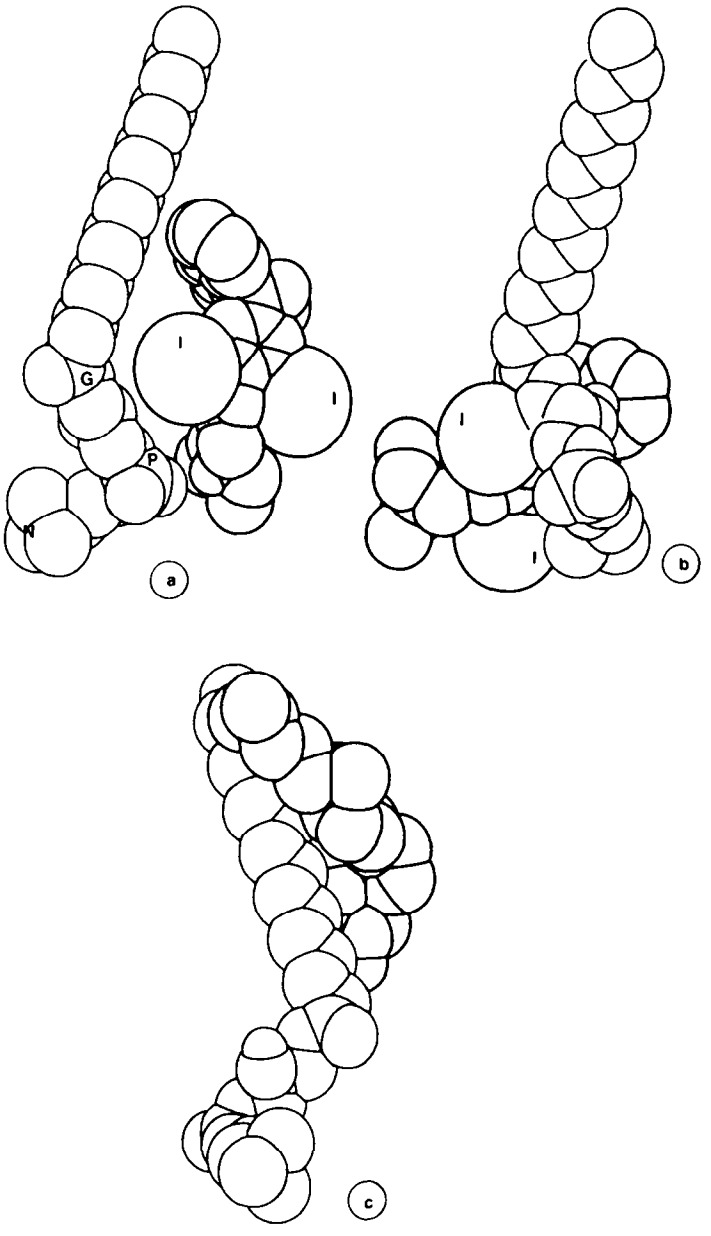


Fig. 2. (a) Space filling model (thick line) of the most probable mode of interaction of lyso PC and P-Am. The phosphate residue and the protonated amine are indicated respectively by P and N. One of the two iodine atoms (indicated by I) interact with the glycerol of the lyso PC (indicated by G). (b) Space filling model (thick line) of the most probable mode of interaction of lyso PC and N-Am. (c) Space filling model (thick line) of the most probable mode of interaction of lyso PC and cholesterol.

energy (−53.9 kJ/mol) [18]. For a given structure, the interaction energy is even higher than with respect to DPPC. The mean molecular area of the hydrophobic and hydrophilic moieties of the 1/1 complex incorporated into the DPPC layer are compiled in Table 2. From the ratio of hydrophobic/hydrophilic surface area, it appears that the essential properties needed to induce a bilayer structure for the P-Am– and cholesterol–lyso PC complexes are retained after incorporation in DPPC. The situation is unchanged in the case of the N-Am–lyso PC complex. It is of note that the mean molecular area is decreased after incorporation into the lipid bilayer (Table 2). Thus, the incorporation of the drug–lyso PC or cholesterol–lyso PC complex into the DPPC matrix stabilizes the complex. The ternary associations (P-Am–lyso PC–DPPC) and (cholesterol–lyso PC–DPPC) are depicted in Fig. 4. The association

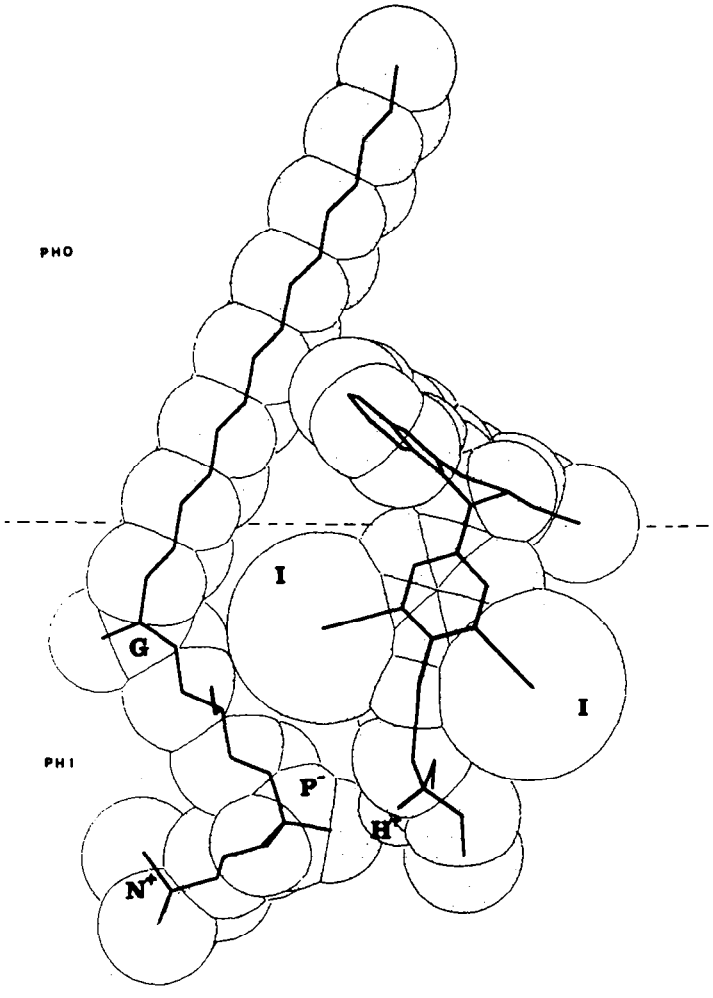


Fig. 3. Combination of space filling, skeleton in perspective and Midas representation showing the mode of interaction of lyso PC and P-Am. The phosphate residue and the protonated amine are indicated respectively by P^- and N^+. One of iodine atoms (I) interacts with the glycerol backbone (G) of the lyso PC. The proton of amiodarone indicated by N^+ interacts with P^- of lyso Pc. The dotted line indicates the position of the interface. The hydrophobic and hydrophilic phases are indicated by PHO and PHI, respectively.

Table 2. Mean molecular area of lyso PC, Ch, P-Am and N-Am and their association in the absence or presence of DPPC

Compound	Hydrophobic moiety	Hydrophilic moiety	$1 - \frac{\text{Hydrophobic}}{\text{Hydrophilic}}$
Lyso PC	0.20	0.59	0.66
P-Am	0.56	0.45	0.24
N-Am	0.58	0.45	0.28
Ch	0.34	0.12	1.83
P-Am–lyso PC	0.61	0.58	0.05
N-Am–lyso PC	0.54	0.74	0.28
Ch–lyso PC	0.58	0.59	0.02
(P-Am–lyso PC)DPPC	0.47	0.50	0.06
(N-Am–lyso PC)DPPC	0.40	0.52	0.76
(Ch–lyso PC)DPPC	0.61	0.62	0.02

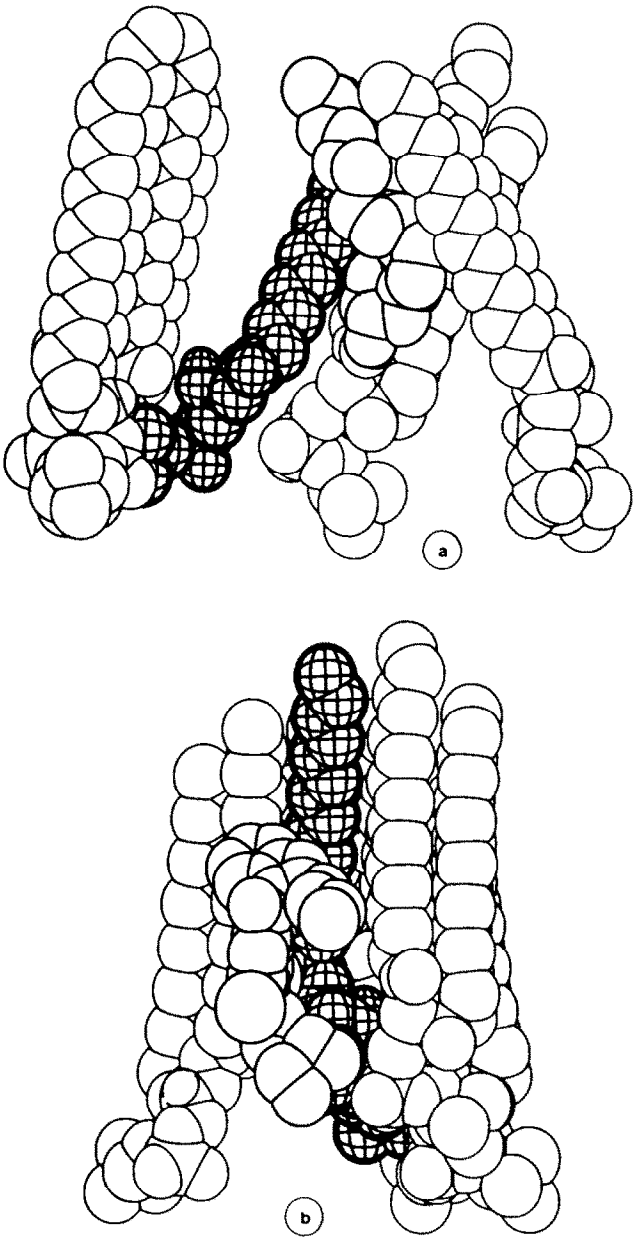


Fig. 4. (a) Space filling model of the most probable mode of interaction of the 1/1 cholesterol–lyso PC complex incorporated into a DPPC layer. The thin line refers to DPPC, the thick line refers to cholesterol, cross hatching refers to lyso PC. (b) Space filling model of the most probable mode of interaction of the 1/1 P-Am–lyso PC complex incorporated into a DPPC layer. The thin line refers to DPPC, the thick line refers to P-Am, cross hatching refers to lyso PC.

of cholesterol or P-Am with lyso PC retained when incorporated into DPPC the general characteristics observed in the binary association (Fig. 2). Both complexes maintained the bilayer structures. However, the cholesterol–lyso PC complex induces a tilting of the phospholipid molecules over the interface whereas P-Am–lyso PC does not (Fig. 4).

DISCUSSION

Amiodarone (and its various analogues) as well as cholesterol have, when interacting with lyso PC, an interaction which is of the same order as the interaction energy with lipids (≈40 kJ/mol). This interaction energy is sufficient to promote a stable association between the various compounds and lyso PC. However, the determination of the hydrophilic and hydrophobic mean molecular areas and the electrostatic energy contours of the various compounds and of lyso PC indicated that two of the former have additional characteristics which reinforce the stability of the association with lyso PC. These two compounds

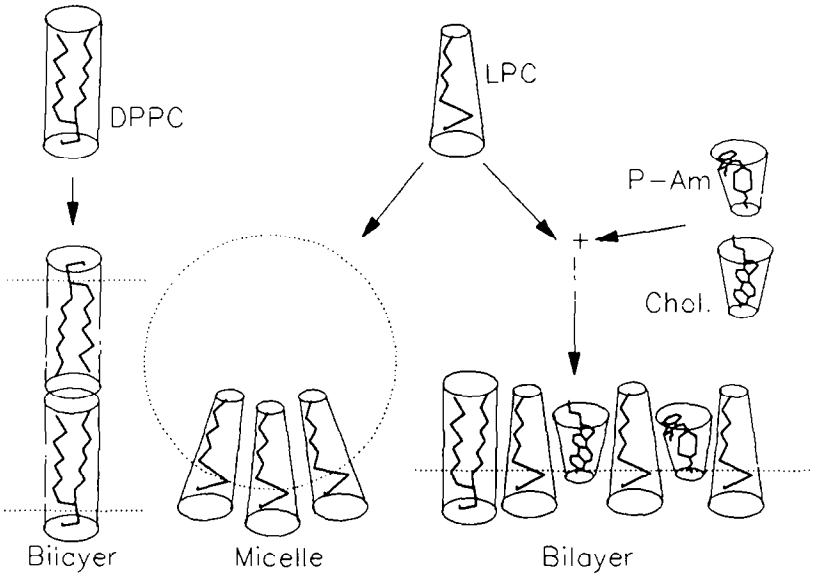


Fig. 5. Schematic view of the cylindrical form of DPPC and the cone-shaped structure of lyso PC. Assembly of DPPC produces bilayer, assembly of lyso PC results in a micelle. The fit between the cone-shaped structure of lyso PC or P-Am and cholesterol inserted in DPPC produces a bilayer organization.

are the protonated form of amiodarone and cholesterol. Both compounds adopt, when interacting with lyso PC, the conformation of an inverted cone which is complementary to the cone-shape of lyso PC. The inverted cones of protonated amiodarone and cholesterol do not exist in the isolated molecule. In the case of protonated amiodarone, the structure of the association with lyso PC allows fit in the electrostatic interaction which is mainly due to an interaction between the negatively charged phosphate of lyso PC and the protonated amine of amiodarone.

Both characteristics (cone-shape and electrostatic complementarity) are maintained and reinforced when the protonated amiodarone- or cholesterol-lyso PC association is incorporated into DPPC layer.

A schematic representation of the interaction of the protonated amiodarone and cholesterol with lyso PC in the absence and the presence of DPPC is depicted in Fig. 5. DPPC has a cylindrical structure and forms a stable bilayer [26]. Lyso PC has a cone structure and forms micelles. Protonated amiodarone and cholesterol have an inverted cone structure which is complementary to the cone formed by lyso PC. Thus, in the presence of protonated amiodarone or cholesterol, the radius of the micelle increases and could ultimately lead to a value large enough to promote a bilayer structure as is the case for cholesterol [14, 15].

Thus, in the present study, we have provided evidence suggesting the association between amiodarone or cholesterol and lyso PC forms highly stable complexes. The properties of lyso PC are generally attributed to their ability to form micelles. Although the lyso PC has a structure comparable to that of phosphatidylcholine, the lack of acyl chain in position 2 on the glycerol backbone enables the formation of micelles. It has been considered [9, 13, 15] that the perturbing effect of the membrane structure is due to the formation of highly curved regions in the presence of increasing concentrations of lyso PC. The characteristics of the association between amiodarone or cholesterol and lyso PC determined in this study suggests that amiodarone or cholesterol could be able to counteract some of the deleterious effects of lyso-PC by modulating the radius of the lyso-PC enriched domains.

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