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MOLECULAR SIMULATION STUDIES OF THE INTERFACIAL REGION IN LIPID BILAYERS

BADANIA OBSZARU INTERFAZY W BŁONACH LIPIDOWYCH METODĄ SYMULACJI DYNAMIKI MOLEKULARNEJ

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Summary: The structure and the dynamics of the membrane/water interface in pure dimyristoylphosphatidylcholine (DMPC) and DMPC-cholesterol (DMPC-Chol) bilayers were studied using a molecular dynamics (MD) simulation method. In this region, an extended network of interactions among polar groups of lipid and water is formed. In the DMPC bilayer, these interactions involve 1) water cross-bridges between negatively charged oxygen atoms of PCs, which link 76% of DMPC molecules in the membrane, 2) charge pairs between positively and negatively charged groups of PCs, which link 93% of DMPC molecules in the membrane. Water bridges and charge pairs together link 98% of all membrane phospholipids at any instant. At the DMPC-Chol bilayer interface, in addition to the PC-PC links, 1) direct hydrogen bonds, 2) water bridges, and 3) charge pairs between PC polar groups and γ Chol are formed. PC-PC and PC-Chol links involve at any instant 97.5% of DMPC and 70% of Chol molecules. Average lifetimes of PC-PC and PC-Chol associations are 1 ns or more. These associations are dynamic and they temporarily break and re-form several times during their lifetime.
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Key words: phosphatidylcholine, cholesterol, hydrogen bonds, water bridges, charge pairs, molecular dynamics

Streszczenie: Badania struktury i dynamiki interfazy błona/woda w czystej błonie dimirystynowo-fosfatydylocholinowej (DMPC) oraz błonie mieszanej DMPC-cholesterol (Chol), prowadzono metodą symulacji dynamiki molekularnej. W rejonie interfazy, grupy polarne lipidów i wody tworzą rozległą sieć połączeń. W błonie DMPC, połączenia są wynikiem oddziaływań: 1) poprzez cząsteczkę wody, która równocześnie tworzy wiązania wodorowe z dwoma ujemnie naładowanymi atomami tlenu PC (pomosty wodne), łączą one 76% cząsteczek DMPC; 2) między dodatnio naładowaną grupą cholinową i ujemnie naładowanym atomem tlenu PC (pary ładunkowe), łączą one 93% cząsteczek DMPC. W każdej chwili, 98% wszystkich cząsteczek DMPC w błonie jest połączonych poprzez pomosty wodne i/lub pary ładunkowe. W obszarze interfazy błony DMPC-Chol, oprócz połączeń PC-PC, występują także analogiczne połączenia PC-Chol poprzez 1) pomosty wodne, 2) pary ładunkowe, 3) bezpośrednie wiązania

wodorowe. W połączeniach tych bierze udział ~98% cząsteczek DMPC oraz 70% cząsteczek Chol. Średnie czasy życia połączeń są rzędu 1 ns lub dłuższe; połączenia te mają charakter dynamiczny i w czasie życia podlegają chwilowym zerwaniom.
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Słowa kluczowe: fosfatydylocholina, cholesterol, wiązania wodorowe, pomosty wodne, pary ładunkowe, dynamika molekularna

INTRODUCTION

Biological membranes are multi-component systems. Their main structural element is a liquid-crystalline lipid bilayer. Other constituents i.e., proteins, sterols, peptides, either intercalate into or loosely attach to the bilayer. The most prevalent molecule that makes up a liquid-crystalline bilayer of eukaryotic cell membranes is phosphatidylcholine (PC). Cholesterol (Chol) often amounts to 50 mol% of the lipids in plasma membranes of these cells. Physico-chemical and biophysical properties that are common to the membranes can be explored by studying the lipid bilayers (model membranes). A molecular dynamics (MD) simulation method is well suited for this purpose because it gives atomic resolution and spans of the observation time (on the time scale of 10^{-8}–10^{-7} s) by a few femtosecond steps.

PC and Chol are amphiphilic molecules that contain distinctly polar and hydrophobic parts. A PC polar part is called headgroup and it contains groups that are positively and negatively charged, whereas its net electrostatic charge is zero. Representative negatively charged groups of PC include the nonester phosphate oxygen atoms (Op) and the carbonyl oxygen atoms in the ester linkages between glycerol and acyl chains (Oc). The positively charged group is the choline moiety. A Chol polar group is the hydroxyl group (OH-Chol).

In the bilayer, the polar groups of PC and Chol are exposed to the water phase and participate in the formation of a multi-component region of the membrane/water interface (Figs 1 and 2). A MD simulation was applied to elucidate the structure and dynamics of the interfacial region in pure dimyristoylphosphatidylcholine (DMPC) [1, 2] and DMPC-Chol [3] bilayers. Due to its complex architecture and dynamic character, the region is particularly difficult to investigate by experimental and theoretical methods, so its contribution to the membrane integrity, stability and functioning has not been well determined so far.

BILAYER MODELS

Computer models of the pure PC bilayer consisted of 72 DMPC and 1622 water molecules (~23 water molecules/DMPC), and of the PC-Chol bilayer consisted of 56 DMPC, 16 Chol (~22 mol% of Chol) and 1622 water molecules. The models were simulated using AMBER 4.0 [4] for the total time of 5 and 4.3 ns, respectively.

Details concerning the construction and equilibration of pure DMPC and DMPC-Chol bilayers have been described in papers [1, 2] and [3], respectively.

STRUCTURAL PROPERTIES OF THE INTERFACE

The pure DMPC bilayer equilibrated within 2 ns [2], and the DMPC-Chol bilayer within 2.3 ns [3]. Thus 3-ns (pure PC) and 2-ns (PC-Chol) MD trajectories generated after the bilayers had reached thermal equilibrium, were used for analyses. The primary aim of the analyses was to investigate short-distance interactions among polar groups of PC, Chol, and water in the interfacial region of the membrane.

In the liquid crystalline DMPC bilayer, each DMPC makes, on average, 5.4 hydrogen (H) bonds with water, but only 4.5 water molecules are H bonded to each DMPC [1, 2]. In the DMPC-Chol bilayer, the average number of H bonds with water/DMPC is 5.7, and that of H bonded water molecules/DMPC is 4.8, thus is ~7% higher than in the pure DMPC membrane [3]. In both membranes, approximately 20% of H bonded water molecules are simultaneously bonded to oxygen atoms of two DMPCs and make water bridges. However, in the bilayer containing 22 mol% Chol, the number of intermolecular water bridges is by ~12% lower than in the pure DMPC bilayer. These bridges link 76 and 64% of DMPCs in pure DMPC and DMPC-Chol bilayers, respectively.

Negatively charged phosphate or carbonyl oxygen atoms of one PC and positively charged methyl groups of the choline moiety ($N\text{-}CH_3$) of another PC interact directly via Coulomb attraction and form relatively stable charge pairs [2]. In the DMPC-Chol bilayer, the number of intermolecular charge pairs is by ~20% lower than that in the pure DMPC bilayer. Charge pairing involves 93 and 91% of DMPCs in pure DMPC and DMPC-Chol bilayers, respectively.

DMPC-DMPC associations *via* both water bridges and charge pairs link 98 and 96% of DMPCs in pure DMPC and DMPC-Chol bilayers, respectively at any instant. On average, each DMPC molecule makes 2.7 and 2.2 links with other DMPCs in pure DMPC and DMPC-Chol bilayers, respectively.

At the interface of the DMPC-Chol bilayer, Chol makes 1.1 H bonds with water molecules, of which 0.5 are bridging H bonds. They link OH-Chol with carbonyl or nonester phosphate oxygen atoms of DMPC. Chol and DMPC can also be linked via charge pairs between OH-Chol and $N\text{-}CH_3$, and direct H bonds between OH-Chol and carbonyl or nonester phosphate oxygen atoms of DMPC. Consequently, 70% of Chol molecules are linked to 21% of DMPC molecules in the membrane at any instant. On average, a Chol molecule forms 0.9 links with DMPC molecules, while a DMPC molecule forms 2.2 and 0.3 links with DMPC and Chol molecules, respectively. Thus, in the DMPC-Chol bilayer, 97.5% of DMPC molecules are linked to either DMPC or Chol. When both DMPC-DMPC and DMPC-Chol links are included, then the average number of links formed by each DMPC molecule

is 2.5/DMPC. Because Chol forms far fewer links, the overall average of intermolecular links/lipid decreases to 1.9 in the DMPC membrane containing 22 mol% Chol.

DYNAMIC PROPERTIES OF THE INTERFACE

At the membrane/water interface, the polar groups of lipids interact via water bridges and charge-pairs, and, in the case of PC-Chol bilayers, direct H bonds. These pair-interactions between groups have relatively short lifetimes, about 50 ps for water bridges, 150 ps for charge pairs, and 70 ps for PC-Chol H bonds. However, in most cases, more than one polar group in one lipid molecules simultaneously interact with more than one polar group in another lipid molecule; also, if one water bridge or charge pair breaks then it is readily replaced by another one, thus inter-lipid associations have much longer lifetimes than individual pairs of groups. In the pure DMPC bilayer, the average lifetime of DMPC-DMPC associations via charge pairs, water bridges and both, are at least 730, 1400, and over 1500 ps, respectively. In the presence of 22 mol% Chol, these lifetimes are decreased by ~20%. The average lifetime of DMPC-Chol association is 920 ps. As was shown in the previuos section, these inter-lipid links form extended networks of interactions, involving, on average, 98% of all membrane lipids. Nevertheless, PC-PC and PC-Chol associations are dynamic and they break and re-form several times during their lifetime. On average, 70% of Chol molecules are linked to DMPCs at any instant, however, transient DMPC-Chol associations involve all Chol molecules in the membrane. During the analysis time of 2.0 ns, every Chol molecule has been associated with a DMPC for at least 20% of this time and on average, for 70% of this time.

CONCLUSIONS

Our MD simulation studies of the membrane/water interface in pure DMPC [1, 2] and DMPC-Chol [3] bilayers indicate that DMPC and Chol headgroups are linked by water molecules (water bridges) and direct Coulomb attraction (charge pairs). Furthermore, DMPC and Chol interact via direct H bonds, which do not take place between PC headgroups. In both bilayers, ~98% of the lipid molecules are linked via these interactions which form an extended network. However, in the DMPC-Chol bilayer, the average number of interlipid links is 1.9/lipid (either DMPC or Chol), while in the pure DMPC bilayer, it is 2.7/lipid. This indicates that the network of interlipid links in both pure and PC-Chol bilayer, involves almost all membrane molecules, however, in the PC-Chol bilayer, it is less branched. The reasons for that is that Chol is often an „end" molecule in the lipid-lipid

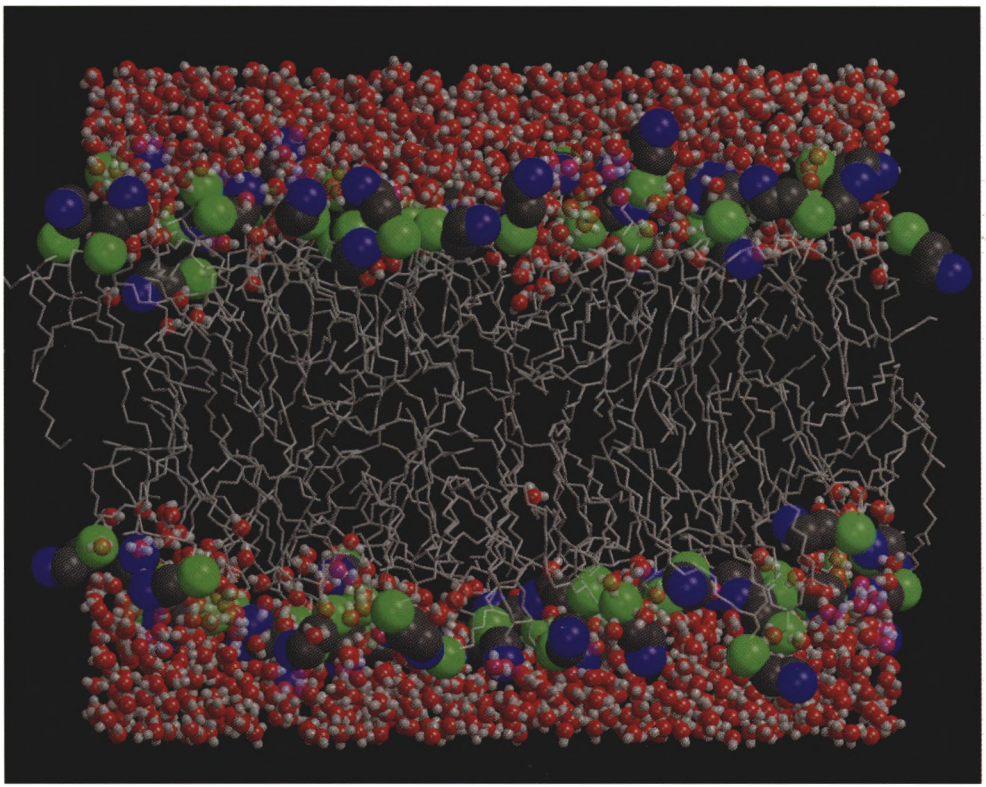

FIGURE 1. Hydrated DMPC bilayer membrane in the liquid-crystalline phase. The DMPC hydrocarbon chains form a hydrophobic core of the bilayer. The polar groups of DMPCs and water molecules form a membrane/water interface. The phosphate and the choline groups are green and blue, respectively, and the carbon atoms of the α-chain are grey. All membrane water molecules are shown, as the CPK model of the reduced size

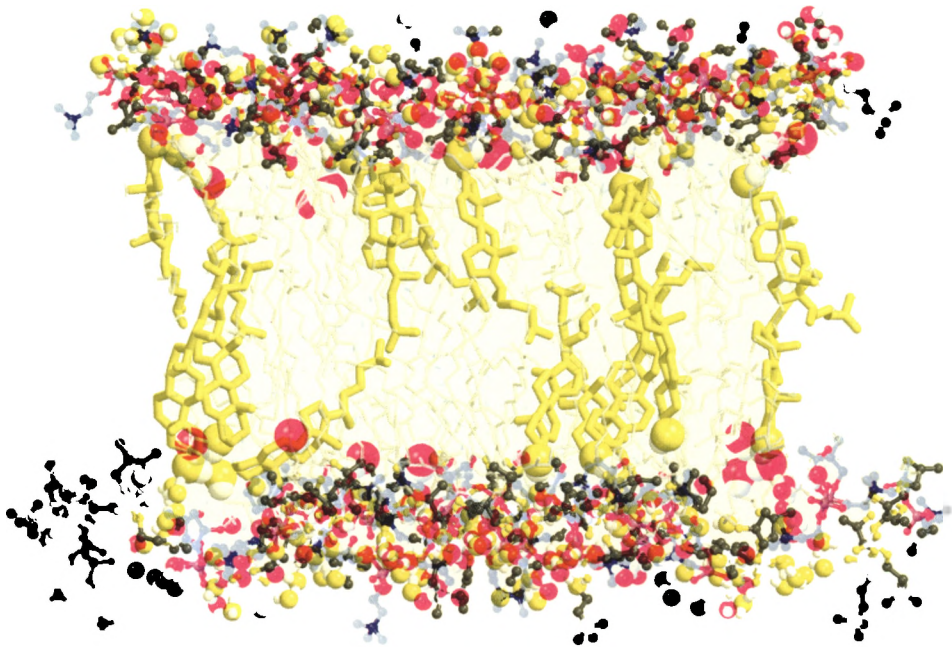

FIGURE 2. DMPC-cholesterol bilayer membrane. The cholesterol molecules are shown as yellow sticks, the cholesterol hydroxyl group is shown in standard colours as the CPK model. The oxygen atoms of DMPC molecule are red. Only water molecules that are hydrogen bonded to DMPCs are included

network, and also, the average distance between PC headgroups is larger in the DMPC-Chol bilayer than in the pure one due to the spacing effect of Chol. These results agree with experimental data [5].

The network of lipid-lipid links in the interfacial region of PC and PC-Chol bilayers observed in MD simulations, has been postulated to make a preferential pathway for proton diffusion along the membrane [6].

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REFERENCES

[1] PASENKIEWICZ-GIERULA M, TAKAOKA Y, MIYAGAWA H, KITAMURA K, KUSUMI A. Hydrogen bonding of water to phosphatidylcholine in the membrane as studied by a molecular dynamics simulation: location, geometry, and lipid-lipid bridging via hydrogen-bonded water. *J Phys Chem* 1997; **101**: 3677–3691.

[2] PASENKIEWICZ-GIERULA M, TAKAOKA Y, MIYAGAWA H, KITAMURA K, KUSUMI A. Charge pairing of headgroups in phosphatidylcholine membranes. A molecular dynamics simulation study. *Biophys J* 1999; **76**: 1228–1240.

[3] PASENKIEWICZ-GIERULA M, RÓG T, KITAMURA K, KUSUMI A. Cholesterol effects on the phosphatidylcholine bilayer polar region: a molecular simulation study. *Biophys J 2000;* **78**: 1376–1389.

[4] PEARLMAN DA, CASE DA, CALDWELL JC, SEIBEL GL, SINGH UC, WEINER PK, KOLLMAN PA. AMBER 4.0. University of California, San Francisco. 1991.

[5] SLATER SJ, HO C, TADDEO FJ, KELLY MB, STUBBS CD. Contribution of hydrogen bonding to lipid-lipid interactions in membranes and the role of lipid order: effects of cholesterol, increased phospholipid unsaturation, and ethanol. *Biochemistry* 1993; **32**: 3714–3721.

[6] TEISSIE J, PRATS M, LEMASSU A, STEWART LC, KATES M. Lateral proton conduction in monolayers of phospholipids from extreme halophies. *Biochemistry* 1990; **29**: 59–65.

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