Molecular dynamics study of binary POPC bilayers: molecular condensing effects on membrane structure and dynamics

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Abstract. Molecular dynamics (MD) simulations of binary 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayers containing cholesterol (CHOL), ceramide (CER), diacylglycerol (DAG), or sphingomyelin (SM) were carried out to investigate effects of these molecules on the structure and dynamics of membranes. Clear condensing effects were observed in all POPC binary bilayers, and those molecular condensing effects were ranked in the order of POPC/CHOL, POPC/CER, POPC/DAG, and POPC/SM bilayer. The differences of condensing effects are attributable to the factors such as molecular shapes, type of polar head groups and hydrogen bond networks in the binary lipid bilayers. The rigid sterol ring of CHOL is due to the ordering of acyl chains of POPC in the membrane. The lack of bulky PC head group of lipids results in the lipids condition in which DAG, CER and CHOL are buried in the hydrophobic region of the bilayer, leading to the condensed membranes. CHOL and CER are closely arranged in each binary bilayer due to the better intermolecular affinities of these lipids. The hydrogen bond network can also be a factor for the condensing of membranes and the decrease of lateral diffusion of lipids in the binary lipid bilayers.

1. Introduction
Addition of different molecules to membranes largely changes the structural and dynamical properties of the membranes, and these changes alter the functions of membranes such as signaling and molecular translocation across the membranes. Especially cholesterol (CHOL) makes membrane dense and changes the membrane state to the liquid-ordered phase above 20-30 mol% of cholesterol [1,
2]. It has been known that such molecular condensing effects also occur by adding other lipids such as ceramide (CER) [3-5], diacylglycerol (DAG) [6, 7] and sphingomyelin (SM)[8, 9]. However, the differences of molecular condensing effects by these lipids on the lipid structure and dynamics, and the molecular mechanisms in membranes have not been well known. In this study, we thus carried out molecular dynamics (MD) simulations of binary 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayers containing the CHOL, CER, DAG, or SM, and systematically investigated the effects of these lipids on structure and dynamics of the membranes. We then discussed the factors to yield the differences of membrane condensing in the binary lipid bilayers.

2. Material and method

2.1. Molecular structures of lipids

Five kinds of lipids, POPC, DAG, SM, CER, and CHOL were used in the MD study. The binary lipid bilayers consist of the POPC and another lipid (i.e. DAG, SM, CER, or CHOL). Figure 1 shows the molecular structures of the lipids used in the study. POPC has a 16:0 palmitic acid (PA) and an 18:1 oleic acid (OA) linking to the glycerol backbone and has a phosphorylcholine (PC) head group. DAG has a hydroxyl group instead of the PC head group of the POPC. SM has an 18:1 sphingosine (SPH) and a PA with an amide and a hydroxyl group at the linkage of the PA and SPH. CER has a hydroxyl group instead of the PC head group of the SM. CHOL has a rigid sterol ring linking to a hydroxyl group and has a short hydrocarbon chain. DAG, SM, CER and CHOL have some hydroxyl or amide groups around the head or linkage to the acyl chains of the lipids, and these can be some donor/acceptor sites to form hydrogen bonds with surrounding POPC or themselves in the membrane.

Figure 1. Molecular structures of (A) 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), (B) diacylglycerol (DAG), (C) sphingomyelin (SM), (D) ceramide (CER), and (E) cholesterol (CHOL).

2.2. Molecular dynamics simulations

Initial bilayer structures of a pure POPC and four binary POPC mixtures were prepared for MD simulations. The pure POPC bilayer was used as a reference system. Each system consists of 128 lipids and 8,192 water molecules. 64 lipids and 4,096 water molecules were packed into each half region of the MD box. In the binary POPC bilayer, thirteen POPC were replaced to the other lipids (CHOL, DAG, CER, or SM) in each leaflet of the bilayer, corresponding to about 20 mol % of molecular addition. The initial coordinates of each system were prepared by CAHRMM-GUI [10].

MD simulations of the pure POPC bilayer and four binary POPC bilayers (POPC/CHOL, POPC/DAG, POPC/DAG and POPC/SM) were run under the constant temperature (T = 303.15 K) and pressure (P = 1 atm) condition for 200 ns. Nose-Hoover thermostat [11, 12] and Parinello-Rahman type borostat [13] were adopted to control the system temperature and the pressure. The periodic lengths of thermostat and barostat were both 1 ps. CHARMM36 force field [14, 15] and TIP3P water
model [16] were used for lipids and water molecule, respectively. Cutoff for the van der Waals (vdW) interaction was applied using a switching scheme, within a range of radius from 10 to 12 Å. Particle mesh Ewald (PME) method [17] was adopted for the calculation of the coulomb electrostatic interactions. The time step for numerical integration of equations of motion was 2 fs, and the coordinates of the systems were stored every 1 ps. Figure 2 shows the area per molecule as a function of MD time for each system. The plots show that all molecular areas are sufficiently equilibrated after 100 ns. Thus, the last 100 ns of the MD runs were used for the analysis. All MD simulations were done by GROMACS 5.1.2 [18].

2.3. Analysis

Time averaged area per molecule $A_{mol}$, membrane thickness $d_{P,P}$, and order parameter $-S_{CD}$ for each system were calculated to investigate the effects of molecular addition on the membrane structure. $A_{mol}$ at each MD step was simply estimated by dividing the $xy$ area of MD box by the lipid number in the leaflet of bilayer. The values of $d_{P,P}$ were calculated as the time averaged distances between positions of the phosphorus atoms in each leaflet of the bilayer. The order parameters $-S_{CD}$ of two acyl chains (sn-1 and sn-2) of POPC in the pure and the binary lipid bilayers were estimated. Some of these parameters on membrane structure can be compared with the corresponding experimental values.

The average number of hydrogen atoms within 3Å cutoff length from the acceptor site for hydrogen bonds of the lipid was defined as a hydrogen coordination number of the site, and this can be calculated by the radial distribution function (RDF). In this study, the hydrogen coordination numbers for all possible acceptors of the lipids were calculated to investigate the hydrogen bond networks and the stabilities of the hydrogen bonds in the membrane. The total number of these hydrogen coordination numbers, which is calculated by summing up all coordination numbers, was evaluated for each binary bilayer and compared each other. The total hydrogen coordination numbers for between additional molecule and POPC and for between additional molecules were also evaluated to investigate the hydrogen bond networks between those lipids in the membrane.

Some experimental observations have shown that the addition of CHOL/CER to lipid bilayer systems causes formation of CHOL/CER enriched micro-domains in the membrane [1-5]. This is because these lipids have better affinity with them than the lipids of host membrane, and this difference of molecular interactions could result in the ununiform distribution of CHOL/CER in the binary POPC bilayer plane. Two-dimensional RDF for the centers of mass of additional lipids was calculated to assess the arrangements of the lipids in the membrane plane.

Mean square displacement (MSD) of the centers of mass of lipids in the membrane plane directions (x-y plane) was calculated to examine the effects of molecular addition on the lateral diffusions of lipids in the binary lipid bilayers. In the estimation of MSD, the lateral diffusions of lipids were sampled in each leaflet of the bilayers, and the center of mass motion of the leaflet was removed. The
Figure 3. Order parameters, $-S_{CD}$, of each acyl chain (left: sn-1 and right: sn-2) of POPC in each binary POPC bilayer.

Table 1. Time averaged area per molecule $A_m$, membrane thickness $d_{PP}$, and order parameters $-S_{CD}$ for pure POPC, and binary POPC bilayers (POPC/CHOL, POPC/CER, POPC/DAG, and POPC/SM). The parenthesis shows the standard deviations of the values. The listed $-S_{CD}$ in the table were average values of $-S_{CD}$ at each carbon of sn-1 and sn-2 chains.

|                | $A_{mol}$ [Å$^2$] | $d_{PP}$ [Å] | $-S_{CD}$  |
|----------------|-----------------|-------------|------------|
| Pure POPC      | 63.5 (1.3)      | 39.1 (0.7)  | 0.15       |
| POPC/CHOL      | 51.8 (1.1)      | 43.3 (0.7)  | 0.21       |
| POPC/CER       | 57.4 (1.2)      | 41.9 (0.7)  | 0.18       |
| POPC/DAG       | 60.4 (1.3)      | 41.2 (0.7)  | 0.17       |
| POPC/SM        | 61.8 (1.1)      | 39.1 (0.6)  | 0.16       |

The time length observed for each lipid lateral diffusion was 20 ns, and the MSD curves were averaged using the MD trajectories for the following 80 ns to obtain the converged MSD curves. The MSD of POPC and additional molecules (DAG, SM, CER, and CHOL) in the binary of lipid bilayer were estimated, and the MSD of POPC evaluated in the pure POPC bilayer was used as a reference MSD data in this study. The lateral diffusion coefficients, $D_s$, of the lipids were estimated by using the gradients of the liner functions which were fitted to the liner portion of the averaged MSD curves for between 10 and 20 ns.

3. Results

Table 1 lists the estimated average of $A_{mol}$, $d_{PP}$, and $-S_{CD}$ for each system. The estimated $A_{mol}$ of pure POPC bilayer was 63.5 Å$^2$, which is comparable with experimental value, 68.3Å$^2$, at 303K [19]. We observed that the all $A_{mol}$ of the binary lipid bilayers decreased from that of pure POPC system, whereas the all $d_{PP}$ of the binary lipid bilayers were increased. We found that the largest changes of those values were shown in the POPC/CHOL bilayer, and then the changes were shown in the order of POPC/CER, POPC/DAG, and POPS/SM. Figures 3 plots the $-S_{CD}$ of each acyl chain (sn-1 and sn-2) of the POPC in each binary lipid bilayer. We observed that all $-S_{CD}$ of acyl chains of POPC in the binary lipid bilayers increased from those in the pure POPC bilayer. The POPC/CHOL system showed the largest $-S_{CD}$ values in the systems, and these values are comparable with the previous MD studies [20]. Generally, the ordered lipid chains by CHOL are tightly packed in the membrane, leading to condensed membrane [21]. In this study, such membrane condensing effects were clearly shown in the other binary lipid bilayer systems containing CER, DAG, or SM, too. The strengths of the condensing effect were ranked in the order of POPC/CHOL, POPC/CER, POPC/DAG, and POPC/PSM in this MD study.
The hydrogen coordination numbers around the acceptor sites of the POPC were calculated to investigate the hydrogen bond networks in the binary lipid bilayers. Figure 4 shows the RDF for the hydrogen of hydroxyl group of CHOL and the oxygen (O22) of the carbonyl group of POPC in the POPC/CHOL bilayer as an example of stable hydrogen bond formation. The peak of RDF at 1.8 Å shows the forming of hydrogen bond around the oxygen (O22) of the carbonyl group. The time averaged coordinated number for this hydrogen bond was evaluated by counting the hydrogens by the distance r of 3 Å and the estimated running coordination number was 3.95 in this graph.

Table 2 summarizes the total coordination numbers of hydrogen in each binary lipid bilayer. In this table, the total coordination numbers for between additional lipid and POPC and for between additional lipids were also listed to assess the contributions of hydrogen bonds to the intermolecular interactions between those lipids in the binary lipid bilayers. The difference of the total coordination numbers depends on the number of possible hydrogen donor/acceptor sites in the systems. While DAG and CHOL have only one hydroxyl group for the formation of hydrogen bond, SM and CER have three and four possible sites for hydrogen bonds, respectively. In the case of CER, the number of hydrogen bonds were much higher in both POPC-CER and CER-CER in the membrane. These properties of hydrogen bond networks should contribute not only to the changes of lipid structure and dynamics but also lipid distribution in the membrane plane.

Table 2. Total hydrogen coordination numbers for between the additional lipid and POPC and for between the additional lipids in the POPC/CHOL, POPC/CER, POPC/DAG and POPC/SM bilayers. The parenthesis shows the standard deviations of the values.

| System       | Additional lipid – POPC | Additional lipid – Additional lipid | Total       |
|--------------|-------------------------|-------------------------------------|-------------|
| POPC/CHOL    | 18.0 (2.0)              | 0.1 (0.2)                           | 18.1 (1.8)  |
| POPC/CER     | 30.4 (0.6)              | 10.0 (1.2)                          | 40.4 (1.2)  |
| POPC/DAG     | 10.5 (0.6)              | 3.6 (0.2)                           | 14.1 (0.6)  |
| POPC/SM      | 21.8 (2.4)              | 1.4 (1.0)                           | 23.1 (1.6)  |

Figure 4. Radial distribution function (RDF) for the hydrogen of hydroxyl of CHOL and the oxygen (O22) of the carbonyl group of POPC in the POPC/CHOL bilayer. The dashed line shows the running coordination number of hydrogen as a function of distance r.

Figure 5. Two-dimensional RDFs between the centers of mass of additional lipids (CHOL, CER, DAG and SM) in each membrane plane.
Figure 5 shows the two-dimensional RDFs between the centers of additional lipids in each binary lipid bilayer. We observed a clear peak of RDF around 6 Å in the POPC/CHOL and POPC/CER bilayer, whereas there are no RDF peaks in the POPC/DAG and POPC/SM bilayer. These results mean that the CHOL and CER rearranged from the randomly arranged initial positions in the membrane plane, and those are closely arranged in each membrane. These should be due to the better intermolecular interaction of those lipids in the membrane.

Figures 6 (a)-(b) show that MSD curves of the POPC and additional lipids in each lipid bilayer. The evaluated diffusion coefficients, $D_l$, of the POPC and additional lipids in each system are listed in Table 3. The evaluated $D_l$ of POPC in pure POPC was $7.49 \times 10^{-8}$ cm$^2$/s, which is very close to the experimental value, $8\sim10 \times 10^{-8}$ cm$^2$/s [22-24]. The calculated diffusion coefficients $D_l$ for the POPC and CHOL in the POPC/CHOL bilayer largely decreased, and those values for each lipid are also in good agreement with the experimental values, $4.46 \times 10^{-8}$ cm$^2$/s and $3.21 \times 10^{-8}$ cm$^2$/s, respectively [23]. We found that the $D_l$ of lipids in the POPC/CER and POPC/SM were also decreased. Such reduction of lipid diffusion has also been known to related to the lipid condensing due to the ordering of lipids in the membrane [25]. Our MD study also shows such ordering of lipid chains in the POPC/CER and POPC/SM, being consistent with the experimental observations. In the case of POPC/DAG bilayer, the calculated $D_l$ of lipids was comparable with that of POPC in the pure POPC bilayer.

### Table 3. Lateral diffusion coefficients, $D_l$, of the POPC and the additional molecules in the pure POPC and the binary POPC binary bilayers. The parenthesis shows the standard deviations of the values. Units are in $10^{-8}$ cm$^2$/s.

|          | Pure POPC | POPC/CHOL | POPC/CER | POPC/DAG  | POPC/SM  |
|----------|-----------|-----------|----------|-----------|----------|
| POPC     | 7.49 (1.28) | 4.51 (0.60) | 5.49 (1.51) | 7.89 (1.17) | 5.78 (1.48) |
| Additional molecule | — | 3.35 (1.34) | 3.59 (1.38) | 7.27 (1.69) | 6.26 (1.4) |

### 4. Discussions

In this section, we summarize the structure and dynamics of each binary POPC bilayer system and discuss the factors which yield the differences of molecular condensing effects.
4.1. POPC/CHOL bilayer

The POPC/CHOL bilayer shows the largest condensing effects in the binary POPC bilayer systems. Such large condensing effect are attributable to the structural characters of CHOL. CHOL has a small polar (hydroxyl) head group and a rigid sterol ring in the body, and the hydroxyl group of CHOL faces the membrane interface region. Thus, the most of CHOL structure is buried in the hydrophobic region of the bilayer [20], also those are oriented to the membrane normal. The acyl chains of POPC next to the sterol ring of CHOL are ordered to avoid the steric hindrance with the CHOL in the membrane. These highly ordered lipid chains results in the decrease of $A_{mol}$ and increase of $d_{n-p}$, leading to the tightly packed membrane. The 2D-RDF analysis of centers of CHOLs in the POPC/CHOL binary bilayer shows that CHOLs are closely arranged in the membrane plane. This should be due to that the system gets energetically stable by facing the ordered rigid sterol rings of CHOLs in the membrane. Such arrangement of CHOLs has been observed in the previous DOPC/CHOL and DPPC/CHOL MD simulations [20]. We also observed that the lateral diffusions of both POPC and CHOL were largely decreased due to the tight packing of POPC/CHOL bilayer. These results correspond to the previous MD studies [26] and experimental observations [23].

4.2. POPC/DAG bilayer

Since the structural difference between DAG and POPC is only seen in the head group of the lipids, a simple effect of replacements of a PC head group with a hydroxyl group can be observed in the POPC/DAG bilayer. We observed that the POPC/DAG bilayer showed a moderate condensing effect in the binary lipid bilayers. The condensing of the POPC/DAG bilayer can be mainly caused by the lack of bulky PC head group, DAG lipids are buried in the hydrophobic region of the bilayer, leading to the condensed packing of the membrane. This result was comparable with the previous MD study [26]. The RDF analysis showed that DAG can form some hydrogen bonds between the hydroxyl group of and surrounding POPC in the membrane. However, since the total number of the hydrogen coordination was the smallest in the binary lipid bilayer system, the contribution to the membrane condensing and the diffusion of lipids in the membrane should be minor. In fact, the diffusion constant of POPC and DAG in the POPC/DAG bilayer were comparable with that of POPC in the pure POPC bilayer. The decrease of bulky PC head groups in the system could also be a factor to determine the lateral diffusion of lipids in the POPC/DAG bilayer.

4.3. POPC/SM bilayer

The POPC/SM bilayer shows the smallest condensing effect in the binary lipid bilayers. This is because that the SM has a PC head group, whereas the CHOL, DAG and CER have a small polar (hydroxyl) head group. Since the PC head group of SM has a comparable area with that of POPC, the change of membrane area of the POPC/SM bilayer results in minor. On the other hand, SM has an amide, hydroxyl and carbonyl groups at the linkage of the PA and SPH, and these can be donor/acceptor sites for forming of hydrogen bonds. The total number of hydrogen coordinate around the sites was larger than those of POPC/CHOL and POPC/DAG systems. These hydrogen bonds could contribute to the condensing of the membrane and the decrease of the lateral diffusion of lipids in the membrane.

4.4. POPC/CER bilayer

A simple effect of replacements of a PC head group of SM with a hydroxyl group can be observed in the POPC/CER bilayer. The POPC/CER bilayer showed the second largest condensing effect in the binary lipid bilayers. Since the lack of bulky PC head group, the most of CER structure is also buried in the hydrophobic region of the bilayer. This “Umbrella effect” in the POPC/CER should be a factor of the condensing of the membrane [20, 26]. Also, CER has four donor/acceptor sites for forming hydrogen bonds. Thus, the POPC/CER bilayer has the largest hydrogen coordination number both between POPC-CER and CER-CER. These hydrogen bonds should contribute to the condensing of the membrane and the large decrease of lateral diffusions of lipids in the membrane. The 2D-RDF analysis
of centers of CERs showed that the CERs were arranged closely in the membrane. This should be due to the buried condition of CER in the membrane and the hydrogen bond networks between CERs. Such arrangement of CERs could be a seed for the CER enriched micro-domain in biomembrane [3-5].

5. Conclusion

Molecular dynamics (MD) simulations of the binary POPC bilayers containing cholesterol (CHOL), ceramide (CER), diacylglycerol (DAG), or sphingomyelin (SM) were carried out to investigate the effects of these lipids on structure and dynamics of POPC bilayer. The membrane structure parameters such as area per lipid ($A_{lipid}$), membrane thickness ($d_{P-P}$) and order parameter ($-S_{CD}$) for each system were calculated and compared with each other. The clear condensing effects (the decreases of $A_{lipid}$, and the increases of $d_{P-P}$ and $-S_{CD}$) were observed in all binary POPC bilayers, and the strengths of the condensing effect were ranked in the order of POPC/CHOL, POPC/CER, POPC/DAG, and POPC/PSM in this MD study.

We found that the differences of condensing effects are attributable to the factors such as molecular shape, type of polar head group of the lipids the hydrogen bond networks in the system. The lipid chains surrounding the rigid sterol ring of CHOL in the membrane are highly ordered to avoid the steric barrier, resulting in the large decrease of $A_{lipid}$ and increase of $d_{P-P}$ of the POPC/CHOL bilayer. The DAG, CER and CHOL have a small polar (hydroxyl) head group instead of the PC head group. Due to the lack of bulky PC head group, these molecules are buried in the hydrophobic region of the bilayer, leading to the condensed packing of membranes. The POPC/CER bilayer has the largest hydrogen coordination number both between POPC-CER and CER-CER. These hydrogen bond networks would contribute to the membrane condensing and the arrangement of CER in the membrane. We observed that the condensing of membranes leads to the decrease of lateral diffusion of lipids in the binary lipid bilayer. Such decreased lateral diffusions of the lipids were good agreement with the experimental observations. The lateral diffusion of lipids in the POPC/DAG bilayer were comparable with that in the pure POPC bilayer. This could be determined with the small number of hydrogen bonding, the moderate condensing effect, and the decrease of bulky PC head groups in the POPC/DAG bilayer.

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