Effects of phospholipid unsaturation on the bilayer nonpolar region: a molecular simulation study

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Abstract Molecular dynamics simulations of two monounsaturated phosphatidylcholines (PCs), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC; cis-unsaturated) and 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC; trans-unsaturated) were carried out to investigate the effects of a double bond in the PC β-chain and its conformation on the bilayer core. Four nanosecond trajectories were used for analyses. A fully saturated 1,2-dimyristoyl-phosphatidylcholine (DMPC) bilayer was used as a reference system. In agreement with experimental data, this study shows that properties of the PEPC bilayer are more similar to those of the DMPC than to the POPC bilayer. The differences between POPC and PEPC bilayers may be attributed to the different ranges of angles covered by the torsion angles β10 and β12 of the single bonds next to the double bond in the oleoyl (O) and elaidoyl (E) chains. Broader distributions of β10 and β12 in the E chain than in the O chain make the E chain more flexible. In effect, the packing of chains in the PEPC bilayer is similar to that in the DMPC bilayer, whereas that in the POPC bilayer is looser than that in the DMPC bilayer. The effect of the cis-double bond on torsions at the beginning of the O chain (β4 and β5) is similar to that of cholesterol on these torsions in a myristoyl chain.—Róg, T., K. Murzyn, R. Gurbici, Y. Takaoka, A. Kusumi, and M. Pasenkiewicz-Gierula. Effects of phospholipid unsaturation on the bilayer nonpolar region: a molecular simulation study. J. Lipid Res. 2004. 45: 326–336.

Supplementary key words phosphatidylcholine • cis double bond • trans double bond • skew conformation • chain packing

Phospholipids with two asymmetric hydrocarbon chains, of which one is fully saturated in the γ position and the other is mono-cis or poly-cis-unsaturated in the β position, are the most common in nature (1). Among mono-cis-unsaturated phosphatidylcholines (PCs), 1-palmitoyl-2-oleoyl-PC (POPC) is the most abundant. In the past, phosphatidylcholines (PCs), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC); 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC); 1-palmitoyl-2-oleoyl-phosphatidylcholine; PSPC, 1-palmitoyl-2-stearoyl-phosphatidylcholine; RAF, reorientational autocorrelation function; RDF, radial distribution function; SA, surface area.

Abbreviations: DMPC, 1,2-dimyristoyl-phosphatidylcholine; E, elaidoyl; M, myristoyl; MD, molecular dynamics; O, oleoyl; PC, phosphatidylcholine; PEPC, 1-palmitoyl-2-elaidoyl-phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; PSPC, 1-palmitoyl-2-stearoyl-phosphatidylcholine; RAF, reorientational autocorrelation function; RDF, radial distribution function; SA, surface area.

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rated bilayers than in saturated bilayers. The introduction of a double bond into the alkyl chain decreases water penetration of the bilayer; the effect is greater for cis-unsaturated than for trans-unsaturated bilayers (26).

Among several reports on computer simulations of unsaturated PC bilayers (27–38), cis- and trans-unsaturated bilayers were compared in two of them (27, 36). In a Langevin dynamics study, Pearce and Harvey (27) showed that structural and dynamic properties of PCs with a trans double bond are similar to those of saturated PCs, whereas PCs with a cis double bond behave differently. In a comparative molecular dynamics (MD) simulation study, Murzyn et al. (36) showed that numbers of inter-lipid interactions via water bridges and charge pairs in cis- and trans-unsaturated PC bilayers are similar and that both are smaller than in a saturated 1,2-dimyristoyl-PC (DMPC) bilayer.

Scarc experimental (10, 39) and molecular modeling (40–42) studies of mixed-chain phospholipids indicate that in mono-cis-unsaturated chains, torsion angles of the single bonds next to the double bond are highly distributed around skew (120°), skew’ (240°), and trans (180°) conformations. The estimated distribution of angles ranges from 90 to 175° and −90 to −175° for the skew and skew’ conformations (10). This follows from the steric energy profile (41), which has two broad minima centered at ±110°, a relatively narrow and low-energy barrier centered at 180°, and a broad, higher energy barrier extending from −60° (gauche−) to 60° (gauche+) with a small maximum at 0°. Thus, torsion angles of the single bonds next to the double bond are unlikely to have conformations from the range (gauche− to gauche+). Similar results were obtained from quantum chemical calculations of two model compounds, each containing two cis-unsaturated bonds, as well as from MD simulations of the poly-cis-unsaturated docosahexaenoic chain (32). Molecular modeling calculations indicate that the gauche probability of the torsion angles second next to the cis double bond is higher than that in fully saturated chains (39, 40). Both experimental (43) and molecular modeling (40) studies indicate that a single cis double bond in the phospholipid β-chain has practically no effect on the fully saturated γ-chain. To our knowledge, the effects of the trans double bond in the β-chain on the neighboring torsion angles or the γ-chain have not been determined either experimentally or from simulations.

The aim of the present MD simulation study was to determine the effect of PC monounsaturation and the conformation (cis or trans) of the double bond in the β-chain on hydrocarbon chain order, packing, and dynamics in the membrane. Two PC bilayers were studied: POPC (mono-cis-unsaturated) and 1-palmitoyl-2-elaidoyl-PC (PEPC; mono-trans-unsaturated) together with a third, DMPC (fully saturated), used as a reference system. It is true that a bilayer made of fully saturated 1-palmitoyl-2-stearoyl-PC (PSPC) would constitute a better reference system for the POPC and PEPC bilayers (the length of corresponding alkyl chains of the three lipids is the same), but there is a serious problem in doing this. Unfortunately, there are very few experimental data for a PSPC bilayer, and this makes the generation of a computer model for this bilayer uncertain and unreliable and therefore unsuitable as a comparison. By contrast, reliable data for the DMPC bilayer are available, making this a safe reference system for comparative studies. The much lower temperature of the main phase transition of DMPC compared with PSPC is an additional advantage for this choice, because the three bilayers are all now in the liquid crystalline phase at the physiological temperature of 37°C. DMPC and POPC bilayers were simulated for 15 ns and the PEPC bilayer for 8 ns. Analyses of the trajectories generated in the MD simulations confirmed that the cis double bond (torsion β11) promotes the conformational variability of the neighboring torsion angles (β10 and β12), consistent with both the experimental and MD simulation data. In addition, they showed that the trans double bond has a similar effect, although the range of angles that β10 and β12 assume is broader. Indeed, β10 and β12 of the oleoyl (O; cis) chain cover angles between 60 and 300°, whereas those of the elaidoyl (E; trans) chain cover the whole range of angles, i.e., between 0 and 360°.

**Methods**

**Simulation systems**

POPC, PEPC, and DMPC bilayers used in this study consisted of 72 (6 × 6 × 2) PC molecules. POPC and PEPC bilayers were hydrated with 1,922 water molecules; the DMPC bilayer was hydrated with 1,622 water molecules (in each bilayer, water constituted ~39% by weight). The structure, numbering of atoms, and torsion angles in POPC, PEPC, and DMPC molecules are shown in Fig. 1.

The β-chain of POPC and PEPC has one double bond between C9 and C10 (Fig. 1). In POPC, the double bond is in the cis conformation (O chain), and in PEPC, it is in the trans conformation (E chain), so the torsion angle for the double bond, β11, is 0° for POPC and 180° for PEPC. The β- and γ-chains of DMPC [myristoyl (M) chain] and the γ-chain of POPC and PEPC are fully saturated. Details concerning the construction of the POPC and PEPC molecules and subsequently bilayers, as well as the initial simulations of these bilayers, were described in Murzyn et al. (36). Details concerning the DMPC bilayer were described in Pasenkiewicz-Gierula et al. (44, 45).

**Simulation parameters**

For PCs, optimized potentials for liquid simulations (OPLS) parameters (46), and for water, TIP3P parameters (47), were used. The united-atom approximation was applied to the CH2, CH2, and CH3 groups of PCs. The atomic charges on head groups of DMPC, POPC, and PEPC were practically the same; details are given in Pasenkiewicz-Gierula et al. (45). Procedures for supplementing the original OPLS base with the missing parameters for the PC head group were described in Pasenkiewicz-Gierula et al. (45), and those for the β-chain sp2 carbon atoms were described in Murzyn et al. (36).

**Simulation conditions**

POPC and PEPC bilayers were simulated for 15 and 8 ns, respectively, initially using the MD AMBER 4.0 (48) and then (last 5 ns) AMBER 5.0 (49) packages. Three-dimensional periodic boundary conditions with the usual minimum image convention were used. The SHAKE algorithm (50) was used to preserve the bond lengths of the water molecule, and the time step was set at 2 fs (51). Restraints of a flat-bottom harmonic potential as de-
Results

Details concerning the equilibration and validation of POPC and PEPC bilayers were described in Murzyn et al. (36), and those concerning the DMPC bilayer were described in Pasenkiewicz-Gierula et al. (45). For the analyses described below, the last 4 ns fragments of the generated trajectories were used. Errors in the derived average values are standard error estimates obtained from the block-averaging procedure. Because the torsion angles β3 and γ3 (Fig. 1) are not in well defined, stable conformations (trans or gauche) (53), when calculating conformation-related quantities, β3 and γ3 as well as the third segmental vector were not considered.

Cross-sectional area per PC

The average surface area (SA) per PC is 64.3 ± 0.6 Å² in POPC, 63.5 ± 0.6 Å² in PEPC, and 60.2 ± 0.6 Å² in DMPC bilayers. The values for the POPC and DMPC bilayers are in good agreement with those published in the literature. SA/PC in the POPC bilayer was estimated to be 63 Å² from POPC monolayer studies at the surface pressure of 30 mN/m (54) and 66 Å² at the surface pressure of 20 mN/m (55). For the DMPC bilayer, the best estimate for SA/PC is ~60 Å² (56). For the PEPC bilayer, there are no published experimental data.

Molecular order parameter of PC alkyl chains

The molecular order parameter, Smol, profiles [for definition, see Róg and Pasenkiewicz-Gierula (53)] along the β- and γ-chains in the POPC, PEPC, and DMPC bilayers are shown in Fig. 2. Shapes of the profiles for the O and E chains agree well with those given in Seelig and Waespe-Šarčević (18). For the POPC γ-chain, the Smol profile agrees with that of Holte et al. (43) and Seelig and Waespe-Šarčević (18). As can be seen from Fig. 2, Smol profiles for β-chains as well as γ-chains of POPC, PEPC, and DMPC are similar, except for segments 10 and 11 of the O chain (i.e., segments that include the cis double bond), for which Smol Values are substantially lower. Average Smol values for the POPC and PEPC chains are only slightly lower than those for the DMPC chains (Table 1), so saturated, mono-cis-unsaturated, and mono-trans-unsaturated chains are similarly ordered.

Tilt of PC alkyl chains

The tilt angle of a PC chain as well as the Δ segment (C-C bonds 4–9 above the double bond) and the ω segment (C-C bonds 10–17 below the double bond) of the O and E chains were derived as shown in

\[ \text{arccos} \left( \sqrt{\cos^2 \theta} \right) \]  

(Eq. 1)

where \( \theta \) is the angle between the bilayer normal and the average segmental vector (averaged over appropriate segmental vectors \( \approx 4 \)) (the \( n \)th segmental vector links \( n \) and \( n + 1 \) carbon atoms in the alkyl chain), and \( \langle \rangle \) denotes both the ensemble and the time average. The distributions of tilt angles of β- and γ-chains in the POPC, PEPC, and DMPC bilayers are shown in Fig. 3A, B and those of the Δ and ω segments are shown in Fig. 3C, D. Average tilts of the PC chains, given in Table 1, are similar in the three bilayers. Also, tilts of the Δ and ω segments are

![Figure 1](image) Molecular structures with numbering of atoms and torsion angles of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) (A), 1-palmitoyl-2-elaidoyl-phosphatidylcholine (PEPC) (B), and 1,2-dimyristoyl-phosphatidylcholine (DMPC) (C). The chemical symbol for carbon atoms (C) is omitted.
similar in the POPC and PEPC bilayers, but in both bilayers, the $\Delta$ segment is significantly less tilted than the $\omega$ segment (Table 1). A large difference between the O and E chains is seen in distributions (Fig. 4) and average values (Table 1) of tilt angles of the double bonds. The average tilt of the cis double bond is $11^\circ$ larger than that of the trans double bond, which, on the other hand, is similar to that of the single C9-C10 bond in the M chain.

### Table 1. Average values of parameters

<table>
<thead>
<tr>
<th>PC</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$\omega$</th>
<th>$\Delta$</th>
<th>$\pi$</th>
<th>$\beta^c$</th>
<th>$\gamma^c$</th>
<th>Lifetime trans/gauche</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>0.24</td>
<td>0.22</td>
<td>25</td>
<td>26</td>
<td>41</td>
<td>49</td>
<td>28</td>
<td>3.2, 3.0</td>
<td>180/65</td>
</tr>
<tr>
<td>PEPC</td>
<td>0.24</td>
<td>0.20</td>
<td>26</td>
<td>26</td>
<td>39</td>
<td>38</td>
<td>29</td>
<td>3.1, 3.0</td>
<td>180/65</td>
</tr>
<tr>
<td>DMPC</td>
<td>0.33</td>
<td>0.26</td>
<td>27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>28</td>
<td>2.5, 2.4</td>
<td>180/55</td>
</tr>
</tbody>
</table>

The molecular order parameter ($S_{mol}$); tilt angles of the $\beta$- and $\gamma$-chains, the $\Delta$- and $\omega$-segments, and the double bond (II); numbers of gauche rotamers per phosphatidylcholine (PC) chain; and lifetimes of trans and gauche conformations in 1-palmitoyl-2-oleoyl-PC (POPC), 1-palmitoyl-2-elaidoyl-PC (PEPC), and 1,2-dimyristoyl-PC (DMPC) bilayers. The errors in the average values are SEM estimates.

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For a detailed analysis of the conformational changes in the PC bilayers, please refer to Figs. 2, 3, and 4. These figures illustrate the distribution of molecular order parameters ($S_{mol}$) along the $\beta$- and $\gamma$-chains in POPC (closed squares), PEPC (closed triangles), and DMPC (closed circles) bilayers. The error bars indicate SEM. Average values of $S_{mol}$ are given in Table 1.

**Fig. 2.** Profiles of the molecular order parameter ($S_{mol}$) along the $\beta$-chain (A) and $\gamma$-chain (B) in the POPC (closed squares), PEPC (closed triangles), and DMPC (closed circles) bilayers. The error bars indicate SEM. Average values of $S_{mol}$ are given in Table 1.

**Fig. 3.** Distribution of tilt angles of the $\beta$-chain (A) and $\gamma$-chain (B) of POPC (thick line), PEPC (dotted line), and DMPC (thin line) as well as the $\Delta$-segment (C) and $\omega$-segment (D) of the $\beta$-chain of POPC (thick line) and PEPC (dotted line). Average tilt angles are given in Table 1.

**Fig. 4.** Distribution of tilt angles of the double bond in the $\beta$-chain of POPC (thick line) and PEPC (dotted line).
fect was brought about in the DMPC β-chain by cholesterol (53). The gauche probability of β10 and β12 (next to the double bond) is zero, and that of β9 and β13 (second next to the double bond) is higher than that in the M chain. The trans double bond does not influence torsion angles at the beginning of the β-chain but, like the cis double bond, increases the gauche probability of β9 and β13, although to a lesser extent. The gauche probability for β10 and β12 in the E chain is nonzero but is distinctly lower than that in the M chain. The double bond (cis or trans) has practically no effect on the gauche probability along the γ-chain.

Populations of conformations of torsion angles that are first (β10 and β12) and second (β9 and β13) neighbors of the double bond in the POPC and PEPC bilayers are illustrated in Fig. 6. β9 and β13 of both the O and E chains assume discrete low-energy conformations (trans or gauche) in 99% of cases (Fig. 6A, B, G, H), whereas β10 and β12 of both the O and E chains continuously populate angles between 60° and 300° and 0° and 360°, respectively, with an apparent single maximum at 180° (Fig. 6C–F). Detailed inspection reveals that the distributions of conformations of β10 and β12 in the O chain have shoulders indicating nonuniform populations of the angles. Indeed, time profiles of the β12 conformation in some cases have a bimodal character (Fig. 7A).

Correlations between values of torsion angles for pairs of angles neighboring the trans double bond in the PEPC bilayer are shown as contour plots in Fig. 8. For comparison, a contour plot for a pair of “typical” torsion angles (γ7 and γ8) in the γ-chain is also shown (Fig. 8A). For the γ7-γ8 pair, five regions on the plot can be recognized, one for trans-trans conformations and four for trans-gauche conformations (Fig. 8A). Gauche-gauche conformations are much less populated, so they do not appear on the plot. Torsion angles β10 and β12 do not reside in the low-energy conformations typical for single C-C bonds; therefore, they cover different regions on the plot. For discrete trans, gauche+, and gauche− conformations of β9 and β13, the values of β10 and β12 cover nearly the whole range of angles (Fig. 8B, C) (relatively less populated conformations do not appear on the plot). The contour plot for the β12-β10 pair shows that conformations of these torsion angles are uncorrelated (Fig. 8D); the region covered on the plot is a simple superposition of these angle distributions (cf. Fig. 6D, F). Similar results were obtained for torsion angles next to the cis double bond (data not shown).

Conformation lifetimes

Figure 6C, D shows that β10 and β12 of the O and E chains have a nonzero probability to populate any angle in the range between 60° and 300° and 0° and 360°, respectively. This means that these torsion angles do not have stable conformations. Figure 7A, B well illustrates the instability of the conformational states of β12 in the O and E chains. Other single C-C bonds in PC hydrocarbon chains assume low-energy trans and gauche conformations, as illustrated in Fig. 7C. Lifetime profiles of these conformations along the β- and γ-chains in the POPC, PEPC, and DMPC bilayers are shown in Fig. 5C, D. Lifetimes of gauche conformations for POPC β10 and β12 were set to zero because these angles are never gauche. Lifetimes of trans conformations for POPC β10 and β12 as well as trans and gauche conformations for PEPC β10 and β12 are very
short, which indicates that these conformations are very unstable. For \( \beta9 \) and \( \beta13 \) of the O and E chains, lifetimes of the gauche conformation increase by the same amount relative to those of the M chain, whereas lifetimes of the trans conformation increase for \( \beta9 \) and \( \beta13 \) of only the E chain. This is the likely reason why the probability of gauche for \( \beta9 \) and \( \beta13 \) of the O chain is higher than that of the E chain (Fig. 5A). For torsion angles other than \( \beta9 \), \( \beta10 \), \( \beta12 \), and \( \beta13 \), lifetimes of both the trans and gauche conformations are similar in all three bilayers.

**Fig. 7.** Time profiles of conformations of the torsion angles \( \beta12 \) of arbitrarily chosen POPC (A) and PEPC (B) molecules and \( \gamma12 \) of an arbitrarily chosen PEPC molecule (C). Trans, gauche\(^+\), and gauche\(^-\) are indicated at right as t, g\(^+\), and g\(^-\), respectively. The dashed lines indicate the ranges of angles that characterize a given conformation.
Chain packing in the hydrophobic core
Radial distribution functions (RDFs) calculated for the center of mass of the PC alkyl chains belonging to different PC molecules in the POPC, PEPC, and DMPC bilayers are shown in Fig. 9A. A broad first maximum and the lack of a second maximum in the RDF for the POPC bilayer indicate that the cis double bond disrupts a regular chain packing observed in the DMPC and PEPC bilayers (Fig. 9A).

The RDFs were decomposed into RDFs for the \( \beta \)-chains (a \( \beta-\beta \) RDF; Fig. 9B), \( \beta \)- and \( \gamma \)-chains (a \( \beta-\gamma \) RDF; Fig. 9C), and \( \gamma \)-chains (a \( \gamma-\gamma \) RDF; Fig. 9D). As indicated in Fig. 9B, saturated \( \beta \)-chains pack much better than unsaturated \( \beta \)-chains, but trans-unsaturated chains pack better than cis-unsaturated chains. The \( \gamma-\gamma \) RDFs in Fig. 9D show that the \( \gamma \)-chains in the DMPC and PEPC bilayers are arranged regularly, whereas the \( \gamma \)-chains in the POPC bilayer are not. In contrast, as the \( \beta-\gamma \) RDFs in Fig. 9D show, the arrangement of the unsaturated \( \beta \)-chains relative to \( \gamma \)-chains in both the POPC and PEPC bilayers is more regular than that of the saturated \( \beta \)-chains relative to \( \gamma \)-chains in the DMPC bilayer. One can conclude that in the vicinity of a cis-unsaturated \( \beta \)-chain in the POPC bilayer, there are mainly saturated \( \gamma \)-chains, whereas in the DMPC bilayer, a \( \beta \)-chain is surrounded by \( \beta \)-chains and a \( \gamma \)-chain is surrounded by \( \gamma \)-chains.

Rotational diffusion
Reorientational autocorrelation functions (RAFs) were calculated for the Legendre polynomial P1 for \( \beta \)-chain and \( \gamma \)-chain vectors (Fig. 10A, B) in the POPC, PEPC, and DMPC bilayers. The \( \beta \)-chain (\( \gamma \)-chain) vector is a vector linking the middle of the C21-C22 (C31-C32) bond (Fig. 1) and the center of gravity of the chain. The RAF curves could not be satisfactorily fitted to a sum of exponentials; thus, the results presented are only qualitative. The effect of the double bond on the alkyl chain rotation is weak, independent of the bond conformation. A similar conclusion was drawn from spin-label studies of bilayers made of saturated, cis-unsaturated, and trans-unsaturated PCs (21). In those studies, however, the effects of unsaturation on the chain reorientational motion were monitored indirectly via the reporter group of the spin-label molecule.
DISCUSSION

In our previous paper (36), no significant effect of the conformation (cis or trans) of the double bond in the PC β-chain was found on the organization of the bilayer-water interface. In this paper, details of the effect of the double bond and its conformation on the organization of the bilayer core were investigated. In the MD simulation study, properties of the hydrocarbon core of the mono-cis-unsaturated (POPC), mono-trans-unsaturated (PEPC), and fully saturated (DMPC) bilayers were compared.

Average parameters characterizing monounsaturated PC bilayers in the liquid crystalline state obtained in this study are similar to those derived experimentally, in spite of the limited size of the computer models. In particular, the mean SA per PC in the POPC (and PEPC) bilayer of ~64 Å² agrees with experimental estimates (54, 55) and, as the experiments predict (13), is greater than that in the DMPC bilayer of ~60 Å². Profiles of the order parameter for the O and E chains have shapes similar to those obtained from NMR spectroscopy (18). In accord with the experimental data of Subczynski and Wisniewska (21), saturated, mono-cis-unsaturated, and mono-trans-unsaturated chains are similarly ordered, and as Holte et al. (43) showed, mono-cis-unsaturation in the β-chain has a minor effect on the order of the γ-chain.

The effect of the cis double bond on the β4 and β5 torsions angles in the pure POPC bilayer is similar to that of cholesterol in the DMPC-cholesterol bilayer (53). In both bilayers, the gauche probability of the β4 is significantly higher and that of the β5 is significantly lower than the respective probabilities for the DMPC β-chain in the pure DMPC bilayer. This is a very interesting but puzzling result, because in the first case, the effect is caused by an intrinsic molecular factor, whereas in the second case, it is driven by intermolecular interaction. The changes in the gauche probability of β4 and β5 are most likely attributable to steric effects, but in the framework of the present study, their origin cannot be clearly indicated. Similar effects of the cis double bond and cholesterol may explain why water penetration through the POPC and DMPC-cholesterol bilayers is decreased similarly compared with that in the pure DMPC bilayer (26). The trans double bond has no effect on the β4 and β5 torsions angles.

Detailed conformational analyses of mono- and poly-cis-unsaturated chains indicate that torsion angles of saturated C-C bonds next to the double bond have broad distributions around the skew, skew, and trans conformations, with a low probability of conformations between gauche and gauche (10, 32, 41). On the other hand, molecular mechanics calculations indicated that the gauche probability for the second next torsions to the double bond is higher than that in a fully saturated chain (39–41). To our knowledge, the effect of the mono-trans-unsaturated bond on the conformation of the neighboring single bonds has not been described in the literature.

Our MD simulation study demonstrated that both cis and trans double bonds strongly modify conformational states of the next (β10 and β12) and second next (β9 and β13) torsion angles. The distributions of β10 and β12 are continuous and broad, with maxima at 180° (Fig. 6). The range of angles covered by β10 and β12 depends on the conformation of the double bond.
β12 in the O chain are between 60 and 300° (Fig. 6C, E), whereas in the E chain, they cover the whole range of angles between 0 and 360° (Fig. 6D, F). The result for the O chain is in accord with results from single crystal studies by Keneko, Yano, and Sato (10) and molecular mechanics calculations by Li et al. (41). The result for the E chain is an evidently new result of the present study; unfortunately, no experimental data are available to verify it.

Different ranges of angles covered by β10 and β12 in the O and E chains are the most likely explanation for the experimentally observed differences in the properties of the POPC and PEPC bilayers. A wider distribution of β10 and β12 in the E chain makes the chain more flexible. Moreover, the distribution of gauche rotamers along the E chain and the inclination of the C9=C10 bond are more similar to those of the M than of the O chain (Fig. 5A). Thus, the E chain is more adaptive than the O chain and, in many respects, displays properties similar to those of the M chain. This result is in agreement with the reported similarity in the subcell structure and the occurrence of polytypic structures of mono-trans-unsaturated and fully saturated fatty acid crystals (57). Also, it is in agreement with the conclusion drawn from experimental observation that cholesterol mixes well with saturated and mono-trans-unsaturated phospholipids but not with mono-cis-unsaturated phospholipids (24, 58).

A greater similarity of the E chain to the M than to the O chain is also reflected in chain-chain RDFs (Fig. 9). The RDFs indicate that both mono-trans-unsaturated and fully saturated chains pack more regularly in the bilayer than the less adaptive mono-cis-unsaturated chains (Fig. 9A). Thus, the generally higher main phase transition temperatures of saturated and mono-trans-unsaturated bilayers than mono-cis-unsaturated bilayers may be attributed mainly to the observed differences in the chain packing. The RDFs shown in Fig. 9B, D indicate that, in contrast to the DMPC and PEPC bilayers, the β-chains as well as the γ-chains in the POPC bilayer are not arranged regularly relative to each other. Nevertheless, spatial ordering of β-chains relative to γ-chains is more apparent in the POPC and PEPC bilayers than in the DMPC bilayer (Fig. 9C).

Our MD simulation study confirmed an earlier Langevin dynamics simulation study of Pearce and Harvey (27) that showed that structural and dynamic properties of PCs with a trans double bond are similar to those of saturated PCs. Most likely, as a result of this similarity, phospholipids with trans-unsaturated hydrocarbon chains are much less abundant in nature than those with cis-unsaturated hydrocarbon chains.

In membranes of some bacteria, the relative proportion of trans-unsaturated fatty acids increases under physiologically stressful conditions (7–9). The increase results from an enzymatically controlled direct cis-trans isomerization of the double bond (7). cis-trans Isomerization was proposed as a biological mechanism of the regulation of bacterial membrane fluidity (5). The cis bond would increase fluidity, whereas the trans bond would decrease it. Unfortunately, experimental results (21–23) and the MD simulation results presented here do not support this hypothesis. They indicate that the lateral self-diffusion of cis-unsaturated lipids in the bilayer is slower than that of trans-unsaturated lipids, whereas the rotational diffusion is similar. Furthermore, S_{mol} profiles for cis- and trans-unsaturated chains have similar overall shapes. Thus, at temperatures above the main phase transition temperature for trans-unsaturated chains, the double bond in either the cis or the trans conformation has little effect on membrane fluidity and order. However, the biological role of cis-trans isomerization of the double bond in bacterial fatty acids might stem from differences in the interactions of other membrane components with cis- and trans-unsaturated chains. It has been demonstrated that membranes of bacteria living in extreme conditions contain polar carotenoids (59, 60). Carotenoids and cholesterol have similar effects on alkyl chains of phospholipids (61, 62) and both affect cis-unsaturated chains less strongly than saturated chains (26, 61, 62); in the case of cholesterol, the effect is also less strong than that of trans-unsaturated chains (26). Because of the similarity of saturated and trans-unsaturated chains discussed above, one can expect that the effect of carotenoids on these chains is similar to that of cholesterol. Therefore, environmentally induced cis-trans isomerization of the double bond should result in a stronger effect of carotenoids (or similar molecules) on the hydrocarbon chains in the bacterial membrane whenever it contains carotenoid-like molecules. The structure of the lipid matrix of the membrane would then become more rigid and hydrophobic and thus less permeable for polar molecules and ions. In this way, bacterial cell membrane integrity would be better preserved and the cell could better sustain stressful external conditions.

CONCLUSIONS

This MD simulation study confirmed numerous experimental results, particularly that the order and rotational diffusion of mono-cis-unsaturated, mono-trans-unsaturated, and saturated chains do not differ significantly from one another. This study also confirmed both experimental and computer simulation results that torsion angles of saturated C-C bonds next to the cis double bond are broadly distributed (in the range between 60 and 300°) and the gauche probability for the second next torsions to the cis double bond is higher than in a fully saturated chain. This study provided the following new results: (1) Torsion angles of saturated C-C bonds next to the trans double bond continuously populated the whole range of angles between 0 and 360°. This makes the mono-trans-unsaturated chain more adaptive than the mono-cis-unsaturated chain and in many respects similar to a fully saturated chain. (2) The intrinsic effect of the cis double bond on β4 and β5 torsions angles is very similar to the extrinsic effect of cholesterol on these angles in a fully saturated chain. Both in the POPC and DMPC-cholesterol bilayers, the gauche probability of β4 and β5 is much higher and lower, respectively, than that in the pure DMPC bilayer. (3) The packing of the alkyl chains in a mono-trans-unsat-
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