Importance of the Sphingosine Base Double-Bond Geometry for the Structural and Thermodynamic Properties of Sphingomyelin Bilayers

Lorant Janosi* and Alemayehu Gorfe*
Department of Integrative Biology and Pharmacology, University of Texas-Health Science Center, Houston, Texas

ABSTRACT The precise role of the sphingosine base trans double bond for the unique properties of sphingomyelins (SMs), one of the main lipid components in raftlike structures of biological membranes, has not been fully explored. Several reports comparing the hydration, lipid packing, and hydrogen-bonding behaviors of SM and glycerophospholipid bilayers found remarkable differences overall. However, the atomic interactions linking the double-bond geometry with these thermodynamic and structural changes remained elusive. A recent report on ceramides, which differ from SMs only by their hydroxyl headgroup, has shown that replacing the trans double bond of the sphingosine base by cis weakens the hydrogen-bonding potential of these lipids and thereby alters their biological activity. Based on data from extensive (a total of 0.75 μs) atomistic molecular dynamics simulations of bilayers composed of all-trans, all-cis, and a trans/cis (4:1 ratio) racemic mixture of sphingomyelin lipids, here we show that the trans configuration allows for the formation of significantly more hydrogen bonds than the cis. The extra hydrogen bonds enabled tighter packing of lipids in the all-trans and trans/cis bilayers, thus reducing the average area per lipid while increasing the chain order and the bilayer thickness. Moreover, fewer water molecules access the lipid-water interface of the all-trans bilayer than of the all-cis bilayer. These results provide the atomic basis for the importance of the natural sphingomyelin trans double-bond conformation for the formation of ordered membrane domains.

INTRODUCTION

Sphingomyelins (SMs) are one of the most abundant phospholipids (1,2) important for the formation of ordered lipid domains and rafts in the cell membranes of vertebrates (3–9). Ordered membrane domains play critical roles in many signaling processes (10–13). Moreover, SM metabolic products, such as ceramides, sphenogenes, and sphingosine 1-phosphates, are involved in the regulation of cell proliferation, differentiation, and apoptosis (14–17), as well as in endocytosis and inflammation (16–20).

SM has a phosphocholine polar headgroup, an N-acyl chain, and a base. The most common base in mammalian SM is sphingosine (1,3-dihydroxy-2-amino-4-octadecene), which contains a trans double bond between C₄ and C₅ (Fig. 1). Recent studies found that changing the natural trans double bond to cis or to a saturated bond has strong influence on the structural properties of sphingomyelin and ceramide (Cer) bilayers (21–23). (Cer differs from SM only by its hydroxyl headgroup.) Although similar to diacyl-glycerophosphatidylcholines (diacyl-PCs from here on) in many regards, SMs exhibit a more complex behavior (24). For example, four distinct gel phases have been identified for 18:0-SM (25) whereas SMs with large mismatch in length between their N-acyl and sphingosine chains have multiple transitions (26). Most natural SMs exhibit a nonlinear dependence on the acyl-chain length (27), with their main phase transition temperature being close to the physiological temperature. For instance, for pure palmitoyl SM (16:0-SM), the transition is ~314 K (26), which is similar to that of DPPC (di-16:0-PC).

Compared with diacyl-PCs, insertion of cis double bonds in the acyl chain of SMs has little impact on their high melting temperatures. This is likely due to the intermolecular hydrogen-bonding networks that stabilize the sphingomyelin bilayers (27). Furthermore, despite the same headgroup, the hydration behavior of SM bilayers is significantly different from that of diacyl-PCs (28,29). The source of this difference is believed to involve hydrogen-bonding interactions, which in turn are affected by the stereochemistry and geometry of the double bond at the sphingosine base (30,31). For instance, studies on Cers found that saturation of the sphingosine trans double bond or its replacement with the cis isomer leads to the loss of the H-bond network between Cer’s hydroxyl groups (both the headgroup and C3–OH) and the bound water molecules (21,23,32).

To gain atomistic insight into the influence of the sphingosine double-bond geometry on the structural, dynamic, and thermodynamic properties of SM bilayers, we performed extensive molecular dynamics simulations of palmitoyl sphingomyelin (PSM) bilayers of pure trans (all-trans), pure cis (all-cis), and mixed trans and cis isomers of the sphingosine double bond. We found that the cis isomer significantly reduces the formation of intermolecular hydrogen bonds. This disrupts the high ordering of the sphingosine chain, resulting in reduced packing of the bilayer. The C₄/C₅ trans and cis unsaturations had opposing effects on the bilayer structures, similar to effects expected for trans/cis isomerization in the midsection of a tail: the trans double bond maintains large values of the order

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*Correspondence: janosi.lorant@uth.tmc.edu or alemayehu.g.abebe@uth.tmc.edu

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The structures of PSM trans and cis isomers are shown in Fig. 1. Each molecule consists of three main parts: the phosphocholine headgroup, the sphingosine (SPH) base, and the palmitoyl (PA) chain. VMD (57) was used to render the images. The representation used for the PSM is the GROMOS96 united-atom model (36); i.e., only the hydrogen atoms of donors (like the O–H and N–H) are explicit.

parameter (i.e., close to the value of a saturated tail), whereas the cis isomer lowers it dramatically (33). We also found that the number of waters solvating the all-trans lipid-water interface is reduced by ~1.13 waters per lipid compared with the all-cis bilayer. These results are discussed in terms of their impact onto the elastic properties of SM bilayers.

MODEL AND METHODS

The structures of PSM trans and cis isomers are shown in Fig. 1. Each molecule consists of three main parts: the phosphocholine headgroup, the sphingosine (SPH) base, and the palmitoyl (PA) chain. The enantiomeric configuration of both molecules is D-erythro (2S, 3R). The first part of the SPH base (Cl–C5) bridges the headgroup to the hydrophobic tails of the lipid, and contains a double bond between C4 and C5 as well as two groups capable of hydrogen bonding: the N–H acting as donor and the O–H both as donor and acceptor. Bilayers made up of PSM lipids with the C5–C6 double bond in the trans and the cis conformations, as well as a racemic mixture of 80% trans and 20% cis, were investigated. To speed up system construction, we began from a previously fully equilibrated DOPC bilayer at 323 K (in the liquid-disordered phase). The cis and trans configurations were generated by imposing an improper dihedral potential around the double bond with minimum at 0° and 180°, respectively. The dihedral was used only during a standard steepest descent energy minimization procedure of 2500 steps. At the end of the minimization, we rigorously tested that the three systems have 1), all-trans, 2), all-cis, and 3), a mixture of 80% trans and 20% cis geometries of the sphingosine double bond. All three systems contained 128 PSM lipids fully solvated with 3735 water molecules, summing up to a total of 17,605 atoms. Each system was then subjected to 250 ns of free equilibration simulations in the NpT (constant temperature and pressure) ensemble at 323 K, and having a proper dihedral potential around the sphingosine double bond as described by Chiu et al. (34). The equilibrated all-trans and all-cis systems are shown in Fig. 2.

All simulations were performed with GROMACS 4.0 (35) and the Gromos96 ffG43A1 force field (36). Parameters proposed by Chiu et al. (34) were used to describe interactions of the PSM lipids with themselves and the surrounding environment. The van der Waals interactions were evaluated up to 1.2 nm, with twin-range cutoff neighbor search approach, with a short-range neighbor-list radius of 1.0 nm. Coulomb interactions were evaluated using the particle-mesh Ewald method with short-range interaction cutoff of 1.0 nm. The bilayer and the water were separately coupled to a Nosé-Hoover heat bath. All systems were semiisotropically (in membrane plane and perpendicular to it) coupled to a reference pressure of 1 bar with compressibility constant 4.6 × 10⁻³ bar⁻¹.

RESULTS AND DISCUSSION

Double-bond isomerization alters lipid packing of PSM bilayer

The structure of a lipid bilayer can be characterized by a number of equilibrium quantities, including the area per lipid (A_L) and the bilayer thickness (d_{P-P}). Because both A_L and d_{P-P} are directly related to lipid packing, we used them to examine the effect of the SM double-bond isomerization on the lateral and transverse organization of the all-trans, all-cis, and trans-cis bilayers. The time evolution of the areas per lipid A_L(t), calculated as the ratio between the (fluctuating) area of the simulation box and the number of lipids in one leaflet, are shown in Fig. 3 a. Fig. 3 b displays the corresponding d_{P-P} (t), calculated as the average

FIGURE 1 Atomistic structures of trans (left) and cis (right) isomers of the PSM lipid. PSM is composed of a phosphocholine headgroup, the sphingosine base (SPH), and a palmitoyl chain (PA). VMD (57) was used to render the images. The representation used for the PSM is the GROMOS96 united-atom model (36); i.e., only the hydrogen atoms of donors (like the O–H and N–H) are explicit.

FIGURE 2 Equilibrated all-trans (a) and all-cis (b) systems at 323 K. PSM lipids are represented in licorice green, phosphorus atoms in van der Waals tan spheres, and water in cyan continuous surface. Snapshots were rendered using VMD (57).
TABLE 1  List of simulated PSM bilayers with the reported equilibrated area per lipid molecule \(A_L\), simulation temperature \(T\), and time \(t\).

<table>
<thead>
<tr>
<th>Ref.</th>
<th>(\langle A_L \rangle [\text{nm}^2])</th>
<th>(T [\text{K}])</th>
<th>(t [\text{ns}])</th>
</tr>
</thead>
<tbody>
<tr>
<td>(40)</td>
<td>0.565</td>
<td>325</td>
<td>8.0</td>
</tr>
<tr>
<td>(40)</td>
<td>0.573</td>
<td>325</td>
<td>8.0</td>
</tr>
<tr>
<td>(40)</td>
<td>0.599</td>
<td>325</td>
<td>8.0</td>
</tr>
<tr>
<td>(41)</td>
<td>0.515</td>
<td>310</td>
<td>5.4</td>
</tr>
<tr>
<td>(42)</td>
<td>0.520</td>
<td>323</td>
<td>50.0</td>
</tr>
<tr>
<td>(44)</td>
<td>0.525</td>
<td>323</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Although not always explicitly stated, we presume that all of these simulations were done with the sphingosine base double bond in the \(\text{trans}\) conformation.

A more direct comparison between the simulated and experimental data can be made for the bilayer thickness, because it is directly measured in experiments (37,38).

The equilibrated portions of our trajectories yield thicknesses of \(d_{p-P}^{\text{trans}} = 4.16 \text{ nm}\), \(d_{p-P}^{\text{cis}} = 3.84 \text{ nm}\), and \(d_{p-P}^{\text{trans/cis}} = 4.02 \text{ nm}\). The calculated bilayer thickness for the all-\(\text{trans}\) system \((d_{p-P} = 4.16 \text{ nm})\) matches reasonably well with the most recent experimental value reported by Maulik and Shipley (4.44 nm) (38).

To evaluate the significance of the differences in the area and thickness of the \(\text{trans}\) and \(\text{cis}\) systems to the bilayers’ elastic properties, we computed the isothermal area compressibility modulus, \(K_A\), from the average area per lipid \(\langle A_L \rangle\) and its variance \(\sigma_{A_L}^2\) (45,46),

\[
K_A = \frac{1}{N_L} \frac{\langle A_L \rangle k_B T}{\sigma_{A_L}^2},
\]

where \(N_L\) is the number of lipids per leaflet. We obtained \(K_A^{\text{trans}} \sim 2700 \text{ mN/m}\), which is more than twice larger than \(K_A^{\text{cis}} \sim 1200 \text{ mN/m}\). Although no experimental data is found for sphingomyelin, this result is in qualitative agreement with previously reported value for 18:0-SM simulated bilayer of \(K_A^{\text{trans}} = 4400 \text{ mN/m}\) (34). However, insufficient fluctuations in the simulation box (possibly due to limitations of the force field) led to area compressibility moduli that are likely overestimated (47) for both the \(\text{cis}\) and \(\text{trans}\) isomers. Nonetheless, their relative values, which are most relevant for the purposes of this work, should hold.

The data described above clearly show that the conformation of the sphingosine base double bond plays a key role in determining the equilibrium structure of the resulting bilayers. In the following sections, therefore, we will discuss the details of the atomic interactions that underlie this role.

**Organization of PSM lipids in bilayers**

The differences in \(d_{p-P}\) and \(A_L\) indicate that the \(\text{trans}\) PSM forms a more compact and thicker bilayer than the \(\text{cis}\). Much more detailed structural information can be obtained from the distribution of atoms along the bilayer transverse...
dimension. Fig. 4 shows the number density distributions for the most important lipid atoms (or groups of atoms) at the lipid-water interface, with solid lines for the all-trans and dashed lines for the all-cis. The reference is set to be the average position of the C2 carbon of the sphingosine base.

For ease of comparison, we divided the lipid-water interface into four regions:

I. Bulk water, defined by the bulk density of the water molecules.
II. Headgroup region, between region I and the average position of the phosphate group.
III. Interfacial region, between the phosphate group and the end of the wet region.
IV. Dry lipid tails region, beyond the sphingosine double bond.

Region III makes the transition from the hydrophilic regions I and II to the hydrophobic region IV. The C2 carbon of the sphingosine base lies approximately in the middle of region III and can be used to calculate the hydrophobic thickness of the bilayer.

The z positions of the peaks for the distributions in Fig. 4 suggest that the thickness difference at the interfacial region between all-trans and all-cis is small, with ~80% of the total difference coming from the hydrophobic thickness (Table 2).

The trans-PSM lipids are more stretched than the cis, a conclusion also supported by analysis of the chain order parameters (discussed later). The cause of the small difference in the thickness of the interfacial region, however, is not as straightforward. The compactness of the trans system allows for less water penetration into the interfacial region (see Ow in Fig. 4) and repositions the phosphate groups such that the C2-P vectors are oriented ~6° closer to the bilayer normal. Hence, the phosphate atoms move further from the interface, slightly increasing the interfacial thickness of the lipid bilayer (see Fig. 1).

Fig. 4 also indicates that the way the water density decays from the bulk value of >32 nm−3 to zero in the hydrophobic region differs from a sigmoidal shape typically observed in diacyl-PCs (e.g., DPPC) (48); it contains a relatively flat region between z = −0.5 nm and z = 0.0 nm. As a result, the number of water molecules in this region (~0.5 nm < z < 0.0 nm) is constant, with the value for the all-trans system being ~1 water molecule (per nm3) less than for the all-cis system. This remarkable result indicates that, due to the better packing of the trans lipids, the overall number of waters that access the all-trans lipid-water interface is reduced by ~1.13 waters per lipid compared with the all-cis bilayer. Hence, the total number of perturbed (interfacial) waters interacting with the trans lipids is also reduced. Consequently, the number and distribution of lipid-water and lipid-lipid hydrogen bonds in region III of the all-trans bilayer differs from that of the all-cis bilayer, as discussed below.

**Hydrogen bonding**

Evolution with time of the total number of hydrogen bonds (HBs) is reported in Fig. 5 for the all-trans and all-cis systems. This number, clearly larger for the trans, is a summation of inter- and intramolecular hydrogen bonds listed in Table 3. The most important intramolecular HBs in both bilayers involve the hydroxyl group as a donor and the carbonyl and phosphoryl oxygens as acceptors. The intermolecular HBs, on the other hand, are dominated by the amide group acting as donor to the carbonyl and hydroxyl groups of the neighboring lipids, in agreement with deuterium-NMR experimental findings (49). It is worth noting that the amide group participates in intermolecular HBs only, strengthening the interlipid interactions and thereby playing a crucial role in the tight packing of the PSM bilayers.

The most significant difference between the trans and cis systems in terms of intramolecular HBs is in the hydroxyl-carbonyl interaction, where there are six more HBs in the trans bilayer. In terms of intermolecular HBs, hydroxyl-amide and hydroxyl-hydroxyl interactions...
Role of Sphingosine Double Bond

The stronger interlipid interactions in the all-\textit{trans} system (than in the all-\textit{cis}) is accompanied by the loss of lipid-water HBs (dark-colored curve in the inset of Fig. 6). This is partially caused by the elimination of \~140 water molecules from the interfacial region of the \textit{trans} bilayer (as calculated from Fig. 4), but also due to the exchange of lipid-water HBs for lipid HBs (light gray curve in the inset of Fig. 6). As a consequence, phosphoryl oxygens in the \textit{trans} system become less hydrated and lose at least 12 HBs with water; carbonyl oxygens lose 16 HBs with water but gain six more intra-PSM HBs; hydroxyl groups exchange 12 HBs with water molecules for 10 inter-PSM HBs and 7.5 intra-PSM (of which six involve carbonyl groups). Finally, as previously noted, the amide groups intensify the intermolecular interactions in the \textit{trans} bilayer by making 5.5 more HBs, but they also form eight more HBs with water molecules. These data clearly demonstrate that one of the major consequences of the presence of the \textit{trans} double bond is to enable tighter lipid packing through the formation of larger number of HBs within the bilayer.

**Water order parameter**

Structural properties of the interfacial water may also be characterized by means of orientational order parameters (OPs). Orientational OPs can be defined in a general way using the so-called Wigner rotational D-matrices (see the Supporting Material).

Rank 1 orientational OP (OP1) is equal to \( \langle \cos \beta \rangle \), where \( \beta \) is the angle between the \( O_w \rightarrow H \) vector and the bilayer normal (\( z \) axis). Plot of OP1 as a function of the \( O_w \)'s distance from the average \( z \) position of the \( C_2 \) atom (\textit{open symbols} in Fig. 7) shows practically no difference in the orientation of the interfacial waters in the all-\textit{trans} and all-\textit{cis}. As water approaches the bilayer, it tries to align its dipole toward the phosphoryl oxygens of the lipid headgroup (51). Past the phosphate group, this water orientation is maintained by donating hydrogen to the C=O and O−H groups of the lipids (see Fig. 7). Further into the bilayer (\( z > \sim 0.1 \) nm), water molecules start to accept HBs from O−H and N−H groups. The latter is located closer to the hydrophobic core and therefore contributes strongly to the water molecule’s dipole reorientation. As a consequence, the average dipole points toward the bilayer core for \( z < 0.1 \) nm, and away from it for \( z > 0.1 \) nm (see distributions in Fig. 4).

Rank 2 OPs are defined similarly to rank one OPs and yield nonzero values only for

\[
S^0 = \left\langle \frac{1}{2}(3\cos^2 \beta - 1) \right\rangle.
\]

This OP (OP2) is particularly important because it is directly related to the quadrupole splitting of a deuterium NMR spectrum (52). Our calculations show that, although the P−N vector population distribution is slightly different in the two systems (data not shown), its average orientation is about the same: \( -81^\circ \) for \textit{trans} and \( -79^\circ \) for \textit{cis}. Because OP2 in the two systems is very similar (see Fig. 7), our findings are in agreement with Aman et al. (48) that a notable change in the water OP2 value would imply a change in the population of the P−N vector orientation along the membrane normal.

Overall, the analysis of the water orientational OPs indicate that, despite differences in the number and interaction of the interfacial water molecules, the average orientation,

**TABLE 3** Number of the most important intra- and interlipid hydrogen bonds, averaged over the last 50 ns of simulation for the pure \textit{trans} and pure \textit{cis} systems

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>( N_{\text{trans}} )</th>
<th>( N_{\text{cis}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>O−H</td>
<td>O−C</td>
<td>18.9</td>
<td>12.9</td>
</tr>
<tr>
<td>N−H</td>
<td>O−C</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N−H</td>
<td>O−H</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>O−H</td>
<td>O−H</td>
<td>—</td>
<td>15.9</td>
</tr>
<tr>
<td>O−H</td>
<td>O_{122}P</td>
<td>9.2</td>
<td>7.7</td>
</tr>
<tr>
<td>N−H</td>
<td>O_{122}P</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28.5</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Hydrogen bonding was defined by an acceptor-hydrogen distance cutoff of 2.5 Å and acceptor-hydrogen donor angle cutoff of 90° (40,42).

FIGURE 5 Time evolution of the total number of hydrogen bonds in the \textit{trans} and \textit{cis} systems.
and hence polarization, remains unaffected by trans/cis isomerization.

**PSM chain order parameter**

The orientational OP of the hydrocarbon chains is best described by the rank 2 OP,

\[
S_{mol} = \frac{1}{2} \langle 3\cos^2 \phi - 1 \rangle,
\]

(2)

calculated at each nonterminal carbon position \(C_n\) in terms of \(\phi\), the angle between the long molecular axis \(\rho\), defined along the vector \(C_{n-1}C_{n+1}\), and the normal to the bilayer.

According to Eq. 2, \(S_{mol}\) will be large (high-ordering) for large \(\langle \cos^2 \phi \rangle\), i.e., small \(\phi\), and small but positive (lower-ordering) for small \(\langle \cos^2 \phi \rangle\), i.e., \(\phi\) less than but close to the magic-angle of 54.74°.

\(S_{mol}\) for the PA and SPH chains in the pure bilayer simulations are plotted in Fig. 8, parts a and b, respectively. \(S_{mol}^{PA}\) is relatively constant throughout the chain in both cases, with lower values toward the tail ends due to their increased mobility at the interface between the two layers. Compared to the cis system, the higher packing of the trans lipids increase the overall ordering of the PA chain. Because all the PA tail bonds are saturated, \(S_{mol}^{PA}\) can be directly related
to the deuterium order parameter $S_{CD}^{PA} \approx -0.5 S_{mol}^{CD}$ (the average orientation of C–H bonds is normal to the molecular axis) (52). Thus, the average $\langle S_{CD}^{PA} \rangle_{3-15} = 0.249$, calculated over carbon positions 3–15, matches very well the experimental value of $\langle S_{CD}^{PA} \rangle_{exp} = 0.221 \pm 0.047$ reported at 321 K (53).

In the case of SPH, the first two carbon atoms have low ordering in both systems, mainly because they have a large $\phi$-angle with respect to the bilayer normal inasmuch as they provide a direct link between the hydrophobic tails and the lipid headgroup (see Fig. 1). The double bond (carbons 4 and 5) and the following C6 are highly ordered in the all-trans system. In the all-cis system, however, C4 is ordered whereas C5 and C6 are highly disordered. Finally, the rest of the chain (C7–C15) exhibits similar ordering to the PA tails. Overall, the trans SPH tails are generally more ordered than the cis SPH tails due to the tighter packing of lipids.

Because $\langle \cos^2 \phi \rangle$ is directly related to the $\phi$-angle probability distribution $p(\phi)$ (see Fig. 9) through the relation

$$\langle \cos^2 \phi \rangle = \int_0^{\phi_{max}} \cos^2 \phi p(\phi) d\phi,$$

the behavior of $S_{mol}$ around the double bond can be explained as follows. For trans double bond, the molecular axes $\rho$ on positions 4 and 5 are expected to be almost parallel to each other, and therefore yield very similar $p(\phi)$ and hence very similar values of $S_{mol}^4$ and $S_{mol}^5$. The orientation of the molecular axis on these bonds influences the nearest neighbor, yielding $S_{mol}^6$ values that are in-between the high ordering of the double bond and the average ordering of the rest of the chain. The cis double bond allows the same molecular orientation on position 4 due to the similar interfacial interactions. However, its different conformation enforces very different $p(\phi)$ on positions 5 and 6. Because $\langle \phi_5 \rangle \approx 46^\circ$ is close to the magic-angle and $p(\phi_5)$ is almost symmetric, $S_{mol}^5$ has a very low value. The change in the orientation of the chain due to the cis double-bond kink is almost always conserved by the next dihedral angle, maintaining a small value for $S_{mol}^5$ (33,47,54).

Fig. 8, c and d, shows $S_{mol}$ calculated for PA and SPH chains in the mixed trans/cis simulation, respectively. Clearly, the $S_{mol}$ values around the SPH double bond extracted separately for each of the two conformations are very similar to $S_{mol}$ from the pure bilayer simulations. Nonetheless, the total $S_{mol}$ computed for the entire bilayer, with trans/cis ratio of 80:20, is dictated by the dominating conformation of the system (see dark curve around the double bond in Fig. 8 d). Because the saturated parts of the chains are less rigid, their profiles are approximately the same for both conformations. In both pure bilayers and the mixture, the coupling between the tails maintains approximately the same average value for $S_{mol}^5$, calculated over positions 7–15 for SPH and 4–12 for PA. Note that the actual positioning of the PA chain (with respect to the bilayer surface) relative to the SPH chain is shifted by 2–3 positions in the trans conformation and by 3–4 positions in the cis (see Fig. 1). These averages,

$$\langle S_{mol} \rangle_{trans} \approx 0.52 > \langle S_{mol} \rangle_{mixed} \approx 0.47 > \langle S_{mol} \rangle_{cis} \approx 0.42,$$

are larger for the better-packed bilayers:

$$\langle A_L \rangle_{trans} < \langle A_L \rangle_{mixed} < \langle A_L \rangle_{cis}.$$

Such a relationship between chain order and area per lipid was found generally valid for previously well-studied lipids (47,54).

**Lipid dynamics**

We studied the dynamics of the lipids in terms of their lateral diffusion and rotational motions. The lateral diffusion coefficients were calculated from the slopes of the
mean-square displacement curves (see Fig. S1 a) at long times:

$$D_L = \lim_{t \to \infty} \frac{1}{4t} \langle [\hat{F}(t)]^2 \rangle. \quad (3)$$

The obtained values are $D_{\text{trans}} = 0.5 \times 10^{-7}$ cm$^2$/s and $D_{\text{cis}} = 1.6 \times 10^{-7}$ cm$^2$/s for all-trans and all-cis bilayers, respectively. The former is in good agreement with the experimental value of $0.6 \times 10^{-7}$ cm$^2$/s obtained by the pulsed field gradient NMR technique (55). Clearly, the all-trans bilayer is not only more compact and ordered, but it is also less fluid than the all-cis.

The autocorrelation functions calculated for an interfacial vector between the two chains (uniting $C_3^{SPH}$ and $C_1^{PA}$) and for a vector along the headgroup (between P and N atoms), shown in Fig. S1 b, indicate that the trans configuration is slower in changing its orientation than the cis. Because of the intermolecular hydrogen bonds in the interfacial region, the decay of the interfacial reorientation ($C_3^{SPH} \to C_1^{PA}$) autocorrelation function is extremely slow. This slow rotational motion is partially transmitted to the headgroup (P $\to$ N) due to the intramolecular contacts between the oxygens of the phosphate group and the hydroxyl/amide groups.

CONCLUSIONS

The conformation of the sphingosine double bond has been reported to play an important role in cholesterol binding to sphingomyelins (22), and to have a strong effect on the structure and hydrogen bonding (23), as well as elastic properties and packing behavior of ceramides (21). In this work we have performed an extensive computational study on the influence of the double-bond geometry on the structural properties of the palmitoyl sphingomyelin lipid bilayer. We found that the sphingosine base double-bond conformation has little impact on the orientation and dynamics of the solvent molecules because the ordering of the interfacial water is mainly regulated by the phosphate group.

On the other hand, we observed tighter packing of the trans lipids, with the average area per lipid being $\sim$10% larger for the all-cis system. This behavior is induced by the larger number of both intra- and intermolecular hydrogen bonds that the trans lipids can form. Additionally, the positioning of the trans phosphate group slightly farther from the hydrophobic core allows the lipids to move closer to each other. Hence, less water enters the interfacial region and hydrogen bonds with the excluded solvent molecules are exchanged for intermolecular hydrogen bonds. The tight hydrogen-bond network in the all-trans bilayer keeps the lipids closely packed, thus reducing the average area per lipid and increasing the ordering and length of the tails, as well as the overall thickness of the bilayer. As a consequence of the strong hydrogen-bond network the all-trans bilayer is less fluid than the all-cis. Finally, the high order around the trans double bond and the disorder around the cis double bond found in the pure bilayers are maintained in the mixed bilayers as well: the ordering of the saturated part of the sphingosine base and of the palmitoyl chain are highest for all-trans, lowest for all-cis, and intermediate for the 4:1 trans/cis mixture. This emphasizes the importance of the double-bond conformation on the overall bilayer behavior with a direct impact on the average thermodynamic properties of bilayers.

An important consequence of these effects of the trans double bond is the resultant changes in bilayer mechanical properties. The isothermal area compressibility modulus is much larger in the all-trans than in the all-cis system, suggesting that stretching bilayers of the naturally occurring trans PSM requires roughly twice the energy needed to stretch the corresponding cis bilayer. Furthermore, using the relationship between the bending rigidity, compressibility modulus, and hydrophobic thickness of the bilayer,

$$K_C = K_A d_{C_2-C_2}/24$$

(56), we can estimate from our data that

$$K_C^{\text{trans}} \approx 2.7 \times K_C^{\text{cis}}.$$

This shows that the trans double bond confers almost three-times’ more resistance to bending of the bilayer than the cis double bond. These mechanical properties may explain the PSM-enrichment of functional lipid rafts in cellular membranes. It is worth noting that while most membrane lipids that are associated with phase separation and lipid rafts have intrinsic curvature, PSM does not. Instead, it appears that its involvement in phase separation arises from a fine balance of intra- and intermolecular hydrogen bonds that are tightly regulated by the trans double bond.

SUPPORTING MATERIAL

Description of orientational order parameters and a figure of mean square displacement and rotational correlation figure are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)01167-7.

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REFERENCES


