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

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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 0.75 million atom) 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, sphingosines, 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
lipid-water interface is reduced by ~1.13 waters per lipid. Also found that the number of waters solvating the all-

trans

configuration of both molecules is

sphingosine (SPH) base, and the palmitoyl (PA) chain. The enantiomeric

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.

MODELS 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 (C1–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 C4–C5 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 Nose-Hoover heat bath. All systems were semiisotropically (in the membrane plane and perpendicular to it) coupled to a reference pressure of 1 bar with compressibility constant $4.6 \times 10^{-3}$ 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. 3a. Fig. 3b displays the corresponding $d_{p-p}(t)$, calculated as the average

![Figure 1](image1.png)

**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](image2.png)

**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).
distance between the centers of mass of the phosphorous atoms at the two leaflets. Both $A_L(t)$ and $d_{P-P}$ ($t$) equilibrated after $\sim$40 ns, 110 ns, and 190 ns in the all-cis, all-trans, and mixed bilayer systems, respectively.

The well-equilibrated portions of the trajectories (last 50 ns) yield average areas per lipid of $\langle A_L \rangle^{\text{trans}} = 0.485 \text{ nm}^2$, $\langle A_L \rangle^{\text{cis}} = 0.531 \text{ nm}^2$, and $\langle A_L \rangle^{\text{trans/cis}} = 0.506 \text{ nm}^2$. The experimental area per lipid for PSM bilayers varies between $0.506 \text{ nm}^2$ at 323 K (37) and $0.47 \text{ nm}^2$ at 328 K (38). This variation is in part due to differences in the specific volume of DPPC was used to approximate the unavailable volume of PSM (37,38). Several computer simulation studies of sphingomyelin bilayers have been reported (34,40–44). Different force-field parameters for SM and water led to variations in the calculated $\langle A_L \rangle$ (40–42,44) (Table 1), though all are within the broad range of the experimental results (37,38). In fact, the variations in the simulated values (including the current ones) are smaller than the variations in the experimental area per lipid.

![Figure 3](image)

**FIGURE 3** Time evolution throughout the three simulations of the (a) area per lipid molecule and of the (b) lipid bilayer thickness measured as the average distance between the phosphorous atoms.

Although not always explicitly stated, we presume that all of these simulations were done with the sphingosine base double bond in the 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-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 trans and 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 underestimated (47) for both the cis and 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.

**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>$T$ [K]</th>
<th>$t$ [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>

**Organization of PSM lipids in bilayers**

The differences in $d_{P-P}$ and $A_L$ indicate that the trans PSM forms a more compact and thicker bilayer than the 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 sphingosine base.

The average density of the water molecules in the system being ~1 water molecule (per nm$^3$) less than for 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 nm$^3$) 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 deuteration-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...
hydroxyl groups exchange 12 HBs carbonyl oxygens lose 16 HBs with water but gain six become less hydrated and lose at least 12 HBs with water; to make the all-trans bond (50), the extra ~10 interlipid hydrogen bonds would contribute ~2 kcal/mol energetic contribution from a single hydrogen elastic strength of bilayers. With a conservative estimate scored by considering their potential contributions to the mechanical energy.

The stronger interlipid interactions in the all-trans system (than in the all-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 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 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 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 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 C₂ atom (open symbols in Fig. 7) shows practically no difference in the orientation of the interfacial waters in the all-trans and all-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. 6). Further into the bilayer (\( z > 0.1 \) 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 orientation. 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° for trans and ~79° for 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 trans and pure cis systems

<table>
<thead>
<tr>
<th>Donor</th>
<th>Acceptor</th>
<th>( N_{\text{trans}} )</th>
<th>( N_{\text{cis}} )</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>
<td>28.8</td>
<td>30.9</td>
</tr>
<tr>
<td>N–H</td>
<td>O=C</td>
<td>—</td>
<td>—</td>
<td>34.6</td>
<td>33.4</td>
</tr>
<tr>
<td>N–H</td>
<td>O–H</td>
<td>—</td>
<td>—</td>
<td>12.4</td>
<td>8.8</td>
</tr>
<tr>
<td>O–H</td>
<td>O–H</td>
<td>—</td>
<td>—</td>
<td>15.9</td>
<td>10.4</td>
</tr>
<tr>
<td>O–H</td>
<td>O_{182}-P</td>
<td>9.2</td>
<td>7.7</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>N–H</td>
<td>O_{182}-P</td>
<td>0.4</td>
<td>1.0</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>28.5</td>
<td>21.6</td>
<td>94.2</td>
<td>84.6</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).
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,

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

![Graphical representation of hydrogen bonding](image-url)

**FIGURE 6** Number of hydrogen bonds in the all-trans and all-cis systems. (Inset) Number of lipid-water hydrogen bonds that were lost when switching from cis to trans, and the number of inter- and intra-PSM H-bonds, as well as their total, gained at the same time. Hydrogen bonding was defined by an acceptor-hydrogen distance cutoff of 2.5 Å and acceptor-hydrogen-donor angle cutoff of 90° (40,42).

![Graphical representation of order parameters](image-url)

**FIGURE 7** First rank (open symbols) and second rank (solid symbols) water order parameters in the all-trans and all-cis systems.

![Graphical representation of molecular order parameters](image-url)

**FIGURE 8** Molecular order parameters for the palmitoyl chain of the pure bilayers (a) and mixture (c) and for the sphingosine chain of the pure bilayers (b) and mixture (d). For the mixed bilayer, the average \( S_{mol} \) calculated over the entire bilayer is also plotted.
to the deuterium order parameter $S_{52}^{PA} ≈ -0.5 S_{mol}^{PA}$ (the
average orientation of C–H bonds is normal to the molec-
ular axis) (52). Thus, the average $\langle S_{CD}^{PA} \rangle_{3-15} = 0.249$, cal-
culated 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 C 6 are highly ordered in the all-
trans system. In the all-cis system, however, C 4 is ordered
whereas C 5 and C 6 are highly disordered. Finally, the rest
of the chain (C 7–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 prob-
ability 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 inter-
facial 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

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Angular distributions of $\phi$ (see text), and of the angle between the bilayer normal and the double bond, $\psi$, for the sphingosine chain in the (a) all-trans and (b) all-cis systems. For clarity, $\phi_1$ and $\phi_2$ were omitted and an average angle distribution of the tail where $S_{mol}$ is relatively constant (see Fig. 8) was calculated for carbons 7–11.}
\end{figure}

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, main-
taining 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 $S_{mol}^{15}$ 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,

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

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:

$$D_L = \lim_{t \to \infty} \frac{1}{4t} \langle |\vec{F}(t)|^2 \rangle.$$  (3)

The obtained values are $D_{trans} = 0.5 \times 10^{-7}$ cm$^2$/s and $D_{cis} = 1.6 \times 10^{-7}$ cm$^2$/s for all-trans and all-cis bilayers, respectively. The former is in good agreement with the experimental value of $\sim 0.6 \times 10^{-7}$ 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 $C_2^{SPH}$ 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 ($C_3^{SPH} \rightarrow C_1^{PA}$) autocorrelation function is extremely slow. This slow rotational motion is partially transmitted to the headgroup (P $\rightarrow$ 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 ~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,

$$K_C = K_A d_{C_2-C_1}/24$$  (56), we can estimate from our data that

$$K_C^{trans} = 2.7 \times K_C^{cis}.$$  (56)

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.

We gratefully acknowledge the Medical School at the University of Texas Health Science Center in Houston for financial support and the Texas Advanced Computing Center for computational resources.

REFERENCES

Role of Sphingosine Double Bond


