Comparison of cholesterol and its direct precursors along the biosynthetic pathway: Effects of cholesterol, desmosterol and 7-dehydrocholesterol on saturated and unsaturated lipid bilayers

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Despite extensive studies, the remarkable structure-function relationship of cholesterol in cellular membranes has remained rather elusive. This is exemplified by the fact that the membrane properties of cholesterol are distinctly different from those of many other sterols. Here we elucidate this issue through atomic-scale simulations of desmosterol and 7-dehydrocholesterol (7DHC), which are immediate precursors of cholesterol in its two distinct biosynthetic pathways. While desmosterol and 7DHC differ from cholesterol only by one additional double bond, we find that their influence on saturated lipid bilayers is substantially different from cholesterol. The capability to form ordered regions in a saturated (dipalmitoyl-phosphatidylcholine) membrane is given by cholesterol > 7DHC > desmosterol, indicating the important role of cholesterol in saturated lipid environments. For comparison, in an unsaturated (dioleoyl-phosphatidylcholine) bilayer, the membrane properties of all sterols were found to be essentially identical. Our studies indicate that the different membrane ordering properties of sterols can be characterized by a single experimentally accessible parameter, the sterol tilt. The smaller the tilt, the more ordered are the lipids around a given sterol. The molecular level mechanisms responsible for tilt modulation are found to be related to changes in local packing around the additional double bonds. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2996296]

INTRODUCTION

Cholesterol is a molecule with many faces. It has numerous biochemical functions which are vital for eukaryotic life, and from the purely physical point of view, it is a small rigid molecule yet it has a major role in determining the structure and, thus, function of eukaryotes. The physical properties of cholesterol are very unique—not even the two molecules which are the immediate precursors of cholesterol are able to produce the same mechanical and structural responses in a membrane; the structural differences are indeed very small, cholesterol differs from desmosterol and 7-dehydrocholesterol (7DHC) by a single double bond (see Fig. 1) only. In this article we focus on the different interaction mechanisms of the structural responses caused by those three sterols, cholesterol, desmosterol, and 7DHC. Those properties largely determine the sterols' ability, or the lack of it, to bind to other molecules in membranes or/and to promote the formation of domains, such as rafts.

Since desmosterol and 7DHC are the immediate precursors of cholesterol along its two different biosynthetic pathways, it is therefore not surprising that the structural differences between them are very minor. Desmosterol differs from cholesterol only by one additional double bond, which resides between carbon atoms 24 and 25 in the tail of the molecule (see Fig. 1). 7DHC also differs from cholesterol by one additional double bond, but in this case it is located in the sterol ring between carbon atoms C7 and C8 (see Fig. 1). These minor structural differences might suggest that desmosterol and 7DHC are equally abundant as cholesterol in cells. This is not the case, however. Yet both desmosterol and 7DHC play an important role in a number of specific situations. For example, in contrast to many other precursors of cholesterol, desmosterol has been identified as an abundant structural membrane component in specific mammalian cell types such as spermatozoa and astrocytes.[1,2] 7DHC, in turn, has been found in high concentrations in rat epididymis.[3] Further, the inability to convert desmosterol or 7DHC to cholesterol leads to human disorders such as desmosterolosis in the case of desmosterol and the Smith–Lemli–Opitz syn-

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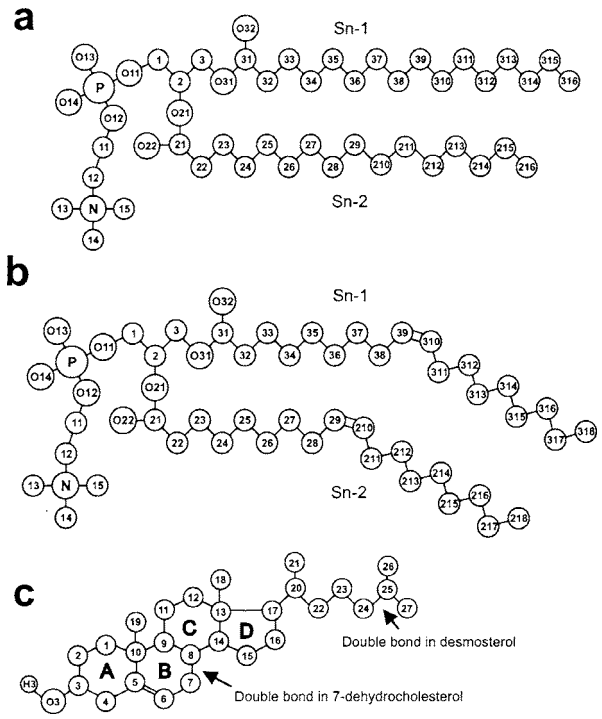

FIG. 1. Molecular structures of (a) DPPC, (b) DOPC, and (c) cholesterol molecules with numbering of atoms. The cholesterol rings are labeled A, B, C, and D. The chemical symbol for carbon atoms C is omitted. In desmosterol the bond C24–C25 is a double bond.

drome in the case of 7DHC.[4] These malformation syndromes are characterized by severe developmental defects and cognitive impairment.

From the biophysical point of view, membrane properties of desmosterol are rather poorly characterized. Experiments have shown that in monounsaturated palmitoyl-oleoyl-phosphatidylcholine (POPC) bilayers, desmosterol and cholesterol are equally effective in promoting the ordering of acyl chains.[5] The case is different in saturated dipalmitoyl-phosphatidylcholine (DPPC) bilayers, where cholesterol has been found to slow down translational diffusion more than desmosterol.[6] Recent atomic-scale simulations in saturated cholesterol-DPPC and desmosterol-DPPC bilayer systems are in line with these findings.[7] In monolayer studies for a mixture of natural unsaturated lipids, desmosterol and cholesterol have condensed membranes to the same extent,[8] while in a saturated DPPC bilayer the effect of desmosterol has been found to be weaker than that of cholesterol.[9] Further experiments have shown recently that desmosterol is characterized by a weaker ability than cholesterol to promote domain formation in model membranes and to increase membrane order and condensation.[7] In the same study, Vainio *et al.* demonstrated that if cholesterol is replaced with desmosterol in raftlike membrane environments, the activity of insulin receptors was inhibited substantially. Nonetheless, monolayer studies have indicated similar but not identical phase behavior of both sterols in DPPC systems, classifying desmosterol as a membrane active sterol.[10]

Similarly, membrane properties of 7DHC are too poorly known. In monolayer studies for a mixture of natural unsaturated lipids, 7DHC has been found to condense membranes less than cholesterol and desmosterol.[8] In saturated DPPC bilayers, the effect of 7DHC has been reported to be weaker than that of cholesterol.[9] Fluorescence studies have shown that the relative influence of cholesterol and 7DHC on membrane properties varies and depends on temperature and sterol concentrations.[11] Yet cholesterol seems to increase membrane rigidity more than 7DHC.[12] As for rafts, the ability of 7DHC to promote raft formation is unclear. Xu *et al.* found evidence that 7DHC promotes raft formation to a degree that is even stronger than the influence of cholesterol,[13] while Keller *et al.* did not observe differences between cholesterol and 7DHC in terms of their raft formation properties.[14] For comparison, Wolf and Chachaty observed destabilization of the lipid microdomain with 7DHC,[15] and the assay used by Rebolj *et al.* suggests weaker ability of 7DHC to form rafts.[16]

Experimental studies of sterol containing membrane systems have recently been complemented by a variety of simulation studies, which have provided a great deal of insights into nanoscale as well as continuum (elastic) properties. The focus of simulation studies has been in the elucidation of the relation between cholesterol structure and its functions in saturated membranes, including a variety of membrane properties such as the ordering at the membrane-water interface,[17] the ordering of hydrocarbon chains,[18,19] membrane condensation,[20,21] free area and volume within a membrane,[21,22] and the influence of cholesterol on membrane dynamics.[19,23] Further work has been done, e.g., to elaborate interactions of cholesterol with unsaturated lipids[24] and sphingolipids,[25,26] to understand the role of cholesterol in the formation of raftlike domains and their properties,[26–28] and to elucidate the influence of cholesterol on the lateral pressure profile of saturated, polyunsaturated, and raftlike membranes.[28–30] More recently, additional work has been conducted on other sterols such as cholesterol sulphate,[31] ergosterol,[32] lanosterol,[32,33] epicholesterol,[34] synthetic cholesterol analogs without methyl groups,[35,36] ketosterol,[35,37] and desmosterol.[7,35] The recent work of Aittoniemi *et al.* is particularly interesting since it showed that sterol tilt is a major determinant of membrane order: the ability of a sterol to order lipid hydrocarbon chains correlates with its tilt with respect membrane normal.[35] This coupling allows one to study how modifications in cholesterol structure lead to changes in sterol tilt and, consequently, to changes in membrane order,[35] thus facilitating aims to find sterols that are distinctly efficient in terms of promoting the formation of raftlike domains.

Despite the rather considerable number of simulation studies of cholesterol containing membranes, the studies of desmosterol and 7DHC are very limited. Desmosterol has been studied in a couple of short reports,[7,35] and for 7DHC there is, to our knowledge, only one previous atomic-scale simulation study.[38]

In this work, we carry out a systematic comparison of cholesterol, desmosterol, and 7DHC through atomic-scale simulations in two-component membranes, where the lipid component is either the saturated DPPC or the unsaturated dioleoyl-phosphatidylcholine (DOPC). A short discussion of the results for desmosterol in terms of sterol orientation[7] and

7DHC (Ref. 38) in terms of lateral pressure profiles has been given elsewhere. Here, we provide a detailed and systematic comparison of the structural properties and interaction mechanisms in those systems. In particular, we aim to clarify the interaction mechanisms that are the key to the different ordering properties of these three sterols, whose structural differences are seemingly marginal. A comprehensive analysis reveals that the subtle structural differences between the sterols give rise to rather significant differences in membrane properties if the membrane matrix is comprised of saturated DPPCs. In a DOPC bilayer, however, all sterols order membranes in an essentially similar manner. The observed changes in the DPPC bilayer take place close to the double bonds not included in cholesterol. The additional double bonds affect the packing close to the sterol and, consequently, change the sterol tilt, which together affect the ordering of lipids around it. In agreement with the previous findings,[35] the sterol tilt is found here to be a relevant measure of sterols' ordering capability.

METHODS

System description and parameters

We have performed atomic-scale molecular dynamics (MD) simulations for eight different membrane systems. The first bilayer system was composed of 128 DPPC molecules. The next three systems included 128 DPPC and 32 sterol molecules (either cholesterol, desmosterol, or 7DHC) (see Fig. 1). Next, the fifth system was comprised of 128 DOPC molecules, and the last three systems consisted of 128 DOPC and 32 sterols (cholesterol, desmosterol, or 7DHC). All systems were hydrated with 3500 water molecules. The initial structures of the DPPC, DPPC-cholesterol, DOPC, and DOPC-cholesterol bilayers were obtained by arranging the PC molecules in a regular array in the bilayer (x,y) plane with an initial surface area of 0.64 nm^2 per PC molecule. An equal number of cholesterol molecules were inserted randomly into each leaflet. Prior to actual MD simulations, the steepest-descent algorithm was used to minimize the energy of the initial structure.[39,40] The other bilayers were constructed by replacing cholesterol with its precursors in the previously simulated systems.

The simulations were performed using the GROMACS software package.[41] The MD simulations of all bilayer systems were carried out over 100 ns. The first 20 ns was considered as an equilibration period,[19] and thus only the last 80 ns of the trajectory was analyzed. Figure 1 shows the structure and the numbering of atoms in DPPC, DOPC, and sterol molecules.

We used the standard force-field parameters for DPPC and DOPC molecules,[42] where the partial charges were taken from the underlying model description.[43] For water, we employed the simple point charge model.[44] For the sterol force

TABLE I. Ordering and condensing effects of sterols. Average values of the molecular order parameter S_{mol}, chain tilt angle, number of *gauche* states per acyl chain, and lifetimes of *trans* conformations. All results are given separately for the *sn*-1 and *sn*-2 chains of DPPC and DOPC. Also given here are the average surface area per DPPC and DOPC and the average surface area per DPPC and DOPC and the membrane thicknesses of all bilayer systems considered in this work. Part of these data has been presented in Refs. 35, 36, and 38. " *" denotes area per all lipids.

Membrane		DPPC	DPPC-CHOL	DPPC-DESMO	DPPC-7DHC	DOPC	DOPC-CHOL	DOPC-DESMO	DOPC-7DHC
S_{mol}	*sn*-1	0.28	0.55	0.45	0.51	0.27	0.42	0.42	0.41
	sn-2	0.29	0.57	0.46	0.54	0.27	0.45	0.42	0.41
±0.05									
	sn-1	23.8	15.7	18.0	16.5	24.0	18.4	18.8	19.2
Tilt (°)	*sn*-2	23.6	16.0	18.6	16.7	23.5	18.0	18.1	18.4
	stero	···	19.7	26.9	21.9	···	24.7	24.9	25.8
±0.2									
	1								
No.	*sn*-1	3.0	2.3	2.7	2.4	3.0	2.7	2.7	2.7
	sn-2	3.0	2.3	2.7	2.4	3.0	2.7	2.7	2.7
gauche ±0.05									
Lifetime (	*sn*-1	87	115	99	108	82	126	126	122
ps) ±4	*sn*-2	85	118	102	110	87	130	129	124
No. of	*sn*-1	32.4	36.8	36.3	37.0	34.5	37.9	38.0	37.7
	sn-2	33.0	37.2	36.8	37.7	34.0	37.5	37.4	37.1
neighbors ±0.2									
Area/PC		0.66	0.60	0.65	0.620	0.69	0.65	0.65	0.66
(nm^2)			(0.50)	(0.56)	(0.53)		(0.56)	(0.56)	(0.56)
±0.05			*	*	*		*	*	*
Thickness (nm) ± 0.1		3.92	4.69	4.22	4.49	3.97	4.54	4.54	4.54

field, we used the description of Holtje *et al.*[45] For the additional double bond in desmosterol chain and 7DHC ring, standard GROMACS parameters were used.

Periodic boundary conditions with the usual minimum image convention were used in all three directions. The LINCS algorithm was used to preserve bond lengths between heavy atoms and hydrogen.[46] The time step was set to 2 fs and the simulations were carried out at constant pressure (1 atm) and temperature (323 K), which is above the main phase transition temperature of DPPC (Ref. 47) and DOPC. The temperature and pressure were controlled using the weak coupling method[48] with relaxation times set to 0.6 and 1.0 ps, respectively. The temperatures of the solute and solvent were controlled independently. For pressure we used semi-isotropic control. The Lennard-Jones interactions were cut off at 1.0 nm. For the electrostatic interactions we employed the particle-mesh Ewald method[49] with a real space cutoff of 1.0 nm, β-spline interpolation (of the order of 5), and direct sum tolerance of 10^{-6}. The list of nonbonded pairs was determined every tenth time step. The simulation protocol used in this study has been successfully applied in various MD simulation studies of lipid bilayers.[7,19,21,22,26,28,35–38,40,50]

Analysis

In the discussion below, we consider various quantities determined from the simulation data. Surface area/PC was calculated by dividing the total area of the membrane by 64, which is the number of PC molecules in a single leaflet (hence the number of sterol molecules was not accounted for in this calculation). The membrane thickness was determined from mass density profiles by considering the points where the mass densities of lipids and water are equal.[40] The molecular order parameter (S_{mol}), described in detail elsewhere,[18] provides essentially the same information as the commonly studied NMR order parameter S_{CD}.[51] For the saturated chains of DPPC, $S_{\mathrm{mol}}=2|S_{\mathrm{CD}}|$. To characterize the orientation of sterols in a bilayer, we calculated the tilt of a sterol defined as the angle between the C3–C17 vector [cf. Fig. 1(c)] and the bilayer normal. To calculate the tilt angles for the acyl chains of DPPC and DOPC, we averaged over the segmental vectors $\geqslant 4$ (the nth segmental vector links carbon atoms $n-1$ and $n+1$ in the acyl chain) to obtain the average segmental vector. The tilt angle for a given acyl chain is then given by $\langle \arccos(\mathrm{sqrt}(\cos^2\theta))\rangle$, where θ is the angle between the bilayer normal and the average segmental vector.[52]

In averaging conformational quantities in terms of *gauche* and *trans* states, only the torsion angles 4–16 were taken into account because the third torsion angle of the *sn*-1 and *sn*-2 chains is not in a well defined, stable conformation (*trans* or *gauche*).[18]

To analyze hydrogen bonding, water bridging, and charge pairing, we employed the same geometrical definitions as in our previous papers.[53,54] Charge pairing, which essentially describes the electrostatic interaction between a positively charged molecular moiety (such as a methyl group in PC choline) and a negatively charged one (such as an oxygen atom in the sterol OH group), complements our studies for atomic-scale interaction mechanisms and is most useful in describing interactions in the head group region.

Errors were calculated via the standard block analysis as described in Ref. 55.

RESULTS

Area per molecule and membrane thickness

The surface area per lipid is easy to calculate in a single-component bilayer by dividing the total area of the bilayer by the number of lipids in a single leaflet. For binary mixtures and many-component systems, this is no longer obvious as has been discussed in recent works.[19,23] In this work we prefer to avoid this subtle issue by considering the total area divided by the number of PC molecules only (see Table I). For our purposes this is completely reasonable since our objective is to compare the influence of the sterols on the membrane system. The surface areas given in Table I show that the presence of all sterols leads to membrane condensation. In the saturated DPPC case, the effect of cholesterol is strongest, followed by 7DHC and desmosterol. In the unsaturated DOPC bilayer, we find no observable differences between the three sterol systems.

The decrease in the surface area is closely associated with an increase in the membrane thickness. As Fig. 2 and Table I illustrate, the effect of cholesterol is stronger than that of desmosterol and 7DHC in DPPC bilayers, and the influence of 7DHC is more significant than that of desmosterol. In DOPC systems we find the effect of all sterols to be similar.

Location and orientation of sterols in the bilayer

It has been shown recently that there is a single experimentally accessible parameter that characterizes the ability of a given sterol type to order lipid acyl chains around it, namely, the tilt of the sterol with respect to membrane normal.[35] The results given in Table I (briefly discussed in a previous study[35]) show very clearly that in the DPPC bilayer the tilt of cholesterol is substantially smaller than the tilt of desmosterol or 7DHC. When the sterols are surrounded by unsaturated lipids in the DOPC bilayer, the subtle differences in sterol structures play a less important role. This is also evident from the results in Table I, which depicts that the tilts of all sterols in DOPC membranes are essentially similar. These results highlight the fact that the differences between different sterols are most evident in domains comprised of saturated lipids. This is also the case in lipid rafts, see for discussion below.

While the orientations of cholesterol, desmosterol, and 7DHC differ from each other in saturated bilayers, Fig. 3 shows that they all reside at the membrane-water interface in a similar manner. Figure 3 illustrates the density profiles of sterol oxygen atoms and PC phosphate oxygen atoms (Op) along the bilayer normal in one of the bilayer leaflets. We find that when the profiles are displayed in such a way that the different membrane thicknesses are accounted for (profiles are shifted to such positions that the Op distributions in

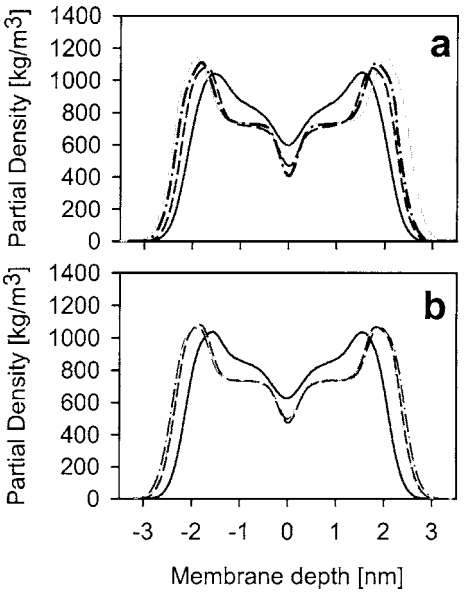

FIG. 2. Partial density profiles along the bilayer normal. All bilayer atoms in PC (black line), PC-cholesterol (gray line), PC-desmosterol (dashed line), and PC-7DHC (dash-dot line); (a) DPPC and (b) DOPC based bilayers. The coordinate $z=0$ corresponds to the membrane center.

all membranes in selected layers overlap), the OH group of the different sterols is at the same distance from the phosphate oxygen atoms.

Order and conformation of acyl chains

One of the most accurate means to gauge changes in membrane properties due to sterols is to consider the ordering of lipid acyl chains by NMR in terms of the S_{CD} order parameter. We employ the same approach through simulations using the molecular order parameter, S_{mol} (see Analysis).

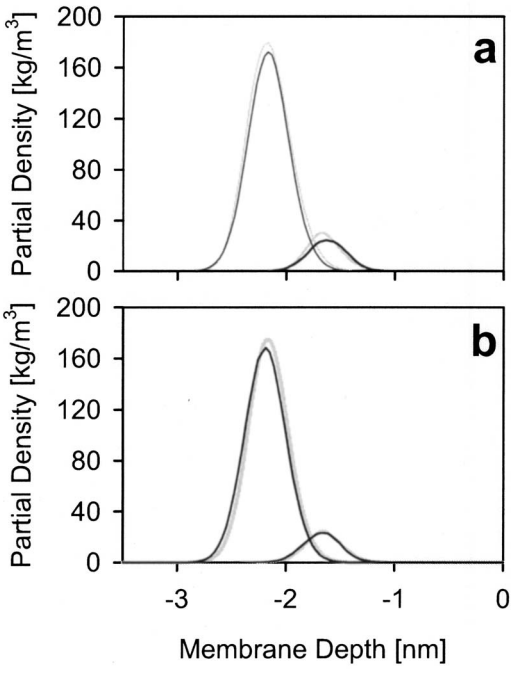

FIG. 3. Profiles of the atom densities of Op (thin line) and the sterol OH groups (thick line) in the (a) DPPC-cholesterol (gray line) and the DPPC-desmosterol (dashed line) an (b) DOPC-cholesterol and the DOPC-desmosterol bilayers. For clarity, the corresponding data for 7DHC are not shown here (the position of the hydroxyl group of 7DHC relative to phosphate oxygen does not differ from the position of hydroxyl group of desmosterol and cholesterol).

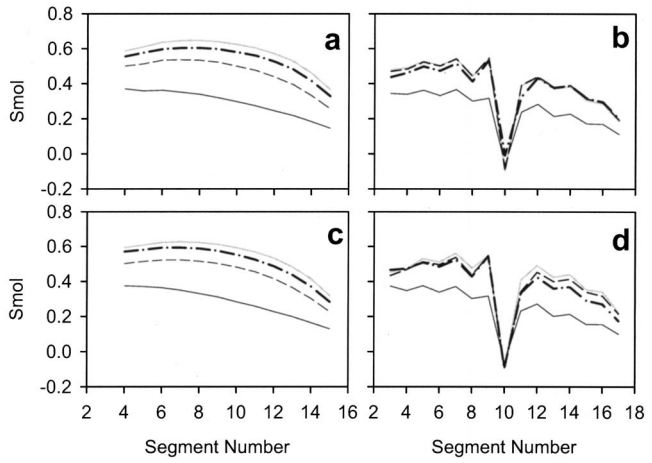

FIG. 4. Molecular order parameter (S_{mol}) profiles calculated for (a) DPPC *sn*-1 chain, (b) DOPC *sn*-1 chain, (c) DPPC *sn*-2 chain, and (d) DOPC *sn*-2 chain. Black line: pure PC, gray line: PC-cholesterol, dashed line: PC-desmosterol, and dash-dot line: PC-7DHC. Errors are not more than 0.002.

The subtle differences in the structures of cholesterol, desmosterol, and 7DHC lead to a rather profound difference in the ordering of DPPC acyl chains. This is illustrated by S_{mol}, whose profiles along the *sn*-1 and *sn*-2 chains of DPPC are shown in Fig. 4. Mean values (averages over segments 4–16) of S_{mol} for the *sn*-1 and *sn*-2 chains are given in Table I. Figure 4 and Table I clearly highlight the stronger ordering effect of cholesterol over those of the other sterols in DPPC. Aside from cholesterol, it seems evident that 7DHC is more able to promote ordering than desmosterol in a DPPC bilayer. In DOPC, the differences are rather marginal and within the error bars (see again Fig. 4).

Distributions of the tilt angles of the *sn*-1 and *sn*-2 chains of DPPC and DOPC are shown in Fig. 5, and the corresponding average values are given in Table I. Using the

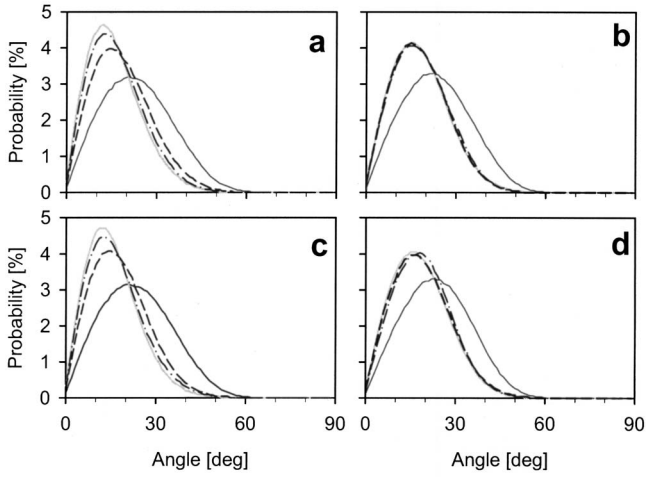

FIG. 5. Distribution of tilt angles of DPPC and DOPC chains in different cases. (a) DPPC *sn*-1 chain, (b) DOPC *sn*-1 chain, (c) DPPC *sn*-2 chain, and (d) DOPC *sn*-2 chain. Black line: pure PC, gray line: PC-cholesterol, dashed line: PC-desmosterol, and dash-dot line: PC-7DHC.

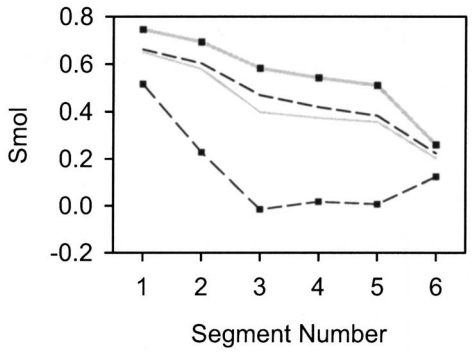

FIG. 6. Molecular order parameter (S_{mol}) profiles for the sterol tail calculated for cholesterol (solid line) and desmosterol (dashed line) in DPPC (black line) and DOPC (gray line) bilayers. Tail segments are numbered as follows: 1 for C13-C17-C20, 2 for C17-C20-C22, 3 for C20-C22-C23, 4 for C22-C23-C24, 5 for C23-C24-C25, and 6 for C24-C25-C26. Errors are not more than 0.002.

single-component DPPC bilayer as a reference, it is evident that cholesterol decreases the average tilt of both the *sn*-1 and *sn*-2 DPPC chains by about $8 \pm 0.4°$. For desmosterol, the same reduction is about $6 \pm 0.4°$ and for 7DHC about $7 \pm 0.4°$. In DOPC, the reduction in the tilt angle is essentially identical (about $6 \pm 0.4°$) in all systems.

As for isomerization and its dependence on the membrane composition, we found that the differences between the average numbers of *gauche* states per chain in all bilayers are small (Table I). In all systems, the average number of *gauche* states per chain is varied between 2.3 and 3.0. Largest numbers are observed in pure DPPC and DOPC bilayers, followed by desmosterol, 7DHC, and cholesterol in order of decreasing amount. The average lifetime of *trans* conformations (Table I) is affected more by cholesterol than desmosterol in DPPC, while in DOPC the effects of both sterols are similar. Again the effect of 7DHC is intermediate and lies between cholesterol and desmosterol.

Order and conformation of sterol tail

The additional double bond in desmosterol's short tail may be expected to change its conformation compared to the case of cholesterol. This is indeed what happens. Figure 6 shows profiles of the molecular order parameter S_{mol} along desmosterol and cholesterol tails in DPPC and DOPC bilayers. In DPPC, the order of the desmosterol tail is considerably lower than the order along the tail of cholesterol. The rapid decrease in S_{mol} of desmosterol's tail is associated with a conformational change in the beginning of the tail,[7] as the double bond between carbon atoms 24 and 25 tilts the chain and makes it more rigid. In DOPC, we find a difference of similar nature, but the quantitative difference is in this case much smaller than in the saturated bilayer; the more disordered nature of the acyl chain environment in DOPC plays a role here. A similar analysis performed for 7DHC (data not shown) did not reveal significant differences between 7DHC and cholesterol. This is not particularly surprising since cholesterol and 7DHC have identical tails.

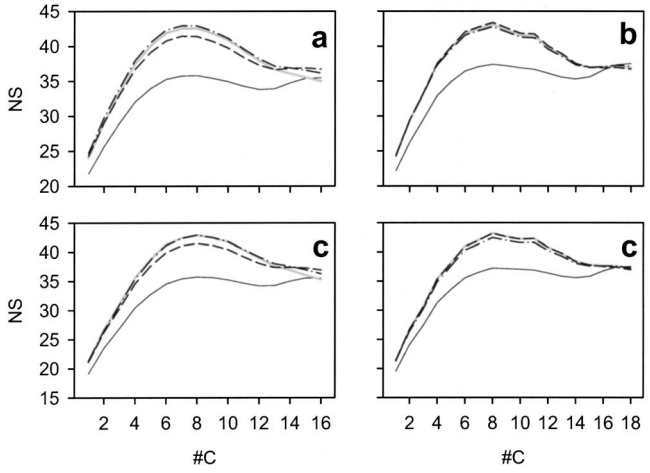

FIG. 7. Profiles of the number of neighbors (NS) along (a) DPPC *sn*-1 chain, (b) DOPC *sn*-1 chain, (c) DPPC *sn*-2 chain, and (d) DOPC *sn*-2 chain in pure PC (black line), PC-cholesterol (gray line), PC-desmosterol (dashed line), and PC-7DHC (dashed-dot line) bilayers. Errors are not more than 0.05.

Packing of atoms relative to acyl chain atoms

To quantify the packing of atoms around the acyl chain atoms in the hydrophobic bilayer core, we computed the number of their neighbors using the method described elsewhere.[20] In essence, for any arbitrarily chosen acyl chain carbon atom in the hydrophobic part of the bilayer, we consider other atoms in their vicinity. If any of those atoms belongs to a different molecule and is located no further than 0.7 nm (the position of the first minimum in the radial distribution function) from the tagged carbon atom in question, we consider the two to be nearest neighbors.

Profiles of the number of neighbors along the *sn*-1 and *sn*-2 chains are shown in Fig. 7. Let us first concentrate on the DPPC case. In DPPC-desmosterol, the number of neighbors is systematically smaller than in the DPPC-cholesterol system for carbons 1–12, and only in the end of the chain the situation becomes opposite. In DPPC-7DHC, the difference with respect to DPPC-cholesterol is much smaller but rather evident, as the number of neighbors in the 7DHC system is consistently larger than in the membrane including cholesterol. Second, in the DOPC systems the packing in the bilayer with 7DHC seems to be slightly smaller than in the other sterol containing membranes, but the differences are very small indeed. Overall, the results for the average number of neighbors given in Table I are in line with the above view. We come back to these findings and interpret them in more detail in the next section.

Packing of atoms relative to sterol ring atoms

Further insight into the packing properties inside the membrane is given by a similar nearest neighbor analysis with respect to carbon atoms in the steroid ring structure.[56]

Considering first the average number of nearest neighbors for cholesterol ring in the DPPC bilayer (cholesterol methyl groups were not included), we found 37.8 ± 0.1 nearest neighbors, of which 21.1 ± 0.1 are located on the α-face and 16.7 ± 0.1 on the β-face. These numbers highlight the

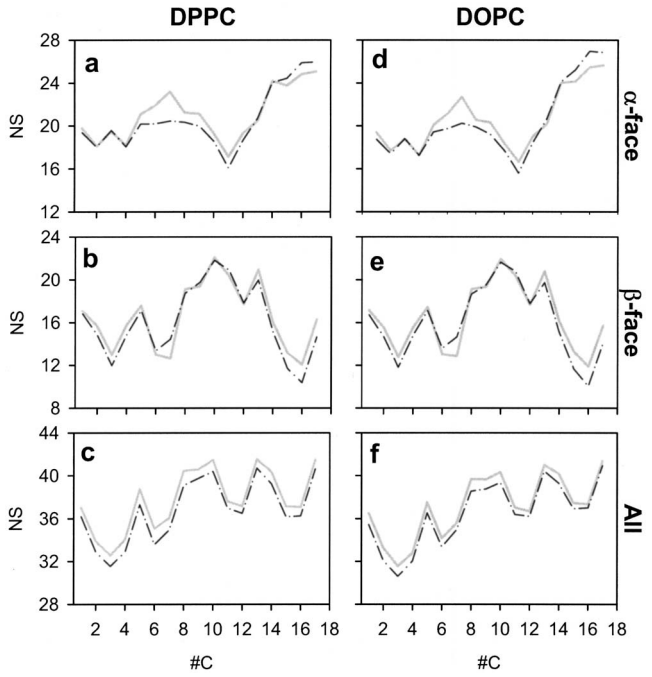

FIG. 8. Profiles of the number of neighbors (NS) along cholesterol (gray line) and 7DHC (dashed-dot line) located at α-face [(a) and (b)] and β-face [(c) and (d)]. The total numbers are given in (e) and (f). The two different bilayers: DPPC [(a), (c), and (e)] and DOPC [(b), (d), and (f)]. Errors are not more than 0.08.

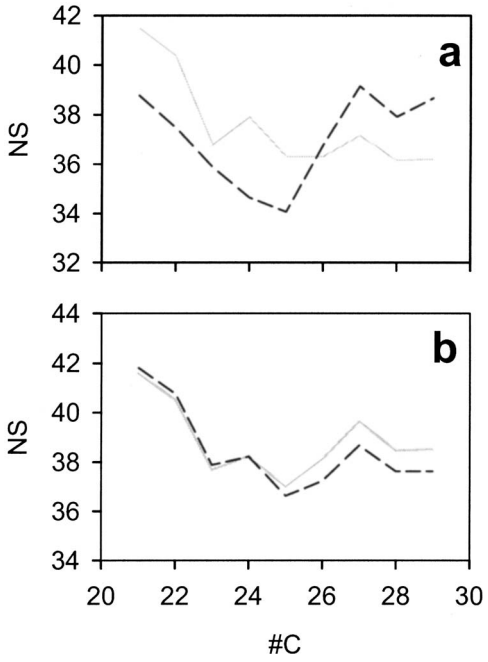

FIG. 9. Profiles of the number of neighbors (NS) along cholesterol (gray line) and desmosterol (dashed line) tail in DPPC (a) and DOPC bilayers (b). Errors are not more than 0.08.

asymmetric nature of the cholesterol ring since the "smooth" α-face has no substituents while the "rough" β-face contains two methyl groups [see Fig. 1(c)]. In a similar manner, we found for the 7DHC ring a nearest neighbor number of 36.8 ± 0.1, of which 20.6 ± 0.1 were located on the α-face and 16.1 ± 0.1 on the β-face. This indicates packing to be less tight around 7DHC. This is clearly illustrated in Fig. 8, where we show the profile for the number of nearest neighbors of cholesterol and 7DHC ring carbon atoms. When 7DHC is compared with cholesterol, the decreased packing is observed mostly on the α-face of rings B and C (carbon atoms 5–13) and on the β-face of rings A and D (carbon atoms 1–5 and 13–17). At the same time, the packing in 7DHC is larger than that in cholesterol on the α-face of ring D (carbons 14–17) and on the β-face of ring B (carbons 6–8). For comparison, the additional double bond in 7DHC is between carbons 5 and 6 in ring B.

The above shows that the packing close to cholesterol and 7DHC is different due to the additional double bond in 7DHC. The packing in a 7DHC-containing DPPC bilayer is decreased substantially close to the double bond on the smooth α-face, but at the same time slightly increased close to the double bond on the rougher β-face. These changes in packing are related to changes in van der Waals interactions in the hydrophobic area and give rise to changes in the sterol tilt, which, in turn, affects the ordering of nearby acyl chains.

In unsaturated DOPC bilayers, we find almost exactly the same kind of behavior for 7DHC and cholesterol (see Fig. 8). The same analysis carried out for desmosterol (data not shown) shows no significant differences between the packing of atoms around the rings of cholesterol and desmosterol. Since the steroid ring structures of cholesterol and desmosterol are identical, this is rather expected.

Packing of atoms relative to sterol tail

To describe packing close to the tails of sterols, we performed a neighbor analysis in a similar way like in the previous sections for the PC acyl chains. This analysis is particularly relevant for desmosterol, whose short tail contains a double bond not included in cholesterol. For the average number of neighbors of the cholesterol tail atoms, we found 37.6 ± 0.1 (38.9 ± 0.1) in DPPC (DOPC). For the desmosterol tail, a similar analysis yielded 37.0 ± 0.1 (38.5 ± 0.1) in a DPPC (DOPC) matrix. These almost identical average numbers are somewhat misleading, though, since the profiles shown in Fig. 9 indicate major differences along the tails in a DPPC matrix. From Fig. 9 we find that the packing around the desmosterol tail is tighter at its end and looser at its beginning compared to cholesterol. The crossover from one of these regimes to another takes place around carbons 24–26. That is precisely where the additional double bond in desmosterol is located at.

In a DOPC bilayer, the packing in the tail region of desmosterol and cholesterol is very similar [see Fig. 9(b)]. This again supports the view that differences between membrane properties due to different sterols are most evident in saturated environments.

The same analysis performed for 7DHC (data not shown) showed no differences between the packing of atoms around cholesterol and 7DHC tails.

Membrane/water interface

To elucidate the effect of desmosterol and 7DHC on the bilayer-water interface, we analyzed the atomic-level inter-

TABLE II. Interactions in the membrane-water interface. Sterol-water and sterol-PC hydrogen bonds, sterol-PC water bridges and charge pairs. Errors are less than ±2% for hydrogen bonds and charge pairs, and less than ±5% for water bridges.

	DPPC-CHOL	DPPC-DESMO	DPPC-7DHC	DOPC-CHOL	DOPC-DESMO	DOPC-7DHC
Sterol-water H-bonds	0.38	0.39	0.38	0.42	0.40	0.39
Sterol-PC H-bonds	0.82	0.81	0.80	0.83	0.82	0.81
OP	0.08	0.08	0.08	0.08	0.06	0.4
O22/O21	0.38/0.20	0.39/0.18	0.44/0.14	0.36/0.20	0.39/0.17	0.38/0.17
O32/O31	0.08/0.08	0.09/0.07	0.08/0.06	0.08/0.08	0.06/0.09	0.09/0.6
Sterol-PC water Bridges	0.32	0.30	0.31	0.32	0.32	0.30
OP	0.08	0.07	0.08	0.08	0.08	0.07
O22/O21	0.14/0.04	0.11/0.04	0.13/0.03	0.13/0.02	0.13/0.03	0.11/0.4
O32/O31	0.08/0.01	0.06/0.01	0.06/0.1	0.06/0.01	0.05/0.01	0.06/0.01
Sterol-PC charge Pairs	1.15	1.10	1.16	1.01	1.08	1.01

actions of these sterols' hydroxyl group (OH–) with PC head groups and water molecules. In particular, we considered the role of OH–on the formation of hydrogen bonds, water bridges, and charge pairs, and compared these results to those induced by the cholesterol hydroxyl group.

The OH group in all sterols participates in hydrogen (H) bonding with water and PC oxygen atoms. The average numbers of hydrogen bonds with water and PC phosphate oxygen atoms are given in Table II. The H-bond pattern is almost the same for all sterols; they make H-bonds predominantly with the ester group of the *sn*-2 chain (56% of all H-bonds). The number of PC-sterol water bridges is about 0.32 for all sterols. More than half (65%) of the water bridges are formed with the ester group of the *sn*-2 chain (O22 and O21). The negatively charged oxygen atom of the sterol hydroxyl group can interact with the positively charged methyl group of the PC choline moiety ($N-CH_3$) through charge pairs. In all bilayers, the average numbers of $O-N-CH_3$ charge pairs per sterol molecule are between 1.01 and 1.16 (Table II).

Summarizing, the interaction patterns of the hydroxyl groups of cholesterol and both of its precursors are essentially similar at the membrane-water interface in both saturated and unsaturated bilayers.

DISCUSSION

In the present work, we have used atomistic simulations to compare the effects of three closely related sterols on the properties of saturated and unsaturated membranes. The sterols considered here have been cholesterol together with desmosterol and 7DHC, which are cholesterol's direct precursors on its two biosynthetic pathways. These precursors differ from cholesterol only by one additional double bond, which in 7DHC is located in the steroid structure and in desmosterol in its short tail. The results obtained in the present study for desmosterol are in good agreement with experimental data. In saturated DPPC bilayers, desmosterol is found to be less effective than cholesterol in terms of increasing membrane order and condensation—this agrees with experimental data on the lateral diffusion of lipids in a DPPC bilayer, which indicates that cholesterol slows down translational diffusion more than desmosterol.[6] The simulation results are also in line with diphenylhexatriene fluorescence polarization measurements[7] and monolayer studies.[9] In unsaturated DOPC bilayers, desmosterol and cholesterol are found to influence membrane order to the same degree, which agrees with experimental data of Huster *et al.*[5] For 7DHC, we found that it condenses saturated bilayers less effectively than cholesterol, and that 7DHC has a weaker condensing property of unsaturated bilayers compared to desmosterol. These results agree with related monolayer studies.[8,9] Also, the weaker ability of 7DHC to increase PC tail's order corresponds well to the reduced ability to increase membrane rigidity.[12] On the other hand, the above discussed data (both experimental and computational) disagree to some extent with the fluorescence studies of Bernsdorff and Winter[11] as well as Megha *et al.*[57] which seem to indicate that the ordering effect of 7DHC in a DPPC bilayer should be similar or a bit higher than in the case of cholesterol. The apparent discrepancies between the experimental results may be related, at least in part, to the sensitivity of 7DHC to oxidation. This, in turn, results from the conjugated double bond structure of 7DHC that is not shared by, e.g., cholesterol and desmosterol.

In a recent work, we compared desmosterol and cholesterol in a DPPC matrix and showed that the lower ability of desmosterol to promote membrane ordering is related to the larger tilt adopted by desmosterol molecules.[7] This guided us to compare the tilts of several sterols with the resulting changes in membrane properties. The comparison showed that sterol tilt is a major determinant characterizing sterol's capability to order and condense a membrane.[35,36] In this paper, this observation was extended also to 7DHC. The results presented here allow us to gain better understanding for explaining the atomic-level mechanisms associated with the modulation of sterol tilt.

Obviously, the underlying reasons for some characteristic sterol tilt angle distributions depend on the interactions between the sterol and its neighborhood. In this respect, there are essentially three possible regions that one may consider important. First, one may consider the head group region.

Here, though, basically all sterols (except for ketosterol) share the same polar hydroxyl group, thus differences due to this part of the molecule are not expected for the molecules considered in this work. Second, the sterols have a rigid steroid structure characterized by subtle structural differences from one sterol to another. In our case, 7DHC belongs to this category due to the additional double bond in ring B. Third, there may be minor but relevant structural differences in the short sterol tail, which is the case in desmosterol.

On the basis of the present work, we can conclude that the additional double bond in 7DHC slightly perturbs the packing of a DPPC bilayer compared to the case where cholesterol would be present. The packing in a DPPC membrane including 7DHC is disturbed mostly around carbon atoms of ring B, where the additional double bond is located. Comparing 7DHC-induced changes to those induced by cholesterol, we found the packing to be decreased substantially close to the double bond on the smooth α-face and slightly increased close to the double bond on the rough β-face. These changes in packing are coupled to changes in van der Waals interactions in the hydrophobic area and give rise to a change in the sterol tilt (compared to cholesterol), which, in turn, affects the ordering of nearby acyl chains.

In the case of desmosterol, the mechanism responsible for an increase in the desmosterol tilt in a saturated DPPC bilayer is associated with the structure of desmosterol tail and its interactions with the surrounding acyl chains. Packing around the end of desmosterol tail (carbons 26–29) is tighter compared to cholesterol and looser in the beginning of the tail [carbons 21–25 (see Fig. 9)]. Since other significant differences between desmosterol and cholesterol in the head group and steroid region were not observed, we can conclude that the perturbed membrane structure and increased desmosterol tilt is due to the sterol tail region.

All the sterols considered in the present work affect membrane ordering and condensation, but differences in their capability to promote these properties are found only in saturated lipid bilayers. In unsaturated DOPC bilayers, all sterols studied here have been found to be essentially equally effective. The tilt of the three sterols in unsaturated bilayers is also found to be almost identical and substantially higher than the tilt of cholesterol in saturated bilayers. These results agree with our previous studies on POPC, where cholesterol tilt was found to be even larger.[24] Similar differences between sterol effects on saturated and unsaturated bilayers have been found for lanosterol.[58,59]

The fact that unsaturated lipid matrices are less sensitive to the details of sterol structure likely results from a higher average sterol tilt (broader tilt distribution) imposed by the increased free volume in the (unsaturated) disordered acyl chain region and the interactions between sterol tail and unsaturated bonds. It seems rather evident that sterol specificity is a characteristic to saturated lipid domains, where the tiny but relevant structural details of different sterols can play the role they have been designed for.

CONCLUSION

We have performed a detailed comparison of interaction mechanisms and structural properties between cholesterol and its two precursors: desmosterol and 7DHC. In line with our previous papers,[7,35] we observed a correlation between the sterol tilt and the sterol's ability to increase membrane order and condensation. The current study shows that a small modification in the sterol structure can influence the atomic packing in the membrane core and thus affect the van der Waals interactions. In the case of 7DHC, we observed weaker packing at the α-face of the steroid ring in the region of additional double bond and better packing at the β-face in the same region. This change in the balance of interactions seems to be responsible for the tilt modulations. In the case of desmosterol, changes in packing are observed in the tail region.

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