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Tilt: Major Factor in Sterols' Ordering Capability in Membranes

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Using extensive atomistic simulations, we show that there is a single experimentally accessible parameter—the sterol tilt—that can be used to determine a sterol's capability to induce order, and thus to promote, e.g., formation of lipid rafts. The observations also facilitate designing new effective sterols.

I. Introduction

Cholesterol is a key molecule governing many membrane properties of animal cells.[1] It is responsible for regulation of membrane permeability for water, gases and small molecules, and it largely determines mechanical properties[2] and fluidity[3] of membranes. It also increases the order of phospholipid acyl chains[4] and membranes' surface density.[5] It has also been shown that cholesterol is involved in signaling, and that it is vital in formation of specialized membrane domains called lipid rafts.[6] As established in experimental studies, the main structural elements of cholesterol are the planar tetracyclic ring system with the 3β-hydroxyl group and a short 8-carbon atom chain attached to C17[7] (Figure 1). Any modification of them weakens the sterol-induced effects on lipid bilayer properties and thus the sterol's biological functions;[8] cholesterol analogs such as desmosterol, ketosterol and 7-dehydrocholesterol differ only slightly from cholesterol but are inferior in promoting formation of lipid rafts.[9,10] This is highlighted by desmosterol, a direct cholesterol precursor on its biosynthetic pathway, which is not able to replace cholesterol in rafts.[10] Despite numerous studies on cholesterol's structure−function relation, the reason for the structural requirements has remained unknown.

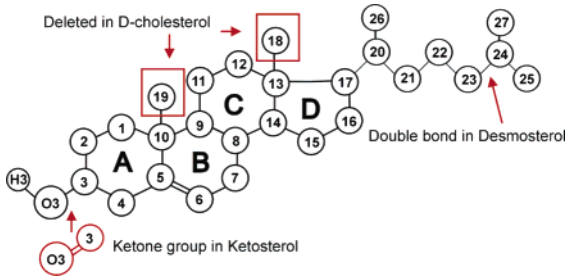

Figure 1. Cholesterol structure with modification sites showing the set of sterols studied here.

Here, we provide evidence that there is a single experimentally measurable parameter that characterizes a sterol's ability to modify membrane properties: the sterol tilt. The smaller the tilt, the stronger the ordering ability of a given sterol. This mechanism differentiates the many sterols and facilitates studies to find sterol molecules comparable to cholesterol in promoting the formation of highly ordered domains such as rafts.

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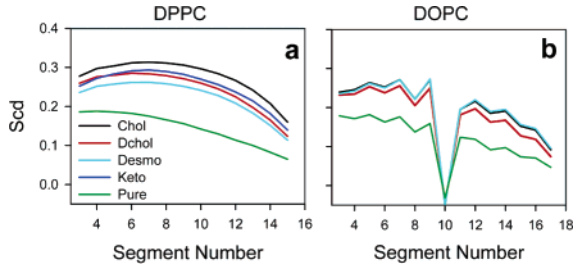

Figure 2. Profiles of the molecular order parameter[10,11] -Scd for (a) the DPPC and (b) the DOPC sn-1 chain in pure and sterol containing bilayers.

TABLE 1: Sterol Tilt, Average Area per PC, and ⟨−Scd⟩

bilayer DPPC/DOPC	sterol tilt [deg] ± 0.5	⟨−Scd⟩ ± 0.01	area [nm^2] ± 0.005
pure		0.15/0.10	0.66/0.69
CHOL	19.7/24.7	0.28/0.18	0.60/0.65
DCHOL	25.2/28.5	0.25/0.16	0.65/0.67
DESMO	27.8/24.4	0.23/0.18	0.62/0.65
KETO	28.1	0.25	0.63

II. Results

We have performed long (100 ns) atomistic molecular dynamics simulations of seven bilayers composed of dipalmitoyl phosphatidylcholine (DPPC) or dioleoyl phosphatidylcholine (DOPC) as the matrix in the liquid crystalline phase (323 K) with 20 mol % concentration of cholesterol or one of its analogs. Details of the simulation protocol are given elsewhere.[11] This choice of lipids allows us to model conditions reminiscent of rafts made of saturated lipids and cholesterol, and less ordered membranes having unsaturated lipids and cholesterol. As cholesterol analogs we chose ketosterol (polar part modified), desmosterol (tail modified), and an artificial sterol (called D-cholesterol) with methyl groups C18 and C19 removed (ring system modified). The structures are shown in Figure 1. Pure DPPC and DOPC bilayers were used as references. This choice of sterols lets us to elucidate the effect of modifying any of the three important parts of the cholesterol molecule.

Figure 2 shows the profiles of the molecular order parameter[10,11] Scd along the sn-1 chain for both DPPC and DOPC. Scd values averaged over the whole sn-1 chain, as well as the area per PC molecule, are given in Table 1. As the table shows, cholesterol affects both quantities more than other sterols do.

Figure 3a,b shows the tilt angle distributions of the sterol ring systems (measured as the angle between the bilayer normal and the vector connecting the ring atoms C3 and C17). Their average values are given in Table 1. The cholesterol tilt found in the DPPC bilayer (19.7°) is in agreement with the experimental value of 16−19°.[12] The tilt of cholesterol is the smallest among all sterols in both saturated and unsaturated bilayers. In general, the sterol tilt is larger in unsaturated bilayers. Figure 3c,d shows the correlation between the instantaneous tilt of the sterol ring and average order parameter ⟨−Scd⟩ (averaged over its sn-1 chain) of a neighboring chain/molecule. A PC molecule was defined to be a sterol neighbor, if the center of mass (CM) of any of its acyl chains was within 0.7 nm of the CM of the sterol rings, as measured in 2D in the bilayer plane. In all cases, a lower sterol tilt promotes higher ordering of the neighboring chains. In saturated bilayers, the effect of cholesterol is strongest, followed by ketosterol, D-cholesterol and desmosterol. In unsaturated bilayers we did not observe significant differences between the sterols.

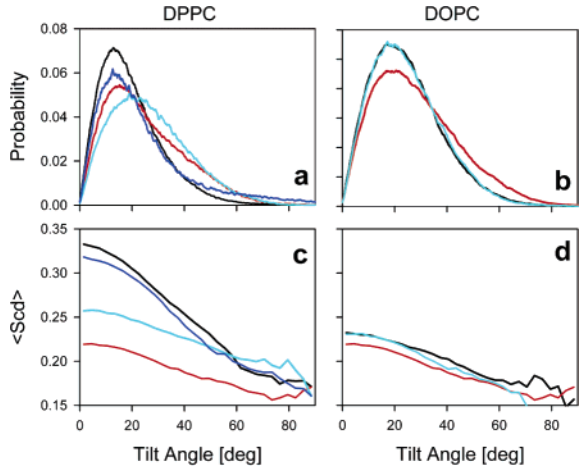

Figure 3. Sterol tilt angle distributions in (a) DPPC and (b) DOPC bilayers. (c) for DPPC and (d) for DOPC illustrate the correlation between the instantaneous tilt angle of a sterol and the corresponding average molecular order parameter ⟨−Scd⟩ of sn-1 chains neighboring the sterol ring. Color scheme as in Figure 2.

III. Discussion and Conclusions

Our results clearly demonstrate a correlation between the sterol tilt and the ability of a given sterol to modify membrane properties. The correlation holds in both saturated and unsaturated bilayers. As shown in Figure 3c,d, the order of the acyl chain neighboring a sterol molecule is strongly correlated with the sterol tilt. In our previous studies we observed that the DMPC chains neighboring cholesterol molecules had a higher order than molecules isolated from cholesterol.[13] Our present results also show that all cholesterol analogues having a higher tilt are less effective in increasing membrane order and condensation. Early simulation studies showed a higher tilt in lanosterol (a biosynthetic precursor of cholesterol) than in cholesterol.[14] However, the time scale of those simulations was too short to allow a detailed study of the ordering effect, and the role of the sterol tilt was not pointed out. Instead, early simulations focused on other aspects such as the role of sterol's vertical location in the bilayer.[14−16]

Our results show a clear difference between a sterol's interaction with saturated and unsaturated lipids. In unsaturated membranes we generally observed higher tilts. Moreover, the differences between the effects of different sterols in unsaturated bilayers are smaller than in saturated membranes. This agrees with experiments: in unsaturated bilayers the effect of desmosterol is practically the same as that of cholesterol,[17] and these two sterols modify the properties of saturated bilayers very differently.[10] Similar observations have been made for lanosterol in saturated[18] and unsaturated bilayers.[19] The above indicates that unsaturated bilayers are less sensitive to the details of sterol structure, and the differences between the ordering effects of different sterols in unsaturated bilayers are entirely related to the sterol tilt; see Figure 3.

In saturated bilayers, the relation between the sterol tilt and the order of a neighboring chain persists, but the degree of correlation varies with the sterol. This indicates that sterol-specific interactions play a role. It is remarkable that cholesterol has not only the lowest tilt but also the best ordering as a function of tilt. It is superior to the other sterols in terms of ordering PCs, condensing the bilayer, and hence promoting the formation of ordered domains. This highlights the fact that the structure of cholesterol has evolved to optimize its interactions with saturated lipids, as is the case in lipid rafts. Studies of ergosterol would also be of interest due to its ordering effect

and importance in fungal cell membranes, where it largely serves the same function as cholesterol in animal membranes.

The tilt of a sterol can be measured through NMR experiments.[12] That provides a means to explore other sterols, either native or synthetic, whose biological functions might be comparable to those of cholesterol. Most importantly, that would allow one to better understand the unique structure−function relationship of cholesterol.

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