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Enantiospecificity of sterol–lipid interactions: first evidence that the absolute configuration of cholesterol affects the physical properties of cholesterol–sphingomyelin membranes

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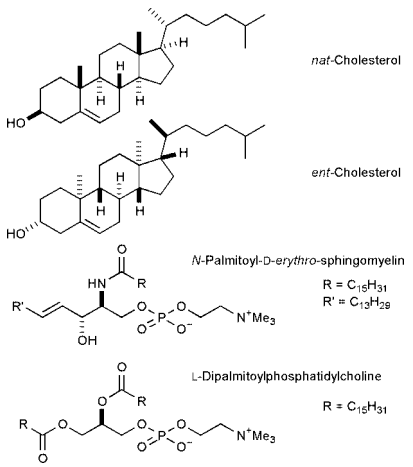
Results from monolayer studies using the enantiomers of cholesterol (*nat*- and *ent*-cholesterol) and egg yolk sphingomyelin show for the first time that enantiospecific interactions between sterols and lipids can affect the physical properties of membranes.

Because the sterols and lipids of cellular membranes occur in enantiomerically pure form, it is possible that the physical properties of the membranes could, in part, be dependent on enantiospecific sterol–lipid interactions. This possibility has seldom been addressed because the unnatural enantiomers of sterols and lipids are not readily available. Nevertheless, in the few studies that have been performed, where interactions of the enantiomers of dipalmitoylphosphatidylcholine, (or its close structural analogues) with natural cholesterol (*nat*-cholesterol)



were investigated, no enantioselective sterol–lipid interactions were detected.[1] Thus, it is perhaps not surprising that enantiospecific sterol–lipid interactions are widely believed to be too weak to influence the physical properties of membranes. Here, we show that this view is unwarranted for certain sterol–lipid interactions. Specifically, we show that enantiospecific interactions between cholesterol and egg yolk sphingomyelin (SPM) influence the physical properties of cholesterol–sphingomyelin monolayers at the air–water interface in a Langmuir–Blodgett trough.

The *ent*-cholesterol was prepared by us as described previously.[2] Enantioselectivity for the interactions of either *nat*- or *ent*-cholesterol with egg yolk sphingomyelin was investigated by examining the well known condensing effect of cholesterol on the packing of monolayers of cellular membrane lipids on a water surface in a Langmuir–Blodgett trough.[3] At low surface pressures, the rigid steroid acts as a template to orient the lipid hydrocarbon chains into fully extended conformations thereby allowing the lipid to occupy less area on the water surface than it would occupy if the steroid were not present. Thus, enantioselectively for the sterol–lipid interactions is detected as a difference in the plots of surface pressure (Π) *vs.* mean molecular area (mmA) during the compression of mixed monolayers containing the same mol% of either cholesterol enantiomer cospread on the surface with SPM.

In data not shown, the Π–mmA compression isotherms of the cholesterol enantiomers and SPM were recorded. The compression isotherms of the cholesterol enantiomers are identical. A gaseous to liquid ordered phase transition ($\Pi \geqslant 0.5$ mN m^{-1}) occurs at mmA ≈ 42 Å^2 molecule^{-1} and the film collapses when $\Pi \approx 49$ mN m^{-1} and mmA ≈ 36 Å^2 molecule^{-1}. In the absence of cholesterol, SPM monolayers compressed on a water subphase undergo a gaseous to liquid ordered phase transition ($\Pi \geqslant 0.5$ mN m^{-1}) at mmA ≈ 82 Å^2 molecule^{-1}. At collapse, the SPM monolayers have $\Pi \approx 55$ mN m^{-1} and mmA ≈ 35 Å^2 molecule^{-1}. These experimental parameters are in agreement with published values for the compression isotherms of similar cholesterol or SPM films.[4]

Results for the Π–mmA compression isotherms of monolayer films containing 30 mol% of either *nat*- or *ent*-cholesterol and 70 mol% SPM are shown in Fig. 1. Differences in mmA values at all Π are observed and are highly significant ($P < 0.00001$).[5] The *ent*-cholesterol has a greater condensing effect on SPM than *nat*-cholesterol.

In experiments not shown, mixed cholesterol–SPM films containing 10–50 mol% of either cholesterol enantiomer were compressed. Enantioselectivity during compression was observed when the mol% of the steroid was varied in 10 mol% increments between 20 and 40 mol%. Enantioselectivity was greatest at 30 mol% sterol and was not observed at either 10 or

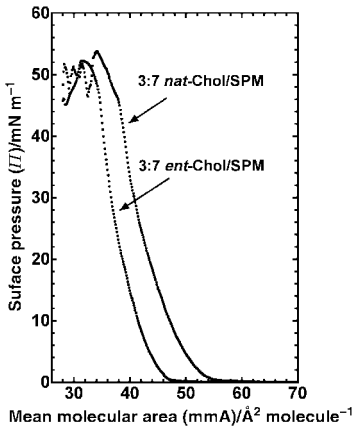


Fig. 1 Pressure–area isotherms for 3:7 mixtures of each cholesterol enantiomer with egg yolk sphingomyelin.

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50 mol% sterol. Also in experiments not shown, mixed cholesterol–DPPC (natural L-dipalmitoylphosphatidylcholine) films containing 10–60 mol% of either cholesterol enantiomer were compressed. These DPPC experiments provide a chirality crosscheck on the earlier reported studies that used DPPC enantiomers. In agreement with the earlier cited studies, we found no enantioselectivity using cholesterol enantiomers and natural DPPC.

The appearance of the 30 mol% *nat*- and *ent*-cholesterol–SPM films during compression was examined using Brewster angle microscopy.[6] Fig. 2 shows micrographs of the films at similar degrees of compression. The SPM film containing *ent*-cholesterol exhibits a greater fraction of the gas (dark) phase than the corresponding SPM film containing *nat*-cholesterol. At this stage of compression the *nat*-cholesterol, but not the *ent*-cholesterol, containing film has undergone the gaseous-to-liquid condensed phase transition (refer to Fig. 1). Thus, the films are visually different at similar degrees of compression. Additionally, there are two phases evident for the *nat*-cholesterol containing film, but three phases present for the *ent*-cholesterol containing film. This difference in the number of phases present also occurs when the films are more expanded and neither film has undergone a phase transition. Further compression of either film after the phase transition has occurred gives uniform films with a similar bright appearance. Although further experiments are needed to characterise the identity of the third phase present in the *ent*-cholesterol containing film, the presence of this additional phase emphasised that the differences between the Π–mmA isotherms of the cholesterol enantiomer–SPM films are not experimental artefacts.

Thus, we confirm that the physical properties of cholesterol–DPPC containing membranes are not measurably influenced by enantiospecific interactions between the sterol and the lipid. In contrast, we report that enantiospecific interactions between cholesterol and SPM do affect the physical properties of these membranes. It is likely that the varying degrees of enantioselectivity observed for DPPC and SPM in their interactions with cholesterol are explained by differences in the hydrogen bonding between the cholesterol hydroxyl group and the polar head groups of the two lipids.

The only chiral center in DPPC is the central carbon in the glycerol backbone of the lipid. Thus, unless the carbonyl oxygen in the ester group attached to this carbon is directly involved in a hydrogen bonding interaction with the hydroxy group attached to the C-3 chiral center in cholesterol, there is little opportunity for the molecules to interact in an enantioselective manner. However, evidence suggests that the DPPC ester carbonyl group and the cholesterol hydroxyl group do not hydrogen bond to each other, but instead hydrogen bond to intervening water molecules.[7,8] Hence, it may not be surprising that cholesterol–DPPC packing interactions are not measurably enantioselective.

The D-*erythro*-sphingosine base of SPM has two chiral centers and a double bond proximate to the zwitterionic choline head group. Additionally, since the polar head group of SPM contains both hydrogen bond donor and acceptor groups it is possible for hydrogen bonding to organise SPM into aggregates in a way that is not possible for DPPC. If hydrogen bonding to cholesterol alters these intermolecular SPM–SPM hydrogen bonding interactions, it seems reasonable that cholesterol could do this in an enantiospecific manner. Further experiments will be needed to investigate this possibility. Nevertheless, the hypothesis seems consistent with published views regarding the idea that intermolecular hydrogen bonding between cholesterol and SPM has important biophysical and biochemical consequences.[8]

This study provides the first evidence that enantioselective interactions between sterols and lipids can affect the physical properties of membranes. In a larger context, *ent*-cholesterol may prove to be a useful reagent for studying cellular cholesterol homeostasis and the role that cholesterol–SPM raft formation plays in the functioning and trafficking of proteins involved in cell-signalling pathways.[9] We have previously used *ent*-cholesterol to demonstrate that the absolute configuration of cholesterol has an effect on the transport of daunomycin by the multidrug resistance transporter P-glycoprotein (Pgp) in human HepG2 and Chinese hamster ovary (CHO) cells.[10] Additional studies to further define the effect that the absolute configuration of cholesterol has on the physical properties of cell membrane lipids and studies of *ent*-cholesterol binding to proteins are in progress.

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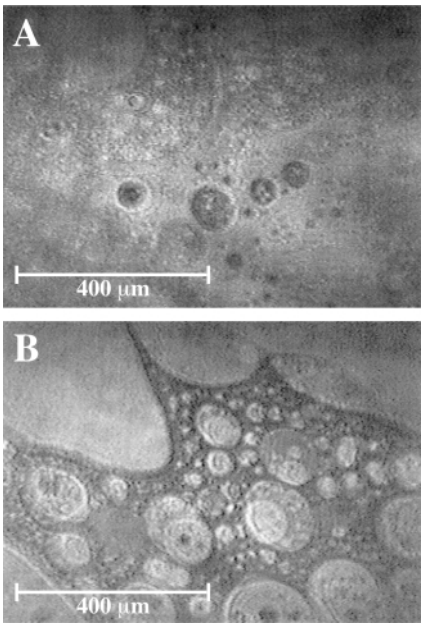

Fig. 2 Brewster angle microscopy of monolayer films. A: *nat*-cholesterol–SPM (3:7); mmA, 50 Å^2 molecule^{-1}; B: *ent*-cholesterol–SPM; mmA, 52 Å^2 molecule^{-1}.

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