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Sterol chemical configuration influences the thermotropic phase behaviour of dipalmitoylphosphatidylcholine bilayers containing 5α-cholestan-3β- and 3α-ol

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ABSTRACT

It is commonly believed that all membrane sterols are rigid all-*trans* ring systems with a fully extended alkyl side-chain and that they similarly influence phospholipid bilayer physical properties. Here, we report the sterol concentration-dependent, thermotropic phase behaviour of binary dipalmitoylphosphatidylcholine (DPPC)/sterol mixtures containing two similar 5α-H sterols with different functional group orientations (3α-OH or 3β-OH), which adopt an ideal all-*trans* planar ring conformation but lack the deformed ring B conformation of cholesterol (Chol) and epicholesterol (Echol), using differential scanning calorimetry (DSC). Our deconvolution of the DSC main phase transition endotherms show differences in the proportions of sterol-poor (sharp) and sterol-rich (broad) domains in the DPPC bilayer with increasing sterol concentration, which delineate gel/liquid-crystalline ($P_{\beta'}/L_\alpha$) and disordered gel (L_β)/liquid-ordered (l_o) phase regions. There are similarities in the DPPC main phase transition temperature, cooperativity and enthalpy for each 3β-ol and 3α-ol pair with increasing sterol concentration and differences in the parameters obtained for both the sterol-poor and sterol-rich regions. The sterol-poor domain persists over a greater concentration range in both 3α-ol/DPPC mixtures, suggesting that either those domains are more stable in the 3α-ols or that those sterols are less miscible in the sterol-rich domain. Corresponding parameters for the sterol-rich domain show that at sterol concentrations up to 20 mol%, the 5α-H,3β-ol is more effective at reducing the phase transition enthalpy of the broad component (ΔH_m^{brd}) than Chol, but is less effective at higher concentrations. Although mixtures containing Echol and 5α-cholestan-3α-ol have similar positive slopes below 7 mol% sterol, suggesting that they abolish the L_β/l_o phase transition equally effectively at low concentrations, Echol is more effective than the saturated 3α-ol at higher sterol concentrations. A comparison of ΔH_m^{brd} obtained for the saturated and unsaturated pairs suggests that the latter sterols stabilize the l_o phase and broaden and abolish the DPPC main phase transition more effectively than the saturated sterols at physiologically relevant concentrations, supporting the idea that the double bond of Chol and Echol promotes greater sterol miscibility and the formation of l_o phase lipid bilayers relative to corresponding saturated sterols in biological membranes.

1. Introduction

Biological membranes contain a wide range of structurally important polar phospho- and glycolipids, as well as many amphiphilic molecules with different chemical configurations and functions. The lipid bilayer is a fundamental structure in biological membranes, acting as a barrier between the cytoplasm and the external environment, dividing the cell into smaller compartments for specific biochemical roles. Sterols, such as cholesterol (Chol), are among the most abundant plasma membrane constituents in eukaryotes. The majority of natural membrane sterols contain an equatorially oriented C3–OH group (β) in ring A of the steroid nucleus (Fig. 1), which is a tetracyclic system containing three cyclohexane and a single cyclopentane ring arranged in an all-*trans* configuration. This ring system may have two or more methyl branches on one or both surfaces and is assumed to be planar and

Abbreviations: DSC, differential scanning calorimetry; FTIR, Fourier-transform infrared; Chol, cholesterol; Echol, epicholesterol; DPPC, dipalmitoylphosphatidylcholine; 5α-H,3α-ol, 5α-cholestan-3α-ol; 5α-H,3β-ol, 5α-cholestan-3β-ol; T_m^{shp}, chain-melting phase transition temperature of the sterol-poor domain; $\Delta T_{1/2}^{shp}$, chain-melting phase transition peak-width at half height of the sterol-poor domain; ΔH_m^{shp}, chain-melting phase transition enthalpy of the sterol-poor domain; T_m^{brd}, chain-melting phase transition temperature of the sterol-rich domain; $\Delta T_{1/2}^{brd}$, chain-melting phase transition peak-width at half height of the sterol-rich domain; ΔH_m^{brd}, chain-melting phase transition enthalpy of the sterol-rich domain; $P_{\beta'}$, tilted ripple gel phase; L_α, lamellar liquid-crystalline phase; L_β, lamellar gel phase; l_o, liquid-ordered phase; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital.

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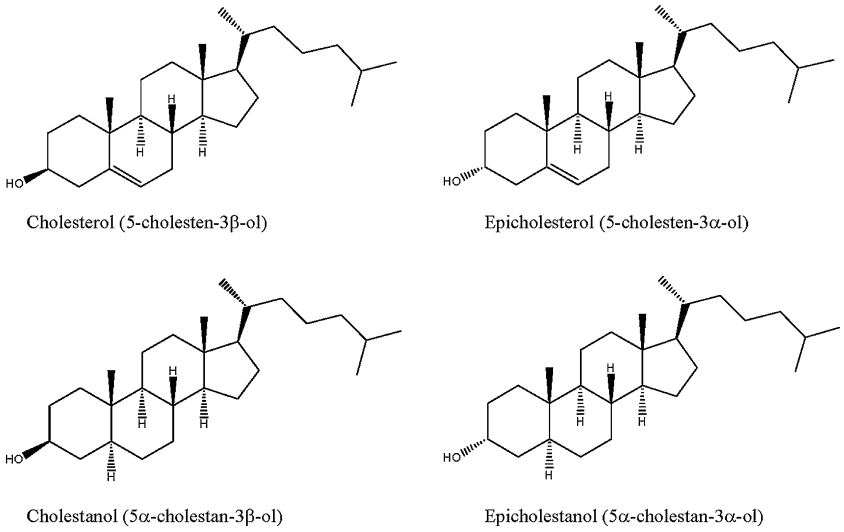

Fig. 1. 2-D structures of the sterols used in this study.

rigid (Barton, 1950, 1970). Typically, one or more double bonds are present in the ring structure, usually in rings A and B and less often in rings C or D. There is also an alkyl side-chain at C17 on the cyclopentane ring, whose structure varies in the number of double bonds and alkyl branches, and their position on the chain. The small polar hydroxyl group and the large hydrophobic ring and side-chain anchor most membrane sterols in the bilayer with the sterol long axis lying parallel to the lipid acyl chains and the sterol hydroxyl group sitting in the phospholipid bilayer interface.

Sterols such as cholestanol (5α-H,3β-ol) and epicholestanol (5α-H,3α-ol) are bacterial reduction products of Chol and epicholesterol (Echol) and have no double bond at Δ^5. The saturated sterols have the same alkyl side-chain configuration as Chol and Echol and have an all-*trans* configuration with a planar ring system conformation (Barton, 1950, 1970). In comparison, ring B in Chol and Echol is slightly deformed by the presence of a double bond at Δ^5, but the overall ring conformation is only slightly changed. Both saturated sterols are found in the soil and in marine sediments (McNamara et al., 1981; Jeng and Han, 1996). The 5α-H,3β-ol is also found in large amounts in all tissues in a sterol storage disorder caused by disruption of bile acid synthesis in humans known as cerebrotendinous xanthomatosis (Federico and Dotti, 1996). A deficiency of the enzyme sterol 27-hydroxylase causes the deposition of cholestanol plaques in the central nervous system, as well as in tendons, the skin, lungs and bones, leading to a wide range of disabling conditions.

1.1. The effect of sterols on the hydrocarbon chain-melting phase transition

There have been many investigations of the effects of Chol on the gel/liquid-crystalline phase transition utilizing model phospholipid bilayers with a range of headgroup structures, containing saturated or unsaturated acyl chains (Nyholm et al., 2003; Holland and Alam, 2008; McMullen et al., 1993, 1994, 2009; Mannock et al., 2003, 2006, 2008, 2010a,b). These studies show that the strength of interactions between Chol and phospholipids varies with the chemical structure of the lipid headgroup and interface. Conversely, the extent and nature of interactions between dipalmitoylphosphatidylcholine (DPPC) and sterols differing in their chemical structure varies with the orientation of the C3–OH group, the number of ring methyl groups, and the alkyl side-chain structure. Not all phospholipids interact strongly with Chol, yet Chol may interact preferentially with lipids such as sphingomyelin (SpM; Mannock et al., 2003; Nyholm et al., 2003; Ramstedt and Slotte, 2006; Holland and Alam, 2008). Thus, changing the sterol structure may raise or lower the overall bilayer chain-melting phase transition temperature (T_m, °C), increase the temperature range of the transition measured at half height ($\Delta T_{½}$, °C), and lower the transition enthalpy (ΔH_m, kcal/mol). Such changes condense and order the host membrane lipids above T_m, creating one or more liquid-ordered (l_o) domains that differ in their bulk physical properties relative to those of the sterol-free bilayer. The extent to which each of these parameters changes is dependent on the sterol concentration and the lipid–sterol chemical composition. However, there are fewer studies of the thermotropic phase behaviour of lipid–sterol bilayers in which the sterol chemical configuration has been systematically varied. This situation arises because of the limited number of commercially available natural sterols and the absence of a substantial pool of sterol structural motifs among those products. Thus, it is difficult to understand the roles played by variations in the functional group number, position and orientation in the tetracyclic ring system and the alkyl side-chain (Baker and Hudec, 1967) in determining the gel/liquid-crystalline phase transition and on the sterols miscibility in the sterol-rich l_o phases seen at higher temperatures.

1.2. The implications of changes in sterol structure in biological membranes

While most current models of lipid–sterol interactions focus on the steric bulk of each bilayer component, steric and electronic contributions to molecular conformation arising from each substructure must also contribute to the bilayer physical properties. This assortment of electronic effects determines the molecular polarization properties, which contribute towards the membrane dipole surface potential (Israelachvili, 1992). Thus, these molecular contributions become part of the properties of the lipid bilayer mixture. The effects of many of these physico-chemical contributions are apparent from thermodynamic and structural measurements of tightly packed gel or lamellar-crystalline lipid bilayers, but are less evident in the "motionally averaged" L_α phase. Nevertheless, those interactions still exert an influence on membrane physical properties, determining acyl chain order, mean area per molecule, sterol lateral diffusion, bilayer permeability, polarity, elasticity, curvature and thus the overall lipid phase morphology. This suite of parameters represents the underlying mechanism regulating biological membrane function in both healthy and diseased cells.