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A calorimetric and spectroscopic comparison of the effects of cholesterol and its immediate biosynthetic precursors 7-dehydrocholesterol and desmosterol on the thermotropic phase behavior and organization of dipalmitoylphosphatidylcholine bilayer membranes

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ABSTRACT

We performed differential scanning calorimetric (DSC) and Fourier transform infrared (FTIR) spectroscopic studies of the effects of cholesterol (CHOL), 7-dehydrocholeterol (7DHC) and desmosterol (DES) on the thermotropic phase behavior and organization of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes. 7DHC and DES are the immediate biosynthetic precursors of CHOL in the Kandutch–Russell and Bloch pathways and 7DHC and DES differ in structure from CHOL only by the presence of an additional double bond at C7 of ring B or C24 of the alkyl side chain, respectively. Our DSC results indicate that the incorporation of all three sterols produces comparable decreases in the temperature of the pretransition of DPPC, but CHOL decreases its cooperativity and enthalpy more strongly than 7DHC and especially DES. These findings indicate that all three sterols decrease the thermal stability of gel phase DPPC bilayers but that 7DHC and especially DES are less miscible in them. However, the incorporation of CHOL and DES produce comparable increases in the temperature of the broad component of the main phase transition of DPPC while 7DHC decreases it, but again CHOL produces greater decreases in its cooperativity and enthalpy then 7DHC and especially DES. These results indicate that CHOL and DES stabilize the sterol-rich domains of fluid DPPC bilayers, but that 7DHC and especially DES are less miscible in them. Our FTIR spectroscopic results indicate that CHOL increases the rotational conformational order of fluid DPPC bilayers to a somewhat and markedly greater degree than DES and 7DHC, respectively, consistent with our DSC findings. Our spectroscopic results also indicate that although all three sterols produce comparable degrees of H-bonding (hydration) of the DPPC ester carbonyl groups in fluid bilayers, CHOL is again found to be fully soluble in gel state DPPC bilayers at low temperatures, whereas 7DHC and especially DES are not. In general, we find that 7DHC and DES incorporation produce considerably different effects on DPPC bilayer membranes. In particular, the presence of an additional double bond at C7 or C24 produces a marked reduction in the ability of 7DHC to order fluid DPPC bilayers and in the miscibility of DES in such bilayers, respectively. These different effects may be the biophysical basis for the reduction of these double bonds in the last steps of CHOL biosynthesis, and for the deleterious biological effects of the accumulation of these sterols in vivo.

Abbreviations: CHOL, cholesterol (5-cholestene-3β-ol); 7DHC, 7-dehydrocholesterol (5,7-cholestadiene-3β-ol); DES, desmosterol (5,24-cholestadiene-3β-ol); LATH, lathosterol (7-cholestene-3β-ol); aCHOL, allocholesterol (4-cholestene-3β-ol); PC, phosphatidylcholine; DPPC, dipalmitoyophosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; SpM, sphingomyelin; ESR, electron spin resonance; NMR, nuclear magnetic resonance; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared; T_p, the pretransition temperature maximum; T_m, the main transition temperature maximum; ΔH, the transition enthalpy; $\Delta T_{1/2}$, the width of the phase transition at half height, inversely related to the cooperativity of the phase transition (the superscripts "shp" and "brd" appended to these thermodynamic parameters refer to the sharp and broad components of the main phase transition of sterol-containing DPPC bilayers, respectively); $L_{\beta'}$ and L_β, lamellar gel phases with tilted and untilted hydrocarbon chains, respectively; $P_{\beta'}$, rippled gel phase with tilted hydrocarbon chains; L_α, lamellar liquid-crystalline phase; L_o, lamellar liquid-ordered phase.

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1. Introduction

Cholesterol (CHOL) is an abundant and essential lipid component particularly of the plasma membranes of the cells of higher animals (Finegold, 1993; Nes and McKean, 1977; Yeagle, 1988) and is known to have many effects on the thermotropic phase behavior and organization of lipid bilayers in both model and biological membranes (Demel and De Kruyff, 1976; Finegold, 1993; McMullen and McElhaney, 1996; Nes and McKean, 1977; Vist and Davis, 1990; Yeagle, 1988). These effects include a broadening and eventual elimination of the cooperative gel/liquid-crystalline phase transition and the concomitant progressive replacement of the L_β and L_α phases by a state with an intermediate degree of organization (the L_o phase). This L_o phase is characterized by a higher phospholipid hydrocarbon chain ordering, a restricted rate of lateral diffusion, and a reduced area per molecule compared to the liquid-crystalline state which would exist at physiological temperatures in the absence of CHOL. As well, the presence of CHOL increases the thickness and mechanical strength of the phospholipid bilayer and reduces its permeability. In addition, the simultaneous presence of the phospholipid-rich L_α and sphingolipid- and CHOL-rich L_o phases in lipid bilayers formed in certain unsaturated phospholipid/sphingomyelin (SpM)/CHOL ternary lipid mixtures has prompted some investigators to postulate the existence of specialized detergent-insoluble "lipid rafts" in animal cell membranes (Brown and London, 2000; Silvius, 2003; Simons and Ikonen, 2000), although this hypothesis remains controversial (Edidin, 2003; McMullen et al., 2004; Munro, 2003). Nevertheless, there is a great deal of evidence that the presence of CHOL does modulate a number of different membrane functions, either directly or through its general effects on the structure, physical properties and possibly on the lateral organization of phospholipid bilayers, in both model and biological membranes (Dahl and Dahl, 1988; McElhaney, 1992a,b; Yeagle, 1988).

CHOL is the only sterol found in significant amounts in the cell membranes of higher animals and late metabolic intermediates in the lengthy and energetically expensive processes of CHOL biosynthesis do not normally accumulate in the membranes of healthy cells (Nes and McKean, 1977; Porter and Herman, 2011; Yeagle, 1988). Specifically, there are two major metabolic pathways for the biosynthesis of CHOL from lanosterol, the first cyclic intermediate and product of the first committed step in sterol biosynthesis. In the Kandutch–Russell pathway (Kandutsch and Russell, 1960), the reduction of the *trans* double at C24 of the alkyl side chain occurs in the first step of biosynthesis and the final intermediate in this pathway is 7-dehydrocholesterol (7DHC), which differs in chemical structure from CHOL only in the presence of an additional double bond at C7 of ring B (Fig. 1). In contrast, in the Bloch pathway (Frantz and Schroepfer, 1967), the reduction of the C24 double bond occurs in the last step of CHOL biosynthesis and the final intermediate is DES, which differs in structure from CHOL only by the presence of a double bond at C24 (Fig. 1). A deficiency in the enzyme 7-dehydrocholesterol reductase in the Kandutch–Russell pathway, or of 24-dehydrocholesterol reductase in the Bloch pathway, results in the accumulation of 7DHC and DES, respectively, and a marked reduction in CHOL levels. Both CHOL metabolic defects result in serious and even fatal congenital malformation diseases, termed the Smith–Lemli–Opitz and desmosterolosis syndromes, respectively (Porter and Herman, 2011). Since CHOL, in addition to its biophysical role in biological membranes, has multiple biological functions, including as a biosynthetic precursor of steroid hormones, neuroactive steroids and oxysterols, it is not surprising that CHOL homeostasis is critical for normal growth and development. However, it is not yet clear whether the primary mechanism triggering the development of these and similar malformation syndromes arising from defects in CHOL biosynthesis are caused by alterations in the membrane lipid bilayer structure and physical properties induced by the accumulation of these late CHOL biosynthetic intermediates, or by alterations in sterol biochemical and cell signaling processes. However, as discussed below, there is evidence that at least some of the physical effects of 7DHC and DES on model lipid bilayers membranes differ somewhat from those of CHOL.

A considerable number of biophysical studies of the comparative effects of 7DHC and CHOL on the structure and organization of phospholipid monolayer and bilayer model membranes have been

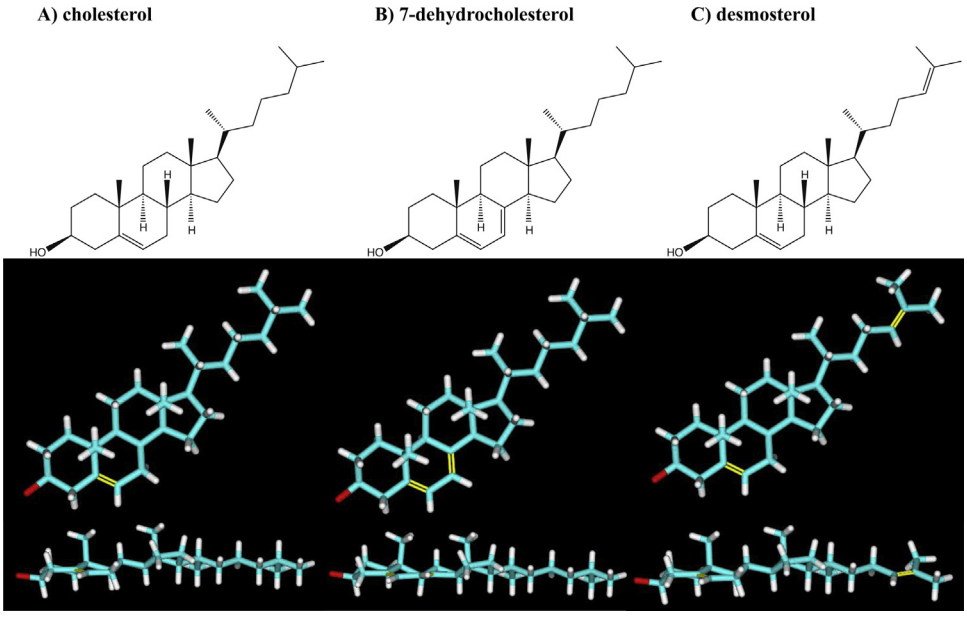

Fig. 1. Molecular models for sterols used in this study. The top figure in each panel shows views normal to the plane of the sterol ring to highlight differences between the structures of cholesterol (A), 7-dehydrocholesterol (B) and desmosterol (C). The middle row shows 3D views normal to the plane of the ring and the bottom view shows 3D views parallel to the plane of the sterol ring. The functional group at C3 is red and double bonds are yellow. The molecules were minimized using Accelyrs Discovery Study 3.5 (Accelyrs, Inc., San Diego, CA). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)