Chemistry and Physics of Lipids 195 (2016) 21–33

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

A comparative differential scanning calorimetry study of the effects of cholesterol and various oxysterols on the thermotropic phase behavior of dipalmitoylphosphatidylcholine bilayer membranes

Matthew G.K. Benesch, Ronald N. McElhaney*

Department of Biochemistry, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2H7, Canada

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form 12 November 2015
Accepted 14 November 2015
Available online 24 November 2015

Keywords:
Cholesterol
Oxysterols
Dipalmitoylphosphatidylcholine
Sterol/phospholipid interactions
Lipid bilayer membranes
Thermotropic phase behavior

ABSTRACT

We have carried out a comparative differential scanning calorimetric (DSC) study of the effects of cholesterol (C) and the eight most physiologically relevant oxysterols on the thermotropic phase behavior of dipalmitoylphosphatidylcholine (DPPC) bilayer membranes. The structures of these oxysterols differ from that of C by the presence of additional hydroxyl, keto or epoxy groups on the steroid ring system or by the presence of a hydroxyl group in the alkyl side chain. In general, the progressive incorporation of these oxysterols reduces the temperature, cooperativity and enthalpy of the pretransition of DPPC to a greater extent than C, indicating that their presence thermally destabilizes and disorders the gel states of DPPC bilayers to a greater extent than C. Similarly, the incorporation of these oxysterols either increases the temperature of the broad component of the main phase transition to a smaller extent than C or actually decreases it. Again, this indicates that the presence of these compounds is less effective at thermally stabilizing and ordering the sterol-rich domains of DPPC bilayers than is C itself. Moreover, the incorporation of these oxysterols decrease the cooperativity and enthalpy of the main phase transition of DPPC to a smaller extent than C, indicating that they are somewhat less miscible in fluid DPPC bilayers than is C. Particularly notable in this regard is 25-hydroxycholesterol, which exhibits a markedly reduced miscibility in both gel and fluid DPPC bilayers compared to C itself. In general, the effectiveness of these oxysterols in stabilizing and ordering DPPC bilayers decreases as their rate of interbilayer exchange and the polarity of the oxysterol increases. We close by providing a tentative molecular explanation for the results of our DSC studies and of those of previous biophysical studies of the effects of various oxysterol on lipid bilayer model membranes.

1. Introduction

Cholesterol (C) is an abundant and essential lipid component particularly in 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 C. As well, the presence of C increases the thickness and

Abbreviations: C, cholesterol (5-cholestene-3β-ol); 4β-HC, 4β-hydroxycholesterol (5-cholesten-3β,4β-diol); 7α-HC, 7α-hydroxycholesterol (5-cholesten-3β,7α-diol); 7β-HC, 7β-hydroxycholesterol (5-cholesten-3β,7β-diol); 25-HC, 25-hydroxycholesterol (5-cholesten-3β,25-diol); 5α,6β-HC, 5α,6β-hydoxycholesterol (cholestan-3β,5α,6β-triol); 7-KC, 7-ketocholesterol (5-cholesten-3β-ol-7-one); 5α,6α-EC, 5α,6α-epoxycholesterol (cholestan-5α,6α-epoxy-3β-ol); 5β,6β-HC, 5β,6β-epoxycholesterol (cholestan-5β,6β-epoxy-3β-ol); PC, phosphatidylcholine; DPPC, dipalmitoyophosphatidylcholine; DSC, differential scanning calorimetry; 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.

* Corresponding author. Fax: +780 492 0886.
E-mail addresses: benesch@ualberta.ca (M.G.K. Benesch), rmcelhan@ualberta.ca (R.N. McElhaney).

http://dx.doi.org/10.1016/j.chemphyslip.2015.11.003
0009-3084/

mechanical strength of the phospholipid bilayer and reduces its permeability. In addition, the simultaneous presence of the phospholipid-rich L_α and sphingolipid- and C-rich L_o phases in lipid bilayers formed in certain unsaturated phospholipid/sphingomyelin/C 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 C 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).

The oxysterols are a heterogeneous group of C-derived compounds with one or sometimes more additional oxygen substitutions, most commonly hydroxyl, carbonyl or epoxy groups, either on the steroid ring or the alkyl side chain (Schroepfer, 2000) (Fig. 1 and Supplemental Fig. 1). The oxysterols can be formed by the autoxidation of C or by a number of enzyme-catalyzed reactions, which typically take place in microsomes or mitochondria and usually involve cytochrome P-450. Although normally present in cells and plasma at low levels, various oxysterols have been shown to have a diverse array of important regulatory functions, most prominently in the control of C biosynthesis, but also in sphingolipid metabolism, platelet aggregation, and apoptosis, among many other roles. Although many oxysterols have been shown to exert their major biological effects through binding to various specific protein receptors, some evidence has been presented that certain of these compounds may act at least in part by inserting into and modifying the physical properties of the lipid bilayers of biological membranes (Olkkonen and Hynynen, 2009; Olsen et al., 2012).

One common property of oxysterols is that the additional oxygen-containing groups present in these molecules makes them considerably more hydrophilic than C itself. Moreover, the presence of additional oxygenated functions can alter the 3-dimensional conformation of the oxysterol and particularly the distribution of polarity over the molecular surface, and thus its packing in phospholipid bilayers. For these reasons, oxysterols such as 7β-HC, 7-KC and 25-HC transfer between lipid bilayer model membranes at rate orders of magnitude faster than C itself

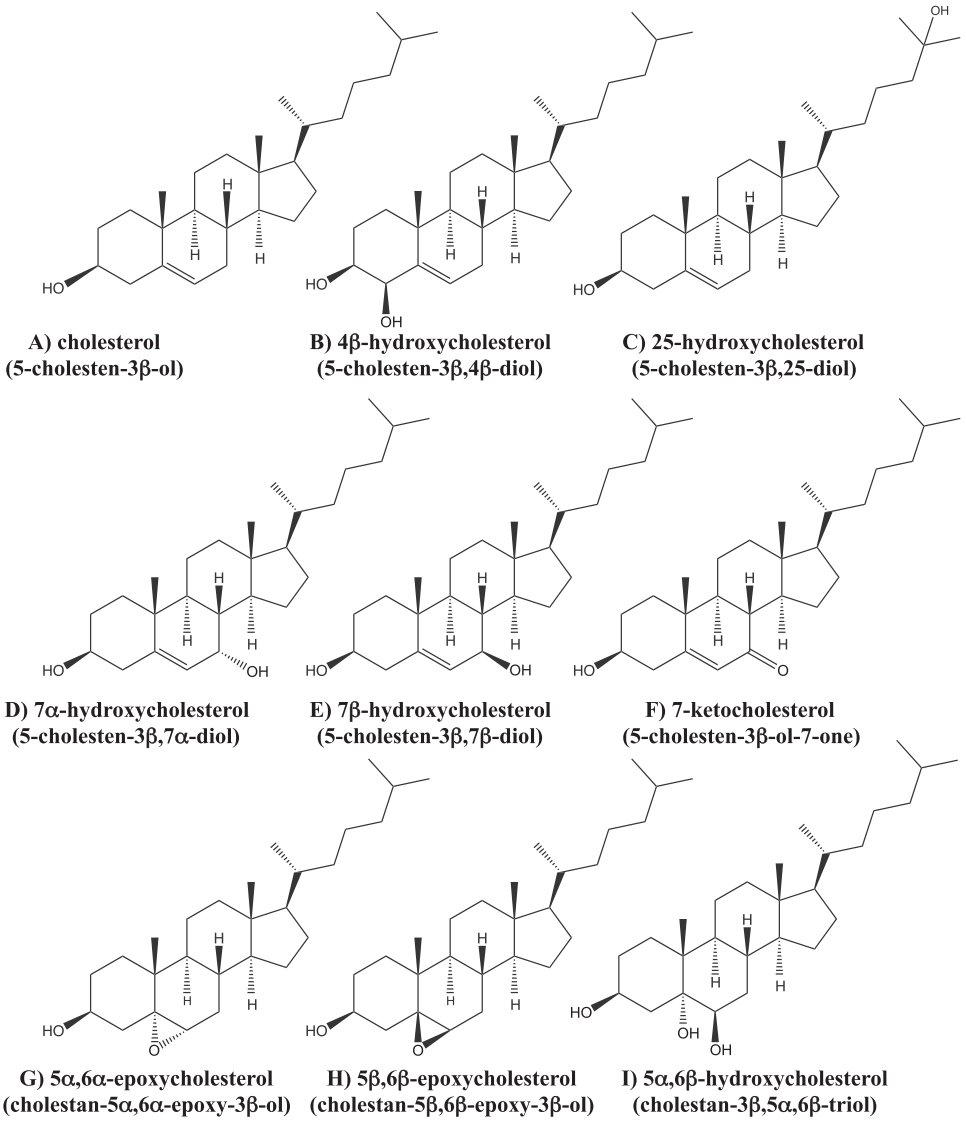

Fig. 1. Structure of cholesterol and oxysterols used in this study. Refer to Supplemental Fig. 1 for 3D views of structures normal and parallel to the plane of the sterol ring.