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Original Contribution

Cholesterol under oxidative stress—How lipid membranes sense oxidation as cholesterol is being replaced by oxysterols

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ABSTRACT

The behavior of oxysterols in phospholipid membranes and their effects on membrane properties were investigated by means of dynamic light scattering, fluorescence spectroscopy, NMR, and extensive atomistic simulations. Two families of oxysterols were scrutinized—tail-oxidized sterols, which are mostly produced by enzymatic processes, and ring-oxidized sterols, formed mostly via reactions with free radicals. The former family of sterols was found to behave similar to cholesterol in terms of molecular orientation, roughly parallel to the bilayer normal, leading to increasing membrane stiffness and suppression of its membrane permeability. In contrast, ring-oxidized sterols behave quantitatively differently from cholesterol. They acquire tilted orientations and therefore disrupt the bilayer structure with potential implications for signaling and other biochemical processes in the membranes.

Introduction

Cellular membranes consist of a bilayer composed primarily of phospholipids and sterols, in which a large amount of proteins and other molecules is embedded. Sterols are known to affect, e.g., the conformational order of the lipid acyl chains [1,2], membrane permeability [2], hydrophobic thickness [1], and lateral organization of the bilayers [1,3–5]. In contrast to the vast abundance of phospholipid moieties, most biological membranes contain only one major sterol type. Cholesterol is the most prevalent and essential sterol in mammalian cellular membranes and is required for diverse cellular functions, including binding to sterol-sensing domains to regulate protein function [6,7], participating in the formation of lipid rafts [8,9], and serving as a precursor for bile acid and steroid hormone synthesis [10]. Because the presence of cholesterol affects so many important functions in human cells, it is not surprising that

Abbreviations: LXR, liver X receptors; HDL, high-density lipoproteins; MD, molecular dynamics; NMR, nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPC-d31, POPC deuterated at the *sn*-1 chain; chol, cholesterol; 7α-OH-chol, 7α-hydroxycholesterol; 7β-OH-chol, 7β-hydroxycholesterol; 7-keto-chol, 7-ketocholesterol; 3β5α6β-3OH-chol, 3β,5α,6β-trihydroxycholestane; 25-OH-chol, 25-hydroxycholesterol; 27-OH-chol, 27-hydroxycholesterol; 24-OH-chol, 24-hydroxycholesterol; 24S-OH-chol, 24*S*-hydroxycholesterol; Hepes, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; POPOP, 2,2′-(1,4-phenylene)bis[5-phenyl-oxazole]; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-lauroyl-2-dimethylaminonaphthalene; LUVs, large unilamellar vesicles; DLS, dynamic light scattering; UV, ultraviolet; SS, steady-state; TCSPC, time correlated single photon counting; GP, generalized polarization; TDFS, time-dependent fluorescence shift; τ_r, integrated relaxation time; $\Delta\nu$, total spectral shift; TRES, time-resolved emission spectra; OPLS, optimized parameters for liquid simulation; NPT, isobaric–isothermic ensemble; PME, particle mesh Ewald scheme; APM, area per molecule; $|S_{CD}|$, deuterium order parameters; Aβ, amyloid beta peptide; 2D, two-dimensional; *FWHM*(*t*), full-width at half-maximum

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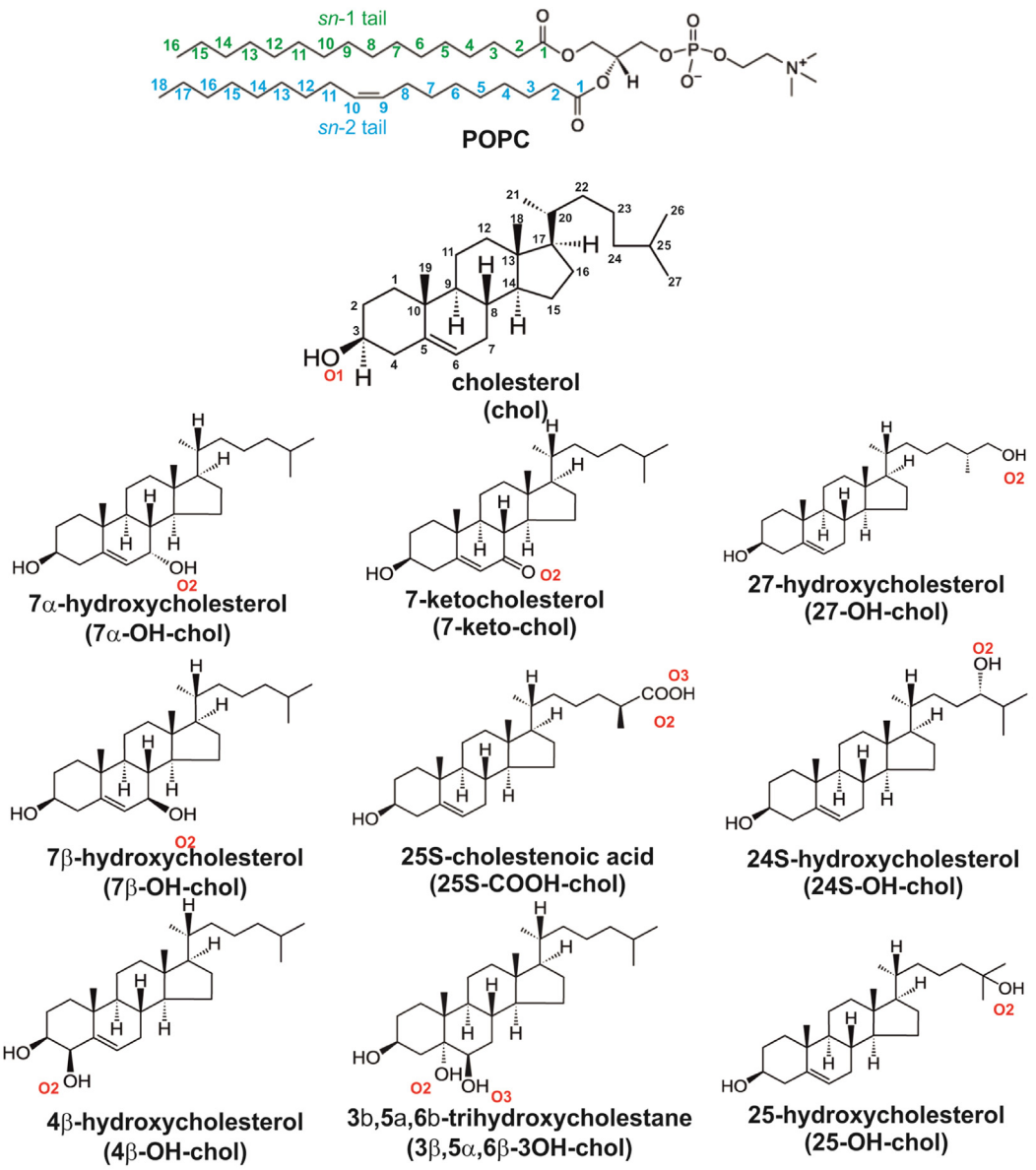

Fig. 1. Chemical structures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), cholesterol, 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 4β-hydroxycholesterol, 3β,5α,6β-trihydroxycholestane, 27-hydroxycholesterol, 24*S*-hydroxycholesterol, 25-hydroxycholesterol, and 25*S*-cholestenoic acid. Abbreviations used in this study are presented in parentheses.

even a small change in chemical structure of cholesterol may significantly alter membrane functions and dynamics [11–13].

Oxysterols (see Fig. 1) are oxidized derivatives of cholesterol with one or more additional oxygen-containing functional groups (hydroxyl, carbonyl, carboxyl, or epoxy). The most abundant oxysterols in human body (27-, 24(*S*)-, 7α-, 4β-hydroxycholesterol) are generated in cells catalyzed by mitochondrial or endoplasmic reticulum cholesterol hydroxylases (part of the cytochrome P450). Oxysterols may also arise through nonenzymatic, free radical, and lipid peroxide oxidative processes, usually referred to as cholesterol autoxidation processes. The most common oxysterols produced during cholesterol autoxidation are 7β-hydroxycholesterol and 7-ketocholesterol. Oxysterols are present in healthy human and animal tissues at low concentrations (as compared to cholesterol), but are found to be profoundly enriched in pathologic conditions such as macrophage foam cells, atherosclerotic lesions, and cataracts [14–17].

Oxysterols play a crucial role in many regulatory processes in the human body. Oxysterols act as intermediates in cholesterol catabolism and especially in bile acid synthesis. Therefore, they are involved in the elimination of excess cholesterol from the body [18,19]. They regulate lipid metabolism as ligands of liver X receptors (LXR) [20,21], key receptors in hepatic lipogenesis, synthesis of nascent high-density lipoproteins (HDL), and biliary neutral sterol secretion [22,23]. Oxysterols also take part in pro-inflammatory signaling [24,25] and modulation of the estrogen receptor function [26–28]. Some oxysterols (e.g., 7-ketocholesterol) show cytotoxic and pro-apoptotic properties [29–31]. Pathogenic effects of oxysterols have also been described in cardiovascular diseases [16,32], diabetes [33] type 2, and degenerative disorders such as the Alzheimer disease [34–36], osteoporosis [37], and age-related macular degeneration [38,39]. There are also reports describing the role of oxysterols in neurological diseases [25,40]. Oxysterols might play a pathological role in the Smith-Lemli-Opitz syndrome [41].

The role of oxysterols in the above-noted natural and pathological situations has been confirmed, but the molecular mechanisms governing oxysterols' behavior in the human body are still