Membrane and protein interactions of oxysterols

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Purpose of review

Oxysterols, oxidation products of cholesterol, mediate numerous and diverse biological processes. The objective of this review is to explain some of the biochemical and cell biological properties of oxysterols based on their membrane biophysical properties and their interaction with integral and peripheral membrane proteins.

Recent findings

According to their biophysical properties, which can be distinct from those of cholesterol, oxysterols can promote or inhibit the formation of membrane microdomains or lipid rafts. Oxysterols that inhibit raft formation are cytotoxic. The stereospecific binding of cholesterol to sterol-sensing domains in cholesterol homeostatic pathways is not duplicated by oxysterols, and some oxysterols are poor substrates for the pathways that detoxify cells of excess cholesterol. The cytotoxic roles of oxysterols are, at least partly, due to a direct physical effect on membranes involved in cholesterol-induced cell apoptosis and raft mediated cell signaling. Oxysterols regulate cellular functions by binding to oxysterol binding protein and oxysterol binding protein-related proteins. Oxysterol binding protein is a sterol-dependent scaffolding protein that regulates the extracellular signal-regulated kinase signaling pathway. According to a recently solved structure for a yeast oxysterol binding protein-related protein, Osh4, some members of this large family of proteins are likely sterol transporters.

Summary

Given the association of some oxysterols with atherosclerosis, it is important to identify the mechanisms by which their association with cell membranes and intracellular proteins controls membrane structure and properties and intracellular signaling and metabolism. Studies on oxysterol binding protein and oxysterol binding protein-related proteins should lead to new understandings about sterol-regulated signal transduction and membrane trafficking pathways in cells.

Keywords

7-ketocholesterol, liquid ordered phase, oxysterol, oxysterol binding protein, oxysterol binding protein-related proteins

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Abbreviations

ACAT1	acyl-coenzyme A : cholesterol acyltransferase 1
l_o	liquid ordered phase
ER	endoplasmic reticulum
ERK1/2	extracellular signal-regulated kinase 1/2
ORD	oxysterol binding protein-related domain
ORP	oxysterol binding protein-related protein
OSBP	oxysterol binding protein
SCAP	sterol regulatory element-binding protein cleavage-activating protein
SREBP	sterol regulatory element-binding protein

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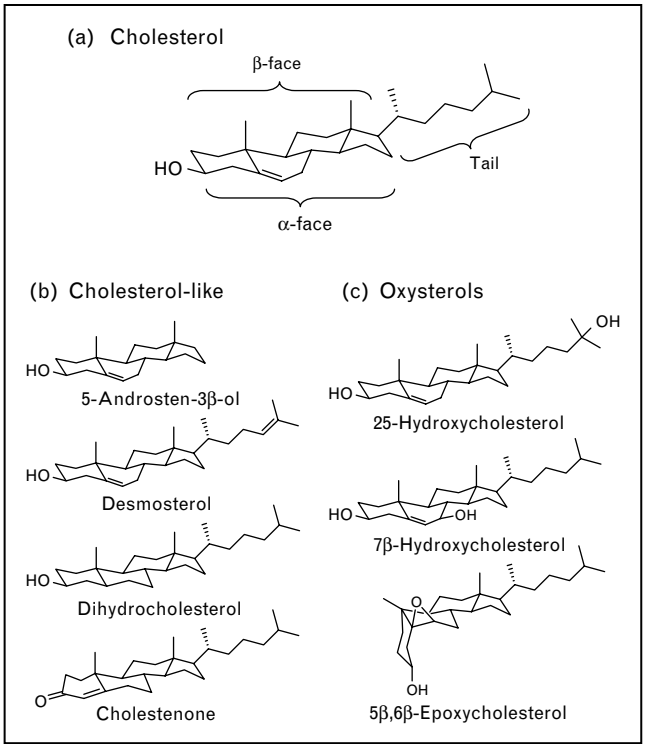
Introduction

Oxysterols are derivatives of cholesterol that contain a second oxygen atom as a carbonyl, hydroxyl, or epoxide group. Enzymatically formed oxysterols are intermediates in the biosynthesis of bile acids and steroid hormones, and are also signaling lipids that regulate cholesterol biosynthesis, cellular uptake and efflux, and intracellular trafficking [1–3]. Cytotoxic oxysterols formed by non-specific oxidative mechanisms can affect many cellular processes that contribute to the pathogenesis of disease. Cholesterol has a hydrophobic and planar fused tetracyclic ring structure that forms a flat α-face, a bulky β-face with a β-oriented hydroxyl group at position 3, and a branched iso-octyl side chain at position 17 (Fig. 1). Enzymatic oxidation of cholesterol occurs at various positions along the iso-octyl side chain and at the 7α-position of the sterol nucleus. Non-enzymatic oxidations occur primarily at the 7-position on the sterol nucleus and at the 5, 6 double bond. The molecular functions of different oxysterols can be revealed in part by comparative structure-function studies of a series of oxysterols. Cholesterol and its structural analogues have been compared in many studies of membrane function. 7-Ketocholesterol, 7β-hydroxycholesterol, and 5β,6β-epoxycholesterol are formed non-enzymatically, and are the major cytotoxic sterols associated with pathological states [4,5]. 25-Hydroxycholesterol, the most widely studied oxysterol, has many biological activities. The objective of this review is to rationalize the biochemistry and cell biology of oxysterols on the basis of their biophysical properties and interactions with integral and peripheral membrane proteins.

Membrane biophysical properties of sterols

Liquid disordered (l_d) and liquid ordered phases (l_o) are fluid membrane phases thought to coexist in cells. According to studies in model membranes, l_d and l_o phases can coexist in the same bilayer and membrane

Figure 1 Structure of cholesterol, cholesterol-like sterols, and oxysterols



(a) Cholesterol has a planar fused tetracyclic ring structure that forms a flat α-face, a bulky β-face with a β-oriented hydroxyl group at position 3, and a branched iso-octyl side chain at position 17 ('cholesterol-like tail'). (b) Cholesterol-like sterols have changes in the length and chemical nature of the side chain (5-androsten-3β-ol and desmosterol), in the number and position of double bonds in the tetracylic ring (dihydrocholesterol), and in the stereochemistry and chemical nature of the group at the 3-position (cholestenone). (c) Oxysterols contain a second oxygen atom as a carbonyl, hydroxyl, or epoxide group. Oxidation in the cholesterol side chain (25-hydroxycholesterol) occurs by enzymatic mechanisms and can generate oxysterols that are regulators of sterol metabolism. Oxidation in the tetracyclic ring structure can occur by both enzymatic (7α-hydroxycholesterol) and non-enzymatic (7β-hydroxycholesterol and 5β,6β-epoxycholesterol) mechanisms. Non-enzymatically formed oxysterols are cytotoxic to cells. The second oxygen moiety changes the three-dimensional shape of the molecule, the polarity, and the orientation of the molecule in a membrane.

proteins and lipids partition between the two phases on the basis of their respective affinities for lipid order [6,7••]. The formation of l_o microdomains, also termed lipid rafts, is driven by tight packing between cholesterol and sphingomyelin or phosphatidylcholines with saturated acyl chains. Sterols can be classified as promoters of the formation or stability of ordered lipid domains and those that are inhibitors [8–10]. Promoter sterols have the following characteristics: (1) flat, fused rings; (2) a hydroxyl or other small polar group at position 3; (3) a 'cholesterol-like' tail; and (4) a small area per molecule [9]. Dihydrocholesterol promotes domain formation as well as cholesterol, whereas desmosterol is less effective, and 5-androsten-3β-ol and cholestenone are inhibitors. 25-Hydroxycholesterol is a promoter; 7-ketocholesterol can be a promoter or inhibitor depending on the phospholipid composition, and 7β-hydroxycholesterol is an inhibitor [8,9,11]. The location and chemical nature of the oxygen substitutions determines whether a given oxysterol would be a lipid-domain forming or disrupting sterol. Insertion of a polar oxygen moiety into the hydrophobic portion of the bilayer is thermodynamically unfavorable [12]; in a membrane bilayer, oxysterols can be tilted with respect to the orientation of cholesterol, so that both of its oxygen moieties are solvated at the lipid–water interfacial region [11,13]. For 7β-hydroxycholesterol, both the 7β-hydroxyl and 3β-hydroxyl groups can be solvated, thereby tilting the sterol nucleus with respect to the plane of the bilayer so that it does not penetrate as far into the bilayer as cholesterol and does not form ordered bilayers. Similarly, the tilt angle of cholesterol is smaller than that of desmosterol which is less effective in forming a l_o phase [14]. Due to poor membrane packing and their intrinsic higher polarity, 7β-hydroxycholesterol, 7-ketocholesterol, 25-hydroxycholesterol, and cholesterol-derived hydroperoxides spontaneously transfer between biological membranes at rates that are orders of magnitude faster than cholesterol [15]. Due to its affinity for sphingolipid-enriched microdomains, the majority of free cholesterol in cells ($\sim$65–80%), is in the plasma membrane [16,17]. Sterols that do not form l_o phases would not be located within rafts and, for oxysterols, could rapidly transfer to cellular membrane compartments where low sterol levels are normally maintained.

Whereas cholesterol and some oxysterols dramatically enhance the rate of apolipoprotein A-I (apoA-I) microsolubilization of phospholipid membranes, yielding discoidal HDL particles, other oxysterols inhibit this process [11,18]. The probable basis for the differences among sterols is their differential ability to induce the formation of l_o phases. Although the rates of microsolubilization were a function of sterol chemical structure, the structures of the resulting discoidal particles were a function of sterol concentration.

Methods to study the roles of cholesterol and oxysterols in cells

The role of cholesterol-rich domains in signal transduction pathways are often studied by cellular depletion of cholesterol [6,7••]. This can be achieved by cyclodextrins, which physically remove cholesterol, statins, which lower cholesterol metabolically, polyene antibiotics, which sequester cholesterol, and cholesterol oxidase, which converts cholesterol to a raft-destabilizing sterol, cholestenone. The limitations of these methods are that levels of cholesterol depletion are often non-physiological, and nonspecific effects can be caused by severe cholesterol depletion. An alternative methodology is to first deplete the cell of cholesterol with cyclodextrins and then replete the cell with a different sterol using

sterol-cyclodextrin complexes [6]. Substitution should reduce alterations of cellular behavior that arise from a decrease in bulk membrane volume upon cholesterol depletion. Substitution experiments with a series of sterols having a range of raft-promoting and raft-inhibiting tendencies could be used to reveal the involvement of rafts in biological processes [6,8].

The activities of receptor tyrosine kinases such as the epidermal growth factor (EGF) receptor, which can be located within lipid rafts, and the insulin receptor, which is found in caveolae, can be modulated by changes in cellular cholesterol content [19]. Different signaling pathways initiated by EGF can be either activated or inhibited upon cholesterol removal, and normal activity is restored upon repletion with cholesterol [20]. EGF stimulation of phosphatidylinositol 4,5-bisphosphate turnover is inhibited by cholesterol depletion, but the effects of repletion with various oxysterols varied according to their structure. Turnover was not restored by 25-hydroxycholesterol, and restoration of turnover by 7-ketocholesterol and 5α,6α-epoxycholesterol were respectively less and more than produced by cholesterol [21]. Similarly, desmosterol, which does not form lipid rafts as well as cholesterol, impaired raft-dependent signaling via the insulin receptor, while non-raft dependent protein secretion was not affected [14]. These studies define an important role for cholesterol in the formation of microdomains involved in signal transduction and show how small changes in sterol structure can alter cell function.

Sterol specificity of proteins with a sterol-sensing domain

A key event in cholesterol homeostasis is the sterol-induced binding of SCAP (sterol regulatory element-binding protein cleavage-activating protein) to the resident endoplasmic reticulum (ER) proteins Insigs to control the transport and proteolytic activation of sterol regulatory element-binding proteins (SREBPs) [22–24]. SCAP is regulated by free cholesterol through a sterol-sensing domain which consists of approximately 180 amino acids that form five predicted membrane-spanning helices with short intervening loops. Although cholesterol, desmosterol, dihydrocholesterol, and 5-androsten-3β-ol bind to SCAP, sterols containing a keto, chloride, or α-hydroxyl group at the 3-position, and hydroxycholesterols with the hydroxyl group at positions 7α, 7β, 19, 25, and 27, do not bind to SCAP. 7-Ketocholesterol is the only oxysterol that binds to SCAP. 5-Androsten-3β-ol can bind to SCAP, but it cannot induce l_o raft formation. Sterol binding to SCAP is highly stereospecific for cholesterol and very similar sterols. When it is added to intact cells, 25-hydroxycholesterol, which does not directly bind to SCAP, enhances SCAP binding to Insig. Oxysterol binding protein (OSBP) was identified as a cytosolic 25-hydroxycholesterol binding protein that was postulated to regulate cholesterol biosynthesis. OSBP is not however involved in the regulation of cholesterol metabolism or SREBP processing [24]. 25-Hydroxycholesterol is thought to act indirectly through another ER protein that has not been characterized.

Acyl-coenzyme A : cholesterol acyltransferase 1 (ACAT1), which catalyzed the intracellular esterification of cholesterol, has two distinct sterol-binding sites; a substrate-binding site and an allosteric-activator site [25,26]. The substrate-binding site accommodates a wide variety of sterols while the activator site may only recognize cholesterol. 5α,6α-Epoxycholesterol, 7α-hydroxycholesterol, and cholesterol are comparable substrates, whereas the cytotoxic sterols, 7-ketocholesterol, 7β-hydroxycholesterol, and 5β,6β-epoxycholesterol, are very poor substrates. Dihydrocholesterol is an allosteric-activator whereas 7-ketocholesterol is not. Sterol activation of ACAT1 and sterol binding to SCAP are highly stereospecific and indicate that cells control their membrane composition by sensing cholesterol directly rather by than monitoring the biophysical properties (e.g. thickness and fluidity of phospholipid membranes).

Effect of oxysterols on cell function

Cytotoxic oxysterols have been implicated in the pathophysiology of atherosclerosis, where they induce apoptosis in cells of the vascular wall and in monocytes/macrophages; a key cell type in all stages of atherogenesis. Among oxysterols, 7β-hydroxycholesterol, 7-ketocholesterol, and 5β,6β-epoxycholesterol are the most cytotoxic, whereas the α-isomers and 25-hydroxycholesterol are less so [4,5]. Free cholesterol-induced macrophage cell death involves the accumulation of cholesterol within ER membranes, which decreases bilayer fluidity and decreases the activities of integral membrane proteins such as the sarco(endo)plasmic reticulum calcium ATPase [7$^{\bullet\bullet}$,27]. The activity of this protein is conformationally regulated and is inhibited in membranes with free cholesterol induced membrane order. Perturbation of ER functions activates the unfolded protein response and other stress pathways that trigger apoptosis. 7-Ketocholesterol induced apoptosis in smooth muscle cells also appears to be mediated by the unfolded protein response where the activation of the ER associated non-phagocytic NADPH oxidase Nox-4 to induce oxidative stress is also important [28]. 7β-Hydroxycholesterol and 25-hydroxycholesterol may induce apoptosis in smooth muscle cells by the same mechanism. Thus, the underlying mechanism for oxysterol induced apoptosis in smooth muscle cells may be mediated by the altered biophysical properties of the ER membrane.

In monocyte/macrophages, free cholesterol and 7-ketocholesterol induce apoptosis by different mechanisms [29]. 7-Ketocholesterol induces apoptosis by the

mitochondrial death pathway, which involves a sustained increase in cytosolic free calcium and the calcium-dependent activation of pro-apoptotic and survival pathways [30,31]. Increased cellular calcium is thought to be driven by the movement of the plasma membrane transient receptor potential calcium channel 1 into lipid rafts [32]. 7-Ketocholesterol may also form a downstream signaling intermediate as an arachidonic acid containing sterol ester formed by ACAT [31]. 7-Ketocholesterol, when added alone to macrophages, induces cell death, however when added with other oxysterols cell death is ablated [33], suggesting that oxysterols compete at the level of a NADPH oxidase [33] or ACAT [31]. The oxysterol mixture does however induce the inflammatory response of increased monocyte chemotactic protein-1 (MCP-1) expression by increasing the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) [34]. Although oxysterol inhibition of isoprenoid-based cholesterol biosynthesis is a proposed component of cytotoxicity in lung cells [35], there is no relationship between oxysterol induced cytotoxicity and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity found in a monocytic cell line [4].

ATP binding cassette transporter A1 (ABCA1), ATP binding cassette transporter G1 (ABCG1), and ACAT function to protect cells from free cholesterol-induced cytotoxicity in the ER [36]. ABCA1 and ABCG1 transport cellular cholesterol to apoA-I and HDL, respectively, and ACAT detoxifies cholesterol by converting it to its acyl ester. Unlike cholesterol, cytotoxic oxysterols are poor substrates for ACAT and, in model foam cells, 7-ketocholesterol is not effluxed via ABCA1, and inhibits cholesterol efflux by this pathway [37]. Thus, oxysterol cytotoxicity may be due to impaired cellular sterol detoxification through reduced esterification and/or efflux.

Oxysterols and oxysterol-binding-protein-related proteins

OSBP was identified as a cytosolic protein that binds 25-hydroxycholesterol and is subsequently translocated to the Golgi. The human OSBP-related protein family consists of 12 genes that code for at least 16 different transcripts and can be divided into short and long forms [3]. The short forms consist of an OSBP-related domain (ORD) that contains a sterol ligand domain and membrane docking sequences [38,39]. The long forms contain the ORD and additional protein-protein and protein-phospholipid interaction domains involved in intracellular targeting [40,41]. Functional studies indicate that individual oxysterol binding protein-related proteins (ORPs) are involved in cellular sterol metabolism [42,43•], membrane vesicle transport [38,39], and cell signaling [44••]. Recent studies indicate that individual ORPs may function as sterol-dependent sensors [44••] and as lipid transfer proteins [45••,46].

OSBP is a cholesterol-dependent scaffolding protein that regulates the ERK1/2 signaling pathway [44••]. Cytosolic OSBP forms an oligomer with a tyrosine phosphatase, a serine/threonine phosphatase, and cholesterol that has dual specific phosphatase activity for phosphorylated ERK1/2. Association of OSBP with cholesterol stabilizes oligomers whereas OSBP association with oxysterols disassembles the oligomers. Thus, ERK phosphatase activity is positively regulated by cholesterol but negatively regulated by oxysterols. OSBP is a sterol-dependent sensor that controls a key cell-signaling pathway. OSBP is stated to be essential for embryonic development in mice [45••] and may be involved in the oxysterol-induced up-regulation of MCP-1 expression [34].

Osh4 is a short ORP found in yeast that consists of only the ORD. Its three dimensional structure was recently solved in a sterol free state and in complexes with ergosterol, cholesterol, and 7, 20, and 25-hydroxycholesterol [45••]. In the sterol-bound structures, a single sterol molecule lies within a hydrophobic tunnel where a flexible lid covers the entrance to the tunnel and shields the bound sterol from water. Sections of the lid move slightly depending upon whether 25-hydroxycholesterol or cholesterol are bound. In the sterol-free structure, two bound sulfate ions, which may represent membrane docking sites for phospholipid phosphate groups, are found. Residues that form the lid and membrane docking sites are highly conserved in all ORPs, however, the residues within the hydrophobic tunnel are not conserved suggesting that different ORPs may bind different lipid ligands. Osh4 is a negative regulator of the Sec14p pathway in yeast that is essential for protein trafficking via Golgi secretory vesicle formation [39]. Mutations within the hydrophobic tunnel region of Osh4 inhibit both *in vitro* sterol binding and *in vivo* functional properties in yeast [39,45••]. Similarly, mutations in the conserved regions are associated with a loss of function in cell assays. Thus, both sterol and membrane binding domains are essential for Osh4 function. A biochemical model was proposed where Osh4 is a lipid transfer protein in which sterol and membrane binding promote reciprocal conformational changes that facilitate sterol transfer between donor and acceptor membranes [45••].

ORP2, ORP1S (short), and ORP1L (long) constitute an ORP subfamily [3]. Overexpression of ORP2 reduces cellular free cholesterol levels, stimulates cholesterol transport out of the ER and enhances cholesterol efflux to both apolipoprotein and HDL acceptors [43•]. ORP1S consists of only the ORD and is a functional homologue of Osh4 in yeast [38]. ORP2 is highly homologous to ORP1S but contains an additional ER protein-targeting

motif [47•] and is not a functional homologue of Osh4. ORP1S and ORP1L are splice variants where ORP1L has additional domains that target it to late endocytic compartments [41]. These proteins have different organelle targeting domains but have similar sterol ligand binding domains and, probably, identical lipid ligand specificity. This suggests that they have similar functions in different cellular locations [3]. Cholesterol is transported among organelles and the plasma membrane by both vesicular and non-vesicular mechanisms [16,48,49]. These ORPs may be lipid transfer proteins that interconnect cholesterol trafficking and membrane transport [49,50•].

Conclusion

The molecular mechanism by which cholesterol regulates normal membrane organization and cell function, is trafficked within and out of cells, and induces pathological conditions such as cell death, is of relevance in several human diseases, including atherosclerosis. Thus, it is important to understand how subtle differences in sterol structure so profoundly alter membrane properties and intracellular activities. Recent biophysical and cell biological studies suggest that studies of a series of oxysterols permit the identification of the sterol-membrane and sterol-protein interactions that define normal and dysregulated metabolic states. This approach should also be fruitful in the search for the biochemical connections between ORPs and sterol regulated signal transduction and lipid trafficking.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 313).

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