Progress in Lipid Research 71 (2018) 18–42

Contents lists available at ScienceDirect

Progress in Lipid Research

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

Review

# Sphingolipid hydroxylation in mammals, yeast and plants – An integrated view

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ARTICLE INFO

*Keywords::*
Sphingolipid domains
H-bonding network
Eukaryotic plasma membrane
Membrane dipole potential
Lipid phase behavior
Cholesterol

ABSTRACT

This review is focused on sphingolipid backbone hydroxylation, a small but widespread structural feature with profound impact on membrane biophysical properties. We start by summarizing sphingolipid metabolism in mammalian cells, yeast and plants, focusing on how distinct hydroxylation patterns emerge in different eukaryotic kingdoms. Then, a comparison of the biophysical properties in membrane model systems and cellular membranes from diverse organisms is made. From an integrative perspective, these results can be rationalized considering that superficial hydroxyl groups in the backbone of sphingolipids (by intervening in the H-bond network) alter the balance of favorable interactions between membrane lipids. They may strengthen the bonding or compete with other hydroxyl groups, in particular the one of membrane sterols. Different sphingolipid hydroxylation patterns can stabilize/disrupt specific membrane domains or change whole plasma membrane properties, and therefore be important in the control of protein distribution, function and lateral diffusion and in the formation and overtime stability of signaling platforms. The recent examples explored throughout this review unveil a potentially key role for sphingolipid backbone hydroxylation in both physiological and pathological situations, as it can be of extreme importance for the proper organization of cell membranes in mammalian cells, yeast and, most likely, also in plants.

## Gene and gene products

| | |
|---|---|
| AtFAH1/ AtFAH2 | *Arabidopsis* sphingolipid fatty acid 2-hydroxylases |
| FAPP2 | four-phosphate adaptor protein 2 |
| Isc1p | inositol phosphosphingolipid phospholipase C |
| Ipt1p | inositolphosphotransferase |
| IPUT1 | inositol phosphorylceramide glucuronosyltransferase 1 |
| Lac1p | longevity-assurance gene cognate |
| Lag1p | longevity assurance gene |
| Lcb1p/Lcb2p | serine palmitoyltransferase (SPT) subunits |
| Lcb3p | long chain base-1-phosphate phosphatase |
| Lcb4p and Lcb5p | long chain base kinases |
| Lip1p | lag1p/Lac1p interacting protein |
| LOH | longevity assurance gene one homolog1 |
| Orm | orosomucoid |
| Sac1p | suppressor of actin protein |
| Scs7p | $^{+}$sphingolipid fatty acyl 2-hydroxylase |
| Sur2p | sphinganine C4-hydroxylase |
| *TSC10* | temperature-sensitive suppressor of csg2 mutants 10 gene |
| *TSC3* | temperature-sensitive suppressor of csg2 mutants 3 gene |

*Abbreviations:* 2-OH-FA, 2-hydroxy-fatty acids; 2-OH-OA, 2-hydroxyoleic acid; AFM, atomic force microscopy; CERK, ceramide kinase; CerS, ceramide synthase; CERT, ceramide transport protein; DES, desaturase; DHS, dihydrosphingosine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; DPH, 1,6-diphenyl-1,3,5-hexatriene; ECR, enoyl-CoA reductase; ER, endoplasmic reticulum; FA, fatty acid; FA2H, fatty acid 2-hydroxylase; GalCer, galactosylceramide; GBA2, β-glucosidase; GDGT, Glycerol dialkyl glycerol tetraethers; GIPC, glucosyl inositolphosphoceramide; GlcCer, glucosylceramide; HCD, β-hydroxyacyl-CoA dehydratase; IPC, inositol phosphorylceramide; KCR, β-ketoacyl-CoA reductase; KCS, ketoacyl-CoA synthase; LCB, long chain base; LCFA, long-chain fatty acids; $l_d$, liquid disordered; $l_o$, liquid ordered; LPP, lipid phosphate phosphatases; M$(IP)_2$C, mannosyl diinositol phosphorylceramide; MIPC, mannosyl inositol phosphorylceramide; NADPH, Nicotinamide adenine dinucleotide phosphate; OHM, 1-O-hexadecyl-2-O-methyl-rac-glycero-3-lactose; PC, phosphatidylcholine; phytoPSM, C16-phytosphingomyelin; PHS, phytosphingosine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PSM, C16-sphingomyelin; SMS, sphingomyelin synthase; SPOTS, Serine Palmitoyltransferase Orm1/2p Tsc3p and Sac1p; SPT, serine palmitoyltransferase; *t*-PnA, *trans*-parinaric acid; $T_m$, melting temperature; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; UDP, Uridine diphosphate; VLCFA, very long-chain fatty acids

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https://doi.org/10.1016/j.plipres.2018.05.001
Received 1 February 2018; Received in revised form 11 April 2018; Accepted 4 May 2018
Available online 08 May 2018
0163-7827/ 

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| Ydc | yeast dihydroceramidase |
| Ypc | yeast phytoceramidase |

## 1. Introduction

Sphingolipids are a major and highly diverse lipid category, comprising both structural and bioactive lipids, with properties that were essential for the origin and evolution of eukaryotic cells. It is now widely accepted that knowledge of how sphingolipids impact membrane biophysical properties such as fluidity and order, hydration, lateral diffusion or inter-leaflet coupling is fundamental for understanding their cellular functions [1–5]. Notably, sphingolipids have a major role in the formation and modulation of membrane domains which, ultimately, are co-responsible, with proteins, for membrane compartmentalization, a key regulatory feature in many membrane-associated cellular events [6]. Hence, it is not surprising that the number of potential sphingolipid-associated functions is large and ever growing and, in mammalian organisms, sphingolipids are provenly involved in key cellular processes ranging from cell cycle arrest to proliferation, different types of cell death, autophagy, inflammation, multidrug resistance or cellular motility [7]. To fully understand biological mechanisms of membrane-associated processes and their regulation, spatial and temporal dynamics should be considered. In the case of membrane lipids, the complete elucidation is even more complex because the biophysical properties of the sphingolipid in the context of the other membrane components, both lipids and proteins, with which it interacts, also needs to be considered [8].

The large structural diversity of sphingolipids has been intensely studied and reviewed in the past few years focusing on their three building blocks, headgroup, acyl chain and long chain (sphingoid) base (LCB) [2,3,9–14]. For the latter two, the hydrocarbon chain length and degree of unsaturation have been extensively explored. However, an additional feature appearing throughout all sphingolipid classes, adding more structural diversity, is the possibility of different patterns of hydroxylation. These patterns are due to the presence of additional hydroxyl groups, in both the acyl chain and the LCB, i.e., in the sphingolipid backbone (see Box 1, nomenclature) and considerably less explored [2,15,16]. Although there are at least two reviews dealing with some of the biological consequences of sphingolipid hydroxylation at the C2 of the fatty acyl chain, they are restricted just to mammals [17,18]. However, evidence accumulated in recent years, reviewed in this work, strongly suggests that hydroxylation patterns will emerge in the near future as a key feature in sphingolipids and biomembrane research. As will be described in more detail, the predominant form of sphingolipids in mammals (with some important exceptions) is based on a LCB with two free hydroxyl groups, one of them being involved in the bond to the headgroup in complex sphingolipids, leaving one hydroxyl group still free, and a non-hydroxylated fatty acyl chain. In this article, for the sake of simplicity, we name this group as “non-hydroxylated” sphingolipids. The two most common variations in the sphingolipid backbone are the presence or not of a hydroxyl (-OH) group at the C2 of the acyl chain and/or of a hydroxyl group at the C4 of the LCB base (Box 1). These will be the focus of this review.

Sphingolipidomics has proven essential to improve our understanding of fungal biology either for central processes common to mammalian cells, or for those specific of fungal organisms. A role for sphingolipids has been found in e.g., stress sensing, hyphae formation, spore germination, lipid cell homeostasis, or pathogenesis and virulence [19–21]. Interestingly, fungal glucosylceramides are mostly acylated with 2-hydroxy-fatty acids (2-OH-FA), and in inositolphosphorylceramide-based complex sphingolipids (the only ones present in the yeast *Saccharomyces cerevisiae*) both types of hydroxylation mentioned above are largely predominant. Similarly, in plants, sphingolipids and membrane domains have been implicated in a growing number of important pathophysiological processes, including intracellular protein trafficking, plant-pathogen interactions, as receptors for necrotizing cytotoxic toxins and in the structure and stability of intercellular communication nanopores (plasmosdesmata) [22–25]. Like in fungi, sphingolipids in plants occur mostly in hydroxylated forms [22].

The main biosynthetic and degradation pathways of hydroxylated sphingolipids in yeast, plants and mammals are highly conserved. Through a comparative presentation of these pathways the diverse hydroxylation patterns in different organisms, and how they differ among eukaryotic kingdoms and tissues, will naturally emerge (Section 2). These different hydroxylation patterns have biophysical consequences, owing to the molecular interactions afforded by the presence of additional −OH groups in sphingolipids, which can be evaluated using membrane model systems of increasing complexity (Section 3).

We hope to show with this review that hydroxylation patterns have a major influence on the biophysical properties of sphingolipids, as illustrated e.g., by the significant difference of gel-liquid disordered ($l_d$) phase transition temperature when comparing analogous sphingolipids with different hydroxylation patterns (Section 3.1). More important is, perhaps, the influence of hydroxylation in the way a given sphingolipid interacts in more complex membranes containing other lipid components, namely ester-linked glycerophospholipids (Section 3.2) and also sterols (Section 3.3). This will allow exploration of specific examples of known cellular functions of these lipids, and to suggest how the special biophysical properties conveyed by the different hydroxylation patterns can be invoked as part of the biological mechanism involved in those processes (Section 3.4).

Recently, important studies of membrane-interaction with compounds, other than sphingolipids, which contain -OH groups occupying a transversal position in membranes similar to the sphingolipid backbone hydroxyl groups, were reported. As we will show, analyzing these cases may bring further insights into the way that certain intermolecular interactions can be disrupted or established in membranes due to the different hydroxylation patterns (Section 4). Most of these compounds can have either a therapeutic or cytotoxic activity, establishing a link between the biophysical studies and physiological/pathological roles of hydroxylation patterns, and their potential usefulness towards human health.

In the final part (Section 5) we will present our critical perception from an integrated perspective of the literature. In this view, the strong impact of the sphingolipid backbone hydroxylation patterns on membrane organization and biophysical properties is due to an altered balance of H-bonding interaction near the membrane-water interface. We postulate that, in most situations, those additional −OH groups in sphingolipids compete with the sterol −OH leading to a stronger segregation of membrane components. In this way, we believe that it is possible to establish a bridge connecting the biological consequences of different patterns of hydroxylation to membrane organization and biophysical properties.

## 2. Sphingolipid metabolism in fungi, plants and mammals

Sphingolipid metabolic pathways, the enzymes involved in the catalysis of individual reactions of sphingolipid metabolism, and their specific cellular location are broadly conserved across fungi, plant and animal kingdoms (Fig. 1). Sphingolipids can be formed via two pathways: by a *de novo* pathway, which starts with the condensation of a serine with an acyl-CoA, and by a salvage pathway, where ceramides and LCBs formed from more complex sphingolipids are channeled into the biosynthetic pathway (Figs. 2 and 3) [26–29]. The yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) had a major role in uncovering the reactions of sphingolipid metabolism and in the discovery of all the genes encoding enzymes catalyzing those reactions, not just in yeast, but also in plants and mammalian cells where most of these genes have orthologs. Here, the enzymes and pathways involved in sphingolipid biosynthesis and catabolism in fungi, plants and mammals will be