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1. Chakraborty, A., **Kamat, S. S.*** (2024) Lysophosphatidylserine: a signaling lipid with implications in human diseases, ***Chemical Reviews*** 124 (9), 5470-5504.
   Link: https://doi.org/10.1021/acs.chemrev.3c00701

2. Kelkar, D. S., Ravikumar, G., Mehendale, N., Singh, S., Joshi, A., Sharma, A. K., Mhetre, A., Rajendan, A., Chakrapani, H., **Kamat, S. S.*** (2019) A chemical genetic screen identifies ABHD12 as an oxidized phosphatidylserine lipase, ***Nature Chemical Biology*** 15, 169-178.
   Link: https://doi.org/10.1038/s41589-018-0195-0

3. Khandelwal, N., Shaikh, M., Mhetre, A., Singh, S., Sajeevan, T., Joshi, A., Balaji, K. N., Chakrapani, H., **Kamat, S. S.*** (2021) Fatty acid chain length drives lysophosphatidylserine dependent immunological outputs, ***Cell Chemical Biology*** 28, 1169-1179.
   Link: https://doi.org/10.1016/j.chembiol.2021.01.008

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Sincerely,

Siddhesh Kamat,
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CHEMICAL REVIEWS

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Review

Lysophosphatidylserine: A Signaling Lipid with Implications in Human Diseases

Arnab Chakraborty and Siddhesh S. Kamat*

Cite This: *Chem. Rev.* 2024, 124, 5470−5504

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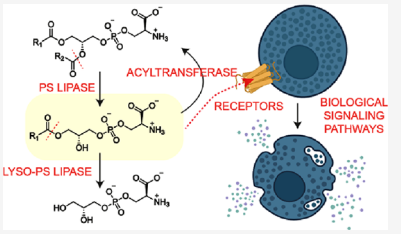
ABSTRACT: Lysophosphatidylserine (lyso-PS) has emerged as yet another important signaling lysophospholipid in mammals, and deregulation in its metabolism has been directly linked to an array of human autoimmune and neurological disorders. It has an indispensable role in several biological processes in humans, and therefore, cellular concentrations of lyso-PS are tightly regulated to ensure optimal signaling and functioning in physiological settings. Given its biological importance, the past two decades have seen an explosion in the available literature toward our understanding of diverse aspects of lyso-PS metabolism and signaling and its association with human diseases. In this Review, we aim to comprehensively summarize different aspects of lyso-PS, such as its structure, biodistribution, chemical synthesis, and SAR studies with some synthetic analogs. From a biochemical perspective, we provide an exhaustive coverage of the diverse biological activities modulated by lyso-PSs, such as its metabolism and the receptors that respond to them in humans. We also briefly discuss the human diseases associated with aberrant lyso-PS metabolism and signaling and posit some future directions that may advance our understanding of lyso-PS-mediated mammalian physiology.



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Article

Fatty acid chain length drives lysophosphatidylserine-dependent immunological outputs

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SUMMARY

In humans, lysophosphatidylserines (lyso-PSs) are potent lipid regulators of important immunological processes. Given their structural diversity and commercial paucity, here we report the synthesis of methyl esters of lyso-PS (Me-lyso-PSs) containing medium- to very-long-chain (VLC) lipid tails. We show that Me-lyso-PSs are excellent substrates for the lyso-PS lipase ABHD12, and that these synthetic lipids are acted upon by cellular carboxylesterases to produce lyso-PSs. Next, in macrophages we demonstrate that VLC lyso-PSs orchestrate pro-inflammatory responses and in turn neuroinflammation via a Toll-like receptor 2 (TLR2)-dependent pathway. We also show that long-chain (LC) lyso-PSs robustly induce intracellular cyclic AMP production, cytosolic calcium influx, and phosphorylation of the nodal extracellular signal-regulated kinase to regulate macrophage activation via a TLR2-independent pathway. Finally, we report that LC lyso-PSs potently elicit histamine release during the mast cell degranulation process, and that ABHD12 is the major lyso-PS lipase in these immune cells.

INTRODUCTION

Lipids have long been known as potent signaling molecules that mediate many important physiological processes in mammals, including humans (Wymann and Schneiter, 2008; Dennis, 2016; Fahy et al., 2005). Prominent among the signaling lipids are the prostaglandins (Dennis and Norris, 2015), the endocannabinoids (2-arachidonoylglycerol [2-AG] and anandamide [AEA]) (Blankman and Cravatt, 2013; Fowler et al., 2005), and the well-studied lysophospholipids, sphingosine 1-phosphate (S1P) (Gonzalez-Cabrera et al., 2014; Rosen et al., 2013) and lysophosphatidic acid (lyso-PA) (Ishii et al., 2004; Contos et al., 2000). Given their physiological importance, the biosynthetic/degradative enzyme(s) and/or cognate receptor(s) of the aforementioned lysophospholipids (S1P and lyso-PA) are pharmacological targets for drugs already in clinical use or under investigation in different phases of clinical trials for an array of human neurological and immunological disorders (Gardell et al., 2006; Yanagida and Valentine, 2020). Recently, the lysophosphatidylserines (lyso-PSs) have emerged as yet another important class of signaling lysophospholipids (Shanbhag et al., 2020), with potent bioactivities in the mammalian central nervous and immune system.

Cellular pharmacological studies have shown that lyso-PSs regulate several immunological processes (Shanbhag et al., 2020) such as macrophage activation to clear apoptotic cells (Frasch and Bratton, 2012), mast cell degranulation (Lloret and Moreno, 1995), leukemic cell stimulation (Park et al., 2005), chemotaxis of human gliomas (Lee et al., 2008), and maturation of regulatory T cells (Barnes et al., 2015), and perhaps signal through Toll-like receptors (TLRs) (Van Der Kleij et al., 2002) and/or G-protein-coupled receptors (GPCRs) (Inoue et al., 2012) in the mammalian nervous and immune system. Interestingly, and of biomedical relevance, mutations to the putative lyso-PS receptors in humans have been linked to different autoimmune diseases (Szymanski et al., 2014; Napier et al., 2015; Chu et al., 2013). Murine studies have recently shown that accumulation of lyso-PS, especially very-long-chain (VLC) lyso-PSs (Blankman et al., 2013), in the mammalian brain is a major cause that drives the pathology of the early-onset human neurological disorder PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract) (Fiskerstrand et al., 2009, 2010).

nature chemical biology

ARTICLES

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A chemical–genetic screen identifies ABHD12 as an oxidized-phosphatidylserine lipase

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Reactive oxygen species (ROS) are transient, highly reactive intermediates or byproducts produced during oxygen metabolism. However, when innate mechanisms are unable to cope with sequestration of surplus ROS, oxidative stress results, in which excess ROS damage biomolecules. Oxidized phosphatidylserine (PS), a proapoptotic 'eat me' signal, is produced in response to elevated ROS, yet little is known regarding its chemical composition and metabolism. Here, we report a small molecule that generates ROS in different mammalian cells. We used this molecule to detect, characterize and study oxidized PS in mammalian cells. We developed a chemical–genetic screen to identify enzymes that regulate oxidized PS in mammalian cells and found that the lipase ABHD12 hydrolyzes oxidized PS. We validated these findings in different physiological settings including primary peritoneal macrophages and brains from *Abhd12*$^{-/-}$ mice under inflammatory stress, and in the process, we functionally annotated an enzyme regulating oxidized PS in vivo.

Oxidative stress is an imbalance between cellular oxidants and antioxidants in favor of oxidants, which leads to the disruption of redox signaling and has been implicated in several human pathophysiologies[1–4]. Under oxidative stress, excess ROS, namely superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radicals, cannot be detoxified through innate coping mechanisms[5], and consequently damage cellular components and cause cell death via apoptosis or necrosis[5]. Lipid membranes serve as cells' first line of defense against ROS by providing a physical barrier to ROS diffusion; therefore, membranes are primary targets for oxidative damage[5]. When ROS are generated near cellular membranes, the constituent lipids, particularly those bearing polyunsaturated fatty acid (PUFA) chains, are oxidized[6,7]. The resulting oxidized lipids disrupt the local membrane structure and integrity, and thus impair cellular functions by modulating the activity of a wide array of important cellular proteins[8,9]. Previously, research groups have focused on the oxidation of a single PUFA[6,7], but little is known regarding the global lipid profile under oxidative stress[8,9] and the enzymatic pathways that metabolize those oxidized lipid products in vivo.

PS, a phospholipid localized to the inner-membrane leaflet, has several critical functions in mammalian biology[10]. Important among these is its role in ROS signaling and apoptosis[11]. Given the asymmetric distribution of PS in the membrane bilayer, the externalization of PS reflects a stressed cell, and this 'flipped' PS is recognized by phagocytes as an 'eat me' signal[12–14]. Several studies have suggested that under oxidative stress, surplus ROS reacts with the *sn*-2-esterified PUFAs of PS, thus producing oxidized PS[6], which has a flipped membrane orientation; the oxidized PS then acts as an apoptotic signal[12,14]. Although these studies have described the production and role of oxidized PS in apoptosis, little is known regarding its metabolism. Physiologically, this metabolism is important, because several cells require high oxygen tension (for example, neurons, macrophages and cancer cells) and consequently have elevated ROS, which produce oxidized PS in a constant flux. However, innate mechanisms within such cells can efficiently metabolize oxidized PS and prevent apoptosis.

In this paper, we synthesized and characterized a small molecule that generates ROS efficiently in mammalian cells and developed mass spectrometry methods to study oxidized PS; using both in tandem, we performed a chemical–genetic screen to identify lipases capable of metabolizing oxidized PS. We found that the serine hydrolase (SH) enzyme ABHD12 (α/β hydrolase domain (ABHD) protein 12) is a major oxidized-PS lipase. We validated these findings through complementary biochemical, pharmacological and genetic assays in different physiological systems. Importantly, we found that ABHD12 controls levels of oxidized PS in the mammalian brain under severe inflammatory stress. Given the central role of ABHD12 in the human neurological disease polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract (PHARC)[15,16], its role in metabolizing oxidized PS adds another avenue toward understanding the PHARC pathophysiology.

Results

Characterization of an esterase-activated ROS probe. 1,4-Dihydroquinones react with oxygen, thereby generating superoxide, which spontaneously dismutates to hydrogen peroxide[17,18]. Among these compounds, the 'juglones' have been found to be effective against several antibiotic-resistant bacteria[19]. Nonetheless, very few probes of this scaffold are used to study ROS signaling in mammalian cells. The lack of intramolecular hydrogen bonding makes these molecules poor ROS generators;[17,18] hence, installing a metabolically cleavable linker that after activation restores this ability provides opportunities to trigger ROS generation on demand in mammalian cells. With this rationale, we synthesized MGR1 (**1**), 5-hydroxy-1,4,4a,9a-tetrahydro-1,4-ethanoanthracene-9,10-dione

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