Chapter 38
Lysophosphatidylserine as an Inflammatory Mediator

Kumiko Makide, Asuka Inoue, and Junken Aoki

Abstract Lysophosphatidylserine (LysoPS), a deacylated form of phosphatidylserine (PS), has been assumed to serve as a bioactive lysophospholipid mediator. However, LysoPS has received little attention because its mode of actions as well as its synthetic pathways have been obscure. Recently G protein-coupled receptors (GPCRs) specific for LysoPS, and enzymes responsible for the generation of LysoPS have been identified. These LysoPS-related molecules are mainly expressed on immune cells, which led us to assume that LysoPS may have some roles in inflammation. Here we summarised the newly discovered LysoPS receptors and enzymes including our recent works.

Keywords Lysophospholipid mediator • GPCR • Lysophosphatidylserine • Phospholipase

38.1 Introduction

Lysophospholipid (LPL, 1-acyl-2-LPLs or 2-acyl-1-LPLs) is a deacylated form of phospholipids with a single fatty acid chain. Various LPLs, including lysophosphatidylcholine (LPC), lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), lysophosphatidylserine (LysoPS), lysophosphatidylinositol (LPI), lysophosphatidylglycerol (LPG), sphingosine-1-phosphate (S1P), and sphingosylphosphorylcholine (SPC) are present. They serve as precursors for diacyl phospholipids, and at least some of them also have roles as lipid mediators. Among

K. Makide (✉) • A. Inoue
Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan

PRESTO, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan
e-mail: makide@m.tohoku.ac.jp

J. Aoki
Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan

AMED-CREST, Sendai 980-8578, Japan

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them, LPA and S1P have been studied intensively on their receptors, producing enzymes, and catabolic enzymes (Mendelson et al. 2014; Yung et al. 2014). Studies using knockout mice and human hereditary diseases of receptors and synthetic enzymes of LPA and S1P have revealed their in vivo significance. Furthermore some of them were found to be drug targets and have received a lot of attention in terms of drug discovery. Notably FTY720 (Fingolimod), an $S1P_1$ functional antagonist, has been approved for treatment of multiple sclerosis (Groves et al. 2013).

On the other hand, lysophosphatidylserine (LysoPS) is known to induce various cellular responses when applied in vivo or in vitro, but has received little attention because its receptors, producing pathways, and its in vivo occurrence have remained unknown. Recently LysoPS receptors and producing enzymes have been identified, which enables us to examine the role of LysoPS in vivo using knockout mice. In addition, the new methods using LC-MS/MS can sensitively detect LysoPS in vivo. In this review we summarise the recent advance in the study of LysoPS in terms of receptors, synthetic enzymes, and detection, with particular focus on the relevance of LysoPS to both physiological and pathological states.

38.2 LysoPS Receptors

Lysophosphatidylserine is known to induce several cellular responses both in vitro and in vivo. The most characterised response has been the stimulatory response of mast cell degranulation. In vitro, LysoPS enhances histamine release from peritoneal rodent mast cells triggered by the crosslinking of high-affinity IgE receptors (FcεRI) (Martin and Lagunoff 1979). It also induces rapid degranulation of mast cells and a consequent anaphylactic shock and hypothermia when administered i.v. in rodents. The mast cell degranulation-stimulating activity is not induced by other LPLs and strictly requires serine residue of LysoPS. This suggests the presence of a receptor for LysoPS on mast cells, although it remains unidentified.

In the course of a ligand fishing study of orphan GPCRs, Sugo et al. found that LysoPS is a ligand for GPR34, which is thought to be a member of P2Y family (Sugo et al. 2006). The P2Y family includes receptors for nucleotide, UDP-glucose, LPA, and the orphan GPCRs. They showed that LysoPS caused a dose-dependent inhibition of forskolin-stimulated cAMP accumulation in human GPR34-expressing Chinese hamster ovary (CHO) cells. They also showed that LysoPS induced phosphorylation of ERK in GPR34-expressing CHO cells. These responses were completely abolished by treatment of pertussis toxin (PTX), which indicates that GPR34 couples to a $G_{i/o}$-type G-protein. Later, Liebscher et al. and Makide et al. demonstrated that GPR34 are conserved in a wide range of vertebrates from fish to mammals by showing that GPR34 from carp and zebrafish react with LysoPS (Liebscher et al. 2011; Makide et al. 2014). Kitamura et al. used two assays for measuring GPR34 activation, a Ca^{2+} mobilisation assay and a newly developed transforming growth factor-α (TGFα) shedding assay, and confirmed that

mammalian GPR34s from human, rat, and mouse origins reacted specifically with LysoPS but not with other LPLs (Kitamura et al. 2012). Notably, GPR34 reacted strongly with LysoPS species with an unsaturated fatty acid at the *sn*-2 position, showing that *sn*-2 unsaturated LysoPS is a preferable ligand for GPR34. GPR34 mRNA is expressed in many tissues but is most highly expressed in mast cells. Thus, it was once proposed that LysoPS enhances mast cell degranulation through GPR34. However, peritoneal mast cells from GPR34-deficient mice still responded to LysoPS, which suggested that GPR34 is not involved in the mast cell degranulation response induced by LysoPS. Until now the LysoPS receptor on mast cells has remained unknown.

Frasch et al. showed that LysoPS was generated in neutrophils by an oxidation-dependent mechanism and that is served as an endogenous anti-inflammatory mediator by stimulating the clearance of recruited neutrophils by macrophages, contributing to the resolution of inflammation (Frasch et al. 2008; Frasch and Bratton 2012). In addition, their results suggest that G2A (GPR132) on macrophages is responsible for the clearance of neutrophils by macrophages, raising the possibility that LysoPS is an endogenous ligand for G2A. G2A was once proposed as a receptor for LPC but the proposal was later retracted. Now many reports have confirmed that G2A is a receptor for protons (Murakami et al. 2004) and for 9-hydroxyoctadecadienoic acid (9-HODE), a kind of oxidised fatty acid (linoleic acid) (Obinata et al. 2005). We observed that G2A responded to 9-HODE, but not to LysoPS using a TGFα shedding assay (unpublished data). Thus, it is currently not clear if G2A directly recognises LysoPS.

Recently, we identified three additional GPCRs (P2Y10, A630033H20, and GPR174) as novel LysoPS receptors through a ligand screening of orphan GPCRs using a TGFα shedding assay (Inoue et al. 2012). These receptors are members of the P2Y family, and are located in tandem in a clustered locus on the X chromosome. We propose that, according to the nomenclature of lysophospholipid receptors, GPR34, P2Y10, A630033H20, and GPR174 be designated as LPS_1, LPS_2, LPS_2-like (LPS_{2L}), and LPS_3, respectively (Fig. 38.1).

P2Y10 is coupled with $G\alpha_{12/13}$ but not with other G proteins. Expression of P2Y10 is restricted to lymphoid organs such as spleen, thymus, and lymph node. The expression of P2Y10 is dependent on PU.1 and Spi-B, which are closely related Ets transcription factors (Rao et al. 1999). In $PU.1^{+/-}$Spi-$B^{-/-}$ mice, the expression of P2Y10 is dramatically reduced. These Ets transcription factors have a role in the signal transduction of B-cell receptors (BCRs), which suggests that P2Y10 has a role in regulating BCR signalling. In addition, $G_{12/13}$ signalling in B cells has a role in regulating the marginal zone B cells and germinal center B cells (Muppidi et al. 2014; Rieken et al. 2006), which raises the possibility that LysoPS functions in B cells through P2Y10.

Although human A630033H20 is truncated in the open reading frame and becomes a pseudogene, mouse and rat A630033H20 function as LysoPS receptors. A630033H20 is the closest homologue of P2Y10, with a 75 % homology to P2Y10 at the amino acid level, coupled with $G_{12/13}$, and its expression pattern (it is highly expressed in lymphoid tissue) is similar to that of P2Y10.

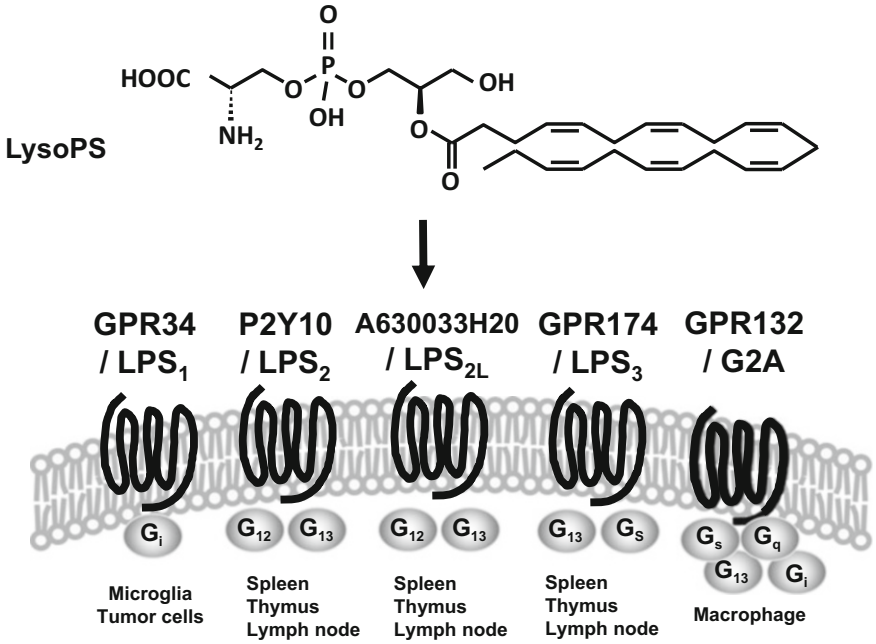

Fig. 38.1 LysoPS receptors

GPR174 shows the highest homology to P2Y10 and A630033H20 with ~50 % identity at the amino acid level. Like P2Y10 and A630033H20, GPR174 is activated by LysoPS. The expression pattern of GPR174 is similar to the expression pattern of P2Y10 and A630033H20 with high expression in lymphoid tissues. However, GPR174 is also strongly expressed in some melanoma cells (Qin et al. 2011). GPR174 mainly coupled with both $G\alpha_s$ and $G\alpha_{13}$. Given that $G\alpha_{13}$ signalling is induced by the three LysoPS receptors (P2Y10, A630033H20, and GPR174), which have similar expression patterns, it is likely that these three LysoPS receptors share redundant functions in activating the $G\alpha_{13}$ pathway. On the other hand, inasmuch as only one LysoPS receptor (GPR174) is coupled with $G\alpha_s$, GPR174 may have a unique role in regulating $G\alpha_s$ signalling. Recent genome-wide association studies show that single nucleotide polymorphisms (SNPs) of GPR174 are associated with the risk for Graves' disease (Chu et al. 2013; Szymanski et al. 2014) or Addison's disease (Napier et al. 2015), autoimmune diseases. The expression of GPR174 is elevated in blood cells in vasovagal syncope patients (Huang et al. 2015). Thus, LysoPS may serve as an immunomodulator through GPR174.

Although P2Y10 was reported to be a receptor for LPA and S1P (Murakami et al. 2008), we and other researchers were unable to confirm it. In recent studies, we throughly examined the ligand specificity of cloned LysoPS receptors, that is, LPS_1/GPR34, LPS_2/P2Y10, and LPS_3/GPR174, using chemically synthesised

LysoPS analogues (Ikubo et al. 2015; Iwashita et al. 2009; Kitamura et al. 2012; Uwamizu et al. 2015). These studies revealed that modifications of the serine residue resulted in the loss of agonistic activity for all three LysoPS receptors, demonstrating that LysoPS receptors strictly recognise the head group of LysoPS, that is, serine. They also revealed that some modifications conferred certain selectivity toward each LysoPS receptor to the LysoPS analogues. For example, deoxy LysoPS, in which an *sn*-2 hydroxy group (−OH) was removed, was found to be selective for LPS_2/P2Y10 and lysophosphatidylallothreonine, in which a methyl group ($-CH_3$) was introduced in the serine residue, was found to be selective for LPS_3/GPR174. These ligand specificities suggest that LysoPS is the real ligand for the LysoPS receptors. This is supported by the fact that all four LysoPS receptors belong to the P2Y family, to which the LPA receptors ($LPA_{4–6}$) belong.

38.3 Generation of LysoPS

The main pathway of LysoPS production is probably the hydrolysis of PS by phospholipase (Figure 38.2). We recently established a method to detect LysoPS with high sensitivity (Okudaira et al. 2014). In this method, acyl migration reaction, in which an acyl chain of 2-acyl-1-LPLs is quickly moved to the *sn*-1 position, generating 1-acyl-2-LPLs, was completely suppressed by lowering the pH. With this method, we detected two types of LysoPS (1-acyl-2-LysoPS and 2-acyl-1-LysoPS) in various tissues and cells. The results, together with previous knowledge, suggest that both phospholipase A_1 (PLA_1) and A_2 (PLA_2) are involved in the production of LysoPS. In fact, rat platelets express two extracellular PLA enzymes, secretory PLA_2 group IIA ($sPLA_2$IIA) and PS-specific PLA_1 (PS-PLA_1), and secrete them upon activation. In the course of activation of rat platelets, $sPLA_2$-IIA and PS-PLA_1 produce 1-acyl LysoPS and 2-acyl LysoPS, respectively.

PS-PLA_1 is stored in α granules of rat platelets and is secreted into the medium when activated. Although PS-PLA_1 is structurally homologous to triglyceride (TG) lipase, it selectively hydrolyses PS and doesn't have lipase activity for TG. PS-PLA_1 expression is dramatically induced at the mRNA and protein levels by various inflammatory stimuli. Under some inflammatory conditions, we detected an increase of LysoPS in parallel with the induction of PS-PLA_1 and found that the increase was partially abolished in PS-PLA_1 knockout mice.

Because secretory PLA_2 and PS-PLA_1 are secreted proteins, they should act on PS extracellularly. On the contrary, PS localises exclusively to the inner leaflet of the plasma membrane. Recently, TMEM16F and Xkr8 were identified as scramblases that trigger exposure of PS in activated platelets and apoptotic cells, respectively (Suzuki et al. 2010; 2013). It is likely that secretory PLAs such as PS-PLA_1 and $sPLA_2$ deacylate PS exposed by TMEM16F or Xkr8 during platelet activation or apoptosis.

Recently ABHD16A was identified as a PS lipase that generates LysoPS (Kamat et al. 2015). ABHD16A, also known as lymphocyte antigen B-associated transcript

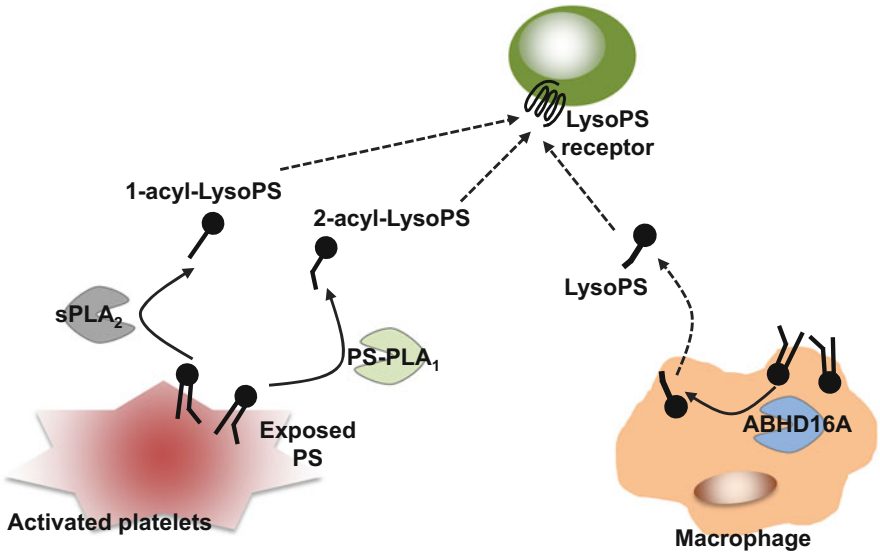

Fig. 38.2 Producing pathways of LysoPS

5 (BAT5), is a member of the alpha beta hydrolase domain (ABHD) enzyme family and is predicted to be a multipass membrane protein. In mice, ABHD16A mRNA is abundantly and ubiquitously expressed with highest expression in skeletal muscle and brain. The number of newborn ABHD16A$^{-/-}$ mice was much less than the value expected from Mendelian ratio. The body weight of ABHD16A$^{-/-}$ mice is smaller than wild-type mice, however, their behavior and survival rate appeared to be normal. The amount of various species of LysoPS in ABHD16A$^{-/-}$ brain were reduced, which suggests that ABHD16A does not discriminate between saturated fatty acids (mainly in the *sn*-1 position) and polyunsaturated fatty acids (mainly in the *sn*-2 position). Thioglycollate-elicited peritoneal macrophages derived from ABHD16A$^{-/-}$ mice have less LysoPS and release fewer inflammatory cytokines following stimulation with lipopolysaccharide. It is not known whether ABHD16A produces LysoPS intracellularly or extracellularly. If LysoPS is produced intracellularly, LysoPS must be released through some transporter on the plasma membrane such as Spns2 for S1P.

The degradation pathways of LysoPS are also important, because degradation is involved in the termination of LysoPS signaling. ABHD12 is reported to have lysoPS lipase activity in the mammalian brain (Blankman et al. 2013). In addition to having elevated brain LysoPS and microglial activation, ABHD12$^{-/-}$ mice show the phenotype of the human neurogenerative disorder PHARC (polyneuropathy, hearing loss, ataxia, retinosis pigmentosa, and cataract). Taken together, these results suggest that the interplay of ABHD12 and ABHD16A regulates the LysoPS level in neuronal diseases.

38.4 Conclusion

The LysoPS field has advanced with an expanding repertoire of receptors and metabolic enzymes. Recent studies strongly suggest that LysoPS plays important roles in processes related to inflammation. Further studies are needed to identify LysoPS-generating cells and enzymes, and LysoPS signaling pathways. The results of these studies will help to develop drugs that target LysoPS enzymes and receptors.

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