論文の内容の要旨

論文題目　Synthesis of Conformationally Constrained Lysophosphatidylserine and Elucidation of Their Bioactive Structures against Specific GPCRs

(配座を固定したリゾホスファチジルセリンの合成と特異的な
G–タンパク質共役型受容体に対する活性構造の探索)

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Introduction

Lysophosphatidylserine (LPS, LysoPS) is reported to be a significant component as a lipid mediator, and derived from phosphatidylserine (PS), which is one of the phospholipids constituting lipid bilayers. There are several reported biological functions of lysoPS: LysoPS can stimulate the mast cell degranulation[1], suppress proliferation of isolated human T lymphocytes[2], and enhance the apoptotic cell engulfment by macrophages[3]. Three specific receptors of lysoPS, GPR34 (LPS1)[4], P2Y10 (LPS2)[5], and GPR174 (LPS3)[5] have been identified recently, and they are G-protein coupled receptors (GPCRs) (Figure 1). According to the research results so far, these receptors are expressed in immune-related tissues and are assumed to be involved in immunological controls.

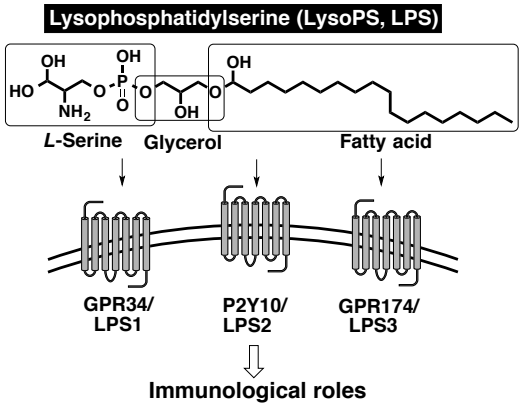

Figure 1. Reported specific receptors of lysoPS

Lysophsophatidylserine has a modular structure containing a single fatty acid, *L*-serine, and a glycerol, connected by a phosphodiester and an ester linkage. This molecule is flexible enough to take various and accessible conformations in its approach to the receptors. Therefore, I focused on the development of conformationally constrained lysoPS analogues which reduce flexibility and finally identified potent and selective analouges. Thus, I proposed the bioactive conformations of LysoPS toward GPR34 and P2Y10, respcetively. Their structures are different from each other.

Results and Discussions

1. Identification of active compounds as agonists for GPR34

In our previous studies, it has been clear that sub-structures of lysoPS have an important role in potency and selectivity of each receptor. While our previous results indicated that elimination of the hydroxyl group at the sn-2 position lost activation of the GRP174, I embeded a tetrahydropyrane ring into the glycerol moiety in two ways. Allowing for the geometrical characteristics of these cyclic lysoPS analogues, all combinations of molecules arising from two possible regioisomers, 1˚-acyl-2˚-phosphoserine and 2˚-acyl-1˚-phosphoserine derivatives, and two possible stereochemistry, trans/cis isomers, are synthesized. In this study, three kinds of fatty acid chain, (A); a component of endogenous lysoPS, (B) and (C); its surrogates, were used.

Agonistic activities of the synthesized compounds toward respective receptors were evaluated by using TGFα shedding assay[5] and EC_{50} values were obtained. From the shedding assay results, the cyclic analogues containing an acyl chain on the primary alcohol (**1**-**3**, **7**-**9**) are more potent than 2˚-acyl compounds (**4**-**6**, **10**-**12**) in both cases of cis and trans isomers (for fatty acid (A) derivatives). This trend is valid in all of the present fatty acid surrogates ((B) and (C)) and acyl chain (C) provided the most potent derivative; that is, **9** is the potent and selective analogue more than lysoPS as an agonist for GPR34.

Suggested acitive conformation of lysoPS for GPR34:

We have a hypothesis that receptor-selective and potent analogues to respective receptors take similar conformations to each other, which might be possible to be an active conformation. From the comparison of calculated distance between the oxygen atom of the phosphodiester and carbon atom of the fatty acid, **9**, the most selective and potent against GPR34, is tend to take folded structures with short distances and we found that accessible folded structure of lysoPS is well superimposed on this conformation of **9.**

2. Identification of active compounds as agonists for P2Y10

Introduction of a double bond into the tetrahydropyran ring is able to restrict conformations of the cyclic analogues. This unsaturated framework is readily obtained from the intermediate in the synthetic procedures. Thus, I synthesized unsaturated cyclic analogues and evaluated their agonistic activities. The results showed that acyl chain (B) is more effective than another fatty acid surrogate (C) for the P2Y10 activation. Additionally, we found that increase in agonistic activity against P2Y10 accompanied decrease in potency toward GPR34 in both cases of unsaturated analogues, trans-isomers **13**-**15** and cis-isomers **19**-**21**. This suggested that increase in planarity on the modified glycerol backbone is favorable to activation of P2Y10.

Suggested acitive conformation of lysoPS for P2Y10: The present results implied that the distance/direction between the acyl chain and phosphoserine is important as well as rigidity of the benzene moiety for the sake of taking a favorable conformer for P2Y10 activation. To evaluate this assumption, we calculated the distance between the two oxygen atoms of the phosphodiester and the ester linkage in most populated conformations. This implies that more extended conformations are

favorable for P2Y10 activation. Virtually, the accessible structure of lysoPS taking a relatively extended glycerol backbone can be well superimposed on **29**. This extended glycerol framework makes the acyl chain and the phosphoserine to locate at distal positions from each other and probably this conformation is favorable for P2Y10 activation.

Summary: The present study of conformationally constrained derivatives of lysophosphatidylserine identified more effective and selective agonists against GPR34 and P2Y10, respectively. The distance between the acyl chain and polar head group may have some link with the potency and selectivity of receptor activation. In this context, I propose different conformations of flexible molecule lysoPS responsible for GPR34 and P2Y10 activation, respectively. The present work will be a fundamental basis for further study of active conformations of lysoPS and design of potent/selective and also synthetically and metabolically robust agonists.

References

[1] Martin, T. *et al*. *Nature* **1979**, *279*, 250-252. [2] Bellini, F. *et al*. *FEBS Lett*. **1993**, *316*(1), 1-4. [3] Frasch, S. C. *et al*. *J*. *Biol*. *Chem*. **2008**, *283*(48), 33736–33749. [4] Sugo, T. *et al*. *Biochem*. *Biophys*. *Res*. *Commun*. **2006**, *341*, 1078-1087. [5] Inoue, A. *et al*. *Nature methods* **2012**, *9*(10), 1021-1029.