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Toward the three-dimensional structure and lysophosphatidic acid binding characteristics of the LPA_4/p2y_9/GPR23 receptor: A homology modeling study

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ABSTRACT

Lysophosphatidic acid (LPA) is a naturally occurring phospholipid that initiates a broad array of biological processes, including those involved in cell proliferation, survival and migration *via* activation of specific G protein-coupled receptors located on the cell surface. To date, at least five receptor subtypes (LPA_{1-5}) have been identified. The LPA_{1-3} receptors are members of the endothelial cell differentiation gene (Edg) family. LPA_4, a member of the purinergic receptor family, and the recently identified LPA_5 are structurally distant from the canonical Edg LPA_{1-3} receptors. LPA_4 and LPA_5 are linked to G_q, $G_{12/13}$ and G_s but not G_i, while LPA_{1-3} all couple to G_i in addition to G_q and $G_{12/13}$. There is also evidence that LPA_4 and LPA_5 are functionally different from the Edg LPA receptors. Computational modeling has provided useful information on the structure–activity relationship (SAR) of the Edg LPA receptors. In this work, we focus on the initial analysis of the structural and ligand-binding properties of LPA_4, a prototype non-Edg LPA receptor. Three homology models of the LPA_4 receptor were developed based on the X-ray crystal structures of the ground state and photoactivated bovine rhodopsin and the recently determined human β_2-adrenergic receptor. Docking studies of LPA in the homology models were then conducted, and plausible LPA binding loci were explored. Based on these analyses, LPA is predicted to bind to LPA_4 in an orientation similar to that reported for LPA_{1-3}, but through a different network of hydrogen bonds. In LPA_{1-3}, the ligand polar head group is reported to interact with residues at positions 3.28, 3.29 and 7.36, whereas three non-conserved amino acid residues, S114(3.28), T187(EL2) and Y265(6.51), are predicted to interact with the polar head group in the LPA_4 receptor models.

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1. Introduction

Lysophosphatidic acid (LPA, **1**, Fig. 1) is an important signaling molecule and a member of the phospholipid growth factor family, exerting its effects *via* class A rhodopsin-like G protein-coupled receptors (GPCRs). Through activation of its receptors, LPA elicits a multitude of biological actions, including cell proliferation, cell survival, cell migration/invasion and regulation of immune responses [1]. LPA is thus involved in diverse physiological effects including neurogenesis, myelination, angiogenesis, spermatogenesis, embryo implantation, platelet aggregation, brain development, smooth muscle contraction, and apoptosis, and a number of biological processes including reproduction, hair growth, neuropathic pain, cardiovascular diseases and cancer have also been linked to the physiological action of LPA and its associated receptors [2]. So far, at least five GPCRs have been cloned and characterized as high-affinity LPA receptors. Three of them, LPA_1, LPA_2 and LPA_3, belong to the endothelial cell differentiation gene (Edg) receptor family [3]. More recently, two orphan GPCRs were found to be additional LPA receptors (LPA4/p2y_9/GPR23 and LPA5/GPR92) [4,5]. However, LPA_4 and LPA_5 are more closely related to the purinergic P2Y receptor family than to the Edg family of LPA receptors. In addition to LPA_4 and LPA_5, other orphan receptors in the purinergic receptor family such as GPR87 and $P2Y_5$ may also be high-affinity receptors for LPA [6,7]. However, independent studies are necessary to confirm their identity as bona fide LPA receptors.

The LPA_4 and LPA_5 receptors share less than 20% amino acid sequence homology with the LPA_{1-3} receptors. There is also a difference in G protein coupling selectivity between the Edg LPA receptors and the novel LPA_4 or LPA_5 receptors. LPA_4 and LPA_5 are primarily linked to G_q, $G_{12/13}$ and G_s but not G_i while LPA_{1-3} couple to G_i as well as G_q and $G_{12/13}$. The expression patterns of LPA_4 and LPA_5 are not coincidental with any of the LPA_{1-3} receptors. Consistent with these differences in structures, G protein coupling, and tissue distribution, these novel LPA receptors may have different functionalities. For example, LPA_4 inhibits

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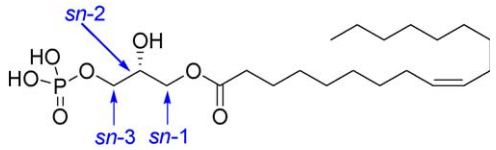

1

Fig. 1. The structure of 1-oleoyl-lysophosphatidic acid (LPA(1:18)), commonly known as LPA. The 'stereospecific numbering' (*sn*) identifiers are shown for the glycerol carbon atoms.

LPA-dependent cell migration and invasion in contrast to the motility-stimulating LPA_{1-3} receptors [8,9]. Therefore, it is important to explore structural and signaling characteristics of the novel LPA receptors.

The LPA receptors are members of a larger rhodopsin-like GPCR subfamily whose members can efficiently bind various functionalized fatty acid derivatives. As noted above, these molecules often act as potent signaling molecules in addition to serving as integral components in lipid membranes [10]. Phylogenetic analysis of related sequences (Fig. 2) shows that the LPA receptors fall into one of two main classes of phospholipidergic receptor. The first group includes the Edg receptors (LPA_{1-3} and sphingosine-1-phosphate $S1P_{1-5}$). The second group, which includes LPA_{4-5}, consists of nucleotide-binding P2Y and cysteinyl-leukotriene (CysLT) receptors. The second group can be further subdivided into those that signal primarily through G_q ($P2Y_{1,2,4,6,11}$) and through G_i ($P2Y_{12-14}$) [11,12]. The ligand selectivity among the receptors depends largely on the degree of similarities in the region(s) comprising the ligand binding site. As will be discussed later, there are both regions of similarities and differences among these closely related receptors.

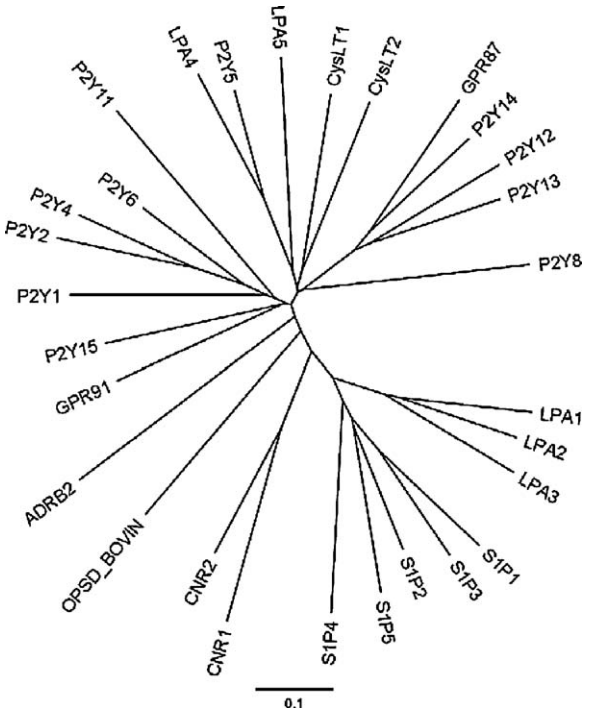

Fig. 2. Tree diagram showing the phylogenetic interrelationships among members of the lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P), cannabinoid (CNR), purinergic nucleotide (P2Y) and Cys-leukotriene (CysLT) receptor families. Also included are the sequences of bovine rhodopsin (OPSD_BOVIN), the β_2-adrenoceptor (ADRB2) and two recently de-orphanized receptors (CPR87 and GPR91). The diagram was generated using the Geneious software package (see Section 2), and the corresponding aligned sequences are presented as Supplemental Figure S2. The scale bar corresponds to a maximum likelihood branch of 0.1 inferred substitutions per site.

In order to visualize which amino acids likely participate in ligand binding, homology models of the LPA_4 receptor would be helpful in inferring the possible interaction between LPA and its receptor and in designing LPA receptor subtype selective ligands. Since an experimentally determined structure of an LPA_4 receptor is not yet available, we may rely on comparative modeling techniques combined with other experimental results to arrive at a plausible solution structure. It is believed that with all the lessons learned from previous experience, GPCR homology modeling based on the bovine rhodopsin and/or beta-adrenergic X-ray crystal structures will help in structure-based drug design and virtual screening for therapeutic applications and in fact such molecular modeling studies have been successfully applied to other GPCRs [13]. Furthermore, molecular modeling combined with site-directed mutagenesis study has aided significantly in our understanding of the binding mode of GPCR ligands to their receptors [14]. One of the challenges in homology modeling of GPCRs is the less predictable conformation of the extracellular loop regions [15]. In this work, we have identified loci on EL2 and the nearby regions on TM3 and TM6 potentially responsible for ligand binding.

There are now several crystal structures of GPCRs that may be used as templates for homology modeling, and the appropriateness of a given template for a particular GPCR model target has begun to be addressed in the literature [16]. The highest resolution (2.2 Å) crystal structure of bovine rhodopsin in its dark state was reported in 2004 [17], and a lower resolution (4.2 Å) photoactivated structure of rhodopsin was reported in 2006 [18]. In this context, we propose the first homology model of the LPA_4 receptor based on the photoactivated rhodopsin crystal structure to simulate an active state of the LPA_4 receptor. In order to compare with the photoactivated model, we further develop a second model of the LPA_4 receptor in an inactive state using the high-resolution (2.2 Å) dark state rhodopsin as a homology template [17]. Two β_2-adrenergic receptor (β_2AR) crystal structures were reported in 2007 [19–21]. Both β_2AR structures are bound with the inverse agonist carazolol, and as such these structures are presumably more representative of an inactive state of β_2AR. However, the reported crystal structures feature broken "ionic locks" [22], and the increased affinity of the β_2AR–T4 fusion protein for agonists compared to the wild type β_2AR suggest that these β_2AR crystal structures may be more closely related to an active state of the β_2AR receptor, or a state between active and inactive, consistent with the binding of a functionally weak inverse agonist. This suggests that a homology model based on β_2AR structure could also be useful as a template to model the LPA_4 receptor.

Therefore, in order to determine which crystal structure would provide the most appropriate template for the modeling of LPA_4 and to gain insight into its interaction with its endogenous ligand LPA, we constructed three separate homology models using (a) the high-resolution "inactive" or dark-state rhodopsin (PDB code = 1U19), (b) the "active" rhodopsin (PDB code = 2I37), and (c) the β_2-adrenoceptor-T4 lysozyme fusion protein (PDB code = 2RH1) [19]. Other GPCR crystal structures, including the recently determined β_1- [23] and β_2-adrenergic [24] receptors and squid rhodopsin [25], are structurally similar to one of these three, and were not considered in this study. The unliganded opsin [26,27] and the A_{2A} adenosine [28] receptors were also not considered as we are more interested in an agonist-occupied receptor model of LPA_4. Automated docking studies of LPA in the three previously mentioned models were then conducted using the GOLD program [29], and the possible binding modes and critical interactions between LPA and the LPA_4 receptor were explored.