Agonist-bound structure of purinergic receptor $P2Y_{12}$

GPCRs activate associated intracellular signal transduction pathways after sensing their endogenous extracellular ligands. Rhodopsin, β_1 and β_2 adrenergic receptors, A_{2A} adenosine receptor and M2 muscarinic receptor have both antagonist- and agonist-bound structures now. Each of these five receptors, however, belongs to the α-group of class A GPCRs [1]. Here, we describe a 2.5 Å structure of human $P2Y_{12}R$ bound to the full agonist 2MeSADP, and a 3.1 Å structure of $P2Y_{12}R$ bound to a potential partial agonist 2MeSATP. Together with the 2.7 Å structure of $P2Y_{12}R$ bound to the antagonist AZD1283 reported in the accompanying paper [2], this allows the first crystallographic assessment of a receptor with both agonist- and antagonist-bound structures in the δ-group of class A GPCRs.

All three $P2Y_{12}R$ structures were determined using the same thermostabilizing construct. Protein sample of $P2Y_{12}R$ was reconstituted into lipidic cubic phase (LCP) for crystallization trials. Their crystals ($80\times50\times5$ μm^3 for 2MeSADP-$P2Y_{12}R$ and $50\times50\times5$ μ^3 for 2MeSATP-$P2Y_{12}R$) diffracted to 2.5 Å and 3.1 Å at maximum on beamline **BL41XU**, respectively. Diffraction data sets were collected by exposing crystals with a 10 μm minibeam for 1 second and 1° oscillation per frame. The structures were solved by molecular replacement and refined in Refmac5 and Buster.

It is observed that the receptor structure of 2MeSADP and 2MeSATP bound complexes are very similar (Fig. 1(a)), indicating that the ligand 2MeSATP could potentially induce similar confirmation change of $P2Y_{12}R$ and activate the receptor to some extent. Thus the tri-phosphate ligands and their derivatives could potentially be partial agonists, instead of antagonists. This hypothesis is further supported by the pharmacological data of AR-C66096, and our finding ends the debate of the pharmacological properties of ATP and 2MeSATP in $P2Y_{12}R$ signaling. In the crystal structure of antagonist bound $P2Y_{12}R$ structure, there is a usual straight conformation and tilted orientation of helix V observed due to the lack of a conserved proline in this helix, which is further confirmed in the agonist bound structures. Considering the facts that the orientations of the fusion protein are different in all three structures, the straight confirmation of helix V is likely a genuine structural feature of the $P2Y_{12}R$ [2].

The agonist bound and antagonist bound $P2Y_{12}R$ structures show remarkable differences at the extracellular end. The inward shift of helices VI and VII in the 2MeSADP complex is striking and the N terminus is moved towards the axis of the helical bundle as well (Fig. 1(b)). The inward movement of these helices is consistent with previous structures, while the scale could not be previously predicted. In the previous structure of PAR1, the authors ran a long simulation try to reveal how the ligand vorapaxar gains access into the binding cavity of the receptor, but without any success [3]. This could be now explained by the very high plasticity of the extracellular region, and this could be a common structural feature of some GPCRs. Interestingly, the intracellular changes of $P2Y_{12}R$ are less prominent than those at the extracellular side (Fig. 1(b)). The $P2Y_{12}R$-2MeSADP structure possibly represents an "agonist-bound inactive state" with respect to the intracellular region, similar to the one observed in agonist-bound β_1AR and β_2AR without G-protein or a G-protein mimic stabilizing their active state [4,5].

The 2MeSADP-binding pocket consists of residues from helices III, IV, V, VI and VII as in the $P2Y_{12}R$-AZD1283 structure [2], but also extensively involves ECL2 and the N-terminus. The adenine group of 2MeSADP forms a similar π-π interaction with the Y105 side chain. The 2-thioether inserts into a hydrophobic pocket and serves as an anchor to maintain the adenine core and the ribose ring in an optimal orientation (Fig. 2). Thus, 2MeSADP binds with greater complementarity, which explains the higher affinity of this ligand compared to ADP.

In the antagonist bound $P2Y_{12}R$ structure, the conserved disulfide bond between TM3 and ECL2 is very flexible and is not built in the structure, while it is

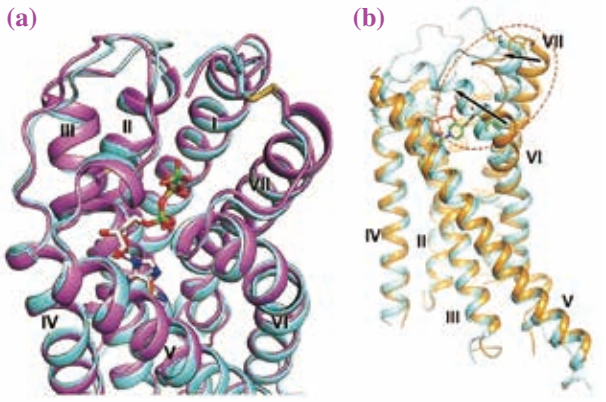

Fig. 1. Structure of the $P2Y_{12}R$-2MeSADP, $P2Y_{12}R$-2MeSATP and $P2Y_{12}R$-AZD1283 complexes. **(a)** Comparison of $P2Y_{12}R$-2MeSADP (cyan) with $P2Y_{12}R$-2MeSATP (violet) complex. 2MeSATP is colored in green. **(b)** Comparison of $P2Y_{12}R$-2MeSADP with $P2Y_{12}R$-AZD1283 complex (receptor: light orange cartoon and ligand: sticks of green carbons).

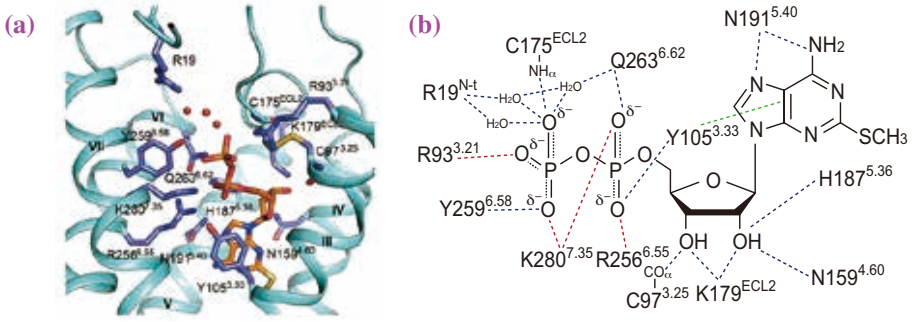

Fig. 2. $P2Y_{12}R$ ligand binding pocket for 2MeSADP. **(a)** The ligand 2MeSADP (orange carbons) and receptor residues (slate carbons) involved in ligand binding are shown in stick representation. The water molecules interacting with 2MeSADP are shown as red spheres. **(b)** Hydrogen bonds are displayed as blue dashed lines and the salt bridges as red dashed lines. The π-π interaction between 2MeSADP and Y105 is indicated as green dashed lines. The NHα and COα indicate the main chain amine and carbonyl groups of the corresponding residue.

observed in two agonist-bound structures. However, to form this disulfide, is the Cα of C97 is required to rotate along the helical path by over 60° and the extracellular tip of helix V is also shifted ~2 Å towards the helical bundle as compared with the AZD1283 complex. This difference indicates a dynamic disulfide of $P2Y_{12}R$, and different state of this disulfide is required by different type of ligand binding.

The mechanism of agonist and antagonist interactions with $P2Y_{12}R$ can be schematically illustrated in Fig. 3. Agonist access to the binding pocket of apo $P2Y_{12}R$ would require plasticity of this extracellular region caused by charge repulsion from the side chains of ~8 arginine and lysine around the pocket. Binding of a nucleotide agonist like 2MeSADP electrostatic attraction between charged phosphates and positively charged residues, and inward position of helices VI and VII and the electrostatic "lid" are stabilized. The additional phosphate group of 2MeSATP is also accommodated by a similar conformation of the lid, though distinct interactions may still impact the 2MeSATP binding and signaling profile. In stark contrast, binding of non-nucleotide antagonists like AZD1283 has an open extracellular side conformation with helices VI and VII away from the pocket and destabilizes the lid.

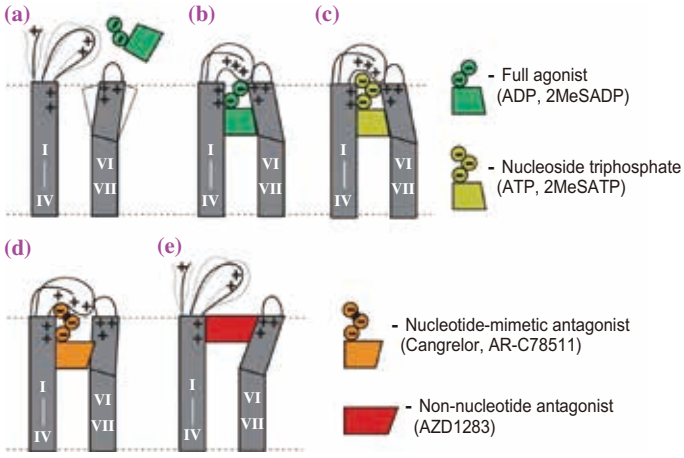

Fig. 3. Schematic illustration of conformational changes in $P2Y_{12}R$ extracellular region.

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