

Crystal structures of the human adiponectin receptors

Adiponectin is an antidiabetic adipokine secreted from adipose tissue. Plasma adiponectin levels are reduced in obesity and type 2 diabetes, while the replenishment of adiponectin reportedly ameliorated the abnormal glucose and lipid metabolism in mice.

We previously reported the expression cloning of the complementary DNAs encoding adiponectin receptors 1 and 2 [1]. Adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) are predicted to contain seven transmembrane helices, with an internal amino terminus and an external carboxy terminus, which is the opposite topology to G-protein-coupled receptors (GPCRs). Therefore, AdipoRs are expected to have different structures from those of GPCRs. AdipoR1 and AdipoR2 serve as the major receptors for adiponectin *in vivo*, with AdipoR1 activating the 5'AMP-activated protein kinase (AMPK) pathways and AdipoR2 activating the peroxisome proliferator-activated receptor (PPAR) α pathways (Fig. 1), leading to the increased expression of target genes such as molecules involved in fatty-acid combustion including acyl-CoA oxidase (ACO) and energy dissipation including uncoupling protein 2 (UCP2) [2]. Thereby, they regulate glucose and lipid metabolism, inflammation, and oxidative stress *in vivo*. Recently, the small-molecule AdipoR agonist AdipoRon was

shown to ameliorate diabetes and increase exercise endurance while simultaneously prolonging the shortened lifespan resulting from obesity [3].

For crystallization, AdipoR1 and AdipoR2 were optimized by deleting their N-terminal tails because the full length human AdipoRs tended to aggregate. We then used the Fv fragment of an anti-AdipoR monoclonal antibody that recognizes a conformational epitope of both AdipoR1 and AdipoR2 and the lipidic mesophase method for crystallization [4]. Finally, we successfully determined the crystal structures of human AdipoR1 and AdipoR2 at 2.9 and 2.4 Å resolutions, respectively [5]. Data collection was performed on beamlines BL32XU at SPring-8, X06SA at the Swiss Light Source, and I24 at the Diamond Light Source. X-ray diffraction data were collected by the helical data collection method with a beam size of $1 \times 10 \mu\text{m}$ (horizontal \times vertical) on beamline BL32XU.

The AdipoR proteins contain the N-terminal intracellular region (NTR), a short intracellular helix (helix 0), a seven transmembrane (7TM) domain, and the C-terminal extracellular region (CTR) (Fig. 2). The Fv fragment is bound to the NTR. The seven transmembrane helices (I–VII) are connected by three intracellular loops (ICL1–3) and three extracellular loops (ECL1–3). ECL3 has a central short α helix, while ICL3 has another short α helix immediately following helix VI.

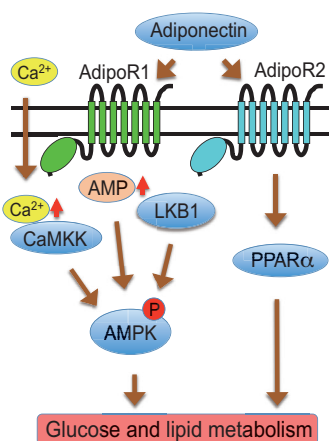


Fig. 1. Schematic representation of adiponectin signaling. Adiponectin induces extracellular Ca^{2+} influx by AdipoR1, which is necessary for the subsequent activation of Ca^{2+} /calmodulin-dependent protein kinase kinase (CaMKK). Adiponectin also increases the concentration of cellular AMP. Both CaMKK and AMP/LKB1 are necessary for adiponectin-induced full AMPK activation via AdipoR1. Adiponectin activates PPAR α pathways via AdipoR2. Thereby, they regulate glucose and lipid metabolism.

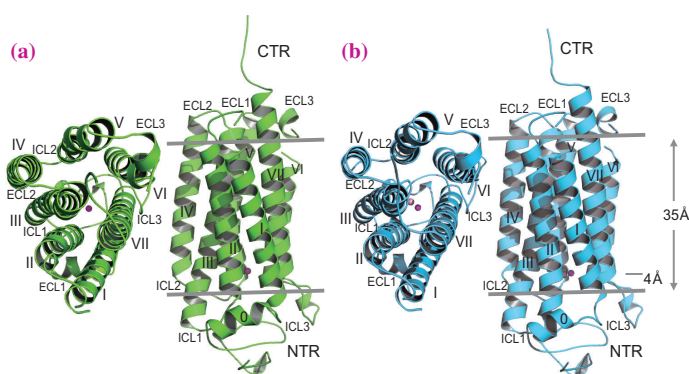


Fig. 2. Overall structures of AdipoR1 and AdipoR2. (a) Structure of AdipoR1 with 2.9 Å resolution. (b) Structure of AdipoR2 with 2.4 Å resolution. The structures of their complexes with Fv fragments were determined, but the Fv fragments are omitted here for clarity. The structures are viewed from the extracellular side (left) and parallel to the membrane (right). The NTR, helix 0, transmembrane helices I–VII, the ECL, the ICL, and the CTR of AdipoR1 (a) and AdipoR2 (b) are indicated. A zinc ion and a water molecule are represented by magenta and pink spheres, respectively.

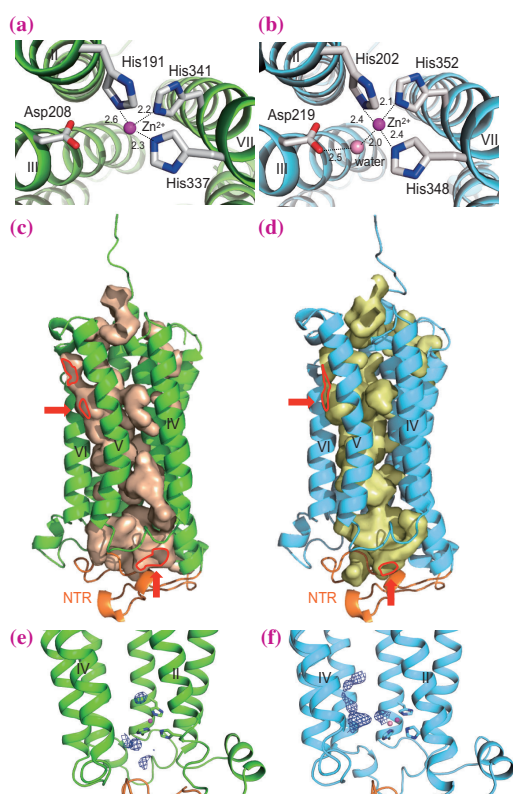


Fig. 3. The zinc-binding sites and cavities of AdipoR1 and AdipoR2. **(a, b)** Coordination of the zinc ion (magenta sphere) by three His residues of AdipoR1 **(a)** and AdipoR2 **(b)**, viewed from the cytoplasmic side. A water molecule (pink sphere) is also coordinated to the zinc ion and is fixed by D219 in AdipoR2. **(c, d)** Cavities of AdipoR1 **(c)** and AdipoR2 **(d)**. The red arrows indicate the openings of the cavities. **(e, f)** Extra electron density maps in the cavities of AdipoR1 **(e)** and AdipoR2 **(f)**, contoured at 0.5 s and 1 s, respectively. The NTR is colored orange. The openings of the cavities are bordered in red.

The seven transmembrane helices of AdipoR form a helix bundle. As viewed from the outside of the cell, the seven transmembrane helices in the helix bundle are arranged circularly in a clockwise manner from helix I to helix VII (Fig. 2). The structures of the AdipoR1•Fv complex and the AdipoR2•Fv complex are quite similar. As compared with the GPCR structures, the AdipoR1/R2 structures lack kinked transmembrane helices, while helix V is slightly curved due to the three Gly residues (Figs. 3(c,d)). In addition, a DALI search revealed that the AdipoR1 and AdipoR2 structures share no similarity with other protein structures in the Protein Data Bank. Consequently, we concluded that the AdipoR1 and AdipoR2 structures are novel.

AdipoR1 and AdipoR2 have two major characteristic structures in their transmembrane domain. One is a zinc ion and the other is a cavity. We found a zinc ion bound within the 7TM domain at a site located in the inner membrane leaflet. The zinc ion is

coordinated by three His residues of AdipoR1/R2 at zinc–nitrogen distances of 2.1–2.6 Å (Figs. 3(a,b)). The zinc ion is thus located approximately 4 Å deep from the inner surface of the plasma membrane (Fig. 2). Furthermore, a water molecule is bound between the zinc ion and the side-chain carboxylate group of D219 of AdipoR2. Thus, the zinc ion has a tetrahedral coordination. We also found a large internal cavity surrounded by the 7TM helices, including the zinc-binding site, in both the AdipoR1 and AdipoR2 structures (Figs. 3(c,d)). The cavities extend from the cytoplasmic surface to the middle of the outer lipid layer of the membrane, and contain unidentified extra electron densities, which are weaker than those of the protein (Figs. 3(e,f)). In the cavity of AdipoR2, extra electron densities are observed along with helices III, V, and VI. In contrast, in the cavity of AdipoR1, even weaker electron densities are observed on the cytoplasmic side of the cavity. These weak electron densities might be relevant to the substrates/products of the hypothesized hydrolytic activities of AdipoR1/AdipoR2.

In summary, this study revealed that the structural and functional characteristics of AdipoR1 and AdipoR2 are completely different from those of GPCRs, and therefore the AdipoRs represent an entirely novel type of receptor. The present crystal structures are expected to provide a strong basis for the development and optimization of adiponectin agonists, such as AdipoRon.

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