Structures of Human PPARα with All Clinically Approved Fibrates and Endogenous Fatty Acids Revealed by X-Ray Crystallography

Fibrates are the most popular class of lipid-lowering medications next to statins; however, how they interact with their molecular target, peroxisome proliferator-activated receptor α (PPARα), had not yet been elucidated. Using sophisticated crystallographic techniques and X-ray crystallography, we succeeded in obtaining 34 novel high-resolution (mostly between 1.23 Å and 2.43 Å) PPARα-ligand domain structures in complexes with 17 PPARα ligands, including all clinically approved fibrates, endogenous fatty acids, and other synthetic PPARα agonists.

PPARα, the master regulator of lipid metabolism (or metabolism in general) that is activated upon fasting in liver, kidney, and other tissues, and regulates the expression of hundreds of genes that encode proteins involved in β-oxidation, ketogenesis, gluconeogenesis, and other metabolic pathways [1]. Most clinically approved fibrates were developed in the 1960s–1980s before their molecular target, PPARα, was identified. As of November 2020, only 21 records of PPARα structures have been deposited in the PDB, in contrast to 224 for PPARδ [2].

Most clinically approved fibrates were α–fibrates co-crystal structures (B) (Fig. 2). As a result, we could obtain 34 PPARα-LBD co-crystals with 17 PPARα ligands, and analyzed them by 1.0 Å X-ray diffraction at four available beamlines (BL-5A, BL-17A, and AR-NE3A at Photon Factory, and BL26B1 at Spring-8) [3]. A single molecule of pematibrate or GW7647 binds to Center, Arm II, and Arm III regions, whereas two molecules of fenofibric acid, ciprofibrate, or clofibrate acid to Arm I and Arm IIX boundary, and a single molecule of bezafibrate or saroglitazar binds to Center and Arm II (Fig. 1A). Among fibrates, only 2-amino benzoic acid moiety of pematibrate, the recently developed PPARα-selective and most potent PPARα agonist, is located at Arm III [Fig. 1B].

Endogenous abundant fatty acids such as palmitic acid and stearic acid that might be released from lipid stores upon nutritional deprivation could be endogenous PPARα ligands. We revealed that a single molecule of palmitic acid, stearic acid, oleic acid, arachidonic acid, and EPA (as well as synthetic PPAR pan fatty acid agonists like TTA and EYA) binds to the similar Center and Arm II regions (Fig. 2A and B). Interestingly, only palmitic acid and stearic acid could activate PPARα in the coactivator recruitment assay [3]. Since both fatty acids are abundant and released to the human circulation with ~100 μM concentrations upon fasting [3], they could be endogenous PPARα ligands.

Thiazolidinedione (glitazone)-class PPARγ agonists are clinically used as anti-diabetic drugs, and PPARγ agonists as well as PPARδ agonists are expected to be used as anti-metabolic disease drugs. Therefore, the present results deepen our understanding of PPARα ligand recognition and will contribute to the molecular design of next-generation PPAR-targeted drugs. All 34 novel structures have been deposited in the PDB.

REFERENCES

BEAMLINES
BL-5A, BL-17A and AR-NE3A

S. Kamata, T. Oyama and I. Ishii (Showa Pharm. Univ., Yamashina Univ.)

Figure 1: Crystal structures of PPARα-ligand-binding domain and PPARα agonists. (A) Magnified views of five fibrates, GW7647 (PPARα-selective agonist), and saroglitazar (PPARα/δ dual agonist) in PPARα-LBD. PDB identities and resolutions are labeled. (B) A superimposed image of five PPARα-LBD-fibrate structures. The ligand-binding pocket (gray) consists of Arm I–III, Arm X, and Center regions, involving at five fibrates in it.

Figure 2: Crystal structures of PPARα ligand-binding domain and fatty acid ligands. (A) Magnified views of five endogenous fatty acids and two synthetic fatty acid ligands (TTA and EYA; PPAR pan agonists) in PPARα-LBD. PDB identities and resolutions are labeled. (B) A superimposed image of five PPARα-LBD-endogenous fatty acid structures. All fatty acids are located at similar positions in Center and Arm II regions of the ligand-binding pocket (gray).