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 crystallization techniques and X-ray crystallography, we succeeded in obtaining 34 novel high-resolution (mostly between 1.23 Å and 2.43 Å) PPARα ligand-binding domain structures in complexes with 17 PPARα ligands, including all clinically approved fibrates, endogenous fatty acids, and other synthetic PPARα agonists.

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptor-type transcription factors with three cognate subtypes (α, δ/β, and γ). PPARα is 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 PPARγ and 44 for PPARδ, probably due to the difficulty in obtaining apo (ligand-free) forms of PPARα-ligand-binding domain (LBD). None of the PPARα–fibrate co-crystal structures had been obtained and structural information regarding their molecular interactions had been completely lacking. With this background, we aimed to obtain the co-crystal structures of PPARα-LBD–all six clinically approved fibrates (pemafibrate, fenofibric acid [active metabolite of fenofibrate], bezafibrate, ciprofibrate, clofibrate, and gemfibrozil) to gain molecular insights into the ligand recognition to facilitate the design of better drugs targeting PPARα, or two or three PPAR subtypes (as dual/pan agonists).

We first prepared human PPARα-LBD recombinant proteins of high purity using three-step column chromatography (cobalt affinity, anion-exchange, and then gel-filtration) according to the method described by Oyama *et al.* [2]. Next, using those purified proteins and various PPARα ligands, we tried various old and new crystallization techniques such as co-crystallization, cross-seeding, soaking, delipidation, coactivator peptide supplementation, and their combinations [3, 4]. First, we prepared the co-crystal with Wy14643 (synthetic PPARα agonist) using the previously described condition [5] and obtained its high-resolution (1.82 Å) structure. Using the co-crystal as seed nuclei, we then obtained co-crystals with pemafibrate and GW7647 (both are high-affinity PPARα ligands) as well as an intrinsic fatty acid (iFA) of *E coli*. origin. By soaking PPARα–iFA co-crystals in the buffer containing other ligands (that have high affinities to PPARα) of high concentrations, we next obtained co-crystals with GW7647 and pemafibrate as well as medium-class affinity PPARα ligands: ETYA (5,8,11,14-eicosatetraynoic acid), TTA (tetradecylthioacetic acid), Wy14643, and saroglitazar.

To obtain co-crystals with lower-class affinity PPARα ligands (lower than iFA), we removed ~80% of lipid components (of *E coli.* origin) from PPARα–iFA preparations by ethanol extraction. Using the delipidized PPARα-LBD (after renaturation), we obtained co-crystals with fenofibric acid, ciprofibrate, and gemfibrozil (only in the presence of GW9662). Furthermore, by applying cross-seeding methods on delipidized PPARα-LBD, we then obtained co-crystals with lower affinity ligands such as endogenous fatty acids: palmitic acid, stearic acid, oleic acid, arachidonic acid, and eicosapentaenoic acid (EPA). To obtain co-crystals with clofibric acid and bezafibrate, we incubated delipidized PPARα-LBD with clofibric acid and the SRC1 coactivator peptide (to further stabilize its active form) and obtained their co-crystals. Finally, using those delipidized PPARα-LBD/clofibric acid/SRC1 co-crystals as seed nuclei, we finally obtained co-crystals with bezafibrate.

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 pemafibrate or GW7647 binds to Center, Arm II, and Arm III regions, whereas two molecules of fenofibric acid, ciprofibrate, or clofibric acid bind to Arm I and Arm II/X boundary, and a single molecule of bezafibrate or saroglitazar binds to Center and Arm II [**Fig. 1(A)**]. Among fibrates, only 2-aminobenzoxazole moiety of pemafibrate, the recently developed PPARα-selective and most potent PPARα agonist, is located at Arm III [**Fig. 1(B)**].

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 ETYA) binds to the similar Center and Arm II regions [**Fig. 2(A)** 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 PPARδ agonists as well as PPAR dual/pan 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.

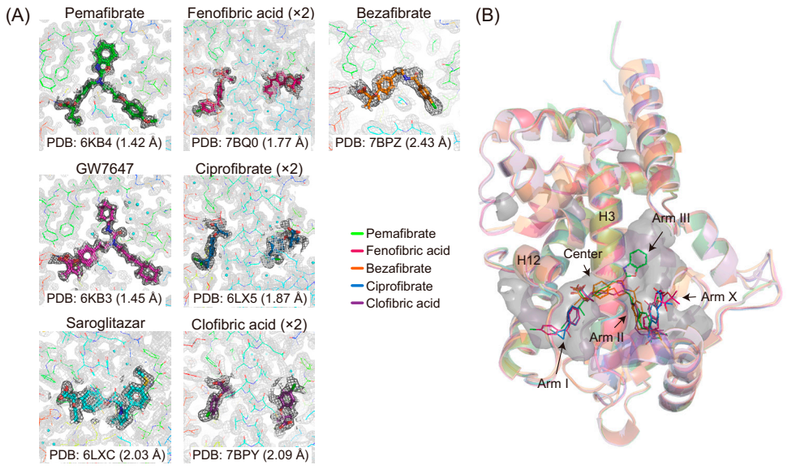

Figure 1: Crystal structures of PPARα ligand-binding domain and PPARα agonists. **(A)** Magnified views of five fibrates, GW7647 (PPARα-selective agonist), and saroglitazar (PPARα/γ dual agonist) in PPARα-LBD. PDB identities and resolutions are labeled. **(B)** A superimposed image of five PPARα-LBD–fibrate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

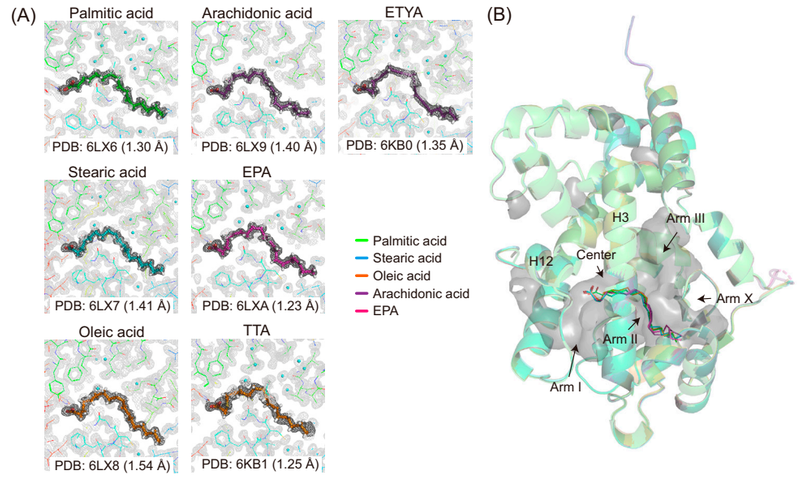

Figure 2: Crystal structures of PPARα ligand-binding domain and fatty acid ligands. **(A)** Magnified views of five endogenous fatty acids and two synthetic fatty acid ligands (TTA and ETYA; PPAR pan agonists) in PPARα-LBD. PDB identities and resolutions are labeled. **(B)** A superimposed image of five 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).

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BEAMLINES

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

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