Structural basis for the activation of PPARgamma by telmisartan

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Abstarcts

Telmisartan, a selective angiotensin recetpor blocker, has been recently shown to act as a partial agonist for peroxisome proliferator-activated receptor-gamma (PPARγ). To understand the activation mechanism of PPARγ by telmisartan, we determined the ternary complex structure of PPARγ, telmisartan and coactivator peptide from SRC1 at 2.25 Å resolution. The overall fold of PPARγ is almost identical to previously-determined complex structures with agonists. However, telmisartan exhibits an unexpected binding mode, devoid of some essential hydrogen bonds for full activation of PPARγ.

Introduction

Peroxisome proliferator-activated receptor-gamma (PPARγ) belongs to the nuclear hormone receptor superfamily of ligand-activated transcription factors [1]. It has been known to play various important roles in regulation of adipose differentiation, metabolism and inflammation. The PPARγ signaling pathway is also involved in the regulation of some biological processes within the cardiovascular system [1]. The agonists of PPARγ improves insulin sensitivity and inhibits vascular oxidative stress and inflammation [2].

The renin angiotensin system (RAS) plays central role in the regulation of blood pressure. Angiotensin II (Ang II) is the main effector of RAS and acts by binding to angiotensin II receptors. The major functions of Ang II in the cardiovascular system are mediated by the angiotensin II type 1 receptor (AT-1R) [3]. Telmisartan is a selective AT-1R blocker (ARB), which is widely used to treat congestive heart failure and diabetic nephropathy [4].

Numerous reports demonstrate that telmisartan has additional PPARγ partial agonist activity independently of AT-1R blocking effects. Indeed, telmisartan reduces glucose, insulin, and triglyceride levels in a rat model of insulin resistance [5]. Telmisartan's dual modes of action are expected to provide additional benefits, such as anti-inflammatory, anti-oxidative, and anti-proliferative effects against atherosclerosis and cardiac infarction.

Molecular modeling studies reported a structural similarity between telmisartan and PPARγ agonists, pioglitazone and rosiglitazone [5]. However, actual structural mechanism of telmisartan's PPARγ agonism is unknown. Here we present a crystal structure of human PPARγ in complex with telimsartan and reveal the mode of activation.

Results and Discussion

To understand the activation mechanism of PPARγ by telmisartan, we determined its crystal structure at 2.25 Å resolution as a ternary complex with a coactivator peptide from SRC1. The structure was solved by the molecular replacement using an in-house structure of the PPARγ-LBD as a search model. Telmisartan was clearly observed in the difference Fo-Fc electron density map after rigid body refinement and first restrained refinement. The overall fold is almost identical to previously-determined complex structures or apo structures [6-10]. Superpositions of all atoms result in an root mean SD of 0.58 Å for the structure complxed with rosiglitazone (2PRG) , 0.66 Å for that with 2-BABA (1WM0), and 0.94 Å for the apo structure (1PRG)

In other known PPARγ-agonist complex structures, carboxylate group or its bioisoster group of agonists has been found to interact with His323, His449 and Tyr473 via hydrogen bonds. These hydrogen bonds have been supposed to play an essential role in an agonistic activity by anchoring helix 12 in a proper position and enabling a coactivator peptide binding. Previous docking simulation also predicted the similar interaction between telmisartan and PPARγ [11]. Surprisingly, the carboxylate group of telmisartan does not interact with these residues but occupies the region close to Arg288 (Fig. 1). Instead of the carboxylate group, the central benzimidazole group of telmisartan forms a hydrogen bond with Tyr473 in helix 12 of PPARγ . On the other hand, hydrogen-bondings with His323 and His449 are not observed in PPARγ-telmisartan. Instead, the side chain of His323 is pushed out from the position it occupies in other complex structures and the propyl moiety in the central benzimidazole group fills the newly formed region. In addition, the other benzimidazole group interacts with Phe363 in helix 9 via π-π stacking. These interactions

seem to make up for the loss of hydrogen bonds with His323 and His449 and increase the affinity between telmisartan and PPARγ.

In conclusion, the ternary complex structure of PPARγ, SRC1 peptide and telmisartan reveals the mechanism of PPARγ activation by telmisartan. The activation is mainly caused by the characteristic structure of telmisartan, two directly-linked benzimidazole groups, which can explain telmisartan's unique dual modes of action.

Materials and Methods

Crystallization

The human PPARγ-LBD (225-505) was expressed in E. coli as N-terminal His tagged protein. Affinity purification was performed with a Ni^{2+}-coupled Sepharose and the His-tag was removed by incubation with thrombin. Further purification was performed by ion exchange chromatography and gel filtration. The purified protein was concentrated to 16 mg/ml. Before crystallization, telmisartan and a short peptide from human SRC1 (residues 685-700, ERHKILHRLLQEGSPS) were added to a final concentration of 0.5mM and 1mM, respectively. After incubation of one hour the solution was mixed to an equal volume of 0.1M Tris-HCl (pH7.0), 0.8M sodium citrate tribasic dihydrate and crystallized using sitting drop-vapor diffusion at 293K. Thick plate-like crystals were appeared after 3-5 days.

Data collection and Structure determination

For data collection, crystals were transferred into cryoprotectant solution containing 30% v/v glycerol and flash frozen at 100K. Data were collected at the Photon Factory AR-NE3A beamline (Tsukuba, Japan) and processed with HKL2000 [12]. The structure of human PPARγ-LBD was solved by molecular replacement using MOLREP [13, 14]. Crystals belong to the orthorhombic space group $P2_12_12$ with cell axes of a = 132.5 Å, b = 53.9 Å, and c = 54.0Å. Data were refined by rigid body and restrained refinement with REFMAC [15, 16] against an existing in-house structure

of the human PPARγ-LBD. Difference electron density was used to place the ligand by real space refinement. Manual rebuilding of protein was done with Coot [17, 18]. The final structure consists of the LBD with the exception of the region between residue 262 and 274, the coactivator peptide (residues 686–696) and 253 water molecules. Data collection and refinement statistics are given in Table 1. The coordinates for the complexes described in the paper have been deposited in the PDB (3AQH).

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Table 1

Diffraction Data	
Crystal	PPARγ/Telmisartan/SRC1 peptide
Source	PFAR-NE3A
Wavelength (Å)	1.000
Space group	P21212
Lattice parameters	a = 132.8 Å, b = 53.5 Å, and c = 53.9 Å
Resolution (Å)	20–2.25
Completeness (%)	93.0
Rsym[a] (%)	6.8
Refinement Statistics	
R_{work}[b] (%)	26.5
R_{free}[b] (%)	32.6
Number of Nonhydrogen Atoms Used in Refinement	
Protein	2,190
Heterogen	39
Solvent	28
Rmsd bonds (Å)	0.023
Rmsd angles (°)	2.0
Average B factor (Å^2)	55.4

R_{work} is calculated from a set of reflections in which 5% of the total reflections have been randomly omitted from the refinement and used to calculate R_{free}.

a $$R_{sym} = \frac{\sum_{hkl}\sum|I(h,k,l,i) - \langle I(h,k,l)\rangle|}{\sum_{hkl}\sum I(h,k,l,i)}$$

b $$R = \frac{\sum_{hkl}||F_{obs}(h,k,l)| - k|F_{calc}(h,k,l)||}{\sum_{hkl}|F_{obs}(h,k,l)|}$$

Figure 1 The binding mode of telmisartan