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Atomic structure of mutant PPARγ LBD complexed with 15d-PGJ$_2$: Novel modulation mechanism of PPARγ/RXRα function by covalently bound ligands

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ABSTRACT

15-deoxy-Δ^{12,14}-prostaglandin J$_2$ (15d-PGJ$_2$) activates a nuclear receptor heterodimer, peroxisome proliferators-activated receptor γ (PPARγ)/ retinoid X receptor (RXRα) through covalent binding to Cys285 in PPARγ ligand-binding domain (LBD). Here, we present the 1.9 Å crystal structure of C285S mutant LBD complexed with 15d-PGJ$_2$, corresponding to the non-covalently bound state. The ligand lies adjacent to a hydrogen-bond network around the helix H2 and the nearby β-sheet. Comparisons with previous structures clarified the relationships between PPARγ function and conformational alterations of LBD during the process of covalently binding ligands, such as 15d-PGJ$_2$, and thus suggested a mechanism, by which these ligands modulate PPARγ/RXRα function through conformational changes of the loop following helix H2′ and the β-sheet.

1. Introduction

Peroxisome proliferators-activated receptor γ (PPARγ), which belongs to a nuclear receptor superfamily, functions as obligate heterodimers with retinoid X receptors (RXRs) [1,2]. This receptor is particularly noteworthy, because it participates in various biological phenomena, such as insulin sensitization, adipogenesis, atherosclerosis, inflammation, and carcinogenesis [1,3–7].

PPARγ is activated by physiological fatty-acid derivatives, including nitrated or oxidized fatty acids [8–13]. Several polyunsaturated lipids, such as 15-deoxy-Δ^{12,14}-prostaglandin J$_2$ (15d-PGJ$_2$), activate the receptor-mediated transcription by covalently binding to a unique cysteine (Cys285) in the ligand-binding domain (LBD) [14,15]. Our recent study revealed the following facts [16]; (1) the covalent binding to endogenous fatty acids, such as 15d-PGJ$_2$, results in the structural alteration of the loop following helix H2′ and in the rearrangement of the side-chain network around Cys285 within LBD; (2) the conformational difference of the loop provides the ligand-type specific effects in term of the distinct degree of the receptor activity; (3) Phe287, one of these repositioned residues, has an important role in receptor activation. However, it has been unclear how the conformational transmission by non-covalent binding to these ligands modulates receptor function before covalent bond formation, since the atomic details of the non-covalently bound state were unavailable.

In this study, we determined the 1.9Å crystal structure of C285S mutant LBD, which is bound to 15d-PGJ$_2$ in the covalent modification-independent manner. The ligand mainly interacts with the residues on helices H3, H5, and H12, and it is located in the vicinity of the network of water molecules formed around the helix H2 and the nearby β-sheet. Comparison of crystal structures of the three distinct binding states to 15d-PGJ$_2$ [16] showed that the conformation of the loop following helix H2′ changes by non-covalent binding, and subsequently the side-chain networks around Cys285, particularly Phe287, are rearranged by covalent binding. This result indicated that covalently bound ligands might sequentially direct the ligand-type specific effects and the switching-on of PPARγ activation, through the two distinct structural alterations. Furthermore, the superposition of the present structure on the crystal structure of intact PPARγ/RXRα complexed with DNA [17] suggested intriguing roles of the 15d-PGJ$_2$ binding pocket in coactivator recruitment and interactions with the DNA-binding domain (DBD) of RXRα.

Abbreviations: PPARγ, peroxisome proliferators-activated receptor γ; RXR, retinoid X receptor; 15d-PGJ$_2$, 15-deoxy-Δ^{12,14}-prostaglandin J$_2$; LBD, ligand-binding domain; LBP, ligand-binding pocket; DBD, DNA-binding domain

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2. Materials and methods

2.1. Protein preparation

The plasmids and recombinant proteins of the wild type and C285S mutant human PPARγ LBDs (hereafter referred to as wtLBD and mtLBD, respectively), were prepared as described previously [15,16]. Using UV absorption spectral analyses [15], we confirmed that the mtLBD did not bind covalently to 15d-PGJ_2 (data not shown).

2.2. Crystallization, data collection, and model refinement

To avoid variability derived from crystallization conditions and different crystal packing, we first crystallized the mtLBD without the ligand, under the conditions reported previously [16], and then soaked the crystals with 15d-PGJ_2. The diffraction data were collected at BL38B1 in SPring-8 (Harima, Japan), as described previously [16]. All data were processed using HKL2000 [18]. The structure was solved by the molecular replacement method, using the wtLBD covalently bound to 15d-PGJ_2 (PDB ID 2ZK1) as a search model [16]. The model refinement was carried out using CNS and O [19,20]. The crystallographic data and refinement statistics are summarized in Supplementary Table 1. All structural figures were made with Chimera [21]. Since helix H12 in monomer B was frequently disoriented, depending upon the crystal packing, we omitted monomer B from the figure. Coordinates and structural factors have been deposited in the Protein Data Bank (PDB ID 2ZVT).

3. Results

3.1. Structure of C285S mutant PPARγ LBD complexed with 15d-PGJ_2

The structure of mtLBD complexed with 15d-PGJ_2 was refined at 1.9Å resolution (Fig. 1A). The final model of the complex presented the clear density of 15d-PGJ_2 (Fig. 1B). This fatty-acid ligand was found to interact mainly with helices H3, H5, and H12, and was located in the close vicinity of the network of water molecules that adjoins helix H2 and the nearby β-sheet consisting of β2 and β3. Notably, most of the water molecules within ligand-binding pocket (LBP) are involved in this network (Fig. 1C and D). This result indicates that the LBP is divided into hydrophobic and hydrophilic regions. Hydrogen/deuterium exchange mass spectrometry experiments revealed that amino acid residues, facing this hydrogen-bond network, generally exhibit higher exchange rates than the other residues [17,19]. The clustering of water molecules may play some role in enhancing the hydrogen/deuterium exchange rates.

3.2. Structural differences among distinct 15d-PGJ_2-binding states of PPARγLBD

To clarify the structure-function relationships of the state with non-covalently bound 15d-PGJ_2, we compared the previously published crystal structures of the unliganded wtLBD (PDB ID 2ZK0) and the wtLBD covalently bound to 15d-PGJ_2 (PDB ID 2ZK1) [16] with the present structure. We first focused on the position and

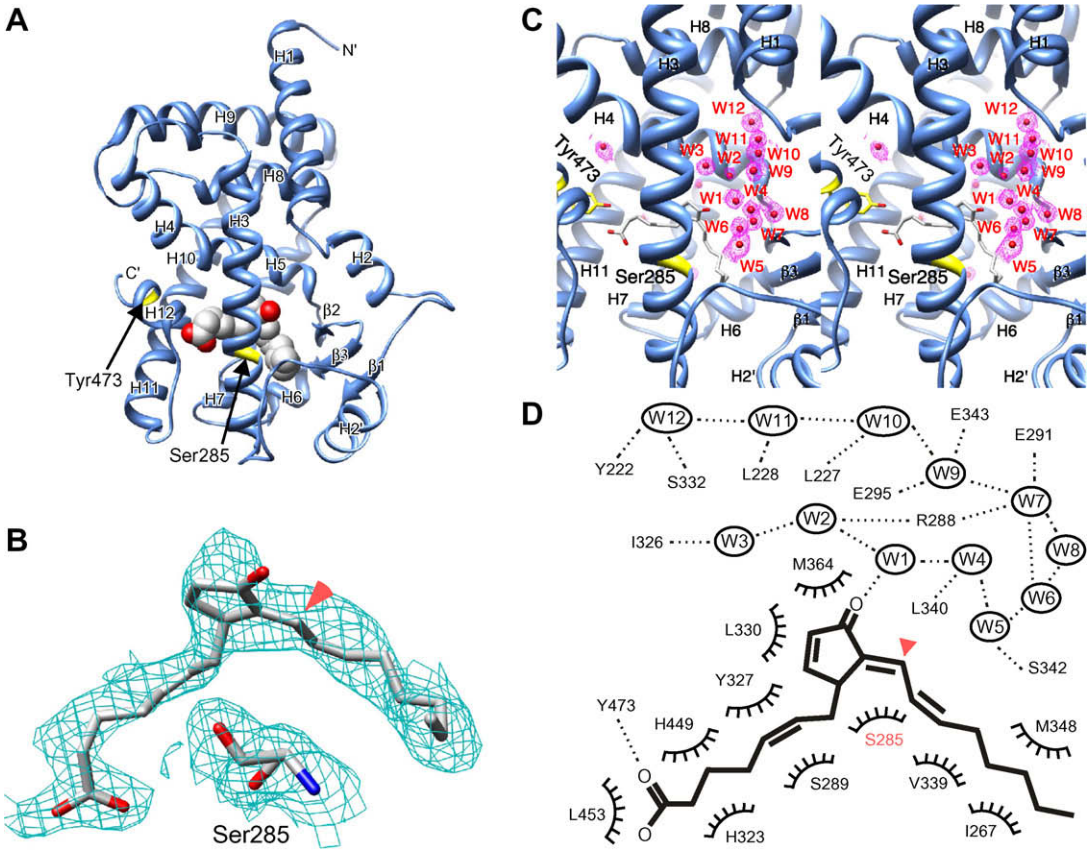

Fig. 1. Structure of C285S mutant PPARγ LBD complexed with 15d-PGJ_2. (A) Overall structure. The Cα atoms of mtLBD are modeled as blue ribbons. The non-covalently bound ligand is depicted as a space-filling model. (B) The composite omit 2|Fo|-|Fc| electron density map of 15d-PGJ_2 (contoured at 1σ). The ligand and the Ser285 residue are represented as stick models. The arrowhead indicates a carbon atom that is potentially covalently bound to Cys285. (C) Close-up stereo view of LBP. Water molecules in LBP are represented as spheres, with the composite omit 2|Fo|-|Fc| electron density map contoured at 1.0σ. (D) Schematic representation of the interaction between mtLBD and 15d-PGJ_2. The ligand is shown by black lines. Hydrophobic interactions are indicated by arcs, and hydrogen bonds are depicted by dashed lines. Ser285 is colored by red. Key water molecules are represented by "w", and are numbered.