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Molecular association model of PPARα and its new specific and efficient ligand, pemafibrate: Structural basis for SPPARMα

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ABSTRACT

Peroxisome proliferator-activated receptor-α (PPARα) is a ligand-activated transcription factor involved in the regulation of lipid homeostasis and improves hypertriglyceridemia. Pemafibrate is a novel selective PPARα modulator (SPPARMα) that activates PPARα transcriptional activity. Here, we computationally constructed the structure of the human PPARα in a complex with pemafibrate, along with that of hPPARα complexed with the classical fenofibrate, and studied their interactions quantitatively by using the first-principles calculations-based fragment molecular orbital (FMO) method. Comprehensive structural and protein-ligand binding elucidation along with the *in vitro* luciferase analysis let us to identify pemafibrate as a novel SPPARMα. Unlike known fibrate ligands, which bind only with the arm I of the Y-shaped ligand binding pocket, the Y-shaped pemafibrate binds to the entire cavity region. This lock and key nature causes enhanced induced fit in pemafibrate-ligated PPARα. Importantly, this selective modulator allosterically changes PPARα conformation to form a brand-new interface, which in turn binds to PPARα co-activator, PGC-1α, resulting in the full activation of PPARα. The structural basis for the potent effects of pemafibrate on PPARα transcriptional activity predicted by the *in silico* FMO methods was confirmed by *in vitro* luciferase assay for mutants. The unique binding mode of pemafibrate reveals a new pattern of nuclear receptor ligand recognition and suggests a novel basis for ligand design, offering cues for improving the binding affinity and selectivity of ligand for better clinical consequences. The findings explain the high affinity and efficacy of pemafibrate, which is expected to be in the clinical use soon.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily comprising three subtypes: PPARα, PPARβ/δ, and PPARγ. They share a common structural organization, composed of a variable N-terminal domain harboring a ligand-independent activation function, a conserved DNA-binding domain, and a C-terminal ligand-binding domain (LBD),

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which contains the ligand-dependent activation function 2 (AF-2) [1]. Activation of PPARα by its agonists triggers conformational changes in PPARα—including stabilization of the extreme C-terminal helix H12 (AF-2 interface) to an active position—and enhances heterodimerization with the RXRα [2], promoting recruitment of nuclear coactivators and ultimately interacts with DNA binding sites designated as PPAR response elements to regulate target gene transcription. Since PPARs are involved in transcription of genes related to the cellular proliferation and differentiation, immune responses and metabolism of carbohydrates and lipids, they are targeted for the treatment of diabetes and metabolic and other related diseases. Thus, PPAR agonists are considered important tools to treat diabetes and metabolic syndrome.

Many synthetic and natural PPAR ligands have been identified [3]. Among others, hypolipidemic fibrate-class drugs ubiquitously activate PPARα, which controls lipid flux in the liver by modulating fatty acid transport and β-oxidation, and improves plasma lipid profiles by decreasing triglyceride (TG) and increasing high-density lipoprotein (HDL) cholesterol levels in patients with hyperlipidemia and type 2 diabetes and can prevent coronary heart disease and stroke [4]. Nevertheless, there are some limitations for efficacy of these fibrates related to their weak activity on PPARα and dose related adverse effects [5].

In contrast, pemafibrate (Fig. 1a), a newly identified novel highly potent selective PPARα modulator (SPPARMα), found enhancing PPARα activity strongly and specifically [6]. It exerted beneficial effects on lipid metabolism, reverse cholesterol transport and inflammation resulting in anti-atherogenic properties and in overall it has higher transcription efficacy than the clinically used fibrates have [7]. This modulator also showed robust TG-lowering effects without increasing adverse drug reactions in dyslipidaemic subjects with elevated TG and low HDL cholesterol [8]. An earlier study [9] revealed that pemafibrate causes higher PPARα activation than other fibrates, therefore, it is designated as SPPARMα. Recently we compared the effects of pemafibrate with those of classical PPARα agonists and found that pemafibrate activates the PPARα transcription activity more effectively than the considered classical agonists [10].

Nonetheless, the structure of the LBD of PPARα (hereafter, referred as structure of PPARα for simplicity) complexed with pemafibrate remains unknown. Due to the presence of flexible Ω-loop in the LBD region that creates the instability, obtaining the structure of PPARα, experimentally, is highly demanding. However, knowing the complex structure is indispensable to understand the structural basis for the mode of action, which ultimately help to design better ligands with improved binding affinity and selectivity.

To uncover the molecular basis of pemafibrate regulating PPARα activity, here we obtained the structure of the pemafibrate-bound PPARα using the *in silico* molecular simulation combined with quantum-mechanics/molecular-mechanics (QM/MM) calculations. Then, by using the first-principles calculations-based fragment molecular orbital (FMO) method [11], we determined the novel binding pattern of this modulator in the LBD of PPARα. FMO calculations helped to obtain the interactions quantitatively. Subsequently, the binding of the complex PPARα-pemafibrate to PPAR gamma coactivator 1 alpha (PGC-1α) was studied in detail. In parallel with these *in silico* investigations, the *in vitro* luciferase analysis was carried out to confirm the theoretical predictions on the *in vivo* cell-basis. For comparison, all the above-mentioned *in silico* and *in vitro* studies were conducted on the fenofibrate-bound PPARα too.

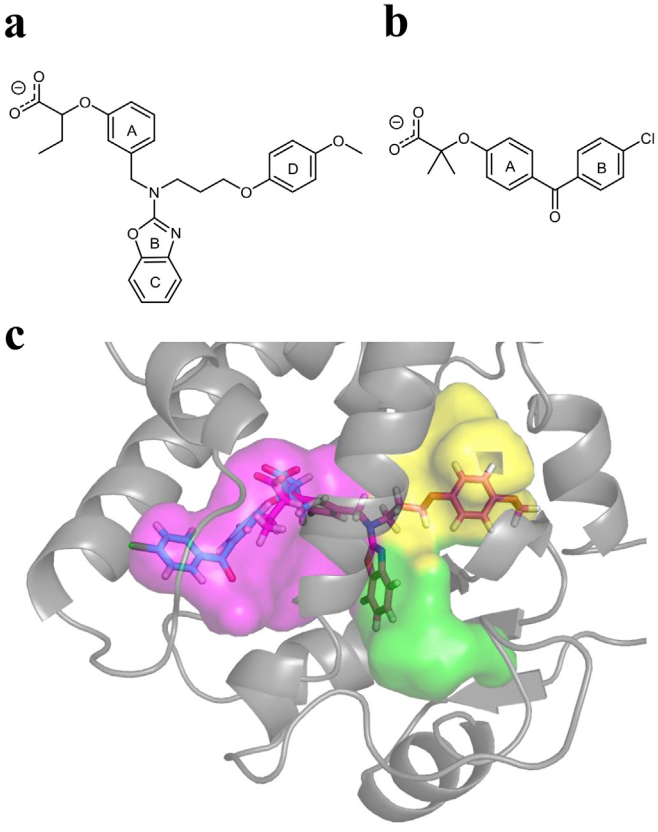

Fig. 1. The structures of **(a)** pemafibrate and **(b)** fenofibrate when bound in PPARα. The rings are labelled as A, B, C, and D. **(c)** Binding mode of the ligands with human PPARα. Pemafibrate in magenta and fenofibrate in blue. The binding pocket is divided into three pharmacophore regions according to the interactions with the ligands.

2. Materials and methods

2.1. Constructing the structural model

The complex structure of the nuclear receptor PPARα and the coactivator PGC-1α was constructed using Molecular Operating Environment (MOE) program [12] by combining the X-ray structures of the complexes PPARα + ligand GW409544 + coactivator motif, LXXLL peptide, derived from the steroid receptor coactivator 1, SRC1 (PDB code 1K7L), [13] and the PPARγ + ligand rosiglitazone + PPARγ coactivator 1α, PGC-1α (PDB code 3CS8), [14]. The original ligand, GW409544, was replaced by either fenofibrate or pemafibrate using careful docking procedures. The preliminary structure optimization was performed using molecular mechanics calculations utilizing the Amber10EHT force-field with solvation energy accounted via the Born model. The constructed structure was subjected to molecular dynamics (MD) simulations up to 100 ns to analyse the stability of the modelled structure. The MD simulations were performed with explicit solvent water molecules. For all the MD simulations, AMBER 14 was used [15] with Amber ff14SB force field for the protein and the TIP3P water model for the solvent. Calculations were run at 300 K and a pressure of 1 bar, with the NPT ensemble.

2.2. QM/MM calculations

The protein-ligand complex structures were fully optimized at QM/MM hybrid level of theory using NWChem program [16]. For