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A single amino acid change humanizes long-chain fatty acid binding and activation of mouse peroxisome proliferator-activated receptor α

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ABSTRACT

Peroxisome proliferator-activated receptor α (PPARα) is an important regulator of hepatic lipid metabolism which functions through ligand binding. Despite high amino acid sequence identity (>90%), marked differences in PPARα ligand binding, activation and gene regulation have been noted across species. Similar to previous observations with synthetic agonists, we have recently reported differences in ligand affinities and extent of activation between human PPARα (hPPARα) and mouse PPARα (mPPARα) in response to long chain fatty acids (LCFA). The present study was aimed to determine if structural alterations could account for these differences. The binding of PPARα to LCFA was examined through in silico molecular modeling and docking simulations. Modeling suggested that variances at amino acid position 272 are likely to be responsible for differences in saturated LCFA binding to hPPARα and mPPARα. To confirm these results experimentally, LCFA binding, circular dichroism, and transactivation studies were performed using a F272I mutant form of mPPARα. Experimental data correlated with in silico docking simulations, further confirming the importance of amino acid 272 in LCFA binding. Although the driving force for evolution of species differences at this position are yet unidentified, this study enhances our understanding of ligand-induced regulation by PPARα and demonstrates the efficacy of molecular modeling and docking simulations.

1. Introduction

Peroxisome proliferator-activated receptor alpha (PPARα) belongs to the nuclear hormone receptor superfamily of ligand-dependent transcription factors and has emerged as one of the central regulators of nutrient–gene interactions. Structurally similar to other members of the nuclear hormone receptor family, the PPARα protein structure consists of an N-terminal ligand-independent transactivation function (AF-1), a highly conserved DNA binding domain (DBD), a hinge region and the C-terminal ligand binding domain (LBD) containing a ligand-dependent transactivation function (AF-2). The LBD of PPARα constitutes a large hydrophobic ligand-binding pocket (1300–1400 Å^3) that allows interaction with a broad range of natural and synthetic ligands [1,2]. PPARα interacts with a variety of endogenous ligands, including fatty acids and fatty acid metabolites, as well as synthetic compounds such as hypolipidemic fibrate drugs, to regulate cellular processes related to fatty acid metabolism, glucose metabolism, inflammation, differentiation and proliferation [3–6].

While long-chain fatty acids (LCFA) serve as major metabolic fuels and important components of biological membranes, they also play a significant role as signaling molecules and gene regulators in response to food intake and nutritional changes. Recently, we have demonstrated that LCFA and their thioesters (long-chain fatty acyl-CoA; LCFA-CoA) constitute high-affinity endogenous ligands of human PPARα (hPPARα) and mouse PPARα (mPPARα). Such ligand binding induces PPARα conformational changes and increases transactivation, consistent with expectations for an endogenous ligand of a ligand-activated nuclear receptor [7]. Thus, PPARα in conjunction with LCFA and their metabolites could serve to regulate metabolic pathways governing fuel utilization, storage, transport and mobilization. However, we also reported differences in binding affinities and the extent of ligand-induced

Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; DBD, DNA binding domain; LBD, ligand binding domain; RXRα, retinoid X receptor alpha; LCFA, long-chain fatty acids; LCFA-CoA, long-chain fatty acyl-CoA; mPPARα, mouse PPARα; hPPARα, human PPARα; F272I mPPARα, mutant form of mPPARα that has a Phe to Ile substitution at position 272; 6xhis, polyhistidine tag; PPRE, peroxisome proliferator response element; ACOX, acyl-CoA oxidase.

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transactivation between mPPARα and hPPARα in response to saturated LCFA [7].

Species differences in PPARα-mediated downstream regulation of target genes have been noted previously [8,9]. Human and mouse PPARα proteins promote transcription to a different extent in response to certain hypolipidemic agents and phthalate monoesters [10,11]. Furthermore, it is well established that long-term administration of PPARα agonists result in hepatic cancer in rats and mice – an effect that does not occur in guinea pigs, canines, non-human primates, or even humans [12]. While a single cause for the existence of such differences is highly unlikely, possible explanations include: differences in expression levels of PPARα or differences in PPARα target genes, alternatively spliced or mutant forms of PPARα protein, mutations or polymorphisms in target gene response elements, increased expression of oncogenes and/or inhibition of apoptosis [12–15]. However, transgenic mice that express human PPARα mainly in the liver do not exhibit hepatocarcinogenesis upon administration of PPARα agonists [16,17]. This observation suggests that structural differences in the PPARα protein could be the underlying cause of such species variation.

Comparison of the PPARα amino acid sequence across species, particularly of the LBD, resulted in >90% homology [18]. However it should be noted that a single amino acid change can result in marked alterations in ligand selectivity of nuclear receptors. For example, a single amino acid change in the mouse PPARα-LBD (E282) results in altered activity of the protein [19], and a valine to methionine substitution in human PPARα (V444M) produced PPARδ ligand binding characteristics, resulting in loss of fibrate responsiveness [20]. While we have reported differences in mPPARα and hPPARα in response to saturated LCFA [7], the goal of this study was to explore the mechanisms underlying such divergence. We have used methods including: molecular modeling and in silico docking, mutagenesis, spectrofluorometry, circular dichroism spectroscopy and transactivation studies to identify a single amino acid change at position 272 that is largely responsible for the altered saturated LCFA binding.

2. Materials and methods

2.1. Molecular modeling simulations

The crystal structure of the ligand binding domain (LBD) of hPPARα complexed with a synthetic agonist (GW409544) was retrieved from RCSB Protein Data Bank (PDB identifier) [2]. This structure was chosen due to the completeness of the crystal structure (no missing amino acid side chains). The apo form of hPPARα-LBD was generated by extracting the ligand (GW409544) from the 1K7L model (using Swiss PDB Viewer, http://www.expasy.org/spdbv/). This structural model was used in all docking simulations. Since the structure of mPPARα has not been crystallized, a homology modeling approach was used to generate the mPPARα-LBD structure. We compared the amino acid sequence of hPPARα to mPPARα and substituted all amino acid residues that were different in the hPPARα-LBD crystal structure. In total, 23 amino acid residues in the hPPARα-LBD were replaced with the corresponding mPPARα residues, followed by energy minimization of the resulting model. This model was used as an initial structure of mPPARα-LBD for all docking simulations. All energy computations were done in vacuo using GROMOS96 43B1 parameters without reaction field, implemented in Swiss PDB Viewer [21]. An energy minimized model of the F272I mPPARα-LBD was also generated using the Swiss PDB Viewer (http://www.expasy.org/spdbv/).

2.2. Molecular docking simulations

In silico docking studies were performed using both AutoDock Vina 1.1.2 [22] and the FlexiDock™ module available on SYBYL®-X 2.0 (Tripos, St. Louis, MO). While AutoDock Vina 1.1.2 allows only the ligand to have flexible/rotatable bonds, the FlexiDock™ module on SYBYL®-X 2.0 permits both protein (sidechains) and ligands to carry flexible/rotatable bonds. For docking with both AutoDock Vina 1.1.2 and FlexiDock™, a search space or putative binding site was defined in a restricted region of the protein. In the present study, the ligand binding pocket was defined based on the experimentally obtained structure of the GW409544 ligand bound to hPPARα-LBD [2]. Once the hPPARα and mPPARα models were energy minimized, docking simulations were conducted using both AutoDock Vina 1.1.2 and FlexiDock™. Docking simulations were first validated using the GW409544 ligand by comparing the X-ray crystal structure 1K7L (hPPARα-LBD + GW409554) with that of the docking output generated for apo-hPPARα with GW409544. Both AutoDock Vina 1.1.2 and FlexiDock™ generated multiple docking poses (differentiated by RMSD relative to the best pose) that were subjected to careful visualization, and only the most energetically favorable conformations were chosen for further analysis.

Docking of LCFA was carried out using both AutoDock Vina 1.1.2 and FlexiDock™. For each binding conformation, the binding energies were calculated using the FlexiDock scoring function based on the Tripos Force Field, as implemented by FlexiDock. The resulting docking conformations were visualized using the PyMOL Molecular Graphics System (Version 1.5.0.4 Schrödinger, LLC) and the program LIGPLOT [23]. In order to determine the volume of each ligand binding pocket, the PVOME algorithm was utilized [24]. Based on the occupancy of GW409544 within the hPPARα ligand binding pocket, the ligand binding pocket was defined using 37 overlapping inclusion spheres. This pocket was visualized using the Visual Molecular Dynamics (VMD) program [25], and volume-grid points near the protein atoms were systematically deleted with a padding variable of 1.09 (radius of a hydrogen atom) or 0.5 (half of a carbon-hydrogen bond length) using POVME [24]. This was followed by volume measurement of the resultant binding pocket. This process was then repeated for mPPARα and F272I mPPARα.

2.3. Chemicals

Fluorescent fatty acid (BODIPY-C16) was purchased from Molecular Probes, Inc. (Eugene, OR). Docosahexaenoyl-CoA and BODIPY C16-CoA were synthesized and purified by HPLC as previously described [26] and found to be >99% unhydrolyzed. Rosiglitazone was generously provided by Dr. Khalid Elased (Wright State University). All other putative ligands were from Sigma–Aldrich (St. Louis, MO).

2.4. Purification of recombinant F272I mutant mPPARα protein

The cloning and purification of wild-type 6xHis-GST-mPPARα has been described [7]. A mutant form of full-length mPPARα (amino acids 1–468) in which the phenylalanine residue at 272 in helix 3 was replaced by isoleucine (F272I; to mimic hPPARα) was used for all experiments presented herein. The F272I mutation was generated by overlap PCR of 6xHis-GST-mPPARα using the following primers: 5′-AAGAGAATCCACGAAGCCTA-3′, 5′-GAAGACAAAGAGGCAGAGGT-CCGAATCTT-3′, 5′-GGACATGCACTGGCAGTGGAAGATTCG-3′ and 5′-CCGGGAGCTGCATGTGTCAGAGG-3′. The PCR product contained internal *Sma* I and *Xho* I sites which were used to replace the 3′ half of wild-type mPPARα in the 6xHis-GST-mPPARα vector with the mutated PCR fragment to produce 6xHis-GST-F272ImPPARα. This plasmid was confirmed by sequencing. The full-length