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The conserved residue Phe273(282) of PPARα(γ), beyond the ligand-binding site, functions in binding affinity through solvation effect

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Abstract

Peroxisome proliferator-activated receptors (PPARs) belong to the members of the nuclear receptor superfamily, and play important roles in lipid and glucose homeostasis. Residue Phe282 in PPARγ (Phe273 in PPARα), beyond the ligand-binding site, is a conserved amino acid across several nuclear receptors and in all PPAR subfamily. In this work, we firstly investigated the influence of Phe282(273)Ala mutation on the binding affinity of PPARγ(α) against a series of agonists by use of surface plasmon resonance (SPR) technique and cellular transcriptional activation analysis. Phe282(273)Ala mutation decreases the binding affinities of the ligands to the receptors in certain degrees, from several to 1000-folds. Phe282Ala mutation dramatically reduced the binding affinity of PPARγ to GI262570, however, this mutation did not affect PPARα binding to this ligand, thereby suggesting that the Phe282 and Phe273 are associated with the selectivity of GI262570 binding to PPARγ and PPARα. The mutation reduced the transcriptional activation activities of the receptors induced by the ligand binding, and the decrease degree is generally in agreement with the binding affinities of the ligands to the receptors. The 5 ns MD simulations for the wild-type and mutated PPARγ showed that the mutation did not influence the flexibility of the receptor. There is no repulsion between Phe282 and the proceeding loop of AF2. However, substitution of Phe282 by alanine enlarged the entrance of the binding pocket and abolished the repulsive interaction between solvent water molecules and this hydrophobic residue, thus more water molecules can enter into the binding pocket. It needs more energy to exclude the extra water molecules for a ligand binding to the mutated receptor. In addition, the extra water molecules abolish some of H-bonds between the ligand and receptors. Therefore, solvent effect may be concluded as the major source of the decrease of binding affinity for the mutated receptors to the ligands, and thereby of the decrease of their transcriptional activation activities.

Keywords: Peroxisome proliferator-activated receptor (PPAR); Mutation; Ligand-binding affinity; Solvation effect; Molecular dynamics; Surface plamon resonance (SPR); Transcriptional activation

1. Introduction

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor subfamily of ligand-activated transcription factors [1]. Up to now, three types of PPARs, PPARα, PPARγ and PPARδ (also called PPARβ), have been cloned from mouse and human [2]. The PPARγ has been extensively studied for its significant effects on intra- and extracellular lipid metabolism, glucose homeostasis and adipocyte differentiation; it has been identified as an attractive molecular target in discovering new drugs for the treatment of a series of diseases such as diabetes, dyslipidemia and hypertension [2,3]. Recently, PPARγ was reported to implicate in the pathophysiology of inflammatory, immune responses and aging [4,5], and PPARγ ligands have been shown to be potent inhibitors of angiogenesis, a process necessary for tumor growth and metastasis, and protect against

Abbreviations: PPAR, peroxisome proliferator-activated receptor; K_D, equilibrium dissociation constant; 15-d-PGJ2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; LBD, ligand-binding domain; PMSF, phenylmethylsulfonyl fluoride; IPTG, isopropyl-β-D-thiogalactoside.

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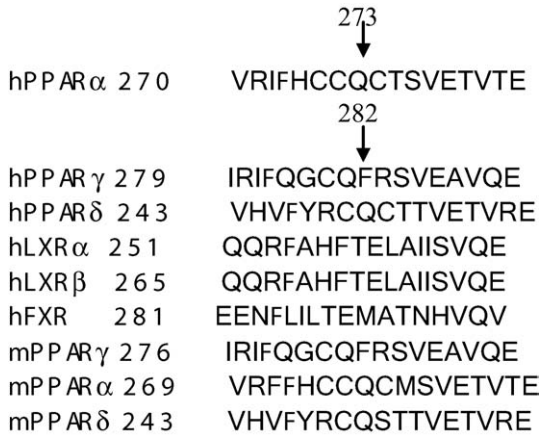
cellular transformation [4,6]. On the other hand, the recent researches have demonstrated that PPARα agonists (e.g., gemfibrozil, fenofibric acid) produce reductions in serum triglycerides and increases in HDL cholesterol [7]. The combined profile of dual PPARα/γ agonists are thus proposed to be promising in treating hyperglycemia together with prevention of cardiovascular disease in type II diabetes [2,8]. The biological activity of PPARδ is relatively obscure [9], even though some roles for PPARδ in cholesterol and fat metabolisms and the development of colon cancer have been reported [1,10,11].

To date, several high-resolution X-ray crystal structures of PPARs in complex with agonists or partial agonists have been reported [1–3,7,10,11]. The crystal structures have shown that PPAR takes a predominant α helix structure with a sandwich architecture (Fig. 1A) [1]. Structurally, the polar head of the bound ligands forms conservative hydrogen bonds with Ser289 and His323 on helix 5 and Tyr473 on AF2 (Fig. 1B) [3], the remainder of the ligand wraps around helix 3 and buries the non-polar tail into a lipophilic pocket formed by helices 2, 3 and the β sheet. Flexibility of C-terminal AF2 was reported to be important as a functional switch [1,12]. The inactive PPAR adopts a "close" conformation, in which the AF2 occupies the position of co-activator, abolishing its normal function. Upon binding with an agonist, PPAR takes an "open" conformation for accommodating the co-activator. The X-ray crystal structures thus reveal the functional helical regions of PPARs and other nuclear receptors (e.g., RXRα and LXRs) for ligand binding, of which helix 3 is involved in ligand entry and AF2 acts as a lid to plug the binding pocket [1].

Phe282 (residues are numbered according to the sequence of PPARγ if not indicated otherwise in this paper) is conserved over all the sequences of PPARs and other nuclear receptors (e.g., FXR and LXRβ) (Scheme 1). This residue is located at the N-terminus of helix 3 in the ligand-binding domain (LBD) that plays an important role for ligand binding (Fig. 1A). Recently, Xu et al. [2] reported that Phe282 of



Scheme 1. The alignment of the helix 3 sequences for several nuclear receptors.
The conserved residue phenylalanine is shown in bold. "h" denotes human and "m" indicates mouse. The mutated residue is indicated by arrow.

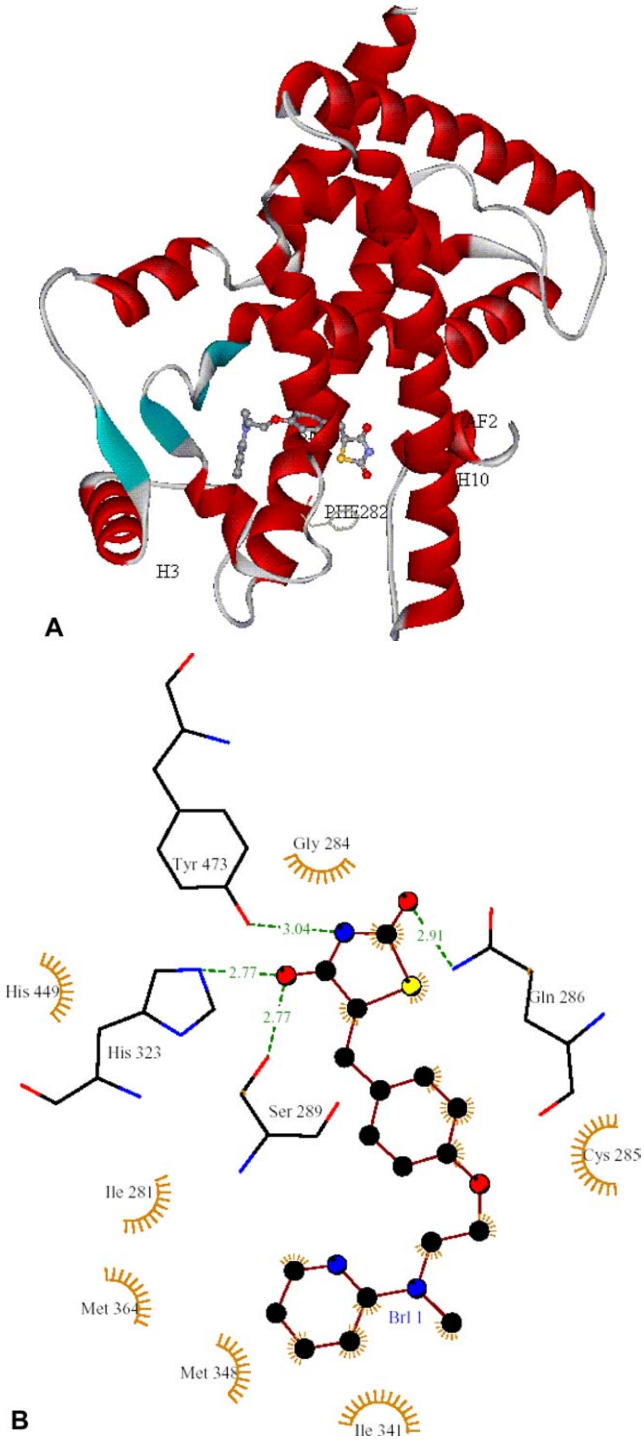


Fig. 1. Structure of hPPARγ-LBD in complex with Rosiglitazone.
(A) Schematic representation of hPPARγ-LDB/Rosiglitazone complex. The receptor is presented as ribbon, Rosiglitazone is shown as ball-and-stick model. (B) Schematic representation of H-bond and hydrophobic interactions between Rosiglitazone and the receptor. Dashed lines represent H-bonds and spiked residues form hydrophobic interactions.

human PPARγ (hPPARγ) participates in the GI262570 selectivity between PPARα and PPARγ. By comparing the crystal structures of the ligand-binding pockets for hPPARα and hPPARγ, it has been found that the Phe273 in PPARα (corresponding Phe282 in PPARγ) sterically clashes with