

INSIGHTS INTO THE MECHANISM OF PARTIAL AGONISM: CRYSTAL STRUCTURES OF PROLIFERATOR-ACTIVATED RECEPTOR γ LIGAND-BINDING DOMAIN IN THE COMPLEX WITH TWO ENANTIOMERIC LIGANDS

> G. Pochetti, F. Mazza

Istituto di Cristallografia, Consiglio Nazionale delle Ricerche, Montelibretti, Roma, ITALY

> C. Godio, N. Mitro, D. Caruso, M. Crestani, A. Galmozzi, S. Scurati

Laboratorio "Giovanni Galli" di Biochimica e Biologia Molecolare dei Lipidi e di Spettrometria di Massa, Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano, ITALY

> F. Liodice, G. Fracchiolla, P. Tortorella, A. Laghezza

Dipartimento Farmaco-Chimico, Università degli Studi di Bari, Bari, ITALY

> A. Lavecchia, E. Novellino

Dipartimento di Chimica Farmaceutica, Università degli Studi di Napoli, Napoli, ITALY

The peroxisome proliferator-activate receptors (PPARs) are transcriptional regulators of glucose and lipid metabolism [1]. They are activated by natural ligands, such as fatty acids. Ligand binding, by promoting the stabilization of the active conformation of the C-terminal helix (H12), triggers the recruitment of co-activator proteins that locally remodel chromatin and activate the cellular transcriptional machinery. The stabilization of H12 is mediated by a direct interaction between the ligand and the important residue Y473, belonging to H12. PPARs are also target of synthetic anti-diabetic and hypolipidemic drugs. In particular, the fibrate class of lipid-lowering drugs are PPAR α ligands. The thiazolidinedione antidiabetic agents are PPAR γ agonists, whose insulin-sensitising action is well established. PPAR ligands with a dual activity on both PPAR α and PPAR γ receptors,

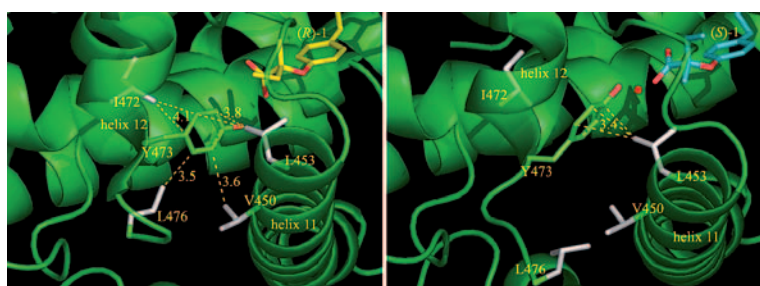
improve hyperglycaemia and dyslipidemic disorders in a coordinate manner. Such compounds may lead to preferred therapies for diabetes, obesity or metabolic syndrome.

We solved the X-ray crystal structures of the PPAR γ ligand-binding domain respectively in complex with the *R*- and the *S*-enantiomer of an ureido-fibrate-like analogue [2] of the well known PPAR α / γ agonist GW2331. The *R*-enantiomer, (*R*)-**1**, is able to activate both PPAR α and PPAR γ showing higher potency on PPAR α . The *S*-enantiomer, (*S*)-**1**, displays a lower efficacy towards PPAR γ and behaves as a partial agonist of this receptor subtype. These model compounds are particularly suitable to study the mechanism of partial agonism because they differ only for the switching of a methyl with an ethyl group on the asymmetric carbon atom, a small structural change that causes a significant difference in the pharmacological profile. The analysis of the two crystal structures shows that the different degree of stabilization of H12 induced by the ligand determines its behaviour as full or partial agonist. In the crystal complex PPAR γ /*(R)*-**1** the active conformation of H12 is stabilized by the following interactions: a) both carboxylate oxygens' of the ligand are engaged in canonical H-bonds with the three residues H323, H449 and Y473 involved in the receptor activation; b) the

Figure 1. (a)

Hydrophobic contacts between Y473 (green) and non-polar residues (white) of the protein complex with the *R*-enantiomer (yellow);

(b) Hydrophobic contacts between Y473 (green) belonging to H12 and apolar residues (white) of the protein complex with the *S*-enantiomer (cyan).



appropriate position of the Y473 aromatic side-chain is ensured by polarization interactions with I472 and L476 on one side, and with V450 and L453 on the other side (Figure 1); c) the ligand methyl and ethyl groups form several favourable hydrophobic interactions with L453 of H11, L469 of H12 and L465 of the loop 11/12 (Figure 2). Thus, the potency of the *R*-enantiomer is a direct consequence of a very effective stabilization of the helix 12, through hydrophobic and electrostatic interactions. Moreover, helix 12 is here stabilized in the proper conformation to recruit the co-activator, the same observed in other crystal structures of complexes with full agonists [3]. In the complex with the *S*-enantiomer, a 1 Å shift of the ligand away from helix 12 is observed. This is probably caused by a steric clash between the ligand ethyl group and the Q286 backbone (Figure 3). Mutation analysis has been made to test the relevance of this steric clash. The Q286G mutation reduces dramatically the basal activity and prevents the activation of the receptor by (*S*)-1, showing that Q286 residue plays a key role in stabilizing helix 12. Because it was not possible to confirm the functional relevance of the steric clash between Q286 and the ethyl group of (*S*)-1 by mutating this residue, we tested a ligand with the shorter methyl group replacing the ethyl group. As expected this substitution improves the potency of this ligand as compared to that of (*S*)-1, thus confirming that this steric clash does play a role in determining the behaviour of the *S*-enantiomer. Moreover, even if the helix 12 conformation only slightly differs from that observed in the complex with the *R*-enantiomer, its stability appears completely different for the

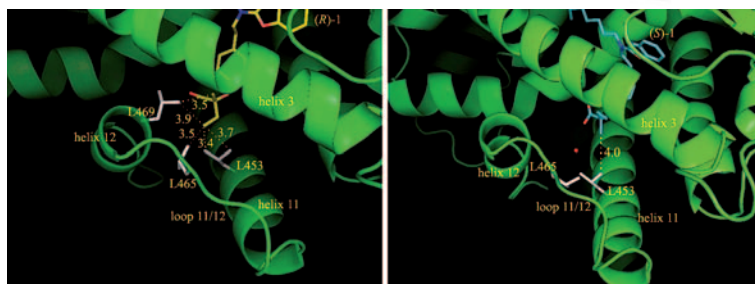


Figure 2. **(a)** Hydrophobic interactions of the *R*-enantiomer (yellow) with Leu residues (white) of LBD; **(b)** Hydrophobic interactions of the *S*-enantiomer (cyan) with Leu residues (white) of LBD.

following aspects: a) only one of the carboxylate oxygens' of the ligand engages H bonds with the three residues H323, H449 and Y473; b) the 1 Å shift of the ligand reduces favourable hydrophobic contacts with helix 12 to only one (Figure 2); c) the Y473 aromatic ring adopts a different orientation forming van der Waals interactions only with L453 of H11 (Figure 1). Finally, another crystal structure of the PPAR γ /*(S)*-1 complex, only differing in the soaking time of the ligand, is also presented. The comparison of the two structures of the complexes with the partial agonist reveals significant differences and is suggestive of the possible co-existence in solution of transcriptionally active and inactive forms of helix 12 in the presence of a partial agonist. This could probably explain the dramatic lack of efficacy in co-activator recruitment and in transactivation activity.

References

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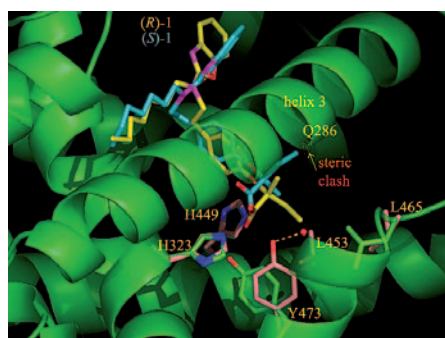


Figure 3. C α superposition of the complexes with the *R*- and the *S*-enantiomer (in yellow and cyan, respectively). Protein side-chains of the

complex with the *R*-enantiomer are shown in green; the correspondent side-chains are in pink for the complex with the *S*-enantiomer.