

fibrates and thiazolidinediones, and in natural ligands, such as fatty acids and eicosanoids. However, while selective ligands have been synthesized, it is still not known whether endogenous ligands possess selectivity in vivo [2]. These ligands differ greatly in their binding affinity and the three-dimensional structures of many nuclear receptors complexed to several ligands have been determined, providing more understanding of the structural basis for the mode of action of agonists and antagonists. In this work, we present the crystal structures of PPAR α ligand-binding domain complexed to two different ligands, the first structure with a potent and widely-used PPAR activator, the WY14643, member of fibrate class of drug which are used in treatment of dyslipidemia, and the second structure in complex with fatty acids, important natural ligands of these receptors. The both structures were refined to a resolution up to 2.5 Å and showed clear electron densities for the ligands interacting with alpha helix twelve, which compel the protein to adopt its active conformation. In the same structures, a second molecule of ligand was observed in different positions of active site. Biophysical and transactivation assays were used to confirm the binding and activation of protein. We concluded the fatty acids and WY14643 are full agonists because they interact with helix twelve and the second site found for WY14643 is functional and could help to modulate the receptor function. These structural studies enabled us to identify the key residues in the ligands recognition and ligand-induced activation. In addition, it can provide us insights about determinants of subtypes selectivity.

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Different binding modes of PPAR γ ligands

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor gene family and regulate the expression of genes. PPAR γ is expressed in a broad range of tissues including gut, colon, heart, skeletal muscle, brain, adipose tissues, vascular cells, immune and inflammatory cells and it has an essential role in the integration of the energy control, glucose and lipid homeostasis [1]. PPAR γ is the key regulator in adipose tissue with expression being induced early in preadipocyte differentiation. The ability of PPAR γ as well other nuclear receptors to promote or suppress transcription of responsive genes depends on the interaction with a ligand that will alter and stabilize the receptor conformation. PPAR γ has a large binding pocket that permits that a wide range of ligands can bind and induce differential interactions with coregulatory factors that will be transduced into a specific biological response. Rosiglitazone and pioglitazone are high-affinity ligands for this receptor and are widely used as insulin-sensitizing agents in the treatment of type 2 diabetes, however this is accompanied by the induction of several undesirable side effects. Recent researches have concentrated on the development of efficacious PPAR γ selective modulators (so-called SPPAR γ Ms) that improve insulin sensitivity and do not promote adipocyte differentiation. Here we describe the crystal structure of PPAR γ in complex the flavonoid luteolin and with myristic acid and in complex with the synthetic agonist indomethacin. Using different cell

based assays and X-ray crystallography we demonstrate that luteolin is a PPAR γ partial agonist differently from indomethacin, which is a full agonist. The crystallographic studies revealed that these ligands interact with PPAR γ in different binding modes. The head group of Indomethacin extends toward the AF2 helix and lock the receptor in an activated conformation to which coactivators can bind and activate the transcriptional machinery. On the other hand, luteolin stabilizes a portion of a Ω loop and is located at a considerable distance from the H12 in a region between H3 and beta sheet. This binding mode can imply a diminished conformational stability, differential receptor-coactivator interactions, attenuated transcriptional activity and improved tolerability as described for SPPAR γ Ms.

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Structure determination (2) of anti-HIV actinohivin in complex with mannobioses

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AIDS/HIV is a major health concern pandemic for the last 30 years and no effective drugs or vaccines have yet been developed. It is known that HIV-envelop glycoprotein gp120 at first binds to human cell CD4⁺ protein in the infection process. We found a lectin actinohivin (AH) from actinomycete *Longispora albida* which specifically binds the high-mannose type glycans (HMTG) bound to the virus envelop. Therefore AH is expected to interfere with the HIV entry into human cell. To modify AH as more effective drugs, it is necessary to reveal the structural basis of the binding affinity and the solubility. Therefore, we have at first determined the crystal structure of the *apo* form [1] and then that in complex with Man- α (1,2)-Man of HMTG by X-ray analyses. The latter AH-Man crystal structure was preliminarily solved based on the orthorhombic space group $P2_12_12_1$ which is one of the two possible space groups. In the present analysis, the space group has been assumed to be $P2_13$ (cubic) and the crystal structure has been successfully refined.

There is no significant difference between R_{sym} values based on the two space groups after data processing. As compared with the $P2_12_12_1$ case, the R -factor after the structure refinement is significantly decreased from 0.21 to 0.15. In addition, several residues invisible on a $P2_12_12_1$ electron density map are reasonably assigned on the corresponding $P2_13$ map. In the latter case, three AH molecules are disordered around the crystallographic 3-fold axis. This can be ascribed to the high molecular 3-fold symmetry in tertiary and primary structures based on the three tandem repeats in the amino-acid sequence, and each folds into a module to compose the *apo*-form of AH. The Man- α (1,2)-Man bound state structure is similar to that of *apo*-form suggesting that AH binds to the target molecules without any large conformational changes, each residue fluctuating within a tolerance (rmsd 0.45Å), estimated from superimposition of the two structures. Such a compact, regular and stable tertiary structure may allow AH exhibiting a high specificity to manno-biose moiety, as seen in Fig. 1.