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 PPARα 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 PPARγ ligands

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Peroxissome proliferator-activated receptos (PPARs) are ligand-activated transcription factors that belong to the nuclear hormone receptor gene family and regulate the expression of genes. PPARγ 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]. PPARγ is the key regulator in adipose tissue with expression being induced early in preadipocyte differentiation. The ability of PPARγ 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. PPARγ 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 PPARγ selective modulators (so-called SPPARγMs) that improve isulin sensivity and do not promote adipocyte differentiation. Here we describe the crystal structure of PPARγ 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 PPARγ partial agonist differently from indomethacin, wich is a full agonist. The crystallographic studies revealed that these ligands interact with PPARγ 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 oher 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 SPPARγMs.

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Keywords: PPARγ, indomethacin, luteolin

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

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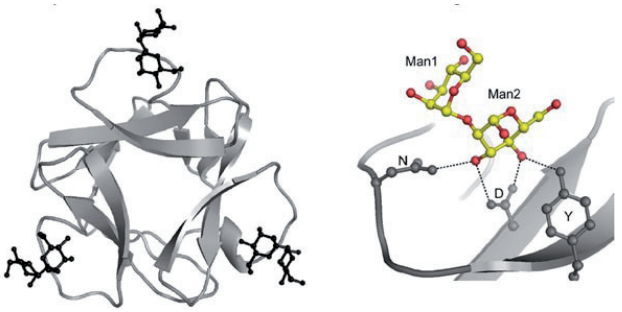
AIDS/HIV is a major health concern pandemic for the last 30 years and no effective drugs or vaccines have yet been developed. It is known that HIV-envelop glycoprotein gp120 at first binds to human cell $CD4^+$ protein in the infection process. We found a lectin actinohivin (AH) from actinomycete *Longispora 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 crystallo-graphic 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.



(*a*) (*b*)

Fig. 1. Actinohivin-mannobiose interaction, three mannobioses being bound symmetrically (*a*) and each recognized by the specific interactions (*b*).

[1] H. Tanaka *et al*., *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15633-15638.

Keywords: X-ray structure, anti-HIV actinohivin, mannobiose binding

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Preliminary Studies on Glucose-6-Phosphate Dehydrogenase from *Trypanosoma cruzi*

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Trypanosoma cruzi is the causative agent of American trypanosomiasis (Chagas' disease). Currently, there are no immunoprophylactic therapies available to combat a disease that affects 14 million people in Latino American countries. Moreover, treatment of the chronic and life-threatening stage of the disease relies on only two drugs that exhibit a high toxicity and poor efficacy. This highlights the urgent need to develop new antichagasic medicines with improved pharmacological properties. In this context, parasite's proteins that play an indispensable cellular function and differ from the human counterpart hold promise as drug-target candidates. Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme of the pentose phosphate pathway, which produces important metabolites (e.g. NADPH, ribose-5-phosphate and glycolytic intermediates). The protein has been rated as a virulence factor in the infective forms of *T. cruzi* [2] and shown to be essential for African trypanosomes [1]. The biological relevance of TcG6PD lies on the critical need for intracellular redox homeostasis (e.g. antioxidant defense) and DNA synthesis (cell proliferation), both processes depending on the continuous supply of NADPH and ribose-5-phosphate, respectively. *T. cruzi* G6PD (TcG6PD) shares only 43 % sequence identity with its human orthologue. The goal of this work is to study TcG6PD at the biochemical and structural levels in order to determine whether the trypanosomal protein can be target of specific drug development. A short form of TcG6PD lacking an N-terminal stretch of 32 residues (ΔN_TcG6PD), absent in the human enzyme, was overexpressed in *Escherichia coli* and purified to homogeneity. In contrast to the human protein that undergoes rapid tetramer-dimer equilibrium, size exclusion chromatography of ΔN_TcG6PD showed that TcG6PD forms a stable non-covalently linked tetramer. High salt concentrations (1M NaCl), ligands (1mM G6P and NADP), a non-competitive inhibitor (dehydroepiandrosterone) as well as a reducing agent (10 mM DTT) did not disrupt the tetrameric structure. ΔN_TcG6PD was crystallized, and its 3D structure solved with X ray diffraction techniques, in its apo form (2.85 Å resolution) as well as in complex with G6P (3.3 Å) and $NADP^+$ (3.2 Å). Data was collected with our home source (Rigaku Micormax007-HF) and at the ALS synchrotron facility (Berkeley, USA), phasing was achieved using the human G6PD as a molecular replacement search probe. Overall, the structural analysis allowed the identification of particular features in the parasite´s enzyme, focusing our interest in the substrate-binding site and inter-subunit contacts. Slight conformational rearrangements were also detected as a result of ligand binding. We will report on the structural and biochemical features that distinguish ΔN_TcG6PD from its human counterpart. To the best of our knowledge, this represents the first report on the 3D structure of a NADPH dependent G6PD from a pathogenic microorganism.

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Keywords: trypanosome, enzyme, drug

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X-Ray Crystal Structure of TEC Family Kinase BMX

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Bone Marrow kinase gene on the X chromosome (BMX) is a member of the Tec family of tyrosine kinases that also includes BTK, ITK, TEC and TXK. Although the structures BTK and ITK are known, the X-ray crystal structures of other Tec family kinases have remained elusive. We have determined the X-ray crystal structure of BMX in complex with ATP binding site ligand. The BMX structure reveals a typical kinase protein fold with well ordered protein conformation that includes an open/extended activation loop conformation and a unique DFG motif conformation that renders the kinase in an inactive conformation. The ligand binds to BMX in the ATP binding pocket and it displays a similar binding mode to that observed in other Tec and Src protein kinases. The structural differences between BMX and other Tec and Src protein kinases suggest significant differences in protein conformation can exist while maintaining critical hinge binding interactions with the ligand. Conformational heterogeneity in the Glycine rich loop, alpha C helix and the DFG motif results in size and shape differences in the ligand binding site and suggest selectivity could be attained within the highly homologous ATP site.

Keywords: tyrosine kinase, ATP Inhibitor-binding, BMX

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Structure analysis of inhibitor complex of OMP decarboxylase from *P.falciparum*

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