structure content when comparing both isoforms and electron paramagnetic resonance approach has identified the presence of an iron-sulfur cluster. In addition, significant differences in specific activities were found for both isoforms. Polyclonal antibodies have also been raised and subcellular localization studies for both isoforms indicated that one enzyme is localized in mitochondria, whereas the second isoform has double cytosol and glycosome localization. Crystallization and structural determination of LmFH isoforms are in progress.

Our results suggest that LmFH isoforms, which share around 60% of sequence identity, are localized in different cell compartments, and also display differences in protein folding and mechanism of action.

The results, here presented, correspond to the first studies on FHs from trypanosomatides, significantly contributing to the understanding of their functional role in *Leishmania*.

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Keywords: fumarase, leishmania, characterization

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Rational design of inhibitors of APE1 supported by crystallographic techniques

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Chemotherapy still constitutes the major pharmacologic approach against cancer. However, the biochemical repair systems of the cancer cell machinery responds, trying to mitigate the cellular damage induced by these agents. As a result, the clinical efficacy of chemotherapeutic agents is often limited. Several advances in the molecular biology of cancer have identified key pathways involved in the DNA repair of the damage induced by chemotherapeutic agents. Between all the mechanisms, we can highlight the base excision repair (BER) pathway. Apurinic/apyrimidinic endonuclease (APE-1) is one of the crucial enzymes in this mechanism. Due to its activity, further studies have been focused in the development of inhibitors for APE-1 enzyme. Here, we report the employment of docking and virtual screening techniques based in the crystallographic models to search for compounds that can act as APE-1 inhibitors. The discovered compounds have shown to be active *in vitro* assays, with activities between low and medium micro-molar range. Currently, we are undertaking *in vivo* assays in order to determine their cytotoxicity and their effects in cancer cell survival. At the same time, we are in the process of solving the crystallographic structure of the complexes formed by APE-1 and each one of the active compounds in order to optimize their affinity for the target.

Key words: inhibitor, complex, structure

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Structural studies of *E.coli* nitroreductase enzymes for use in gene therapy of cancer

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The nitroreductase enzymes of *E.coli* have potential for use in Virus-Directed Enzyme Prodrug Therapy (VDEPT), converting the inactive prodrug CB1954 (5-[aziridin-1-yl]-2,4-dinitrobenzamide) to a potent difunctional alkylating agent [1]. The combination of the minor nitroreductase NfsB and the prodrug CB1954 has been put through Phase I/II clinical trials with promising results. The VDEPT uses an adenovirus vector to insert the gene encoding the nitroreductase into tumour cells, and then the prodrug is separately administered, and upon reaction first with the enzyme and subsequently with cellular thioesters, is transformed into a cell killing agent. This compound is able to transfer into nearby cells and kill those too, causing a 'bystander effect', which is essential for the effectiveness of the treatment. However for treatment to be successful greater efficacy is required.

The major limiting factor is the relatively poor affinity of NfsB for CB1954. Mutants of NfsB have been produced which bind more strongly to the prodrug. It has also been discovered that NfsA, the major *E.coli* nitroreductase, confers 3.5-8 times greater sensitivity to CB1954 than NfsB, in human cells and bacterial cells [2]. The crystal structures of two of the most active NfsB mutants, a double mutant (T41LN71S) and a triple mutant (T41QN71SF124T) are presented here in complex with different inhibitors and compared with wild-type [3]. These structures help us to understand the improvements in binding seen with these mutants. The cooperativity of the T41Q and F124T mutations is shown through a direct H-bonding interaction between the side chains and then to the inhibitor, providing an explanation for why the combination of these mutations has a greater effect than would be expected by the combination of the improvements seen with the single mutants alone.

Also presented are crystal structures of NfsA bound both to inhibitors and to a substrate, the antibiotic nitrofurantoin. The two nitroreductase enzymes are both homodimeric flavoenzymes, with two FMN containing active sites at the dimer interface. Despite having little sequence homology they do share several structural similarities. These structures can be used to guide future rational mutagenesis of the enzymes and design of improved prodrugs, ultimately creating an effective treatment method.

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Crystal Structures of PPARα in Complex with Synthetic and Natural Ligands

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The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptors superfamily, a group of transcription factors. Three subtypes are encoded by separate genes: PPARα, PPARβ/δ, and PPARγ, each with different ligand specificity, very distinct tissue distributions, and different biological functions. They are involved in numerous physiological events in human, including glucose and lipid metabolism. PPAR ligands effectively treat dyslipidemia and have significant anti-inflamatory and antiatherosclerotic activity. These effects and their ligand-dependent activity make nuclear receptor obvious targets for drug design in many therapeutic areas [1]. The ligands may be classified in synthetic compounds, such as

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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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.