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