Poster

Structural characterization of thioredoxin reductase from *Cryptosporidium parvum* and its interaction with auranofin

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Cryptosporidium parvum, an apicomplexan protozoan parasite, is the causative agent of cryptosporidiosis, one of the most common causes of diarrheal disease worldwide. Cryptosporidiosis is strongly related to early childhood mortality, with prevalence in tropical countries, and is also a potential life-threatening complication in individuals with poor health or weakened immune systems (HIV/AIDS patients, cancer, and transplant patients) [1]. Because of its link with malnutrition, poverty and poor hygiene, cryptosporidiosis was included in the WHO Neglected Disease Initiative in 2004 [2]. No fully effective treatments or vaccines are currently available as the only FDA-approved drug, nitazoxanide, is effective in immunocompetent patients while showing reduced efficacy in immunocompromised ones [2].

Over the last decades, the screening of existing drugs has been used as a convenient strategy for new antiparasitic drugs development. Auranofin (AF, Ridaura®), an FDA-orphan drug and a gold-containing compound, has been identified as an antiparasitic drug for the treatment of many human parasitic diseases. Reprofiled auranofin has been shown to be active against several parasites, including *Schistosoma mansoni*, *Brugia malayi*, *Onchocherca volvulus*, *Leishmania* spp., *Trypanosoma brucei*, *Entamoeba histolytica*, and *Giardia lamblia* [3]. Moreover Debnath A. et al. [2] found that auranofin was effective *in vitro* against *C. parvum* in the micromolar range, which was comparable to the aforementioned nitazoxanide. It is well known that auranofin can target thioredoxin reductase (TrxR), a crucial parasite enzyme involved in the detoxification of reactive oxygen species, among other functions [3].

Using X-ray crystallography, we solved the crystal structure of *C. parvum* TrxR (CpTrxR) in the apo form (1.9 Å) and in complex with AF (3.3 Å). The 3D structure classifies CpTrxR as a type II high molecular weight thioredoxin reductase, sharing a characteristic spacer of four residues between the two redox active Cys residues at the C-terminus. This distinctive redox motif characterizes the TrxRs from apicomplexan protozoa, including *Plasmodium falciparum*, a malaria parasite (4), and, to the best of our knowledge, it has been observed for the first time in our crystallographic structure. This result will allow a thorough investigation of the catalytic mechanism of these enzymes. The second structure results from co-crystallization of CpTrxR with AF in reducing conditions. This structure shows the gold atom bound to the protein, providing insights into the molecular mechanism of AF against these parasites.

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