Structural biology of the thiol-dependent redox systems of *Schistosoma mansoni* and *Plasmodium falciparum*

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Schistosomiasis is a widespread tropical parasitic disease, caused by three species of the blood-fluke *Schistosoma*. The disease is debilitating and affects 200 million people in tropical areas; the death toll is estimated at two hundred thousands people per year. Schistosomiasis is currently treated with one drug, Praziquantel, whose precise molecular target is unknown. Several other drugs are known to kill the schistosomes *in vivo* and *in vitro*, but these are seldom employed because of toxicity, high cost, complex administration or other reasons. The improvement of known drugs or the development of entirely new ones is a desirable goal, in view of the fact that strains of *Schistosoma mansoni* with reduced sensitivity to Praziquantel have appeared. In this project we are exploring known or putative macromolecular targets of schistocidal drugs; thus we focus on the biochemistry and molecular biology of the parasite. The rationale of this approach is that drug design may become realistic if the mechanism of action of each drug were known at atomic detail, ideally as the 3D structure of the drug in complex with its target. The enzymes involved in the detoxification of reactive oxygen species (ROS) and other oxidants are potential drug targets. We have already characterized from the structural and functional point of view: Glutathione Transferase; Thioredoxin Glutathione Reductase; and Glutathione Peroxidase from either *S. mansoni* or *S. haematobium*. We have also characterized a Fatty Acid Binding Protein and a Cyclophilin, both from *S. mansoni*, even though these two proteins are not directly related to the ROS detoxification pathway.

Two of the enzymes we characterized are known to be “druggable”, i.e. they can serve as the target of known or putative drugs: Thioredoxin Glutathione Reductase (TGR) and Glutathione Peroxidase. In the case of Thioredoxin Glutathione Reductase an effective inhibitor is available, that is known to kill not only the schistosomes, but also malarial parasites: the gold containing drug Auranofin. TGR is a NADPH-dependent flavoreductase containing a selenocysteine residue (Sec). During its enzymatic cycle thiolates and selenolates that have high affinity for transition metals are generated. Auranofin inhibits TGR (and other selenocysteine-containing flavoreductases) more effectively than non Se-containing ones (glutathione reductase); this preference was traditionally ascribed to the high affinity of selenium for gold. We solved the structure of the gold-TGR complex, and found Au combined to the sulfur of Cys residues, rather than (or in addition to) the Se of Sec. Thus our results challenge the commonly held view. Kinetic measurements have demonstrated that the relative velocity of the reaction rather than the relative affinity, depends on the presence of Sec residues, which appear to dictate AF selectivity. Indeed when an external source of...
selenium (benzeneselenol) was added to the reaction mixture, auranofin was able to inhibit the Se-lacking reductases Glutathione Reductase and Sec->Cys mutated TGR with an efficiency similar to Se-containing ones.

In order to complete the structural characterization of the enzymes belonging to the thiol-mediated detoxification pathway, we solved the high-resolution crystal structure of *S. mansoni* Thioredoxin (SmTrx) in three states, namely: the wild-type oxidized adult enzyme and the oxidized and reduced forms of a juvenile isoform, carrying an N-terminal extension. SmTrx shows a typical thioredoxin fold, highly similar to the other components of the superfamily. SmTrx presents some functional peculiarities, e.g. the ability to reduce oxidized glutathione. Moreover it is one of the few defence proteins expressed in mature eggs and in the hatch fluid, thus confirming an important role in the parasite.

Only one relevant enzyme of the pathway remains to be characterized, namely Peroxiredoxin; this protein has been heterologously expressed in *E. coli*, purified and crystallized and its structure is the next target of our project.

**Publications**


**Research Group**

Adriana Erica Miele, Francesco Angelucci, researchers; Giovanna Boumis, technician, Fulvio Saccoccia, PhD student.

**Collaborations**

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