Luise Krauth-Siegel

The unique trypanothione-based redox metabolism of trypanosomatids

Goal
Aim of our work is to unravel the role of the unusual dithiol trypanothione in trypanosomatids and to contribute to the development of new antiparasitic drugs by identifying target molecules and the analysis of parasite specific enzyme inhibitors.

Background
Trypanosomatids, the causative agents of various tropical diseases, possess a redox metabolism that is based on the bis(glutathionyl)spermidine-conjugate trypanothione. The dithiol is the donor of reducing equivalents for a wide variety of essential pathways (Fig. 1).

Research Highlights
Trypanothione reductase (TR) as a drug target molecule
TR is an attractive drug target molecule (Leroux and Krauth-Siegel 2016). However, its large active site allowing multiple possible ligand orientations renders a structure-based inhibitor design challenging. In a joint effort of several groups, biological testing, kinetic and mutational studies, and virtual docking simulations revealed a new series of small-molecule inhibitors (Persch et al. 2014). The co-crystal structures showed that the ligands interact with the hydrophobic wall of the so-called “mepacrine binding site”, but, remarkably, the binding conformation of the inhibitors varied for TR from Trypanosoma brucei and T. cruzi. The study gives new insight into the molecular recognition of nonpeptidic small-molecule ligands by TR and provides the basis for an ongoing lead optimization.

Fig. 1: The trypanothione metabolism.
Hydroperoxide detoxifying peroxidases play completely different roles in the infectious bloodstream and procyclic insect stage

African trypanosomes express three virtually identical non-selenium glutathione peroxidase-type enzymes which preferably detoxify lipid-derived hydroperoxides. Bloodstream *Trypanosoma brucei* lacking the mitochondrial isoenzyme display only a weak and transient proliferation defect and in vivo studies in mice confirmed the negligible role of the protein. In contrast, parasites that lack the cytosolic peroxidases undergo extremely fast lipid peroxidation and cell lysis. The phenotype, which can be completely rescued by supplementing the medium with the α-tocopherol derivative Trolox, is due to damage of the parasite lysosome (Fig. 2; Hiller et al. 2014).

In the insect form of *T. brucei* selective deletion of the genes revealed that parasites that lack either the cytosolic or mitochondrial peroxidase proliferate nearly as wild type cells whereas the knockout of the complete genomic locus is lethal (Schaffroth et al. 2016). The parasites lose their mitochondrial membrane potential and lyse. The cellular damage is prevented by Trolox, ubiquinone derivatives, and deferoxamine. In glucose-rich medium, cell death is attenuated suggesting that oxidants generated by the respiratory chain contribute to the lethal phenotype. Depending on the developmental stage, the lysosome or the mitochondrion is the origin of iron-mediated oxidative membrane damages. Strikingly, in the insect stage either the cytosolic or the mitochondrial form of the peroxidases is required and sufficient to protect the mitochondrion.

Selected Publications 2014 - 2016


Luise Krauth-Siegel
Phone: +49 (0)6221 54 4187
E-mail: luise.krauth-siegel@bzh.uni-heidelberg.de

Fig. 2 Bloodstream *T. brucei* that lack the cytosolic peroxidases undergo lysosomal disintegration and cell lysis. In the presence of Trolox, the deficient parasites fed with Alexa Fluor-conjugated dextran display a discrete lysosomal staining (0 h) and proliferate like wildtype cells. Yet upon withdrawal of the antioxidant, the signal becomes progressively spread over the whole cell body (1-2 h) and the parasite die. The lethal phenotype is attenuated in the presence of the iron-chelator deferoxamin.

*T. brucei* acquire iron by endocytosis of host transferrin. Supplementing the medium with iron or transferrin induces, whereas the iron chelator deferoxamine and apo-transferrin attenuate lysis of the Px I-II-deficient cells. These data demonstrate that in the infectious form of African trypanosomes, the lysosome is the primary site of oxidative damage and cytosolic trypanothione/tryparedoxin-dependent peroxidases protect the organelle from iron-induced membrane peroxidation.