Genetically encoded biosensors for monitoring redox changes in the trypanothione-based thiol metabolism of trypanosomes

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State of the art/preliminary work

In the majority of organisms, glutathione (GSH) is the main low molecular mass thiol. Despite its exclusive synthesis in the cytosol, GSH occurs in cellular organelles creating separate redox pools. Even under severe oxidative stress conditions, the cytosolic glutathione disulfide (GSSG) concentration in yeast cells remains extremely low and virtually all GSSG that is not immediately reduced is transported into the vacuole (Morgan et al., 2013). The cytosolic glutathione homeostasis is maintained and whole cell glutathione measurements do not necessarily reflect the situation in the cytosol.

Genetically encoded redox biosensors allow specific, quantitative, dynamic, and compartment-specific measurements of thiol redox switches in intact cells (Schwarzlander et al., 2016). Coupling human glutaredoxin 1 (hGrx1) to roGFP2 renders the sensor highly specific for GSH (Gutscher et al., 2008). In yeast, hGrx1-roGFP2 reveals an almost identical GSH redox potential in the cytosol and mitochondrial compartments under physiological conditions. Limiting amounts of glutaredoxin 2 (Grx2) in the intermembrane space (IMS) provide a kinetic barrier for the reduction of proteins by the GSH pool and thus ensure the efficient oxidative protein folding in the reducing environment of this compartment (Kojer et al., 2015).

Trypanosomes and Leishmania have a trypanothione-based thiol redox system (Fig. 1).

Figure 1. **Biosynthesis of trypanothione.** Trypanothione synthetase (TryS) catalyzes the two consecutive steps linking spermidine and two molecules of glutathione to form trypanothione \([T(SH)_2]\), with glutathionylspermidine (Gsp) as intermediate. TryS has also amidase activity and thus is able to catalyze the hydrolysis of the conjugates.

*Trypanosoma brucei* TryS is highly regulated by both its substrate GSH and product T(SH)\(_2\) (Leroux et al., 2013). T(SH)\(_2\) which is kept reduced by TR (Fig. 2) fulfills nearly all of the functions that in other cells depend on either GSH or thioredoxin (Krauth-Siegel and Leroux, 2012; Manta et al., 2013; Rahbari et al., 2015). TryS, TR and tryparedoxin (Tpx) are cytosolic key enzymes of the trypanothione metabolism and depletion of any of the proteins severely affects the thiol redox balance of the parasite (Comini et al., 2004; Comini et al., 2007; Krieger et al., 2000).
African trypanosomes express three virtually identical glutathione peroxidase-type enzymes. Bloodstream (BS) *T. brucei* that lack the mitochondrial isoenzyme are fully viable whereas parasites that are devoid of the cytosolic peroxidases undergo lipid peroxidation and lyse. The lysosome is the primary site of damage and the cytosolic enzymes protect the organelle from iron-induced membrane peroxidation (Hiller et al., 2014). Procyclic (PC) insect stage parasites that lack either the cytosolic or the mitochondrial enzymes proliferate as wild type cells. However, parasites in which the complete genomic locus has been deleted display lipid peroxidation, loss of the mitochondrial membrane potential and lysis (Schaffroth et al., 2016). The peroxidases protect the parasites from iron-mediated oxidative membrane damages that originate at the lysosome or mitochondrion depending on the developmental stage.

In the first funding period of the SPP 1710, we addressed the oxidative stress response of the intact parasite. Challenging BS *T. brucei* with diamide, H$_2$O$_2$ or hypochlorite resulted in reversible protein S-thiolation. Quantitative proteome analyses revealed 84 proteins oxidized in diamide-stressed parasites. Fourteen of them, comprising several thiol redox proteins and chaperones, were probably S-thiolated. Upon exposure to H$_2$O$_2$, other proteins became modified indicating specific stress responses. Depletion of TryS induced protein S-glutathionylation. The data revealed for the first time that trypanosomes employ protein S-thiolation when exposed to exogenous and endogenous oxidative challenges and trypanothione, despite its dithiol character, forms protein-mixed disulfides (Ulrich et al. 2017).

Two dithiol glutaredoxins (Grxs) have been characterized in African trypanosomes. Grx1 is...
located in the cytosol whereas Grx2 appears to occur specifically in the IMS of the mitochondrion (Ceylan et al., 2010). Knockout (KO) of the genes encoding Grx1 or Grx2 in BS T. brucei does not result in any proliferation defect. Grx1 accounts for about half of the total hydroxyethyl disulfide (HEDS) reductase activity of the parasite (Musunda et al., 2015), and Grx1 KO cells show delayed recovery from stress-induced protein S-thiolation (Ulrich et al., 2017). Intriguingly, when the culture temperature is raised from 37 °C to 39 °C, simulating fever conditions in the infected mammalian host, proliferation and morphology of the Grx-deficient parasites are significantly better preserved compared to wildtype cells suggesting a role of the oxidoreductases in regulating the thermo-tolerance of BS T. brucei (Musunda et al., 2015; Ebersoll, unpublished).

Recently we generated several redox sensors for in vitro and in vivo studies. hGrx1-roGFP2 and Tpx-roGFP2, but not roGFP2, displayed a concentration-dependent reduction by GSH and T(SH)₂, in accordance with the catalytic effect of the fused oxidoreductases. In the case of hGrx1-roGFP2, millimolar concentrations of GSH or T(SH)₂ were required to reduce the protein within the observation time. Reduction of Tpx-roGFP2 by T(SH)₂ was 100-times faster compared to GSH. The data obtained so far indicate that the sensors are suited for the analysis of the trypanothione-based redox metabolism of trypanosomes.

Objectives

Aim of this work is to unravel the trypanothione-based thiol redox metabolism of African trypanosomes by use of genetically encoded redox sensors expressed in the cytosol as well as the intermembrane space and matrix of the developmentally regulated single mitochondrion of these protozoa.

The following main questions will be addresses:

1. Does the whole cell thiol status reflect the conditions in the cytosol or do oxidatively challenged trypanosomes sequester low molecular mass disulfides?
2. Is there a cross-talk between the thiol redox metabolism of the cytosol and the mitochondrial matrix or intermembrane space?
3. How does the thiol redox status in the fully elaborated mitochondrion of the insect stage compare with that in the rudimentary organelle of the infectious bloodstream parasite?
4. How does down-regulation of trypanothione biosynthesis or reduction affect the cytosolic and mitochondrial thiol redox status?
5. Do the glutaredoxins of the parasite, that are located in the cytosol and mitochondrial intermembrane space, respectively, act as thiol redox switches?

Cooperations

Cooperations within this priority program include the analysis of roGFP fusion proteins in T. brucei (Tobias Dick), analysis of the physiological role of Grx2 in the intermembrane space of the single mitochondrion of trypanosomes (Jan Riemer) and studies on the trypanothione-dependent iron sulfur cluster formation (Roland Lill).

References


