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Hydrogen peroxide-induced reversible protein S-glutathionylation in African trypanosomes

Fach: Biochemie
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Reversible protein S-glutathionylation protects cysteine residues from irreversible overoxidation, modifies protein functions and is involved in redox signaling. Hydrogen peroxide represents a physiological stress that is known to induce glutathionylation.

Aim of this thesis was to identify proteins that are glutathionylated upon H₂O₂ treatment of African trypanosomes, to quantify protein-bound glutathione depending on the stress exposure time and to investigate the reversibility after stress removal.

Glutathionylated proteins from bloodstream *T. brucei*, either exposed to 1 mM H₂O₂ or kept in PBS, were deglutathionylated by Grx1 and enriched by subsequent Biotin-HPDP labeling. In-gel trypsin digestion and stable isotope dimethyl labeling allowed to quantitatively compare proteins S-glutathionylated in stressed versus untreated parasites by ESI-MS.

As a 5 min exposure to H₂O₂ resulted only in marginal increase of glutathionylation, the incubation time was prolonged to 45 min. In total, 48 proteins were enriched by this procedure. Most of them did not show an H₂O₂-induced increase of glutathionylation suggesting that they are either glutathionylated already under standard culture conditions or became modified during the harvesting procedure. H₂O₂-mediated glutathionylation was reproducibly observed in at least three of five (5 min analyses) or six (45 min analyses) experiments for ten proteins, representing an isomerase, a peptidase, chaperones, and several proteins with so far unknown functions.

Recently, it was shown that Prx, a key enzyme for H₂O₂ detoxification, is glutathionylated upon diamide stress of the parasites. Interestingly, this was not the case in the H₂O₂-treated cells. Western blot analyses of bloodstream *T. brucei* incubated with 0.1 mM H₂O₂ for only 1 min revealed the formation of covalent dimers of Prx. At higher H₂O₂ concentrations and/or longer incubation times, the monomer was the predominant form. Most likely Prx became overoxidized, preventing both formation of intermolecular disulfide bridges and glutathionylation.

In a second approach, the total cellular protein-bound glutathione was measured by NDA derivatization and fluorescence detection. Treatment of bloodstream *T. brucei* with 1 mM H₂O₂ induced protein S-glutathionylation which increased with time up to 45 min. An incubation of 95 min, however, resulted in cell death indicating that the parasites cannot compensate severe oxidative stress. After stress removal, glutathionylation induced by 1 mM H₂O₂ for 5 min was rapidly reversible in HMI-9 medium pre-warmed to 37 °C. In PBS at RT, glutathionylation was not reversible, but the parasites remained intact. However, parasites exposed to 1 mM H₂O₂ for 45 min did not recover but lysed when transferred into medium. Obviously, the cells are no longer able to survive as soon as their metabolism is switched on again under culture conditions. This indicates that deglutathionylation requires an active cell metabolism and the availability of nutrients.

Taken together, the mass spectrometric identification of proteins glutathionylated upon H₂O₂ induction represents a promising starting point to clarify the cellular mechanisms of redox control in *T. brucei*. The results of this thesis show the sensitivity of the parasites towards exogenous H₂O₂ and corroborate the role of Prx as crucial enzyme for H₂O₂ detoxification. At low levels, the enzyme rapidly detoxifies H₂O₂, but is overoxidized and inactivated at high

H₂O₂ concentrations. Thus, Prx is unable to execute its function of H₂O₂ detoxification and the parasites will not survive.