Identification of putative protein interactors of *Escherichia coli* Glutaredoxin 2

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Introduction. Glutaredoxin 2 (Grx2) contributes more that 80 % of glutathione (GSH) mediated redox activity in cellular extracts and protects cells from general oxidative damage (formation of carbonyls[1,2]). The specific substrates of Grx2 however, are unknown. To this aim, immobilized monothiol/athiol Grx2 mutants were used in affinity chromatography with cellular lysates from *Escherichia coli* (*E. coli*). The athiol Grx2 (Grx2 C9S C12S) served as a bait for proteins interacting in a non-thiol manner while the monothiol Grx2 C12S was used to trap dithiol substrates of Grx2 (Figure 1, right column). In addition, lysates from *E. coli* null mutants for *grxB,* encoding Grx2, were compared to those of the wild type. All proteomic analyses were performed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) followed by bioinformatics and gene ontology evaluations.

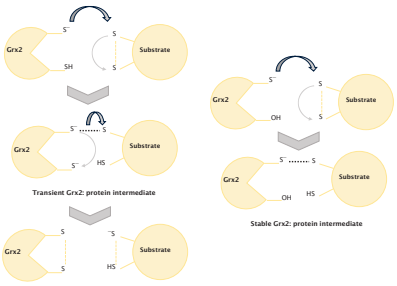

Figure 1. Catalytic mechanism and monothiol Grx substrate trap.

Methodology. Overexpression and purification of *E. coli* Grx2 C9S C12S and Grx2 C12S mutants. Each protein (approximately 6 mg) was immobilized on Affi-Gel 10 beads in chromatographic columns. *E. coli* cells were grown in LB-medium and collected during both exponential and stationary phases. Cell lysates were prepared and chromatographed through columns with of the immobilized Grx2 mutants. Empty resin served as control. Bound proteins were eluted with salt (KCl, step gradients), CH_3COOH/HCOOH and finally DTT. The eluted proteins were analyzed by LC-MS/MS. All cromatographies were performed in triplicates. Furthermore, *E. coli* wild type strain and the null mutant for *grxB* were grown and harvested in their exponential phase of growth. Their total proteins were analyzed by LC-MS/MS.

Results

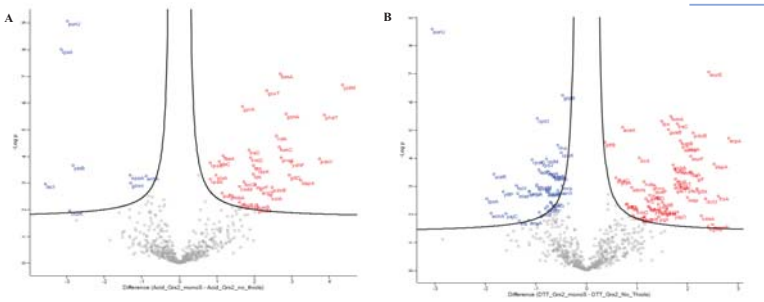

Figure 2. Volcano plot of statistically significant proteins, derived from affinity chromatography by A) Acidic and B) DTT elutions in the exponential phase of growth. Comparison of eluted proteins from monothiol Grx2 (red) and athiol Grx2 (blue).

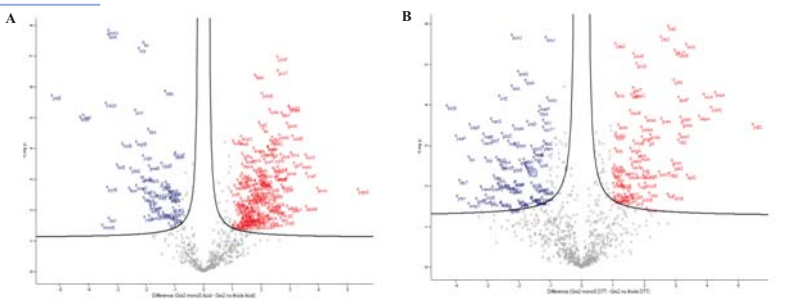

Figure 3. Volcano plot of statistically significant proteins, from affinity chromatographies and A) Acidic, B) DTT elutions in the stationary phase of growth. Eluates were from monothiol Grx2 (red) and athiol Grx2 (blue) columns.

Figure 4. Gene Ontology annotation of the statistically significant proteins, derived from affinity chromatography, performed in Cytoscape. Ontologies were retrieved from the databases Biological process, Molecular function Cellular component and Complexes. A) Exponential phase of growth acidic (left) and DTT (right) elution. B) Stationary phase of growth acidic (left) and DTT (right) elution.

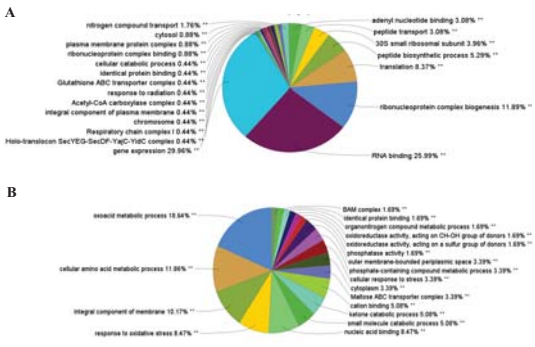

Figure 5. Gene Ontology annotation of the statistically significant proteins, derived from null *grxB* mutant whole proteome analysis, performed in Cytoscape. Ontologies were retrieved from the databases Biological process, Molecular function, Cellular component and Complexes. A) Up-regulated and B) Down-regulated proteins compared to wild-type.

Table 1. Top up and down regulated genes/proteins in *grxB*$^-$ compared to the wild type.

Up regulated	Protein description	Down regulated	Protein description
flu	Antigen 43	*zinT*	Metal-binding protein ZinT
yeeR	Inner membrane protein YeeR	*ykgM*	50S ribosomal protein L31 type B
cysU	Sulfate transport system permease protein CysT	*hyfA*	Hydrogenase-4 component A
ydbL	Uncharacterized protein YdbL	*znuA*	High-affinity zinc uptake system protein ZnuA
hfq	RNA-binding protein Hfq	*ilvN*	Acetolactate synthase isozyme 1 small subunit
ydiE	Uncharacterized protein YdiE	*ilvB*	Acetolactate synthase isozyme 1 large subunit
fumD	Fumarase D	*rraB*	Regulator of ribonuclease activity B
rpmE	50S ribosomal protein L31	*torZ*	Trimethylamine-N-oxide reductase 2
ypeB	Uncharacterized protein YpeB	*yjiM*	Putative dehydratase subunit YjiM
exbD	Biopolymer transport protein ExbD	*yhhW*	Quercetin 2,3-dioxygenase

Conclusions

1. Affinity chromatography experiments revealed that Grx2-interacting proteins may be involved, in translation, metabolism and iron binding.
2. *grxB*$^-$ -wild type whole proteome comparisons showed significant changes in the levels of proteins involved in metabolism, translation, transcription, response to toxic substance and DNA damage.
3. Grx2 appears as a multifunctional protein involved in many more biological pathways than its known general antioxidant function.

References

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2.Ogata F. T., Branco V., Vale F. F., Coppo, L. (2021). Glutaredoxin: Discovery, redox defense and much more. *Redox Biology*, 43, 101975.

Acknowledgements

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