Implications from the updated interactome of Glutaredoxin 1 from *Escherichia coli*

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Introduction

The glutaredoxin (Grx) and thioredoxin systems catalyze the reversible reduction of disulfides in the cytosol of all organisms including viruses. Glutaredoxin 1 (Grx1) from *Escherichia coli* (*E. coli*) is a small (85 amino acids, 9,7 kDa) cytosolic oxidoreductase that catalyzes glutathione (GSH)–dependent thiol–disulfide exchange reactions. Three more Grxs in the *E. coli* cytosol, along with Grx1 maintain redox homeostasis. However, Grx1 was initially discovered, and remains, the major electron donor of ribonucleotide reductase, the key enzyme for the *de novo* biosynthesis of deoxyribonucleotides [1]. All dithiol Grxs have an active site of the type CxxC ($C^{11}PYC^{14}$ for Grx1) to react via a transient covalent disulfide bond with their substrates [2]. Although this redox activity is well established, the specific covalent and non-covalent interaction partners of Grx1 have not been systematically analyzed. Our study aimed at investigating the largely ignored non-covalent interactions of Grx1. The approach used was affinity chromatography of cell extracts through columns with immobilized Grx1, reduced or oxidized. Our findings combined with *in silico* analysis suggest that the formation of a simple disulfide in the active site of the enzyme may modify its binding affinity and specificity to its protein ligands.

Methodology

In a first step, wild type Grx1 (oxidized or reduced) was used as a bait in affinity chromatography. Cellular lysates from *E. coli* were prepared and chromatographed under oxidizing (selenite) or reducing (DTT/GSH) conditions through the Grx1-containing affinity columns. Unsubstituted Affi-Gel 15 resin was used as a control. Bound proteins were eluted sequentially by increasing concentrations of KCl, followed by acidic elutions (CH_3COOH/HCOOH, pH 2.1) and finally reduction by DTT (5 mM). All chromatographies (bait and controls) were performed in triplicates. Collected fractions were precipitated with sodium deoxycholate and TCA, washed with methanol, dried, and resuspended in Tris-SDS. The concentrated eluates from each condition were identified by LC-MS/MS and subjected to bioinformatic processing and functional enrichment evaluation. In a second step, the herein identified protein ligands along with all other known protein ligands from UniProt were subjected to *in silico* docking (Prism) and the binding parameters of the formed complexes were determined by Prodigy. The contacts of the surface residues of reduced and oxidized Grx1 in complexes (by Prism) were detected by Biovia. A percentage representation of all contacts was made after considering all possible contacts of each amino acid of Grx1 with those of the selected protein ligands.

Results

1. Novel protein partners and functions for Grx1

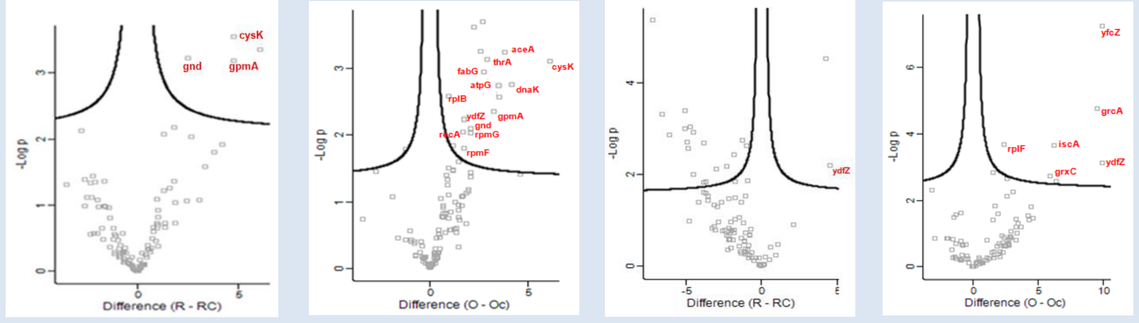


Figure 1. Analysis of the proteomic data by Volcano plots. Four independent two-sample t-tests were performed using the Perseus software to compare each experimental condition with its corresponding control. Volcano plots display the $\log_2$ fold-change between sample and control (x-axis) and the statistical significance $-\log_{10}(p)$ (y-axis). Proteins above the significance thresholds (up right) were considered differentially enriched and were termed as interaction partners of Grx1. Acidic elution under reducing conditions (a) and acidic elution under oxidizing conditions (b). DTT elution following reducing conditions (c) and DTT elution following oxidizing conditions (d).

A

Grx1-S_2 (oxidized)	Elution ACID	Grx1 $(SH)_2$ (reduced)
Log_2 fold difference	*gene*	Log_2 fold difference
6,16	***cysK***	3,546789
3,80	***aceA***	
3,27	***gpmA***	3,185798
3,10	***atpG***	
2,91	***thrA***	
2,75	***fabG***	
2,35	*dnaK*	
2,09	***gnd***	2,491717
2,08	***rpmG***	
2,05	***ydfZ***	
1,76	***rpmF***	
1,702315	*recA*	
0,954770	***rplB***	

B

Grx1-S_2 (oxidized)	Elution DTT	Grx1 $(SH)_2$ (reduced)
Log_2 fold difference	*gene*	Log_2 fold difference
9,885815	***ydfZ***	2,913315
9,33024	***yfcZ***	
9,538066	***grcA***	
6,215289	***iscA***	
5,897476	*grxC*	
2,348083	***rplF***	

Tabe 1. The identified proteins ligands. The specifically eluted protein species from the affinity chromatographies are presented as genes. Eluates from different elution conditions are from columns with immobilized (bait) reduced or oxidized Grx1. Genes in bold represent new findings. *ydfZ* was present both A and B. The specificity of these findings was demonstrated by the fact that no protein whatsoever was detected in the final 500 mM KCl elution step preceding the herein presented elutions by acid and DTT.

Previous art, common, new

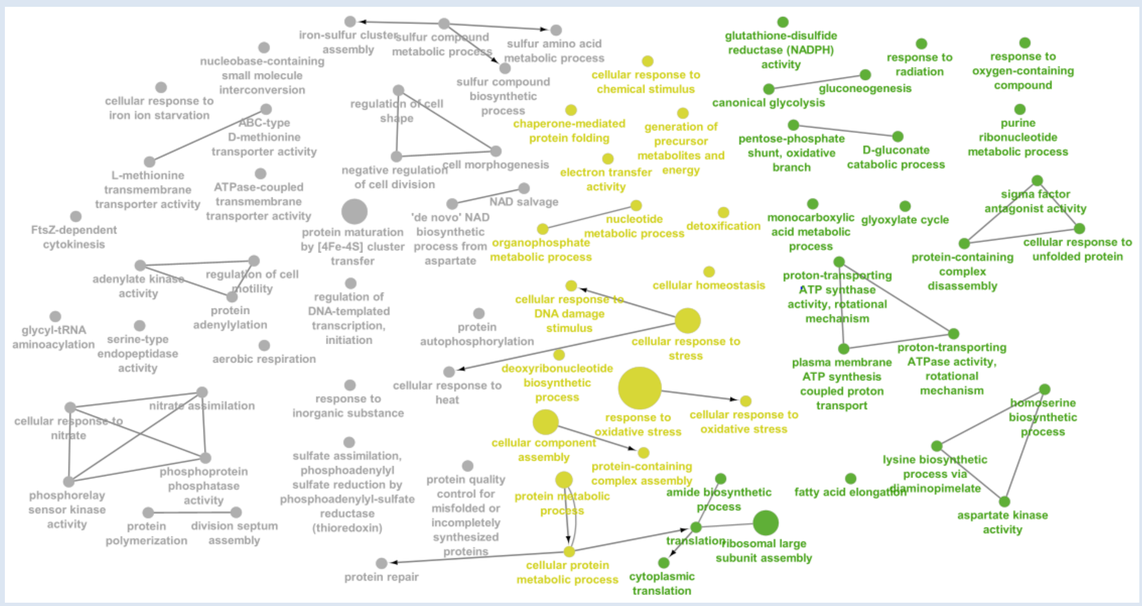


Figure 2. Functional network and Biological Process pathways based on the updated interactome of Grx1. The graph was generated using Cytoscape ClueGO [3]. The presented network enrichment combined the protein substrates uncovered in this study (green) with the known interaction partners of Grx1 from FunCoup, BioGRID and InterAct. Functionally related GO terms are grouped into pathway clusters based on shared gene contributions. Grey nodes represent previously known genes and pathways, green nodes represent newly discovered substrates identified in this study, while yellow nodes indicate pathways in which both newly discovered partners and previously known genes participate.

2. The redox state of Grx1 may affect its contact interfaces with protein ligands

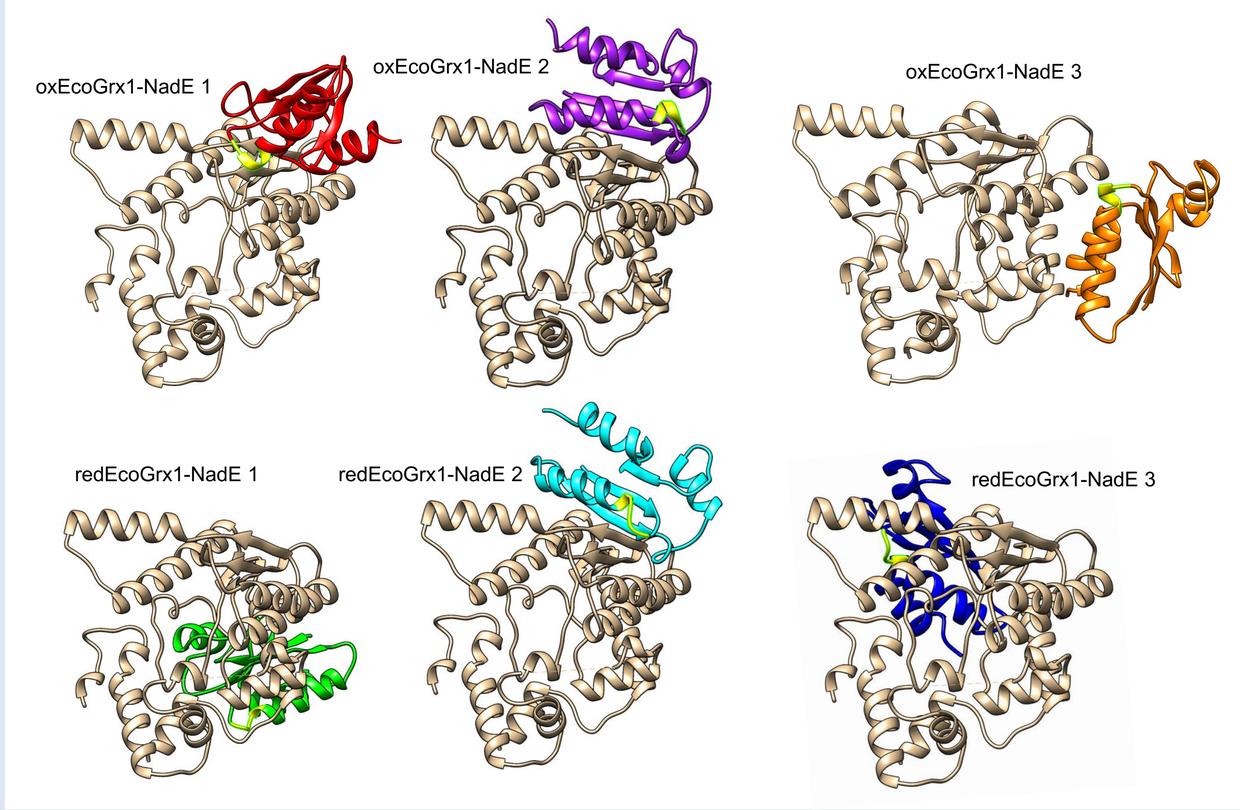


Figure 3. Reduced and oxidized Grx1 may bind to the same ligand but with different interfaces. Reduced and oxidized Grx1 may bind to different proteins (table 1). To examine the structural basis of this differentiation, *in silico* docking (Prism) was performed on identified protein ligands of the reduced and oxidized Grx1 (redEcoGrx1 and oxEcoGrx1 respectively). In the presented example of figure 3, a protein ligand (here NadE, P18843), bound to the two forms of Grx1 but at different places. The active site disulfide of Grx1 is presented by yellow.

A. Ligands interacting only with oxidized Grx1

#No.	UniProt ID	Gene	PDB ID or AF	Docking Score (kcal)	Interface ID	ΔG (kcal mol^{-1})	K_d (M) at 25 °C	Interaction type	Reference
1	P0ABK5	*cysK*	5j43	-7.21	3spuAC	-8.9	3.1e-07	sNC	This work
2	P0A7G6	*recA*	1u94 (chains A and B)	-16.93	3gl9BC	-26.1	7.2e-20		
3	P0AD33	*yfcZ*	AF-P0AD33-F1-model_v6	-13.5	3t5pAH	-12.6	5.3e-10		
				-11.1	2obkAB	-11.2	6.6e-09		
4	P00561	*thrA*	6MX1	-6.51	3ijmAB	-7.6	2.5e-06		
				-4.55	4a9eAB	-10.1	4e-08		
5	P0A9X4	*mreB*	AF-P0A9X4-F1-model_v6	-25.41	3k2vAB	-9.6	8.8e-08	Pull down	IntAct
				-11.63	2hw6AB	-6.6	1.4e-05		
				-10.32	1s5fDE	-7.4	3.7e-06		
6	P09158	*speE*	3o4f (chain A)	-23.42	1z8hAB	-11.6	2.9e-09	Y2H	BioGRID
				-5.04	2wirAB	-6.8	1e-05		
				-1.17	1inlAB	-9.0	2.7e-07		
7	P73728	*prx5_syny3*	AF-P73728-F1-model_v6	-17.53	1ytaCD	-8.4	6.3e-07	Enzymatic	IntAct
8	P77174	*ybdM*	AF-P77174-F1-model_v6	-8.1	2b8nAB	-12.4	8.5e-10	Y2H	
9	P00960	*glyQ*	7eiv	-7.51	1rjdAC	-22.2	5.1e-17	Pull down	
				-4.03	1rjdAC	-22.2	5.3e-17		

B. Ligands interacting only with reduced Grx1

#No.	UniProt ID	Gene	PDB ID or AF	Docking Score (kcal)	Interface ID	ΔG (kcal mol^{-1})	K_d (M) at 25 °C	Interaction type	Reference
10	P62707	*gpmA*	1e58	-31.23	1t1vAB	-9.3	1.5e-07	sNC	This work
				-23.08	3n2lEF	-9.5	1.2e-07		
11	P0AEK2	*fabG*	1q7b	-3.42	1ytaCD	-7.3	4.7e-06		
12	P0A6Y8	*dnaK*	4b9q (chain C)	-9.5	1zwzAB	-8.6	4.9e-07		
				-1.71	3ci6AB	-8.1	1.1e-06		
13	P00350	*gnd*	2ZYA	-10.98	2r6aAC	-7.5	3e-06		
				-8.23	2yvzAB	-9.7	7.3e-08		
14	P0AAC8	*iscA*	1s98	-9.19	3do8AB	-8.7	4.1e-07		
				-3.59	3ijmAB	-8.3	8.1e-07		
15	P75679	*insN1*	AF-P75679-F1-model_v6	-7.43	3ct6AB	-11.7	2.8e-09	Y2H	IntAct
16	P24178	*yffB*	AF-P24178-F1-model_v6	-19.19	3s55BC	-11.0	8.5e-09	Y2H	BioGRID
				-3.82	2b8nAB	-12.5	6.3e-10		
				-2.8	3ky8AB	-12.9	3.6e-10		
17	P0A6Z3	*htpG*	2iop chain A	-6.03	3ijmAB	-10.3	3e-08	Pull down	IntAct
18	P75863	*ycbX*	AF-P75863-F1-model_v6	-13.48	3eo6AB	-13.3	1.8e-10	Y2H	BioGRID
19	P0ACC3	*erpA*	AF-P0ACC3-F1-model_v6	-44.29	1rd5AB	-9.9	5.1e-08		FunCoup
20	P63020	*nfuA*	AF-P63020-F1-model_v6	-26.77	1r1uAB	-8.6	4.9e-07		
				-6.17	2q03AB	-11.6	3e-09		
				-1.76	2ux8BC	-10.0	5e-08		
				-1.08	3ci6AB	-10.4	2.5e-08		

Oxidized Grx1 was from pdb 1ego, reduced Grx1 from pdb 1egr

Tabe 2. Characteristics of the interaction of protein ligands that interact selectively with oxidized (A, 1-9, 16 interfaces) or reduced (B, 10-20, 20 interfaces) Grx1. Interactions were performed by Prism, binding characteristics were determined by Prodigy. Y2H stands for yeast two hybrid, sNC for strong non-covalent interaction (acidic elution).

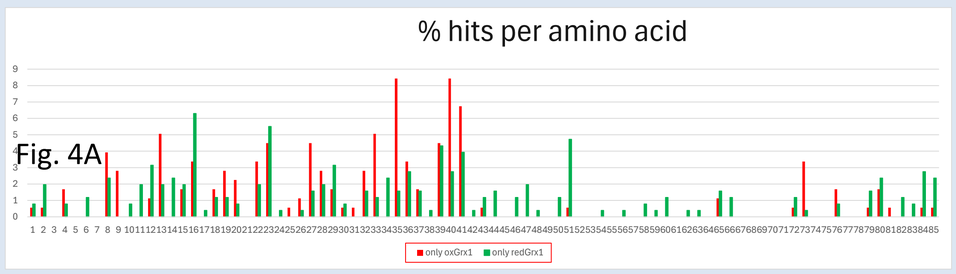


Fig. 4A

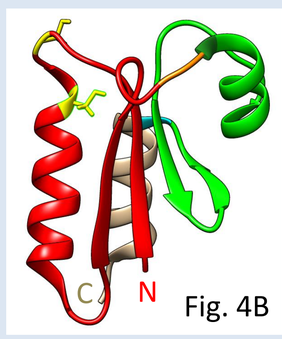
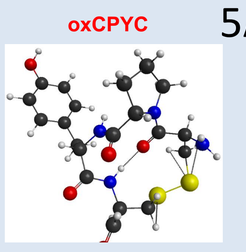


Fig. 4B

Figure 4. Preference of surface residues of the reduced and oxidized Grx1 in their respective interactions with protein ligands. Figure 4A shows the % contribution of residues from the oxidized (red) or reduced (green) residues of Grx1 in their interactions with the ligands of table 2. In figure 4B the red part (residues 1-41) corresponds to the amino acids of both oxidized and reduced Grx1 that interact with substrates. The green part in Fig. 4B (residues 44-71) presents the residues that seems to prefer interactions with exclusively reduced Grx1. The presented molecule in Fig. 4B) is the reduced form of Grx1 with the active site cysteines ($C^{11}PYC^{14}$) shown in yellow, the $G^{42}I^{43}$ in orange and $G^{70}G^{71}$ in cyan.



State	Grx1 segment	Energy (Units in Hartree)	Tm (° C)
oxidised	$C^{11}P^{12}Y^{13}C^{14}$	-2167.8310	55
reduced		-2168.7289	57

5B

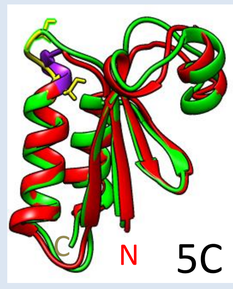


Figure 5. The disulfide of the active site CPYC attains a slightly less stable conformation. Single point energy calculations showed that the oxidized dithiol active site is slightly less stable (A, B). This has been previously observed in heat-induced denaturations of the two forms of the whole molecule [4]. The RMSD between 70 pruned atom pairs of reduced and oxidized Grx is 0.920 angstroms, while across all 85 pairs is 1.628 (Chimera).The structures of the two forms (red: oxidized, green: reduced, figure 5C) appear thus almost identical. Reduced Cys are in yellow.

Conclusions

1. Oxidized and reduced Grx1 may bind to protein ligands very tightly in a non-covalent manner. Oxidized Grx1 favours even tighter binding.
2. The herein novel 16 ligands increase further the present 29 protein-interactome of Grx1 and implicate it in cellular processes such as protein folding, ribosome biogenesis, and metabolic regulation beyond its known functions (redox homeostasis and biosynthesis of deoxyribonucleotides).
3. Oxidized and reduced Grx1 may contact the same protein ligand with different interfaces. The oxidized form tends to interact mostly using the first half of the molecule, containing the active site. The reduced form may use additional contact points from the other half.

Future work aims at finding the fine details that may determine substrate specificity between the so similarly structured reduced and oxidized forms of Grx1.

References

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Acknowledgements

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