Eltayb et al., J Proteomics Bioinform 2017, 10:4
DOI: 10.4172/jpb.1000431

Journal of
Proteomics & Bioinformatics

Research Article Open Access

Number of Cysteine Interactions with the Activity in GRX Family

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Abstract

Grx families are small proteins that are present in eukaryotic and prokaryotic organisms, and in a few viruses, most species have several glutaredoxin isoforms. This study primarily aims a comparative analysis on the duplication of amino acids and its effect on protein function and interaction with the active site. The second active-site cysteine or non-catalytic cysteine affects the reactivity of the catalytic cysteine. Most of the proteins can form an intramolecular the disulfide between cysteines of the two active sites, which could play the role of a protective mechanism for the cell. The non-catalytic cysteine is unnecessary for deglutathionylation reaction or a true catalytic intermediate formed during the reduction of particular disulfide substrates or in particular conditions or compartments where glutathione levels are insufficient to support Grx regeneration. However, all these factors can be influenced by differences in the expression pattern and subcellular localization.

Keywords: Cysteine; Grx; Glutaredoxin; Active-site; Dithiol; Monothiol

Introduction

Glutaredoxins (GRXs or GLRXs) tend to be little pervasive oxidoreductases that belong to the Thioredoxin (Trx) superfamily and commonly contain a CxxC/S active-site motif. By utilizing reduced Glutathione (GSH) as reductants, as well as an NADPH-dependent Glutathione Reductase (GR), Grxs have the ability to reduce the disulfide bridges or glutathionylateproteins [1].

In the whole life kingdom, there are various Grx isoforms that could be categorized into three unique subgroups. The first type, which consists of Grxs along with C[P/G/S][Y/F][C/S] motifs, is actually homologous to the conventional Dithiol Grxs such as Yeast Grx1 and 2, *Escherichia coli* Grx1 and 3, and mammalian Grx1 and 2. The second type has a conserved CGFS active-site sequence consisting of Grxs homologous to *E. coli* Grx4 and yeast Grx3, Grx4, and Grx5. The third type specially exists in plants containing a CC[M/L][C/S] active site [2,3]. Glutaredoxins and thioredoxin follow two distinct reaction mechanisms that require either one or two cysteines in the active-site motif.

Among all organisms, higher plants have a huge number of genes coding for Grxs [4], while a *Drosophila melanogaster* and *Saccharomyces cerevisiae* have only a limited number of Grxs family members (Figure 1).

Glutaredoxins tend to be evolutionarily conserved; glutathione-dependent oxidoreductases are vitally implicated in the maintenance of cellular redox homeostasis [5].

Knowledge about the Grx family is expanding progressively day by day. At the time of writing this review, PubMed listed approximately 1518 entries for glutaredoxin. This study focuses on comparative analysis of the duplication of amino acid in the active site and explains how it affects the active site and protein function.

Method

All the data presented here was collected from the PDB and UniProt database, while the multalin sequence created by ClustalW.

Grx Multiple Sequence Alignment

Figure 2 Grx sequences have been shortened to about 25 amino acids in front of the active site and about 70 amino acids beyond the active site (blue). This keeps the active site and the GSH-binding site intact. In addition, this removes any existing N-terminal signal peptides, after the arrival of Grxs to their destinations. So they do not have any direct impact on Grx function. TVP (green) and GG (red) motif take part as the interface in the substrate GSH and also interact with GSSG, while the pink motif denotes are positively charged residues.

Glutaredoxins are redox proteins and divided into two subclasses: Dithiol and Monothiol. Several Grxs have been shown to form iron-sulfur cluster belonging to both the classes, depending on the presence of one or two cysteines in the active site (CGFS and CPYC). The Dithiol Grx homodimer is proposed to act as a sequestration form and its iron-sulfur cluster as an oxidative stress sensor. While the Monothiol Grx homodimer has been suggested to serve as a scaffold for iron-sulfur cluster delivery [6], different Grxs have a different number of Cys in their sequence (Table 1). Most of the tetramers and dimers have one or two Cys while most of the monomer has more than three Cys. Human GLRX5 has a single cysteine in its CGPS active-site motif; it has been shown to have thiol reductase activity, while the 2nd cysteine Cys-117 is essential for the catalytic activity [7]. Human GLRX2 known to form an iron-sulfur containing dimer, in addition, mutation of non-catalytic cysteine residues annul dimer formation during recombinant bacterial expression, and for these reasons, the non-catalytic cysteine has been suggested to mediate dimerization by coordinating the iron-sulfur cluster [8]. In the situation of a more oxidizing environment, an excessive redox potential for the viral glutaredoxin would be needed to ensure that a major fraction of the enzyme dwells in the active reduced state; in this case, a related adaptation may be the loss of three non-catalytic cysteine residues that are conserved in most mammalians. In humans, Grx-1, Cys8, Cys79, and Cys83 have been suggested to play a redox-sensitive regulatory role. Cys8 and Cys79 are surface-exposed

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Received November 22, 2016; **Accepted** April 07, 2017; **Published** April 11, 2017

Citation: Eltayb WA, Abdalla M, Samad A, EL-Arabey AA, Ghanam AR, et al. (2017) Number of Cysteine Interactions with the Activity in GRX Family. J Proteomics Bioinform 10: 114-118. doi: 10.4172/jpb.1000431

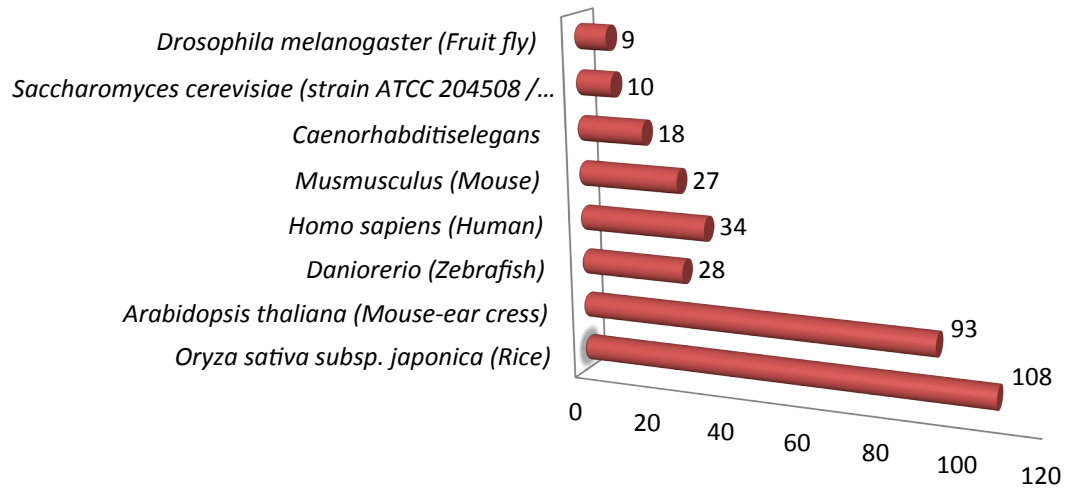

Figure 1: Distribution of Grx proteins in some species.

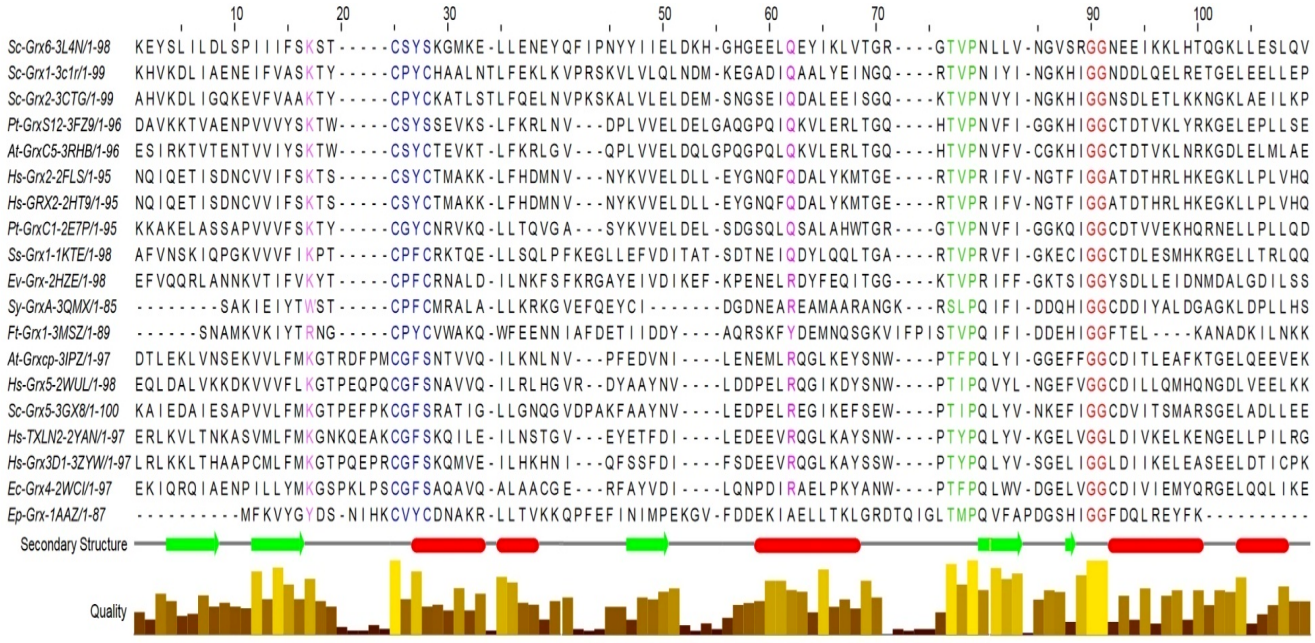

Figure 2: Grx multiple sequence alignment.

and oxidation of Cys8 has been shown to decrease the lifetime of human Grx-1 due to disulfide-linked aggregate formation [9].

It has been reported that human Grx1 consists of extra active-site cysteines (Cys_3) and oxidative treatment led to the identification of several possible post-translational modifications. Disulfide-bonded dimers and oligomers, intramolecular disulfide, glutathione adducts, and nitrosylation play a role in inhibition [10]. Cys_3 was found to be either glutathionylated or nitrosylated or involved into a disulfide bond formation. Similarly, redox changes occur of plant GrxC1 and C2 upon treatment with oxidants that could further provide clues about the possible function of this cysteine. In the presence of H_2O_2, this cysteine was found to be involved in disulfide-bridged homodimers, while in the presence of GSSG or GSNO, Cys_3 is prone to oxidative modification, but in the form of a glutathione adduct [4].

Poplar GrxC1 have four chains A, B, C and D. Chains B and D do not directly interact with the [2Fe–2S] cluster but serve to stabilize the tetramer structure [11]. The cluster was not observed in the poplar Grx C1 C31S variant, showing that the catalytic cysteine (Cys-31) is likely to be a cluster ligand; at the same time, both Cys-34 and Cys-88 play a role in stabilizing the [2Fe–2S] cluster against oxygen degradation. The Cys-28 in addition to Cys-113 in human Grx2 perhaps has a structural role that assists Fe–S cluster assembly instead of providing cluster ligands [11].

In plants GrxC1 and GrxC2 and other eukaryote Grxs, the mutation

PDB ID	Title	Oligo State	Ligands	Active-site motif	Organism	N. Cys
2cq9	GLRX2 protein	Monomer	None	CSYC	*Homo sapiens*	4
3d4m	Glutaredoxin-2, mitochondrial	Monomer	None	CPYC	*Saccharomyces cerevisiae*	2
2fls	Glutaredoxin-2	Monomer	1 × GSH	CSYC	*Homo sapiens*	4
3d5j	Glutaredoxin-2, mitochondrial	Monomer	1 × GSH	CPYS	*Saccharomyces cerevisiae*	1
2e7p	Glutaredoxin	Homo-tetramer	4 × GSH, 1 × FES	CGYC	*Populus tremuloides*	3
2ht9	Glutaredoxin-2 3.05	Hetero-oligomer	2 × GSH, 1 × FES	CPYS	*Homo sapiens*	1
3ctg	Glutaredoxin-2	Monomer	None	CPYC	*Saccharomyces cerevisiae*	2
1z7r	Glutaredoxin	Monomer	None	CGYC	*Populus tremuloides*	3
3c1s	Glutaredoxin-1	Monomer	1 × GSH	CPYC	*Saccharomyces cerevisiae*	2
3rhc	Glutaredoxin-C5, chloroplastic 2.57	Homo-dimer	2 × GSH, 1 × FES	CSYC	*Arabidopsis thaliana*	4
1jhb	GLUTAREDOXIN	Monomer	None	CPYC	*Homo sapiens*	5
4rqr	Glutaredoxin-1	Monomer	2 × COM	CPYC	*Homo sapiens*	5
1z7p	Glutaredoxin	Monomer	None	CGYC	*Populus tremuloides*	3
3d5j	Glutaredoxin-2, mitochondrial	Monomer	1 × GSH	CPYS	*Saccharomyces cerevisiae*	1
2jac.	GLUTAREDOXIN-1	Monomer	1 × GSH	CPYS	*Saccharomyces cerevisiae*	1
3c1r	Glutaredoxin-1	Monomer	1 × MES or 1 × GSH	CPYC	*Saccharomyces cerevisiae*	2
1kte	Thiol transferase	Monomer	None	CPFC	*Sus scrofa*	4
3fz9	Glutaredoxin S12	Monomer	1 × GSH	CSYS	*Populus tremula x*	2
3qmx	Glutaredoxin	Monomer	None	CPFC	*Synechocystis* sp.	4
2hze	Glutaredoxin	Monomer	None	CPFC	*Ectromelia virus*	3
3ipz	Monothiol glutaredoxin-S14	Monomer	None	CGFS	*Arabidopsis thaliana*	3
1aaz	*T4 glutaredoxin*	Monomer	2 × CD	CVYC	*Enterobacteria phage t4 sensu lato*	2
2wul	GLUTAREDOXIN RELATED PROTEIN 5	Homo-tetramer	4 × GSH, 2 × FES	CGFS	*Homo sapiens*	2
3gx8	Glutaredoxin-5	Monomer	None	CGFS	*Saccharomyces cerevisiae*	2
3msz	Glutaredoxin 1	Monomer	1 × CAC, 1 × GSH	CPYC	*Francisella tularensis*	2
2wci	GLUTAREDOXIN-4	Homo-tetramer	4 × GSH, 2 × FES	CGFS	*Escherichia coli*	2
2yan	GLUTAREDOXIN-3	Monomer	1 × GSH, 1 × FE	CGFS	*Homo sapiens*	1
3zyw	Glutaredoxin(Glrx3)	Monomer	None	CGFS	*Homo sapiens*	3
5j3r	Glutaredoxin-6	Dimer	2 × GSH, 1 × FES	CPYS	*Saccharomyces cerevisiae*	1
Cacodylate dimethylarsinate (Synonym) (CAC), Glutathione (GSH), Cadmium (CD), 2-(N-Morpholino) Ethanesulfonic acid (MES), Thioethanesulfonic acid (COM).						

Table 1: Comparison between Grx families member.

of the second cysteine (Cys_2) increased their activity. The data indicates that the presence of Cys_2 slows down the reaction for some reason. It was found that mutation could not affect the activity of plant GrxC3 and GrxC4 [4]. Structural analysis of AtGRX displayed that Cys172 is existing in the α5-helix in the C-terminus. On the molecular surface form an intermolecular disulfide bond with Cys172 of a symmetry-related Grx molecule [9].

E. coli Grx-1 structure demonstrates the covalent intermediate of disulfide reduction by glutaredoxin together with a mixed disulfide between Cys11 of the enzyme and Cys75 of the peptide. Cys14 and Cys75 were mutated to serine to obtain a stable complex [9]. Accumulating evidence shows that Monothiol Glutaredoxins are essential both in yeast and *E. coli*. Their conserved appearance in higher order genomes proposes that any rife functionality in this subfamily is most likely for fundamental biological mechanisms [12]. With the Presence of three cysteines in *E. coil* Grx4; glutathione should be attached to the cysteine in the CXFX tetrad. The remarkable surface conservation among Monothiol proposes that they are all able to employ the same cysteine to form a mixed disulfide with glutathione [13]. It has been noted that the presence of Cys60 and Cys117 in yeast Grx5 resulted in a higher reactivity of the protein toward GSSG [14].

In monothiol glutaredoxins with only one cysteine in addition to that in the CXFX tetrad, a structural change on glutathionylation could encourage mixed disulfide formation with specific targets or in oligomerization events [13]. John and Bart suggest that the replacement of three non-catalytic cysteine residues that are conserved in many mammalian sequences prevents enzyme inhibition in oxidizing environments [15]. Li et al. [9] in comparative studies denote that the active-site motifs in monothiol Grxs are quite likely to be flexible and some conformational changes may occur when a ligand binds to an enzyme. While more of amino acids similar to the active site may make several conformational changes.

From an evolutionary point of view, it is spectacular to observe that the monothiol Grxs show a high degree of homology when compared with the Dithiol Grxs. Proteins having mono cysteine are unable to execute the whole reduction of a disulfide group. They are able to raid a disulfide bridge of model proteins *in vitro*, creating a stable intermediate complex. When the positivity and negativity of non-catalytic cysteine residues are similar to what is in the active site, it will be more difficult to crystal and also it will affect the probability of FES linkage, conformational changes, post-translational modifications, and so on.

Grx Domain Distribution

CGFS and CPYC domains are the most common domains in Grx family (Figure 3). Grx CGFS and CPYC domain might be combined as multidomain with Frataxin-Rhodanese, thioredoxin, Peroxiredoxin domain, pyridine nucleotide-disulfide oxidoreductases, and peptide methionine sulfoxide reductase as well as to other less characterizing protein domains, while Grx CCMC domain is found as a single

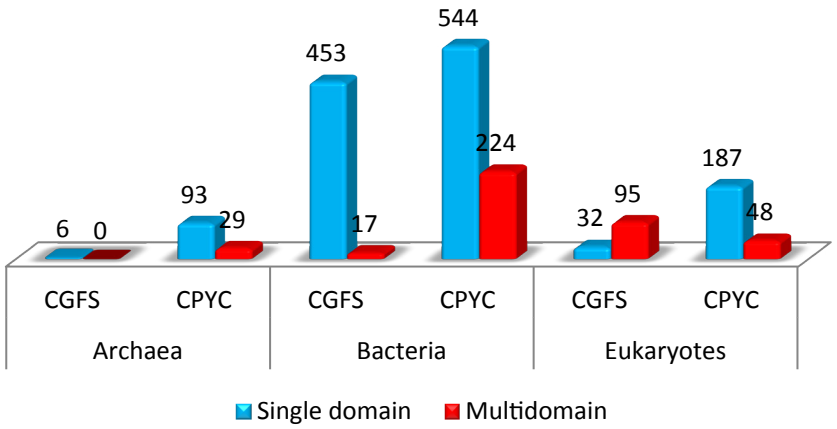

Figure 3: Classification of Grx domains based on the UniProt database.

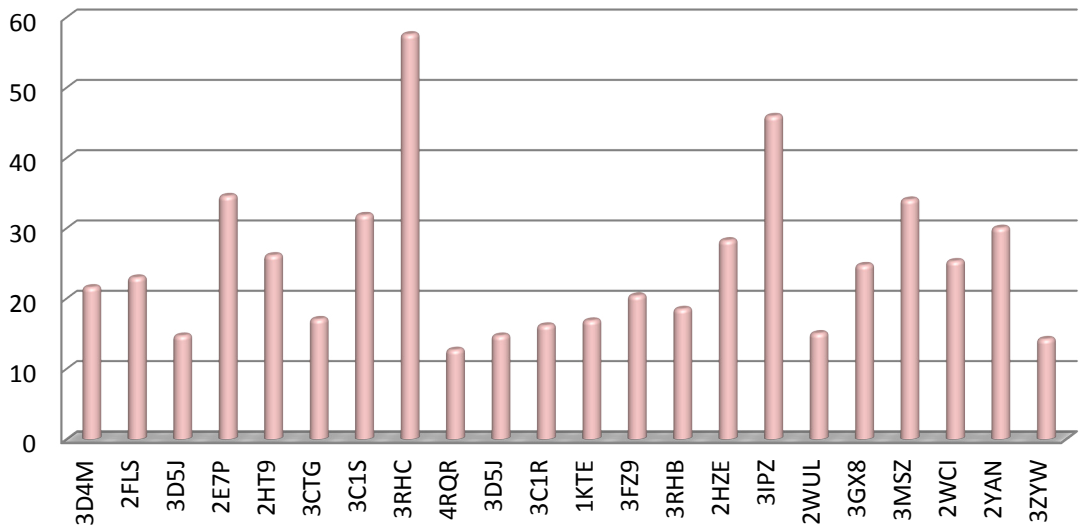

Figure 4: The Grx average temperature factor of the active-site motif. x axis refer protein name using the PDB ID, while y axis refer to the temperature factor.

domain in higher plants. This indicates that Grx widespread function and somehow shares the same function in different stages of the same biological process. Also, the genomes of some organisms encode larger proteins with Grx domains that need to be characterized.

Grx Average Temperature Factor

This Grx average temperature factor of the active-site motif (Figure 4) suggests that the conformation of the active-site motif in Grx has higher B factor and is less stable, which agrees with our conclusion that the active-site motif in monothiol Grxs is more flexible.

Acknowledgements

A. R. Ghanam and Waleed A Almahi, is a recipient of Chinese Scholarship Council (CSC), Mohnad Abdalla, Wafa Ali Eltayb, Abdus Samad, Amr A EL-Arabey, is a recipient of CAS-TWAS President's PhD Fellowship. The authors declare that they have no conflicts of interests.

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