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Structural and Biochemical Characterization of Yeast Monothiol Glutaredoxin Grx6

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Glutaredoxins (Grxs) are a ubiquitous family of proteins that reduce disulfide bonds in substrate proteins using electrons from reduced glutathione (GSH). The yeast *Saccharomyces cerevisiae* Grx6 is a monothiol Grx that is localized in the endoplasmic reticulum and Golgi compartments. Grx6 consists of three segments, a putative signal peptide (M1-I36), an N-terminal domain (K37-T110), and a C-terminal Grx domain (K111-N231, designated Grx6C). Compared to the classic dithiol glutaredoxin Grx1, Grx6 has a lower glutathione disulfide reductase activity but a higher glutathione *S*-transferase activity. In addition, similar to human Grx2, Grx6 binds GSH via an iron–sulfur cluster *in vitro*. The N-terminal domain is essential for noncovalent dimerization, but not required for either of the above activities. The crystal structure of Grx6C at 1.5 Å resolution revealed a novel two-strand antiparallel β-sheet opposite the GSH binding groove. This extra β-sheet might also exist in yeast Grx7 and in a group of putative Grxs in lower organisms, suggesting that Grx6 might represent the first member of a novel Grx subfamily.

Keywords: glutaredoxin; *Saccharomyces cerevisiae*; crystal structure; enzymatic activity; glutathione *S*-transferase

Introduction

Loss of redox homeostasis leads to accumulation of reactive oxygen species that can be harmful to cells. In addition to the thioredoxins (Trxs), a group of glutathione (GSH)-dependent oxidoreductases called glutaredoxins (Grxs) also function in maintaining this homeostasis.[1,2] Primary sequence alignment easily distinguishes most Grxs from Trxs. However, from the viewpoint of three-dimensional (3-D) structure, Grxs share a highly similar fold and topology with the Trx family†,[3] which is characterized by a central β-pleated sheet surrounded by five helices and a CXX(C/S) active-site motif.[1,4] As previously reported, Grxs carry out a number of biological functions, including reduction of ascorbate, activation of ribonucleotide reductase and 3′-phosphoadenylylsulfate reductase, and regulation of the DNA-binding capacity of nuclear factors.[4,5] Recently, however, a variety of Grxs or Grx-like proteins were found that execute distinct functions, such as signal transduction and iron–sulfur (Fe–S) cluster assembly.[5,6]

The genome of the yeast *Saccharomyces cerevisiae* encodes multiple Grx isoforms, making it an ideal model for exploring the diversity of Grx subcellular localizations and molecular functions. To date, eight Grxs have been found in yeast, named Grx1 to Grx8 in chronological order of identification‡. Grx1/YCL035C and Grx2/YDR513W are dithiol cytosolic Grxs that contain a conserved CPYC motif at the active site. Deletion of *GRX1* or *GRX2* will lead to some sort of sensitivity to superoxide anions and

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† http://pfam.sanger.ac.uk

Abbreviations used: β-ME-SG, glutathionylated β-mercaptoehanol; Fe–S, iron–sulfur; GR, glutathione reductase; Grx, glutaredoxin; Grx6C, C-terminal domain of Grx6; GSH, reduced glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; HEDS, bis-(2-hydroxyethyl)-disulfide; NBD-Cl, 4-chloro-7-nitro-2,1,3-benzoxadiazole; PDB, Protein Data Bank; Trx, thioredoxin.

‡ http://www.yeastgenome.org/

hydrogen peroxide, respectively, suggesting their different roles in protecting the yeast cells from oxidative stress.[7] Grx1 exists mostly in the cytoplasm. Upon reduction, its residues around the active site undergo conformational changes that reinforce GSH binding.[8] Grx2 is expressed in two isoforms via alternative translation using different start codons, with the full-length version translocated to the mitochondrial matrix and the truncated version retained in the cytosol.[9,10] In contrast to these two typical dithiol Grxs, the monothiol Grxs, Grx3/YDR098C, Grx4/YER174C, and Grx5/YPL059W, have an active site with the CGFS motif.[11] Grx3 and Grx4 are required for the regulation of the iron-dependent transcriptional factor Aft1 in the nucleus,[12] and Grx5 is involved in the synthesis or assembly of Fe–S clusters in the mitochondrial matrix.[13,14]

In addition to these five well-known yeast Grxs, three new members, Grx6/YDL010W, Grx7/YBR014C, and Grx8/YLR364W were recently identified.[15] Grx6 and Grx7 are monothiol Grxs, with an active-site motif of CSYS in Grx6 and of CPYS in Grx7. These Grxs were proposed to be responsible for regulating the sulfhydryl redox state in the oxidative conditions of early secretory pathway vesicles.[16] Heterogeneously expressed Grx6 and Grx7 have glutathione disulfide (GSSG) reductase activity, as detected by the GSH–HEDS [bis-(2-hydroxyethyl)-disulfide transhydrogenase] assay.[17] However, Grx6 is localized in both the endoplasmic reticulum and Golgi apparatus, while Grx7 is found mostly in the Golgi apparatus. Grx6, but not Grx7, binds two [2Fe–2S] clusters to form a tetramer *in vitro*.[17] In addition to Grx1 and Grx2, Grx8 is a third putative dithiol Grx, encoded by *GRX8/YLR364W*, which does not affect growth rate under oxidative stress conditions when deleted.[18] The apparent K_{cat} for Grx7 in the HEDS assay is about 1000-fold higher than that for Grx8, even though their apparent K_m values are comparable.[18]

To systematically explore structural insights into the diverse functions of Grxs, we initiated a project to solve the 3-D structures of all eight yeast Grxs. In addition to previous reports for Grx1 [under the Protein Data Bank (PDB) codes of 3C1R and 3C1S],[8] Grx2 (PDB codes 3CTF and 3CTG), and Grx5 (PDB code 3GX8),[19] we present here the biochemical characterization of Grx6, and the crystal structure of its C-terminal Grx domain (designated Grx6C). Full-length Grx6 and Grx6C possess similar GSSG reductase activities (EC 1.8.1.7) and glutathione *S*-transferase (GST) activities (EC 2.5.1.18), and the crystal structure of Grx6C at 1.5 Å resolution revealed a novel two-strand antiparallel β-sheet motif inserted into the classical Grx domain. Further structure-based multiple alignments of Grx6 against its bacterial and fungal homologs enabled us to define a new subfamily of Grxs.

Results

The organization of Grx6

Grx6 is composed of three segments, a putative signal peptide (M1-I36), an N-terminal domain

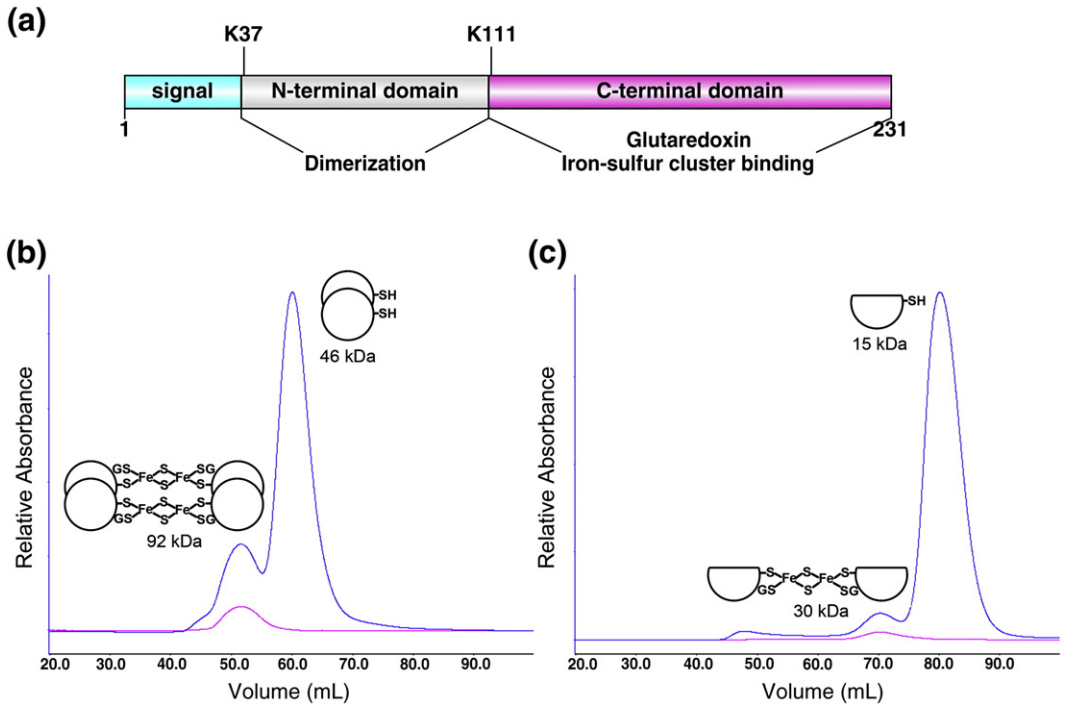

Fig. 1. The organization of Grx6. (a) Three segments of Grx6 drawn by Domain Graph, version 1.0.[20] Signal peptide, N- and C-terminal domains are shown in cyan, gray and pink, respectively. Gel-filtration chromatography of (b) Grx6 and (c) Grx6C. The blue and pink chromograms represent the absorbance at 280 and 430 nm, respectively. A schematic diagram of each oligomer is shown at the corresponding peak.