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# Cloning, overproduction, purification, crystallization and preliminary X-ray diffraction analysis of yeast glutaredoxin Grx5

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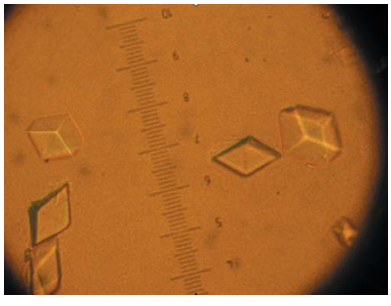
Grx5 from the yeast *Saccharomyces cerevisiae* is a monothiol glutaredoxin that is involved in iron–sulfur cluster biogenesis. Here, yeast Grx5 was cloned and overproduced in *Escherichia coli*. The purified protein was crystallized using the hanging-drop vapour-diffusion method. Diffraction data for Grx5 were collected to 1.67 Å resolution. The crystal of Grx5 belonged to space group *R*3, with unit-cell parameters $a = b = 85.12$, $c = 48.95$ Å, $\alpha = \beta = 90.00$, $\gamma = 120.00^\circ$.

## 1. Introduction

Glutaredoxins (Grxs) are small proteins that catalyze the reduction of protein disulfides or glutathione–protein mixed disulfides using reduced glutathione (GSH) as the electron donor (Holmgren, 1985, 1989). They belong to the thioredoxin-like protein superfamily and are involved in a large number of cellular processes (Martin, 1995). Besides functioning as an antioxidant, yeast glutaredoxin Grx5 plays an essential role in the biogenesis of iron–sulfur (Fe–S) clusters in mitochondria (Rodriguez-Manzaneque *et al.*, 2002). Homologues of yeast Grx5 from other species, including *Escherichia coli* Grx4, *Chlamydomonas* Grx3 and human Grx5, have been reported to possess similar functions (Rahlfs *et al.*, 2001; Picciocchi *et al.*, 2007; Zaffagnini *et al.*, 2008). A lack of Grx5 in mitochondria leads to the shutoff of biosynthesis of the Fe–S cluster, which is used as a cofactor by several enzymes, such as aconitase and succinate dehydrogenase in the tricarboxylic acid (TCA) cycle (Rodriguez-Manzaneque *et al.*, 2002).

The monothiol glutaredoxin Grx5 has significant homology to dithiol glutaredoxins (sequence identity ∼25%), mainly in the carboxyl-terminal region of the molecule (Rodriguez-Manzaneque *et al.*, 1999; Bellí *et al.*, 2002). In addition to Cys60 at the CGFS site, Grx5 has an additional cysteine Cys117 in the C-terminal region (Rodriguez-Manzaneque *et al.*, 1999). This cysteine residue has been reported to be essential for the antioxidative role of Grx5. In addition to Grx5, the other two yeast monothiol glutaredoxins, Grx3 and Grx4, share high sequence homology with Grx5 (sequence identity above 45%) and also play important roles in the Fe–S cluster relevant signal transduction pathway (Kumánovics *et al.*, 2008). However, none of these three proteins has been investigated from a structural point of view. Therefore, the crystal structure of Grx5 should provide insight into its molecular function.





## 2. Materials and methods

### 2.1. Cloning and protein expression

The open reading frame of *GRX5* lacking the sequence coding for the N-terminal mitochondrial targeting residues Met1–Tyr29 was amplified by PCR using the *Saccharomyces cerevisiae* genomic DNA as the template and inserted into a pET29a-derived vector between the *Nde*I and *Not*I sites. The protein was overproduced at 310 K in *E. coli* Rosetta (DE3) strain using 2×YT culture medium (5 g NaCl,

16 g bactotryptone and 10 g yeast extract per litre). *E. coli* Rosetta (DE3) cells were transformed with this plasmid. To induce expression of the desired protein, isopropyl $\beta$-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 m*M* when the $OD_{600}$ was 0.6 and the culture was grown for another 5 h at 310 K. Cells were harvested by centrifugation at 7330*g* for 10 min and then resuspended in lysis buffer (20 m*M* Tris–HCl pH 7.0). The suspension was sonicated and then centrifuged at 29 300*g* for 25 min at 277 K.

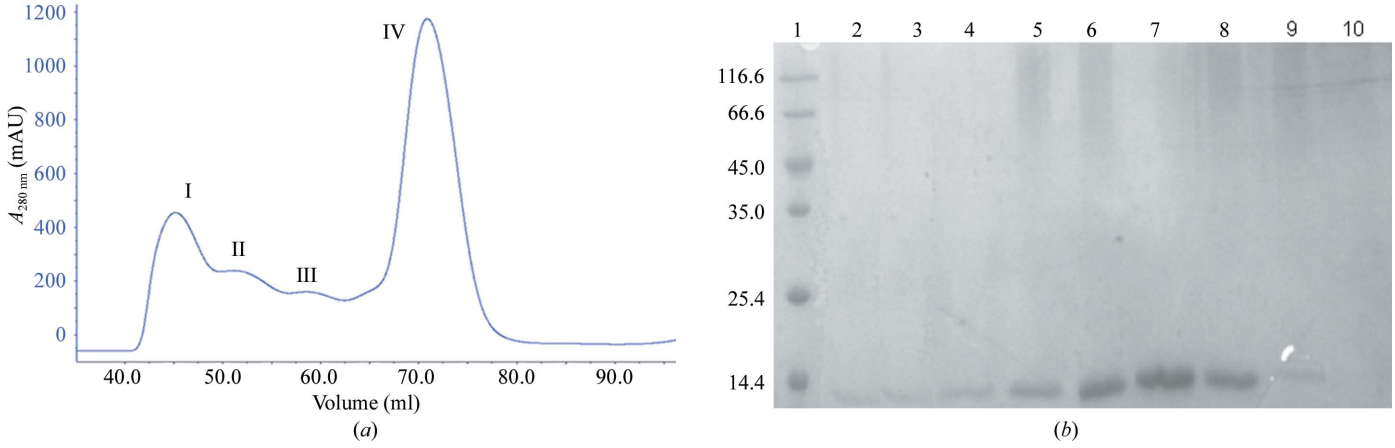



**Figure 1**
(*a*) Gel filtration of Grx5 using a HiLoad 16/60 Superdex 75 column. The flow rate was 1 ml $min^{-1}$. Impurities were contained in peaks I, II and III and target protein was contained in peak IV. (*b*) 15% SDS–PAGE analysis of fractions from gel filtration. Lanes 2–10, fractions from the main peak III; lane 1, low-molecular-mass markers (kDa).

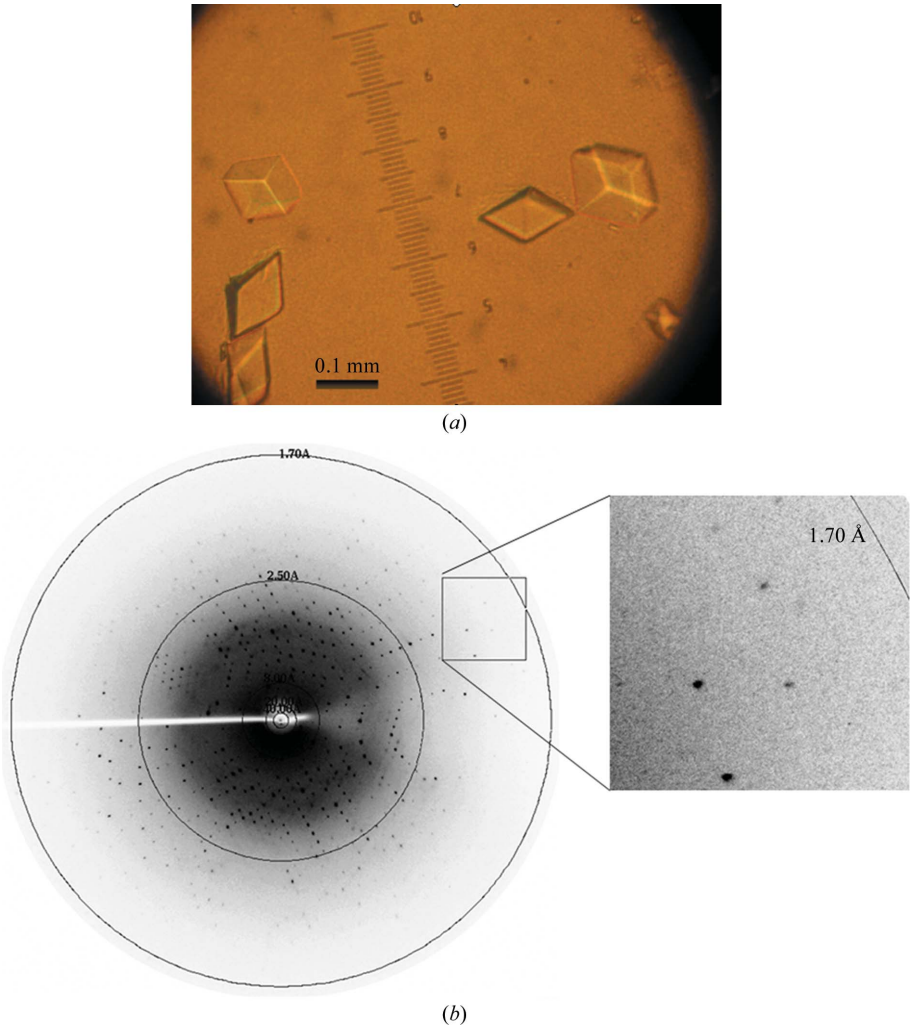



**Figure 2**
(*a*) Crystals of Grx5. (*b*) A diffraction image of a Grx5 crystal.

**Table 1**
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Crystal | Grx5 |
| Space group | *R*3 |
| Unit-cell parameters (Å, °) | $a = b = 85.12$, $c = 48.95$, $\alpha = \gamma = 90.00$, $\beta = 120.00$ |
| Temperature (K) | 100 |
| Wavelength (Å) | 1.54178 |
| Resolution range (Å) | 24.57–1.67 (1.76–1.67) |
| Total No. of observations | 63271 (7915) |
| No. of unique reflections | 15256 (2020) |
| Mean $I/\sigma(I)$ | 21.7 (5.4) |
| Completeness (%) | 98.5 (89.6) |
| Redundancy | 4.1 (3.9) |
| $R_{\rm merge}$ | 0.034 (0.303) |
| $R_{\rm meas}$† | 0.039 (0.352) |

† Calculated according to Evans (2006).

### 2.2. Protein purification

The supernatant was loaded onto an anion-exchange column (QFF-Sepharose, Amersham Biosciences, Sweden) pre-equilibrated with buffer containing 20 m*M* Tris–HCl pH 7.0 and the target protein was eluted with a linear gradient of NaCl from 0 to 1 *M*. The protein was further loaded onto a Superdex75 gel-filtration column (Amersham Biosciences, Sweden) and was eluted with a buffer containing 20 m*M* Tris–HCl pH 7.0, 100 m*M* NaCl. The approximate yield of purified Grx5 was 10 mg per litre of culture. After concentration to 8 mg ml$^{-1}$, the target protein was added to an equal volume of 100% glycerol and frozen at 233 K. The protein concentration was determined by measuring the absorption at 280 nm using a theoretical coefficient of 8605 $M^{-1}$ cm$^{-1}$ (http://www.expasy.org/cgi-bin/protparam).

### 2.3. Crystallization and X-ray data collection

Crystals of Grx5 were obtained by the hanging-drop vapour-diffusion method at 289 K using commercial screens from Hampton Research. Each drop, consisting of 1 µl 8 mg ml$^{-1}$ protein solution (20 m*M* Tris–HCl pH 7.0, 100 m*M* NaCl, 10 m*M* DTT) and 1 µl reservoir solution, was equilibrated against 400 µl reservoir solution. Crystal of Grx5 grew to dimensions of 0.7 × 0.8 × 0.6 mm within one week using a reservoir containing 1.6 *M* ammonium sulfate, 0.1 *M* Tris–HCl pH 8.0. A mixture of 30% glycerol with the reservoir solution described above was used as a cryogenic liquor. The X-ray diffraction data were collected at 100 K in a liquid-nitrogen gas stream using a Rigaku MM007 X-ray generator (λ = 1.54178 Å) with a MAR Research 345 image-plate detector at the School of Life Sciences, University of Science and Technology of China (USTC, Hefei, People's Republic of China). 137 frames were collected with 1° oscillation and 10 min exposure per frame at a crystal-to-detector distance of 120 mm. The diffraction data were indexed and integrated using the program *MOSFLM* 7.0.4 (Leslie, 1992) and scaled using *SCALA* (Evans, 1993).

## 3. Results and discussion

The gel-filtration method was used as the final purification step and the purity of the target protein was further checked by SDS–PAGE (Fig. 1). Crystals of Grx5 appeared in drops obtained using a reservoir containing 1.6 *M* ammonium sulfate, 0.1 *M* Tris–HCl pH 8.0 (Fig. 2*a*). A diffraction image for Grx5 is shown in Fig. 2(*b*). The data-collection statistics are given in Table 1. The crystal of Grx5 belonged to space group *R*3, with unit-cell parameters $a = b = 85.12$, $c = 48.95$ Å, $\alpha = \beta = 90.00$, $\gamma = 120.00°$. Assuming a Matthews coefficient of 2.63 Å$^3$ Da$^{-1}$ and a solvent content of 53.18%, the asymmetric unit contains one molecule (Matthews, 1968). The structure solution has been obtained using the molecular-replacement method with the program *MOLREP* (Vagin & Teplyakov, 1997), using *E. coli* Grx4 (PDB code 1yka, sequence identity 38%) as the search model, and confirmed the presence of a single Grx5 molecule in the asymmetric unit (Fladvad *et al.*, 2005). Structure refinement is in progress.

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