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Expression, purification, crystallization and preliminary X-ray diffraction analysis of thioredoxin Trx1 from *Saccharomyces cerevisiae*

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Thioredoxins play key roles in the cellular response to oxidative stress. Three isoforms of thioredoxin have been identified in *Saccharomyces cerevisiae*: two that are cytosolic (Trx1 and Trx2) and one that is mitochondrial (Trx3). In the present work, the cytosolic form Trx1 was cloned, expressed, purified and crystallized. Crystals were obtained by the hanging-drop vapour-diffusion method. A data set was collected from a single crystal to 1.7 Å resolution. The crystal belongs to space group $P2_12_12_1$, with unit-cell parameters a = 32.29, b = 46.59, c = 64.20 Å, $\alpha = \beta = \gamma = 90°$.

1. Introduction

Thioredoxin (Trx) is a small protein (M_r 12 000) with a conserved sequence (Trp-Cys-Gly-Pro-Cys) in its active site. Trx was originally identified as the hydrogen donor for ribonucleotide reductase (Laurent *et al.*, 1964). It also acts upon a number of metabolic enzymes that form a disulfide as part of their catalytic cycle (Holmgren & Björnstedt, 1995). Trx is an effective antioxidant that is able to reduce hydrogen peroxide (Spector *et al.*, 1988), to scavenge free radicals (Schallreuter & Wood, 1986) and to protect cells against oxidative stress (Nakamura *et al.*, 1994). Eukaryotic thioredoxins have been implicated in various physiological functions, including gene expression, cell growth, apoptosis and so on (Watson *et al.*, 2004).

The yeast *Saccharomyces cerevisiae* contains a cytoplasmic thioredoxin system (Trx1, Trx2 and Trr1; Gan, 1991) as well as an intact mitochondrial thioredoxin system (Trx3 and Trr2; Pedrajas *et al.*, 1998). A sequence alignment of the three thioredoxin isoforms is shown in Fig. 1. Both Trx1 and Trx2 localize in the cytoplasm and/or nucleus, sharing 76% identity and 87% similarity. Although Trx1 and Trx2 appear to be functionally redundant as antioxidants, it seems that Trx2 plays the predominant role. This may be a consequence of differential regulation of Trx1 and Trx2 expression. Trx2 expression can be induced in response to oxidative stress conditions, while Trx1 expression remains largely unchanged (Garrido & Grant, 2002). The different expression profiles of the two isoforms in response to environmental changes support this. In response to different environments, Trx2 was expressed similarly to proteins that participate in

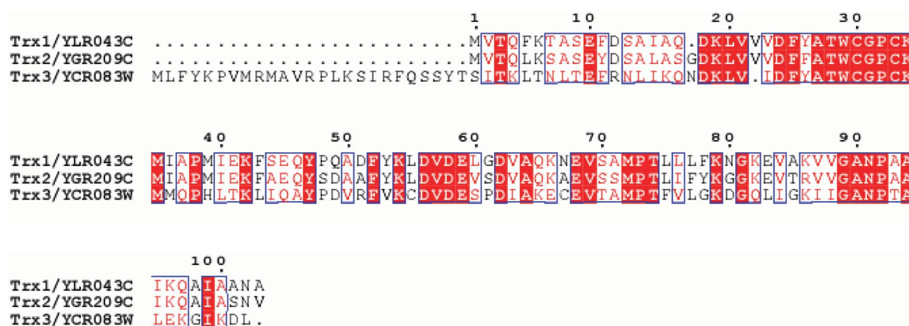

Figure 1
Alignment of the amino-acid sequences of *S. cerevisiae* Trx1, Trx2 and Trx3. Trx3 has an extra N-terminal signal peptide for mitochondrial localization. The figure was produced with *ESPript* v. 2.2 (Gouet *et al.*, 1999) based on an alignment using *ClustalW2* at EBI.

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the oxidative stress response, such as Prx1 and Tsa2. In contrast, Trx1 expression matched well with Vma5 and Cdc10, which are proteins that function in other pathways (Gasch *et al.*, 2000). Also, a yeast two-hydrid experiment in a Trx-knockout strain revealed that Trx2 interacts specifically with the yeast Ahp1 and Tsa1 peroxiredoxins involved in the oxidative stress response, whereas Trx1 preferentially interacts with PAPS reductase (Met16), which is involved in sulfur metabolism (Vignols *et al.*, 2005). All these observations suggest that Trx2 mainly functions in the redox balance, whereas Trx1 may play a salvage role for Trx2 and/or participate in other pathways.

Recently, the crystal structures of Trx2 and Trx3 from the yeast *S. cerevisiae* have been solved by our group (Bao *et al.*, 2006, 2007). In the present work, we report the crystallization and preliminary X-ray diffraction analysis of the last thioredoxin, Trx1, the structure of which remains unknown. This structure will enable us to perform a comprehensive comparison of the three isoforms of thioredoxin in yeast at the atomic level, which will shed light on the diversity and overlap of their functions.

2. Materials and methods

2.1. Cloning and purification

Two primers, the forward primer 5′-GGGG**CATATG**GTTACTC-AAT-3′ and the reverse primer 5′-CCCC**GCGGCCGC**TTAAG CATTAGC-3′, containing *Nde*I and *Not*I restriction sites, respectively (in bold), were employed to amplify the *TRX1*/*YLR043C* open reading frame from *S. cerevisiae* genomic DNA. The PCR product was cloned into a pET28a-derived expression vector, which gives a protein with a hexahistidine tag just after the start codon. The resulting construct was then transformed to the *Escherichia coli* BL21 (DE3) strain; the transformed cells were grown at 310 K in LB containing 10 µg ml^{-1} kanamycin until the OD_{600} reached about 0.6 and were then induced with 0.8 m*M* IPTG at 291 K overnight.

The protein was isolated by sonicating cell pellets resuspended in 40 ml cold lysis buffer (20 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 14 m*M* β-mercaptoethanol). The lysate was cleared by centrifugation at 26 000*g* for 30 min and the supernatant was filtered and loaded onto a 2.5 ml Ni^{2+}-chelating Sepharose Fast Flow column (Amersham Biosciences) equilibrated with lysis buffer. After washing with ten volumes of lysis buffer, a linear gradient of imidazole (10–500 m*M*) was applied to elute the protein. The fractions containing the target protein were further purified by size-exclusion chromatography on Superdex 75 (GE Healthcare; 20 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 14 m*M* β-mercaptoethanol). The purity of the protein was checked by SDS–PAGE (Fig. 2).

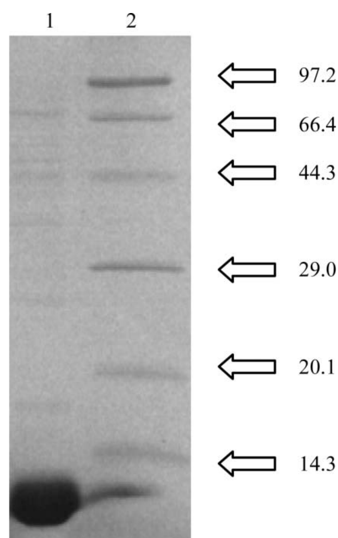
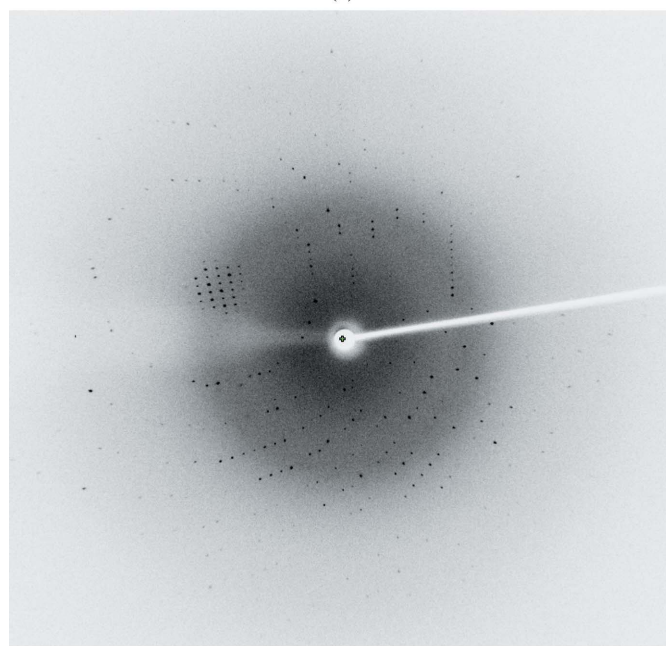


Figure 2
15% SDS–PAGE analysis of Trx1. Lane 1, Trx1 after gel filtration. Lane 2, low-molecular-weight markers (kDa).

2.2. Crystallization experiments

The purified protein was concentrated to 10 mg ml^{-1} by ultra-filtration (Millipore Amicon). Crystallization trials were carried out using the hanging-drop vapour-diffusion method at 291 K using Crystal Screens I and II (Hampton Research) for initial screening. Drops were prepared by mixing 1 µl protein solution with 1 µl precipitant solution and were equilibrated against 500 µl reservoir solution.

(*a*)



(*b*)

Figure 3
(*a*) Crystals of *S. cerevisiae* Trx1 obtained by the hanging-drop vapour-diffusion method. The average dimensions of the crystals were 0.1 × 0.08 × 0.7 mm. (*b*) X-ray diffraction pattern at a resolution of 1.7 Å. The crystal-to-detector distance was 125 mm.

Table 1
Data-collection and refinement parameters.

Nominal resolution range (Å)	23.3–1.7
Space group	$P2_12_12_1$
Temperature (K)	100
Unit-cell parameters (Å, °)	a = 32.29, b = 46.59, c = 64.20, $\alpha = \beta = \gamma$ = 90
Wavelength (Å)	1.54180
No. of reflections with $I > \sigma(I)$	10699
No. of reflections with $I > 3\sigma(I)$	8396
Completeness (%)	97.8
$R_{\rm stand}(F) = \langle\sigma(F)\rangle/\langle F\rangle$	0.019
$I/\sigma(I)$	21.84
$R_{\rm merge}$† (%)	4.1

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the measured reflection and $\langle I(hkl)\rangle$ is the mean intensity of all symmetry-related reflections.

2.3. Data collection

X-ray diffraction data were collected on an in-house Rigaku FRE Cu rotating-anode generator (operated at 50 kV and 100 mA) producing Cu $K\alpha$ radiation (wavelength 1.5418 Å) and equipped with an R-AXIS IV^{++} image-plate detector (Shanghai Institutes for Biological Sciences). The crystal was flash-frozen and maintained at 100 K using nitrogen gas during data collection; 15%(v/v) glycerol solution was added for cryoprotection.

3. Results

The Trx1 protein was expressed in *E. coli* BL21 (DE3) in a soluble form and was purified to homogeneity in two steps. Hampton Research Crystal Screens I and II were used for preliminary screening and produced crystals from several conditions. We chose buffer consisting of 25% PEG 4000, 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate pH 4.6 as the initial condition for optimization. After two weeks of growth, we obtained a single crystal with dimensions of 0.1 × 0.1 × 0.1 mm that was suitable for diffraction experiments using the condition 4% PEG 400, 12% PEG 8000, 0.1 *M* sodium acetate pH 4.6 and 0.2 *M* zinc acetate (Fig. 3*a*). The crystal showed significant diffraction to 1.7 Å resolution (Fig. 3*b*). The data were processed with *CrystalClear*/*d*TREK* (Pflugrath, 1999). Data processing showed that the crystal belonged to space group $P2_12_12_1$, with unit-cell parameters a = 32.29, b = 46.59, c = 64.20 Å, $\alpha = \beta = \gamma = 90°$. The data-collection statistics are listed in Table 1.

4. Discussion and conclusion

The structures of *S. cerevisiae* Trx2 and Trx3 have recently been determined by our group. The space groups of Trx2 and Trx3 are $P4_12_12$ and $P3_1$, with unit-cell parameters $a = b$ = 83.15, c = 64.73 Å, $\alpha = \beta = \gamma = 90°$ (Bao *et al.*, 2007) and $a = b$ = 49.57, c = 94.55 Å, $\alpha = \beta = 90$, $\gamma = 120°$ (Bao *et al.*, 2006), respectively. The data set for Trx1 was indexed in space group $P2_12_12_1$ and calculation of the Matthews coefficient (∼2.15 $Å^3\,Da^{-1}$) indicated that there is only one molecule in the asymmetric unit (Collaborative Computational Project, Number 4, 1994), with a solvent content of 61.3%. Trx1 and Trx2 are both located in the cytoplasm and together with thioredoxin reductase 1 form the cytoplasmic thioredoxin system. The two proteins share high homology, with 76% identity and 87% similarity. The current work should make it possible to compare the two isoforms at atomic resolution, which may illustrate their small differences and explain the reason for their coexistence through evolution.

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