crystallization communications

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Crystallization and preliminary X-ray crystallographic analysis of *Escherichia coli* glutaredoxin 2 in complex with glutathione and of a cysteine-less variant without glutathione

Glutaredoxin 2 (Grx2) from *Escherichia coli* is larger in size than classical glutaredoxins. It is extremely efficient in the catalysis of reduced glutathione-dependent disulfide reduction. A complex of Grx2 with reduced glutathione (GSH) has been crystallized. Data were collected to 1.60 Å. The crystals belong to space group $P3_221$, with one Grx2–GSH complex in the asymmetric unit. The unit-cell parameters are $a = b = 50.10$, $c = 152.47$ Å. A Grx2 mutant, C9S/C12S, which cannot form a disulfide bond with GSH was also crystallized. The crystals diffracted to 2.40 Å and belong to space group $P2_12_12_1$, with one molecule in the asymmetric unit. The unit-cell parameters are $a = 28.16$, $b = 78.65$, $c = 89.16$ Å.

1. Introduction

The glutaredoxin protein family is ubiquitously present in nearly every living organism from bacteria to humans. These enzymes are general GSH-dependent thiol–disulfide oxidoreductases (Fernandes & Holmgren, 2004). They can operate in a dithiol mechanism to reduce protein disulfides or in a monothiol and/or dithiol mechanism to reduce mixed disulfides formed between reduced glutathione (GSH) and proteins or low-molecular-weight thiols (Bushweller *et al.*, 1992). Glutaredoxins have been implicated in a variety of cellular functions, including transcription, signal transduction, cell-cycle control, protection against oxidative stress and the reduction of the enzymes ribonucleotide reductase and 3′-phosphoadenylylsulfate reductase (reviewed in Fernandes & Holmgren, 2004). They are also involved in the glutathionylation of proteins (Ghezzi, 2005) and arsenate reduction (Mukhopadhyay & Rosen, 2002).

To date, four glutaredoxins (Grx1, Grx2, Grx3 and Grx4) have been characterized in *Escherichia coli* (Åslund *et al.*, 1994; Holmgren, 1976; Fladvad *et al.*, 2005). Grx4 has recently been identified as the only monothiol glutaredoxin in *E. coli*, with a suggested CGFS active site (Fladvad *et al.*, 2005). Grx1, Grx2 and Grx3 are all dithiol glutaredoxins, with CPYC as their active site. Grx2 is atypical, differing from other classical glutaredoxins in its larger size and lower amino-acid sequence similarity to other glutaredoxins. It is extremely efficient in the catalysis of GSH-dependent disulfide reduction (Vlamis-Gardikas *et al.*, 1997; Lundstrom-Ljung *et al.*, 1999). Grx2 has been shown to be the most effective hydrogen donor for the reduction of arsenate by plasmid R773 ArsC, an arsenate reductase (Shi *et al.*, 1999). The NMR structure of reduced Grx2 has been obtained (Xia *et al.*, 2001) and the structure shows an overall similarity to glutathione *S*-transferases (GSTs), with the additional C-terminal residues forming a second larger domain that is lacking in Grx1 or Grx3. The NMR structures of the GSH complexes of human (Yang *et al.*, 1998) and *E. coli* Grx1 (Bushweller *et al.*, 1994) and *E. coli* Grx3 (Nordstrand *et al.*, 1999) have been reported. The structure of the complex of human Grx2 (which, despite its name, is not closely related to *E. coli* Grx2) with GSH has been deposited with PDB code 2fls (C. Johansson *et al.*, unpublished results). However, information on the GSH-complexed or oxidized structures of *E. coli* Grx2 is still lacking. Here, we describe crystallization of the Grx2–GSH complex
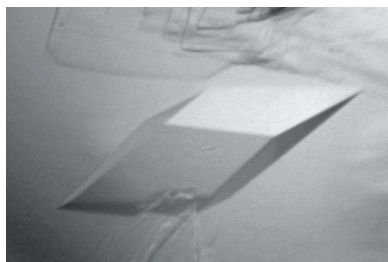
Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	Grx2–GSH	Grx2 C9S/C12S
Wavelength (Å)	1.0000	1.5418
Space group	$P3_221$	$P2_12_12_1$
Unit-cell parameters (Å)	$a = b = 50.10$, $c = 152.47$	$a = 28.16$, $b = 78.65$, $c = 89.16$
Resolution range (Å)	22.47–1.60 (1.69–1.60)	29.49–2.40 (2.49–2.40)
No. of observations	185447 (4270)	26609 (2816)
No. of unique reflections	28685 (2853)	8139 (785)
Completeness (%)	94.8 (67.0)	98.7 (99.1)
$\langle I\rangle/\langle\sigma(I)\rangle$	20.6 (4.7)	5.0 (1.8)
R_{merge}† (%)	6.2 (10.0)	15.0 (40.7)

† $R_{\mathrm{merge}} = \sum_i |I_{hkl,i} - \langle I_{hkl}\rangle|/\sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the intensity of the ith measurement of the reflection with Miller indices hkl and $\langle I_{hkl}\rangle$ is the mean intensity of that reflection.

and of a derivative lacking cysteine that is unable to form a complex with GSH.

2. Materials and methods

2.1. Protein expression and purification

The *grx2* gene was cloned into pET28a vector (Novagen) and expressed in *E. coli* strain BL21(DE3) (Shi *et al.*, 1999). The protein with a six-histidine tag was purified as described previously (Shi *et al.*, 1999), with the addition of a final step of gel-filtration chromatography using Superdex G-75. The six-histidine-tag fusion peptide was removed using a Thrombin Cleavage Capture Kit (Novagen) before crystallization, leaving three residues (Gly-Ser-His) from the vector at the N-terminus. A Grx2 C9S/C12S derivative (Shi *et al.*, 1999) was purified similarly.

2.2. Crystallization

Purified Grx2 was buffer-exchanged into a buffer consisting of 10 m*M* Tris–HCl pH 7.5 containing 2 m*M* disodium ethylenediamine tetraacetate (EDTA), 5 m*M* dithiothreitol (DTT). Crystals were grown using hanging-drop vapor diffusion at 277 K with VDX plates (Hampton Research). About 200 solution conditions were tried before crystals were obtained. After incubation with 4 m*M* GSH and 20 m*M* sodium arsenate on ice for 10 min, 2 µl purified Grx2 (20 mg ml^{-1}) was mixed with 2 µl well solution containing 0.1 *M* sodium citrate, 0.2 *M* ammonium acetate and 30%(*w/v*) PEG 4000 at a final pH of 5.9. The drop was then equilibrated against 0.5 ml well solution. The Grx2 C9S/C12S mutant was crystallized under similar conditions but in the absence of GSH and sodium arsenate. Crystals appeared in approximately two weeks (Fig. 1).

2.3. Data collection

Grx2–GSH or Grx2 mutant crystals were transferred to a cryoprotectant solution (well solution with an additional 5% PEG 4000) and flash-cooled in nitrogen gas and/or stored in liquid nitrogen. The Grx2–GSH data set was collected under cryogenic conditions at Argonne National Laboratory Advanced Photon Source (ANL-APS) beamline 32-ID-B. It was indexed and integrated using *MOSFLM* and scaled and merged using *SCALA* (Collaborative Computational Project, Number 4, 1994; Table 1). The Grx2 C9/C12S mutant data set was collected using an in-house Rigaku/MSC FR-D rotating-anode X-ray source equipped with an R-AXIS HTC image-plate detector at a crystal-to-detector distance of 150 mm. The X-rays

were focused with the MSC Blue-2 Optics configuration with the focal point fixed at 120 mm beyond the crystal. A collimator with a 2 mm rear and 0.5 mm front aperture was used. The data were integrated, scaled and merged with the program *CrystalClear* 1.3.6 (Pflugrath, 1999).

2.4. Initial phasing

The Grx2–GSH crystal structure was solved by molecular replacement using the program *AMoRe* (Navaza, 2001) implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The first conformer of the Grx2 NMR structure (Xia *et al.*, 2001) was used as an input probe. The best solution gave a correlation coefficient of 0.46. The second highest peak only had a correlation coefficient of 0.28. The Grx2 mutant structure was also solved by molecular replacement using the partially refined Grx2–GSH structure as the search model.

3. Results and discussion

We have crystallized the Grx2–GSH complex and the cysteine-free derivative C9S/C12S. The crystals of the Grx2–GSH complex diffracted to 1.6 Å. The Grx2–GSH crystals belong to space group $P3_221$, with one complex in the asymmetric unit, giving a V_{M} of 2.3 Å^3 Da^{-1} (Matthews, 1968). The unit-cell parameters are $a = b = 50.10$, $c = 152.47$ Å. It is interesting to note that attempts to crystallize this complex in the absence of arsenate were unsuccessful, even though arsenate is not visible in the final structure. One possibility is that arsenate serves as an oxidant for disulfide-bond formation between Grx2 and GSH. Of relevance is the observation that the ectromelia virus glutaredoxin reduces dimethylarsenate to dimethyl-

(a)

(b)
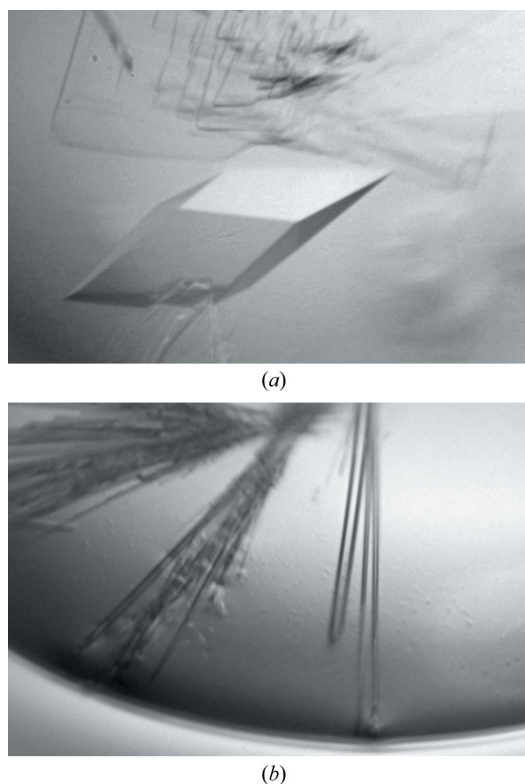
Figure 1
Crystals of (*a*) Grx2–GSH and (*b*) Grx2 C9S/C12S. Approximate dimensions are 0.3 × 0.3 × 0.2 and 0.8 × 0.05 × 0.05 mm for the Grx2–GSH and Grx2 C9S/C12S crystals, respectively.

arsenite and a cysteine thiolate complex with dimethylarsenite was observed in the glutaredoxin structure (Bacik & Hazes, 2007).

Although the NMR structure of Grx2 in the reduced form has been determined (Xia *et al.*, 2001), the region between residues 124 and 134 in the C-terminal domain was not well defined and the structure of the GSH-bound form was not determined. Our goal is to identify the conformational changes that occur in Grx2 as a result of interaction with GSH (and eventually with the ArsC arsenate reductase). This requires comparison of the structures of the reduced and oxidized forms in which all of the residues are well defined. However, we were only able to produce crystals of Grx2 in complex with GSH and not in either the reduced or the oxidized form. As an alternative, a C9S/12S derivative that is unable to support GSH-dependent ArsC-catalyzed arsenate reduction was utilized (Shi *et al.*, 1999). Presumably, this protein is unable to form a complex with GSH and its structure may mimic that of the reduced form. Crystals of Grx2 C9S/C12S were obtained that diffracted to 2.4 Å. The C9S/C12S crystals belong to space group $P2_12_12_1$ with one molecule in the asymmetric unit, giving a V_M of 2.0 $Å^3 Da^{-1}$. The unit-cell parameters are $a = 28.16$, $b = 78.65$, $c = 89.16$ Å. In contrast to wild-type Grx2, neither arsenate nor GSH were required for crystallization of C9S/C12S.

Of the four *E. coli* glutaredoxins, only Grx1 and Grx3 have been extensively characterized structurally and functionally. The three-dimensional structures of Grx1 and Grx3 have been obtained by NMR in both the reduced, oxidized and mixed-disulfide forms (Bushweller *et al.*, 1994; Sodano *et al.*, 1991; Xia *et al.*, 1992; Foloppe *et al.*, 2001; Nordstrand *et al.*, 1999, 2000). Although the NMR structure of Grx2 in the reduced form is available, considering the substantial differences in size and number of domains between Grx2 and Grx1 or Grx3, comparison of the crystal structures of Grx2 with and without GSH will be of considerable value in elucidating the mechanism of this atypical glutaredoxin. The refined structures of Grx2–GSH and the Grx2 C9S/C12S mutant and the implications for Grx2 function will be presented elsewhere.

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