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Crystallization and preliminary X-ray analysis of a Trx domain of human thioredoxin-like protein

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The Trx domain of human thioredoxin-like protein has been purified and crystallized using ammonium sulfate as precipitant. The crystal belongs to space group $C2$, with unit-cell parameters $a = 87.5$, $b = 48.5$, $c = 29.8$ Å, $\beta = 99.59^\circ$. It has one molecule per asymmetric unit and diffracts beyond 2.2 Å under cryoconditions (100 K) using an in-house Cu rotating-anode X-ray generator.

1. Introduction

Thioredoxin is a small thiol-containing protein of approximately 100 amino-acid residues which is widely distributed and evolutionarily conserved from prokaryotes to higher eukaryotes (Holmgren, 1984, 1985, 1989). It is characterized by the active-site amino-acid sequence -Cys-Gly-Pro-Cys-, which has been conserved throughout evolution. The active site of thioredoxin is located in a protrusion of its three-dimensional structure as determined in *Escherichia coli* thioredoxin by NMR and X-ray crystallography (Jeng *et al.*, 1994; Qin *et al.*, 1994; Katti *et al.*, 1990; Weichsel *et al.*, 1996). Thioredoxin can exist in either a reduced form or an oxidized form (Holmgren, 1985). The reduced form contains two thiol groups and can efficiently disrupt the disulfide bridge of target proteins. In its oxidized form, the two cysteine residues are linked by an intramolecular disulfide bond which can be reduced by a thioredoxin reductase *in vivo* (Clancey & Gilbert, 1987; Chivers *et al.*, 1996, 1997; Schultz *et al.*, 1999). It participates in various redox reactions *via* the reversible oxidation and reduction of the two cysteine residues in the active centre.

In *E. coli*, thioredoxin was first identified as an electron donor for ribonucleotide reductase (Laurent *et al.*, 1964). It can also function as a hydrogen donor (Tsang & Schiff, 1976; Ejiri *et al.*, 1979) and as the essential subunit of T7 DNA polymerase (Chamberlin, 1974; Russel & Model, 1985; Lim *et al.*, 1985). In eukaryotic cells, besides acting as a hydrogen donor (Holmgren, 1985) and as an efficient anti-oxidant (Spector *et al.*, 1988; Schallreuter & Wood, 1986; Nakamura *et al.*, 1994), thioredoxin has been shown to facilitate the refolding of disulfide-containing proteins (Lundstrom & Holmgren, 1990), to modulate the activity of some transcription factors and to stimulate the growth of human T cells (Tagaya *et al.*, 1989).

A number of related proteins of similar size and highly similar structure to thioredoxin have been described and most of them appear to belong to the protein-disulfide isomerase (PDI) family (Freedman *et al.*, 1988, 1994; Kivirikko *et al.*, 1989). All members of the PDI family contain two or three Trx domains per PDI molecule (Eklund *et al.*, 1991) and are primarily retained within the ER lumen by the recognition system.

The novel 32 kDa human thioredoxin-like protein designated hTrxl contains one Trx domain at its N-terminal (GenBank accession No. AF051896). It is widely expressed in human tissues, with the highest expression in stomach, testis and bone marrow (Miranda-Vizuete *et al.*, 1998). Our work proves it to be a development-related protein and it also possesses a similar reducing activity to *E. coli* thioredoxin for insulin disulfide bonds (J. Jin *et al.*, unpublished results). Unlike other proteins of the PDI family, it is localized in the cytoplasm (Lee *et al.*, 1998). Human and mouse Trxl proteins are 99% identical and, in particular, the Trx domains are completely homologous. However, the C-terminus of this protein shows no similarity to any other proteins in the public databases and no putative homologue in the *E. coli* or yeast genome sequences could be identified. Further work on the X-ray structure of this protein may provide some structural evidence to elucidate the probable function of the protein.

The 12 kDa Trx domain of hTrxl, designated hTrxN, consists of 105 amino-acid residues. It is localized at the N-terminus of hTrxl and shares 42% identity and 55% similarity with human thioredoxin. It also contains the conserved active-site sequence CGPC (Cys-Gly-Pro-Cys). A similar reducing activity for insulin disulfide bonds, with kinetics similar to those of hTrxl but slower than those of *E. coli* thioredoxin (Sigma), has also been detected and used to characterize the expression of this recombinant domain (J. Jin *et al.*, unpublished work).

2. Experimental

2.1. Purification of hTrxN

The 105 amino-acid recombinant hTrxN was expressed in *E. coli* strain BL21 as a fusion protein with glutathione S-transferase (expression vector pGEX-4T) and induced with 1 m*M* IPTG when OD_{600} reached 0.6 at 303 K. Purification was performed using glutathione affinity chromatography and the exogenous glutathione S-transferase was cleaved from the fusion protein with thrombin (Pharmacia Biotech) at 277 K (using the protocol of the GST Gene Fusion System, Pharmacia Biotech). The purified protein from the GST column in PBS buffer (140 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* Na_2HPO_4, 1.8 m*M* KH_2PO_4 pH 7.4) was concentrated to 10–15 mg ml^{-1} using a 5K ultrafiltration membrane and was immediately used for crystallization after filtration. Prior to crystallization, homogeneity was demonstrated by SDS–PAGE and native PAGE.

2.2. Crystallization

Crystallization trials were set up using the hanging-drop vapour-diffusion method on Linbro crystallization plates at 291 K. Initial screening was performed using the sparse-matrix method (Jancarik & Kim, 1991) with commercially available buffers (Hampton Research). Crystals of the recombinant hTrxN proteins emerged from several conditions around pH 4.6. These conditions were refined and the best crystals were obtained with sodium acetate buffer pH 4.9, 1.7–2 *M* ammonium sulfate at 291 K. In each trial, a hanging drop of 1 µl of protein solution (10–15 mg ml^{-1} in PBS buffer) mixed with 1 µl of well solution (20 m*M* sodium acetate, 1.7–2 *M* ammonium sulfate pH 4.9) was equilibrated against a reservoir containing 200 µl of well solution. They grew to their full size in about 5 d (Fig. 1).

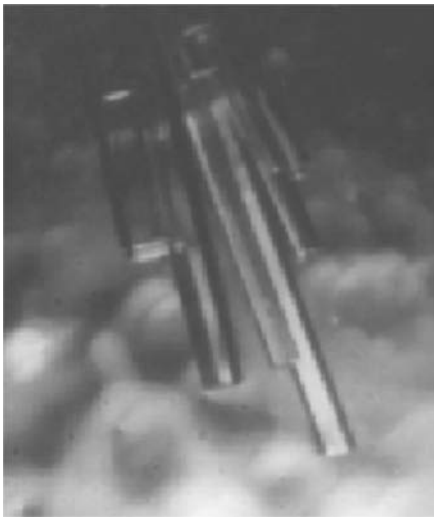

Figure 1
Crystals of hTrxN grown in an ammonium sulfate system at pH 4.9. Dimensions are approximately 0.1 × 0.1 × 1 mm.

2.3. X-ray analysis

Diffraction data were collected with a MAR345 image-plate detector mounted on an in-house Rigaku rotating Cu anode X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å). Data collection was performed at 100 K; cryocooling required the crystal to be immersed in cryoprotectant [crystallization buffer containing 30%(*v*/*v*) glycerol] prior to mounting and freezing. The hTrxN crystal diffracted beyond 2.0 Å and a data set (180 frames in total) was collected from a single crystal at 2.2 Å with an oscillation range of 1° per image (Fig. 2). The exposure time was 300 s per frame. All diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) (Table 1).

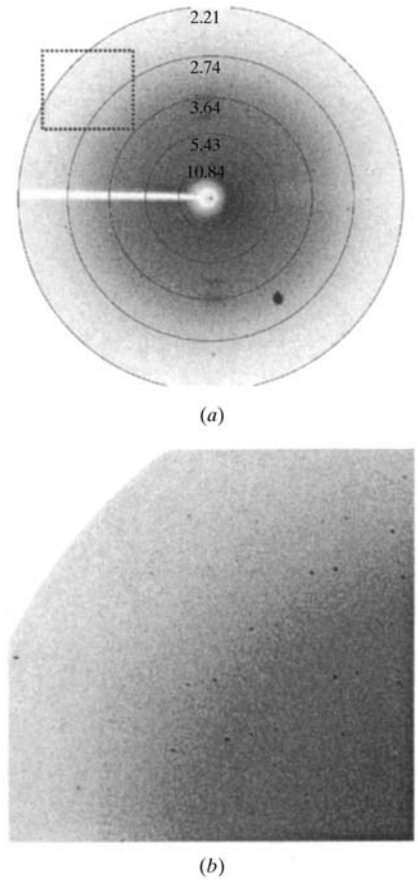

Figure 2
X-ray diffraction image of hTrxN. (*a*) A 1° oscillation image taken from a crystal of hTrxN at 100 K. This image was taken in-house on a MAR345 detector. The resolution is 2.2 Å at the edge of the plates. (*b*) Enlargement of one corner showing the spots at the highest resolution

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the highest resolution shell (2.30–2.22 Å).

Space group	*C*2
Unit-cell parameters (Å, °)	a = 87.5, b = 48.5, c = 29.8, β = 99.59
Resolution range (Å)	15–2.2
No. of reflections	6710
R_{merge} (%)	0.089 (0.316)
Completeness (%)	99.8 (98.7)
Average $I/\sigma(I)$	8.4 (3.9)
Mean redundancy	7.4 (7.2)
Mean mosaicity	0.4

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