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Expression, purification, crystallization and preliminary X-ray crystallographic studies of *Mycobacterium tuberculosis* thioredoxin reductase

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column. The column was washed with 50 mM phosphate buffer pH 8 containing 300 mM NaCl, 5% glycerol and 20 mM imidazole. The protein was eluted with the same buffer supplemented with 250 mM imidazole. The purity was checked on Coomassie-stained SDS-PAGE, where it appeared as a single band (Fig. 1).

2.2. Crystallization

The purified protein was dialyzed against 10 mM Tris-HCl pH 8.0 buffer and then concentrated to 30 mg ml⁻¹. Before crystallization, 10 mM reduced DTT was added to the protein solution and the solution was incubated for 30 min on ice. Initial crystallization trials were performed using a random screening of chemical conditions in our laboratory. The screening was conducted using the hanging-drop vapour-diffusion technique in 24-well plates. A 2 µl droplet of protein mixed in a 1:1 ratio with reservoir solution was equilibrated against 500 µl reservoir solution at room temperature. Crystals suitable for X-ray diffraction (Fig. 2) were obtained using 0.1 M sodium phosphate-citrate buffer pH 5.0 and 15% PEG 3350 at room temperature. Crystals grew within 36 h to dimensions of 0.5 × 0.5 × 0.3 mm.

2.3. X-ray data collection and processing

Crystals were soaked in cryoprotectant solution for a few seconds prior to freezing in a nitrogen cold stream. The cryoprotectant solution contained 11% ethylene glycol, 20% PEG 3350 and 0.1 M sodium phosphate-citrate buffer pH 5.0. X-ray diffraction data were collected at 100 K on beamline XRD-1 (wavelength = 0.927 Å) at the ELETTRA synchrotron facility, Trieste,

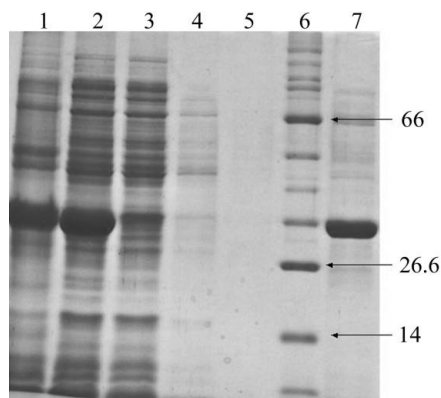


Figure 1
10% SDS-PAGE gel showing protein purification. Lane 1, cell supernatant after sonication; lane 2, flowthrough; lanes 3, 4 and 5, washthrough from Ni²⁺-NTA column; lane 6, molecular-weight markers (kDa); lane 7, purified TrxR.

Italy using a MAR CCD 165 detector. The crystal-to-detector distance was maintained at 200 mm with an oscillation range per image of 1°, covering a total oscillation range of 100° (Fig. 3). Determination of the unit-cell parameters and integration of reflections were performed using the programs *DENZO/SCALEPACK* (Otwinowski & Minor, 1997). The intensities were then converted to the corresponding structure-factor amplitudes using the *TRUNCATE* program from the *CCP4* suite (Collaborative Computational Project, Number 4, 1994).

Molecular replacement was attempted using the *AMoRe* program (Navaza, 1994) from the *CCP4* suite. The homologous *E. coli* TrxR (dimeric) crystal structure (PDB code 1tde), which has 46% sequence identity (Waksman *et al.*, 1994), was used as the search model.

3. Results and discussion

The *trxR* gene encoding a polypeptide of 335 amino acids was cloned and expressed in *E. coli* and the protein was purified to homogeneity. The protein purity was observed to be better than 98% as shown in Fig. 1. On gel-filtration chromatography, the purified TrxR protein eluted in a dimeric form, which is consistent with the physiological quaternary state of the *E. coli* TrxR (Moore *et al.*, 1964; Russel & Model, 1988) (data not shown). Crystallization trials using the mycobacterial TrxR yielded bright yellow coloured crystals (Fig. 2) of dimensions ~0.5 × 0.5 × 0.3 mm. These crystals were not stable when data collection was attempted at room temperature. However, the crystals appeared to be comparatively stable under cryoconditions. Diffraction data were collected at the ELETTRA synchrotron source, Italy in the presence of the cryoprotectant ethylene glycol. The completeness of the data set was found to be

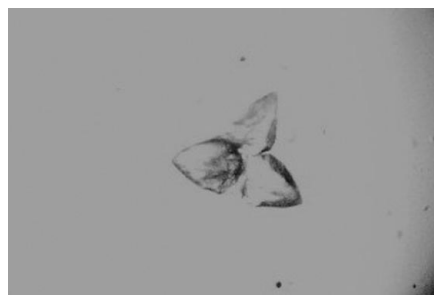


Figure 2
A crystal of reduced TrxR grown using 15% PEG 3350 and 0.1 M sodium-phosphate citrate pH 5.0. The size of one of the crystals shown is approximately 0.5 × 0.5 × 0.3 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the last resolution shell (3.11–3.0 Å).	
Wavelength (Å)	0.927
Space group	<i>P</i> 4 ₁ 2 ₁ 2
Unit-cell parameters (Å)	<i>a</i> = 107.4, <i>c</i> = 118.2
Resolution limits (Å)	50.0–3.0
Unique reflections	14186 (1368)
<i>I</i> / σ (<i>I</i>)	16.2 (4.1)
<i>R</i> _{merge} † (%)	8.1 (36.9)
Completeness (%)	97.8 (97.9)
Probable solvent content (%)	51.4
No. molecules in AU	2
Matthews coefficient (Å ³ Da ⁻¹)	2.5

† $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ are the intensities of symmetry-redundant reflections and $\langle I(hkl) \rangle$ is the average intensity over all observations.

97.8% with acceptable statistical parameters. The 3 Å resolution data were processed using the *HKL2000* program and the space group was determined to be *P*4_x2_x2, with unit-cell parameters *a* = 107.4, *c* = 118.2 Å. Analysis of the systematic absences was not conclusive because only a few reflections of the relevant form were present. The *R*_{merge} values increased rapidly beyond 3 Å resolution and hence data were only processed to 3 Å resolution. Assuming the presence of two monomers in the asymmetric unit, a value for the Matthews coefficient of 2.5 Å³ Da⁻¹ was obtained, which corresponds to a solvent content of 51.4% (Matthews, 1968). Data-collection and resolution statistics are shown in Table 1. The self-rotation function plot did not show any peaks for the expected twofold non-crystallographic symmetry. The Patterson calculated using native intensities also did not show any significant peak apart from the origin, ruling out the possibility of pseudo-translation. The *N*(*Z*) test and the $\langle I^2 \rangle / \langle I \rangle^2$

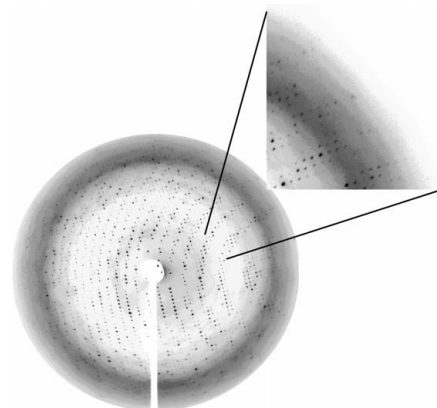


Figure 3
A 1° oscillation image collected at the ELETTRA synchrotron at 100 K temperature from a native TrxR crystal. The inset clearly shows the presence of reflections beyond the solvent ring.

distribution clearly indicated that the intensity data were not twinned.

The *AMoRe* (Navaza, 1994) program was used for molecular-replacement calculations using *E. coli* dimeric TrxR (PDB code 1tde) as the search model. The screw axes of the space group (which could not be identified earlier) were determined during translation searches in *AMoRe*. The best molecular-replacement solution was obtained in space group $P4_12_12$. The correctly oriented and positioned molecule of TrxR showed a correlation coefficient of 64.2 and an *R* factor of 47.5%. In comparison, the next best solution showed a correlation coefficient of 47.1 and an *R* factor of 55.1%. The placement of the model using the molecular-replacement solution did not show any unfavourable overlaps between symmetry-related molecules.

Currently, refinement of the molecular-replacement solution is in progress. The crystal structure of *M. tuberculosis* TrxR will contribute to the rational design of inhibi-

tors. The structure will also increase the understanding of the catalytic mechanisms of Trx/TrxR in *M. tuberculosis*.

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