Purification, crystallization and preliminary X-ray diffraction analysis of the glyoxalase II from *Leishmania infantum*

In trypanosomatids, trypanothione replaces glutathione in all glutathione-dependent processes. Of the two enzymes involved in the glyoxalase pathway, glyoxalase I and glyoxalase II, the latter shows absolute specificity towards trypanothione thioester, making this enzyme an excellent model to understand the molecular basis of trypanothione binding. Cloned glyoxalase II from *Leishmania infantum* was overexpressed in *Escherichia coli*, purified and crystallized. Crystals belong to space group C2221 (unit-cell parameters a = 65.6, b = 88.3, c = 85.2 Å) and diffract beyond 2.15 Å using synchrotron radiation. The structure was solved by molecular replacement using the human glyoxalase II structure as a search model. These results, together with future detailed kinetic characterization using lactoyltrypanothione, should shed light on the evolutionary selection of trypanothione instead of glutathione by trypanosomatids.

1. Introduction

Trypanosomatids, the causal agents of several human and animal diseases worldwide, present two characteristics that set them apart from all other living organisms. Firstly, glycolysis, the most fundamental biochemical pathway, occurs in these organisms within a specific organelle, the glycosome (Hannaert et al., 2003). Secondly, the unique thiol N1,N8-bis(glutathionyl)spermidine (trypanothione) replaces glutathione in similar eukaryotic glutathione-dependent reactions (Müller et al., 2003; Fairlamb & Cerami, 1992). These differences may be exploited in the development of novel therapeutic strategies, considering that diseases caused by trypanosomatids have no effective curative therapies and are often lethal. Synergistic effects of simultaneous inhibition of multiple trypanothione-dependent enzymes might prove to be the best option, given the absolute need for this thiol during the life cycle of the parasite (Oza et al., 2003).

The glyoxalase pathway is one of the important systems that depend on trypanothione. In trypanosomatids, this system is composed of the two enzymes glyoxalase I (lactoylglutathione lyase; EC 4.4.1.5) and glyoxalase II (hydroxyacylglutathione hydrolase; EC 3.1.2.6), which are specific for trypanothione and lactoyltrypanothione, respectively (Sousa Silva et al., 2005; Irsch & Krauth-Siegel, 2004). Glyoxalase II shows absolute specificity towards trypanothione thioester, in contrast to glyoxalase I, which can also react with glutathione-methylglyoxal hemithioacetal (Sousa Silva et al., 2005). Thus, it is an excellent model to understand the molecular basis of trypanothione specificity.

*L. infantum* glyoxalase II is a monomeric protein with 295 residues (molecular weight 32.5 kDa) and shares 35% homology with the human glutathione-dependent glyoxalase II (Cameron et al., 1999). Here, we report the purification, crystallization and preliminary X-ray diffraction analysis of *L. infantum* glyoxalase II. Its structure determination will shed some light onto the structure–function relationships of the trypanothione-dependent glyoxalase II.

2. Materials and methods

2.1. Cloning, expression and purification

The *LiGLO2* gene was amplified from *L. infantum* (clone MHOM/MA67ITMAP263) genomic DNA. The PCR product was cloned into
the NdeI/XhoI-digested expression vector pET-28a (Novagen), which was then transformed into *Escherichia coli* BL21-Codon Plus (Stratagene). For overexpression of His$_6$-glyoxalase II, BL21-Codon Plus transformants were grown in LB medium containing 50 µg ml$^{-1}$ kanamycin and 100 µg ml$^{-1}$ chloramphenicol at 310 K. When the culture reached an OD$_{600}$ of 0.6, expression was induced with 0.2 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 3 h at 310 K. The fusion protein, with an N-terminal tail of six histidines and a thrombin cleavage site (the total tag sequence including linker and cleavage site is HHHHHHSSGLVPRGSH), was purified at 277 K by chromatography on a His-Bind resin (Novagen) column. His$_6$-glyoxalase II was eluted with an imidazole gradient from 5 mM to 1 M at a flow rate of 2.5 ml min$^{-1}$. Fractions containing glyoxalase II (confirmed by SDS–PAGE) were pooled and the buffer was exchanged to 1× PBS pH 7.4 using PD-10 columns (Amersham Biosciences). The purity of the recombinant glyoxalase II was estimated by SDS–PAGE. The His$_6$-LiGLO II was concentrated to 25 mg ml$^{-1}$ in 10 mM HEPES pH 7.0 using Amicon Ultra-4 filters (10 000 NMWL; Millipore Corporation).

### 2.2. Crystallization

Crystallization conditions were obtained with an in-house screen of 80 conditions at 293 K, applying the hanging-drop vapour-diffusion method using the crystallization tools from Nextal Biotechnology. Drops were prepared by mixing 2 µl protein solution with 2 µl of each precipitant solution equilibrated over 700 µl reservoir solution. Several conditions in the crystallization setup were tested. Crystals were obtained from various conditions including PEGs of molecular weight ranging from 400 to 8000, MES or MOPS with pH between 5.5 and 7.0 and sodium or ammonium acetate as salt components. The best crystals were obtained by mixing 1 µl protein solution with 2 µl reservoir solution containing 30% (w/v) PEG 8000, 0.2 M magnesium chloride and 0.1 M sodium acetate buffer pH 5.5. This final crystallization condition resulted in thick plates, which grew within 2 d to maximum dimensions of approximately 0.16 × 0.06 × 0.02 mm at 288 K (Fig. 1).

![Crystals of *L. infantum* glyoxalase II.](image)

### 2.3. Data collection and processing

Crystals were directly flash-cooled in the cryostream without any additional cryoprotectant. All data were collected at 100 K. A low-resolution data set was collected in-house using a Cu Ka Enraf–Nonius rotating-anode generator operated at 5 kW and a MAR Research image-plate detector (Table 1). A high-resolution native data set was measured at beamline ID14-1 at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France) using an ADSC Quantum 4R CCD detector. The crystal diffracted beyond 2.15 Å.

The diffraction experiments showed that glyoxalase II crystals belong to space group C222₁, with unit-cell parameters $a = 65.7$, $b = 88.3$, $c = 85.2$ Å.

The data were processed using MOSFLM v.6.2.5 (Leslie, 1992) and scaled using SCALA from the CCP4 program package v.6.0 (Collaborative Computational Project, Number 4, 1994). In order to estimate the crystallographic data, the Matthews coefficient $V_m$ (Matthews, 1968) and solvent content were calculated based on a subunit molecular weight of 32.5 kDa (predicted from the sequence). The structure was solved by molecular replacement using PHASER (Read, 2001) from the CCP4 suite.

### 3. Results and discussion

The *L. infantum* LiGLO2 gene (GenBank accession No. DQ294972) was cloned and expressed in *E. coli* BL21 Codon Plus cells. The protein was expressed with an N-terminal His-tag fusion. A nickel-affinity column was used for purification. The purity of the recombinant *L. infantum* His-glyoxalase II was ≥95% as estimated by SDS–PAGE, which showed a single band corresponding to a molecular weight of about 32 kDa.

After optimization of the crystal-growth process, the purified protein produced thick plate crystals within 2 d (Fig. 1) which diffracted beyond 2.15 Å resolution using synchrotron radiation. The crystals belong to space group C222₁, with unit-cell parameters $a = 65.7$, $b = 88.3$, $c = 85.2$ Å. The calculated Matthews coefficient is 1.88 Å$³$Da$^{-1}$, corresponding to a solvent content of ~35%, assuming the presence of one molecule in the asymmetric unit (Matthews, 1968). Data-collection and processing statistics are shown in Table 1.

Molecular replacement was performed using the human glyoxalase II structure (Cameron et al., 1999; PDB code 1qh3; the full model was used), which shows 35% sequence identity with the *L. infantum* glyoxalase II, as a search model and the molecular-replacement program Phaser (Read, 2001). One clear solution was obtained and the calculated phases were improved using Pirate (Cowtan, 2000). A preliminary C$_s$ trace was manually built from the search model and is currently being rebuilt using COOT (Emsley & Cowtan, 2004) and refined with REFMAC5 from the CCP4 suite (Collaborative Computational Project, Number 4, 1994).

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<th>Table 1 Crystal data and data-collection statistics.</th>
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<td><strong>Space group</strong></td>
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<td><strong>Unit-cell parameters (Å)</strong></td>
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<td><strong>Source</strong></td>
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<td><strong>Wavelength (Å)</strong></td>
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<td><strong>No. of observed reflections</strong></td>
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<td><strong>Resolution limits (Å)</strong></td>
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<td><strong>Completeness (%)</strong></td>
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<td>(I/σ(I))</td>
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† $R_{sym} = \sum_h |I_h - \langle I_h \rangle|/\sum_h I_h$, where $I_h$ is the observed intensity for reflection $h$ and $\langle I_h \rangle$ is the weighted average intensity for all observations $l$ of reflection $h$.
The structure of L. infantum glyoxalase II, together with complete biochemical studies, will provide important insights into the molecular basis of trypanothione specificity.

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References