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Crystallization and preliminary crystallographic analysis of the soluble α-glycerophosphate oxidase from *Streptococcus* sp.

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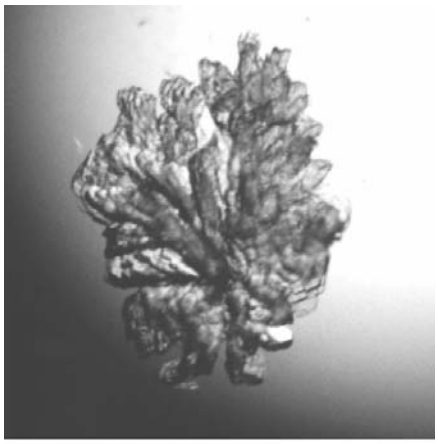
Single crystals of soluble FAD-dependent α-glycerophosphate oxidase (GlpO) from *Streptococcus* sp. were obtained using the microseeding and hanging-drop vapor-equilibrium methods. Synchrotron X-ray radiation was used to collect diffraction data to 2.4 Å resolution from these crystals. GlpO shares >30% identity with several bacterial and mitochondrial α-glycerophosphate dehydrogenases, although the GlpOs contain a 50–52-residue unique insert that appears to be important for efficient flavin reduction. The present work is an important first step in determining the structure of GlpO, which should provide insights on the function of this interesting flavoenzyme and its homologs.

1. Introduction

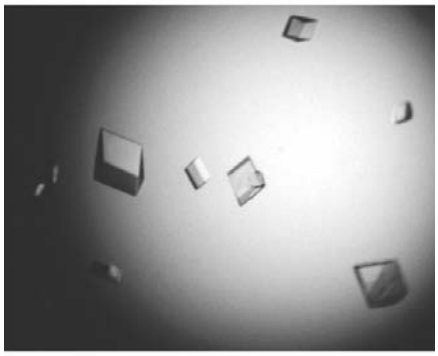
In most common bacteria capable of glycerol catabolism (*e.g. Escherichia coli*, *Bacillus subtilis*) and in mitochondria, the oxidation of α-glycerophosphate (Glp) to dihydroxyacetone phosphate (DHAP) is catalyzed by a membrane-associated flavoprotein α-glycerophosphate dehydrogenase (GlpD), which transfers electrons to a quinone component of the respiratory chain (Austin & Larson, 1991). This redox process is not possible in the heme-deficient lactic acid bacteria (*e.g. Enterococcus casseliflavus*, *Streptococcus* sp.) since they lack the equivalent of a membrane-associated electron-transport chain (Dolin, 1961); instead, the soluble FAD-dependent α-glycerophosphate oxidase (GlpO) catalyzes the direct reduction of O_2 to H_2O_2 (Parsonage *et al.*, 1998). Still, the GlpO sequences compare favorably (>30% identity) with those of several bacterial and mitochondrial GlpDs, and considerable attention has been focused on the role of the 50–52-residue insert that is found exclusively in the GlpOs. Recent results from a limited proteolysis study with the *Streptococcus* sp. GlpO (Charrier *et al.*, 2000) indicate that elements of this insert present a flexible surface region that plays an important role in mediating efficient flavin reduction. A crystallographic study of GlpO would not only provide much-needed information on the function of this surface-exposed region, but it would also yield the structure of a new flavoprotein oxidase not closely related in sequence to other well defined members of this class (Parsonage *et al.*, 1998). The *Streptococcus* sp. GlpO was used for the preliminary crystallographic characterization reported here. This enzyme has a subunit molecular mass of 67.6 kDa (without FAD) and, by analogy to the GlpO from *E. casseliflavus* (Claiborne, 1986), it is most likely to occur as a dimer. A high-resolution structure for the *Streptococcus* sp. GlpO would also provide a good basis for probing structure–function relationships in the related GlpDs and for investigating the catalytic mechanism of Glp oxidation.

2. Methods and results

Purified *Streptococcus* sp. GlpO (Charrier *et al.*, 2000) at a concentration of 16.5 mg ml^{-1} in 10 m*M* HEPES pH 7.0 was screened for crystallization using the hanging-drop method in a sparse-matrix screen (Jancarik & Kim, 1991) based on the commercially available Crystal Screens I and II (Hampton Research, Inc.). Initial screening used drops containing 2 µl protein sample and 2 µl reservoir solution. Crystals were observed in conditions using polyethylene glycol 400 and polyethylene glycol 1000 (PEG 1000) as precipitant. Optimization demonstrated that the best condition for the growth of large crystals was 18–20% PEG 1000, 0.1 *M* Tris pH 8.0 and 0.2 *M* $MgCl_2{\cdot}6H_2O$ at room temperature. In both the early screens and optimization, however, the crystals grew in clusters that were unsatisfactory for data collection (Fig. 1*a*). Single crystals



(*a*)



(*b*)

Figure 1
(*a*) Large (~0.3 mm) GlpO crystal cluster, unsuitable for data collection, grown by the hanging-drop method. This morphology was typical of the crystals observed during initial screening and optimization. (*b*) Single crystals grown from microseeds and used for data collection. Larger crystals measure approximately 0.15 × 0.3 × 0.3 mm.

Table 1
GlpO CHESS data statistics.

	Overall (28.89–2.40 Å)	Highest resolution bin (2.53–2.40 Å)
No. of reflections	57965	8692
R_{meas}	0.064	0.298
Multiplicity	2.4	2.4
Completeness (%)	94.8	97.4
Reflections with $I/\sigma(I) > 3$ (%)	73.6	37.9
Mean $I/\sigma(I)$	9.9	2.8

were obtained by microseeding. Crystals grown under the conditions described above were harvested, crushed and serially diluted in artificial mother liquor (35% PEG 1000, 0.1 *M* Tris pH 8.0, 0.2 *M* $MgCl_2{\cdot}6H_2O$). Recipient drops containing 4 µl of protein solution and 4 µl of reservoir solution (12–14% PEG 1000, 0.1 *M* Tris pH 8.0, 0.2 *M* $MgCl_2{\cdot}6H_2O$) were allowed to equilibrate for 24 h before 2 µl of diluted crystal seeds were added. Within one to two weeks single crystals measuring approximately 0.15 × 0.3 × 0.3 mm on a side were observed (Fig. 1*b*).

X-ray diffraction data were collected using synchrotron radiation at the Cornell High Energy Synchrotron Source (CHESS) F-1 station using an Area Detection Systems Corporation Quantum-4 CCD detector. Crystals used for data collection were serially transferred to artificial mother liquor containing 5, 10 and finally 15% glycerol as cryoprotectant prior to freezing in a liquid-nitrogen stream.

A 94.8% complete data set to 2.4 Å resolution was collected from a single GlpO crystal using a crystal-to-detector distance of 200 mm (Fig. 2). Data processing was performed using an interactive GUI based on several crystallographic algorithms (Nielsen *et al.*, 1998). *DPS* (Steller *et al.*, 1997) was used for indexing and *MOSFLM* (Leslie, 1991) was used for refinement and integration of the images. The crystal had the symmetry of space group *P2*, with unit-cell parameters $a = 58.89$, $b = 106.78$, $c = 128.36$ Å, $\beta = 99.18^\circ$. A total of 141 187 observations were merged into 57 965 unique observations using the *CCP4* program suite (Collaborative Computational Project, Number 4, 1994), with an overall multiplicity of 2.4 and an R_{meas} of 6.4%. The highest resolution bin had a completeness of 97.4%, an R_{meas} of 29.8% and a mean $I/\sigma(I)$ of 2.8 (Table 1).[1]

The Matthews coefficients (Matthews, 1968) for one, two or three GlpO molecules per asymmetric unit are 5.9, 2.9 and 2.0 $Å^3\ Da^{-1}$, respectively, corresponding to solvent contents of 79, 58 or 37%. GlpO possesses 12 methionine residues per 607-residue subunit, suggesting that a selenomethionyl derivative of GlpO should have sufficient anomalous signal for MAD phasing. We have recently obtained a selenomethionyl derivative of GlpO and plan to solve the structure of this interesting flavoenzyme using MAD phasing.

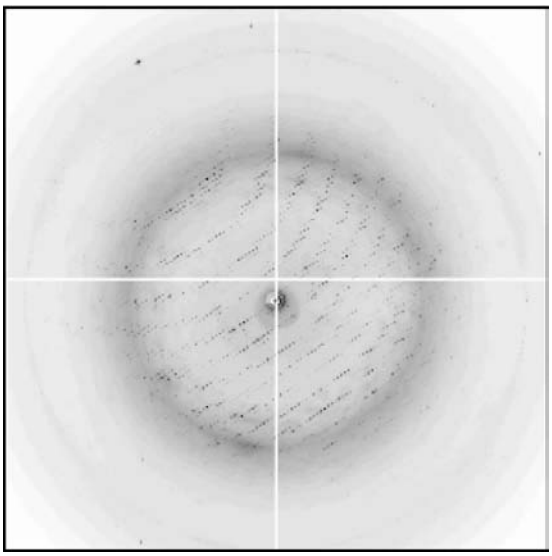


Figure 2
GlpO crystal diffraction image taken at the Cornell High Energy Synchrotron Source, station F-1. Wavelength, 0.9296 Å; $\Delta\varphi$, 1°; crystal-to-detector distance, 200 mm; exposure time, 40 s.

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[1] Supplementary data have been deposited in the IUCr electronic archive (Reference: gr2182). Details on how to access these data are available at the back of the journal.

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