ESRF	**Experiment title:** Structure determination GlpE, a single domain sulfurtransferase from *Escherichia coli*	**Experiment number**: **LS1803**
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Introduction

Rhodanese domains are structural modules occurring in the three major evolutionary *phyla*. They are found as single-domain proteins, as tandemly-repeated modules where the C-terminal domain only bears the properly structured active site, or as members of multidomain proteins. Although *in vitro* assays show sulfurtransferase or phosphatase activity associated with rhodanese or rhodanese-like domains, specific biological roles for most members of this homology superfamily have not been established. The *Escherichia coli* K-12 genome contains eight ORFs coding for proteins consisting of (or containing) a rhodanese domain, bearing the potentially catalytic Cys at the expected position. One of them codes for the 12 kDa protein GlpE, a member of the , a member of the *sn*-glycerol 3-phosphate (*glp*) regulon (1).

Structure determination of GlpE

The crystal structure of GlpE was determined at 1.06 Å resolution (2), using the SIRAS (single isomorphous replacement with anomalous scattering) method on a single heavy atom derivative obtained by soaking GlpE native crystals in $HoSO_4$ solutions collected at ESRF. A single GlpE molecule is observed in the crystallographic asymmetric unit of the trigonal crystal form analyzed ($P3_2$). The initial phases, calculated at 2.0 Å resolution, were improved by solvent flattening and phase extension to the resolution limit of 1.06 Å. The GlpE model (108 amino acids) could then be entirely built in the experimental electron density map, which resulted of outstanding quality, and refined to a final R-factor of 12.8 % (free-R-factor = 15.1 %).

As anticipated by the weak but significant amino acid sequence homology (17% identical reisdues) to other sulfurtransferase enzymes, GlpE adopts the three-dimensional fold typical of a single α/β rhodanese/Cdc25 phosphatase domain, based on a central parallel β-sheet composed of five β-strands surrounded by α-helices (3,4).

Enzymatic activity

To determine, on a structural basis the *in vitro* observed thiosulfate:cyanide sulfutransferase activity, native GlpE crystals were treated with Na_2SO_3 and polysulfide (S_x) previous to data collection.

Data collection statistics

	$SO3^{2-}$	S_x
Space group	$P3_2$	$P3_2$
Unit cell (Å)	$a = b = 53.85$, $c = 30.32$	$a = b = 53.93$, $c = 30.37$
Mosaicity (°)	0.34	0.17
Resolution (Å)	2.0	1.4
Measurements	91,607	195,336
Unique reflections	6378	17301
Completeness (%)	96.1	89.3
R_{sym} (%)	3.5	4.8

References

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