BM16 (ESRF) ANNUAL REPORT

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Structural Biology of Enzymes of the L-ascorbate Utilization Metabolic Pathway

The structural analysis of bacterial metabolic enzymes by X-ray crystallography has proven a powerful analytic and discovery tool both for fundamental biology and for drug discovery programmes. In this context, our group is studying the structure-function relationship of enterobacteria enzymes of the L-ascorbate utilization pathway. One of the most intriguing enzymes is UlaG, a putative L-ascorbate-6-phosphate lactonase for which no convincing substrate or enzymatic activity has been reported to date. To shed light on the enzymatic activity and structure of this remarkable enzyme, we have expressed, purified, crystallized and determine the structure of full-length UlaG. A native diffraction dataset was collected from UlaG crystals at ID14-3 up to 3 Å resolution (Garces et al 2008). Since no homology model was available, we grew crystals of selenomethionine-containing UlaG and collected 3-wavelength multiwavelength anomalous diffraction (MAD) data on BM16 (Table 1). The quality of the data on BM16 was high, improving the maximum resolution up to 2.6 Å. Consequently, the anomalous signal was sufficiently strong to allow all of the selenium atoms to be located by standard methods and an almost complete model to be built. At present refinement and final modelling of UlaG structure is under progress.

Beamline	BM16
Wavelength (Å)	0.97909
Spacegroup	*C*2
Unit cell dimensions (Å, °)	103.6, 178.6, 112.4, 90, 103.8, 90
Resolution (Å)	2.6 (25)
Rmerge	0.16 (0.55)
Rpim	0.08 (0.32)
Total number of observations	437,714 (34,718)
Total number unique	60,575 (7,831)
Mean((I)/sd(I))	11.0 (2.6)
Completeness (%)	98 (87)
Multiplicity	7.2 (4.4)
Anomalous completeness (%)	97.3 (82)
Anomalous multiplicity	3.7 (2.3)

Table 1. Crystallographic statistics.

References

Garces F, Fernández FJ, Pérez-Luque R, Aguilar J, Baldomà L, Coll M, Badía J, Vega MC. (2008). Overproduction, crystallization and preliminary X-ray analysis of the putative L-ascorbate-6-phosphate lactonase UlaG from Escherichia coli. Acta Crystallogr Sect F Struct Biol Cryst Commun. Jan 1;64(Pt 1):36-8. Epub 2007 Dec 20.