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Purification, crystallization and preliminary X-ray diffraction analysis of *S*-formylglutathione hydrolase from *Arabidopsis thaliana*: effects of pressure and selenomethionine substitution on space-group changes

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S-Formylglutathione hydrolase (SFGH) has activity toward several xenobiotic carboxyesters and catalyses the final step of formaldehyde detoxification: the hydrolysis of *S*-formylglutathione to formate and glutathione. The *Arabidopsis thaliana* enzyme (*At*SFGH) was crystallized in space group *C*2, with unit-cell parameters a = 128.5, b = 81.1, c = 94.3 Å, β = 93.3° and three molecules in the asymmetric unit. A second crystal form of *At*SFGH could be obtained by pressurizing the monoclinic crystals at 2 MPa for 30 min. The resulting space group is either $P3_121$ or $P3_221$, with unit-cell parameters a = 75.1, c = 92.8 Å and one molecule in the asymmetric unit. Crystallographic data have been collected for both crystal forms to resolutions of 1.7 Å for the monoclinic crystal and 1.6 Å for the trigonal crystal. The structure has been solved by MAD phasing using a three-wavelength data set collected from a monoclinic crystal of selenomethionine-labelled *At*SFGH.

1. Introduction

S-Formylglutathione hydrolase (EC 3.1.2.12) catalyses a key step in the metabolism of formaldehyde which, as well as being a ubiquitous pollutant, is also a common by-product of primary metabolism arising from the spontaneous dissociation of 5,10-methylene tetrahydrofolate and from protein-repair and oxidative-demethylation reactions (Hanson *et al.*, 2000). Once released, formaldehyde spontaneously reacts with glutathione to form *S*-hydroxymethylglutathione, which is oxidized to *S*-formylglutathione by formaldehyde dehydrogenase (Martinez *et al.*, 1996; Shafqat *et al.*, 1996). The *S*-formylglutathione is then hydrolysed to formic acid and glutathione by SFGH (Hanson *et al.*, 2000). It has been suggested that this shunt pathway of C1 metabolism is essential to all cells (Harms *et al.*, 1996). Microbial SFGHs have been purified and characterized in detail from *Paracoccus denitrificans* and *Saccharomyces cerevisiae* (Harms *et al.*, 1996; Degrassi *et al.*, 1999). The component enzymes appear to have essential functions in both eukaryotes and prokaryotes and are conserved in sequence from microbes through to *Caenorhabditis elegans*, *Drosophila melanogaster* and man (Hanson *et al.*, 2000). We have recently reported that SFGH is also conserved in sequence in plants and have cloned and characterized the functional enzyme from *Arabidopsis thaliana*, terming it *At*SFGH (Kordic *et al.*, 2002). In addition to its activity toward glutathione thioesters, SFGH also hydrolyses carboxyesters of xenobiotics and was originally termed esterase D (Lee & Lee, 1986). In animals, esterase D is highly active toward xenobiotic carboxyesters, notably 4-methylumbelliferyl acetate (4MUA), with polymorphisms in its expression serving as a useful biochemical marker for several diseases including retinoblastoma and Wilson's disease (Lee & Lee, 1986). It was subsequently determined that esterase D was in fact SFGH (Uotila, 1984). *At*SFGH has similar catalytic activities to its mammalian and bacterial counterparts (Kordic *et al.*, 2002), being able to hydrolyse a diverse range of carboxyesters (4MUA, naphthyl acetate and *p*-nitrophenyl acetate). However, unlike other SFGHs the *Arabidopsis* enzyme is highly active toward *S*-acetylglutathione in addition to *S*-formylglutathione, suggesting some species-dependent variation in the respective active sites of the enzymes. In common with other SFGHs, the *Arabidopsis* enzyme is highly sensitive to inhibition by *N*-ethylmaleimide and heavy metals. It has therefore been suggested that SFGHs are cysteinyl hydrolases (Kordic *et al.*, 2002), with the most likely candidate for a catalytic residue being a conserved cysteine (Cys59). We now report the crystallization of

*At*SFGH, which forms part of an ongoing study of the enzyme's catalytic mechanism and functions in plant metabolism.

2. Methods

2.1. Protein expression, purification and selenomethionine labelling of SFGH

The PCR amplification, cloning and expression of *At*SFGH with a C-terminal hexahistidine tag using pET24a (Novagen) in *Escherichia coli* BL21 (DE3) cells were as described by Kordic *et al.* (2002). *At*SFGH synthesis was induced by the addition of 1 m*M* IPTG to the LB culture medium when the cells were in the mid-log phase of growth at 310 K, with cells grown for a further 5 h prior to harvesting. *At*SFGH was purified using nickel-chelate chromatography. Cells were resuspended in binding buffer (20 m*M* Tris, 20 m*M* imidazole, 500 m*M* NaCl pH 7.8) containing 1 mg ml^{-1} lysozyme and 5 µg ml^{-1} DNase I. After lysis using a French press and centrifugation, the clarified supernatant was applied to a Probond nickel-chelate affinity column (Invitrogen) and washed with buffer containing 40 m*M* imidazole; *At*SFGH was then eluted with buffer containing 300 m*M* imidazole. Protein was dialysed against 10 m*M* Tris pH 8.0, 1 m*M* dithiothreitol (DTT) overnight and concentrated with a Centricon 10 (Amicon).

Selenomethionine-labelled *At*SFGH (SeMet-*At*SFGH) was produced using the methionine-auxotrophic strain *E. coli* 834 (DE3) (Novagen) by a modification of the protocol described by Ramakrishnan and Graziano (http://alf1.mrc-lmb.cam.ac.uk/~ramak/madms/segrowth.html). Cells containing the expression construct were grown overnight in 100 ml of 2×M9 minimal medium, 0.4% glucose with vitamins and all amino acids including L-methionine at 40 µg ml^{-1}. After collection by centrifugation, cells were washed twice with water, resuspended in 1.0 ml water and inoculated into 1 l of the above medium supplemented with 40 µg ml^{-1} L-selenomethionine (Fisher), which had been pre-warmed to 310 K. After 2 h incubation, IPTG was added to 1.0 m*M* and the culture was continued for a further 5 h. SeMet-*At*SFGH was purified as described for the native protein and selenomethionine incorporation was confirmed by electrospray mass spectrometry.

2.2. Crystallization

*At*SFGH was crystallized using the hanging-drop vapour-diffusion technique at 293 K. Initial crystallization conditions were found using a screen based on the method of Jancarik & Kim (1991). The optimized precipitant solution contained 16% PEG 4000, 0.2 *M* magnesium acetate, 0.2 *M* imidazole–malate pH 6.5, 3% methanol. Protein solution at 30 mg ml^{-1} (1.5 µl) was mixed with 1.5 µl precipitant and suspended as a hanging drop above precipitant solution (500 µl). Optimal crystals were obtained by microseeding into freshly prepared drops. Crystals of SFGH appeared 1–5 d after seeding and grew to approximate dimensions of 0.3 × 0.2 × 0.1 mm. A typical crystal of SFGH is shown in Fig. 1. Selenomethionine-substituted *At*SFGH was crystallized under similar conditions, but the precipitant solution contained 10% PEG 4000, 0.2 *M* magnesium acetate, 0.2 *M* imidazole–malate pH 6.5, 3% methanol, 10 m*M* DTT.

Table 1
Data-collection statistics for native and pressurized crystals.

Values in parentheses refer to the highest resolution shell.

	Native	Xe pressurized	N_2 pressurized
Data-collection source	SRS 9.6	SRS 14.1	SRS 9.6
Wavelength (Å)	0.87	1.488	0.87
Temperature (K)	100	100	100
Space group	*C*2	$P3_121$	$P3_121$
Unit-cell parameters			
a (Å)	128.5	75.1	74.1
b (Å)	81.1	75.1	74.1
c (Å)	94.3	92.8	91.4
α (°)	90	90	90
β (°)	93.3	90	90
γ (°)	90	120	120
V_M (Å^3 Da^{-1})	2.6	2.4	2.4
Solvent content (%)	52	48	48
Resolution (Å)	35–1.84 (1.91–1.84)	35–1.7 (1.73–1.7)	25–1.6 (1.63–1.6)
R_{sym} (%)	4.2 (25.3)	9.3 (43.1)	3.9 (39.3)
$\langle I/\sigma(I)\rangle$	26.4 (5.4)	35.2 (4.4)	31.4 (2.8)
Unique reflections	83066	33724	37786
Redundancy	4.1	15.6	4.6
Completeness	99.2 (99.4)	99.9 (99.8)	96.5 (72.6)

3. Results

Crystals of *At*SFGH were cryoprotected by brief soaking in a solution containing mother liquor supplemented with 15% ethylene glycol and then flash-frozen in a liquid-nitrogen stream. A native data set was collected at SRS Daresbury to a resolution of 1.84 Å; the data-processing statistics are given in Table 1. Data were indexed, integrated and scaled using the *HKL* suite (Otwinowski & Minor, 1997). The crystals belong to space group *C*2, with unit-cell parameters a = 128.5, b = 81.1, c = 94.3 Å, β = 93.3°. Assuming the presence of three molecules of *At*SFGH in the asymmetric unit, the Matthews coefficient (V_M) is 2.6 Å^3 Da^{-1}, corresponding to a solvent content of 52% (Matthews, 1968).

Attempts to phase the data by molecular replacement in *AMoRe* (Navaza, 1994) using a search model (PDB code 1jjf) with 25% sequence identity failed and so a search for derivatives was initiated. A xenon-treated crystal was prepared by placing a crystal, soaked in cryoprotectant and held in a cryoloop, into a xenon pressure chamber (http://www.oxfordcryosystems.co.uk/cryo/xcell) and pressurizing with xenon gas at 2 MPa for 30 min. The crystal was then flash-cooled in liquid nitrogen within 15 s of being removed from the pressure cell.

A change in space group was observed for the xenon-treated crystal. The new space group is trigonal, either $P3_121$ or $P3_221$, with unit-cell parameters a = 75.1, c = 92.8 Å. To determine whether the space-group change arose as a result of xenon binding or of pressurization, a second crystal was placed in the xenon pressure chamber and pressurized under similar conditions, but this time with nitrogen gas rather than xenon. Again, the space group changed from monoclinic to trigonal. Two further data sets were collected, one to a resolution of 1.7 Å for the xenon-treated crystal and the other

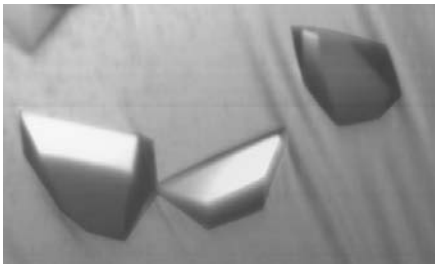

Figure 1
Crystals of SeMet-*At*SFGH. The crystals are approximately 0.3 mm in the longest dimension.

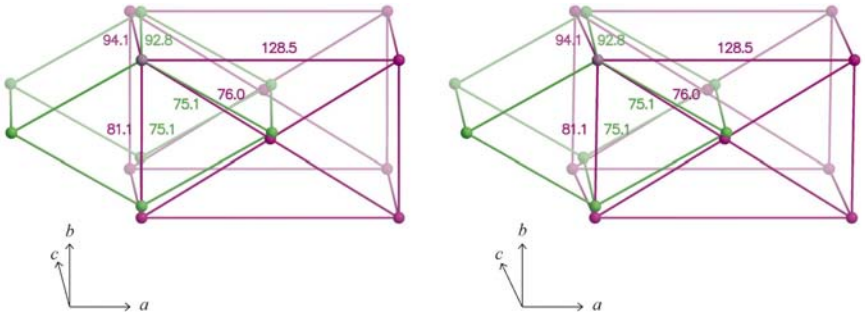

Figure 2
A stereoview comparison of the monoclinic (magenta) and trigonal (green) space groups. The unit-cell parameters are shown in Å.

Table 2
Data-collection statistics for SeMet SFGH.

All data were collected from one crystal in space group *C*2. Values in parentheses refer to the highest resolution shell.

	Peak	Edge	Remote
Wavelength (Å)	0.9790	0.9792	0.8856
Unit-cell parameters (Å, °)	a = 128.5, b = 80.7, c = 93.8, β = 93.2		
Resolution (Å)	30–2.32 (2.40–2.32)	30–2.32 (2.40–2.32)	30–1.7 (1.76–.70)
R_{sym} (%)	7.4 (12.8)	5.7 (11.0)	6.9 (47.5)
$\langle I/\sigma(I)\rangle$	19.4 (9.5)	20.5 (10.9)	22.4 (2.7)
Unique reflections	40855	40846	104555
Redundancy	4.3	4.3	5.6
Completeness	98.0 (81.5)	98.0 (81.8)	99.3 (94.7)

to a resolution of 1.6 Å for the nitrogen-treated crystal (Table 1).

Fig. 2 illustrates the change in space group and unit-cell parameters that occurs after pressurization of the crystals. The overall crystal packing is very similar in the two crystal forms. The biggest change is in *b*, which is reduced by 8% from 81.1 Å in the *C*2 setting to 75.1 Å in the trigonal form. The other unit-cell parameters undergo a much smaller change of approximately 1% and there is a change in the angle to the *ab* plane of 3.3° to make it perpendicular. Overall, there is a reduction in the volume of the unit cell of approximately 8% from 9.8×10^5 Å^3 for the *C*2 cell to 9.1×10^5 Å^3 for double the volume of the trigonal cell. A reduction in unit-cell volume (1.8–2.6%) has also been observed for rhombohedral insulin crystals when subjected to moderate pressures (Nicholson *et al.*, 1996), but in this case there was not an accompanying change in the space group.

While the search for derivatives was ongoing, selenomethionine-labelled protein was prepared and crystallized. The SeMet-*At*SFGH crystals were also pressurized under nitrogen, but they remained in the *C*2 space group with unit-cell parameters nearly identical to those of the unpressurized native crystals. A three-wavelength MAD experiment was performed on BM14 at the ESRF and the data-collection and processing statistics are shown in Table 2. The structure was solved using MAD phasing and refinement of the data is in progress.

4. Conclusion

In our attempts to solve the structure of *At*SFGH, we have discovered a pressure-induced space-group change. The change could be brought about by pressurizing the crystal with either xenon or nitrogen gas, which rules out the possibility that changes in lattice interactions are caused by the specific binding of xenon to *At*SFGH. The lack of signal in the difference or the anomalous difference Patterson maps calculated for the xenon derivative again implies there is no specific binding of xenon to the protein. The selenomethionylated protein crystals do not undergo the same space-group change when subjected to high pressure and so we can deduce that one or more methionines are either involved in the formation of crystal contacts or are in close proximity to the intermolecular interfaces.

The space-group change can be explained using Le Chatelier's principle, which specifies that when pressure is applied to a system at equilibrium the system adjusts by trying to reduce its volume. Therefore, when protein crystals are pressurized there is a reduction in the volume of the unit cell. This can occur by shortening of the unit-cell parameters as happens in the example of rhombohedral insulin given above (Nicholson *et al.*, 1996). However, in our case we observe a much larger reduction in unit-cell volume, which is accommodated by a phase transition from the monoclinic crystal form to the trigonal crystal form.

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