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Crystallization and preliminary X-ray analysis of chicken-liver glutathione S-transferase CL 3-3

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Abstract

Five different crystal forms of recombinant chicken-liver glutathione S-transferase CL 3-3 have been obtained by the vapor-diffusion method. The form *A* crystals are monoclinic $C2$, $a = 125.56$, $b = 85.81$, $c = 52.71$ Å and $\beta = 114.64°$, and diffract to 4 Å resolution. The form *B* crystals are monoclinic $P2_1$, $a = 105.13$, $b = 118.54$, $c = 62.62$ Å and $\beta = 124.74°$, and diffract to 2.8 Å resolution. The form *C* crystals are orthorhombic $C222_1$, $a = 101.69$, $b = 115.46$, $c = 95.40$ Å, and diffract to 2.8 Å resolution. The form *D* crystals are tetragonal, $P4_12_12$ or $P4_32_12$, $a = b = 115.31$, $c = 171.20$ Å and diffract to 3.5 Å resolution. The form *E* crystals are hexagonal, $P6_1$ or $P6_5$, $a = b = 104.23$, $c = 114.35$ Å, diffract to 3.5 Å resolution. Forms *A*, *C* and *E* have one dimer of molecular weight 50 kDa, while forms *B* and *D* have two dimers per asymmetric unit, respectively.

1. Introduction

Glutathione S-transferases (GST's, E.C. 2.5.1.18) comprise a large group of multifunctional enzymes that catalyze the conjugation of glutathione to a wide variety of electrophiles and xenobiotics. They have been implicated as one of the causes for multiple drug resistance (Rushmore & Pickett, 1993). These isozymes can be distinguished by their physical, chemical, immunological, enzymatic and structural properties. The cytosolic enzymes from vertebrates have been grouped into four classes called alpha, mu, pi (Mannervik *et al.*, 1985) and theta (Meyer *et al.*, 1991). A separate membrane-bound transferase, designated microsomal GST, has been described by Morgenstern *et al.* (1985). An isozyme with significant difference in primary structure from members of the other classes has also been isolated from the digestive gland of a cephalopod (Harris *et al.*, 1991). In addition, a murine GST expressed in lung and liver tissues has been reported to have enzymatic and immunological properties which differ from that of the four major classes of GST's (Medh & Awasthi, 1990; Medh, Saxena, Singhal, Ahmad & Awasthi, 1991). Together with rat GST 8-8 (Stenberg, Ridderstrom, Engstrom, Pemble & Mannervik, 1992) and chick CL 3-3 (Chang, Fan, Liu, Tsai & Tam, 1992). Zimniak *et al.* (Zimniak, Eckles, Saxena & Awasthi, 1992) have proposed that these murine, rat and chicken isozymes should form a subgroup of class-alpha GST's.

X-ray crystal structures have been determined for mammalian (Ji, Zhang, Armstrong & Gilliland, 1992; Reinemer *et al.*, 1992; Sinning *et al.*, 1993), insect (Wilce, Board, Feil & Parker, 1995), parasitic helminth (McTigue, Williams & Twiner, 1995), and a cephalopod (Ji *et al.*, 1995) GST's. However, a structure of an avian GST is yet to be reported. We present here the purification, crystallization, and preliminary X-ray diffraction analyses of recombinant GST CL 3-3 from chicken liver. Elucidation of the three-dimensional structure of CL 3-3 would help to confirm the existence of a subgroup of class-alpha GST's and understand its substrate specificities and reactivities. Interestingly, this protein crystallized in many different forms, while enzymatic assays of protein solutions before crystallization showed no difference in substrate specificities. Crystals with axial lengths that are multiples of each other can be found in all forms (such as $c = 62$, 124 and 186 Å). These crystals provide an opportunity to compare structures of the same GST molecule crystallized with different sets of crystal-packing interactions. In this paper we reported the preliminary crystallographic studies of these five basic crystal forms of GST CL 3-3.

2. Experimental

2.1. *Expression and purification of CL 3-3 GST*

The Bluescript phagemid (pGCL301), containing the coding region of CL3 subunit has been described (Chang *et al.*, 1992). Using site-directed mutagenesis, a unique *Nde*I restriction endonuclease site was introduced onto pGCL301 at the location of the ATG start codon for the *CL3* gene. The *Nde*I–*Sal*I (720 bp) fragment, containing the *CL3* coding region, was inserted into a pBAce expression vector and transformed into *E. coli* TG1 cells for protein expression. The transformed bacteria were grown in Luria broth to exponential phase before dilution (10 000 fold) into the induction medium (Craig, Yuan, Kuntz, McKerrow & Wang, 1991). Cells were allowed to grow at 303 K until the culture reached an absorbance at 600 nm of 1.2 (about 18 h) for maximum induction of recombinant protein expression.

Harvested cells were suspended in homogenization buffer [50 m*M* Tris–HCl, pH 8.0, 1 m*M* ethylenedinitrilotetra-acetic acid, 15%(*w*/*v*) sucrose, 6 m*M* β-mercaptoethanol and 0.5 m*M* phenylmethane-sulfonyl fluoride] at 100 ml l^{-1} of cell culture. Cells were lysed by sonication after incubating with lysozyme (0.1 mg ml^{-1}) for 20 min. Cell debris was removed by centrifugation at 205 600*g* for 1 h. The supernatant was dialyzed overnight against the equilibrium buffer (10 m*M* Tris–HCl, pH 8.0, 1 m*M* ethylenedinitrilotetra-acetic acid, 0.2 m*M* dithiothreitol and 0.5 m*M* phenylmethane-sulfonyl fluoride) before loading onto a GSH–Sepharose affinity column. After sample loading, the affinity column was washed with the equilibrium buffer containing 0.2 *M* NaCl. The GST CL 3-3 protein was eluted off the column with 0.2 *M* NaCl and 5 m*M* S-hexylglutathione in equilibrium buffer.

Table 1. *Crystal parameters of glutathione S-transferase CL 3-3*

	A	*B*	*C*	*D*	*E**
Space group	*C*2	$P2_1$	$C222_1$	$P4_12_12$ or $P4_32_12$	$P6_1$ or $P6_5$
a (Å)	125.56	105.133	101.69	115.31	104.23
b (Å)	85.81	118.54	115.46	115.31	104.23
c (Å)	52.71	62.62	95.40	171.20	114.35
α (°)	90	90	90	90	90
β (°)	114.64	124.74	90	90	90
γ (°)	90	90	90	90	120
Size (mm)	0.1 × 0.1 × 0.4	0.2 × 0.2 × 0.4	0.1 × 0.3 × 1.0	0.1 × 0.1 × 0.6	0.1 × 0.1 × 0.3
Crystallization temperature (K)	301	301	297	277	297
Resolution (Å)	4.0	2.8	2.8	3.5	3.5
No. of dimers/a.u.†	1	2	1	2	1
Solvent content‡	0.53	0.62	0.56	0.57	0.66
Completeness (%)	62	89	97	90	—
No. of measured reflections	4832	88264	82570	62154	—
No. of unique reflections	2679	27638	13560	13580	—
R_{merge}§	5.1	10.5	6.8	8.6	—

* Perfect twin or disorder along *xy* plane of the crystals causes failure on data processing. † Number of dimer molecules in asymmetric unit is calculated according to the equation of Mathews (Mathews, 1969). ‡ The solvent content is calculated according to the equation of Mathews (Matthews, 1968). § $R_{merge} = \sum |I - \langle I \rangle| / \sum I$, where I is the intensity for a detection of a multiply observed reflection.

2.2. *Crystallization*

Crystallization conditions were screened by using the hanging-drop vapor-diffusion method (Wlodawer & Hodgson, 1975). Initial crystallization conditions were screened by using the sparse-matrix sampling technique (Jancarik & Kim, 1991) by mixing 2 µl of protein samples with equal volume of the reservoir solutions. Five different crystal forms were obtained (Fig. 1). Form *A* crystals were obtained from a protein preparation with a concentration of about 10 mg ml^{-1} in 0.1 *M* 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), pH 10.5, against a solution of 21%(*w*/*v*) PEG 8000 and 8%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD) in the same buffer. Plate crystals appeared after 5 d at 301 K and reached maximum dimensions after three weeks. This form of crystals decays easily during data collection. Form *B* crystals were acquired with a protein concentration of about 10 mg ml^{-1} in 0.2 *M* CAPS, pH 10.2, against a solution of 11.6%(*w*/*v*) PEG 8000 and 10%(*v*/*v*) MPD in the same buffer. Pencil-like crystals appeared after 3 d at 301 K and reached maximum dimensions after two weeks. However, twin crystals were mostly obtained. Form *C* crystals were procured with a protein concentration of about 10.7 mg ml^{-1} in 0.1 *M* citrate buffer, pH 5.6, against a solution of 15.3%(*w*/*v*) PEG 8000, 0.09 *M* ammonium sulfate, 0.22 *M* magnesium acetate and 0.4 m*M* glutathione in the same buffer. Long plate crystals appeared after 5 d at 297 K and reached maximum dimensions after two weeks. Form *D* crystals were obtained with a protein concentration of about 11 mg ml^{-1} in 0.1 *M* cacodylate buffer, pH 6.5, against a solution of 10.2%(*w*/*v*) PEG 8000, 6%(*v*/*v*) MPD, 0.1 *M* ammonium sulfate and 0.05 m*M* glutathione in the same buffer. Tetragonal bipyramidal column crystals appeared after 12 d at 277 K and reached maximum dimensions after two months. Form *E* crystals were crystallized with a protein concentration of about 11 mg ml^{-1} in 0.1 *M* cacodylate buffer, pH 6.5, against a solution of 18%(*w*/*v*) PEG 8000, 0.1 *M* ammonium sulfate and 0.4 m*M* glutathione in the same buffer. Hexagonal plate crystals appeared at 10 d at 297 K and reached maximum dimensions after two months. However, twin crystals were mostly obtained. The crystallization conditions mentioned

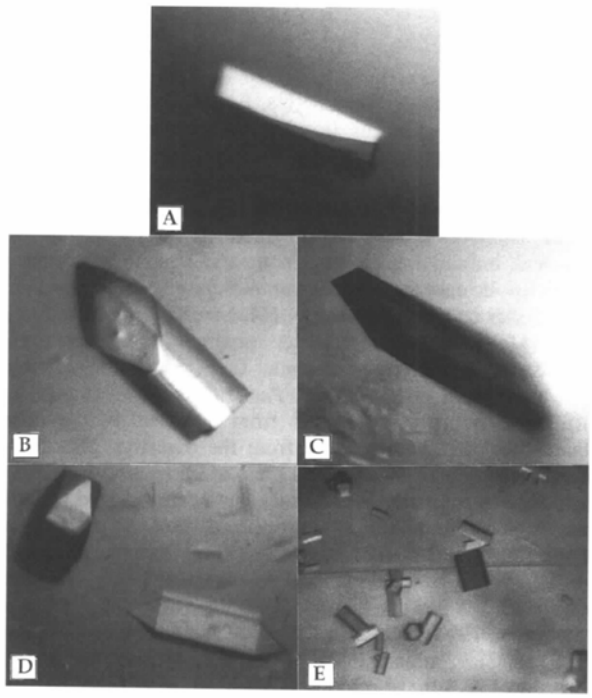

Fig. 1. Five crystal forms of glutathione S-transferase CL 3-3. Form *A*: plate-like crystals have approximate dimensions of 0.1 × 0.1 × 0.4 mm. Form *B*: pencil-like crystals have approximate dimensions of 0.2 × 0.2 × 0.4 mm. Form *C*: long plate crystals have approximate dimensions of 0.1 × 0.3 × 1.0 mm. Form *D*: tetragonal bipyramidal column crystals have approximate dimensions of 0.1 × 0.1 × 0.6 mm for the largest crystal. Form *E*: hexagonal plate crystals have approximate dimensions of 0.1 × 0.1 × 0.3 mm for the largest crystal. See text for detailed crystallization conditions.

above were fine tuned for best crystal formation. However, crystals can be obtained in the presence or absence of extrinsically added glutathione or inhibitors. All these crystal forms decay to some degree during data collection.

3. Results

All the data sets were collected at room temperature on an R-AXIS II imaging-plate detector system using Cu $K\alpha$ radiation generated by a Rigaku RU-300 rotating anode at 50 kV and a current setting of 80 mA. The detector-to-image plate distance is 110 mm with the swing angle set to 0.0°. Three still photographs (0, 45 and 90°) were recorded to a maximum of 2.5–2.8 Å resolution for each crystal form. The cell parameters were deduced from the reflections extracted from these images. Upon complete data-set collection, the cell parameters were further refined using a software package supplied by Molecular Structure Corporation. The space group of these five crystal forms were deduced from systematic absence. The space groups and cell parameters are listed in Table 1. Homology model building of GST CL 3-3 using human class-alpha GST (Sinning *et al.*, 1993) as a template reveals that a total of six intramolecular hydrogen bonds and salt bridges in human class-alpha GST are missing in GST CL 3-3 (Lee, Liaw & Tam, manuscript in preparation). This might account for the low crystal qualities as well as low tolerance to X-ray exposure. Attempts to solve these crystal forms by combination of molecular replacement and multiple isomorphous replacement are in progress.

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