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Purification, crystallization and preliminary X-ray diffraction analysis of human Gadd45γ

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Gadd45, MyD118 and CR6 (also termed Gadd45α, Gadd45β and Gadd45γ, respectively) comprise a family of proteins that play important roles in negative growth control, maintenance of genomic stability, DNA repair, cell-cycle control and apoptosis. Recombinant human Gadd45γ and its selenomethionine derivative were expressed in an *Escherichia coli* expression system and purified; they were then crystallized using the hanging-drop vapour-diffusion method. Diffraction-quality crystals were grown at 291 K using PEG 3350 as precipitant. Using synchrotron radiation, the best diffraction data were collected to 2.3 Å resolution for native crystals at 100 K; selenomethionyl derivative data were collected to 3.3 Å resolution. All the crystals belonged to space group $I2_13$, with approximate unit-cell parameters $a = b = c = 126$ Å.

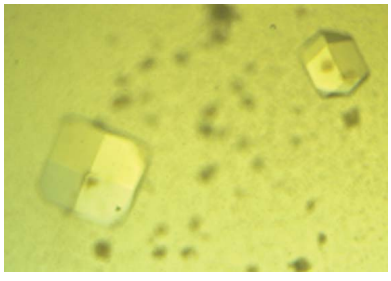
1. Introduction

The human growth-arrest and DNA damage-inducible protein family Gadd45 has three members: Gadd45α, MyD118/Gadd45β and CR6/OIG37/Gadd45γ. The transcription levels of the genes encoding these products increase under stressful growth-arrest conditions or treatment with DNA-damaging agents and are regulated by a distinct subset of environmental and physiological stresses (Liebermann & Hoffman, 2007). The three isoforms of human Gadd45 proteins are all small (18 kDa), highly acidic (pI $\simeq$ 4.0–4.2), evolutionarily conserved and predominantly localized within the cell nucleus. They are often coordinately expressed and can function cooperatively in inhibiting cell growth.

The Gadd45 proteins play important roles in maintaining genomic stability and control of the cell cycle and cell fate (Abdollahi *et al.*, 1991; Amanullah *et al.*, 2003; Hildesheim *et al.*, 2002; Hollander *et al.*, 1999; Liu *et al.*, 2005; Smith *et al.*, 1994; Tong *et al.*, 2001; Vairapandi *et al.*, 1996; Wang *et al.*, 1999; Yang *et al.*, 2001; Yoo *et al.*, 2003; Zhang *et al.*, 1999). Gadd45γ has also been found to be a functional tumour suppressor and is frequently disrupted epigenetically in multiple tumours (Ying *et al.*, 2005). At the same time, Gadd45α relieves epigenetic gene silencing by promoting DNA repair, which erases methylation marks (Barreto *et al.*, 2007).

At the molecular level, the Gadd45 family of proteins can interact directly with a wide range of proteins such as cdc2/cdk1, the cdc2–cyclin B1 complex, PCNA, $p21^{WAF1/Cip1}$, MEKK4/MTK1, MKK7/JNKK2, p38, Bcl-xL, histone, CRIF1/Gadd45GIP1, Aurora-A, B23, the DNA-repair endonuclease XPG and so on. Moreover, they can interact with each other to form homo and hetero complexes *in vivo* as well as *in vitro* (Kovalsky *et al.*, 2001).

To date, the precise roles of the human Gadd45 proteins remain unclear. In order to explore their detailed mechanisms, it is important to determine the three-dimensional structures of the human Gadd45 family. Here, we report our results on the cloning, expression,



purification, crystallization and preliminary X-ray crystallographic analysis of recombinant native and selenomethionyl (SeMet) human Gadd45γ. From this structure, we expect to understand more about the cell cycle and apoptosis pathway in mammals and to shed light on the structure and function of other members of this family.

2. Materials and methods

2.1. Expression and purification of human Gadd45γ

A full-length cDNA fragment encoding human Gadd45γ was cloned into the GST-tagged expression plasmid pGEX-6P-1 vector (Amersham Biosciences) between the *Eco*RI and *Xho*I restriction sites. Comparing the sequence of our construct with the originally submitted sequence of human Gadd45γ (GenBank accession No. NP_006696), in this gene product Gadd45γ and GST are connected by a short linker sequence containing a PreScission protease cleavage site, which after cleavage yields Gadd45γ with eight additional residues (GPLGSPEF) at its N-terminus (17.91 kDa and 167 amino-acid residues in total). The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3) and transformed cells were plated onto LB plates containing 100 µg ml^{-1} ampicillin. A single colony was picked and grown overnight at 310 K in 10 ml LB medium containing 100 µg ml^{-1} ampicillin. The next day, 10 ml of the overnight culture was added to 1 l LB medium containing 100 µg ml^{-1} ampicillin. When the culture density reached 0.6–0.8 (A_{600}), induction with 1 m*M* IPTG (isopropyl β-D-1-thiogalactopyranoside) was performed and cell growth continued for 3–4 h at 310 K.

After harvesting by centrifugation (4000 rev min^{-1}, 30 min, 277 K), the cells were resuspended in PBS buffer (140 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* $Na_2HPO_4.12H_2O$, 1.8 m*M* KH_2PO_4 pH 7.4) and sonicated. After centrifugation at 15 000 rev min^{-1} for 30 min at 277 K, the clarified supernatant was passed through a glutathione-Sepharose 4B column (equilibrated with PBS buffer). The GST-fusion protein-bound column was washed with ten column volumes of PBS buffer. The GST-fusion proteins were cleaved by PreScission protease overnight at 277 K in cleavage buffer (50 m*M* Tris–HCl, 150 m*M* NaCl, 1 m*M* DTT, 1 m*M* EDTA pH 8.0). The target protein was then eluted with buffer *A* (25 m*M* Tris–HCl, 150 m*M* NaCl pH 8.0) and loaded onto a Mono Q (Amersham Pharmacia, USA) ion-exchange chromatography column run in buffer *A*. After washing away unbound protein with two bed volumes of buffer *A*, a linear gradient of 0.15–1 *M* NaCl in the same buffer was applied. The target protein was further purified by gel filtration on a Superdex75 HiLoad 16/60 (GE Healthcare, USA) column run in 25 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl. The purity of the protein was then analyzed on SDS–PAGE (better than 95% purity) and was judged to be suitable for crystallization.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

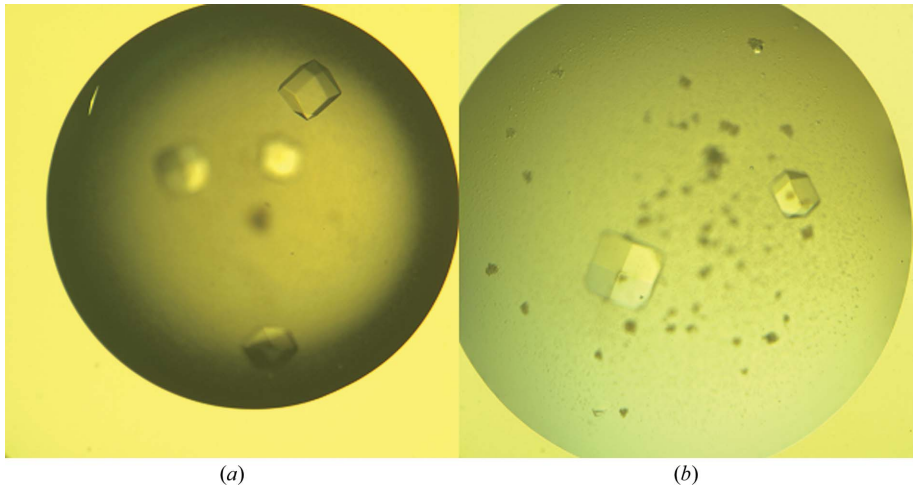
Data set	Native	Native	SeMet derivative
Space group	$I2_13$	$I2_13$	$I2_13$
Unit-cell parameters (Å, °)	$a = b = c = 126.413$, $\alpha = \beta = \gamma = 90.0$	$a = b = c = 126.079$, $\alpha = \beta = \gamma = 90.0$	$a = b = c = 126.073$, $\alpha = \beta = \gamma = 90.0$
Wavelength (Å)	1.000	1.000	0.9803
Resolution (Å)	2.3 (2.35–2.30)	3.0 (3.08–3.0)	3.3 (3.47–3.3)
Total observations	305532	554955	443337
Unique reflections	15070 (991)	6790 (660)	5194 (611)
Data completeness (%)	99.8 (99.1)	100.0 (99.9)	98.4 (88.6)
$R_{\rm merge}$†	0.050 (0.755)	0.070 (0.533)	0.119 (0.869)
$\langle I/\sigma(I)\rangle$	44.1 (2.28)	34.9 (5.02)	15.1 (1.60)
Redundancy	10.6 (6.5)	15.1 (12.5)	13.0 (5.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the mean intensity of the reflections.

The SeMet human Gadd45γ protein was also expressed in *E. coli* strain BL21 (DE3). After incubation overnight in LB medium containing 100 µg ml^{-1} ampicillin, the cells were diluted with adaptive medium (20% LB medium, 80% M9 medium) and grown at 310 K to an OD_{600} of 0.6–0.8. The cells were harvested, resuspended in M9 medium, transferred into restrictive medium [5%(*w*/*v*) glucose] and then grown to an OD_{600} of 0.6–0.8 before induction. L-Selenomethionine at 60 mg l^{-1}, lysine, threonine and phenylalanine at 100 mg l^{-1}, leucine, isoleucine and valine at 50 mg l^{-1} and 1 m*M* IPTG were added and incubation continued at 289 K for about 20 h. The cells were harvested and the SeMet-labelled human Gadd45γ was purified and crystallized by the same method as used for the native protein except for the addition of a reducing environment provided by 5 m*M* DTT and 0.5 m*M* EDTA. The incorporation of selenium was confirmed by mass-spectrometric analysis.

2.2. Crystallization

The purified protein was exchanged into crystallization buffer (25 m*M* Tris–HCl pH 8.0 containing 50 m*M* NaCl) and concentrated



(*a*) (*b*)

Figure 1
(*a*) Typical crystals of native human Gadd45γ (approximate dimensions 0.3 × 0.3 × 0.4 mm). (*b*) Typical crystals of SeMet human Gadd45γ (approximate dimensions 0.25 × 0.25 × 0.3 mm).

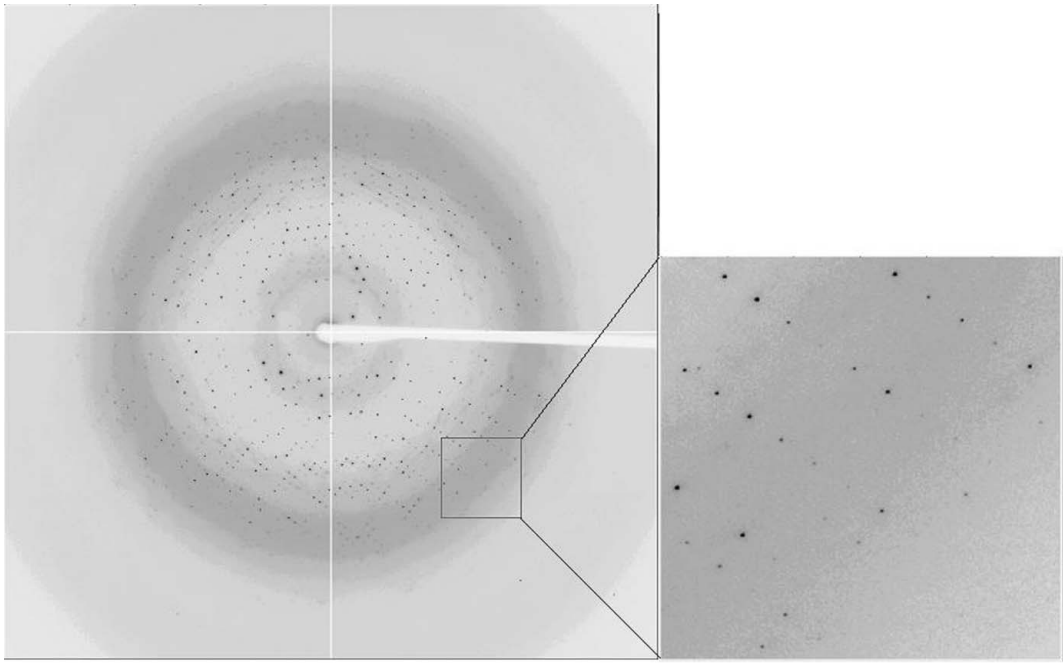


Figure 2
(*a*) A typical diffraction pattern of human Gadd45γ crystals diffracting to 2.3 Å resolution. The diffraction image was collected on an ADSC Q315 e-coupled display detector with a crystal-to-film distance of 275 mm. The oscillation range is 1°. (*b*) An enlarged image of the area indicated in (*a*).

to 20–40 mg ml^{-1} using a 5K ultrafiltration tube. Preliminary crystallization conditions were obtained by the sparse-matrix sampling technique (Jancarik & Kim, 1991) with the hanging-drop vapour-diffusion method using Index and PEG/Ion Screen reagent kits (Hampton Research). Each drop was formed by mixing equal volumes (1–2 µl) of protein and reservoir solutions and was allowed to equilibrate *via* vapour diffusion over 200 µl reservoir solution at 291 K. Crystals suitable for X-ray diffraction were obtained from 22%(*w*/*v*) PEG 3350, 0.1 *M* bis-tris pH 5.5, 0.2 *M* NaCl. Crystals of 0.3 × 0.3 × 0.4 mm in size (Fig. 1) were obtained in about four weeks.

The purified selenomethionine derivative was concentrated to 15–40 mg ml^{-1}. Crystallization trials were set up based on the optimum conditions used for the native protein.

2.3. Data collection and processing

Native data for human Gadd45γ were collected to 3.0 Å resolution using X-rays of wavelength 1.000 Å. Anomalous data were collected to 3.3 Å resolution from the SeMet human Gadd45γ derivative using X-rays of wavelength 0.9803 Å. All data were collected at 100 K using a MAR Research CCD detector on beamline 3W1A of the Beijing Synchrotron Radiation Facility (BSRF; Beijing, People's Republic of China). The cryoprotectant solution contained 25%(*w*/*v*) PEG 3350, 1 *M* NaCl and 0.1 *M* bis-tris pH 5.5. A more complete data set for native human Gadd45γ was collected to 2.3 Å resolution at 100 K on beamline BL-5A of the Photon Factory (KEK, Japan) using an ADSC Q315 e-coupled display detector (Fig. 2). Data processing and scaling were performed with *HKL*-2000.

3. Results

Diffraction data from the native human Gadd45γ crystal were observed to a Bragg spacing of at least 2.3 Å. The unit-cell parameters were determined to be $a = b = c = 126.4$ Å, $\alpha = \beta = \gamma = 90.0°$ in space group $I2_13$. The crystal is predicted to contain one molecule in the asymmetric unit, with a solvent content of about 73% and a high Matthews coefficient (V_M) of 4.7 Å^3 Da^{-1} (Matthews, 1968). Detailed statistics for the X-ray data collection and processing are shown in Table 1. Attempts to determine the structure by SAD phasing based on selenomethionyl substitution are currently in progress.

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