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Crystallization and preliminary X-ray crystallographic studies of human FAIM protein

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Fas apoptosis inhibitory molecule (FAIM), an antagonist of Fas-induced cell death, is highly conserved and is broadly expressed in many tissues. It has been found that FAIM can stimulate neurite outgrowth in PC12 cells and primary neurons. However, the molecular mechanisms of action of FAIM are not understood in detail. Here, full-length human FAIM and two truncation constructs have successfully been cloned, expressed and purified in *Escherichia coli*. FAIM (1–90) was crystallized and diffracted to a resolution of 2.5 Å; the crystal belonged to space group $P3_1$, with unit-cell parameters $a = b = 58.02$, $c = 71.11$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. There were two molecules in the asymmetric unit.

1. Introduction

Programmed cell death, termed apoptosis, plays an important role in the regulation of cellularity in various types of cell and tissue. Cell apoptosis may be influenced by various factors, including cytokines, viral infection, cell stress and damage (Nagata, 1997; Geng, 2001). As such a stimulus, Fas is known to be critically involved in the regulation of immune responses (Chinnaiyan *et al.*, 1995). Several anti-apoptotic proteins, such as Bcl-xL and c-FLIP, have been found to modulate Fas signalling for cell death and their overexpression protects primary B cells from Fas killing (Foote *et al.*, 1996; Schneider *et al.*, 1997; Yoshikawa *et al.*, 2000). Moreover, a completely novel gene that encodes a Fas apoptosis inhibitory molecule (FAIM) was detected in Fas-resistant primary B cells but not in Fas-sensitive primary B cells (Schneider *et al.*, 1999; Zhong *et al.*, 2001). The human *faim* gene localizes to chromosome 3q22 and encodes a 179-amino-acid protein (Hemond *et al.*, 2009; Kaku & Rothstein, 2009*a*). FAIM is conserved from *Caenorhabditis elegans* to *Homo sapiens*; the amino-acid sequence of *C. elegans* FAIM is 49% identical to that of *H. sapiens* FAIM (Rothstein *et al.*, 2000; Sole *et al.*, 2004). Through the NF-κB and MAPK/ERK signal pathways, overexpression of FAIM stimulates neurite outgrowth in PC12 (pheochromocytoma cell line) cells and primary neurons (Sole *et al.*, 2004; Segura *et al.*, 2007). Furthermore, FAIM is also regulated by IRF4 by a feed-forward mechanism in B cells (Kaku & Rothstein, 2009*b*). To date, some efforts have been made to understand the cellular function of FAIM, but its molecular mechanisms are not yet understood. Because there is no notable sequence homology between FAIM and any protein of known structure (Hemond *et al.*, 2009), determination of the crystal structure of FAIM will help us to further understand its molecular mechanism.

In this study, we cloned, expressed, purified and crystallized full-length FAIM and two truncation constructs and finally obtained preliminary X-ray crystallographic data for FAIM (1–90).

2. Experimental results

2.1. Gene cloning, protein expression and purification

Full-length human *faim* cDNA (GeneID 55179) was cloned into an in-house-constructed pET21-DEST expression vector (provided by Professor Ming Luo at University of Alabama at Birmingham) with

an N-terminal His_5 tag using the Gateway cloning system (Invitrogen, USA; Walhout *et al.*, 2000). The *faim* sequence in the expression vector was confirmed by DNA sequencing.

The recombinant FAIM protein was overexpressed and purified in *Escherichia coli* BL21 (DE3) as follows. The expression plasmid was transformed into BL21 (DE3) cells and plated on a Luria–Bertani broth (LB) agar plate with 100 mg l^{-1} ampicillin. A single colony was cultured in 20 ml LB medium supplemented with 100 mg l^{-1} ampicillin overnight at 310 K and then transferred into 1 l LB medium and incubated until the OD_{600} reached 0.6–0.8. The expression of FAIM was induced with 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG; Sigma) for 20 h at 291 K. Cells were harvested by centrifugation at 5000 rev min^{-1} for 10 min and then suspended in binding buffer (20 m*M* HEPES pH 7.5, 500 m*M* NaCl and 5 m*M* imidazole) and sonicated (on for 5 s, off for 10 s; 99 cycles). The lysate was centrifuged at 18 000 rev min^{-1} for 30 min and the supernatant was filtered through a 0.22 µm filter and subsequently loaded onto a 5 ml HiTrap Ni^{2+}-affinity column (GE Healthcare) attached to an ÄKTA fast protein liquid chromatography (FPLC) system (GE Healthcare). The protein was eluted in a linear gradient of 5–500 m*M* imidazole and the protein peak was harvested in 200 m*M* imidazole. The peak fractions were concentrated to 2 ml and loaded onto a Superdex-75 column (GE Healthcare) equilibrated with buffer consisting of 10 m*M* HEPES pH 7.5, 200 m*M* NaCl and 5 m*M* DTT to further purify the protein. The peak fractions from the elution were pooled and concentrated in a Amicon Ultra-15 (Millipore, USA). All purification procedures were performed at 277 K.

The full-length FAIM was unstable after purification and degraded into two bands (Fig. 1, lane 3). FAIM consists of two independently folding domains (Hemond *et al.*, 2009); two truncation constructs FAIM (1–90) and FAIM (91–179) were therefore designed in order to obtain more stable proteins. The specific primer sequences used for FAIM (1–90) were 5′-GGAATTC**CATATG**ATGACAGATCTCG and 5′-CCC**AAGCTT**CTACATATACTTCTTGAGAC and the specific primer sequences used for FAIM (91–179) were 5′-GGAATTC**CATATG**GAGGACAGATCAAA and 5′-CCC**AAGCTT**TTAACTTGCAATCTCT. *Nde*I and *Hin*dIII restriction sites are shown in bold. The truncation constructs were amplified by PCR, ligated into pET28a vector (Novagen) with an N-terminal His_6 tag and purified using the same protocol as described above (Fig. 1, lanes 1 and 2). The recombinant full-length FAIM and its truncated proteins were used in subsequent crystallization. Protein concentrations were measured using the Bradford dye assay. The final yields of full-length FAIM, FAIM (1–90) and FAIM (91–179) were 12, 15 and 10 mg l^{-1}, respectively, and their purity was over 95%.

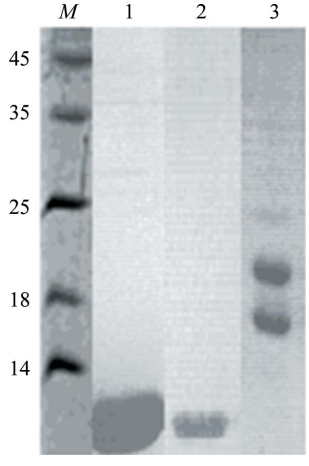

Figure 1
SDS–PAGE analysis of purified recombinant FAIM. Lane *M*, protein molecular-weight markers (kDa); lanes 1, 2 and 3, purified FAIM truncation constructs FAIM (1–90), FAIM (91–179) and full-length FAIM with an N-terminal tag, respectively.

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the last resolution shell.

Wavelength (Å)	1.04361
Resolution (Å)	30–2.5 (2.54–2.50)
Completeness (%)	100 (99.8)
R_{merge}† (%)	12.0 (35.6)
$I/\sigma(I)$	9.0 (2.68)
Space group	$P3_1$
Unit-cell parameters (Å, °)	a = 58.02, b = 58.02, c = 71.11, α = 90.00, β = 90.00, γ = 120.00
No. of observed reflections	15590
No. of unique reflections	9214
Molecules per asymmetric unit	2
V_M (Å^3 Da^{-1})	3.46
Solvent content (%)	64.4

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

2.2. Protein crystallization

The protein solution was centrifuged at 14 000 rev min^{-1} for 30 min at 277 K before crystallization setup. Crystallization screening experiments were performed at 293 K by the hanging-drop vapour-diffusion method using Crystal Screen, Crystal Screen 2, Grid Screen, PEG/Ion and Index (Hampton Research, USA). Typically, each drop was prepared by mixing 1.2 µl protein solution with 1.2 µl reservoir solution. Microcrystals were obtained for the FAIM (1–90) truncation construct under many initial conditions. After optimizing the pH and the protein concentration, larger crystals (diamond-shaped; 0.5 × 0.4 × 0.2 mm) that yielded the best diffraction quality were obtained from 0.67 *M* sodium phosphate and 0.73 *M* potassium phosphate pH 6.5 with a protein concentration of 19 mg ml^{-1} within 7 d (Fig. 2).

2.3. Diffraction data collection

X-ray diffraction data were collected on a MAR 345 image-plate detector at a wavelength of 1.04361 Å on beamline 3W1A at Beijing Synchrotron Radiation Facility. For crystal data collection, crystals were transferred into the corresponding reservoir solution containing 20% glycerol as a cryoprotectant. The crystal was mounted in a cryoloop and flash-cooled in a nitrogen stream at 100 K during data

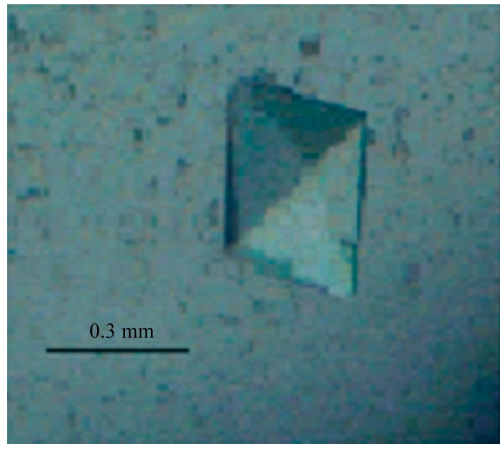

Figure 2
Crystal of FAIM (1–90) obtained using 0.67 *M* sodium phosphate and 0.73 *M* potassium phosphate pH 6.5.

collection. The crystal-to-detector distance was 160 mm. A total of 180 frames of 1° oscillation were collected with 60 s exposure per frame. Data were processed with *MOSFLM* (Powell, 1999) and *CCP*4 (Collaborative Computational Project, Number 4, 1994).

The FAIM (1–90) crystal diffracted to 2.5 Å resolution and belonged to space group $P3_1$, with unit-cell parameters $a = b = 58.02$, $c = 71.11$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. We predicted the presence of two molecules per asymmetric unit. The crystallographic parameters and data-collection statistics are listed in Table 1. Because there is no known sequence homology to any protein of known structure, we have therefore started screening heavy-atom derivatives and the structure of FAIM (1–90) will be reported in the future.

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