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Crystallization and preliminary X-ray crystallographic analysis of human FAF1 UBX domain

Wonchull Kang, Hwa Young Shin and Jin Kuk Yang*

Department of Chemistry, Soongsil University, Seoul 156-743, Republic of Korea

Correspondence e-mail: jinkukyang@ssu.ac.kr

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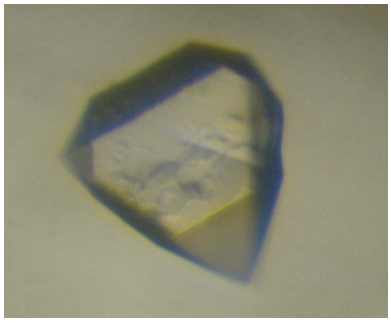
Fas-associated factor 1 (FAF1) is a multifunctional pro-apoptotic protein that is involved in Fas-mediated apoptosis, NF-κB signalling and the ubiquitin–proteasome pathway. In the ubiquitin–proteasome pathway, FAF1 binds to the N domain of p97/VCP, a molecular chaperone that acts in complex with the proteasome, through its C-terminal UBX domain and inhibits the proteasomal protein-degradation process. In an effort to elucidate the structural basis of the function of FAF1 in modulating p97/VCP activity related to proteasomal protein degradation, crystallographic analysis of the FAF1 UBX domain and the p97/VCP N domain was initiated. Following the recently reported crystallization of the FAF1 UBX domain bound to the p97/VCP N domain, the unbound FAF1 UBX domain was also crystallized for purposes of structural comparison. X-ray data were collected to 3.00 Å resolution and the crystals belonged to space group $F4_132$, with unit-cell parameters $a = b = c = 176.40$ Å. The Matthews coefficient and solvent content were estimated to be 3.04 Å^3 Da^{-1} and 59.5%, respectively, assuming that the asymmetric unit contained two molecules of the UBX domain, which was subsequently confirmed by molecular-replacement calculations.

1. Introduction

Fas-associated factor 1 (FAF1) was initially identified as a pro-apoptotic protein that binds to Fas in the death-inducing signalling complex (DISC) and potentiates Fas-mediated apoptosis (Chu *et al.*, 1995; Ryu *et al.*, 1999, 2003). Subsequent work revealed that FAF1 is also involved in NF-κB signalling. It interacts with the NF-κB p65 subunit and inhibits NF-κB nuclear translocation (Park *et al.*, 2004). FAF1 also binds to the IκB kinase (IKK) β subunit, thereby disrupting IKK complex assembly and inhibiting IKK activation (Park *et al.*, 2007). In addition to Fas-mediated apoptosis and NF-κB signalling, FAF1 is also involved in the ubiquitin–proteasome pathway and inhibits the protein-degradation process. FAF1 interacts with ubiquitinated client proteins and also with p97/VCP, which acts as a chaperone that presents client proteins to the proteasome for degradation (Song *et al.*, 2005). Overexpression of FAF1 inhibits the degradation of the ubiquitinated proteins and results in their accumulation, leading to cell death (Song *et al.*, 2005).

Presumably *via* these multiple functions, FAF1 can act as a tumour suppressor and its loss or downregulation contributes to oncogenesis (Menges *et al.*, 2009). Loss of the *FAF1* gene or downregulation of its expression has been observed in various types of cancers: gastric carcinoma, uterine cervix carcinoma, malignant mesothelioma and mantle cell lymphoma (Bjorling-Poulsen *et al.*, 2003; Hidalgo *et al.*, 2005; Altomare *et al.*, 2009; Bea *et al.*, 2009). In addition, FAF1 has also been implicated in neuronal cell death in Parkinson's disease and its pro-apoptotic activity may be relevant (Betarbet *et al.*, 2008).

Human FAF1, which is composed of 650 amino acids, has been revealed to contain four types of ubiquitin-related domain: UBA, UBL, UAS and UBX domains (Song *et al.*, 2005; Menges *et al.*, 2009). The UBX domain at the C-terminus is responsible for interaction with p97/VCP. In the ubiquitin–proteasome pathway, p97/VCP acts as



a chaperone that presents client proteins to the proteasome for degradation; FAF1 inhibits the degradation process by interacting with ubiquitinated client proteins through its UBA domain and also with p97/VCP through the UBX domain (Song *et al.*, 2005). The pro-apoptotic function of FAF1 seems to be accounted for at least in part by inhibition of proteasomal degradation in addition to Fas-mediated enhancement of apoptosis and NF-κB signalling inhibition.

In an effort to elucidate the structural details of the interaction between FAF1 and p97/VCP, we initiated structural analysis of the FAF1 UBX domain bound to the p97/VCP N domain and have recently reported its crystallization (Shin *et al.*, 2010). The refined crystal structure (not yet published) raises questions about the precise conformations of key residues within the interface of the complex that require additional examination of FAF1 UBX in the unbound state. We have initiated crystallographic analysis of the unbound FAF1 UBX even though an NMR structure of the unbound UBX domain is available (Buchberger *et al.*, 2001); here, we report its crystallization and X-ray data collection.

2. Experimental procedures

2.1. Protein overproduction and purification

Construction of the expression vector and protein overproduction and purification have been reported in Shin *et al.* (2010). In summary, the FAF1 UBX domain (residues 571–650) was expressed in *Escherichia coli* in fusion with His-tagged thioredoxin, which was cleaved off by TEV protease during the purification process. After TEV cleavage, Gly-Ser-Glu-Phe remained attached to the N-terminal end of the FAF1 UBX. The purified FAF1 UBX was concentrated to 9.5 mg ml^{-1}, aliquoted and stored at 203 K. The protein solution also contained 20 m*M* Tris–HCl, 5%(*v*/*v*) glycerol, 0.1 m*M* TCEP, 100 m*M* sodium chloride pH 8.0. The protein concentration was determined by measuring the absorbance at 280 nm using a calculated extinction coefficient of 0.74 mg^{-1} ml cm^{-1} (http://www.expasy.org).

2.2. Crystallization and X-ray data collection

Crystallization conditions were initially screened using commercial screening kits from Hampton Research, Emerald BioSystems and Qiagen. Crystals were grown by the hanging-drop vapour-diffusion method. 2 µl protein solution was mixed with an equal volume of reservoir solution and the drop was equilibrated against 0.5 ml reservoir solution at 295 K. Refinement of the composition of the reservoir solution was carried out by varying the concentrations of the component chemicals. Crystals were soaked for 1–2 s in a 1 µl droplet of a cryoprotective solution and were flash-cooled in liquid nitrogen before data collection.

X-ray diffraction data were collected at 100 K on synchrotron beamline 4A at Pohang Light Source, Republic of Korea. The intensity data were processed, merged and scaled with *MOSFLM* and *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994; Powell, 1999).

3. Results and discussion

The human FAF1 UBX domain was overproduced in *E. coli* and purified as described previously (Shin *et al.*, 2010). The purified protein sample was concentrated to 9.5 mg ml^{-1} and subjected to initial crystallization trials using commercial screening kits. Crystals of 200–300 µm in all three dimensions were grown in 2 d using condition No. 5 of the Qiagen Classics Suite II (Fig. 1). The crystals were reproduced using a reservoir solution with the same composition: 2.0 *M* ammonium sulfate and 0.1 *M* HEPES pH 7.5. A crystal was soaked for 1–2 s in a 10 µl droplet of a cryoprotective solution consisting of 3.6 *M* ammonium sulfate and 0.1 *M* HEPES pH 7.5. Immediately after soaking, the crystal was flash-cooled in liquid nitrogen for X-ray data collection.

The crystal diffracted to 3.00 Å resolution on beamline 4A at Pohang Light Source, Republic of Korea (Fig. 2). The crystal belonged to the *F*-centred cubic system, with unit-cell parameter a = 176.40 Å. The diffraction data set was collected as 180 frames; each frame was recorded with 1° oscillation. Systematic absences of reflections were not sufficiently clear to indicate the correct space group from *F*432 and $F4_132$, but the subsequent molecular-replacement calculation gave a solution with the choice of space group $F4_132$. If two molecules of the FAF1 UBX domain were assumed to be present in the asymmetric unit, the Matthews coefficient was 3.04 Å^3 Da^{-1} and the corresponding solvent content was 59.5 (Matthews, 1968). The diffraction data set, which was 99.8% complete, contained 5071

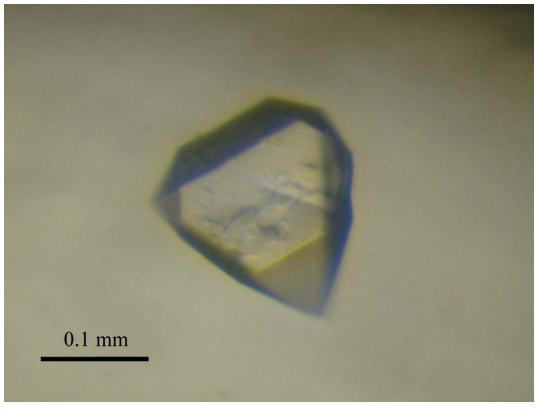


Figure 1
Crystal of the FAF1 UBX domain.

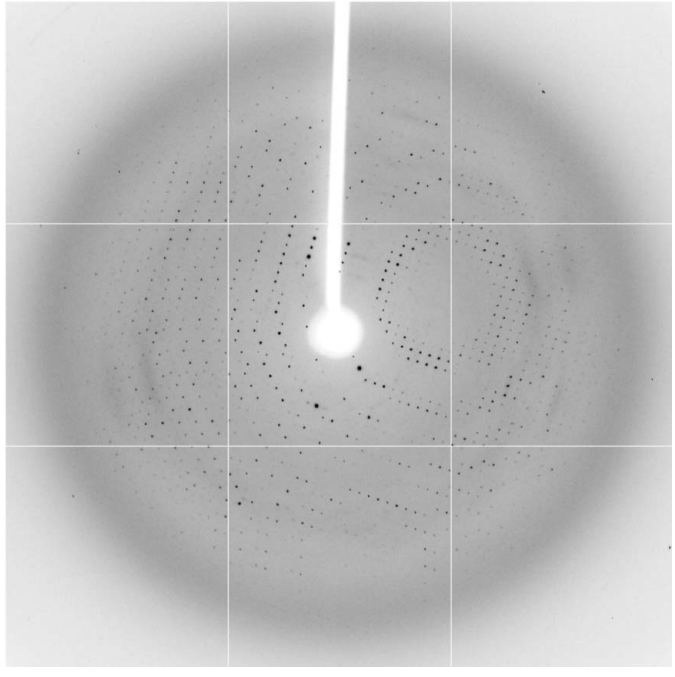


Figure 2
Diffraction image obtained on Pohang Light Source beamline 4A. The crystal-to-detector distance was 400 mm and the wavelength was 0.9999 Å.

Table 1
Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$F4_132$
Unit-cell parameters (°)	$a = b = c = 176.40$
Resolution (Å)	30.00–3.00 (3.16–3.00)
No. of measured reflections	196544
No. of unique reflections	5071
R_{merge}† (%)	6.1 (29.9)
Completeness (%)	99.8 (100.0)
$\langle I/\sigma(I)\rangle$	8.3 (2.5)
Redundancy	38.8 (41.3)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

unique reflections with an average redundancy of 38.8 and an R_{merge} of 6.1%. Table 1 summarizes the statistics of the data collection.

Structure solution was attempted using molecular replacement with *Phaser* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994; McCoy *et al.*, 2007). The search model was the FAF1 UBX part of the crystal structure of FAF1 UBX bound to the p97/VCP N domain which was recently solved by our group using the crystal reported by Shin *et al.* (2010). Two molecules of FAF1 UBX were successfully located in the asymmetric unit and the correct space group was proven to be $F4_132$ and not $F432$. The RFZ and TFZ scores from *Phaser* were 3.9 and 11.2, respectively, and the initial R factor before bulk-solvent correction was 55.38%. Direct refinement of the coordinates of the solution without any manual rebuilding using *REFMAC*5 (Vagin *et al.*, 2004) lowered the R factor to 38.45% and the free R factor to 41.34% after 20 cycles of refinement. Model rebuilding and structure refinement are under way.

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