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Crystallization of the AAA domain of the ATP-dependent protease FtsH of *Escherichia coli*

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FtsH is a membrane-anchored ATP-dependent protease that degrades misfolded or misassembled membrane proteins as well as a subset of cytoplasmic regulatory proteins. It belongs to the family of AAA$^+$ ATPases with roles in diverse cellular processes. The ATPase domain of FtsH from *Escherichia coli* has been crystallized from ammonium sulfate solutions and crystals diffracting to 1.5 Å resolution have been obtained.

1. Introduction

Intracellular proteolysis is important for eliminating harmful abnormal proteins and for modulating the activities of authentic proteins such as short-lived regulatory factors. In eukaryotes, substrates for degradation are targeted to a single ATP-dependent protease, the 26S proteasome, whereas in prokaryotes the situation is more complex, with *E. coli* possessing five ATP-dependent proteases: Lon, ClpXP, ClpAP, HslUV and FtsH (Schmidt *et al.*, 1999). Each contains an AAA (*A*TPases *a*ssociated with cellular *a*ctivities) domain and forms large multisubunit assemblies in which ATP hydrolysis is coupled to the degradation of substrates. AAA domains are Walker-type ATPases made up of ~250 residues and contain Walker A and B motifs as well as an SRH (*s*econd *r*egion of *h*omology) element (Karata *et al.*, 1999). In contrast, the protease domains/subunits have quite different evolutionary origins, with serine proteases (Lon, ClpAP, ClpXP), Ntn hydrolases (26S proteasome, HslUV) and a Zn^{2+}-metalloprotease (FtsH) represented. FtsH has two special characteristics: (i) it is the only membrane-bound ATP-dependent protease and (ii) it is the only one which is essential for *E. coli* cell growth and viability, making it a potential target for the design of antimicrobial drugs.

FtsH is a 647-residue protein of M_r = 71 000, with two putative transmembrane segments towards its N-terminus which anchor the protein to the membrane, giving rise to a periplasmic domain of 70 residues and a cytoplasmic segment of 520 residues containing the ATPase and protease domains. Mutations in *ftsH* (*f*ilamentation *t*emperature-*s*ensitive *H*) have pleiotropic effects. This is because of the diverse roles of the substrates of FtsH, which include regulatory factors such as the heat-shock RNA polymerase sigma factor σ^{32} (Tomoyasu *et al.*, 1995), the *lpxC* gene product, which plays a pivotal role in lipopolysaccharide biosynthesis (Ogura *et al.*, 1999), SsrA-tagged proteins (Herman *et al.*, 1998) and bacteriophage λ CII (Shotland *et al.*, 1997). FtsH is also responsible for degrading membrane proteins which have not been appropriately assembled into functional complexes (Kihara *et al.*, 1995).

2. Expression and purification

An AAA-domain fragment of FtsH from *E. coli*, comprising residues 126–398 with an MGSSHHHHHHSGLVPRGSH tag at its N-terminus and two additional residues LE at its C-terminus, was expressed from a pET15b plasmid derivative in *E. coli* strain AR5088. Cells were grown in LB medium supplemented with ampicillin to A_{595} = 0.6. Expression of recombinant FtsH(126–398) was induced by the addition of IPTG to a final concentration of 1 m*M*. 3 h later the cells were harvested by centrifugation and resuspended in a solution containing 50 m*M* sodium phosphate buffer pH 7.5, 0.5 *M* NaCl (buffer *A*) and 1 mg ml^{-1} lysozyme. The cells were frozen and thawed twice at 193 K and lysis was completed with 10 × 1 min sonication pulses delivered over a period of 20 min with an MSE SoniPrep 150 sonicator. The lysate was clarified by centrifugation.

The soluble lysis fraction was loaded onto a nickel-chelation chromatography column equilibrated in buffer *A* and the column was washed with four column volumes of buffer *A* plus 25 m*M* imidazole and developed with a 25–500 m*M* imidazole gradient in buffer *A*. Fractions containing His-tagged FtsH(126–398) were identified by denaturing gel electrophoresis, pooled and dialysed against 20 m*M* Tris–HCl, 150 m*M* NaCl, 1 m*M* $CaCl_2$. 0.05 units of thrombin (Boehringer) were added per milligram of protein and the N-terminal His tag was removed by overnight digestion at 277 K.

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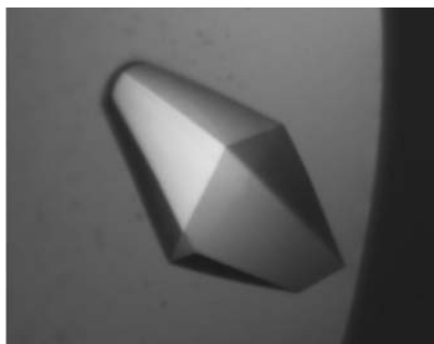

Figure 1
A crystal of FtsH(126–398) with approximate dimensions of 0.2 × 0.1 × 0.1 mm.

FtsH(126–398) was further purified by chromatography on a Superdex S75 16/60 gel-filtration column run in 20 m*M* ethylamine pH 9.0, 0.2 *M* NaCl. The pooled fractions were exchanged into buffer *B* (20 m*M* ethanolamine pH 9.0, 20 m*M* NaCl) and loaded onto a MonoQ ion-exchange chromatography column run in buffer *B* and developed with a 20–500 m*M* NaCl gradient. Individual FtsH(126–398) fractions from this column were exchanged into 20 m*M* Tris–HCl pH 8.5 buffer containing 1 m*M* dithiothreitol (DTT).

3. Crystallization

Crystallization experiments utilized the hanging-drop vapour-diffusion method exploiting a variety of screening kits (Brzozowski & Walton, 2001; Jancarik & Kim, 1991). Protein was dissolved in 20 m*M* Tris–HCl buffer pH 8.5, 50 m*M* NaCl and 1 m*M* DTT at a concentration of 10 mg ml^{-1} prior to the crystallization experiments. Crystals grew from drops composed of 1 µl of protein and 1 µl of 0.1 *M* Tris–HCl pH 8.5, 1.5 *M* $(NH_4)_2SO_4$ (condition No. 1, Clear Strategy Screen II, Molecular Dimensions) over a period of 2–3 d (Fig. 1). The crystals have the sharp appearance of tetragonal bipyramids and typical dimensions of 0.2 × 0.1 × 0.1 mm.

4. Data collection

A single crystal was soaked in a solution of mother liquor supplemented with 25% glycerol and flash-frozen in liquid N_2. Diffraction data were collected on beamline ID14-2 (λ = 0.933 Å) at the ESRF on a CCD ADSC Quantum 4 detector. High-, medium- and low-resolution sweeps were collected. The data were processed using *DENZO*, *SCALEPACK* and programs from the *CCP*4 suite (Otwinowski & Minor, 1997; Collaborative Computational Project Number 4, 1994). The crystals belong to space group $P4_{1(3)}2_12$, with unit-cell parameters $a = b = 53.15$, $c = 189.07$ Å. If it is assumed that there is one monomer in the asymmetric unit, the calculated solvent content is 45% and the Matthews coefficient is 2.25 $Å^3\,Da^{-1}$. 913 258 observations of 44 655 reflections in the resolution range 40–1.5 Å were recorded. The mean overall redundancy is 4.4 and is 3.5 in the highest resolution shell (1.55–1.50 Å). The data are 98.4% (91.1% in the highest resolution shell) complete, with an overall R_{merge} (on intensities) of 0.064 (0.261); the mean $I/\sigma(I)$ value is 18.3 (2.6).

5. Discussion

The structures of several AAA domains are known (reviewed in Ogura & Wilkinson, 2001), with monomers and hexamers featuring in the various crystals. The functional forms of AAA-domain proteins are generally large homo- or hetero-oligomeric ring-shaped assemblies. Electron-microscopy images reveal that FtsH forms circular structures, although the number of subunits involved is not known (Shotland *et al.*, 1997). Hexamers of FtsH(126–398) in our crystals are unlikely given the 422 symmetry and the presence of just one molecule in the asymmetric unit. We have not succeeded in solving the structure of FtsH(126–398) by molecular replacement using various AAA-domain search models, including the D1 domain of p97, which has the closest sequence similarity (Zhang *et al.*, 2000). We are therefore searching for heavy-atom derivatives to enable structure solution by either isomorphous replacement or anomalous dispersion methods. The structure of the AAA domain of FtsH from *E. coli* will be a first step towards a structure of the intact protein. Crystallographic and electron-microscopy studies of the proteasome core and of HslUV have provided detailed insights into the structure and assembly of these remarkable enzymes, which appear to unfold their substrates and translocate them into an enclosed cavity where proteolysis takes place (Bochtler *et al.*, 1997, 1999; Ishikawa *et al.*, 2000; Sousa *et al.*, 2000; Wang *et al.*, 2001). In the case of FtsH, there is an additional level of interest, as the unfolding step for many of its substrates must involve extraction of a polypeptide from the cytoplasmic membrane.

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