Junso Fujita,^a Yuma Miyazaki,^a
Mika Hirose,^b Chioko Nagao,^c
Eiichi Mizohata,^a Yoshimi
Matsumoto,^d Kenji Mizuguchi,^c
Tsuyoshi Inoue^a and Hiroyoshi
Matsumura^{a*}

^aDepartment of Applied Chemistry, Graduate
School of Engineering, Osaka University,
2-1 Yamadaoka, Suita, Osaka 565-0871, Japan,

^bDepartment of Chemistry, Graduate
School of Science, Osaka University,
1-1 Machikaneyama-cho, Toyonaka,

Osaka 560-0043, Japan, ^cNational Institute of
Biomedical Innovation, 7-6-8 Saito-Asagi,
Ibaraki, Osaka 567-0085, Japan, and

^dLaboratory of Microbiology and Infectious
Diseases, Institute of Scientific and Industrial
Research, Osaka University, 8-1 Mihogaoka,
Ibaraki, Osaka 567-0047, Japan

Correspondence e-mail:
matsumura@chem.eng.osaka-u.ac.jp

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Expression, purification, crystallization and preliminary crystallographic study of FtsA from methicillin-resistant *Staphylococcus aureus*

FtsA from methicillin-resistant *Staphylococcus aureus* (MRSA) was cloned, overexpressed and purified. The protein was crystallized using the sitting-drop vapour-diffusion technique. A cocrystal with β - γ -imidoadenosine 5'-phosphate (AMPPNP; a nonhydrolysable ATP analogue) was grown using PEG 3350 as a precipitant at 293 K. X-ray diffraction data were collected to a resolution of 2.3 Å at 100 K. The crystal belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 75.31$, $b = 102.78$, $c = 105.90$ Å, $\beta = 96.54^\circ$. The calculated Matthews coefficient suggested that the asymmetric unit contained three or four monomers.

1. Introduction

FtsA is a highly conserved bacterial cell-division protein that belongs to the actin/HSP70/sugar kinase superfamily (Bork *et al.*, 1992; Sánchez *et al.*, 1994). In the initial stage of the division process, FtsA acts as a membrane anchor for the FtsZ polymer (Z-ring) at the nascent division site (Pichoff & Lutkenhaus, 2002). FtsA, FtsZ and the other cell-division proteins (*e.g.* ZipA, FtsK and FtsN) form a multi-protein complex known as the 'divisome', which allows the physical separation of daughter cells (Aarsman *et al.*, 2005). The recruitment of the cell-division proteins is also dependent on localization of FtsA and FtsZ (de Boer, 2010), suggesting that FtsA plays a critical role in cytokinesis. FtsA is also an attractive target for the development of an antibacterial agent because it is essential for cytokinesis in many bacterial species.

Previous functional studies have indicated that FtsA may be functionally distinct from actin, the ATP-hydrolysis activity of which is correlated with its polymerization (Oda *et al.*, 2009; Murakami *et al.*, 2010). It is unclear whether FtsA can hydrolyse ATP and whether such ATP hydrolysis is relevant *in vivo*. ATPase activity of FtsA has been detected *in vitro* for *Bacillus subtilis* (Feucht *et al.*, 2001) and *Pseudomonas aeruginosa* (Paradis-Bleau *et al.*, 2005), whereas no ATPase activity has been detected for *Streptococcus pneumoniae* (Lara *et al.*, 2005), *Escherichia coli* (Martos *et al.*, 2012) or *Thermotoga maritima* (Szwedziak *et al.*, 2012). Furthermore, the manner of FtsA polymerization *in vivo* is still unclear. An electron cryotomography study indicated that the FtsAs from *T. maritima* (TmFtsA), *E. coli* and *B. subtilis* assemble into long straight polymers on lipid monolayers (Szwedziak *et al.*, 2012), whereas *S. pneumoniae* FtsA polymerizes to form helical filaments (Lara *et al.*, 2005). The biological relevance of the polymerization to the function of FtsA remains uncertain.

High-resolution structural data are only available for TmFtsA (van den Ent & Löwe, 2000; Szwedziak *et al.*, 2012). These data indicate that TmFtsA shares three homologous domains (1A, 2A and 2B) with actin, but lacks one domain (1B). Instead, TmFtsA conserves the swapped domain (1C), which may mediate the recruitment of downstream divisome components. A nucleotide-binding cleft is formed by domains 1A, 2A and 2B, similar to actin. The TmFtsA crystals contain pairs of antiparallel straight protofilaments related by a crystallographic twofold screw axis (all TmFtsA crystals belonged to space group $P2_1$). The protofilaments are composed of transla-

tionally aligned monomers, and intermonomer interactions are mediated by the 1A–1C and 2A–2B domains. The longitudinal axes of the protofilaments are parallel to the crystallographic *a* or *c* axes. Whether the crystal structure of TmFtsA represents the *in vivo* state correctly is unknown, owing to the limited availability of FtsA structures.

Here, we cloned, overexpressed, purified and crystallized FtsA from methicillin-resistant *Staphylococcus aureus* (SaFtsA) and performed a preliminary crystallographic study. Future structure determination of SaFtsA is expected to provide a further structural basis for FtsA function and a useful tool for the development of new antimicrobial agents.

2. Materials and methods

2.1. Cloning and expression

The *ftsA* gene was amplified from the genomic DNA of *S. aureus* (ATCC accession No. 43300) using primers *ftsA*-for (5'-GCCATA-TGGAAGAACATTACTACGTAAG-3') and *ftsA*-rev (5'-GCGGA-TCCATTCATCAAATAGAGATTTTCATTAG-3'), and was subcloned into the vector pCR4-TOPO (Invitrogen) with a TOPO TA cloning kit (Invitrogen). The sequence was verified by sequencing. Recloning into the vector pCold I (TaKaRa) was performed *via* the restriction sites *Nde*I and *Bam*HI, resulting in the addition of an N-terminal hexahistidine tag and a factor Xa protease cleavage site with the sequence MNHKVHHHHHHIEGRH. The resulting vector was transformed into *E. coli* DH5 α and successful plasmids were selected by colony PCR and extracted using a QIAprep Spin Miniprep Kit (Qiagen). pCold I (TaKaRa) harbouring the *ftsA* gene was transformed into *E. coli* BL21 (DE3) cells and the cells were cultured in 10 l Luria Broth medium supplemented with 100 μ g ml⁻¹ ampicillin at 310 K. Expression was induced at an OD₆₀₀ of ~0.5 with cooling to 288 K and the addition of isopropyl β -D-1-thiogalactopyranoside to a final concentration of 0.5 mM. Cells were harvested by centrifugation for 20 min at 12 000g and 277 K after 24 h of expression and were stored at 193 K.

Figure 1
12.5% SDS-PAGE used to confirm the purity of FtsA from methicillin-resistant *S. aureus* (54.7 kDa). The left lane contains molecular-mass markers (labelled in kDa).

2.2. Protein purification

FtsA cell pellets were resuspended in lysis buffer (50 mM Tris–HCl pH 7.5, 300 mM NaCl, 20 mM imidazole) with one tablet of protease-inhibitor cocktail (EDTA-free, Roche) per 50 ml of buffer, lysed using an EmulsiFlex-C3 homogenizer (Avestin Inc., Canada) and centrifuged for 30 min at 100 000g and 277 K. The supernatant was filtered using a 0.45 μ m syringe filter (Sartorius) and applied onto a 5 ml HisTrap HP column (GE Healthcare). FtsA with an N-terminal histidine tag was eluted using a 45–310 mM imidazole gradient. Fractions containing FtsA were diluted ten times with a buffer consisting of 50 mM Tris–HCl pH 7.5 and loaded onto a 5 ml HiTrap Q HP column (GE Healthcare). FtsA was eluted with a 30–750 mM NaCl gradient. The FtsA fractions were further purified using a HiLoad 16/60 Superdex 200 prep-grade column (GE Healthcare) equilibrated with gel-filtration buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl). The histidine tag was not cleaved. The purity of the product was confirmed by SDS-PAGE (Fig. 1) and matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry. The purified protein was concentrated to 10 mg ml⁻¹, flash-frozen in liquid nitrogen and immediately stored at 193 K.

2.3. Crystallization

The initial crystallization assays were performed in a 96-well plate incubated at 293 K by the sitting-drop vapour-diffusion technique using the following screening kits: Crystal Screen, Crystal Screen 2, Crystal Screen Lite, Crystal Screen Cryo, SaltRx, MembFac, PEG/Ion, PEG/Ion 2, PEGRx 1, PEGRx 2 (Hampton Research), Wizard I

(a)

(b)

Figure 2
(a) Needle-like crystals of SaFtsA obtained in the initial crystallization screening. (b) Diffraction-quality crystal of SaFtsA obtained after optimizing the crystallization conditions.

and II (Emerald BioSystems), MemStart and MemSys (Molecular Dimensions). Crystallization drops consisting of 0.5 μl 10 mg ml^{-1} protein solution and 0.5 μl reservoir solution were equilibrated against 60 μl reservoir solution. A needle-like crystal was obtained by mixing protein solution consisting of 10 mg ml^{-1} FtsA, 1 mM AMPPNP (β - γ -imidoadenosine 5'-phosphate), 2 mM MgCl_2 with PEG/Ion 2 condition No. 46: 0.2 M NaBr, 20% (w/v) PEG 3350 (Fig. 2a). This condition was further optimized using the sitting-drop vapour-diffusion technique. A diffraction-quality FtsA crystal was obtained after two weeks by mixing 1 μl of the same protein solution with 1 μl of a precipitant solution consisting of 0.15 M NaBr, 14.3% (w/v) PEG 3350, 0.075 M Tris pH 7.8 and equilibrating against 1 ml of a reservoir solution consisting of 0.2 M NaBr, 19% (w/v) PEG 3350, 0.1 M Tris pH 7.8 (Fig. 2b).

2.4. Data collection and processing

The crystal was cryoprotected by changing the concentration of PEG 3350 to 37% (w/v) and adding 15% (w/v) PEG 8000. X-ray diffraction data were collected at 100 K using an ADSC Quantum 270 detector on beamline BL-1A of the Photon Factory (PF) at the High Energy Acceleration Research Organization, Tsukuba, Japan (Fig. 3). The raw data were indexed, integrated and scaled using the *HKL-2000* program package (Otwinowski & Minor, 1997). Further data analysis was performed using the *CCP4* program suite (Winn *et al.*, 2011).

3. Results and discussion

SaFtsA was expressed in *E. coli* BL21 (DE3) cells with a hexahistidine tag and a factor Xa protease cleavage site and was purified by Ni-affinity, anion-exchange and size-exclusion chromatography. The recombinant protein was >95% pure as judged by SDS-PAGE (Fig. 1)

Table 1

Crystal parameters and X-ray diffraction data-collection statistics.

Values in parentheses are for the outermost resolution shell.

X-ray source	BL-1A, PF
Wavelength ($\text{\AA}$)	1.0000
Space group	$P2_1$
Unit-cell parameters ($\text{\AA}$, $^\circ$)	$a = 75.31$, $b = 102.78$, $c = 105.90$, $\beta = 96.54$
Resolution range ($\text{\AA}$)	46.83–2.30 (2.34–2.30)
No. of molecules per asymmetric unit	3–4
V_M ($\text{\AA}^3 \text{Da}^{-1}$)	2.48–1.86
V_{solv} (%)	50.46–33.95
No. of observed reflections	256293
No. of unique reflections	70121 (3498)
$R_{\text{merge}}^\dagger$ (%)	6.8 (30.3)
Completeness (%)	98.7 (98.8)
$\langle I/\sigma(I) \rangle$	19.76 (2.59)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the value of the i th measurement of the intensity of reflection hkl and $\langle I(hkl) \rangle$ is the mean value of the intensity of that reflection.

and the identity of the protein was confirmed by MALDI-TOF mass spectrometry. The yield of purified FtsA was 2.8 mg per litre of culture. A diffraction-quality AMPPNP cocrystal was obtained within two weeks using precipitant solution consisting of 0.15 M NaBr, 14.3% (w/v) PEG 3350, 0.075 M Tris pH 7.8 equilibrated against reservoir solution consisting of 0.2 M NaBr, 19% (w/v) PEG 3350, 0.1 M Tris pH 7.8 (Fig. 2b). Diffraction data were collected from one SaFtsA crystal to 2.3 $\text{\AA}$ resolution (Fig. 3 and Table 1). The crystal belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 75.31$, $b = 102.78$, $c = 105.90$ $\text{\AA}$, $\beta = 96.54^\circ$. The Matthews coefficient (Matthews, 1968) suggested the presence of either three monomers per asymmetric unit with a solvent content of 50.46% ($2.48 \text{ \AA}^3 \text{Da}^{-1}$) or four monomers per asymmetric unit with a solvent content of 33.95% ($1.86 \text{ \AA}^3 \text{Da}^{-1}$). Although *T. maritima* FtsA (TmFtsA) monomers are oriented similarly (or translationally

Figure 3
X-ray diffraction image from the cocrystal of FtsA and AMPPNP. The data were collected to a resolution of 2.3 $\text{\AA}$.

aligned) in the crystals (PDB entry 1e4g; van den Ent & Löwe, 2000), the native Patterson map for SaFtsA calculated using data in several resolution ranges (*e.g.* 20–4, 10–3 and 8–4.5 Å) did not have any significant peaks. Furthermore, the self-rotation function for SaFtsA calculated using data in these resolution ranges indicated no obvious noncrystallographic symmetry elements. Attempts to determine the structure by molecular replacement with the available atomic coordinates of TmFtsA (PDB entries 1e4f, 1e4g, 4a2a and 4a2b; van den Ent & Löwe, 2000; Szwedziak *et al.*, 2012) failed, probably because of the low sequence identity of 17.7% to TmFtsA. We have grown crystals of selenomethionine-substituted FtsA and phase determination by the single-wavelength anomalous dispersion method is in progress.

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