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Crystal structure of FtsA from *Staphylococcus aureus*

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ABSTRACT

The bacterial cell-division protein FtsA anchors FtsZ to the cytoplasmic membrane. But how FtsA and FtsZ interact during membrane division remains obscure. We have solved 2.2 Å resolution crystal structure for FtsA from *Staphylococcus aureus*. In the crystals, SaFtsA molecules within the dimer units are twisted, in contrast to the straight filament of FtsA from *Thermotoga maritima*, and the half of S12–S13 hairpin regions are disordered. We confirmed that SaFtsZ and SaFtsA associate in vitro, and found that SaFtsZ GTPase activity is enhanced by interaction with SaFtsA.

Structured summary of protein interactions:
SaFtsA and **SaFtsZ** bind by comigration in non denaturing gel electrophoresis (View interaction)
SaFtsZ and **SaFtsA** bind by molecular sieving (View interaction)
SaFtsA and **SaFtsA** bind by x-ray crystallography (View interaction)

1. Introduction

During bacterial cell division, more than 20 proteins constitute a multi-protein complex called the divisome at the midcell [1]. FtsZ, a divisome protein, assembles to form the ring-like structure, the Z-ring, that allows the daughter cells to physically separate [2]. Polymerized FtsZ is tethered to the cytoplasmic membrane by FtsA through its C-terminal membrane-targeting sequence (MTS) [3]. Optical microscopic studies have indicated that yellow fluorescent protein (YFP) and MTS-fused FtsZ (FtsZ–YFP–MTS) dents a liposome membrane, but does not cause the liposome to divide [4], whereas the combination of FtsZ–YFP and FtsA* (an FtsA gain-of-function mutant [5]) causes the formation of a continuous septum in a GTP/ATP-dependent manner [6]. Therefore, FtsA appears to tether FtsZ to the membrane and may be necessary for downstream cell division. Both FtsZ and FtsA are essential for cell division and viability and they are highly conserved in many bacterial species, making them attractive targets for the development of antibacterial agents.

FtsZ and FtsA tend to form filamentous structures [7–9]. FtsZ forms straight protofilaments in the presence of GTP, thereby forming a GTPase active site between two FtsZ molecules. Hydrolysis of GTP to GDP leads straight-to-curved conformational change of FtsZ protofilaments [9]. Crystal structures of a mesophilic and a thermophilic FtsZ have been solved [10], and the recent crystallographic study of *Staphylococcus aureus* FtsZ (SaFtsZ) has proposed the mechanism how the straight-to-curved conformational change of FtsZ polymers is coupled to its GTPase activity [11,12].

FtsA belongs to the actin/MreB family of proteins, and actin/MreB possess ATPase activity coupled to their polymerization [13,14]. Although FtsA forms protofilaments similar to those of actin and MreB [7,8], the role of its ATPase activity remains to be clarified. ATPase activity has been detected in vitro for *Bacillus subtilis* [15] and *Pseudomonas aeruginosa* FtsAs [16], but it has not been found for FtsA from *Streptococcus pneumoniae* [7], *Escherichia coli* [17] and *Thermotoga maritima* [8]. Recent microscopic study shows that FtsZ and FtsA self-organize into a rapidly reorganizing filament network [18], but understanding of the molecular function of FtsA during cell division has been hampered by the limited structural information available for a mesophilic FtsA; the only crystal structure available is that of the FtsA from the thermophile *T. maritima* (TmFtsA) [8,19]. Therefore, a three-dimensional structure for a mesophilic FtsA is needed, but, until this report,

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difficulties had been encountered when preparation of a mesophilic FtsA was attempted, e.g., refolding of *P. aeruginosa* and *E. coli* FtsA from inclusion bodies [16,17] and the inability to crystallize the folded product.

We previously reported that full-length FtsA from mesophilic methicillin-resistant *S. aureus* (SaFtsA) could be purified in a tractable form and crystallized [20]. Herein, we report the crystal structure of SaFtsA-β-γ-imidoadenosine 5′-phosphate (AMPPNP; a non-hydrolyzable ATP analog) complex. In the crystal, the SaFtsA molecules stack head to tail forming a continuous filament, as does crystalline TmFtsA and TmMreB. However, SaFtsA dimer in the filament is twisted, and the torsion angles of the neighboring dimers within the asymmetric unit are different. In addition, we show that SaFtsZ and SaFtsA form a complex in vitro and that SaFtsZ GTPase activity is promoted by its interaction with SaFtsA, which sheds light on the coordinated regulation of FtsA and FtsZ during cell division.

2. Materials and methods

2.1. Protein expression and purification

SaFtsA (UniProt ID: Q6GHQ0) was expressed and purified as described [20]. SaFtsZ (UniProt ID: Q6GHP9) was cloned, expressed, and purified as reported for SaFtsA except that the cloning primers were: *ftsZ*-for (5′-GCCATATGTTAGAATTTGAACAAGG ATTTAATC-3′) and *ftsZ*-rev (5′-GCGGATCCTTAACGTCTTGTTCTTCT TGAACG-3′). Selenomethionine-labeled SaFtsA (SeMet-SaFtsA) was expressed and purified with the same protocols as wild-type (WT) SaFtsA, except that the expression system was *E. coli* B834 (DE3) (Invitrogen) and that the cells were cultured in LeMaster medium, 50 mg/L SeMet (QIAGEN), 100 μg/ml ampicillin (Wako), 5 ml/L vitamin solution as reported [21,22]. As assessed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry, six of the possible seven methionines are present in SeMet-SaFtsA (SaFtsA: observed, 54679 Da; calculated, 54703 Da; SeMet-SaFtsA: observed, 54962 Da; full substitution: calculated, 55031 Da). Site-directed mutagenesis of SaFtsZ (D210A) was performed by inverse-PCR using KOD-plus (TOYOBO) and the primer 5′-GAAGTAAACTTAGcaTTTGCAGACGTTAAGAC-3′. (The changed nucleotides are shown in small letters, and the codons corresponding to the amino acid residues to be changed are underlined). SaFtsZ D210A was expressed and purified with the same protocols as WT SaFtsZ. All the proteins have N-terminal extra residues of MNHKVHHHHHHIEGRH.

2.2. Crystallization, data collection and structure determination

SaFtsA was crystallized in the presence of AMPPNP as reported [20]. SaFtsA was also crystallized in the presence of ADP by mixing an equal volume (1 μl) of protein solution (10 mg/ml) containing 1 mM ADP and 2 mM $MgCl_2$ with 0.2 M lithium nitrate, 20% (w/v) PEG3350, 0.1 M sodium cacodylate, pH 7.0 (reservoir solution) and equilibrating against 1 ml of the reservoir solution. The crystallization conditions for SeMet-SaFtsA were the same as those for crystallization of SaFtsA in the presence of AMPPNP. Crystals were individually mounted in a loop and then flash frozen in a stream of nitrogen at 100 K. Synchrotron-radiation diffraction data were collected at 100 K at the SPring-8 BL44XU and Photon Factory BL-1A beamlines (Japan). The diffraction datasets were processed using HKL2000 [23]. Data-collection statistics are summarized in Table 1.

The structure of SaFtsA was determined by the single-wavelength anomalous diffraction method using SeMet-labeled-SaFtsA. Of the 24 possible selenium atoms in an asymmetric unit, 17 were found by SHELXD [24] using the anomalous signals in the SeMet-SaFtsA peak datasets. Initial phases were calculated and refined using SHELXE [24] and the graphical interface HKL2MAP [25]. Model refinement used CNS [26] and PHENIX [27], with manual inspection and modification in conjunction with the CCP4 program COOT [28]. Phasing and refinement statistics are given in Table 1. PROCHECK [29] indicated that >85% of residue ϕ–φ values for each of structures are in the most favored regions of the corresponding Ramachandran plots. Chain interactions were assessed at the PISA server [30], and interface interactions were identified by LIGPLOT [31]. Figures were prepared using Pymol (www.pymol.org) and ESPript [32]. The final atomic coordinates and structure factor amplitudes (PDB codes 3WQT for with AMPPNP and 3WQU for with ATP) have been deposited in the Worldwide Protein Data Bank (wwPDB; http://www.wwpdb.org), and the Protein Data Bank Japan at the Institute for Protein Research in Osaka University (PDBj; http://www.pdbj.org/).

Table 1
Data collection and refinement statistics.

Data set	SaFtsA (AMPPNP)	SaFtsA (ATP)	SaFtsA (SeMet)
PDB code	3WQT	3WQU	
X-ray source	Photon factory BL1A	SPring-8 BL44XU	SPring-8 BL44XU
Space group	$P2_1$	$P2_1$	$P2_1$
Unit-cell parameters			
a (Å)	75.26	82.53	74.54
b (Å)	102.74	122.02	101.87
c (Å)	105.86	107.00	105.37
β (°)	96.54	95.66	96.23
Wavelength (Å)	1.00	0.900	0.979
Resolution (Å)	38.0–2.2 (2.24–2.20)[a]	39.7–2.8 (2.85–2.80)[a]	50.0–2.7 (2.75–2.70)[a]
Total reflections	234088	196794	334143
Unique reflections	78940	47837	84991
Completeness (%)	97.4 (97.4)	92.3 (86.5)	100.0 (100.0)
R-merge (%)[b]	6.7 (39.6)	7.5 (59.3)	10.0 (58.5)
I/σ	18.6 (2.1)	18.7 (2.2)	14.7 (1.6)
Phasing			
No. of sites			17
FOM[c]			0.32
Refinement			
Resolution	38.0–2.2	39.7–2.8	
R_{work}/R_{free}	0.234/0.289	0.207/0.254	
No. of atoms			
Protein	11051	11487	
Ligand	139	128	
Water	428	48	
R.m.s. deviation[d]			
Bond length (Å)	0.007	0.009	
Bond angles (°)	1.2	1.3	

[a] Values in parentheses are for the highest resolution shells.
[b] *R*-merge$(I) = \sum | I(k) - < I > | / \sum I(k)$, where $I(k)$ is the value of the k th measurement of the intensity of a reflection,<*I*> is the mean value of the intensity of that reflection and the summation is over all measurement.
[c] FOM – figure of merit = $|F(hkl)\text{best}|/|F(hkl)|$; $\boldsymbol{F}(hkl)\text{best} = \Sigma_\alpha P(\alpha)\boldsymbol{F}_{hkl}(\alpha)/\Sigma_\alpha P(\alpha)$.
[d] R.m.s.deviation – root-mean-square deviation.

2.3. GTPase/ATPase activity assay

GTPase or ATPase activity was measured by quantification of free inorganic phosphate using Malachite Green Phosphate Assay kit reagents (BioAssay Systems), according to manufacturer's instruction. The reaction mixture (final volume 200 μl) contained 50 mM MOPS, pH 7.2, 5 mM $MgCl_2$, 200 mM KCl, 0–1500 μM GTP or ATP and was incubated at 37 °C for 15 min. The reaction mixture (32 μl) was diluted 25-fold with 50 mM MOPS, pH 7.2. The prepared working reagent (200 μl) was added, and the mixture was incubated at 20 °C for 30 min. The absorbance at 620 nm was