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STRUCTURE NOTE

The first crystal structure of an archaeal metallo-β-lactamase superfamily protein; ST1585 from *Sulfolobus tokodaii*

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INTRODUCTION

The metallo-β-lactamase superfamily is a large protein superfamily composed of about 20 families, such as glyoxalase II, cyclase, arylsulfatase, alkylsulfatase, flavoprotein, and ribonuclease.[1] They all use the metallo-β-lactamase fold (a four-layered β-sandwich fold with two mixed β-sheets flanked by α-helices) as a catalytic unit with one or two Zn or Fe ions bound at the active site.[2] The substrate specificity of each family is achieved by the structures flanking the active site, which are not included in the common metallo-β-lactamase fold.[2] For example, ribonucleases of the metallo-β-lactamase superfamily have an additional domain which helps to form a positively charged cleft in the vicinity of the active site, which is suitable for binding of polynucleotides with negatively charged phosphate backbones.[3] The metallo-β-lactamase superfamily proteins are wide-spread over all three domains of life. However, in archaea, few structural and functional analyses of metallo-β-lactamase superfamily proteins are reported.

In this study, we determined the crystal structure of ST1585, a hypothetical protein that belongs to the metallo-β-lactamase superfamily, from the hyperthermophilic archaeon *Sulfolobus tokodaii*. To our best knowledge, this is the first report of the structure of the archaeal metallo-β-lactamase superfamily protein. It had the common metallo-β-lactamase fold and an additional α-helix. The additional α-helix made a narrow and deep tunnel structure around the active site, which was similar to that of PqsE, a protein from *Pseudomonas aeruginosa* involved in quorum sensing using quinolone as a signaling factor.[4,5] The structural similarity between the two proteins raises the possibility that ST1585 is involved in a quorum sensing using quinolone. There was no report of a quorum sensing system in most archaea except for a few species including *Natronococcus*. The wide distribution of ST1585 homologs in archaea suggests the presence of a yet uncharacterized quorum sensing system in the archaea.

METHODS

Cloning, expression, and purification

The open reading frame of *S. tokodaii* ST1585 was cloned into the pET-21a expression vector (*Nde* I-*Bam* HI sites) (Novagen, Madison, WI). A selenomethionine-substituted protein was produced in the *Escherichia coli* BL21-CodonPlus (DE3)-RIL-X strain (Agilent, Santa Clara, CA). The transformed *E. coli* was cultured in

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L-broth containing 0.13 m*M* selenomethionine. The cells overexpressing ST1585 were harvested by centrifugation and resuspended in buffer comprised of 20 m*M* Tris-HCl (pH 8.0), 500 m*M* NaCl, 5 m*M* 2-mercaptoethanol, and 1 m*M* phenylmethylsulfonyl fluoride. The resuspended cells were disrupted by sonication, and the cell lysate was heat-treated at 70°C for 13 min. The soluble fraction was recovered by centrifugation at 34,000*g* at 4°C for 30 min. The fraction was applied to a HiPrep 26/10 column (GE Healthcare Biosciences, Uppsala, Sweden) for desalting. The desalted sample was applied to a TOYOPEARL SuperQ-650M column (TOSOH, Tokyo, Japan) equilibrated with 20 m*M* Tris-HCl (pH 8.0). The fractions containing ST1585 were eluted using a linear gradient of 0–0.3*M* NaCl. The fractions were dialyzed two times against 5 L of 20 m*M* Tris-HCl (pH 8.0). The dialyzed solution was loaded onto a Resource Q column (GE Healthcare Biosciences) equilibrated with 20 m*M* Tris-HCl (pH 8.0). The fractions containing target protein were eluted with a linear gradient of 0–0.3*M* NaCl, and then the fractions were diluted to a threefold volume with 10 m*M* potassium phosphate (pH 7.0). The diluted sample was applied to a Bio-Scale CHT5-I column (BIO-RAD, Hercules, CA) equilibrated with 10 m*M* potassium phosphate (pH 7.0). The fractions containing target protein were eluted using a linear gradient of 0.01–0.5*M* potassium phosphate (pH 7.0). The fractions were loaded onto a HiLoad 16/60 Superdex 200 column (GE Healthcare Bioscience) equilibrated with 20 m*M* Tris-HCl (pH 8.0) and 200 m*M* NaCl. The purified protein was concentrated to 17.3 mg/mL using a VIVASPIN (10 kDa molecular weight cutoff, Vivascience, Hannover, Germany).

Crystallization, data collection, and structure determination

Crystallization of ST1585 was performed at 20°C by the hanging drop vapor diffusion method. One microliter of the protein solution was mixed with an equal volume of a reservoir solution comprising 0.1*M* HEPES (pH 7.2), 10% PEG 8000, and 8% ethylene glycol. The crystal in the mother liquor was cryocooled in a nitrogen-gas steam. X-ray diffraction data were obtained at the RIKEN Structural Genomics Beamline I (BL26B1) at SPring-8 (Hyogo, Japan). The diffraction data were obtained to a 1.8-Å resolution at three wavelengths determined from an X-ray fluorescence spectrum for the selenium multiple-wavelength anomalous dispersion (MAD) method. The collected data was processed with the HKL2000 program suite.[6] The positions of the selenium atoms in the asymmetric unit of the crystal were determined with the program SOLVE,[7] and then density modification was performed with program RESOLVE.[8] The automatic tracing procedure in program ARP/wARP[9] was utilized to build the initial model. Model refinement, initial picking, and manual verification of the water molecules were carried out using the programs CNS and XtalView/Xfit.[10,11] The stereochemistry of the structure was checked using the program MOLPROBITY.[12] In the Ramachandran plot, no residue was found in the disallowed regions. The atomic coordinates are deposited in the Protein Data Bank with the PDB ID 3ADR.

RESULTS AND DISCUSSION

The *S. tokodaii* ST1585 protein is comprised of 261 amino acid residues with a molecular mass of 34 kDa, and belongs to the metallo-β-lactamase superfamily. The highly conserved amino acid residues, which coordinate metal ions, in metallo-β-lactamase superfamily proteins are also detected in the ST1585 amino acid sequence [Fig. 1(A)]. The crystal structure of ST1585 was determined at 1.8-Å using multiple wavelength anomalous dispersion. Data collection, phasing, and refinement statistics are summarized in Table I. The overall structure of ST1585 contains thirteen β-strands and nine α-helices [Fig. 1(B)]. The asymmetric unit of the crystal contains two monomers of ST1585, and one *cis* peptide bond is observed between Pro181 and Val182. The overall structure of ST1585 is made up of an αββα structure, which is a common fold observed in the structure of metallo-β-lactamase superfamily proteins [Fig. 1(B)]. The two-metal ion-binding center is located at the external edge of the ββ sandwich, and two zinc ions are coordinated with conserved amino acid residues located in segments 2 to 5 [Fig. 1(A,D)].

A structural similarity search using the DALI server[15] revealed that the tertiary structure of ST1585 was very similar to that of PqsE (PDB ID, 2Q0J; Z-score 22.3) from *P. aeruginosa*, CphA (PDB ID, 3FAI; Z-score 19.0) from *Aeromonas hydrophila*, and TTHA1623 (PDB ID, 2ZWR; Z-score 18.9) from *Thermus thermophilus*. Although there was no significant difference in the *Z*-scores of the three structures, a big difference was observed in the structures around the active sites. ST1585 and PqsE had a narrow tunnel-like structure around the active site [Fig. 1(B,C)], whereas the others had the shallow and wide active site cavities opened to the solvent [Fig. 1(G)]. In comparison to other members of the metallo-β-lactamase superfamily, ST1585 and PqsE possess additional α-helices at their C-terminus. Both α-helices cover the active sites to make up the tunnel structure [colored red in Fig. 1(B,C)], and therefore metal ions in active sites of ST1585 and PqsE were buried in the narrow tunnel structure [Fig. 1(E,F)]. Such architecture seems to be unique to ST1585 and PqsE.

The active sites of ST1585 and PqsE are almost identical to each other, although two ferric ions are coordinated in PqsE structure instead of two zinc ions in ST1585 [Fig. 1(B,C)]. At the active site of PqsE, there was a copurified ligand, which was assigned to be a benzoate from the shape

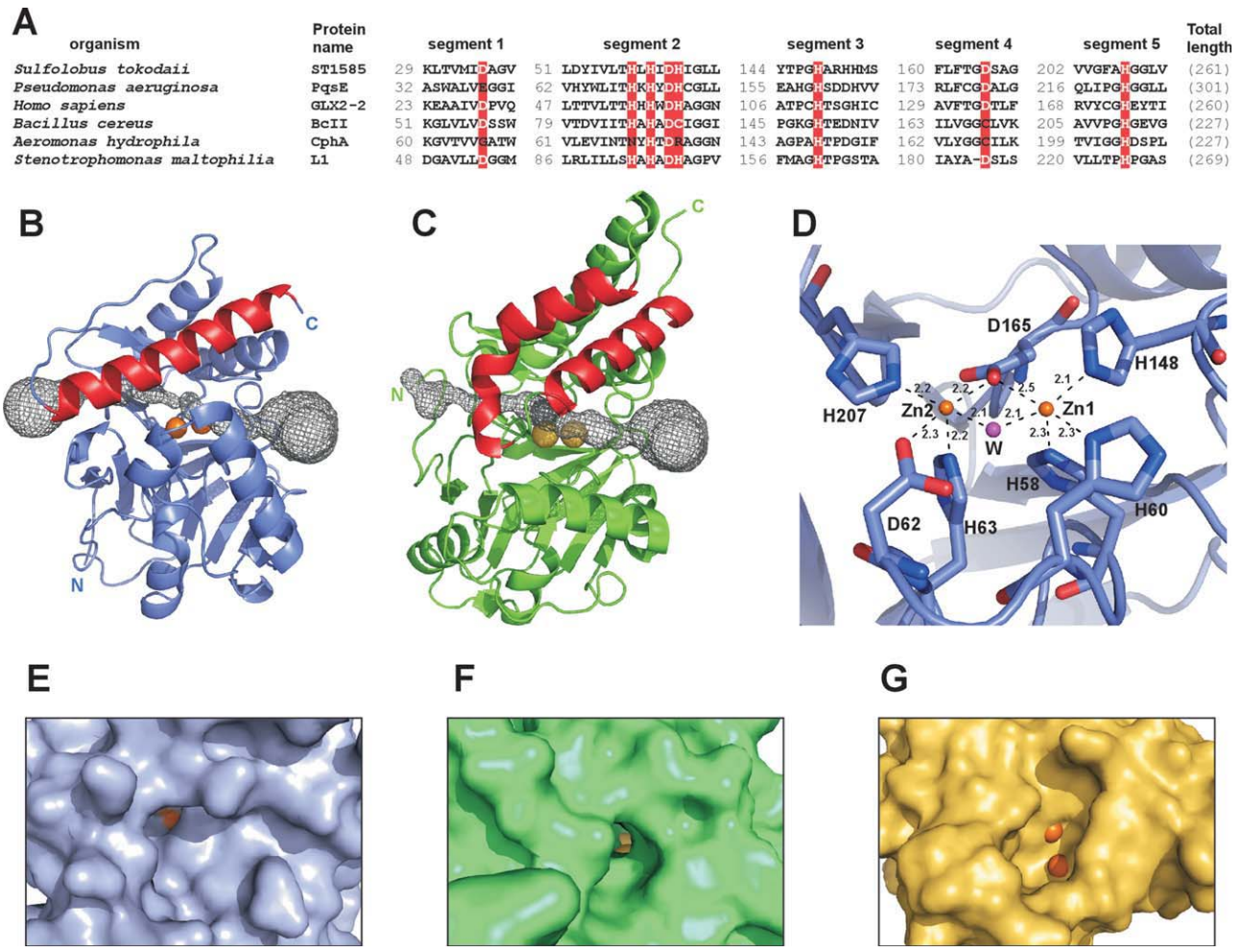

Figure 1

(**A**) The conserved amino acid residues coordinating the metal ions in metallo-β-lactamase superfamily proteins. PqsE, GLX2-2, BcII, CphA and L1 are metallo-β-lactamase superfamily proteins that have been structurally determined (PDB ID; 2Q0J, 1QH5, 2BC2, 3FAI, and 1SML respectively). Only the conserved segments are shown. The numbers on the left of the segments indicate the distances from the protein N-termini. The amino acid residues coordinating the metal ions are indicated by white letters on red background. (**B, C**) Overall structures of ST1585 (B) and PqsE[4] (C). The N- and C-termini are labeled N and C, respectively. Two tunnels approaching the putative active site are shown in gray mesh. In ST1585, two zinc ions in the putative active site are colored orange, and in PqsE, two iron ions are colored yellow. The unique α-helices observed in ST1585 and PqsE are colored red. (**D**) The zinc-binding site in ST1585. The water molecule is shown in a magenta sphere. (**E, F, G**) Surface diagrams of ST1585 (E), PqsE (F), and CphA[20] (G). Metal ions are colored as in Figure 1 (B, C). These figures were prepared using PyMOL (http://pymol.sourceforge.net/) and with CAVER.[21]

of the electron density.[4] Similarly, we observed some extra electron density in the active site of ST1585, but could not assign a particular ligand. Thus, the structures of ST1585 and PqsE are quite similar, despite the fact that they have only 10% sequence identity. Although the natural substrate of PqsE has yet to be identified, it was reported that PqsE was involved in *Pseudomonas* quinolone signal system,[4,5] which is one of the multiple systems of quorum sensing. Based on the similarity between ST1585 and PqsE, ST1585 is hypothesized to be involved in quorum sensing in *S. tokodaii*. In bacteria, quorum sensing regulates the synthesis of virulent factors and the generation of biofilm.[16–18] In archaea, quorum sensing was reported in only a limited number of species of *Natrialba*[19] and *Natronococcus*.[20,21] The wide distribution of ST1585 homologs in many archaea suggests the presence of a novel and universal quorum sensing system using quinolone in archaea.

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Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession number BA000023. The three-dimensional structures reported in this article are available in the Protein Data Bank under the PDB ID 3ADR.

Table I
X-ray Data Collection and Refinement Statistics

	Se-edge	Se-peak	Se-remote
Data collection			
Wavelength (Å)	0.9794	0.9791	0.9000
Resolution (Å)		50.00–1.80 (1.86–1.80)	
No. of observed reflection	399,949	403,722	390,816
No. of unique reflection	56,067	56,293	55,592
Completeness (%)	98.3 (84.7)	98.6 (87.5)	97.4 (79.5)
Redundancy	7.1 (4.6)	7.2 (4.8)	7.0 (4.2)
I/σ(I)	28.2 (3.3)	28.9 (4.0)	27.8 (3.1)
R_{merge}[a] (%)	0.097 (0.289)	0.101 (0.272)	0.098 (0.290)
Space group		C2	
Unit cell parameters (Å, °)		a = 163.97, b = 45.23, c = 110.89, α = γ = 90, β = 131.64	
Refinement			
Resolution (Å)		20.0–1.8	
R_{factor}[b] (%)/R_{free}[c] (%)		19.9/21.6	
No. of protein atoms/water atoms		4048/303	
r.m.s.d bond length (Å)		0.005	
r.m.s.d bond angle (°)		1.3	
Wilson B factor (Å^2)		15.0	
Average overall B factor for protein (Å^2)		22.7	
Ramachandran plot (%)			
Most favored		96.97	
Allowed		3.03	
Disallowed		0.00	

Values in parentheses are for the highest-resolution shell.
[a] $R_{merge} = \sum_{hkl} [(\sum_I |I_i - <I>|)/\sum_i |I_i|]$.
[b] $R_{factor} = (\sum_{hkl} ||F_o| - |F_c||)/\sum_{hkl} |F_o|$.
[c] R_{free} is the R_{factor} calculated using 5% of the data that were excluded from the refinement.

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