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Purification, crystallization and preliminary X-ray diffraction analysis of the thiaminase type II from *Staphylococcus aureus*

Thiaminase type II (TenA) catalyzes the deamination of aminopyrimidines, including the cleavage of thiamine to 4-amino-5-hydroxymethyl-2-methylpyrimidine and 5-(2-hydroxyethyl)-4-methylthiazole in the metabolism of thiamine (vitamin B₁), in *Staphylococcus aureus* (*Sa*). *Sa*TenA was crystallized by the vapour-diffusion method and the resulting crystal diffracted to 2.6 Å resolution using synchrotron radiation. The crystal is orthorhombic, belonging to space group $P2_12_12_1$ with unit-cell parameters $a = 103.5$, $b = 104.1$, $c = 109.6$ Å. With four molecules in the asymmetric unit, the Matthews coefficient is $2.85 \text{ \AA}^3 \text{ Da}^{-1}$. Initial attempts to solve the structure by molecular-replacement techniques were successful.

1. Introduction

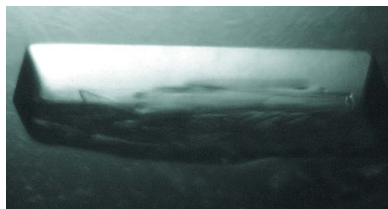
Staphylococcus aureus can cause a wide spectrum of human and animal diseases ranging from benign skin infections to severe diseases such as pneumonia, meningitis and osteomyelitis (Fey *et al.*, 2003; Lowy, 1998). This human pathogen has developed specific strategies for persistence in humans (Kauffman *et al.*, 1993; Powers *et al.*, 1990). Although not traditionally considered as an intracellular pathogen, the bacterium can survive in a variety of cells, hiding from the human immune system (Lowy, 1998). Furthermore, *S. aureus* has developed resistance against various antibiotics, which makes treatment of this bacterial infection difficult, especially in immune-deficient people (Tumbarello *et al.*, 1998; Weinke *et al.*, 1992).

Because of this, investigations to identify novel drug targets for the development of new chemotherapeutics against this pathogen are unceasing in the pharmaceutical industry. In this context, pathogen-specific vitamin syntheses represent an ideal drug target because the corresponding pathways are not present in human metabolism and thus potential cross-reaction with the host is avoided; also, their novelty as targets should minimize latent drug resistance.

Thiamine pyrophosphate (TPP), the active form of vitamin B₁, is present in all organisms as an essential cofactor of a variety of key enzymes (Pohl *et al.*, 2004). In contrast to plants, some protozoa and bacteria, humans and other mammals are unable to synthesize thiamine and are completely dependent on uptake of this essential nutrient from their diet. In humans, a deficiency of exogenous vitamin B₁ can result in Wernicke's disease and beriberi (Ogershok *et al.*, 2002; Platt & Lu, 1936).

Bacteria possess an enzyme known as TenA that has been shown to be a type II thiaminase. The biological function of TenA is involved in the salvage of degraded products of thiamine and promotes the production of degradative enzymes in *Bacillus subtilis* (Jurgenson *et al.*, 2009).

Crystal structures of TenA enzymes from *Helicobacter pylori* HP1287 (Barison *et al.*, 2009), *B. subtilis* (Toms *et al.*, 2005), *Sulfolobus solfataricus* (PDB code 2qzc; Joint Center for Structural Genomics, unpublished work) and *Staphylococcus epidermidis* (PDB code 3no6; Joint Center for Structural Genomics, unpublished work) have been determined and analyzed. Here, we report the crystallization and data collection to 2.6 Å resolution of TenA from *S. aureus* (*Sa*TenA). Its highest degree of sequence identity is 57.7% with TenA from *S. epidermidis*.

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2. Materials and methods

2.1. Protein expression and purification

The open reading frame of *SaTenA* was previously cloned into the *E. coli* expression plasmid pASK-IBA3 (Müller *et al.*, 2009), which encodes a C-terminal *Strep*-tag that allows one-step purification of the recombinant fusion protein using *Strep*-Tactin Sepharose. The construct was transformed into *Escherichia coli* BLR (DE3) expression cells (Stratagene, Germany). Briefly, single colonies were picked and grown overnight in Luria–Bertani medium. The bacterial culture was diluted 1:100 and grown at 310 K until the A_{600} reached 0.5. Expression was initiated with 200 ng ml^{-1} anhydrotetracycline and the cells were grown for 4 h at 310 K before being harvested. The cell pellet was resuspended in 100 mM Tris–HCl pH 8.0, 100 mM NaCl, sonicated and centrifuged at 75 000g for 1 h at 277 K. The recombinant *Strep*-Tag fusion protein was purified according to the manufacturer's recommendation (Institut für Bioanalytik, Germany). The eluted proteins from the affinity chromatography were analyzed by SDS–PAGE and the protein was visualized by Coomassie staining (Sambrook *et al.*, 1989).

Eluted samples were concentrated to 10 mg ml^{-1} in resuspension buffer. The isolated enzyme consists of 229 amino-acid residues and has a molecular weight of 26 kDa. The C-terminal *Strep*-Tag comprises ten additional residues (SAWSHPQFEK) and was not cleaved off.

2.2. Crystallization of *SaTenA*

The purified *SaTenA* was dialyzed against 100 mM Tris–HCl buffer pH 8.0; it was then concentrated to 10 mg ml^{-1} using an Amicon Ultra centrifugal filter device (Millipore; 3 kDa molecular-weight cutoff) and used for initial screening of crystallization conditions. Preliminary crystallization experiments were carried out at 293 K in 96-well crystallization plates (NeXtal QIA1 μ plates, Qiagen) using the following commercially available crystallization screening kits: JCSG+ Suite, ComPAS, Classics and Cryo (Qiagen). A total of 384 crystallization conditions were screened by the sitting-drop vapour-diffusion method using a Honeybee 961 robot (Genomic Solutions Ltd). Droplets of 600 nl in volume (300 nl protein solution plus 300 nl reservoir solution) were equilibrated against 35 μ l reservoir solution. Initial crystals appeared after 5 d using a solution consisting of 0.2 M sodium acetate, 0.1 M Tris–HCl pH 8.5 and 26% (w/v) polyethylene glycol (PEG) 4000. The condition that yielded crystals was further optimized in order to obtain crystals that were suitable for X-ray analysis. A drop containing 2 μ l protein solution was mixed with 2 μ l precipitant and equilibrated against 1 ml reservoir solution consisting of 0.2 M sodium acetate, 0.1 M Tris–HCl pH 8.5 and 28% (w/v) PEG 3350 using the hanging-drop technique in 24-well Linbro plates. Crystals grew to dimensions of $0.1 \times 0.1 \times 0.4 \text{ mm}$ after 7 d (Fig. 1).

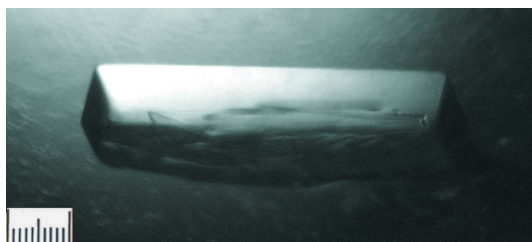


Figure 1
A crystal of *SaTenA* ($0.1 \times 0.1 \times 0.4 \text{ mm}$; the scale bar is 0.1 mm in length) obtained using 0.2 M sodium acetate, 0.1 M Tris–HCl pH 8.5 and 28% (w/v) PEG 3350.

2.3. Diffraction experiment

Diffraction data were collected to a resolution of 2.60 Å using synchrotron radiation of wavelength 0.8123 Å on the consortium's fixed-wavelength beamline X13 at HASYLAB/DESY, Hamburg, Germany (Fig. 2). For cryocooling, crystals were transferred to reservoir solution containing 10% (v/v) glycerol before flash-freezing them in a nitrogen stream at 100 K prior to X-ray diffraction analysis. Data were collected at a crystal-to-detector distance of 256 mm and with 0.8° oscillation per image. Initial crystal characterization and space-group assignment were performed using the *HKL*-2000 software package (Otwinowski & Minor, 1997) and scaling was performed using *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

SaTenA was cloned with an affinity tag and expressed in *E. coli* BLR (DE3) cells. The protein consists of 229 amino acids with a molecular weight of 26 840 Da as calculated from the primary sequence. Sequence comparison of *SaTenA* within the PDB revealed high homology to the related protein from *S. epidermidis* (PDB code 3no6; Joint Center for Structural Genomics, unpublished work). The sequence identity between the two models is 57.7%. The sequence alignment and the putative active-site residues Asp44, Cys137, Tyr167 and Glu208 are shown in Fig. 3 (Jurgenson *et al.*, 2009). The crystallization procedure is described above. A complete diffraction data set was collected to 2.60 Å resolution from a single crystal. Data-collection statistics are reported in Table 1. A total of 424 031 measured reflections in the resolution range 30–2.6 Å merged to 31 741 unique reflections with an overall R_{merge} of 4.2%. Analysis of the diffraction intensities confirmed the space group to be the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 103.5$, $b = 104.1$, $c = 109.6 \text{ Å}$. With four molecules in the asymmetric unit, the calculated Matthews coefficient is $2.85 \text{ Å}^3 \text{ Da}^{-1}$ (Matthews, 1968), corresponding to a solvent content of 56%. A preliminary solution of

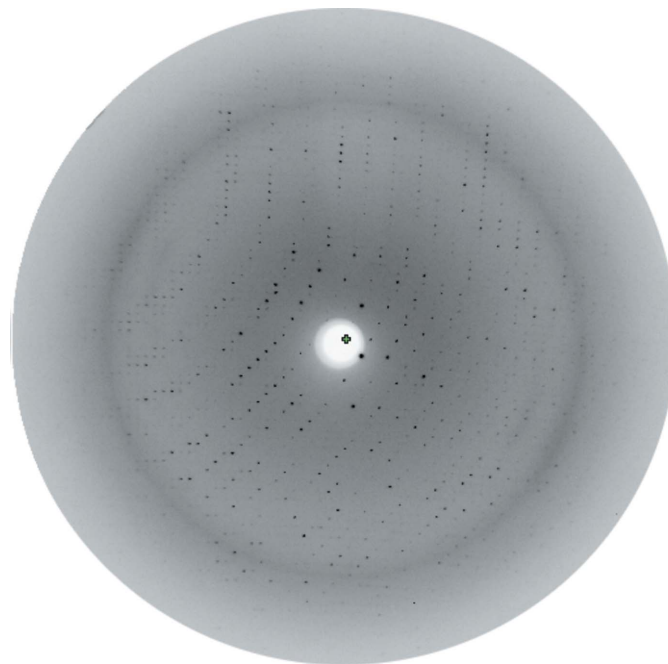


Figure 2
X-ray diffraction pattern of an *SaTenA* crystal recorded using a MAR CCD detector.

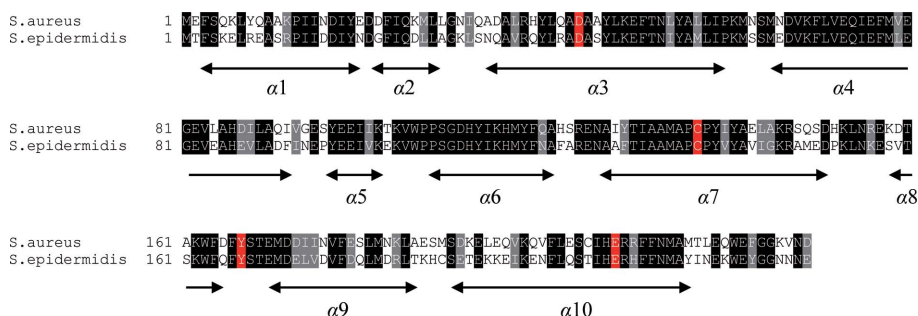


Figure 3 Sequence alignment of *S. aureus* and *S. epidermidis* TenA. The secondary-structure regions are indicated and the active-site residues are shown in red.

Table 1 Summary of data-collection statistics.

Values in parentheses are for the highest resolution shell.

Data-collection parameters	
Wavelength (Å)	0.8123
Temperature (K)	100
Oscillation range (°)	0.8
Crystal-to-detector distance (mm)	256
Data-integration statistics	
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 103.5, b = 104.1, c = 109.6$
Resolution limits (Å)	30–2.60
Total No. of reflections	424031
No. of unique reflections	31741
Multiplicity	5.1 (4.9)
Completeness (%)	95.3 (95.0)
R_{merge}^\dagger	0.042 (0.48)
Mean $I/\sigma(I)$	23.0 (3.3)
Molecules per asymmetric unit	4
V_M^\ddagger (Å ³ Da ⁻¹)	2.85
Solvent content (%)	56

$^\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $\langle I(hkl) \rangle$ is the mean intensity of the observations $I_i(hkl)$ of reflection hkl . ‡ For four molecules in the asymmetric unit.

the structure was obtained by molecular replacement with the program *MOLREP* (Vagin & Teplyakov, 2010) using TenA from *S. epidermidis* as the search model. The best results gave a correlation coefficient of 51.8% and an *R* factor of 45.8% at 15–3.5 Å resolution for four molecules in the asymmetric unit. Examination of the best solution revealed good crystal packing and no clashes between symmetry-related molecules. Model building with *Coot* (Emsley & Cowtan, 2004), sequence adaptation and further refinement of the model continue.

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