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Protein preparation and preliminary X-ray crystallographic analysis of a putative glucosamine 6-phosphate deaminase from *Streptococcus mutans*

The SMU.636 protein from *Streptococcus mutans* is a putative glucosamine 6-phosphate deaminase with 233 residues. The *smu.636* gene was PCR-amplified from *S. mutans* genomic DNA and cloned into the expression vector pET-28a(+). The resultant His-tagged fusion protein was expressed in *Escherichia coli* and purified to homogeneity in two steps. Crystals of the fusion protein were obtained by the hanging-drop vapour-diffusion method. The crystals diffracted to 2.4 Å resolution and belong to space group $P2_12_12_1$, with unit-cell parameters $a = 53.83$, $b = 82.13$, $c = 134.70$ Å.

1. Introduction

The Gram-positive bacterium *Streptococcus mutans* is the leading pathogen in human dental caries (tooth decay; Loesche, 1986) and is associated with non-oral infections such as subacute bacterial endocarditis (Ullman *et al.*, 1988). The genome of *S. mutans* UA159, a serotype strain, was completely sequenced in 2002. It is composed of 2 030 936 base pairs containing 1963 ORFs, about 63% of which have been assigned putative functions (Ajdic *et al.*, 2002).

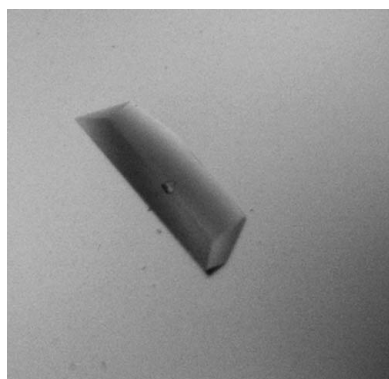
The *smu.636* gene of *S. mutans* encodes a putative protein of 233 residues with a molecular weight of 25.5 kDa. Primary sequence alignment shows that the SMU.636 protein belongs to the glucosamine 6-phosphate deaminase (NagB; EC 3.5.99.6) family (Fig. 1). NagB performs the deamination and isomerization reactions that convert glucosamine 6-phosphate (GlcN-6-P) to fructose 6-phosphate (F6P) and release ammonia (Warren, 1972), which is the final step in the specific pathway of GlcNAc utilization. Lying at the central position in peptidoglycan formation and glycolysis, NagB decides the metabolic fate of GlcNAc (Komatsuzawa *et al.*, 2004). To date, NagB structures from *Escherichia coli*, *Bacillus subtilis* and human have been determined (Oliva *et al.*, 1995; Vincent *et al.*, 2005; Arreola *et al.*, 2003). *E. coli* and human NagBs exist as hexamers and are allosterically regulated, while *B. subtilis* NagB exists as a monomer. SMU.636 protein shows a higher sequence identity to *B. subtilis* NagB. Structural analysis of *B. subtilis* NagB indicates that Thr36, Thr39, Asp67, His138, Gly140 and Arg167 are conserved and compose the catalytic active centre (Vincent *et al.*, 2005); these residues are also conserved in SMU.636 protein (Fig. 1).

The enzymes participating in the biosynthesis of peptidoglycan have long been considered attractive targets for antibacterial agents. The determination of the crystal structure of SMU.636, which is a potential 'gate' in this pathway, will help us understand its biological function and provide clues for the design of antimicrobial agents against this dental pathogen.

2. Materials and methods

2.1. Cloning and expression

The SMU.636 gene was amplified by polymerase chain reaction (PCR) from *S. mutans* genomic DNA using primers 5'-CGCGGAT-



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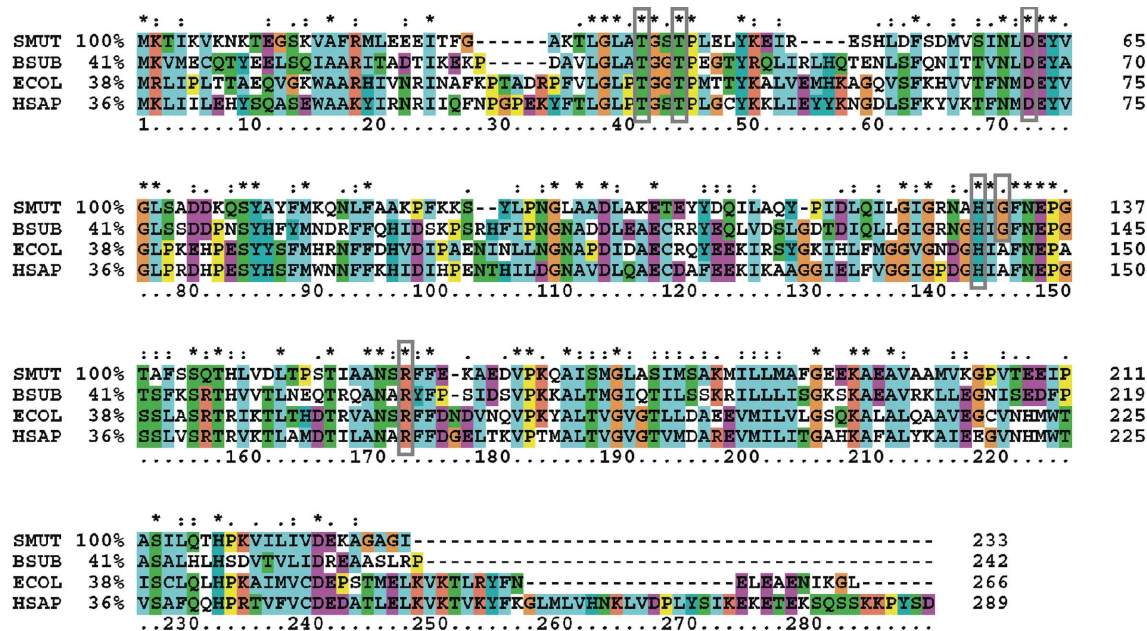


Figure 1 Multiple sequence alignment of SMU.636 homologues. The sequences of the glucosamine 6-phosphate deaminases of known structure from *B. subtilis* (Vincent *et al.*, 2005), *E. coli* (Oliva *et al.*, 1995) and human (Arreola *et al.*, 2003) are aligned with SMU.636. The conserved residues composing the active centre are indicated by grey boxes and the percentage sequence identities with SMU.636 are indicated after the organism names; asterisks indicate totally conserved residues. The alignment was performed using the program CLUSTALX (Thompson *et al.*, 1997). BSUB, *B. subtilis*; ECOL, *E. coli*; HSAP, *Homo sapiens*.

CCCATGAAAAC TATTAAGTAAAAAAT-3' and 5'-CCGCTC-GAGTTAAATTCCTGCTCCTGCTTTT-3'. The amplified fragment was inserted into the *Bam*HI/*Xho*I-digested expression vector pET-28a(+) (Novagen). An N-terminal His₆ tag of 34 amino-acid residues and sequence MGSSHHHHHSSGLVPRGSHMASMTGGQQM-GRGS was added to the gene product. Transformed *E. coli* strain BL21 (DE3) cells were cultured in lysogeny broth (LB) medium supplemented with 50 µg ml⁻¹ kanamycin at 310 K until the OD₆₀₀ reached 0.6–0.8. Gene expression was then induced with 1.0 mM isopropyl β-D-thiogalactoside (IPTG) and the culture was incubated for a further 5 h at 303 K. The cells were then harvested by centrifugation at 6700g for 10 min and resuspended in lysis buffer containing 20 mM Tris–HCl, 500 mM NaCl pH 7.5.

2.2. Protein purification and crystallization

The cells were disrupted by sonication on ice. The lysate was centrifuged at 34 700g at 277 K for 30 min twice to exclude the debris. The supernatant was loaded onto a 5 ml HiTrap Ni column (GE Healthcare) equilibrated with buffer A (20 mM Tris–HCl, 0.5 M NaCl pH 7.5). Unbound proteins were eluted with buffer A and loosely bound proteins were eluted with 5% buffer B (20 mM Tris–HCl, 0.5 M NaCl, 0.5 M imidazole pH 7.5) in buffer A. The tightly bound proteins were then eluted with buffer B. The protein was further purified to homogeneity using a HiLoad Superdex 75 column (GE Healthcare) with buffer C (20 mM Tris–HCl, 150 mM NaCl pH 7.5). The purity of the protein was examined by SDS–PAGE at each step.

The purified protein from gel filtration was concentrated to 10 mg ml⁻¹ in the final elution buffer by ultrafiltration (Ultra-15, 10 kDa cutoff, Millipore Amicon) and used in crystallization experiments. Initial crystallization experiments were performed at 289 K by the hanging-drop vapour-diffusion method using Crystal Screen I, Crystal Screen II and Index Screen (Hampton Research, CA, USA). 1 µl protein solution was mixed with 1 µl reservoir

solution and the droplets were equilibrated against 500 µl reservoir solution.

2.3. X-ray data collection and processing

The crystal was flash-cooled and maintained at 100 K in a cold nitrogen-gas stream during data collection; no cryoprotectant was needed. X-ray diffraction data were collected on a Bruker SMART 6000 CCD detector using Cu Kα radiation from a Bruker–Nonius FR591 rotating-anode generator. A total of 800 frames were collected with 0.3° φ oscillation per frame; the exposure time per frame was 100 s. Data were processed using the Bruker PROTEUM online software suite.

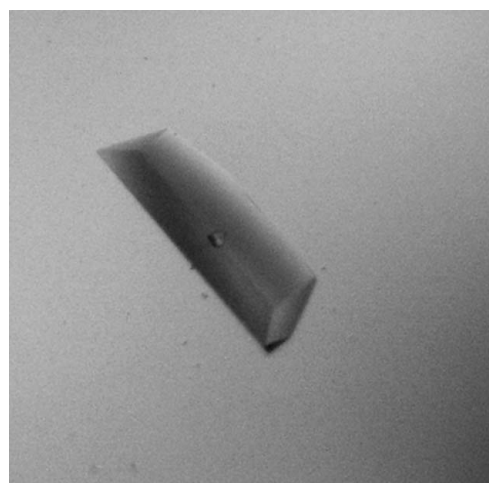


Figure 2 Crystal of *S. mutans* SMU.636. Approximate dimensions are 0.1 × 0.2 × 0.8 mm.

Table 1

Data-collection statistics of SMU636.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	50–2.4 (2.54–2.4)
Completeness (%)	95.6 (89.5)
R_{sym}^{\dagger} (%)	9.1 (32.5)
Mean $I/\sigma(I)$	5.5 (2.0)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 53.83, b = 82.13, c = 134.70$
No. of observed reflections	196175
No. of unique reflections	23007

$\dagger R_{\text{sym}} = \sum_h [\sum_i |I(h)_i - \langle I(h) \rangle| / \sum_i I(h)_i]$, where $I(h)_i$ is the i th observation of reflection h and $\langle I(h) \rangle$ is the mean intensity of all observations of h .

3. Results

The target protein was purified to homogeneity after gel filtration; only one band was visible on the SDS–PAGE gel. Microcrystals were observed under several conditions and further optimization was applied based on condition No. 66 of Index Screen, which consisted of 0.2 M ammonium sulfate, 0.1 M bis-Tris pH 5.5, 25% (w/v) PEG 3350. After reducing the protein concentration to 5 mg ml⁻¹, diffraction-quality crystals appeared within 2 d under the original conditions (Fig. 2).

The crystal diffracted to 2.4 Å and belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 53.83, b = 82.13, c = 134.70$ Å. There could be two or three molecules in each asymmetric unit, which

would give V_M values of 3.1 or 2.0 Å³ Da⁻¹, corresponding to solvent contents of 59% and 39%, respectively (Matthews, 1968). Statistics of data collection are summarized in Table 1.

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References

- Ajdic, D. *et al.* (2002). *Proc. Natl Acad. Sci. USA*, **99**, 14434–14439.
- Arreola, R., Valderrama, B., Morante, M. L. & Horjales, E. (2003). *FEBS Lett.* **551**, 63–70.
- Komatsuzawa, H., Fujiwara, T., Nishi, H., Yamada, S., Ohara, M., McCallum, N., Berger-Bachi, B. & Sugai, M. (2004). *Mol. Microbiol.* **53**, 1221–1231.
- Loesche, W. J. (1986). *Microbiol. Rev.* **50**, 353–380.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Oliva, G., Fontes, M. R., Garratt, R. C., Altamirano, M. M., Calcagno, M. L. & Horjales, E. (1995). *Structure*, **3**, 1323–1332.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). *Nucleic Acids Res.* **25**, 4876–4882.
- Ullman, R. F., Miller, S. J., Strampfer, M. J. & Cunha, B. A. (1988). *Heart Lung*, **17**, 209–212.
- Vincent, F., Davies, G. J. & Brannigan, J. A. (2005). *J. Biol. Chem.* **280**, 19649–19655.
- Warren, L. (1972). In *Glycoproteins*, edited by A. Gottschalk. Amsterdam: Elsevier.