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# Preliminary X-ray crystallographic analysis of SMU.2055 protein from the caries pathogen *Streptococcus mutans*

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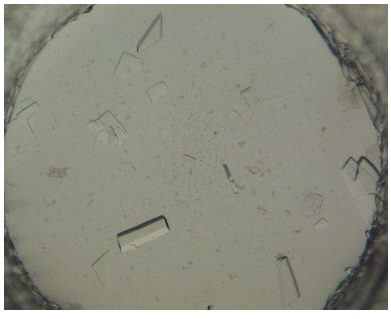
The SMU.2055 gene from the major caries pathogen *Streptococcus mutans* is annotated as a putative acetyltransferase with 163 amino-acid residues. In order to identify its function *via* structural studies, the SMU.2055 gene was cloned into the expression vector pET28a. Native and SeMet-labelled SMU.2055 proteins with a $His_6$ tag at the N-terminus were expressed at a high level in *Escherichia coli* strain BL21 (DE3) and purified to homogeneity by $Ni^{2+}$-chelating affinity chromatography. Diffraction-quality crystals of SeMet-labelled SMU.2055 were obtained using the sitting-drop vapour-diffusion method and diffracted to a resolution of 2.5 Å on beamline BL17A at the Photon Factory, Tsukuba, Japan. The crystals belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a$ = 92.0, $b$ = 95.0, $c$ = 192.2 Å. The asymmetric unit contained four molecules, with a solvent content of 57.1%.

## 1. Introduction

Dental caries is one of the most prevalent diseases afflicting humans. It is an infectious disease that not only affects the calcified tissue of teeth (Shivakumar *et al.*, 2009) but can also lead to a series of diseases such as pulpitis (Nguyen & Martin, 2008) and periapical abscesses (Mueller & Lowder, 1998). The marked increase in the prevalence of dental caries signals a pending public health crisis (Bagramian *et al.*, 2009). *Streptococcus mutans* has been recognized as the leading causative agent of human dental caries (Loesche, 1986) and recent studies on the cariogenesis mechanism have focused on biofilm formation (Bleiweis *et al.*, 1992), acid tolerance (Quivey *et al.*, 2001), virulence factors and bacterial adhesion to the tooth (Islam *et al.*, 2007). In addition, *S. mutans* can cause subacute bacterial endocarditis (Ullman *et al.*, 1988).

The genome of *S. mutans* UA159, a serotype C strain, has been sequenced and contains about 1960 open reading frames, 63% of which have been assigned putative functions (Ajdic *et al.*, 2002). An *S. mutans* structural genomics project aimed at solving the structures of the majority of the cytosolic proteins was initiated in the People's Republic of China in 2005 (Su *et al.*, 2006). One of the selected targets, SMU.2055 (gi:24380392; Gene ID 1029233), has successfully been crystallized. The SMU.2055 gene from the *S. mutans* genome encodes a putative acetyltransferase protein with 163 amino-acid residues, a theoretical molecular weight of 18.6 kDa and an isoelectric point of 7.7. A *BLAST* search showed that SMU.2055 belongs to the Gcn5-related *N*-acetyltransferase (GNAT) superfamily. The GNAT superfamily is widespread in nature and contains numerous members that use acyl-CoAs to acylate their cognate substrates (Vetting *et al.*, 2005). SMU.2055 and its closest homologue in the Protein Data Bank (PDB), a GNAT-family acetyltransferase from *Enterococcus faecalis* V583 (PDB code 2ae6; Y. Kim, C. Hatzos, S. Moy, F. Collart & A. Joachimiak, unpublished work), share only 27% sequence identity. As it may be difficult to solve the SMU.2055 structure by molecular



replacement with such a low sequence identity, we therefore prepared selenomethionine-labelled SMU.2055 for use in phase determination (Doublié, 1997). Structure determination of SMU.2055 will help us to better study its biological function.

## 2. Experimental procedures

### 2.1. Gene cloning

To construct the expression plasmid, two primers containing restriction sites were designed: SMU.2055-F (5′-CGCGGATCCATGAAAATAAGCCCTATGTTA-3′) and SMU.2055-R (5′-CCGCTCGAGTTATTTGGCATAGGCAGCCT-3′). The SMU.2055 gene was amplified by polymerase chain reaction (PCR) from *S. mutans* genomic DNA (Saiki *et al.*, 1988). After digestion with *Bam*HI and *Xho*I overnight at room temperature, the PCR product was cloned into the vector pET28a(+)(Novagen) by the conventional cloning method with an N-terminal fusion $His_6$ tag (MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS). The recombinant vector containing the target gene SMU.2055, which was verified by DNA sequencing, was transformed into *Escherichia coli* strain BL21 (DE3) for protein expression.

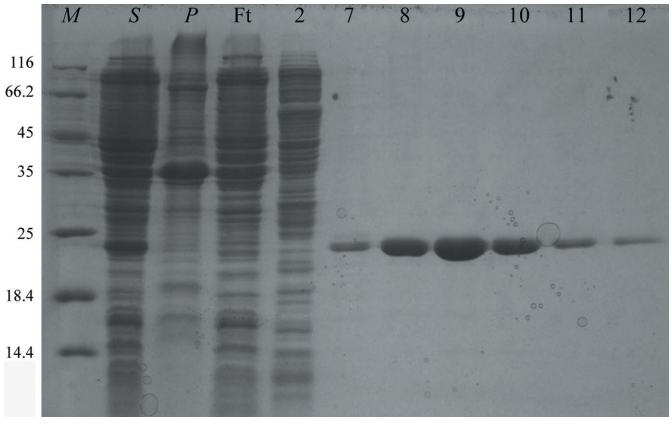



**Figure 1**
15% SDS–PAGE analysis of SMU.2055 protein purified by $Ni^{2+}$-affinity column chromatography (with Coomassie Brilliant Blue staining). Lane *M*, molecular-weight markers (kDa). Lanes *S* and *P*, soluble and insoluble material after inducing expression of SMU.2055, respectively. Lane Ft, unbound material from the $Ni^{2+}$-affinity column; lane 2, fraction eluted with 10% elution buffer (20 m*M* Tris–HCl pH 8.5, 500 m*M* NaCl and 500 m*M* imidazole) in lysis buffer (20 m*M* Tris–HCl pH 8.5, 500 m*M* NaCl). Lanes 7–12, fractions of the target protein eluted with a linear gradient of elution buffer from 10 to 100% in lysis buffer.

### 2.2. Protein expression and purification

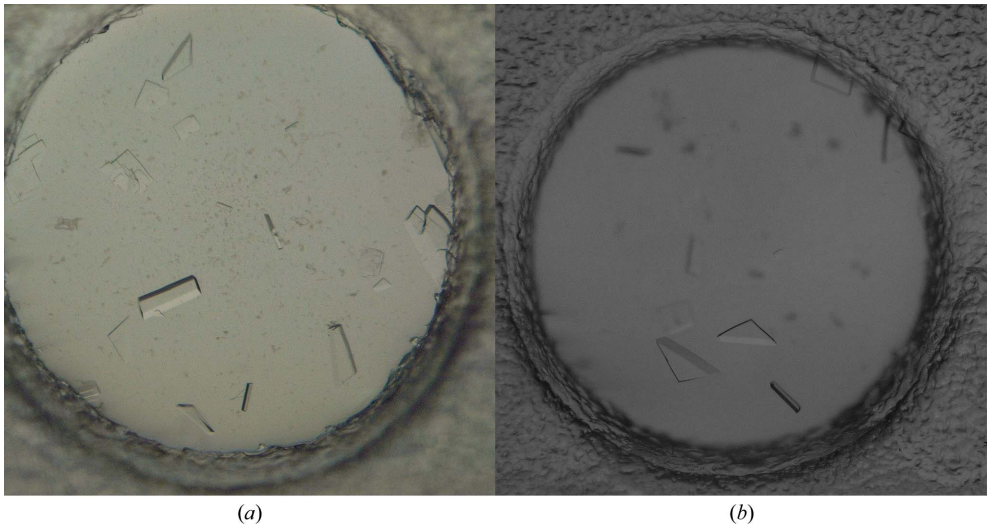
*E. coli* strain BL21 (DE3) cells containing pET28a-SMU.2055 were grown overnight in 20 ml Luria–Bertani (LB) medium containing 50 µg ml$^{-1}$ kanamycin at 310 K. The overnight culture was transferred into 1 l LB medium containing 50 µg ml$^{-1}$ kanamycin and growth continued at 310 K until the $OD_{600}$ reached 0.6. The cells were then induced with 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) for 8 h at 303 K.

The cells were harvested by centrifugation for 10 min at 8600*g* and 277 K, resuspended in 20 ml lysis buffer (20 m*M* Tris–HCl, 500 m*M* NaCl pH 8.5) and lysed by sonication on ice. After centrifugation at 48 400*g* and 277 K for 20 min twice to remove debris, the supernatant was filtered and loaded onto a 5 ml HiTrap $Ni^{2+}$-chelating affinity column (GE Healthcare, USA) equilibrated with lysis buffer. The unbound material was first washed with lysis buffer. The low-nickel-affinity proteins were eluted with 10% elution buffer (20 m*M* Tris–HCl pH 8.5, 500 m*M* NaCl and 500 m*M* imidazole) in lysis buffer. Subsequently, the bound target protein was eluted with a 10–100% linear gradient of elution buffer in lysis buffer and examined by SDS–PAGE (Fig. 1). The fractions containing the target protein were pooled and concentrated by ultrafiltration using a Millipore centrifugal ultrafiltration device (Amicon Ultra, 10 kDa cutoff) at 277 K. The target protein was buffer-exchanged into buffer solution (20 m*M* Tris–HCl pH 8.5, 500 m*M* NaCl) by ultrafiltration.

At the same time, SeMet-labelled SMU.2055, which was used for phase determination, was prepared and purified using a similar procedure.

### 2.3. Protein crystallization

For crystallization trials, the purified native and SeMet-labelled SMU.2055 proteins were concentrated to 19 and 27 mg ml$^{-1}$, respectively, in buffer solution (20 m*M* Tris–HCl pH 8.5, 500 m*M* NaCl) by ultrafiltration without removal of the N-terminal fusion



(*a*) (*b*)

**Figure 2**
Crystals of (*a*) native and (*b*) SeMet-labelled SMU.2055 protein. Both were obtained using a reservoir solution consisting of 30%(*v*/*v*) pentaerythritol ethoxylate (15/4 EO/OH), 0.05 *M* bis-tris pH 6.5 and 0.05 *M* ammonium sulfate.

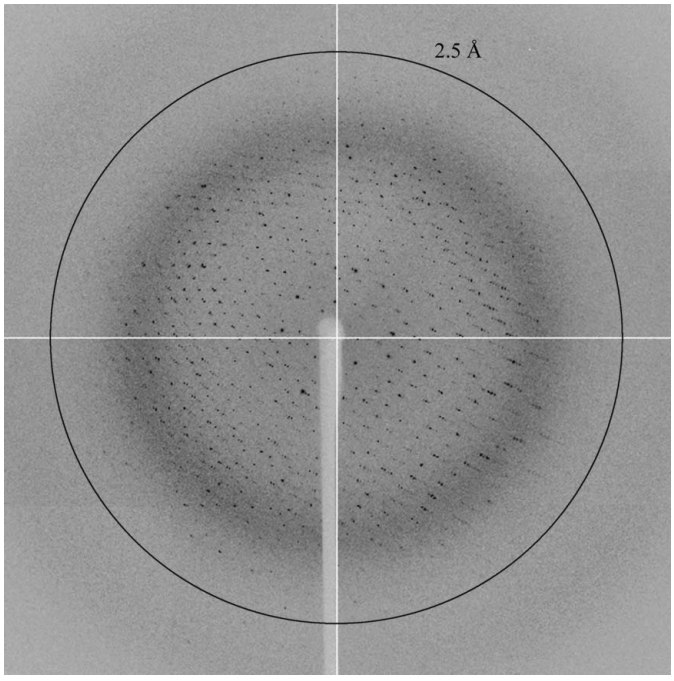



**Figure 3**
Diffraction image from a SeMet-labelled SMU.2055 protein crystal.

$His_6$ tag. The protein concentration was determined using a Bio-Rad protein-assay kit (Bio-Rad Laboratories, USA) based on the method of Bradford. Initial crystallization screening was carried out by the sitting-drop vapour-diffusion method with an XtalQuest48[2] crystallization plate (XtalQuest Inc., Beijing, People's Republic of China) at 289 K, using several commercially available crystallization screening kits: Index, Crystal Screen, Crystal Screen 2 and Natrix (Hampton Research, USA) and BioXtal (XtalQuest Inc., Beijing, People's Repubic of China). 1 µl protein solution was mixed with an equal volume of reservoir solution and equilibrated against 130 µl reservoir solution.

### 2.4. X-ray diffraction data collection and processing

Native and SeMet-labelled SMU.2055 protein crystals were quick-soaked (~30 s) in reservoir solution containing 25%(*v*/*v*) glycerol as a cryoprotectant and flash-cooled in liquid nitrogen. The native crystal diffracted to a maximum resolution of 2.3 Å using a Bruker SMART 6000 CCD and Cu $K\alpha$ radiation from a Bruker MICROSTAR-H rotating-anode generator operated at 45 kV and 40 mA. 800 frames were collected with 0.3° oscillation per image. The diffraction data were processed using the *PROTEUM* software suite (Bruker). Diffraction data from the SeMet-labelled SMU.2055 protein crystal were collected on beamline BL17A at the Photon Factory (Tsukuba, Japan) at the peak wavelength of 0.97909 Å. The crystal-to-detector distance was set to 276.4 mm. 360 frames were collected with 1° oscillation per image. The diffraction data were processed using the *XDS* program suite (Kabsch, 1993).

## 3. Results

The typical yield of the native protein was about 46 mg pure protein per litre of cell culture; the SeMet-labelled protein showed a similar yield. The SDS–PAGE results showed that the purified protein had a molecular mass of about 22.6 kDa, which is consistent with the calculated molecular mass of the SMU.2055 protein of 18.6 kDa plus a 4 kDa $His_6$ tag.

**Table 1**
Data-collection statistics for native and SeMet-labelled SMU.2055 protein crystals.

Values in parentheses are for the highest resolution shell.

| | Native | SeMet-labelled |
|---|---|---|
| Wavelength (Å) | 1.54 | 0.97909 |
| Resolution (Å) | 50–2.3 (2.4–2.3) | 50.0–2.5 (2.6–2.5) |
| Completeness (%) | 82.2 (38.9) | 99.4 (97.0) |
| $R_{merge}$† (%) | 7.7 (36.7) | 9.1 (49.2) |
| Mean $I/\sigma(I)$ | 8.9 (1.8) | 18.8 (4.4) |
| Space group | $C222_1$ | $C222_1$ |
| Unit-cell parameters (Å) | $a$ = 92.0, $b$ = 94.6, $c$ = 193.9 | $a$ = 92.0, $b$ = 95.0, $c$ = 192.2 |
| No. of observed reflections | 223053 (14746) | 405912 (32609) |
| No. of unique reflections | 31240 (1739) | 56047(6048) |
| No. of molecules in the asymmetric unit | 4 | 4 |
| $V_M$ (Å$^3$ Da$^{-1}$) | 2.84 | 2.86 |
| Solvent content (%) | 56.7 | 57.1 |

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection $hkl$ and $\sum_i$ is the sum over all $i$ measurements of reflection $hkl$.

Crystals of the native and SeMet-labelled protein suitable for diffraction were obtained using the condition 30%(*v*/*v*) pentaerythritol ethoxylate (15/4 EO/OH), 0.05 *M* bis-tris pH 6.5 and 0.05 *M* ammonium sulfate (BioXtal screen condition No. 95). The crystal dimensions of the native SMU.2055 protein crystals were about 0.1 × 0.1 × 0.5 mm (Fig. 2*a*); the SeMet-labelled SMU.2055 protein crystals were not easily obtained but grew to dimensions of about 0.1 × 0.2 × 0.3 mm (Fig. 2*b*).

The SeMet-labelled SMU.2055 protein crystals diffracted to a resolution of 2.5 Å and belonged to space group $C222_1$ as indicated by systematic absences, with unit-cell parameters $a$ = 92.0, $b$ = 95.0, $c$ = 192.2 Å. Assuming the presence of four molecules per asymmetric unit, the $V_M$ value is 2.86 Å$^3$ Da$^{-1}$, corresponding to a solvent content of 57.1% (Matthews, 1968). Fig. 3 shows a typical diffraction pattern for the SeMet-labelled crystals. The native crystal belonged to the same space group, with very similar unit-cell parameters. The crystallographic parameters and data-collection statistics for both the native and SeMet-labelled proteins are listed in Table 1. The phases of the structure have been determined using the single-wavelength anomalous dispersion method and refinement of the structure is in progress.

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## References

Ajdic, D. *et al.* (2002). *Proc. Natl Acad. Sci. USA*, **99**, 14434–14439.
Bagramian, R. A., Garcia-Godoy, F. & Volpe, A. R. (2009). *Am. J. Dent.* **22**, 3–8.
Bleiweis, A. S., Oyston, P. C. & Brady, L. J. (1992). *Adv. Exp. Med. Biol.* **327**, 229–241.
Doublié, S. (1997). *Methods Enzymol.* **276**, 523–530.
Islam, B., Khan, S. N. & Khan, A. U. (2007). *Med. Sci. Monit.* **13**, RA196–RA203.
Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
Loesche, W. J. (1986). *Microbiol. Rev.* **50**, 353–380.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mueller, P. O. & Lowder, M. Q. (1998). *Vet. Clin. North Am. Equine Pract.* **14**, 349–363.
Nguyen, D. H. & Martin, J. T. (2008). *Am. Fam. Physician*, **77**, 797–802.

Quivey, R. G., Kuhnert, W. L. & Hahn, K. (2001). *Crit. Rev. Oral Biol. Med.* **12**, 301–314.
Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988). *Science*, **239**, 487–491.
Shivakumar, K. M., Vidya, S. K. & Chandu, G. N. (2009). *Indian J. Dent. Res.* **20**, 99–106.
Su, X.-D., Liang, Y., Li, L., Nan, J., Bröstromer, E., Liu, P., Dong, Y. & Xian, D. (2006). *Acta Cryst.* D**62**, 843–851.
Ullman, R. F., Miller, S. J., Strampfer, M. J. & Cunha, B. A. (1988). *Heart Lung*, **17**, 209–212.
Vetting, M. W., de Carvalho, L. P. S., Yu, M., Hegde, S. S., Magnet, S., Roderick, S. L. & Blanchard, J. S. (2005). *Arch. Biochem. Biophys.* **433**, 212–226.