

Preparation, Crystallization and Preliminary X-ray Crystallographic Analysis of Smu.776 From Caries Pathogen *Streptococcus mutans**

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Abstract The gene *smu.776* encodes a possible S-adenosylmethionine-dependent methyltransferase of 385 residues in *Streptococcus mutans*, a primary pathogen for human dental caries. The DNA fragment of *smu.776* was cloned into pET28a and expressed in good amount from the *E. coli* strain BL21 (DE3). Smu.776 protein was purified to homogeneity in a two-step procedure of Ni²⁺ chelating and size exclusion chromatography. Crystals were obtained by hanging-drop vapor diffusion method and diffracted to 2.0 Å resolution. The crystal belongs to orthorhombic space group C2 with cell dimension of $a=168.47$ Å, $b=50.66$ Å, $c=53.96$ Å, $\beta=104.22^\circ$. The asymmetric unit is expected to contain one molecule with solvent content of 51.3%.

Key words *Streptococcus mutans*, dental caries, Smu.776, protein crystallography

Smu.776 is a hypothetical protein of 385 amino acid residues from *Streptococcus mutans*, the principal etiological agent in human dental caries^[1] and a primary pathogen of infective endocarditis as well^[2]. Bioinformatics studies showed that the protein represented a family of proteins (Figure 1), most of which are from gram-positive bacteria, especially *Streptococcus* genus. The annotations for the homologous proteins of Smu.776 are all hypothetical, but some of them are predicted to have potential S-adenosylmethionine-dependent methyltransferase (SAM-dependent MT) activities, which can play an important role in synthesis of many bio-molecules by utilizing the methyl donor S-adenosylmethionine (SAM or AdoMet) to methylate substrates, such as hormones, neurotransmitters, lipids, proteins and nucleic acids^[3]. Conserved domain search^[4] in NCBI COG (Clusters of Orthologous Groups) database also confirmed that Smu.776 contains conserved domain of predicted SAM-dependent methyltransferases (COG1092). And some proteins from the family contain RNA binding motifs, which is a hint that Smu.776 could have RNA methyltransferase activity. But the exact biological function of Smu.776 can only be

provided by the experiments. The structure determination of Smu.776 will give us clues on the study of the protein, and will help us to find inhibitors of the protein, if the protein is functionally important to *S. mutans* metabolism and a potential drug design target.

1 Materials and methods

1.1 Cloning, expression and purification

To form a His-tag fusion protein, two primers contain *Bam*H I and *Not* I digestion sequence respectively are designed as follows: SMU776-F 5' cgcgatccatgattaaacttatggtgggttc 3' and SMU776-R 5' ccgctcgagctaaccctttattgttaaactttt 3'. *smu.776* gene was

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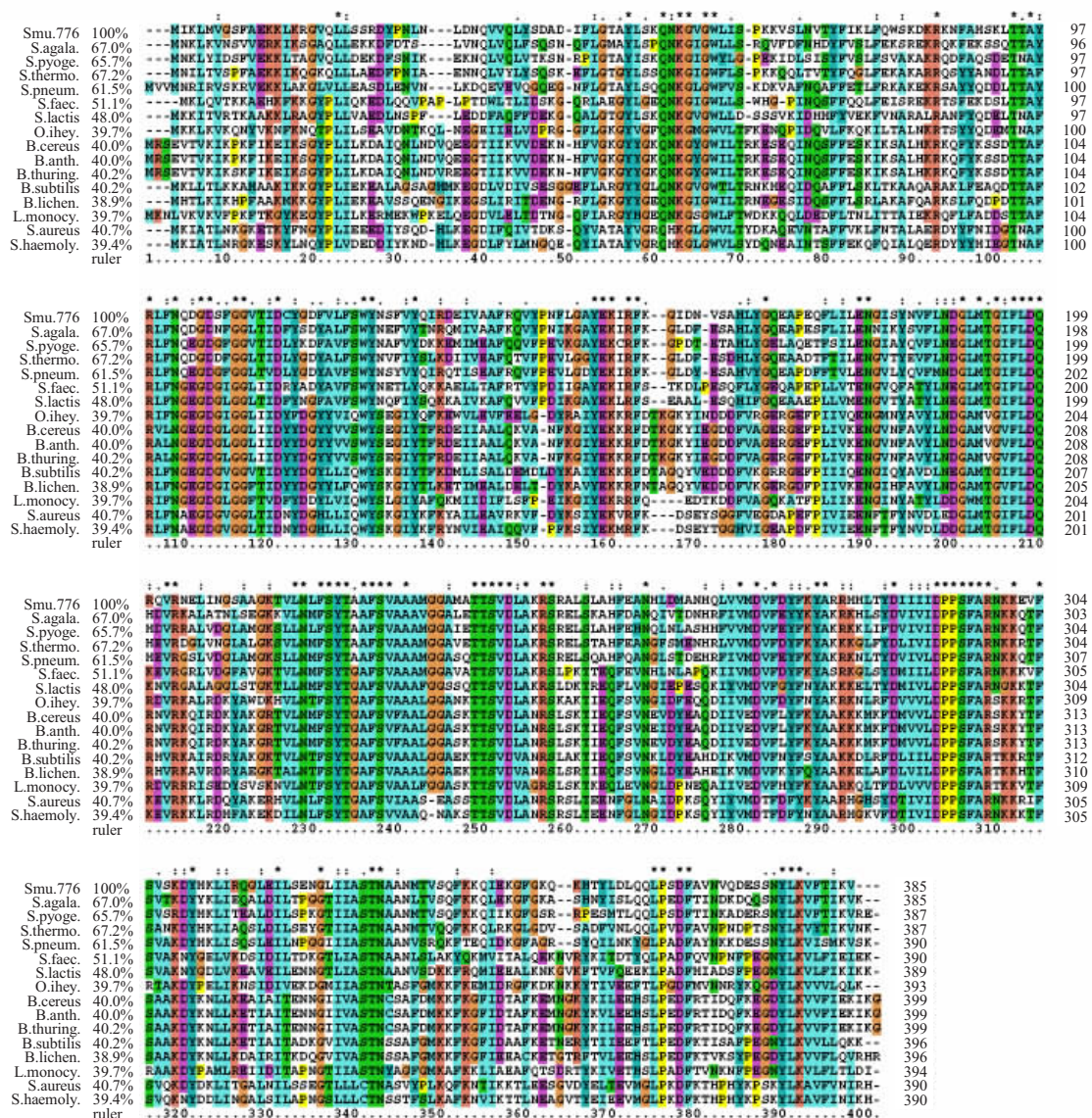


Fig. 1 Multiple sequence alignment of some Smu.776 homologous

The alignment is performed using the program Clustal X^[5]. The percentage identities with *S. mutans* Smu.776 are indicated after the organism names. The asterisks indicate the strict conserved residues.

PCR amplified from chromosomal DNA of *Streptococcus mutans* ATCC25175 and the PCR product was cloned into a pET28a (Novagen) expression vector using conventional cloning method. *E. coli* cells strain BL21 (DE3) (Novagen) were transformed with this expression vector and grown at 310K in 1L Luria-Bertan medium containing 50 mg/L kanamycin. The cultures were grew to an A_{600} of 0.6, and chilled to 291K for an additional half hour before induction. Isopropyl- β -D-thiogalactopyranoside (IPTG) was added to an ultimate concentration of 0.5 mmol/L, and induction continued for 24 h.

Cells were harvested by centrifugation at 6 000 g

for 10 min. The pellet was re-suspended with 20 ml buffer A (20 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.5) and lysed by sonication on ice. The cell lysate was centrifuged at 40 000 g for 40 min to get rid of cell debris and the supernatant was applied to a Ni²⁺ chelating column (HiTrapTM, GE). The impurity was removed by 10% buffer B (20 mmol/L Tris-HCl, 500 mmol/L NaCl, pH 7.5, 500 mmol/L imidazole) and the target protein was eluted using a linear gradient of 10% to 30% buffer B. Then size exclusion chromatography (Hiload Superdex 75, column volume 120 ml, GE, USA) was chosen for the further purification, above mentioned buffer A. Protein purity

was examined in each step by SDS-PAGE using Coomassie Blue staining. Purified protein was directly concentrated with Millipore centrifugal filter device with a molecular-weight cutoff of 10 000 u and concentrated protein was stored at 191K.

1.2 Protein crystallization

Crystallization of Smu.776 protein was performed at 289K using hanging-drop vapor-diffusion method. The initial crystallization conditions were screened using Crystal Screen, Crystal Screen 2 and Index Kits (Hampton Research, CA, USA). In each trail, a hanging drop of 1 μ l of protein solution mixed with 1 μ l of reservoir solution was equilibrated against 500 μ l of reservoir solution.

1.3 X-ray diffraction data collection and processing

X-ray diffraction data were collected on a Bruker SMART 6000 CCD detector using Cu K α radiation from a Bruker Nonius FR591 rotating anode generator operated at 45 kV and 100 mA. The crystal to detector distance was set to 4 cm, and 2 θ angle of the detector was set to 5°. 1 800 frames were collected with 0.2° ϕ oscillation and 90 s exposing time per frame. During data collection, the crystal was flash-cooled and maintained at 100 K using nitrogen gas. Diffraction data were processed using the Bruker on-line software PROTEUM suite.

2 Result and discussion

Smu.776 gene was successfully cloned into pET28a expression vector, and protein was expressed at a soluble form when induced at a lower temperature for a relatively long time. Recombinant protein with 6-histidine tag was first purified by 5 ml Ni chelating column (Amarsham). During gradient washing with imidazole, protein was washed out together with nucleic acid, which is indicated by a higher absorbance at 254 nm than 280 nm and agarose gel (data not shown). This confirmed the prediction that Smu.776 could be a RNA methyltransferase. Further experiments to discover the nature of this protein are still on the way. After gel filtration from Superdex S75 column (in 500 mmol/L NaCl), protein purity was monitored by SDS-PAGE using Coomassie blue staining. The protein would aggregate at lower salt concentration buffer (200 mmol/L NaCl etc.). About 20 mg purified protein can be obtained from 1L culture. After concentration with Millipore centricon tube, protein concentration reached 30 g/L.

Micro-crystals of Smu.776 were found under several conditions (conditions 41, 43, 45, 47 and 62 from Index Kit, conditions 18, 37 and 38 from Crystal Screen) at the first round of screening. Crystals from all conditions except condition 18 of Crystal Screen were needle like and not suitable for data collection, so the optimization of crystallization was performed around condition 18 of Crystal Screen. The crystals suitable for X-ray diffraction were obtained in an optimized condition containing 20% PEG8000, 0.1 mol/L Sodium Cacodylate buffer pH 6.5 and 0.2 mol/L Magnesium Acetates (Figure 2). The crystal diffracted to better than 2.0 Å and belonged to space group C2, with cell dimensions of $a=168.47$ Å, $b=50.66$ Å, $c=53.96$ Å, $\beta=104.22^\circ$. There is one molecule per asymmetric unit, which gives a V_M value of $2.52 \text{ Å}^3 \cdot \text{Da}^{-1}$ [6], corresponding to a solvent content of 51.3%. The crystallographic parameters and data collection statistics are listed in Table 1.

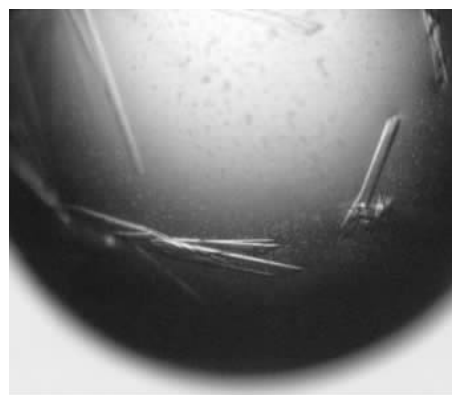


Fig. 2 Crystals of Smu.776 protein
0.05 mm $\times$ 0.05 mm $\times$ 0.4 mm.

Table 1 X-ray data collection and processing statistics

Resolution range /Å	50~2.0 (2.09~2.0) ¹⁾
Data completeness /%	98.66 (100)
R_{sym}^2 /%	4.95 (9.47)
Average $I/\sigma I$	11.56 (5.06)
Space group	C2
Unit cell parameters/(Å, °)	$a=168.47$, $b=50.66$, $c=53.96$, $\beta=104.22$
No. of Observed reflections	159 435
No. of Unique reflections	29 673
Mol/Asym.	1
$V_M / \text{Å}^3 \cdot \text{Da}^{-1}$	2.52
Solvent content /%	51.3

¹⁾Values in parentheses refer to the highest resolution shell.

²⁾ $R_{\text{sym}} = \sum |I_{\text{obs}} - I_{\text{avg}}| / \sum I_{\text{obs}}$

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口腔致病菌变形链球菌 Smu.776 蛋白的 制备、结晶及初级晶体学分析*

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摘要 变形链球菌 *smu.776* 基因编码一段含有 385 个氨基酸的蛋白质, 其功能可能为依赖于腺苷甲硫氨酸的甲基转移酶. *smu.776* 的 DNA 片段被克隆到表达载体 pET28a 后, 在大肠杆菌 BL21(DE3)菌株中过量表达得到很好的产量. 产物 Smu.776 蛋白通过 Ni²⁺ 亲和柱和分子筛柱层析两步得到纯化. 采用悬滴气相扩散法得到了 Smu.776 蛋白的晶体. X 射线衍射分辨率达到 2.0 Å, 晶体属单斜空间群 C2, 晶格参数为 $a=168.47$ Å, $b=50.66$ Å, $c=53.96$ Å, $\beta=104.22^\circ$. 每个最小不对称单元内含有一个蛋白质分子, 溶剂含量为 51.3%.

关键词 变形链球菌, 龋齿, Smu.776, 蛋白晶体学

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