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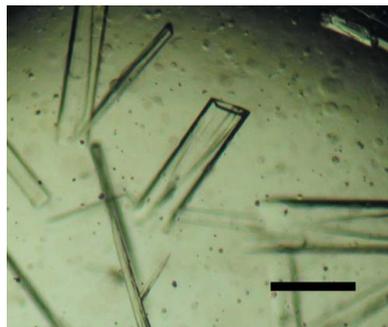
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Crystallization and preliminary X-ray analysis of *Streptococcus mutans* dextran glucosidase

Dextran glucosidase from *Streptococcus mutans* is an exo-hydrolase that acts on the nonreducing terminal α -1,6-glycosidic linkage of oligosaccharides and dextran with a high degree of transglucosylation. Based on amino-acid sequence similarity, this enzyme is classified into glycoside hydrolase family 13. Recombinant dextran glucosidase was purified and crystallized by the hanging-drop vapour-diffusion technique using polyethylene glycol 6000 as a precipitant. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 72.72$, $b = 86.47$, $c = 104.30$ Å. A native data set was collected to 2.2 Å resolution from a single crystal.

1. Introduction

The cariogenic bacterium *Streptococcus mutans* synthesizes extracellular glucose polymers (Hamada & Slade, 1980). Glucan synthesis from sucrose involves sucrose phosphorylase and three extracellular glycosyltransferases (Yamashita *et al.*, 1993). These enzymes are encoded by *gtfA*, *gtfB*, *gtfC* and *gtfD*, respectively. The glucans are applied as an adhesion material for bacterial accumulation and aggregation on smooth surfaces of the tooth in dental caries. One of the major components of the glucans is dextran, which is made of α -1,6-glycosidic linkages with some degree of branched chains, mainly via α -1,3 bonding (Loesche, 1986). In addition, the synthesis of glucans provides an ample source of hexoses for the bacterium. Eight proteins encoded by the multiple sugar metabolism operon are responsible for the metabolism and uptake of isomaltooligosaccharides, sucrose, dextran and related carbohydrates (Russell *et al.*, 1992). One component of the operon is *dexB*, which encodes dextran glucosidase.

The dextran glucosidase from *S. mutans*, SMDG, exclusively hydrolyzes the α -1,6-glycosidic linkage at the nonreducing end of panose, isomaltooligosaccharides and dextran to liberate α -glucose (Walker & Pulkownik, 1973; Saburi *et al.*, 2006). SMDG is classified into glycoside hydrolase family 13 (GH family 13) by amino-acid sequence-based classification of glycosidases and transglycosylases (Russell & Ferretti, 1990; Coutinho & Henrissat, 1999*a,b*). The similarity of the entire amino-acid sequences of GH family 13 enzymes is very low, but these enzymes share four short conserved regions, I–IV, that include the catalytic amino acids and important amino acids for stabilization of the transition state (MacGregor *et al.*, 2001). Each enzyme of GH family 13 has one glutamic acid and two aspartic acid residues that are necessary for activity in conserved regions III, II and IV, respectively. GH family 13 consists of about 30 types of α -retaining glycosidases and transglycosylases such as α -amylase, α -glucosidase, cyclodextrin glucanotransferase and amylosucrase (MacGregor *et al.*, 2001). All enzymes of analyzed three-dimensional structure in GH family 13 have a common multi-domain structure composed of three domains: A, B and C (MacGregor *et al.*, 2001). Domain A is a $(\beta/\alpha)_8$ -barrel domain bearing the catalytic site. Domain B is a long protruding loop connecting α -strand 3 and α -helix 3 of domain A. Domain C, which follows domain A, is

made up of antiparallel β -sheets. The catalytic nucleophile in conserved region II and the general acid/base catalyst in conserved region III are located at the C-termini of β -strand 4 and β -strand 5 of domain A, respectively.

SMDG has a particularly high similarity to *Bacillus oligo-1,6-glucosidases* (O16Gs) in GH family 13. SMDG shares 52% sequence identity with O16G based on analysis using the pairwise global and local alignment tool *EMBOSS* (Rice *et al.*, 2000). The molecular weight of native SMDG is 62 001. O16Gs also specifically hydrolyze the α -1,6-glucosidic linkage at the nonreducing end of substrates, but their preference for substrate chain length is distinct from that of SMDG. O16Gs only act on disaccharides and trisaccharides, but SMDG hydrolyzes isomaltooligosaccharides with a high degree of polymerization and even dextran (Suzuki *et al.*, 1979, 1982, 1987; Suzuki & Tomura, 1986; Saburi *et al.*, 2006). In our previous study, it was demonstrated that the chain-length preference was a consequence of distinct structural features in subsite +2 and $\beta \rightarrow \alpha$ loop 4, and a possible mechanism of long-chain substrate binding was proposed: Trp238 in subsite +2 leads a long-chain substrate to the space formed by the short $\beta \rightarrow \alpha$ loop 4 (Saburi *et al.*, 2006). Another feature of SMDG is a relatively high level of transglucosylation (Saburi *et al.*, 2006), which would be applicable to oligosaccharide synthesis.

The substrate-free structure of *B. cereus* O16G is the only structure of the O16Gs and α -glucosidases available (Watanabe *et al.*, 1997). To gain a better understanding of SMDG and the exo-glucosidases from a structural point of view, we crystallized SMDG for X-ray structure analysis in order to obtain detailed information about its enzyme-substrate interactions.

2. Materials and methods

2.1. Protein production and purification

Recombinant SMDG was produced in *Escherichia coli* BL21(DE3) CodonPlus RIL (Stratagene, USA) with a pET-23d-derived expression system. For the recombinant SMDG, the Asn at the C-terminus of the native enzyme was replaced by Leu, Glu and a histidine tag. A transformant of *E. coli* containing the SMDG expression plasmid (Saburi *et al.*, 2006) was cultured in 1 l Luria-Bertani medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) containing 100 $\mu\text{g ml}^{-1}$ ampicillin and 50 $\mu\text{g ml}^{-1}$ chloramphenicol at 310 K until the absor-

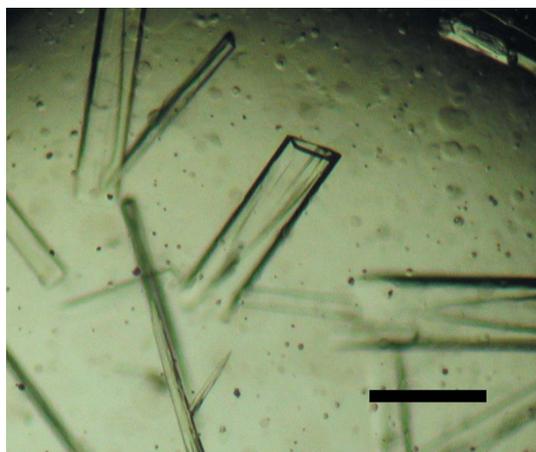


Figure 1
Crystals of dextran glucosidase. The longest dimension is approximately 1.0 mm. The length of the scale bar is 0.5 mm.

Table 1

Data-collection statistics for *S. mutans* dextran glucosidase.

Values in parentheses are for the highest resolution shell.

No. of crystals used	1
Wavelength (\AA)	1.5418
Resolution range	36.37–2.20 (2.32–2.20)
No. of reflections	189588
No. of unique reflections	32892
Space group	$P2_12_12_1$
Unit-cell parameters	
<i>a</i> (\AA)	72.72
<i>b</i> (\AA)	86.47
<i>c</i> (\AA)	104.30
Completeness (%)	96.8 (88.7)
$R_{\text{r.i.m.}}$ † (%)	6.8 (18.4)
Average $I/\sigma(I)$	26.8 (7.6)
Multiplicity	5.8 (4.7)

† $R_{\text{r.i.m.}} = \sum_{hkl} [N/(N-1)]^{1/2} \sum_i |I_i(hkl) - \overline{I(hkl)}| / \sum_{hkl} \sum_i I_i(hkl)$, where N is the redundancy and I_i is the intensity of the i th observation.

bance of the culture at 600 nm reached 0.5, meaning that the cells had entered the exponential phase of growth. Production of the recombinant enzyme was then induced with 0.1 mM isopropyl β -thiogalactoside (Wako Pure Chemical Industries, Japan) at 291 K for 24 h. Recombinant SMDG was purified by nickel-chelating chromatography with Chelating Sepharose FF (Amersham Biosciences, UK) from a cell-free extract prepared by sonication. DEAE Toyopearl chromatography was performed in 20 mM sodium phosphate buffer pH 6.2 for further purification, in which absorbed protein was eluted using a 0–0.5 M linear gradient of NaCl. Purified SMDG was dialyzed against 10 mM sodium acetate buffer pH 6.2.

2.2. Crystallization

Recombinant SMDG with a histidine tag was concentrated to 10 mg ml^{-1} in 10 mM sodium acetate buffer pH 6.2 by ultrafiltration with Centriprep YM30 filters (Millipore, USA) and subjected to crystallization trials. Crystallization was carried out by the hanging-drop vapour-diffusion method at 293 K. Cover slips coated with fluoro resin using New TFE coat spray (Fine Chemical Japan) were used to obtain suitable water repellency. Hampton Research Crystal Screen kits were used to determine the initial crystallization conditions. Drops consisting of 5 μl protein solution and 3 μl reservoir solution were equilibrated over 1 ml reservoir solutions. Crystals were obtained using solution No. 46 [100 mM sodium cacodylate pH 6.5, 200 mM calcium acetate hydrate, 18% (w/v) polyethylene glycol (PEG) 8000] of Crystal Screen I. The concentration of PEG and the pH of the buffer solution were first optimized and the molecular weight and concentration of PEG were then refined. After optimization, large rectangular-shaped crystals (1 \times 0.3 \times 0.1 mm) were obtained (Fig. 1). The reservoir solution consisted of 100 mM Tris-HCl buffer pH 7.5, 200 mM calcium chloride and 12% (w/v) PEG 6000. The dextran glucosidase crystals grew to full size within one week.

2.3. Data collection and processing

Crystals were soaked in cryoprotectant solution [50 mM Tris-HCl buffer pH 7.5, 200 mM calcium chloride, 20% (w/v) polyethylene glycol 6000, 30% (w/v) glycerol] before flash-cooling in a nitrogen-gas stream. A native data set was collected from a single crystal at 100 K using an R-AXIS IV⁺⁺ image-plate detector with Cu $K\alpha$ radiation from a Rigaku Ultra-X 18 rotating-anode generator equipped with an Osmic mirror system. A total of 180 images were collected with 1° oscillation. All data were processed using *MOSFLM* (Leslie, 1992)

and scaled using *SCALA* (Evans, 1993) from the *CCP4* suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The dextran glucosidase crystals diffracted to better than 2.2 Å resolution. The statistics of the corrected data are summarized in Table 1. A total of 189 588 measured reflections were merged into 32 892 unique reflections with an $R_{\text{r.i.m.}}$ (redundancy-independent merging R factor) of 6.8%. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 72.72$, $b = 86.47$, $c = 104.30$ Å. The Matthews coefficient calculation suggests the presence of one molecule in the asymmetric unit, with a V_M value of $2.6 \text{ \AA}^3 \text{ Da}^{-1}$ and a solvent content of 31% (Matthews, 1968). Structure determination is in progress using molecular replacement with the reported structure of the oligo-1,6-glucosidase from *B. cereus* (PDB code 1uok; Watanabe *et al.*, 1997) as a search model. The substrate-complex crystals will be prepared by cocrystallization and soaking methods.

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