HOKKAIDO UNIVERSITY

Title	Crystallization and preliminary crystallographic analysis of dextranase from Streptococcus mutans
Author(s)	Suzuki, Nobuhiro; Kim, Young-Min; Fujimoto, Zui et al.
Citation	Acta Crystallographica Section F: Structural Biology and Crystallization Communications, 67(12), 1542-1544 https://doi.org/10.1107/S1744309111038425
Issue Date	2011-12
Doc URL	https://hdl.handle.net/2115/47973
Type	journal article
File Information	ACF67-12_1542-1544.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

electronic reprint

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091
Editors: **H. M. Einspahr and M. S. Weiss**

Crystallization and preliminary crystallographic analysis of dextranase from *Streptococcus mutans*

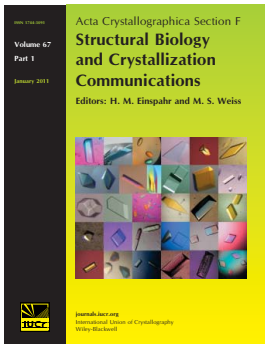
Nobuhiro Suzuki, Young-Min Kim, Zui Fujimoto, Mitsuru Momma, Hee-Kwon Kang, Kazumi Funane, Masayuki Okuyama, Haruhide Mori and Atsuo Kimura

Acta Cryst. (2011). F**67**, 1542–1544

Copyright © International Union of Crystallography

Author(s) of this paper may load this reprint on their own web site or institutional repository provided that this cover page is retained. Republication of this article or its storage in electronic databases other than as specified above is not permitted without prior permission in writing from the IUCr.

For further information see http://journals.iucr.org/services/authorrights.html



Acta Crystallographica Section F: Structural Biology and Crystallization Communications is a rapid all-electronic journal, which provides a home for short communications on the crystallization and structure of biological macromolecules. Structures determined through structural genomics initiatives or from iterative studies such as those used in the pharmaceutical industry are particularly welcomed. Articles are available online when ready, making publication as fast as possible, and include unlimited free colour illustrations, movies and other enhancements. The editorial process is completely electronic with respect to deposition, submission, refereeing and publication.

Crystallography Journals Online is available from **journals.iucr.org**

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary crystallographic analysis of dextranase from *Streptococcus mutans*

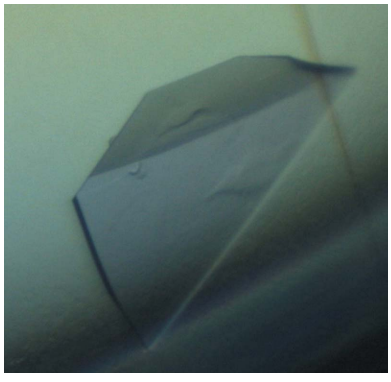
Nobuhiro Suzuki,[a]‡ Young-Min Kim,[a,b]‡§ Zui Fujimoto,[a]* Mitsuru Momma,[a] Hee-Kwon Kang,[b] Kazumi Funane,[c] Masayuki Okuyama,[b] Haruhide Mori[b] and Atsuo Kimura[b]

[a]Biomolecular Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan, [b]Research Faculty of Agriculture, Hokkaido University, Kita-9 Nishi-9, Kita-ku, Sapporo 060-8589, Japan, and [c]Applied Microbiology Division, National Food Research Institute, 2-1-12 Kannondai, Tsukuba 305-8642, Japan

‡ These authors contributed equally to this work.
§ Present address: Eco-Friendly Biomaterial Research Center and AI Control Material Research Center, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup-si, Jeonbuk 580-185, Republic of Korea.

Correspondence e-mail: zui@affrc.go.jp

Received 10 August 2011
Accepted 19 September 2011



© 2011 International Union of Crystallography
All rights reserved

Streptococcus mutans dextranase hydrolyzes the internal α-1,6-linkages of dextran and belongs to glycoside hydrolase family 66. An N- and C-terminal deletion mutant of *S. mutans* dextranase was crystallized by the sitting-drop vapour-diffusion method. The crystals diffracted to a resolution of 1.6 Å and belonged to space group $P2_1$, with unit-cell parameters a = 53.2, b = 89.7, c = 63.3 Å, β = 102.3°. Assuming that the asymmetric unit of the crystal contained one molecule, the Matthews coefficient was calculated to be 4.07 Å^3 Da^{-1}; assuming the presence of two molecules in the asymmetric unit it was calculated to be 2.03 Å^3 Da^{-1}.

1. Introduction

Dextranases (EC 3.2.1.11) hydrolyze the internal α-1,6-linkages of dextran in an endo reaction mode, producing isomaltooligosaccharides of various sizes. Based on the similarities in their amino-acid sequences, dextranases are mainly classified into glycoside hydrolase family (GH) 49 and GH 66 in the CAZY database (Henrissat & Bairoch, 1996; Cantarel *et al.*, 2009; http://www.cazy.org/). Dextranases belonging to GH 49 are found in bacteria and fungi, and a crystallographic study has revealed that the catalytic domain of Dex49A from *Penicillium minioluteum* is composed of a right-handed parallel β-helix (Larsson *et al.*, 2003). GH 66 enzymes consist of dextranases and cycloisomaltooligosaccharide glucanotransferases (CITases; EC 2.4.1.248) and these enzymes are mainly found in bacteria. Both enzymes utilize dextran as a substrate, but CITases synthesize cycloisomaltooligosaccharide (a cyclic saccharide linked by α-1,6-glucosyl units) from dextran by intramolecular transglycosylation (Oguma *et al.*, 1993, 1994; Funane *et al.*, 2011). Amino-acid sequence comparison among GH 66 enzymes revealed that they are composed of four regions: an N-terminal variable region, a conserved region, a glucan-binding region and a C-terminal variable region (Igarashi *et al.*, 2002, 2004; Morisaki *et al.*, 2002), together with a CITase-specific region which is only observed in CITases (Funane *et al.*, 2011). Although no three-dimensional structures of GH 66 enzymes have been determined, the similarity of the amino-acid sequence of the conserved region to members of GH 13, GH 27, GH 31 and GH 36 implies that GH 66 enzymes share a common $(\beta/\alpha)_8$-barrel core with these families (Rigden, 2002). GH 49 enzymes act with an inverting catalytic mechanism, but GH 66 enzymes, as well as GH 13, GH 27, GH 31 and GH 36 enzymes, are known to employ a retaining mechanism.

Dextranase from *Streptococcus mutans* (SmDex), a bacterium found in human dental plaque, is a well studied enzyme in GH 66. *S. mutans* synthesizes extracellular α-glucans from sucrose using glucosyltransferases and hydrolyzes the α-1,6-linkages in the glucans using SmDex to produce isomaltooligosaccharides for metabolic utilization (Walker *et al.*, 1981; Colby *et al.*, 1995). Several isoforms of SmDex, as well as other dextranases belonging to GH 66, have been detected (Igarashi *et al.*, 1995; Khalikova *et al.*, 2005) and our recent work has also shown that recombinant full-length SmDex (Ser25–Asp850; 95 kDa) expressed in *Escherichia coli* is susceptible to protease digestion after long storage (Kim *et al.*, 2011). Since the

electronic reprint

production of stable enzymes is a prerequisite for further biochemical studies and industrial applications, we have constructed a truncation mutant tolerant to protease digestion which is devoid of the N- and C-terminal variable regions (SmDexTM; Gln100–Ile732).

In biochemical studies of SmDex and two other GH 66 proteins, Asp385 of SmDex (Igarashi *et al.*, 2002), Asp270 of CITase from *Bacillus circulans* T3040 (Yamamoto *et al.*, 2006) and Asp243 of endodextranase from *Thermotoga lettingae* TMO (Kim & Kim, 2010) have been implicated as catalytic residues. However, the detailed catalytic mechanism of GH 66 enzymes is as yet unclear owing to the lack of three-dimensional structures. We recently reported that ω-epoxyalkyl α-glucopyranosides inactivate SmDex irreversibly, but the mechanism of this suicide inhibitor needs to be further elucidated (Kang *et al.*, 2010). In order to address these questions and to obtain a structural basis for protein engineering applicable for use in isomaltooligosaccharide production, we attempted the crystallization of SmDexTM.

2. Materials and methods

SmDexTM was overexpressed with the pET-28 expression system (Novagen, Madison, Wisconsin, USA) using *E. coli* BL21 (DE3) cells in LB medium by induction with 0.1 m*M* isopropyl β-D-1-thiogalactopyranoside overnight at 298 K as reported previously (Kim *et al.*, 2011). The protein was purified from the soluble fraction of the bacterial cell lysate by Ni^{2+}-charged HiTrap chelating HP column chromatography (GE Healthcare, Buckinghamshire, England) in a buffer consisting of 50 m*M* sodium phosphate pH 6.8, 300 m*M* NaCl, 200 m*M* imidazole. After desalting the solution using PD-10 column chromatography (GE Healthcare) into a buffer consisting of 20 m*M* Tris–HCl pH 8.0, the protein was further purified by anion-exchange chromatography using HiTrap Q-Sepharose HP (GE Healthcare) using a buffer consisting of 1 *M* NaCl, 20 m*M* Tris–HCl pH 8.0.

The protein solution was desalted and concentrated to 9.8 mg ml^{-1} by ultrafiltration using a YM-30 membrane (Millipore, Billerica, Massachusetts, USA) and filtered through a 0.1 µm membrane (Millipore). The pooled solution without buffer reagents was used in crystallization trials. Sparse-matrix crystal screening was performed using Crystal Screen HT, Index HT (Hampton Research, Aliso Viejo, California, USA), Wizard I and II and Cryo I and II (Emerald BioSystems, Bainbridge Island, Washington, USA). Sitting-drop vapour-diffusion trials were set up using the Thermo Scientific Matrix Hydra II (Thermo Fisher Scientific Inc., Hudson, New Hampshire, USA) automated liquid-handling system in 96-well Intelli-Plates (Art Robbins Instruments, Sunnyvale, California, USA) at 293 K using 50 µl reservoir solution; each drop consisted of 0.3 µl protein solution and 0.3 µl reservoir solution. Within two weeks, several tiny plate-shaped crystals were observed under several conditions using polyethylene glycol 600 or polyethylene glycol monomethyl ether 2000 as a precipitant. Crystallization conditions were optimized manually by refinement of the protein and/or precipitant concentrations and buffer solution using CrystalClear Strips 96-well sitting-drop plates (Douglas Instruments, Berkshire, England).

Native diffraction data were collected from a single crystal on beamline BL-17A of the Photon Factory (PF), High Energy Accelerator Research Organization, Tsukuba, Japan. The crystal was scooped in a nylon CryoLoop (Hampton Research) and then flash-cooled in a nitrogen-gas stream at 95 K. Diffraction data were collected with 5 s exposures for 1° oscillations over a total of 360° at a wavelength of 0.970 Å with a Quantum 270 CCD detector (ADSC, Poway, California, USA). Data were integrated and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

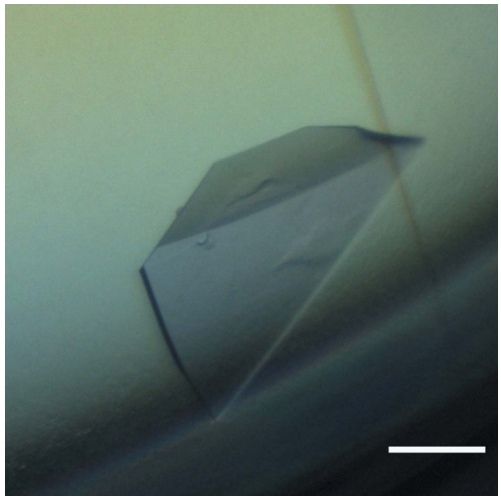


Figure 1
The crystal of SmDexTM. The scale bar represents 100 µm.

Table 1
Data-collection statistics for the SmDexTM crystal.

Values in parentheses are for the highest resolution shell.

Beamline	PF BL-17
Detector	ADSC Quantum 270
Wavelength (Å)	0.970
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 53.2, b = 89.7, c = 63.3, β = 102.3
Resolution range (Å)	50.0–1.6 (1.66–1.60)
R_{merge}†	0.063 (0.175)
Completeness (%)	100.0 (100.0)
Multiplicity	7.5 (7.5)
Average $I/\sigma(I)$	39.2 (11.5)
Unique reflections	76683 (7641)
Total reflections	571898
Mosaicity (°)	0.77
Overall B factor from Wilson plot (Å^2)	13.5

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all i observations of reflection hkl.

3. Results and discussion

We optimized the crystallization condition for SmDexTM, which was composed of 30%(*w*/*v*) polyethylene glycol monomethyl ether 2000, 100 m*M* phosphate–citrate buffer pH 4.2. The plate-shaped crystals were obtained in a drop consisting of 2 µl protein solution with a protein concentration of 3.6 mg ml^{-1} and 2 µl reservoir solution equilibrated against 50 µl reservoir solution at 293 K. The largest crystal grew to dimensions of 300 × 150 × 30 µm within one month (Fig. 1).

The crystal diffracted to a maximum resolution of 1.6 Å using synchrotron radiation at PF. The crystal belonged to space group $P2_1$, with unit-cell parameters a = 53.2, b = 89.7, c = 63.3 Å, β = 102.3°. The processing statistics of the collected data are summarized in Table 1. Assuming that the asymmetric unit of the crystal contained one SmDexTM molecule, the Matthews coefficient was calculated to be 4.07 Å^3 Da^{-1} (Matthews, 1968); assuming two molecules to be present in the asymmetric unit it was calculated to be 2.03 Å^3 Da^{-1}.

Although three-dimensional structures of GH 66 enzymes have not been reported, several crystal structures of enzymes belonging to

electronic reprint

GH 13, GH 27 and GH 31, which share a common catalytic $(\beta/\alpha)_8$-barrel, are available. We performed molecular-replacement calculations with the program *MOLREP* (Vagin & Teplyakov, 2010; Winn *et al.*, 2011) using polyalanine structures of the conserved regions, including those of two GH 13 α-amylases from *B. subtilis* (PDB entry 1bag; Fujimoto *et al.*, 1998) and from *Geobacillus stearothermophilus* (PDB entry 1hvx; Suvd *et al.*, 2001), two GH 27 α-galactosidases from rice (PDB entry 1uas; Fujimoto *et al.*, 2003) and from *Umbelopsis vinacea* (PDB entry 3a5v; Fujimoto *et al.*, 2009) and GH 31 α-xylosidase (PDB entry 1we5; Kitamura *et al.*, 2005). All of the trials gave a single solution, indicating that the crystal contains one SmDexTM molecule in the asymmetric unit, with a Matthews coefficient of 4.07 Å^3 Da^{-1} corresponding to 69.8% solvent content. However, the solutions were insufficient to solve the structure because of their low amino-acid sequence identities and the lack in these structures of the extra domains possessed by SmDexTM. We are currently preparing a selenomethionine-substituted protein crystal (17 methionines per molecule) for phase determination using the multiple anomalous dispersion technique.

We would like to thank the beamline researchers and staff at PF. This work was partially supported by the Program for Promotion of Basic and Applied Research for Innovations in Bio-oriented Industry (BRAIN) and a grant-in-aid from JSPS (Japan Society for the Promotion of Science) Fellows relating to a JSPS Postdoctoral Fellowship for Foreign Researchers (17-05467).

References

Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V. & Henrissat, B. (2009). *Nucleic Acids Res.* **37**, D233–D238.
Colby, S. M., Whiting, G. C., Tao, L. & Russell, R. R. (1995). *Microbiology*, **141**, 2929–2936.
Fujimoto, Z., Kaneko, S., Kim, W.-D., Park, G.-G., Momma, M. & Kobayashi, H. (2009). *Biosci. Biotechnol. Biochem.* **73**, 2360–2364.
Fujimoto, Z., Kaneko, S., Momma, M., Kobayashi, H. & Mizuno, H. (2003). *J. Biol. Chem.* **278**, 20313–20318.
Fujimoto, Z., Takase, K., Doui, N., Momma, M., Matsumoto, T. & Mizuno, H. (1998). *J. Mol. Biol.* **277**, 393–407.
Funane, K., Kawabata, Y., Suzuki, R., Kim, Y. M., Kang, H.-K., Suzuki, N., Fujimoto, Z., Kimura, A. & Kobayashi, M. (2011). *Biochim. Biophys. Acta*, **1814**, 428–434.
Henrissat, B. & Bairoch, A. (1996). *Biochem. J.* **316**, 695–696.
Igarashi, T., Morisaki, H. & Goto, N. (2004). *Microbiol. Immunol.* **48**, 155–162.
Igarashi, T., Morisaki, H., Yamamoto, A. & Goto, N. (2002). *Oral Microbiol. Immunol.* **17**, 193–196.
Igarashi, T., Yamamoto, A. & Goto, N. (1995). *Microbiol. Immunol.* **39**, 387–391.
Kang, H.-K., Kim, Y.-M., Nakai, H., Kang, M.-S., Hakamada, W., Okuyama, M., Mori, H., Nishio, T. & Kimura, A. (2010). *J. Appl. Glycosci.* **57**, 269–272.
Khalikova, E., Susi, P. & Korpela, T. (2005). *Microbiol. Mol. Biol. Rev.* **69**, 306–325.
Kim, Y.-M. & Kim, D. (2010). *Appl. Microbiol. Biotechnol.* **85**, 581–587.
Kim, Y.-M., Shimizu, R., Nakai, H., Mori, H., Okuyama, M., Kang, M.-S., Fujimoto, Z., Funane, K., Kim, D. & Kimura, A. (2011). *Appl. Microbiol. Biotechnol.* **91**, 329–339.
Kitamura, M., Ose, T., Okuyama, M., Watanabe, H., Yao, M., Mori, H., Kimura, A. & Tanaka, I. (2005). *Acta Cryst.* F**61**, 178–179.
Larsson, A. M., Andersson, R., Ståhlberg, J., Kenne, L. & Jones, T. A. (2003). *Structure*, **11**, 1111–1121.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Morisaki, H., Igarashi, T., Yamamoto, A. & Goto, N. (2002). *Lett. Appl. Microbiol.* **35**, 223–227.
Oguma, T., Horiuchi, T. & Kobayashi, M. (1993). *Biosci. Biotechnol. Biochem.* **57**, 1225–1227.
Oguma, T., Tobe, K. & Kobayashi, M. (1994). *FEBS Lett.* **345**, 135–138.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rigden, D. J. (2002). *FEBS Lett.* **523**, 17–22.
Suvd, D., Fujimoto, Z., Takase, K., Matsumura, M. & Mizuno, H. (2001). *J. Biochem.* **129**, 461–468.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Walker, G. J., Pulkownik, A. & Morrey-Jones, J. G. (1981). *J. Gen. Microbiol.* **127**, 201–208.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.
Yamamoto, T., Terasawa, K., Kim, Y.-M., Kimura, A., Kitamura, Y., Kobayashi, M. & Funane, K. (2006). *Biosci. Biotechnol. Biochem.* **70**, 1947–1953.

electronic reprint