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X-ray Crystallographic Study of Glucodextranase from a Gram-positive Bacterium, *Arthrobacter globiformis* I42

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Abstract: Glucodextranase (GDase) hydrolyzes α-1,6-glucosidic linkages of dextran from the non-reducing end to produce β-D-glucose. GDase is classified under GH15, whose major member is glucoamylase (GA) that hydrolyzes α-1,4-glucosidic linkages of starch. We have cloned a GDase gene from the Gram-positive bacterium *Arthrobacter globiformis* I42 and determined the crystal structure at 2.42-Å resolution. The structure of GDase is composed of four domains N, A, B and C. Domain N consists of 17 antiparallel β-strands and domain A forms an $(\alpha/\alpha)_6$ barrel structure, which is conserved between GAs. Furthermore, the complex structure with acarbose was also determined at 2.42-Å resolution. The structure of GDase complexed with acarbose revealed that the positions and orientations of the residues at subsites −1 and +1 are nearly identical for GDase and GA; however, Glu380 and Trp582 located at subsite +2, which form the entrance of the catalytic pocket, and the position of the open space and constriction of GDase are different from those of GAs. On the other hand, domains B and C are not found in GAs. The primary structure of domain C is homologous with the surface layer homology (SLH) of pullulanases from Gram-positive bacteria, and the three-dimensional structure of domain C resembles the carbohydrate-binding domain of some glycohydrolases. The hydrophobicity of domain B is higher than that of the other three domains. These findings suggest that domains B and C serve as cell wall anchors and contribute to the effective degradation of dextran at the cell surface.

Key words: glucodextranase, glucoamylase, glycoside hydrolase family 15, X-ray crystallography, S-layer homology

Dextran is a homo polysaccharide composed of α-D-glucose, in which the main chain is formed by α-1,6-glucosidic linkages and may be branched via α-1,2-, α-1,3- and α-1,4-glucosidic linkages. Dextran-hydrolyzing enzymes are divided into two groups based on their reaction patterns, endo- and exo-type dextranases. Endo-type dextranase [EC 3.2.1.11] finally produces isomaltose and isomaltotriose and is found in two glycoside hydrolase families (GH),[1)] 49 and 66, with no primary structural similarity.[2)] Each dextranase belonging to GH49 and GH 66 produces β- and α-anomeric products via a different catalytic mechanism. On the other hand, three types of exo-type dextranases have been reported, glucodextranase (GDase) [EC 3.2.1.70],[3)] isomalto-dextranase (IMD) [EC 3.2.1.94][4,5)] and dextran 1,6-α-isomaltotriosidase (IMTD) [EC 3.2.1.95],[6)] which produce only glucose, isomaltose and isomaltotriose by splitting the non-reducing end of dextran, and belong to GH 15, 27 and 49, respectively.

Glucodextranase (GDase) is an inverting exo-glucan hydrolase which hydrolyzes α-1,6-glucosidic linkages of dextran and related isomaltooligosaccharides from the non-reducing ends to release β-D-glucose. Sawai *et al*. described GDase from a Gram-positive bacterium *Arthrobacter globiformis* I42[3)] and enzymatic properties of GDase have been investigated.[7−9)] Oguma *et al*. isolated a GDase from a different strain, *A. globiformis* T-3044, for the purification of cyclodextrans in industrial production.[10)] The deduced primary structure of GDase from *A. globiformis* T-3044 shows 38% identity to a glucoamylase (GA) [EC 3.2.1.3] from *Clostridium* sp. G0005.[11)]

While GDase hydrolyzes α-1,6-glucosidic linkages of dextran from the non-reducing end, GA hydrolyzes α-1,4-glucosidic linkages of starch, glycogen and maltooligosaccharides from the non-reducing end to produce β-D-glucose. These two enzymes, GDase and GA, are classified under GH15 with clan-L. GDase and bacterial GAs have the ability to hydrolyze both α-1,4- and α-1,6-glucosidic linkages of small oligosaccharides like maltose and isomaltose,[11,12)] whereas fungal GAs scarcely hydrolyzes isomaltose. In this article, we introduce our recent achievement of X-ray crystallographic analysis of GDase,

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focusing on the molecular differences between GDase and GA and functions of the C-terminal region containing SLH.[13]

Primary structure analysis.

The GDase gene of *A. globiformis* I42 consists of 3147 bp (DDBJ/EMBL/GenBank™ database accession no. AB 033333) and, the deduced primary structure of the GDase gene is composed of 1049-aa residues. N-terminal analysis using a protein sequencer revealed that the first 29-aa residues were identified as a signal peptide and GDase composed of 1020-aa residues was secreted as a mature form. From the results of a homology search of the deduced primary structure, GDase was roughly divided into two regions of N (1−689) and C (690−1020)-terminal regions. The N-region shows high similarity to bacterial GAs from *Clostridium* sp. G0005[11] (36% in a 680-aa overlap) and *Thermoanaerobacterium thermosaccharolyticum*[14] (36% in a 660-aa overlap). Although the similarity of eukaryotic GAs, for example *Aspergillus awamori* var. X-100 and *Saccharomycopsis fibuligera*, was lower than that of bacteria (15 and 13% similarities, respectively), the five regions proposed for GA[15] were conserved in GDase. On the other hand, the C-terminal region of GDase shows similarity to that of amylopullulanase from *Thermus thermophilus* HB8 (GeneBank accession no. AP008226) (32% in a 244-aa overlap) and from *Thermococcus hydrothermalis*[16] (31% in a 227-aa overlap). These homologous sequences found in C-terminal regions of GDase and amylopullulanase contain surface layer homology (SLH), which has been reported to serve as a cell wall anchor.[17]

Three-dimensional structure of GDase.

Although the expression system using *Escherichia coli* was constructed, the expression level was extremely low, probably due to the high G+C content of the gene (69.4%) and the high molecular weight of GDase (120 kDa). In this study, we prepared GDase from a culture medium of *A. globiformis* I42 according to the method of Okada *et al*.[9] Crystals of GDase grew under the conditions of 1.5% PEG8000 and 40 mM KH_2PO_4 in 25 mM sodium acetate buffer (pH 5.1) using the hanging drop vapor diffusion method (Fig. 1A). The diffraction measurement was carried out at SPring8 (BL38B1) at 100 K in a cold nitrogen stream. The structure of GDase was solved by the molecular replacement method with GA form *T. thermosaccharolyticum* (TthGA)[18] as a search model.

The three-dimensional structure of GDase was finally determined at 2.42-Å resolution with overall dimensions of 118 Å×64 Å×82 Å, as shown in Fig. 1B.[13] The structure can be divided into four domains. The first domain (domain N) forms an anti-parallel β-sandwich containing 17 β-strands. One of the sheets was wrapped by an extended polypeptide chain containing the first 20 residues. The second domain (domain A), containing the catalytic site, forms an $(\alpha/\alpha)_6$ barrel. This domain is connected to domain N by a linker region consisting of two α-helices (237−252 and 253 and 271) that are connected by a short turn with 90°. Like GDase, enzymes of several GH families (8, 9, 15, 48, 65, 88 and 94) also have an $(\alpha/\alpha)_6$ barrel structure as their catalytic domains.

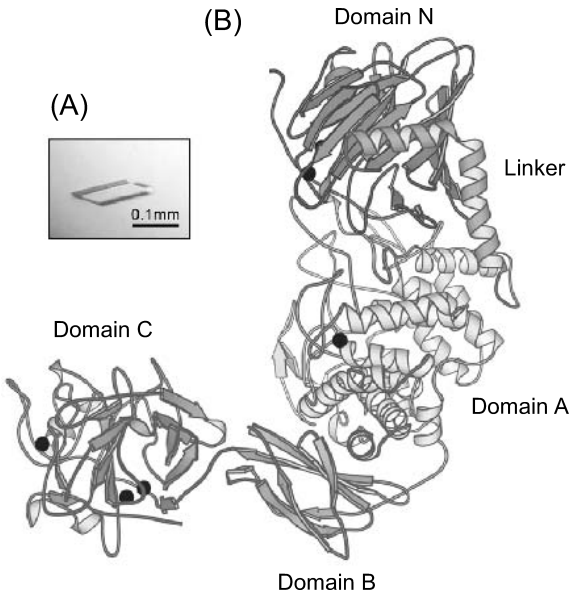

Fig. 1. Crystal structure of GDase from *A. globiformis* I42.

(A) Crystal of GDase. (B) Four domains, N, A, B and C are shown by different gray scales. The six bound calcium ions are shown as black spheres.

The third domain (domain B) consists of 8 antiparallel β-strands which resemble a type I immunoglobulin-like fold in which the first strand is divided between the two β-sheets due to a bulge caused by a *cis*-peptide bond. The fourth domain (domain C) consists of 17 β-strands and 3 short α-helices. The core structure of this domain is an antiparallel β-strand composed of a five-stranded sheet and a six-stranded sheet. GDase has six Ca^{2+} binding sites located at domains N, A and C, as shown in Fig. 1B. The F_o-F_c electron densities for all calcium ions were clearly observed at the contour level of 6 σ. The coordination of all the calcium ions is a tetragonal bi-pyramid structure with six coordinating atoms.

The structure of GDase in the N-terminal region, which is composed of domains N and A and two helix linkers, is similar to maltose phosphorylase from *Lactobacillus brevis* (PDB 1H54)[19] and chitobiose phosphorylase from *Vibrio proteolyticus* (PDB 1V7V),[20] which belong to GH65 and GH94, respectively. Although substrate specificities of these enzymes are absolutely different, these enzymes share domain configuration and are classified into clan-L.

Complex structure with acarbose.

Although acarbose is a mimic of α-1,4-glucan, the structure of the catalytic pocket of GDase was almost identical with that of glucoamylase. The inhibited activity of acarbose for dextran-hydrolyzing activity was measured and IC_{50} was 0.17 mM, which was the same inhibitory order as bacterial GA from *Thermoactinomyces vulgaris* R-47 (0.10 mM). Thus the crystals of GDase were soaked in a solution containing 1.0 mM acarbose for 10 min and then X-ray diffraction was collected at PF-AR (NW12). The complex structure was finally determined at 2.42-Å resolution by the molecular replacement method with native GA as a search model. The conformation of the whole structure was almost identical to that of the native structure (rms deviation is 0.8 Å). An electron density of trisaccharide unit occupied subsites −1, +1 and +2, ex-

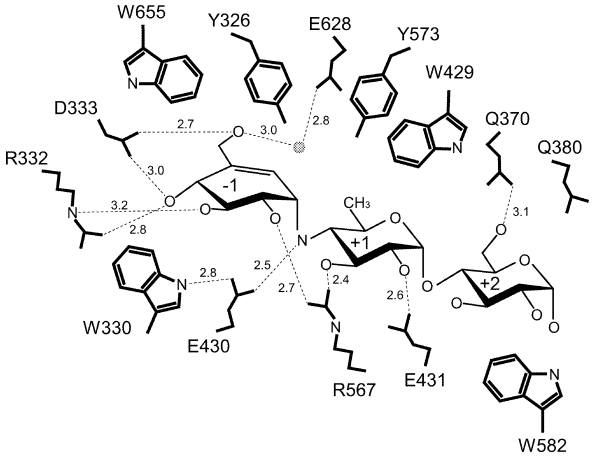

Fig. 2. Schematic representation of the interactions between acarbose and GDase.

The glucose unit of the reducing end was disordered in the complex structure. The water molecule is drawn as a sphere. The dotted lines show hydrogen bonds, and their distances are indicated in angstroms.

cept for a glucose unit at the reducing end of acarbose, whose electron density was disordered. The interactions between acarbose and GDase are shown in Fig. 2. The non-reducing end of acarbose (valienamine moiety) is taken into subsite −1 and tightly bound by hydrogen bonds with Arg332 and Asp333 located at the bottom of the active pocket. The substrate-binding pocket seemed to be roughly divided into two parts. One side, which mainly consists of Glu431, Gln370, Gln380 and a catalytic residue, Glu430, participates in the hydrophilic interaction between the enzyme and acarbose. The other side, containing the other catalytic residue, Glu628, is mainly composed of four aromatic amino acid residues, Tyr326, Tyr573, Trp655 and Trp582, which appear to contribute to the formation of a large hydrophobic wall. The carbohydrate unit (6-deoxyglucoside moiety) occupying subsite +1 makes a stacking interaction with the aromatic ring of Tyr573 and is further stabilized by hydrogen bonds between the O2 and O3 hydroxyl groups with Glu431 and Arg567 at distances of 2.6 and 2.4 Å, respectively. Although 6-deoxyglucose was bound closer to the hydrophobic wall in the acarbose complex, the subsite +1 has a comparatively wider space around the opposite side of the

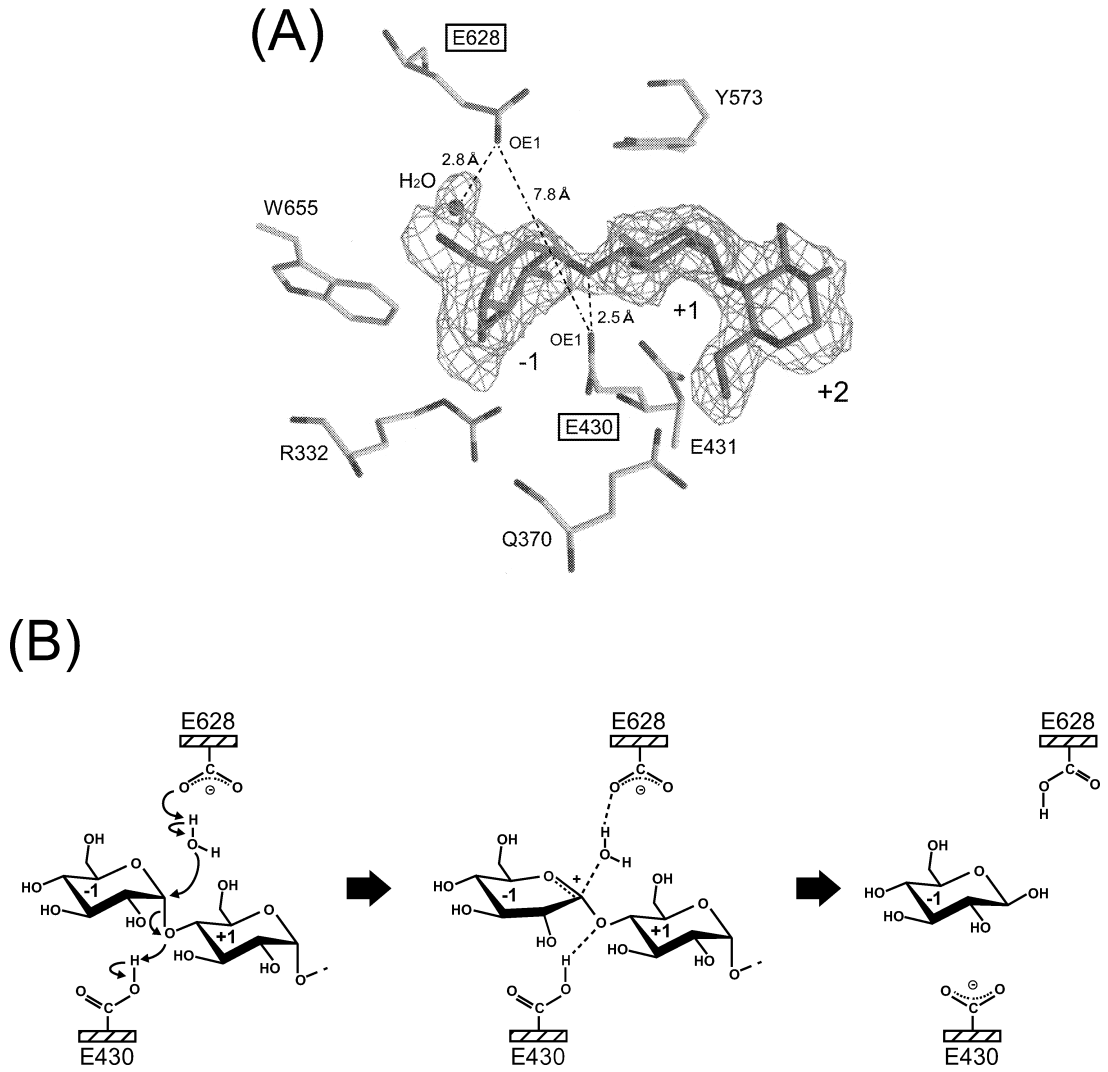

Fig. 3. Catalytic mechanism of GDase.

(A) $2F_o$–F_c electron density map at 1.0 σ of acarbose bound in the catalytic pocket. Catalytic residues, Glu430 and Glu628, are surrounded by a box. The distances between atoms show as dotted lines. (B) Scheme of the reaction mechanism of GDase via single replacement reaction mechanism.

hydrophobic wall for binding the substrate. The O6 hydroxyl group of the glucose moiety occupying subsite +2 makes only a weak hydrogen bond with Gln370 at a distance of 3.1 Å. Average B-factors of each carbohydrate moiety of acarbose are 26.5 Å^2 (valienamine moiety), 27.0 Å^2 (6-deoxyglucoside moiety) and 38.4 Å^2 (glucose moiety), which support the contention that the substrate affinity of subsite +2 is weaker than those of other subsites.

Catalytic mechanism.

The enzymatic hydrolysis of glucosidic linkages by glycoside hydrolases takes place via general acid catalysis that requires two critical residues, a proton donor and a nucleophile/base.[21] In the inverting enzymes, the distance between the side chains of the acidic residues is approximately 10 Å because of accommodation of a water molecule between the base and the sugar.[21] In GDase, the catalytic residues have not been confirmed by site directed mutagenesis or other methods. However, the primary structure analysis shows that two acidic residues identified as the catalytic residues in GAs are also conserved as Glu430 and Glu628 in GDase. In the structure complexed with acarbose, the side chain of Glu430 orients to form a hydrogen bond with the nitrogen atom of acarbose at distance of 2.5 Å. There is a putative nucleophilic water molecule which forms hydrogen bonds to Glu628 OE1 and to the 6OH of valienamine at subsite +1 (Fig. 3A). We conclude that the hydrolysis of GDase proceed via a single nucleophilic displacement. The protonation of glucosidic oxygen by Glu430 and the break of glucosidic linkage are accompanied by a concomitant nucleophilic attack of a water molecule activated by Glu628 at the anomeric carbon of subsite −1 (Fig. 3B).

Comparison of the catalytic pocket between GDase and GA.

The complex structure of GDase with acarbose was superimposed on those of a prokaryotic TthGA[17] and a eukaryotic AawGA.[22] The amino acid residues involved in subsites −1 and +1 are highly conserved among these three enzymes. However, two residues, Gln380 and Trp582, located at the entrance of the active pocket are relatively poorly conserved (Fig. 4). Gln380 of GDase does not directory interact with acarbose. In TthGA, Trp 390 corresponding to Gln380 of GDase forms subsite +2 and interacts with glucose unit +2 of acarbose through the stacking effect. In AawGA, an extended loop is inserted in the N-terminus of this region and protrudes from the entrance of the active pocket. Although the Cα position of Trp120 of AawGA is different from those of GDase and TthGA, the side chain of this residue is located in almost identical position as that of TthGA and stacks to glucose unit +2. Thus, in GAs, bulky aromatic side chains like tryptophan form subsite +2, and play an important role in the binding of α-1,4-glucan. On the other hand, Gln380 of GDase contributes to form a wide and shallow active pocket, which may be favorable for accommodating the α-1,6-glucosidic linkage of dextran. Trp582 of GDase is located at the opposite side of the active pocket to Gln380 and the top of the hydrophobic wall. The bulky side chain of Trp582 forms a constriction at the entrance of the active pocket. However, in TthGA, the constriction of this position is smaller than in GDase and no corresponding residue to Trp582 of GDase is seen in this region in AawGA (Fig. 4). It is likely that the differences around subsite +2 are responsible for the determination of the substrate specificities of these enzymes.

Predicted functions of domains B and C.

No bacterial GA has additional domains at the C-terminal end of the $(\alpha/\alpha)_6$ barrel domain, whereas some fungal GAs, for example AawGA, have a starch-binding domain (SBD) at the C-terminus.[23] In the case of GDase, the primary structure of the C-terminal region is weakly homologous with a surface layer homology (SLH) conserved in the C-terminal region of pullulanase from *Ther-*

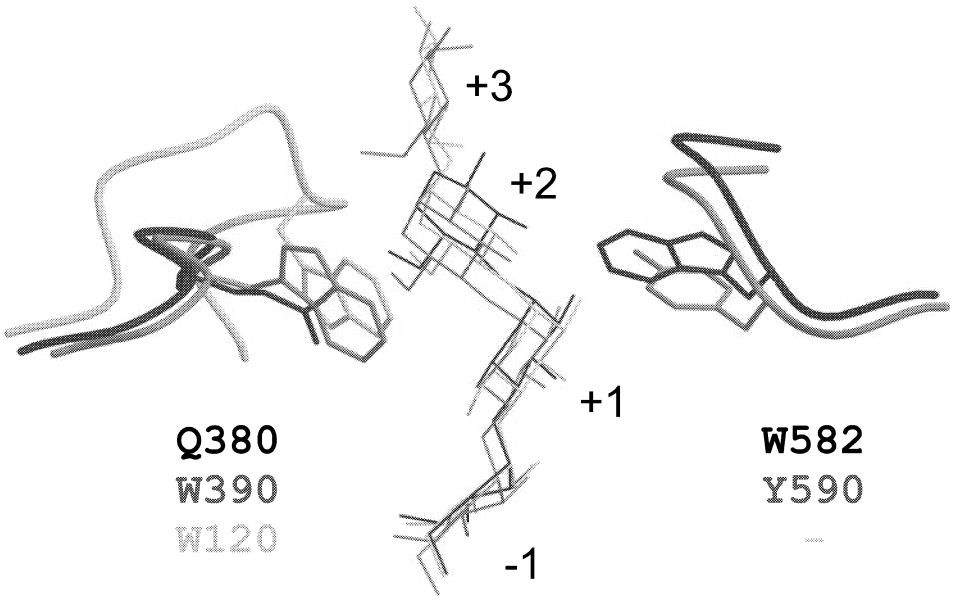

Fig. 4. Two distinctive residues located at subsite +2.

The interactions of GDase between these two residues and carbohydrate unit +2 are different from those of GAs. GDase, TthGA and AawGA are colored in black, dark gray and light gray, respectively.

mococcus hydrothermalis[15] and amylopullulanase from *Pyrococcus abyssi* (GenBank accession no. AJ248283) belonging to GH57. The SLH sequence has been reported to serve as a cell wall anchor, and has also found in other several exocellular proteins.[16] The outside of the peptidoglycan layer in some Gram-positive bacteria is covered by an S-layer that is a regular array of proteins or glycoproteins. The hydrophobicity of the S-layer is generally high (~40–60mol%).[24] The SLHs have also been identified from various exocellular carbohydrate polymer-metabolizing enzymes such as *Clostridium thermocellum* 1,4-β-cellobiohydrolase (GenBank accession no. M67438), *Bacillus* sp. strain KSM-635 alkaline cellulase (M27420), *Thermoanaerobacter saccharolyticum* endo-1,4-β-xylanase A (M97882), *Bacillus* sp. strain XAL601 α-amylase-pullulanase (D28467), and *Thermoanaerobacterium thermosulfurigenes* EM1 pullulanase (M57692).

In addition to the primary structural homologies, we also investigated the structural homologies of domains B and C. Dali structural similarity search using the three dimensional structure of GDase found numerous hits to various functional proteins for each domains B and C, respectively. Domain B especially resembles members of the immunoglobulin superfamily, most of which are found in immunoglobulins and adhesion proteins located on the cell surface. The function of these proteins is to bind to some other proteins by the hydrophobic interaction. The hydrophobicity of the primary structure of GDase was calculated using a Protscale tool at the ExPASy server. The profile showed that domain B is more hydrophobic than any other domain, N, A or C (Fig. 5A). These observations led us to the conclusion that domain B interacts with the S-layer via hydrophobicity. On the other hand, domain C shows weak homology to the carbohydrate-binding units of some glycosidases, endo-1,4-β-D-xylanase (PDB 1I82) and exo-1,4-β-D-glycanase (PDB 1EXG). The SLH domain of pullulanase from *T. thermosulfurigenes* EM1 binds accessory cell wall polymers, which are covalently bound to the peptidoglycan layer.[25] In *Bacillus stearothermophilus* PV/p2, the accessory cell wall polymer contains carbohydrates, *N*-acethylglucosamin and *N*-acethylmannosamin.[26] These findings suggest that domain C of GDase plays a part in attachment to cell surface as a carbohydrate-binding domain.

To our knowledge, this is the first time that the three-dimensional structure of a full-length polypeptide containing the SLH domain has been analyzed. Based on the picture depicted by Brechtel and Bahl,[25] we propose a model for the cell wall attachment via domains B and C of GDase (Fig. 5B), although the mechanisms of anchoring in the S-layer and the secretion outside *A. globiformis* I42 cells have not been investigated in detail. Domain B is buried in the S-layer, and the flexible linker located between domains A and B confers motion to the catalytic unit composed of domains N and A, which is capable of efficient hydrolysis of substrates located close to the cell surface. It is likely that domains B and C serve as cell wall anchors.

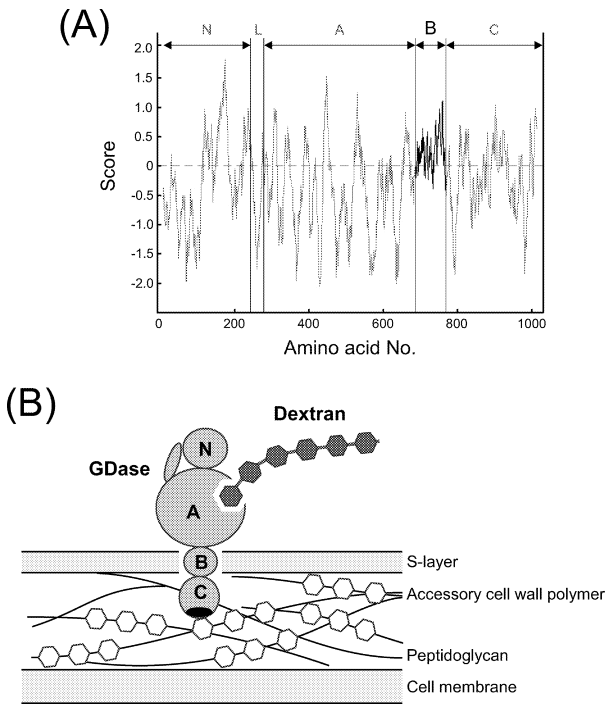

Fig. 5. Estimated function of domains B and C of GDase.

(A) Primary structure analysis of GDase. Diagram of hydrophobicity and hydrophobicity scales of the primary structure of GDase calculated by ProtScale (ExPASy). (B) Hypothetic model for the attachment of GDase to the cell surface via domains B and C. The drawing is not to scale. Because of the high hydrophobicity of domain B, it appears to be needed for passage through the S-layer. Domain C might be bound with secondary cell wall polymers in peptidoglycan, based on the model of Brechtel and Bahl.[26]

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REFERENCES

1) P.M. Coutinho and B. Henrissat: Carbohydrate-active enzymes: an integrated database approach. in *Recent Advances in Carbohydrate Bioengineering*, H.J. Gilbert, G. Davies, B. Henrissat, and B. Svensson, eds., The Royal Society of Chemistry, Cambridge, pp. 3–12 (1999).

2) H. Aoki and Y. Sakano: A classification of dextran-hydrolyzing enzymes based on amino-acid-sequence similarities. *Biochem. J*., **323**, 859–861 (1997).

3) T. Sawai, T. Yamaki and T. Ohya:. Purification and some properties of *Arthrobacter globiformis* exo-1,6-α-glucosidase. *Agric. Biol. Chem*., **40**, 1293–1299 (1976).

4) T. Sawai, K. Toriyama and K. Yano: A bacterial dextranase releasing only isomaltose from dextrans. *J. Biochem*., **75**, 105–112 (1974).

5) Y. Hatada, Y. Hidaka, Y. Nogi, K. Uchimura, K. Katayama, Z. Li, M. Akita, Y. Ohta, S. Goda, H. Ito, H. Matsui, S. Ito and K. Horikoshi: Hyper-production of an isomalto-dextranase of an *Arthrobacter* sp. by a proteases-deficient *Bacillus subtilis*: sequencing, properties, and crystallization of the recombinant enzyme. *Appl. Microbiol. Biotechnol*., **65**, 583–592 (2004).

6) T. Mizuno, H. Matsui, H. Ito, H. Mori, A. Kimura, M. Honma and S. Chiba: Reinvestigation of isomaltotrio-dextranase from *Brevibacterium fuscum* var. *dextranlyticum. J. Appl. Glycosci*., **43**, 347–353 (1996).

7) T. Ohya, T. Sawai, S. Uemura and K. Abe: Some catalytic properties of an exo-1,6-α-glucosidase (glucodextranase) from *Arthrobacter globiformis* I42. *Agric. Biol. Chem*., **42**, 571–577 (1978).
8) T. Takayanagi, G. Okada and S. Chiba: Quantitative study of the anomeric forms of isomaltose and glucose produced by isomalto-dextranase and glucodextranase. *Agric. Biol. Chem*., **51**, 2337–2341 (1987).
9) G. Okada, T. Unno and T. Sawai: A simple purification procedure for and further characterization of a glucodextranase from *Arthrobacter globiformis* I42. *Agric. Biol. Chem*., **52**, 2169–2176 (1988).
10) T. Oguma, T. Kurokawa, K. Tobe, S. Kitao and M. Kobayashi: Cloning and sequence analysis of the gene for glucodextranase from *Arthrobacter globiformis* T-3044 and expression in *Escherichia coli* cells. *Biosci. Biotechnol. Biochem*., **63**, 2174–2182 (1999).
11) H. Ohnishi, H. Kitamura, T. Minowa, H. Sakai and T. Ohta: Molecular cloning of a glucoamylase gene from a thermophilic *Clostridium* and kinetics of the cloned enzyme. *Eur. J. Biochem*., **207**, 413–418 (1992).
12) R. Uotsu-Tomita, T. Tonozuka, H. Sakai and Y. Sakano: Novel glucoamylase-type enzymes from *Thermoactinomyces vulgaris* and *Methanococcus jannaschii* whose genes are found in the flanking region of the α-amylase genes. *Appl. Microbiol. Biotechnol*., **56**, 465–473 (2001).
13) M. Mizuno, T. Tonozuka, S. Suzuki, R. Uotsu-Tomita, S. Kamitori, A. Nishikawa and Y. Sakano: Structural insights into substrate specificity and function of glucodextranase. *J. Biol. Chem*., **279**, 10575–10583 (2004).
14) A. Ducki, O. Grundmann, L. Konermann, F. Mayer and M. Hoppert: Glucoamylase from *Thermoanaerobacterium thermosaccharolyticum*: Sequence studies and analysis of the macromolecular architecture of the enzyme. *J. Gen. Appl. Microbiol*., **44**, 327–335 (1998).
15) P.M. Coutinho and P.J. Reilly: Structure-function relationships in the catalytic and starch binding domains of glucoamylase. *Protein Eng*., **7**, 393–400 (1994).
16) M. Erra-Pujada, P. Debeire, F. Duchiron and M.J. O'Donohue: The type II pullulanase of *Thermococcus hydrothermalis*: molecular characterization of the gene and expression of the catalytic domain. *J. Bacteriol*., **181**, 3284–3287 (1999).
17) A. Lupas, H. Engelhardt, J. Peters, U. Santarius, S. Volker and W. Baumeiste: Domain structure of the *Acetogenium kivui* surface layer revealed by electron crystallography and sequence analysis. *J. Bacteriol*., **176**, 1224–1233 (1994).
18) A.E. Aleshin, P.H. Feng, R.B. Honzatko and P.J. Reilly: Crystal structure and evolution of a prokaryotic glucoamylase. *J. Mol. Biol*., **327**, 61–73 (2003).
19) M.P. Egloff, J. Uppenberg, L. Haalck and H. van Tilbeurgh: Crystal structure of maltose phosphorylase from *Lactobacillus brevis*: unexpected evolutionary relationship with glucoamylases. *Structure*, **9**, 689–697 (2001).
20) M. Hidaka, Y. Honda, M. Kitaoka, S. Nirasawa, K. Hayashi, T. Wakagi, H. Shoun and S. Fushinobu: Chitobiose phosphorylase from *Vibrio proteolyticus*, a member of glycosyl transferase family 36, has a clan GH-L-like $(\alpha/\alpha)_6$ barrel fold. *Structure*, **12**, 937–947 (2004).
21) G. Davies and B. Henrissat: Structures and mechanisms of glycosyl hydrolases. *Structure*, **3**, 853–859 (1995).
22) A.E. Aleshin, L.M. Firsov and R.B. Honzatko: Refined structure for the complex of acarbose with glucoamylase from *Aspergillus awamori* var. *X100* to 2.4 Å resolution. *J. Biol. Chem*., **269**, 15631–15639 (1994).
23) R. Evans, C. Ford, M. Sierks, Z. Nikolov and B. Svensson: Activity and thermal stability of genetically truncated forms of *Aspergillus* glucoamylase. *Gene*, **91**, 131–134 (1990).
24) U.B. Sleytr: I. Basic and applied S-layer research: an overview. *FEMS Microbiol. Rev*., **20**, 5–12 (1997).
25) E. Brechtel and H. Bahl: In *Thermoanaerobacterium thermosulfurigenes* EM1 S-layer homology domains do not attach to peptidoglycan. *J. Bacteriol*., **181**, 5017–1023 (1999)
26) W. Ries, C. Hotzy, I. Schocher, U.B. Sleytr and M. Sára: Evidence that the N-terminal part of the S-layer protein from *Bacillus stearothermophilus* PV72/p2 recognizes a secondary cell wall polymer. *J. Bacteriol*., **179**, 3892–3898 (1997).

グラム陽性菌 *Arthrobacter globiformis* 由来グルコデキストラナーゼの X 線結晶構造解析

水野正浩[1]，殿塚隆史[1,2]，市川和洋[2]，神鳥成弘[3]
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グルコデキストラナーゼ（GDase）は，デキストランのα-1,6-グルコシド結合を非還元末端側からエキソ型に加水分解して，β-D-グルコースを生成するアノマー反転型酵素であり，グルコアミラーゼ（GA）と共に GH ファミリー 15 に分類される．われわれはグラム陽性菌 *Arthrobacter globiformis* I42 株より GDase のクローニングを行い，2.42 Å 分解能での X 線結晶構造を決定した．GDase は N 末端に 17 本の β-ストランドからなるドメイン N と GA と同様に $(\alpha/\alpha)_6$ バレル構造からなる活性ドメイン A を有していた．さらに，GDase の C 末端領域には β-ストランドに富んだ二つのドメイン B と C が付加されていた．また，阻害剤アカボースとの複合体構造を 2.42 Å 分解能で決定した．GA のアカボース複合体構造と比較した結果，両酵素ではサブサイト−1 と+1 におけるアカボースとの相互作用はほぼ同じであったが，触媒ポケットの入り口に相当するサブサイト+2 の構造に大きな違いがあった．GDase ではサブサイト+2 は Gln370 のみが水素結合を形成しているが，GA では Try が位置しておりサブサイト+2 のグルコース環と相互作用をしている．一方，活性部位を挟んで Gln370 の反対側に位置する Trp582 はアカボースとはスタッキングしていないが活性部位の内側に向かって突き出しているが，GA ではこの張り出しは存在しない．このように，活性部位入り口近傍の違いが，GDase と GA 基質特異性の違いの一つになっていることが考えられた．ドメイン C にはグラム陽性細菌の表層に存在する S-layer に結合するのに必要な S-layer 相同性配列(SLH)が認められるが，ドメイン B の高い疎水性やドメイン C の糖結合ドメインとの相同性から，これらのドメインが菌体表層への結合に何らかの関与を示すことが示唆された．

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〔質　問〕　林原生化研　久保田

1）膜への結合についての実験データはあるのか？

2）基質結合について，マルトトリオース，パノース，イソパノース，イソマルトトリオースの実験結果と構造との結果が一致するのか？

〔答〕

1）現段階では得ておりません．今後の課題として考えております．

2）現在，それらの基質を用いて解析を行っている最中ではあります．各基質への認識に最も関与していると思われるのがGln380であると考えると構造的なデータと一致いたしますが，詳細につきましては別途論文にまとめているところであります．

〔質　問〕　食総研　北岡

1）マルト系オリゴ糖とイソマルト系オリゴ糖の違いは，結合様式(プロダクティブとノンプロダクティブ）で説明されるのか？

2）酵素はどの程度培地に出ているのか？多量に培地に出ていることと菌体結合であるという結論に矛盾しないか？

〔答〕

1）廣海先生のサブサイト親和力に基づくイソマルト系オリゴ糖とマルト系オリゴ糖の違いはまだ調べておりません．

2）菌体に結合した量を測っていないため，定量的なお答えをすることができませんが，他のグラム陽性細菌でも菌体結合型のものが培養上清に分泌されている例もありますので，生産される全ての酵素が菌体結合型ではないと考えております．