Crystal Structure of GH54 α -L-Arabinofuranosidase and Unique Function of CBM42 Attached to It

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Abstract: As the first known structures of a glycoside hydrolase family 54 enzyme, we determined the crystal structures of free and arabinose-complex forms of α -L-arabinofuranosidase from *Aspergillus kawachii* IFO 4308 (AkAbf54). AkAbf54 comprises two domains: a catalytic domain and an arabinose-binding domain. The catalytic domain has a β -sandwich fold slightly similar to those of clan-B glycoside hydrolases. The arabinose-binding domain has a β -trefoil fold similar to that of carbohydrate-binding module (CBM) family 13. However, the arabinose-binding domain shows a number of distinctive characteristics from those of CBM family 13. Therefore, it was classified into a new CBM family, CBM42, and was referred to as AkCBM42. In the arabinose-complex structure, one of three arabinofuranose molecules bound to the catalytic domain through many interactions. Interestingly, a disulfide bond formed between two adjacent cysteine residues recognized the arabinofuranose molecule. From the location of this arabinofuranose and the results of a mutation study, the nucleophile and acid/base residues were determined to be Glu221 and Asp297, respectively. The other two arabinofuranose molecules bound to AkCBM42. The O1 atoms of both these arabinofuranose molecules are exposed to the solvent, indicating that AkCBM42 binds arabinofuranose residues linked to the xylan backbones of arabinoxylans. Binding assay and affinity gel electrophoresis analysis with insoluble polysaccharides, and ITC analysis with mono- or oligosaccharides revealed a unique function of AkCBM42. This is the first example of a CBM that can specifically recognize the side-chain moieties of branched polysaccharides.

Key words: crystallography, glycoside hydrolase family 54, α -L-arabinofuranosidase, arabinose-binding domain, carbohydrate-binding module family 42

The plant cell wall has a composite structure mainly consisting of celluloses, hemicelluloses, and lignins. Degradation of cellulose and hemicellulose is dependent on carbohydrate-active enzymes, such as glycoside hydrolases (GHs). GHs have been classified into about 100 families based on amino acid sequence similarity (Carbohydrate Active enZymes server at <http://www.cazy.org/CAZY>).¹⁾ GHs are frequently associated with comparatively small non-catalytic carbohydrate-binding modules (CBMs) besides the catalytic domain. CBMs enhance the hydrolysis of insoluble carbohydrates, such as celluloses and hemicelluloses, by binding them.²⁾ CBMs keep the catalytic domain of enzymes in proximity with the carbohydrates through their sugar-binding activity. At present, they are classified into more than 40 families based on sequence and structural similarity (See CAZy website).¹⁾ α -L-Arabinofuranosidases (EC 3.2.1.55) catalyze the hydrolysis of α -1,2-, α -1,3- and α -1,5-L-arabinofuranosidic bonds in L-arabinose-containing hemicelluloses such as arabinoxylan and L-arabinan.^{3,4)} Arabinoxylan, a major component of hemicellulose, has a β -1,4-linked xylopyranose backbone, and generally contains heterogeneous substituents such as L-arabinose, *O*-acetyl, ferulic acid, *p*-coumaric acid and 4-*O*-methylglucuronic acid.⁵⁾

Arabinan has an α -1,5-linked arabinose backbone that is branched via α -1,2- or α -1,3-linked arabinofuranose side-chains.⁶⁾ α -L-Arabinofuranosidases belong to five GH families, 3, 43, 51, 54 and 62. The enzymatic hydrolysis of glycosidic bonds causes retention or inversion of the anomeric configuration. GH3, GH51 and GH54 are retaining enzymes, and GH43 inverting ones.^{7,8)}

Aspergillus kawachii is an industrially important fungus in *shochu* (traditional Japanese liquor) brewing. *A. kawachii* IFO4308 produces various hemicellulases including an endo- β -1,4-xylanase,⁹⁾ feruloyl esterase and two different α -L-arabinofuranosidases, GH51 arabinofuranosidase A (AkAbf51) and GH54 arabinofuranosidase B (AkAbf54).¹⁰⁾ AkAbf51 and AkAbf54 act synergically with xylanase in the degradation of arabinoxylan. These two enzymes also help the release of ferulic acid, which are converted to the phenolic flavor compounds in *shochu* brewing,¹¹⁾ by feruloyl esterase. In fact, these arabinofuranosidases contribute to the degradation of cereals and formation of aroma in *shochu* brewing. AkAbf51 and AkAbf54 are acidophilic enzymes which have an optimum pH of 4.0 and are stable at temperature up to 60°C. The substrate specificities of α -L-arabinofuranosidases I and II (GH51 AwAbfI and GH54 AwAbfII) from *Aspergillus awamori* IFO 4033, which are highly homologous to AkAbf51 and AkAbf54, respectively, have been elucidated.³⁾ Both enzymes exhibited broad specificity, acting

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on α -1,2-, α -1,3- and α -1,5-L-arabinofuranosidic bonds. However, AwAbfI preferentially hydrolyzed the α -1,5-linkage of branched arabinotrisaccharide, whereas AwAbfII preferentially hydrolyzed an α -1,3-linkage in the same substrate and hydrolyzed the arabinosyl side-chain linkage of arabinan. As to GH51, the crystal structure and catalytic residues of *Geobacillus stearothermophilus* T-6 α -L-arabinofuranosidase A (GsAbfA) have been determined.¹²⁾ On the other hand, the catalytic residues and 3-D structure of GH54 enzymes had been completely unknown, until we reported the AkAbf54 crystal structures.^{13,14)}

Crystallography.

Recombinant AkAbf54 protein without the native signal sequence region was expressed in *Escherichia coli* BL21 (DE3) CodonPlus RIL (Stratagene) and *Pichia pastoris* GS115 (Invitrogen). *E. coli* cells, however, did not produce sufficient AkAbf54 enzyme for crystallization. On the other hand, *P. pastoris* cells produced a sufficient amount of the enzyme. The samples produced from *E. coli* and *P. pastoris* migrated as single bands on SDS-PAGE corresponding to approximately 60 and 64 kDa, respectively. The band of the sample from *P. pastoris* was stained red with the periodic acid-Schiff base (PAS) stain method,¹⁵⁾ which detects protein glycosylation. The result indicates that the recombinant AkAbf54 expressed in *P. pastoris* has glycosyl chains at both of its potential *N*-glycosylation sites (Asn83 and Asn202). The contribution of each *N*-glycosyl chain to the thermostability was examined by using site-directed mutagenesis of two asparagine residues (Koseki, unpublished results). N83Q mutant enzyme did not affect the thermostability. On the other hand, N202Q mutant enzymes showed a considerable decrease of thermostability.

The recombinant AkAbf54 produced by *P. pastoris*

was used for subsequent crystallization experiments. AkAbf54 native crystals were grown with a reservoir solution comprising 30% PEG 6000 and 0.1 M MES-NaOH buffer (pH 6.0). The AkAbf54 native structure was determined at 1.75 Å, using xenon, platinum and mercury derivatives for the MIR phase determination. The crystal belongs to space group $P2_12_12_1$, and contains one monomer of AkAbf54 per asymmetric unit. Complex crystals with arabinose and arabinofuranosyl- α -(1,2)-xylobiose (AXX) complex were obtained by soaking native crystals in 50 mM L-arabinose for 90 min and 10 mM AXX for 30 min, respectively (Miyanaga, unpublished results for AXX complex). The complex structures with arabinose and AXX were determined at 2.07 and 2.3 Å, respectively.

Overall structure.

AkAbf54 is an almost all- β protein and is organized into two domains: an N-terminal catalytic domain (residues 18–335) and a C-terminal arabinose-binding domain (residues 336–499) (Fig. 1). The catalytic domain and arabinose-binding domain are connected to each other by many interactions. In the complex structure with arabinose, three arabinofuranose molecules were found: one (Araf1) in the catalytic domain (Fig. 2A) and two (Araf2 and Araf3) in the arabinose-binding domain (Fig. 2B and 2C). There are three disulfide bonds (Cys21–Cys31, Cys81–Cys86 and Cys176–Cys177) in the catalytic domain and one (Cys401–Cys439) in the arabinose-binding domain. The electron density of two *N*-acetylglucosamine units linked to Asn202 has been clearly visible. This glycoside chain is interacted with the arabinose-binding domain, consistent with the result that it contributes to the thermostability. On the other hand, the electron density linked with Asn83 was not observed.

Fig. 1. Overall structure of the AkAbf54 in a complex with arabinose.

The catalytic domain and arabinose-binding domain (AkCBM42) are shown in light and dark gray, respectively. Three arabinofuranose molecules are shown as black spheres. Asn202 and the glycoside chain are shown as gray wireframe models.

Fig. 2. Electron density maps of AkAbf54.

F_o-F_c electron density maps were constructed prior to incorporation of each ligand into the model structures. (A) F_o-F_c electron density map at the 3σ contour level in the catalytic center. (B) F_o-F_c electron density map at the 4σ contour level in the β sub-domain pocket of AkCBM42. (C) F_o-F_c electron density map at the 4σ contour level in the γ sub-domain pocket of AkCBM42. (D) $2F_o-F_c$ electron density map at the 2σ contour level around the two adjacent cysteine residues.

Catalytic domain.

The catalytic domain possesses a β -sandwich fold (Fig. 1). The β -sandwich fold consists of two anti-parallel β sheets with seven and six strands and one extra anti-parallel β sheet with seven strands. In addition, there are four helices outside the β -strands. The β -sandwich strands are closely packed and curved with a jelly-roll topology, creating a concave catalytic pocket. Acidic residues in this pocket make it highly charged to negative. The catalytic domain of AkAbf54 shows slight structural similarity with clan-B GHs, *e.g.* GH16 κ -carrageenase¹⁶ and GH7 cello-biohydrolase.¹⁷ Both enzymes have a long substrate binding tunnel. When AkAbf54 is superimposed on these enzymes, the substrate binding pocket of AkAbf54 lies near these substrate binding tunnels. Both the catalytic residues of AkAbf54 (Glu221 and Asp297, described later) are located in this pocket. However, one of the catalytic residues (Asp297) does not superimpose on any of the clan-B enzymes, although the other catalytic residue (Glu221) does. Moreover, AkAbf54 does not contain the E-X-D-X-X-E motif of the clan-B enzymes. Thus, the catalytic domain of GH54 AkAbf54 is fairly distinct from those of clan-B enzymes, but they may have evolutionary relationships.

Active site.

In the complex structure of AkAbf54 with arabinose, one arabinofuranose molecule (Araf1) was found in the negatively charged pocket of the catalytic domain. Because AkAbf54 cleaves α -1,2- and α -1,3-arabinofuranosidic bonds, this site is assigned as subsite -1. Araf1 was recognized due to its many hydrogen bonds and hydrophobic interactions with AkAbf54 (Fig. 3A). The +1 subsite appears to be located outside of the catalytic pocket, although it could not be confirmed. The side-chains of Glu221 and Asp297 are located on either side of the anomeric C1 carbon of Araf1, indicating that they are catalytic residues (Fig. 3A). As expected for a retaining enzyme, these two residues are separated by 5.6 Å. The E221A mutant showed no detectable activity, but the D297A mutant exhibited 10^{-3} fold the activity. When the active sites of AkAbf54 and GH51 GsAbfA¹² were superimposed, the relative positions of arabinofuranose and the two catalytic residues were the same (Fig. 3B). Therefore, we conclude that Glu221 is the nucleophile and Asp297 is the acid/base residue. It is interesting that a disulfide bond between adjacent cysteine residues (Cys176 and Cys177) is formed with an uncommon *cis*-peptide bond between them (Fig. 2D). This disulfide bond seems to recognize the C4/C5 atoms of Araf1 through hydrophobic interaction. In GH51 GsAbfA,¹² a tryptophan side-chain replaces this disulfide bond, and similarly recognizes the carbon atoms of arabinofuranose through hydrophobic interaction. A disulfide bond between adjacent cysteines joined by a *cis*-peptide bond has been rarely reported, *i.e.* only for methanol dehydrogenase,¹⁸ and human ribonuclease inhibitor.¹⁹ An analogous example is the interaction between a disulfide bond formed by Cys241-Cys242 of GH43 arabinanase A from *Cellvibrio japonicus* and the arabinose moiety of the bound arabinan.²⁰ The C1 atom of arabinose is located near the S γ atoms of Cys241 and

Fig. 3. The active sites of AkAbf54 and GH51 GsAbfA.

The O1 atom of the arabinofuranose molecule in AkAbf54 is omitted, because its electron density was not observed. (A) The catalytic center of AkAbf54 in a complex with arabinofuranose. Hydrogen bonds are shown as dotted lines. (B) Superimposition of AkAbf54 in a complex with Araf1 (light gray) and GsAbfA (heavy gray) with Ara- α (1,3)-Xyl. GsAbfA in a complex with Ara- α (1,3)-Xyl was constructed by combining the structures of the native enzyme (1PZ3) and Ara- α (1,3)-Xyl complex (1QW8).

Cys242. However, these two adjacent cysteines are connected by normal *trans*-peptide bonds.

Arabinose-binding domain (AkCBM42).

The arabinose-binding domain possesses a β -trefoil fold (Fig. 1), having an internal triple repeated sequence, and comprises three sub-domains (α sub-domain: 348–399, β sub-domain: 400–446, and γ sub-domain: 447–499) (Fig. 4A). Each sub-domain is assembled with a pseudo-3-fold-axis, so there is structural similarity between each pair of them. The side-chains of hydrophobic residues of each sub-domain assemble at the center of the β -trefoil fold, forming a hydrophobic core. Such a structural scaffold of the arabinose-binding domain is similar to that of CBM13.²¹ However, three different characteristics between CBM13 and the arabinose-binding domain were found. First, the arabinose-binding domain does not contain the G-X-X-Q-X(W/Y) motif of CBM13 (Fig. 4A). Second, tyrosine residues are located at the position of the comparatively conserved disulfide bond of CBM13 in each sub-domain. Third, the sugar binding sites of

Fig. 4. CBM42 and CBM13.

(A) Alignment of the amino acid sequences of AkCBM42 and CBM13 from *S. lividans*. Arrows indicate the residues forming the hydrophobic core. Triangles indicate G-X-X-XQX(W/Y) motif. Cysteine residues forming disulfide bonds and residues involved in substrate binding are highlighted in light and dark gray, respectively. (B) A ribbon drawing diagram of AkCBM42. Araf2 and Araf3 are shown as black wireframe models. (C) A ribbon drawing diagram of CBM13 from *S. lividans*. The xylobiose molecules are shown as black wireframe models.

arabinose-binding domain are completely different from those of CBM13 (described later). Considering these points, the arabinose-binding domain, the non-catalytic β -trefoil domain of GH54, was classified as a new CBM, CBM42. Therefore, this arabinose-binding domain is referred to as AkCBM42. All known GH54 open reading frames are expected to contain CBM42, because they exhibit high amino acid sequence similarity in their C-terminal regions. In the CAZy database, CBM42 can also be found in the members of GH43, SAV1043 and SAV1115 in the genome sequence of *Streptomyces avermitilis*.

Arabinose binding sites in AkCBM42.

In the complex structure of AkAbf54 with arabinose, two arabinofuranose molecules, Araf2 and Araf3, were found in pockets of the β and γ sub-domains of AkCBM42, respectively, but not in the α sub-domain. Araf2 and Araf3 are strictly recognized in the same manner (Fig. 2B and 2C). An aspartate residue, Asp435 (β sub-domain) or Asp488 (γ sub-domain), forms hydrogen bonds with the O2 and O3 atoms. A histidine residue forms a hydrogen bond with the O5 atom. Moreover, arabinofuranose is stacked between two tyrosines. In the α sub-domain pocket, the residues corresponding to the aspartate and two tyrosines are replaced by one glutamate and two threonines, respectively (Fig. 4A). This replacement seems to prevent an arabinofuranose molecule from binding to the α sub-domain pocket. The locations of the sugar binding sites of AkCBM42 are shifted about 8 Å in the direction of the next sub-domain and the structures around them are completely different, compared with those of CBM13.²²⁻²⁴⁾ The O1 atoms of both Araf2 and Araf3 were exposed to the solvent. Therefore, AkCBM42 was presumed to bind arabinose residues linked to the xylan backbones of arabi-

noxylans with two binding sites.

In the complex structure of AkAbf54 with AXX, two AXX molecules were found in pockets of the β and γ sub-domains of AkCBM42. The arabinose moiety occupies the binding pocket of AkCBM42, while the xylobiose moiety is exposed to the solvent. The mode of recognition of the arabinose moiety of AXX is similar to that found for the complex structure with arabinose. Asp435 and Asp488 interact with the arabinose moiety through bifurcated hydrogen bonds, like in the arabinose complex structure. On the other hand, the xylobiose moiety is weakly interacted with AkCBM42. The mode of binding of AXX to AkCBM42 also indicates that AkCBM42 specifically recognizes side-chain arabinose of arabinoxylan.

Characterization of AkCBM42.

To evaluate the role of AkCBM42, mutant proteins, D435A, D488A and D435A/D488A, were produced, and their catalytic activities toward an insoluble substrate, wheat arabinoxylan, were compared with that of wild-type AkAbf54 (Miyanaga, unpublished results). The catalytic activities of these mutants against wheat arabinoxylan were significantly decreased. This result shows that AkCBM42 facilitates the hydrolysis of insoluble arabinoxylan. ITC analysis showed that the association constants (K_a) of both binding sites against an arabinooligosaccharide are measurable but very low, being about $2 \times 10^3 \text{ M}^{-1}$. On the other hand, it exhibited no detectable affinity to xylo-oligosaccharides. The results of binding assay and affinity gel electrophoresis showed that AkCBM42 interacts with arabinose-substituted hemicelluloses but not with unsubstituted ones. These results reinforce the idea that AkCBM42 binds to the non-reducing-end arabinose moiety of hemicellulose. A number of

CBMs that can recognize the hemicellulose main-chain, or a part of it including the side-chain, has been reported.^{2,24)} To our knowledge, however, no CBM similar to CBM42, which specifically recognizes the side-chain moiety of branched polysaccharide, is known to date.

Classification of CBM42.

A classification of CBMs based on structural and functional similarities, in which these protein modules are grouped into three types,²⁾ has been proposed. Type A CBMs have a flat hydrophobic surface to interact with adjacent chains on the surface of crystalline cellulose. Type B CBMs have a cleft to accommodate the single glycan chains of poly/oligosaccharide ligands. These CBMs show relatively high affinity with an association constant of more than 10^5 . On the other hand, Type C CBMs have surface pockets and indentations to accommodate oligosaccharide ligands. These CBMs exhibit the lectin-like property of binding optimally to mono-, di- or tri-saccharides with relatively weak affinity. In this context, AkCBM42 could be an extreme example of Type C, because it recognizes a monosaccharide unit only. Moreover, it exhibits novel ligand specificity; specific binding to the side-chain of branched polysaccharides. It might be appropriate to categorize it as a subclass of Type C, for example, Type C'.

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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) A.B. Boraston, D.N. Bolam, H.J. Gilbert and G.J. Davies: Carbohydrate-binding modules: fine-tuning polysaccharide recognition. *Biochem. J.*, **382**, 769-781 (2004).
- 3) S. Kaneko, M. Arimoto, M. Ohba, H. Kobayashi, T. Ishii and I. Kusakabe: Purification and substrate specificities of two α -L-arabinofuranosidases from *Aspergillus awamori* IFO 4033. *Appl. Environ. Microbiol.*, **64**, 4021-4027 (1998).
- 4) B.C. Saha: α -L-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. *Biotechnol. Adv.*, **18**, 403-423 (2000).
- 5) J. Puls and J. Schuseil: Chemistry of hemicelluloses: relationship between hemicellulose structure and enzymes required for hydrolysis. in *Hemicellulose and Hemicellulases*, M.P. Coughlan and G.P. Hazlewood, eds., Portland Press, London, pp. 1-27 (1993).
- 6) G. Beldman, M.J.F. Searle-van Leeuwen, G.A. DeRuiter, H.A. Siliha and A.G.J. Voragen: Degradation of arabinans by arabinanases from *Aspergillus aculeatus* and *Aspergillus niger*. *Carbohydr. Polym.*, **20**, 159-168 (1993).
- 7) S.M. Pitson, A.G. Voragen and G. Beldman: Stereochemical course of hydrolysis catalyzed by arabinofuranosyl hydrolases. *FEBS Lett.*, **398**, 7-11 (1996).
- 8) J.N. Varghese, M. Hrmova and G.B. Fincher: Three-dimensional structure of a barley β -D-glucan exohydrolase, a family 3 glycosyl hydrolase. *Structure*, **7**, 179-190 (1999).
- 9) S. Fushinobu, K. Ito, M. Konno, T. Wakagi and H. Matsuzawa: Crystallographic and mutational analyses of an extremely acidophilic and acid-stable xylanase: biased distribution of acidic residues and importance of Asp37 for catalysis at low pH. *Protein Eng.*, **11**, 1121-1128 (1998).
- 10) T. Koseki, M. Okuda, S. Sudoh, Y. Kizaki, K. Iwano, I. Aramaki and H. Matsuzawa: Role of two α -L-arabinofuranosidases in arabinoxylan degradation and characteristics of the encoding genes from shochu koji molds, *Aspergillus kawachii* and *Aspergillus awamori*. *J. Biosci. Bioeng.*, **96**, 232-241 (2003).
- 11) T. Koseki, Y. Ito, S. Furuse, K. Ito and K. Iwano: Conversion of ferulic acid into 4-vinylguaiaicol, vanillin and vanillic acid in model solutions of shochu. *J. Ferment. Bioeng.*, **82**, 46-50 (1996).
- 12) K. Hovel, D. Shallom, K. Niefind, V. Belakhov, G. Shoham, T. Baasov, Y. Shoham and D. Schomburg: Crystal structure and snapshots along the reaction pathway of a family 51 α -L-arabinofuranosidase. *EMBO J.*, **22**, 4922-4932 (2003).
- 13) A. Miyanaga, T. Koseki, H. Matsuzawa, T. Wakagi, H. Shoun and S. Fushinobu: Expression, purification, crystallization and preliminary X-ray analysis of α -L-arabinofuranosidase B from *Aspergillus kawachii*. *Acta Crystallogr. Sect. D Biol. Crystallogr.*, **60**, 1286-1288 (2004).
- 14) A. Miyanaga, T. Koseki, H. Matsuzawa, T. Wakagi, H. Shoun and S. Fushinobu: Crystal structure of a family 54 α -L-arabinofuranosidase reveals a novel carbohydrate-binding module that can bind arabinose. *J. Biol. Chem.*, **279**, 44907-44914 (2004).
- 15) R.M. Zacharius, T.E. Zell, J.H. Morrison and J.J. Woodlock: Glycoprotein staining following electrophoresis on acrylamide gels. *Anal. Biochem.*, **30**, 148-152 (1969).
- 16) G. Michel, L. Chantalat, E. Duee, T. Barbeyron, B. Henrissat, B. Kloareg and O. Dideberg: The kappa-carrageenase of *P. carrageenovora* features a tunnel-shaped active site: a novel insight in the evolution of clan-B glycoside hydrolases. *Structure*, **9**, 513-525 (2001).
- 17) C. Divne, J. Stahlberg, T.T. Teeri and T.A. Jones: High-resolution crystal structures reveal how a cellulose chain is bound in the 50 angstrom long tunnel of cellobiohydrolase I from *Trichoderma reesei*. *J. Mol. Biol.*, **275**, 309-325 (1998).
- 18) C.C.F. Blake, M. Ghosh, K. Harlos, A. Avezoux and C. Anthony: The active-site of methanol dehydrogenase contains a disulfide bridge between adjacent cysteine residues. *Nat. Struct. Biol.*, **1**, 102-105 (1994).
- 19) B.M. Kim, L.W. Schultz and R.T. Raines: Variants of ribonuclease inhibitor that resist oxidation. *Protein Sci.*, **8**, 430-434 (1999).
- 20) D. Nurizzo, J.P. Turkenburg, S.J. Charnock, S.M. Roberts, E.J. Dodson, V.A. McKie, E.J. Taylor, H.J. Gilbert and G.J. Davies: *Cellvibrio japonicus* α -L-arabinanase 43A has a novel five-blade beta-propeller fold. *Nat. Struct. Biol.*, **9**, 665-668 (2002).
- 21) Z. Fujimoto, A. Kuno, S. Kaneko, S. Yoshida, H. Kobayashi, I. Kusakabe and H. Mizuno: Crystal structure of *Streptomyces olivaceoviridis* E-86 β -xylanase containing xylan-binding domain. *J. Mol. Biol.*, **300**, 575-585 (2000).
- 22) Z. Fujimoto, A. Kuno, S. Kaneko, S. Kobayashi, I. Kusakabe and H. Mizuno: Crystal structures of the sugar complexes of *Streptomyces olivaceoviridis* E-86 xylanase: Sugar binding structure of the family 13 carbohydrate binding module. *J. Mol. Biol.*, **316**, 65-78 (2002).
- 23) V. Notenboom, A.B. Boraston, S.J. Williams, D.G. Kilburn and D.R. Rose: High-resolution crystal structures of the lectin-like xylan binding domain from *Streptomyces lividans* xylanase 10A with bound substrates reveal a novel mode of xylan binding. *Biochemistry*, **41**, 4246-4254 (2002).
- 24) Z. Fujimoto, S. Kaneko, A. Kuno, S. Kobayashi, I. Kusakabe and H. Mizuno: Crystal structures of decorated xylooligosaccharides bound to a family 10 xylanase from *Streptomyces olivaceoviridis* E-86. *J. Biol. Chem.*, **279**, 9606-9614 (2004).

GH54 アラビノフラノシダーゼの結晶構造解析

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アラビノキシランは、植物細胞壁の成分の一つであり、 β -1,4 キシラン主鎖に α -1,2 または α -1,3 結合でアラビノースが側鎖として結合している構造をとっている。 α -L-アラビノフラノシダーゼは、そのアラビノキシランのキシラン主鎖と側鎖アラビノース間の結合を切断する酵素である。*Aspergillus kawachii* 由来の α -L-アラビノフラノシダーゼ B (AkAbf54) は、構造未知である糖質分解酵素ファミリー 54 に属しており、反応機構なども不明であった。われわれはこの AkAbf54 の X 線結晶構造解析を行った。AkAbf54 を *Pichia pastoris* で大量発現させ、結晶構造解析を行った。全体構造を明らかにしたところ、AkAbf54 は触媒ドメインとアラビノース結合ドメインの二つのドメインから構成されていた。アラビノースとの複合体構造では、アラビノースが触媒ドメインに 1 分子、アラビノース結合ドメインに 2 分子結合していた。複合体構造および変異体を用いた実験などから、求核残基が Glu221、酸/塩基触媒が Asp297 であることを明らかにした。興味深いことに隣り合ったシステイン残基 Cys176 および Cys177 がジスルフィド結合を形成し、アラビノースを疎水的に認識していた。アラビノース結合ドメインは、糖質結合ドメイン (CBM) ファミリー 13 と似たフォールドをとっていた。しかし、糖リガンド結合部位やモチーフが異なるなどいくつか異なる点がみられた。これらのことを考慮に入れた結果、アラビノース結合ドメインは新規な糖質結合ドメインである CBM42 に分類された。われわれは CBM42 の詳細な機能解析を行い、CBM42 はヘミセルロースの側鎖の糖であるアラビノースのみを認識して結合するという、これまでには例のない新規な糖質結合ド

メインであることを示した。

〔質 問〕

長岡技科大 森川

1) 酵素は基質アラビノキシランとどのように結合して活性を示すのでしょうか？

2) 触媒ドメインとアラビノース結合ドメインの折りたたみの協同性についてはどうでしょうか？

〔答〕

1) アラビノキシランは、セルロースなどと一緒に密集して存在しています。アラビノース結合ドメインは、その密集した表面にあるアラビノキシラン鎖や表面から出ている独立したアラビノキシラン鎖に、そのアラビノース側鎖を認識して、結合すると考えられます。アラビノース結合ドメインは、弱い結合力のため、すぐに解離します。そして、再び、アラビノース側鎖を含むアラビノキシラン鎖を認識し、結合します。このようにして、アラビノース結合ドメインが、結合と解離を繰り返しながら、アラビノース側鎖を多く含むアラビノキシラン鎖の近傍に存在し続けることにより、触媒ドメインが基質であるアラビノース側鎖を捕らえる確率が上昇し、反応が進みやすくなっているのではないかと考えられます。

2) 確かに、触媒ドメイン単独、アラビノース結合ドメイン単独では発現しませんので、二つのドメインが協同して折りたたまれている可能性は大いに考えられます。今後の研究課題と考えさせていただきます。

〔質 問〕

北大 木村

アラビノースの複合体構造を得ているが、アラビノースを加えてアラビノースが結合していたのか、それともはじめからアラビノースが結合していたのか？

〔答〕

ネイティブ結晶の構造にはアラビノース分子は確認できませんでした。アラビノースをネイティブ結晶にソーキングさせることにより、アラビノースが結合した複合体構造を得ました。