

立体構造に基づくアルカリキシラナーゼの機能ドメイン解析と好アルカリ性向上

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Analysis of Functional Domains and Improvement of Alkaliphily of an Alkaline Xylanase on the Basis of Its Three-dimensional Structure

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Abstract: Xylanase J (XynJ) from alkaliphilic *Bacillus* sp. strain 41M-1 is a multi-domain enzyme consisting of a glycoside hydrolase (GH) family 11 catalytic domain and a carbohydrate-binding module family 36 xylan-binding domain (XBD). Structural comparison of the GH family 11 catalytic domains indicated that there were several specific salt bridges in the catalytic cleft of XynJ. Mutant enzymes were prepared by substituting several amino acid residues responsible for the characteristic salt bridges. Elimination of the salt bridges caused an acidophilic shift in optimum pH, suggesting that the characteristic salt bridges contributed to the alkaliphily of XynJ. On the other hand, reinforcing one of the characteristic salt bridges in the catalytic domain shifted the optimum pH of XynJ from 8.5 to 9.0. Furthermore, introducing excess Arg residues on the protein surface was also effective to improve the alkaliphily of XynJ. Xylan-binding properties of various XBD mutants were investigated as fusion proteins with glutathione-S-transferase. Furthermore, the same substitutions were introduced into the XBD region of XynJ and insoluble xylan-hydrolyzing activity was measured. The results indicated that some Asp, Trp and Tyr residues were important for xylan-binding activity of the XBD, and that xylan-hydrolyzing activity of XynJ was closely correlated to xylan-binding activity of the XBD region.

Key words: glycoside hydrolase family 11, xylanase, carbohydrate-binding module family 36, xylan-binding domain, alkaliphily

Xylan, the major component of hemicellulose, has a backbone of β -1,4-linked xylopyranose units. Xylanase (1,4- β -D-xylan xylanohydrolase; EC 3.2.1.8) catalyzes hydrolysis of β -1,4 bonds of xylan to generate xylo-oligosaccharides and xylose. Various applications of xylanases in the bioconversion, food, and feed industries have been suggested.^{1–3)} Since the solubility of xylan in aqueous solutions increases at alkaline pHs, xylanases active under alkaline conditions are required for industrial applications.

We have isolated alkaliphilic *Bacillus* sp. 41M-1 from a soil sample.⁴⁾ The strain produced a xylanase that had an alkaline pH optimum.⁵⁾ The enzyme, named xylanase J (XynJ), consists of two functional domains: a glycoside hydrolase (GH) family 11 catalytic domain (CatD) and a xylan-binding domain (XBD) belonging to carbohydrate-binding module family 36 (<http://www.cazy.org/index.html>) (Fig. 1).⁶⁾ Mutational analysis of the CatD revealed some amino acid residues that were important for catalytic activity.⁶⁾ The alkaline adaptation mechanism of XynJ, however, had not been elucidated. On the other hand, an XBD-truncated XynJ derivative (Δ XBD), and a fusion protein of glutathione-S-transferase (GST) and the XBD (GST-XBD) were prepared.^{6,7)} GST-XBD bound tightly to insoluble xylan and Δ XBD showed far lower hydrolyzing activity toward insoluble xylan than full-length XynJ. These results indicated that the XBD

would bind to insoluble xylan and enhance hydrolyzing activity of the adjacent CatD. The XBD was successfully displayed on the surface of filamentous phage. Analysis of the xylan-binding activity-deficient mutant phages revealed that some Asp, Phe and Trp residues might contribute to xylan-binding activity of the XBD.⁷⁾

Recently, we have solved the crystal structure of XynJ (Ihsanawati *et al.*, manuscript in preparation). Structure comparison between CatDs of neutral and alkaline xylanases indicated that there were several salt bridges specific in alkaline xylanases, including XynJ. These salt bridges lie in the catalytic cleft, suggesting that the salt bridges might contribute to the alkaliphily of XynJ. In this article, we describe our recent findings from the analysis of functional domains and successful improvement of the alkaliphily of XynJ.

Structural comparison of CatDs from GH family 11 xylanases.

Structural comparison of GH family 11 alkaline xylanase (Xyn11) from *Bacillus agaradhaerens* with neutral xylanases revealed that there was a characteristic salt bridge network in Xyn11.⁸⁾ We also solved the crystal structures of XynJ in two crystalline forms obtained from pH 6.5 and 8.0 (PDB ID: 2DCJ and 2DCK, respectively). There was also the same characteristic salt bridge network (formed by Glu16, Arg48, Lys52 and Glu177) found in Xyn11. Moreover, an additional salt bridge (formed by Asp14 and Lys51) characteristic in alkaline xylanases was discovered in the CatD region of XynJ (Fig. 2).^{9,10)} All

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these salt bridges lay in the catalytic cleft. Previous work showed that mutant enzymes XynJD14N (Asp14 of XynJ was replaced by Asn), XynJE16Q and XynJE177Q exhibited acidophilic shifts in optimum pH.⁶⁾ These results suggested that the characteristic salt bridges existing in the catalytic cleft might contribute to the alkaliphily of XynJ.

Mutations of the residues responsible for the characteristic salt bridges found in the CatD.

The amino acid residues Arg48, Lys51 and Lys52, which were responsible for the characteristic salt bridges, were targeted for further mutational analysis. Δ XBD, consisting of the CatD only and corresponding to Ala1-Pro222 of XynJ, was used as a parent enzyme for the mutational study. To eliminate the salt bridges, Arg48, Lys51 and Lys52 were replaced by Gln according to our previous work (XynJE16Q and XynJE177Q).⁶⁾ To reinforce the salt bridges, the concerned Lys51 and Lys52 were re-

placed by Arg. Salt bridges of Δ XBD should be eliminated in mutants Δ XBDR48Q, Δ XBDK51Q and Δ XBDK52Q, whereas they should be reinforced in Δ XBDK51R and Δ XBDK52R.

Wild-type Δ XBD, and mutants Δ XBDD14N, Δ XBDE16Q, Δ XBDR48Q, Δ XBDK51Q, Δ XBDK51R, Δ XBDK52Q, Δ XBDK52R and Δ XBDE177Q were assayed for xylanase activity at various pHs. Wild-type Δ XBD showed a broad pH profile and the highest activity at pH 8.5 (Fig. 3(A)). The pH optima of Δ XBDE16Q, Δ XBDR48Q, Δ XBDK52Q and Δ XBDE177Q were changed to 5.0–6.0, while Δ XBDD14N and Δ XBDK51Q exhibited almost the same optimum pH as wild-type Δ XBD.⁹⁾ These results suggested that the characteristic salt bridges contributed to the alkaliphily of XynJ.

Unlike Δ XBDK51Q, Δ XBDK51R exhibited somewhat higher specific activity than wild-type Δ XBD in a broad range of pH and had a pH optimum of 9.0 (Fig. 3(B)).¹⁰⁾ On the other hand, Δ XBDK52R showed lower activity at alkaline pHs compared to wild-type Δ XBD, and had a pH optimum of 8.0. These results indicated that reinforcement of the characteristic salt bridge formed by Asp14 and Lys51 caused an alkaliphilic shift in optimum pH. The reinforcement of this salt bridge might increase the stability of the cleft structure at alkaline pHs, and thus result in improvement of the alkaliphily of XynJ. In the case of Δ XBDK52R, the salt bridges might not be formed correctly, especially at alkaline pHs. As a result, Δ XBDK52R exhibited an acidophilic shift in optimum pH. Further studies need to be conducted to clarify why Δ XBDK52R has rather higher activity at neutral pHs compared to wild-type Δ XBD.

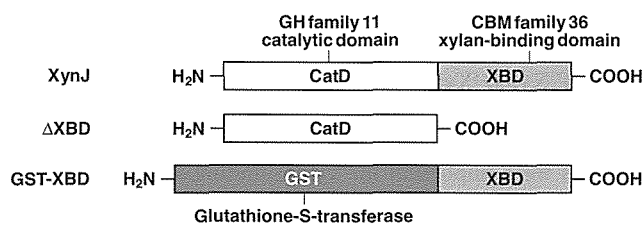


Fig. 1. Molecular architectures of XynJ, Δ XBD and GST-XBD.

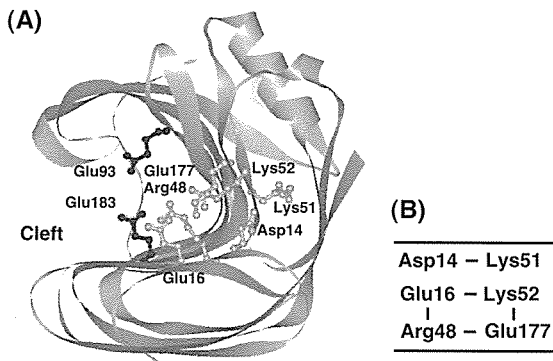


Fig. 2. Three-dimensional structure of the CatD region of XynJ (A) and characteristic salt bridges found in XynJ (B).

The catalytic residues and the amino acid residues responsible for the characteristic salt bridges are shown in panel (A).

Introduction of excess Arg residues on the protein surface of the CatD.

Amino acid sequence comparison of some alkaline serine proteases revealed that high-alkaline adaptation is accompanied by an increase in the number of Arg on the protein surface.¹¹⁾ Some mutant enzymes were prepared from *Trichoderma reesei* xylanase II by introducing excess Arg residues on the Ser/Thr surface.¹²⁾ The Ser/Thr surface forms a major part of the outer surface of the double-layered β -sheet and contains a large number of Ser and Thr residues. These mutations shifted the optimum

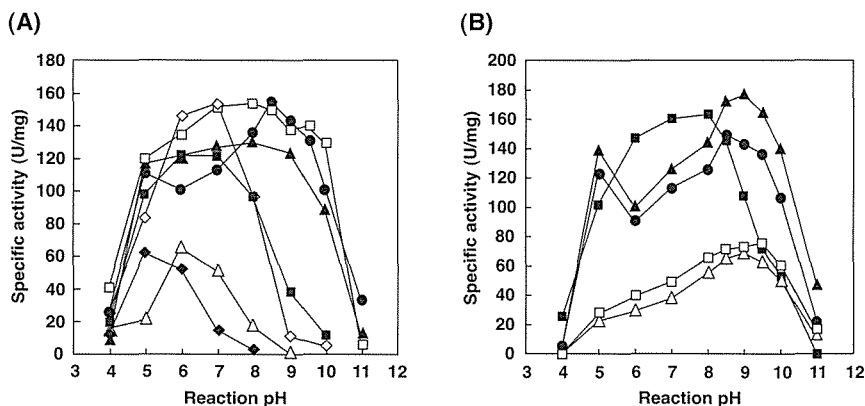


Fig. 3. Effect of reaction pH on activity of wild-type and mutant Δ XBDS.

The reaction was carried out at 37°C for 10 min using birchwood xylan as a soluble substrate. Xylanase activity was determined by measuring the amount of liberated reducing sugars by the conventional method. One unit (U) was defined as amount of enzyme that released reducing sugars equivalent to 1 μ mol of xylose per min. (A) ●, wild-type Δ XBD; ▲, Δ XBDD14N; ■, Δ XBDE16Q; ◆, Δ XBDE177Q; △, Δ XBDR48Q; □, Δ XBDK51Q; ◇, Δ XBDK52Q. (B) ●, wild-type Δ XBD; ▲, Δ XBDK51R; ■, Δ XBDK52R; △, Δ XBDR5; □, Δ XBDR5/K51R.

pH to the alkaline region by 0.5–1.0 pH unit. Moreover, the temperature optima of these mutants increased by 5°C compared to the wild-type enzyme.

The amino acid residues Ser26, Thr34, Asn74, Asn76 and Asn192 at the Ser/Thr surface were selected for substitutions according to one of the *T. reesei* xylanase II mutants.¹²⁾ Amino acid substitutions of these five residues by Arg were introduced into Δ XBD and Δ XBDK51R to construct Δ XBDR5 and Δ XBDK51R/R5, respectively. The positions of the substituted amino acids (Ser26, Thr34, Lys51, Asn74, Asn76 and Asn192) are shown on the three-dimensional structure of the CatD region of XynJ (Fig. 4). Δ XBDR5 and Δ XBDK51R/R5 showed about 50% of the activity of wild-type Δ XBD and had pH optima of 9.0 and 9.5, respectively (Fig. 3(B)).¹⁰⁾ These results suggested that the introduction of five Arg residues on the Ser/Thr surface could also improve the alkaliphily of Δ XBD. It is noteworthy that the optimum pH of Δ XBD has been successfully shifted from 8.5 to 9.5 by combining the two methodologies presented in this study.

Wild-type Δ XBD, and mutants Δ XBDK51R, Δ XBDR5 and Δ XBDK51R/R5 were assayed for xylanase activity at various temperatures. As shown in Fig. 5, wild-type Δ XBD was most active at 55°C. Δ XBDK51R exhibited al-

most the same temperature profile and temperature optimum as wild-type Δ XBD. On the other hand, the temperature optima of Δ XBDR5 and Δ XBDK51R/R5 were both 60°C.¹⁰⁾ Although these mutants showed lower specific activity than wild-type Δ XBD at 37–60°C, they showed apparently higher activity at 65°C. These results indicated that the introduction of excess Arg residues on the protein surface increased the thermostability of Δ XBD.

Structure modeling of the XBD/xylotriose complex.

In the crystal structure of XynJ, the XBD region has also the β -jelly-roll fold well observed in other CBMs. It contains two Ca^{2+} on the side and top of the fold, referred to as Ca-1 and Ca-2. The structure of CBM family 36 XBD of *Paenibacillus polymyxa* xylanase 43 A (*Pp*CBM36) in complex with xylotriose has already been reported.¹³⁾ *Pp*CBM36 also contains two Ca^{2+} and one of the xylose residues is coordinated to the Ca^{2+} on the top of the fold via its O2 and O3 hydroxyls, suggesting that *Pp*CBM36 should be a Ca^{2+} -dependent XBD. The XBD structure of XynJ (2DCJ) was superimposed with that of *Pp*CBM36 to construct the structure model of the XBD/xylotriose complex (Fig. 6).¹⁴⁾ It seemed that the XBD might follow the same Ca^{2+} -dependent xylan-binding mechanism as *Pp*CBM36 and that Ca-2 might interact

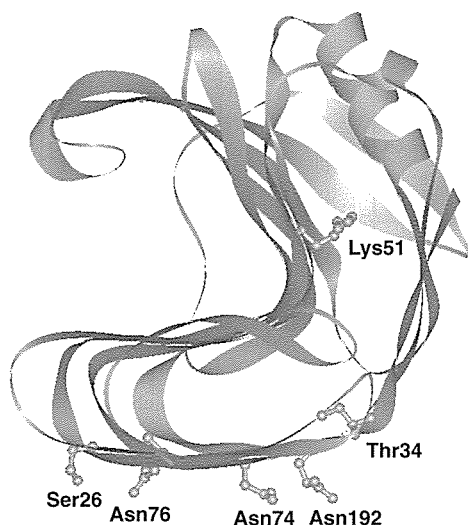


Fig. 4. Three-dimensional structure of the CatD region of XynJ.

The positions of substituted amino acid residues in mutant Δ XBDK51R/R5 are shown.

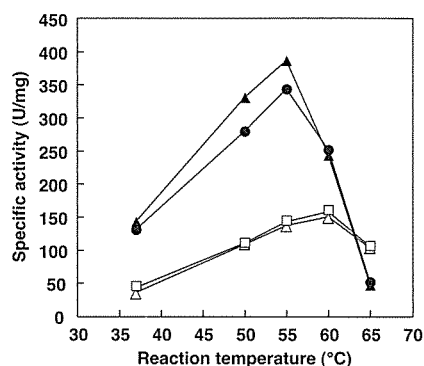


Fig. 5. Effect of reaction temperature on activity of wild-type and mutant Δ XBDs.

The reaction was carried out at pH 8.5 for 10 min using birchwood xylan as a soluble substrate. ●, wild-type Δ XBD; ▲, Δ XBDK51R; △, Δ XBDR5; □, Δ XBDK51R/R5.

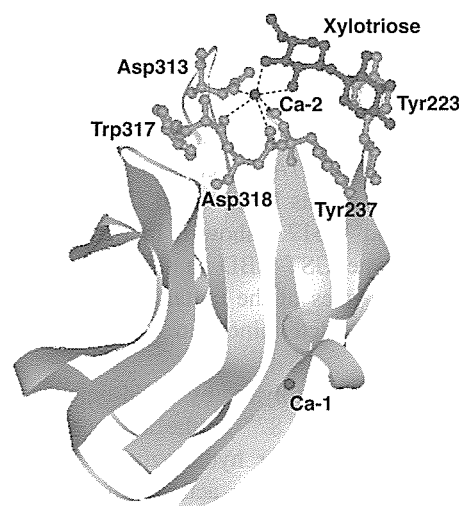


Fig. 6. Three-dimensional structure model of the XBD/xylotriose complex.

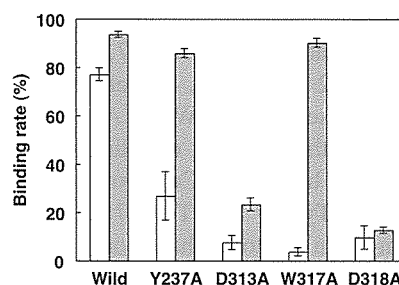


Fig. 7. Insoluble xylan-binding of wild-type and mutant GST-XBDs in the absence (open bar) or presence (shaded bar) of 10 mM CaCl_2 .

GST-XBDs were incubated with insoluble oat spelt xylan at room temperature and pH 7.5 for 3.0 h. The residual GST activity in the supernatant was determined. The decrease of activity in the supernatant corresponds to the protein bound to xylan. The mean $\pm$ SD of triplicate experiments is shown.

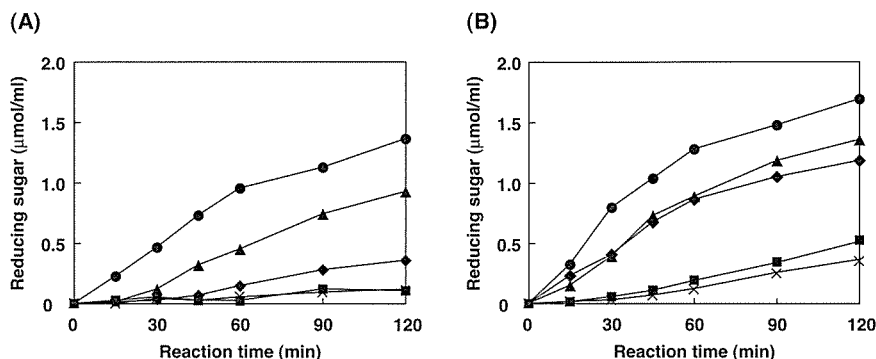


Fig. 8. Insoluble xylan-hydrolysis by wild-type and mutant XynJs in the absence (A) or presence (B) of 10 mM CaCl_2 .

The reaction was carried out at 37°C and pH 7.5 using oat spelt xylan as an insoluble substrate. An increase of reducing sugars was monitored at several points by the conventional method and expressed as xylose equivalent. ●, wild-type XynJ; ▲, Y237A; ■, D313A; ◆, W317A; ×, D318A.

with Tyr237, Asp313, Trp317 and Asp318 as well as the second xylose residue. In addition, it seemed that the xylose ring at the nonreducing end might be wedged between the aromatic side chains of Tyr223 and Tyr237.

Mutations of the residues that coordinate to a Ca^{2+} of the XBD.

In the XBD/xylotriase complex structure model, Ca-2 seemed to interact with Tyr237, Asp313, Trp317 and Asp318, as well as a xylose. These residues were replaced with Ala. Insoluble xylan-binding of wild-type and mutant GST-XBDs is shown in Fig. 7.¹⁴⁾ In the absence of CaCl_2 , all the mutants showed lower binding rates than wild-type GST-XBD. This result indicated the importance of these residues in insoluble xylan-binding. Binding rates of mutants Y237A and W317A increased by adding CaCl_2 , although those of mutants D313A and D318A did not. Mutants Y237A and W317A showed higher K_d value compared to wild-type GST-XBD in the presence of CaCl_2 , although binding rates of the mutants D313A and D318A were too low to determine the K_d values (data not shown).

Insoluble xylan-hydrolysis by wild-type and mutant XynJs is shown in Fig. 8.¹⁴⁾ In the absence of CaCl_2 , all the mutants showed lower hydrolyzing activity than the wild-type XynJ. Hydrolyzing activity of mutants Y237A and W317A was improved by adding CaCl_2 , although that of mutants D313A and D318A was not. These results corresponded to those obtained with mutant GST-XBDs.

Figure 9 shows the coordination bonds to Ca^{2+} . Ca-2 seemed to coordinate to O of Tyr237, Oδ1 of Asp313, O of Trp317 and Oδ2 of Asp318, as well as O2 and O3 hydroxyls of a xylose residue. When Asp313 and Asp318 were replaced with Ala, the coordinate bonds to Ca^{2+} would completely break and Ca^{2+} would not be taken in any more. When Tyr237 and Trp317 were replaced with Ala, the main-chain O atoms that coordinated to Ca^{2+} still existed. It was thought, therefore, that Ca^{2+} was compensated and binding of the mutants Y237A and W317A increased by adding CaCl_2 .

Mutations of the residues in the XBD that stack against a xylose ring.

The aromatic side chains of Tyr223 and Tyr237 might make stacking interactions with a xylose ring. These resi-

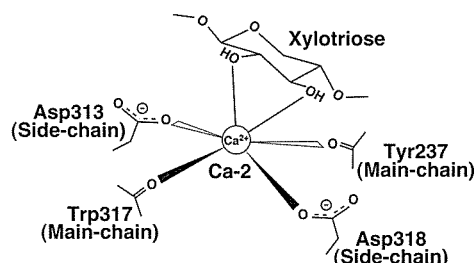


Fig. 9. Schematic diagram of the coordinate bonds to Ca^{2+} .

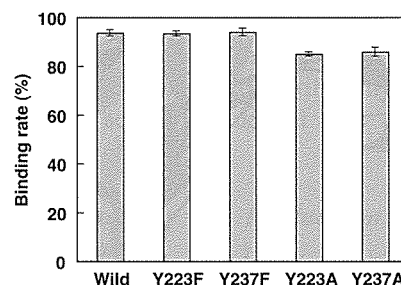


Fig. 10. Insoluble xylan-binding of wild-type and mutant GST-XBDs in the presence of 10 mM CaCl_2 .

GST-XBDs were incubated with insoluble oat spelt xylan at room temperature and pH 7.5 for 3.0 h.

dues were replaced with Phe or Ala. Insoluble xylan-binding of wild-type and mutant GST-XBDs in the presence of CaCl_2 is shown in Fig. 10.¹⁴⁾ Mutants Y223F and Y237F showed the same binding rate as wild-type GST-XBD. On the other hand, mutants Y223A and Y237A showed a rather lower rate than wild-type GST-XBD. The K_d values of mutants Y223F and Y237F were the same as that of wild-type GST-XBD in the presence of CaCl_2 (data not shown). On the other hand, mutants Y223A and Y237A showed higher K_d compared to wild-type GST-XBD. These results indicated the importance of the stacking interactions between the xylose ring and the aromatic side chains of Tyr223 and Tyr237.

Insoluble xylan-hydrolysis by wild-type and mutant XynJs in the presence of CaCl_2 is shown in Fig. 11.¹⁴⁾ Mutants Y223F and Y237F showed almost the same hydrolyzing activity as wild-type XynJ. On the other hand, mutants Y223A and Y237A showed rather lower activity than wild-type XynJ. These results corresponded to those obtained with mutant GST-XBDs.

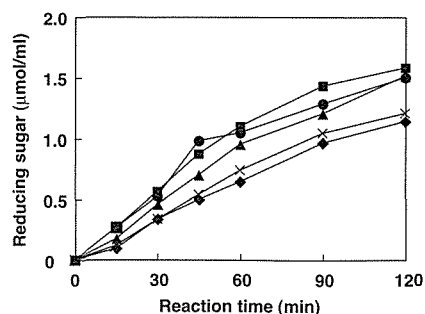


Fig. 11. Insoluble xylan-hydrolysis by wild-type and mutant XynJs in the presence of 10 mM CaCl₂.

The reaction was carried out at 37°C and pH 7.5 using oat spelt xylan as an insoluble substrate. ●, wild-type XynJ; ▲, Y223F; ■, Y237F; ◆, Y223A; ×, Y237A.

Conclusions.

The characteristic salt bridges found in the CatD are shown to contribute to the alkaliphily of XynJ. Reinforcing the salt bridge formed by Asp14 and Lys51 caused an alkaliphilic shift in optimum pH. Introducing five Arg residues on the Ser/Thr surface is also effective to improve the alkaliphily of XynJ. The amino acid residues Tyr237, Asp313, Trp317 and Asp318 of the XBD are important for xylan-binding activity, because the XBD binds to xylan via Ca²⁺ that is coordinated to by these residues. The aromatic side chains of Tyr223 and Tyr237 also play an important role in xylan-binding. Xylan hydrolyzing activity of XynJ is closely correlated to xylan-binding activity of the XBD region.

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REFERENCES

- 1) K.K.Y. Wong, L.U.L. Tan and J.N. Saddler: Multiplicity of β -1,4-xylanase in microorganisms: function and applications. *Microbiol. Rev.*, **52**, 305–317 (1988).
- 2) L. Viikari, A. Kantelinen, J. Sundquist and M. Linko: Xylanases in bleaching: from an idea to the industry. *FEMS Microbiol. Rev.*, **13**, 335–350 (1994).
- 3) Q.K. Beg, M. Kapoor, L. Mahajan and G.S. Hoondal: Microbial xylanases and their industrial applications: a review. *Appl. Microbiol. Biotechnol.*, **56**, 326–338 (2001).
- 4) S. Nakamura, K. Wakabayashi, R. Nakai, R. Aono and K. Horikoshi: Production of alkaline xylanase by a newly isolated alkaliphilic *Bacillus* sp. strain 41M-1. *World J. Microbiol. Biotechnol.*, **9**, 221–224 (1993).
- 5) S. Nakamura, K. Wakabayashi, R. Nakai, R. Aono and K. Horikoshi: Purification and some properties of an alkaline xylanase from alkaliphilic *Bacillus* sp. strain 41M-1. *Appl. Environ. Microbiol.*, **59**, 2311–2316 (1993).
- 6) S. Nakamura: Structure and function of a multidomain alkaline xylanase from alkaliphilic *Bacillus* sp. strain 41M-1. *Catal. Surveys Asia*, **7**, 157–164 (2003).
- 7) T. Sakata, H. Miyakubo, Y. Osada, R. Wada, H. Takahashi, R. Yatsunami, T. Fukui and S. Nakamura: Functional improvement of xylanase by introducing mutated xylan-binding domain. *J. Appl. Glycosci.*, **53**, 131–136 (2006).
- 8) F. De Lemos Esteves, T. Gouders, J. Lamotte-Brasseur, S. Rigali and J.M. Frère: Improving the alkaliphilic performances of the Xyl1 xylanase from *Streptomyces* sp. S38: structural

comparison and mutational analysis. *Protein Sci.*, **14**, 292–302 (2005).

- 9) H. Umemoto, Ihsanawati, M. Inami, R. Yatsunami, T. Fukui, T. Kumasaka, N. Tanaka and S. Nakamura: Contribution of salt bridges to alkaliphily of *Bacillus* alkaline xylanase. *Nucleic Acids Symp. Ser.*, **51**, 461–462 (2007).
- 10) H. Umemoto, Ihsanawati, M. Inami, R. Yatsunami, T. Fukui, T. Kumasaka, N. Tanaka and S. Nakamura: Improvement of alkaliphily of *Bacillus* alkaline xylanase by introducing amino acid substitutions both on catalytic cleft and protein surface. *Biosci. Biotechnol. Biochem.*, **73**, 965–967 (2009).
- 11) T. Shirai, A. Suzuki, T. Yamane, T. Ashida, T. Kobayashi, J. Hitomi and S. Ito: High-resolution crystal structure of M-protease: phylogeny aided analysis of the high-alkaline adaptation mechanism. *Protein Eng.*, **10**, 627–634 (1997).
- 12) O. Turunen, M. Vuorio, F. Fenel and M. Leisola: Engineering of multiple arginines into the Ser/Thr surface of *Trichoderma reesei* endo-1,4- β -xylanase II increases the thermotolerance and shifts the pH optimum towards alkaline pH. *Protein Eng.*, **15**, 141–145 (2002).
- 13) S. Jamal-Talabani, A.B. Boraston, J.P. Turkenburg, N. Tarbouriech, V.M. Ducros and G.J. Davies: *Ab initio* structure determination and functional characterization of CBM36; a new family of calcium-dependent carbohydrate binding modules. *Structure*, **12**, 1177–1187 (2004).
- 14) R. Yazawa, J. Takakura, T. Sakata, H. Miyakubo, Ihsanawati, H. Umemoto, R. Yatsunami, T. Fukui, T. Kumasaka, N. Tanaka and S. Nakamura: Mutational analysis of CBM Family 36 xylan-binding domain of alkaline xylanase. in *Biotechnology of Lignocellulose Degradation and Biomass Utilization*, K. Sakka, S. Karita, T. Kimura, M. Sakka, H. Matsui, H. Miyake and A. Tanaka, eds., Ito Print Publishing Division, Mie, pp. 448–454 (2009).

立体構造に基づくアルカリキシラーゼの

機能ドメイン解析と好アルカリ性向上

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好アルカリ性 *Bacillus* sp. 41M-1 株が分泌するキシラーゼ J (XynJ) は, 反応の最適をアルカリ性域に有する新規な好アルカリ性のキシラーゼである. 本酵素はマルチドメイン酵素であり, 糖質加水分解酵素 (GH) ファミリー 11 に属する触媒ドメインおよび糖質結合モジュール (CBM) ファミリー 36 に属するキシラン結合ドメイン (XBD) からなる. GH ファミリー 11 中性キシラーゼとの立体構造比較の結果, XynJ の触媒クレフト内部にはいくつかの特徴的塩橋が存在することがわかった. 特徴的塩橋を構成する残基にアミノ酸置換を導入した変異型酵素を調製し, 性質検討を行った. 塩橋を破壊した変異型酵素においては反応最適 pH の酸性側へのシフトが観察され, 特徴的塩橋は XynJ の好アルカリ性に重要であることが明らかとなった. 一方, 触媒クレフトに存在する特徴的塩橋の強化により, 野生型酵素では pH 8.5 にあった反応の最適が pH 9.0 にまでシフトした. さらに, タンパク質分子表面への過剰の Arg の導入も XynJ の好アルカリ性向上に効果的であった. グルタチオン-S-トランスフェラーゼと XBD との融合タンパク質を用い, アミノ酸置換を導入した変異型 XBD のキシラン結合能を調べた. さらに, XynJ の XBD 領域に同様なアミノ酸置換を導入し, それらの不溶性キシラン加水分解活性を調べた. その結果, いくつかの Asp, Trp および Tyr 残基が XBD のキシラン結合活性に重要であることがわかり, 不溶性キシラン加水分解活性はキシラン結合活性とよく相関していた.

〔質問〕

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1) XBD の有無が反応最適 pH に影響を与えているが, ΔXBD への変異導入による pH プロファイルの変化は, XBD を含む全領域の酵素とした場合においても同様に反映されるか.

2) ΔXBD への変異導入による pH プロファイルの変化は, XBD を含む全領域の酵素と不溶性基質を用いた場合にも同様に反映されるか.

〔答〕

1) 可溶性キシランを基質とした場合, XBD を含む全領域の野生型 XynJ の反応最適 pH は 9.0 である. 一方, ΔXBD 反応最適 pH は 8.5 であり, XBD の有無により反応最適 pH により差が生じる. 反応最適 pH が酸性側にシフトしたいくつかの変異体においても, XBD の有無により反応最適 pH に 0.5 ユニット程度の違いが生じることが確認されている. 反応最適 pH がアルカリ性側にシフトした変異体についてはまだ調べていないが, 同様な結果が得られるものと考えている.

2) pH プロファイルの作成に際しては反応初期速度の測定が必須であるため, 基質にはもっぱら可溶性キシランを用いてきた. 基質に不溶性キシランを用いた場合, 反応初期速度の測定に誤差が生じることが予想される. したがって, 可溶性キシランを用いた場合とまったく同一の結果となるかは不明であるが, 少なくとも同様な傾向は得られるものと考えている.

〔質問〕

信州大・工 天野

種々の変異体について加水分解反応の pH プロファイルを見ていたが, タンパク質の構造変化に伴って基質との結合が変化し, そのことによる生成物の変化が結果に影響を与えている可能性は考えられないか.

〔答〕

十分に考えられる. 反応 pH を広範囲に変化させた場合, タンパク質 (酵素) だけでなく基質のキシランも構造変化することが予想される. 野生型 XynJ の pH プロファイルは二峰性を示すが, これは pH 変化に伴って酵素と基質の両者が構造変化を起こし, 両者間の相互作用が最適となる pH において活性が極大となることによると考えている. ただし, 野生型 XynJ による加水分解生成物は主としてキシロビオースおよびキシロトリオースであり, これは pH 6.0 および pH 9.0 のいずれの反応 pH においても同様であった. したがって, 酵素-基質間の相互作用は変化しても, 反応産物は変化しないようである. アミノ酸置換を導入した変異型酵素についても, pH 変化に伴い酵素-基質間相互作用が変化することが予想される. 一方で, 反応最適 pH が変化した変異型酵素による反応生成物に関しては調べておらず, 今後の課題と考える.