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The Role of Carbohydrate-Binding Module (CBM) Repeat of a Multimodular Xylanase (XynX) from *Clostridium thermocellum* in Cellulose and Xylan Binding

Thangaswamy Selvaraj[1], Sung Kyum Kim[1], Yong Ho Kim[1], Yu Seok Jeong[1], Yu-Jeong Kim[1], Nguyen Dinh Phuong[1], Kyung Hwa Jung[2], Jungho Kim[1], Han Dae Yun[3], and Hoon Kim[1]*

[1]*Department of Agricultural Chemistry, Sunchon National University, Suncheon 540-742, Republic of Korea*
[2]*Amicogen, Inc., 694-4 Sangchon, Jinsung, Jinju 660-852, Republic of Korea*
[3]*Division of Applied Life Science, and Research Institute of Life Science, Gyeongsang National University, Chinju 660-701, Republic of Korea*

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A non-cellulosomal xylanase from *Clostridium thermocellum*, XynX, consists of a family-22 carbohydrate-binding module (CBM22), a family-10 glycoside hydrolase (GH10) catalytic module, two family-9 carbohydrate-binding modules (CBM9-I and CBM9-II), and an S-layer homology (SLH) module. *E. coli* BL21(DE3) (pKM29), a transformant carrying *xyn*X', produced several truncated forms of the enzyme. Among them, three major active species were purified by SDS-PAGE, activity staining, gel-slicing, and diffusion from the gel. The truncated xylanases were different from each other only in their C-terminal regions. In addition to the CBM22 and GH10 catalytic modules, $XynX_1$ had the CBM9-I and most of the CBM9-II, $XynX_2$ had the CBM9-I and about 40% of the CBM9-II, and $XynX_3$ had about 75% of the CBM9-I. The truncated xylanases showed higher binding capacities toward Avicel than those toward insoluble xylan. $XynX_1$ showed a higher affinity toward Avicel (70.5%) than $XynX_2$ (46.0%) and $XynX_3$ (42.1%); however, there were no significant differences in the affinities toward insoluble xylan. It is suggested that the CBM9 repeat, especially CBM9-II, of XynX plays a role in xylan degradation in nature by strengthening cellulose binding rather than by enhancing xylan binding.

***Keywords*:** *Clostridium thermocellum* XynX, truncated xylanases, gel-elution, carbohydrate-binding module, cellulose and xylan binding

Xylan, a highly branched β-1,4-linked D-xylose polymer, is a major component of hemicellulose in plant cell walls and can be degraded by many xylanolytic bacteria and fungi. Complete degradation of xylan requires the actions of several types of enzymes such as endo-β-1,4-xylanase (1,4-β-D-xylan xylanohydrolase: EC 3.2.1.8), β-xylosidase (1,4-β-D-xylan xylohydrolase: EC 3.2.1.37), α-arabinofuranosidase, and α-glucuronidase. Among many xylanolytic bacteria and fungi, *Clostridium thermocellum*, an anaerobic thermophilic bacterium, is one of the most active cellulose-degrading microorganisms and is of great significance to the production of biofuel from cellulosic biomass (Bayer *et al*., 2004; Demain *et al*., 2005). *C. thermocellum* secretes non-cellulosomal xylanases in addition to a cellulosome that contains at least one noncatalytic scaffolding protein (CipA) and up to 41 different polypeptides including endoglucanases, exoglucanases, xylanases, and cell-surface anchors (Gold and Martin, 2007; Bayer *et al*., 2008). Although much is known about cellulases associated with the cellulosome, there is only a limited amount of information available regarding the xylanases of *C. thermocellum*. Xylanases often exhibit a multimodular structure consisting of catalytic domains (CDs) linked to one or more non-catalytic domains such as cellulose-binding domains (CBDs), thermostabilizing domains (TSDs), and S-layer-like domains (SLDs) (Hall and Gilbert, 1988). Nine xylanase genes have been characterized from *C. thermocellum*: *xyn*Z (Grepinet *et al*., 1988), *xyn*10B (formerly *xyn*Y) (Fontes *et al*., 1995; Charnock *et al*., 2000), *xyn*C (Hayashi *et al*., 1997), *xyn*X (Jung *et al*., 1998; Kim *et al*., 2000), *xyn*A and *xyn*B (Hayashi *et al*., 1999), *xyn*U and *xyn*V (Fernandes *et al*., 1999), and *xyn*D (Xyn10D) (Zverlov *et al*., 2005). A unique xyloglucanase gene *xgh*A (Xgh74A) was cloned by a targeted DNA amplification (Zverlov *et al*., 2005). Previously, we cloned a xylanase gene, *xyn*X, of *C. thermocellum* (Jung *et al*., 1998; Kim *et al*., 2000). The xylanase gene encoded a multidomain xylanase, XynX, which is composed of a TSD, a CD, two repeats of CBD, and an SLD; the TSD was located between the signal peptide sequence and the CD, in the order TSD-CD. XynX is thought to be a non-cellulosomal xylanase and is bound directly to the cell surface because the SLD of XynX has no similarity to the other cellulosomal enzymes of *C. thermocellum* and XynX has no dockerin domain (Beguin and Lemaire, 1996). XynX was not listed in a recent study on the quantitative proteomic analysis of *C. thermocellum* cellulosome (Gold and Martin, 2007). In a study with a domain-transpositioned enzyme, the TSD of XynX was found to have dual thermostabilization and xylan-binding functions, $XynX'_{CD\text{-}TSD}$ with CD-TSD in the order (Shin *et al*., 2002). The transposetioned enzyme was degraded into several species in *Escherichia coli* DH5α or BL21(DE3), and the pattern of proteolytic cleavage was dependent on the presence of a host protease(s) and the arrangement of the domains in the enzyme. A TSD of XynX was renamed to a family-22 carbohydrate-binding module

* For correspondence. E-mail: hoon@sunchon.ac.kr; Tel: +82-61-750-3294; Fax: +82-61-752-8011

(CBM22), a CD to a family-10 glycoside hydrolase (GH10) catalytic module, two CBDs to two family-9 carbohydrate-binding modules (CBM9), and an SLD to an S-layer homology (SLH) module (Bayer *et al*., 2006). The CBM9 from *Thermotoga maritima* xylanase 10A was able to bind to amorphous cellulose, crystalline cellulose, and insoluble xylan, as well as to soluble saccharides (Boraston *et al.*, 2001). Cellooligosaccharides longer than two glucose units did not bind with improved affinity, indicating that cellobiose was sufficient to occupy the entire binding site. The addition of CBM9 to a catalytic module of *Clostridium stercorarium* Xyn10B did not significantly change the catalytic activity of the enzyme toward xylan (Zhao *et al.*, 2005).

Here, we describe the purification of the three truncated xylanase species of *C. thermocellum* XynX expressed in *E. coli* BL21(DE3), the domain structures of the three truncated enzymes determined from their N-terminal sequences and molecular masses, and the role of the CBM9 repeat, CBM9-I and CBM9-II, of XynX in cellulose and xylan binding.

Materials and Methods

Bacterial strains, plasmids, and culture conditions

E. coli BL21(DE3) (pKM29) was used as the source of xylanase from *C. thermocellum* ATCC 27405 (Jung *et al*., 1998; Kim *et al*., 2000). Plasmid pKM29 contains the signal peptide, CBM22, GH10, and CBM9 repeat (CBM9-I and CBM9-II) of *xyn*X, but not the SLH portion of the gene. *E. coli* BL21(DE3) (pKM29) was grown in LB broth supplemented with ampicillin (50 μg/ml) for 15 h at 37°C for the xylanase production.

Separation and purification of the truncated xylanase species

Crude extracts of *E. coli* (pKM29) cells were prepared as described previously (Shin *et al*., 2002), heat-treated for 30 min at 60°C, and then centrifuged at 8,000×g for 15 min at 4°C. The proteins in the supernatant were precipitated with ammonium sulfate at 40-80% saturation and the precipitate was dissolved in a minimum volume of 50 mM sodium citrate buffer (pH 5.5) and then dialyzed against 25 mM Tris-HCl buffer (pH 8.0). The truncated xylanase species in the dialysate were separated by SDS-PAGE on a large gel (15×15 cm). Activity staining for xylanase in the gel was carried out as described previously (Kim *et al*., 2000) with slight modifications. The washed gel was soaked for 30 min in a fresh buffer containing 1 mM 4-methylumbelliferyl-β-D-cellobioside (MUC) (Sigma, USA) at 4°C, placed on a glass plate, and then incubated for 30 min at 55°C. After the detection of active xylanase bands under UV-light, portions of the gel corresponding to each truncated enzyme species were separately cut into slices. The xylanase species in each gel slice was recovered by grinding the gel slice with a minimum volume of 50 mM sodium citrate buffer (pH 5.5), shaking the gel paste in a glass tube overnight at 4°C, filtering through a membrane (0.45 μm, Millipore, USA), and then centrifuging the filtrate at 12,000×g for 15 min at 4°C. Alternatively, the proteins were eluted using an electroeluter (Bio-Rad, USA) according to the manufacturer's instructions. The solution obtained from each truncated xylanase band was lyophilized and used for further study.

N-Terminal amino acid sequence analysis of the truncated xylanases

The lyophilized samples were electrophoresed on an SDS-PAGE gel, the proteins were transferred to a PVDF membrane, and the visualized bands were then cut out for the analysis of the N-terminal amino acid sequence. Amino acid sequences were analyzed using an automatic sequencer (Applied Biosystems, Model 476A-01-120, USA) at the Korea Basic Science Institute (Daejon, Korea).

Determination of enzyme activity and protein concentration

Xylanase activity was determined using a dinitrosalicylic acid (DNS) method (Jung *et al*., 1998) with 0.5% (w/v) oat spelt xylan (Sigma) as the substrate. Reaction was carried out for 30 min at 50°C in 50 mM sodium citrate buffer (pH 5.5). One unit of the enzyme activity was defined as the amount of the enzyme that liberated 1 μmol of reducing sugar per min under the reaction conditions. To determine the optimum temperature, the enzymes were reacted with the substrate in 50 mM sodium citrate buffer (pH 5.5) for 30 min at temperatures ranging from 40 to 70°C at 5°C intervals. The heat stability of the enzyme species was determined by measuring the residual activity after heat treatment for 20 min at 70°C in the absence of the substrate. The protein concentration was determined using the method of Lowry *et al*. (1951).

Determination of polysaccharide binding ability and electrophoretic mobility of the truncated xylanases

Soluble and insoluble fractions of xylan were prepared by stirring 5% (w/v) oat spelt xylan (Sigma) in deionized water as described previously (Shin *et al*., 2002). The supernatant and the pellet were used as soluble and insoluble xylan fractions, respectively. To measure the binding capacities of the xylanase species to insoluble polysaccharides, approximately 0.12 U of the enzyme species were mixed with 0.5 ml of 1.5% Avicel or insoluble xylan in 50 mM sodium citrate buffer (pH 5.5). After shaking for 1 h at 4°C, the mixtures were centrifuged at 15,000×g for 20 min at 4°C to remove the insoluble polysaccharides and the enzymes bound to the polysaccharides (Shin *et al*., 2002). The amount of the unbound enzyme was determined by assaying xylanase activity in the supernatant. Changes in the electrophoretic mobility of the xylanase species caused by binding to soluble xylan were analyzed by denaturing SDS-PAGE on an 11.5% gel containing 0.1% soluble xylan, using a gel without soluble xylan as a control (Shin *et al*., 2002). The positions of the truncated enzymes were visualized by activity staining as described above.

Results

Isolation and purification of the truncated xylanases

The specific activity of the xylanase in the crude cell extract of *E. coli* BL21(DE3) (pKM29) was 6.7 U/mg protein. After heat treatment for 30 min at 60°C, the specific activity was increased by about 2.4-fold, and 81% of the total activity was recovered (data not shown). Three major forms of active truncated xylanase were produced by *E. coli* BL21(DE3) (pKM29) and were named, from the largest to the smallest: $XynX_1$, $XynX_2$, and $XynX_3$ (lanes 1 in Figs. 1A and 1B). The molecular masses of $XynX_1$, $XynX_2$, and $XynX_3$ were estimated to be about 97, 85, and 60 kDa, respectively (Fig. 1A).

The truncated xylanase species were purified from the SDS-PAGE gel after renaturation and activity staining. Although XynX hardly cleaved cellulosic substrates, it could be stained with MUC because it was screened from a transformant with an $MUC^+CMC^-pNPG^-$ phenotype during the cloning procedure (Jung *et al.*, 1998). The bands corresponding to $XynX_1$, $XynX_2$,

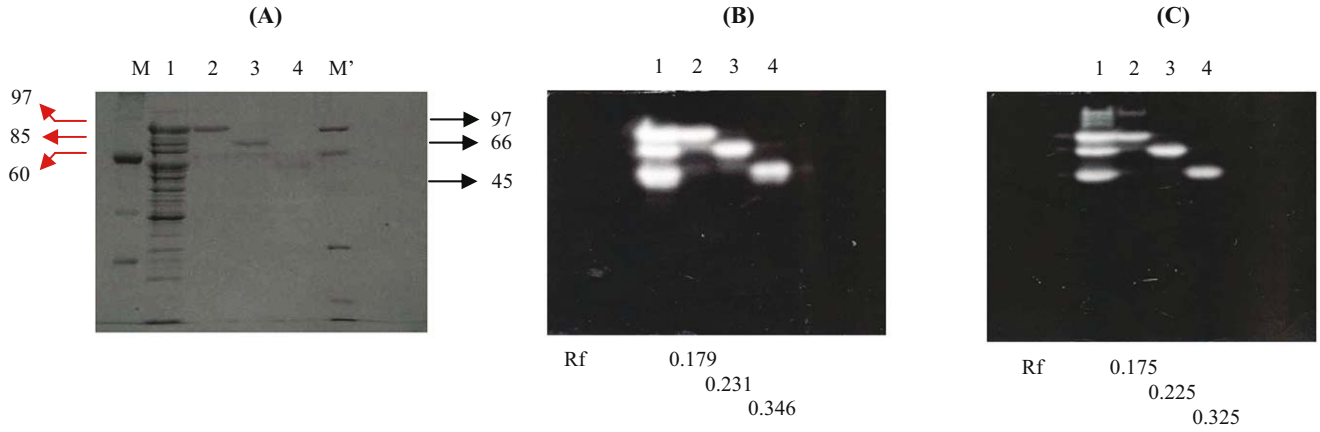

Fig. 1. SDS-PAGE and activity staining of the truncated xylanases of *Clostridium thermocellum* XynX produced from *E. coli* BL21(DE3) (pKM29) in the absence or in the presence of soluble xylan. (A) SDS-PAGE in the absence of soluble xylan, Coomassie stained. (B) SDS-PAGE in the absence of soluble xylan, activity stained. (C) SDS-PAGE in the presence of 0.1% soluble xylan, activity stained. Lanes: M, protein size marker; 1, heat-treated crude enzyme; 2, $XynX_1$; 3, $XynX_2$; 4, $XynX_3$; M', BR_6 protein size marker. The numerals in the left side of Fig. 1A represent the molecular masses of $XynX_1$, $XynX_2$, and $XynX_3$, respectively. The numerals in the right side represent the molecular masses of protein size markers. The values under the Fig. 1-b and 1-c represent the Rf values of $XynX_1$, $XynX_2$, and $XynX_3$, where Rf=distance

and $XynX_3$ were cut out separately, and the truncated xylanases were eluted from the gel using a gel-slicing and diffusion method. Almost all the proteins loaded onto the gel (4.08 mg) were recovered by this method, and the amounts of proteins for $XynX_1$, $XynX_2$, and $XynX_3$ were 1.4, 1.1, and 1.3 mg, respectively (Table 1). The amounts of recovered xylanase activity for $XynX_1$, $XynX_2$, and $XynX_3$ were 27.6, 70.8, and 49.4 U, respectively (Table 1). The purified truncated enzymes appeared to be homogeneous in the protein stained gel (Fig. 1A), and the molecular masses of $XynX_1$, $XynX_2$, and $XynX_3$ were exactly the same as those of the corresponding enzymes in the crude enzyme preparation (Figs. 1A and B).

N-terminal amino acid sequences and domain structures of the truncated xylanases

The N-terminal amino acid sequences of $XynX_1$, $XynX_2$, and $XynX_3$ were the same, i.e., DDNNA, indicating that the three truncated xylanase species are different from each other only in their C-terminal regions. The N-terminal amino residue corresponded to the 33rd amino acid residue of XynX based on the nucleotide sequence (GenBank accession no. M67438) (Kim *et al*., 2000).

The amino acid sequence of XynX revealed a multidomain structure consisting of a TSD (CBM22), a CD (GH10), a duplicated CBD (CBM9-I and CBM9-II), and an SLD (SLH) (Kim *et al*., 2000). From the estimated molecular masses and N-terminal amino acid sequences of the three truncated xylanases, $XynX_1$, $XynX_2$, and $XynX_3$ were predicted to be composed of approximately 882, 773, and 545 amino acid residues, respectively. All three truncated xylanases had CBM22 and GH10. In addition to the CBM22 and GH10; $XynX_1$ had the CBM9-I and most of the CBM9-II; $XynX_2$ had the CBM9-I and about 40% of the CBM9-II; and $XynX_3$ had about 24% of the CBM9-I, but completely lacked the CBM9-II (Fig. 2).

Table 1. Purification of the truncated XynX species by gel-slicing and diffusion methods from *E. coli* BL21(DE3) (pKM29)

Xylanase species	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)
$XynX_1$	27.6±9.0	1.44±0.67	20.9±6.8
$XynX_2$	70.8±21.4	1.07±0.67	55.1±10.2
$XynX_3$	49.4±17.6	1.31±0.94	45.2±15.5

The total amount of the enzyme loaded onto the gel was 110 U of activity and 4.08 mg of protein. The values represent the average of the results from triplicate experiments.

Polysaccharide binding abilities and electrophoretic mobilities of the truncated xylanases

The largest form of the truncated xylanases, $XynX_1$, showed a higher binding ability (70.5%) toward Avicel, microcrystalline

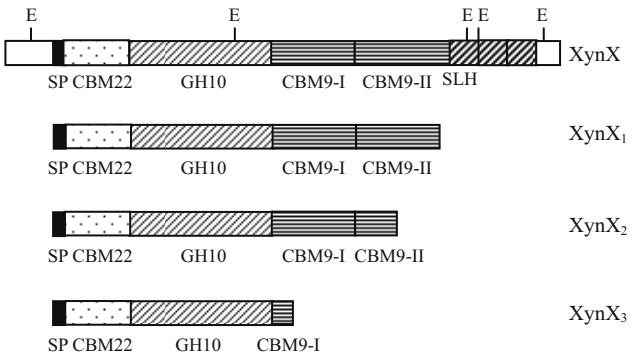

Fig. 2. Module organizations of the truncated xylanases, $XynX_1$, $XynX_2$, and $XynX_3$, predicted from their N-terminal amino acid sequences and the estimated molecular masses. Abbreviations: SP, signal peptide; CBM22, family-22 carbohydrate-binding module; GH10, family-10 glycoside hydrolase catalytic module; CBM9, family-9 carbohydrate-binding module; SLH, S-layer homology module. The sizes of the presented boxes in the figures are not proportional to the actual lengths of the modules. E, *Eco*RI site, is included to indicate the relative positions.

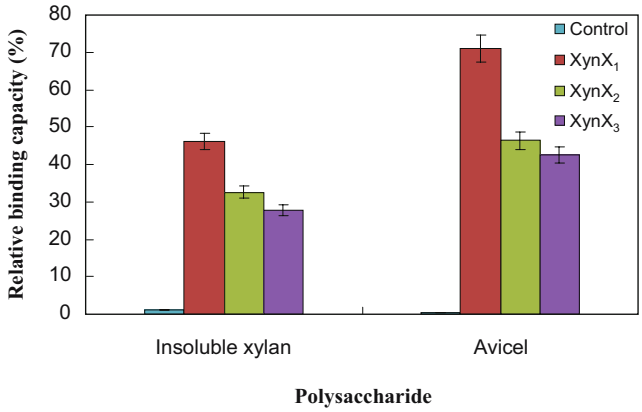

Fig. 3. Binding abilities of the truncated xylanases toward insoluble xylan and Avicel. Polysaccharides were used at their final concentrations of 1.5% (w/v). The activity of the same amount of each enzyme mixed only with the buffer was considered to be 100%. The values represent the average of the results from triplicate experiments. For more details, refer to 'Materials and Methods'.

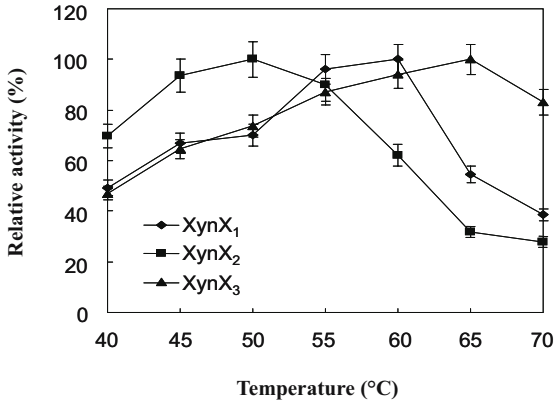

Fig. 4. Optimum temperatures of the truncated xylanases, $XynX_1$, $XynX_2$, and $XynX_3$, for xylanase activity. The activity of each enzyme at its optimum temperature was considered to be 100%. The values represent the average of the results from triplicate experiments.

cellulose, than toward insoluble xylan (45.0%). $XynX_1$ showed a higher binding ability to Avicel (70.5%) than $XynX_2$ (46.0%) or $XynX_3$ (42.1%) (Fig. 3). $XynX_1$ also showed a higher binding capacity toward insoluble xylan (45.0%) than $XynX_2$ (31.5%) or $XynX_3$ (36.7%), but the differences were not as great as with Avicel.

When the crude extract was electrophoresed in the presence of soluble xylan in the gel, aggregated forms of xylanase with higher molecular masses were observed in the upper region of the gel. The aggregated form was also observed with the purified $XynX_1$, but not with the purified $XynX_2$ or $XynX_3$ (Fig. 1C). The electrophoretic mobilities of $XynX_1$, $XynX_2$, and $XynX_3$ were slightly retarded by the inclusion of soluble xylan in the gel, and the decrease in the relative mobility (Rf) of $XynX_3$ (0.346 to 0.325) was greater than those of $XynX_1$ and $XynX_2$ (Figs. 1B and 1C).

Thermostabilities and optimum temperatures of the truncated xylanases

$XynX_1$, the largest truncated enzyme, consisted of CBM22, GH10, CBM9-I, and CBM9-II and showed 50.3% residual activity after 20 min of pre-incubation at 70°C (Table 2). Smaller forms of the truncated enzymes, $XynX_2$ with CBM22, GH10, CBM9-I, and a part of CBM9-II and $XynX_3$ with CBM22, GH10, and only a small portion of CBM9-I, showed higher residual activity and the residual activities for $XynX_2$ and $XynX_3$ were 75.0% and 84.0%, respectively. The optimum temperatures of $XynX_1$, $XynX_2$, and $XynX_3$ were 60, 50, and 65°C, respectively (Fig. 4).

Table 2. Heat stability of the truncated $XynX_1$, $XynX_2$, and $XynX_3$ enzymes

Xylanase species	Heat stability (%)
$XynX_1$	50.3±6.2
$XynX_2$	75.0±7.8
$XynX_3$	84.0±8.6

* Heat stability was determined by preincubating the enzymes for 20 min at 70°C in the absence of the substrate and then assaying the residual activities as described in Materials and Methods. The activity of the nonpreincuabted enzyme was considered to be 100%. The values represent the average of the results from triplicate experiments.

Discussion

XynX is a non-cellulosomal multidomain xylanase produced by *C. thermocellum*. The role of CBM9-I and CBM9-II in XynX was investigated using the truncated enzymes produced by *E. coli* BL21(DE3) (pKM29). Plasmid pKM29 carries the signal peptide, CBM22, GH10, and CBM9 of *xynX*; lacks the SLH; and encodes 946 amino acid residues. After cleavage of the signal peptide of 32 amino acids, the xylanase had 914 amino acid residues. When the xylanase gene was expressed in *E. coli* BL21(DE3) or DH5α, several active truncated xylanases were produced due to the internal proteolytic cleavage of the protein, similar to the production of several active species by *E. coli* DH5α or JM83 transformants carrying the full length XynX gene (Kim *et al*., 2000). This phenomenon has also been reported with a *Bacillus subtilis* endoglucanase, which was produced as smaller truncated forms in *E. coli* or *B. megaterium* transformants (Kim *et al*., 1991, 1995). Active cleavage products produced by the strains BL21(DE3) and DH5α carrying the plasmid pKM29 were different from each other in their numbers and sizes, and the largest truncated XynX of 97 kDa was produced by the strain BL21(DE3) (data not shown).

Major truncated xylanase species, $XynX_1$, $XynX_2$, and $XynX_3$, (XynX), were purified from the crude extract of *E. coli* BL21(DE3) (pKM29) by gel slicing and diffusion. Much of the undesired proteins in the crude extract could be removed by the heat treatment due to the thermostable nature of XynX. The diffusion method was much more efficient than the electroelution method in recovering the truncated enzymes; the recovery rates were more than 90% and less than 30%, respectively (data not shown). The N-terminal amino acid sequences of the truncated xylanases were identical, and the truncated enzymes were predicted to differ from each other only in the C-terminal regions of the proteins. The molecular masses of the truncated enzymes indicated that about 60% of the CBM9-II was deleted in

$XynX_2$ and that the CBM9-II and 75% of the CBM9-I of XynX were deleted in $XynX_3$. The truncated xylanases were used to study the role of the deleted domains CBM9-I and CBM9-II in polysaccharide binding.

All three truncated xylanases showed significantly higher binding abilities toward Avicel than toward insoluble xylan, though XynX had negligible cellulolytic activity. Based on the observation that the binding capacity of $XynX_1$ toward Avicel was the strongest among the truncated xylanases and that the binding abilities of $XynX_2$ and $XynX_3$ were almost same, it is likely that the CBM9-II portion of XynX has a more important role in cellulose binding than CBM9-I. Although $XynX_1$ had the highest binding capacity toward insoluble xylan, the differences in the binding abilities of the truncated enzymes toward insoluble xylan were much smaller than those toward Avicel. $XynX_3$ that lacks both CBM9-I and CBM9-II showed a higher binding capacity toward insoluble xylan than $XynX_2$, the value being close to the mean value of $XynX_1$ and $XynX_2$. The differences in the binding abilities of $XynX_3$ toward insoluble xylan and Avicel were much smaller than those of $XynX_1$ and $XynX_2$. These observations suggest that the CBM9 repeat is not the major component of the enzyme responsible for xylan-binding, and that CBM22, the domain existing in all the three truncated forms, plays a greater role in xylan-binding. Involvement of CBM22 in xylan-binding has been reported in a study with a CBM22-transpositioned mutant (Shin *et al*., 2002), as well as in other studies (Grepinet *et al*., 1988; Meissner *et al*., 2000; Sunna *et al*., 2000). In a database search, the carbohydrate binding sites of both the CBM9-I and CBM9-II were found on the same seven amino acid residues (Marchler-Bauer *et al*., 2009). However, the CBM9-I and CBM9-II of XynX did not show the same degree of influence on the cellulose-binding ability of the enzyme.

When electrophoresed in the presence of soluble xylan, aggregated form(s) of the enzyme were observed with the crude extract and with the purified $XynX_1$, but not with $XynX_2$ and $XynX_3$, indicating that aggregation was caused mainly by the weak interaction of CBM9-II, rather than CBM9-I, to soluble xylan. The relative mobilities (Rf) of the truncated xylanases were slightly decreased by the presence of the soluble xylan as the substrate. Substrates of several enzymes or related compounds were known to interact with the enzyme in the gel matrix during electrophoresis and slow the migration of the enzyme (Charnock *et al*., 2000; Shin *et al*., 2002).

The deletion of the domains in XynX showed different effects on heat stability and optimum temperature of the enzyme. The heat stability of $XynX_1$ was lower than those of $XynX_2$ and $XynX_3$, whereas the optimum temperature of $XynX_1$ was higher than that of $XynX_2$ and lower than that of $XynX_3$. It is thought that the CBM9 of XynX exerts its influence on the temperature dependent reactions in the presence of the substrate to some extent rather than on the heat stability of the enzyme in the absence of the substrate; it is also thought that the complete CBM9-I and CBM9-II in $XynX_1$ exerts more influence than the CBM9-I and partially deleted CBM9-II in $XynX_2$. The CBM22, existing in all the three truncated enzymes, is thought to be the major factor that governs the heat stability of XynX, especially that of the smallest $XynX_3$. The CBM22 of XynX was found to have dual functions of substrate binding and thermostabilization (Shin *et al*., 2002), although it has been reported that the CBM of xylanase 10B of *C. thermocellum* is not a thermostabilizing domain (Dias *et al*., 2004).

XynX of *C. thermocellum*, which has a CBM9 repeat, CBM9-I, and CBM9-II, is a non-cellulosomal xylanase with no significant cellulase activity. It is suggested that the CBM9 repeat of XynX, especially CBM9-II, plays a role in xylan degradation in nature by strengthening cellulose-binding rather than by enhancing xylan-binding. The presence of the complete CBM9 repeat is necessary for effective binding of the enzyme to cellulose. Considering the complexity of cellulosic materials in nature, the binding of the CBM9 repeat to cellulose may make XynX more accessible to the actual substrate, xylan, which coexists with cellulose. The CBM22 existing in all the truncated xylanases is thought to be a major factor, with a minor contribution of CBM9-II, that affects the heat stability and optimum temperature of the enzymes. Detailed studies to elucidate how the binding of the xylanase to cellulose enhances the enzyme activity are needed to better understand of the action of the enzyme.

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