Homologous xylanases from *Clostridium thermocellum*: evidence for bi-functional activity, synergism between xylanase catalytic modules and the presence of xylan-binding domains in enzyme complexes

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Clostridium thermocellum produces a consortium of plant-cell-wall hydrolases that form a cell-bound multi-enzyme complex called the cellulosome. In the present study two similar xylanase genes, *xynU* and *xynV*, were cloned from *C. thermocellum* strain YS and sequenced. The deduced primary structures of both xylanases, xylanase U (XylU) and xylanase V (XylV), were homologous with the previously characterized xylanases from *C. thermocellum* strain F1. Truncated derivatives of XylV were produced and their biochemical properties were characterized. The xylanases were shown to be remarkably thermostable and resistant to proteolytic inactivation. The catalytic domains hydrolysed xylan by a typical endo-mode of action. The type VI cellulose-binding domain (CBD) homologue of XylV bound xylan and, to a smaller extent, Avicel and acid-swollen cellulose. Deletion of the CBD from XylV abolished the capacity of the enzymes to bind polysaccharides. The polysaccharide-binding domain was shown to have a key role in the hydrolysis of insoluble substrates by XylV. The C-terminal domain of XylV, which is absent from XylU, removed acetyl groups from acetylated xylan and acted in synergy with the glycosyl hydrolase catalytic domain of the enzyme to elicit the hydrolysis of acetylated xylan.

Key words: carbohydrate-binding domain, xylan deacetylation.

INTRODUCTION

Enzymes from anaerobic micro-organisms associate to form high-molecular-mass plant-cell-wall-degrading multi-enzyme complexes, exemplified by the corresponding *Clostridium thermocellum* complex, termed the cellulosome [1]. In addition to cellulases, the *C. thermocellum* cellulosome contains xylanases, which have very complex molecular architectures and contain various non-catalytic modules in addition to one or possibly two catalytic domains [2–6]. Examples of the non-catalytic modules found in clostridial xylanases are type IX cellulose-binding domains (CBDs) [5], thermostabilizing domains [3] (which were also found in enzymes from mesophilic bacteria), SLH domains (for S-layer homology) which promote the binding of individual enzymes to the cell envelope [7], and domains homologous with a *Rhizobium*-derived protein which is involved in generating plant-specific nodulation signals (NodB). The function of NodB in nitrogen-fixing bacteria such as *Rhizobium* is to deacetylate the non-reducing end of chito-oligosaccharides, which are then appended to acyl moieties and function as signalling molecules in the nodulation process in leguminous plants. NodB homologues have been identified in some xylanases; in one of these enzymes, xylanase D from *Cellulomonas fimi*, the domain deacetylates acetylated xylan [8]. Although the cellulosome binds tightly to the plant cell wall via the CBD present on the scaffolding protein (CipA), some of the polysaccharidases, including one xylanase, bind cellulose via the non-catalytic modules [5,9]. However, CBDs from aggregated cellulases and xylanases exhibit lower affinities for crystalline cellulose than do the corresponding modules from non-aggregated enzymes or CipA [9,10]. It remains to be established whether the polysaccharide-binding domains from the different cellulosome components act in synergy to elicit attachment to the plant cell wall. The functions of some cellulosomal xylanase non-catalytic domains remain to be established, although they could constitute additional catalytic domains involved in the removal of xylan side-chains. In fact, accessory enzymes that cleave bonds other than the β-1,4-glycosidic linkages of the xylan backbone have not been described in anaerobic thermophilic bacteria, although they are known to be important in xylan hydrolysis by mesophilic organisms [11].

From the foregoing it is apparent that there are several unresolved questions about xylanases from *C. thermocellum*. For example, the efficient hydrolysis of xylans requires the synergistic interaction of β-1,4-xylanases with side-chain-cleaving enzymes, which have yet to be identified in this thermophilic bacterium. It remains unclear whether these catalytic modules are discrete individual enzymes or are encoded by modular xylanases comprising multiple catalytic domains [11]. Additionally, domains that mediate the binding of polysaccharidases to xylan have yet to be discovered in cellulosomal enzymes.

Here we analyse the role of the non-catalytic modules of xylanases U and V (XylU and XylV), homologous with xylanase A and B from *C. thermocellum* strain F1 [6], in enzyme function. We show that the two xylanases are components of the

Abbreviations used: CBD, cellulose-binding domain; CD, family 11 catalytic domain; CDCBD, CD fused to TVI; FL, full-length enzyme; Nod, NodB homologue; NodB, a *Rhizobium*-derived protein which is involved in generating plant-specific nodulation signals; NodCBD, Nod plus TVI; TVI, type VI CBD; XBD, xylan-binding domain; XylU, xylanase U; XylV, xylanase V.

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cellulosome and contain a type VI polysaccharide-binding domain that binds strongly to xylan. The xylan-binding domain (XBD) potentiates the activity of the enzymes against insoluble substrates. In addition, the NodB homologue of XylV removes acetyl groups from acetylated xylan and acts in synergy with the family 11 catalytic domain (CD) of the enzyme during the hydrolysis of acetylated xylan.

MATERIALS AND METHODS

Sequence analysis

A 5.3 kb DNA fragment, derived from a xylanase-positive clone isolated from a *C. thermocellum* strain YS genomic library was sequenced by methods described previously [3]. Two contiguous open reading frames, sharing considerable identity, were identified; the genes were named *xynU* and *xynV*. The sequences of *xynU* and *xynV* were assigned the accession number AF047761 in the EMBL/GenBank/DDBJ nucleotide sequence data libraries.

Production of truncated derivatives of *xynV* and protein purification

To hyperexpress *xynV* and its truncated derivatives, the appropriate DNA sequences were amplified by PCR and the resulting fragments were cloned into the prokaryotic expression vector pET21a. PCRs were performed as described previously [3]. DNA was amplified by using primers with *Nde*I and *Xho*I restriction sites, enabling the PCR products to be cloned into *Nde*I/*Xho*I-restricted pET21a. Full-length and truncated derivatives of XylV were purified by metal-chelate affinity chromatography from cell-free extracts derived from 200 ml cultures, as described previously, with a His Trap column (Pharmacia). Fractions recovered from the column were analysed by SDS/PAGE [12]; those containing pure polypeptides were dialysed twice against 1000 vol. of 50 mM sodium phosphate buffer, pH 7.4.

Enzyme assays

All enzyme assays were performed at 60 °C in 50 mM sodium phosphate buffer, pH 7.4, with 0.2 % (w/v) of the appropriate plant structural polysaccharide, unless stated otherwise [13]. Soluble and insoluble xylans were prepared from oat spelt or birchwood acetylated xylan as described by Sun et al. [14]. Reducing sugar was measured with the dinitrosalicylic acid reagent [15]. Enzyme activity is expressed in katals per molecule of enzyme. Protein was measured by the Lowry method with BSA as standard. The affinity of the xylanases for insoluble polysaccharides was measured as follows: purified enzyme (0.5 g/l) was mixed with an equal volume of 5 % (w/v) polysaccharide in 50 mM sodium phosphate buffer, pH 7.0, for 15 min at 0 °C. Unbound material was quantified by measuring protein concentration in the filtrate after the insoluble xylan had been recovered by filtration. Polysaccharide pellets were resuspended in SDS/PAGE buffer and analysed by electrophoresis. Acetylated xylan was prepared as described by Ferreira et al. [16]; the liberation of acetic acid was assayed as described previously [8]. Proteolysis and thermostability experiments were performed as described by Fontes et al. [3].

RESULTS

Molecular architecture of XylU and XylV

XylU and XylV from *C. thermocellum* strain YS are almost identical with xylanases A and B respectively, which were recently characterized from *C. thermocellum* strain F1 [6]. XylU/xylanase B and XylV/xylanase A have a modular architecture, being composed of three and four distinct domains respectively (Figure 1). XylU and XylV shared almost complete identity (97.8 %) between the corresponding modules. On the basis of comparison with translated sequences in the GenBank database, residues 29–222 of XylU and XylV correspond to xylanase catalytic domains belonging to glycosyl hydrolase family 11. Downstream of the catalytic domains was a 132-residue sequence that exhibited a high degree of similarity to type VI CBD (TVI). The C-terminal domain of XylU and the internal domain of XylV, located between residues 389 and 451, correspond to classic *C. thermocellum* dockerin domains. The C-terminal 205 residues of XylV, not present in XylU, exhibited extensive identity with NodB proteins. The interdomain regions of XylU and XylV were typical proline/threonine linker sequences found in other polysaccharidases [17].

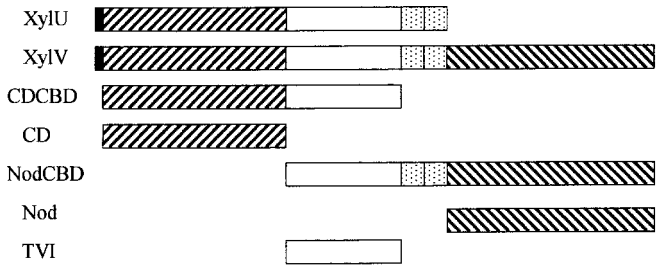

Figure 1 Molecular architecture of XylU, XylV and truncated XylV derivatives from *C. thermocellum*

The functional domains in XylU, XylV and XylV derivatives are shown as follows: black, signal peptide; upward hatching, CD; white, TVI; stippling, dockerin domain; downward hatching, NodB.

Biochemical properties of XylU and XylV

After incubation for 2 h with pancreatic proteinases, both XylU and XylV retained more than 90 % of their catalytic activity, demonstrating a high degree of resistance to proteolytic inactivation. Analysis of the substrate specificities of the two xylanases revealed that both enzymes hydrolysed oat spelt xylan (soluble and insoluble forms) and also displayed catalytic activity against lichenan and β-glucan. Neither xylanase was able to depolymerize laminarin, Avicel or carboxymethylcellulose.

Binding of full-length and truncated derivatives of XylV to polysaccharides

To evaluate the roles of the different XylU and XylV domains, truncated forms of XylV were constructed consisting of full-length enzyme (FL), CD, the NodB homologue (Nod), TVI, Nod plus TVI (NodCBD) and CD fused to TVI (CDCBD) (see Figure 1 for the molecular architecture of the enzymes). The six recombinant enzymes, which were highly expressed in *Escherichia coli*, were purified to apparent homogeneity (Figures 2A and 2C). Purified XylV (Figure 2B) had a broad pH optimum between 5 and 8 and a maximum specific activity at 75 °C. The enzyme was also remarkably thermostable because significant thermal inactivation occurred only at temperatures above 80 °C.

To determine the ligand specificity of TVI, the capacity of the purified proteins to bind insoluble xylan was studied. The results (Figure 2B and Table 1) show that all the proteins containing TVI could bind to insoluble xylan, whereas truncated derivatives

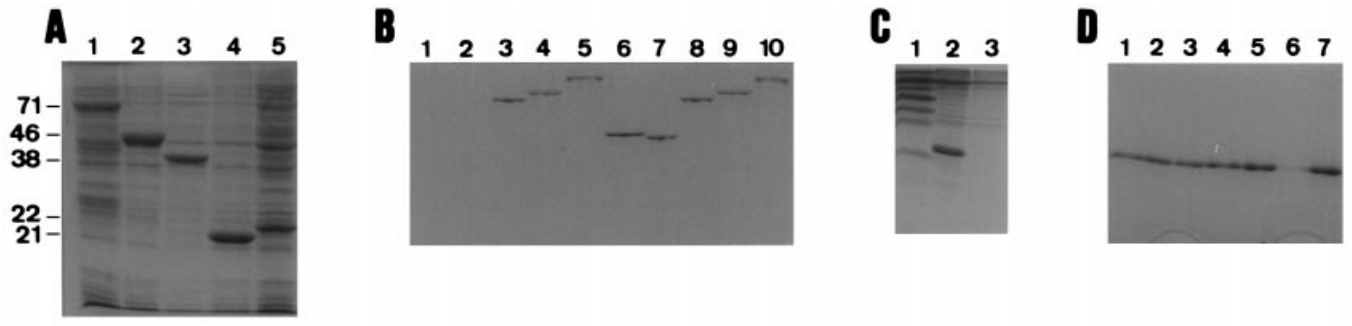

Figure 2 Hyperexpression (A, C), purification (B) and affinity for plant cell wall polysaccharides (B, D) of XylV and its truncated derivatives

(**A**) Cell-free proteins extracted from *E. coli* strains harbouring the recombinant genes encoding FL (lane 1), NodCBD (lane 2), CDCBD (lane 3), CD (lane 4) and Nod (lane 5) were subjected to SDS/PAGE. The sizes of prominent polypeptides, corresponding to the recombinant proteins, are shown (in kDa) at the left. (**B**) SDS/PAGE of purified Nod (lane 6), CD (lane 7), CDCBD (lane 8), NodCBD (lane 9) and FL (lane 10) and of material released from xylan, which had been incubated with the corresponding polypeptides (lanes 1–5). (**C**) SDS/PAGE of cell-free extract proteins from the *E. coli* strain expressing TVI (lane 2) and of corresponding polypeptides not retained by the chelate affinity column (lane 3). Lane 1 contained Sigma low-molecular-mass standards. (**D**) Affinity of TVI for various polysaccharides. TVI was incubated with Avicel (lanes 1 and 2), acid-swollen cellulose (lanes 3 and 4) and xylan (lanes 5 and 6). Bound (lanes 1, 3 and 5) and unbound (lanes 2, 4 and 6) materials were analysed by SDS/PAGE. Lane 7 contained purified TVI.

Table 1 Binding of XylV and its truncated derivatives to xylan and other cellulosic polysaccharides

Purified proteins (0.5 g/l) were incubated with 1 vol. of 5% (w/v) insoluble polysaccharide. The quantity of protein that bound to the polymers was evaluated by determining protein concentration in the filtrate. Results are means ± S.E.M. ($n = 3$). Abbreviation: n.d., not determined.

	Relative binding (%)		
Enzyme	Insoluble xylan	Avicel	Acid-swollen cellulose
FL	88 ± 3	n.d.	n.d.
CDCBD	86 ± 4	n.d.	n.d.
CD	12 ± 2	8 ± 3	6 ± 2
TVI	90 ± 4	58 ± 7	56 ± 8
NodCBD	91 ± 5	n.d.	n.d.
Nod	9 ± 1	n.d.	n.d.

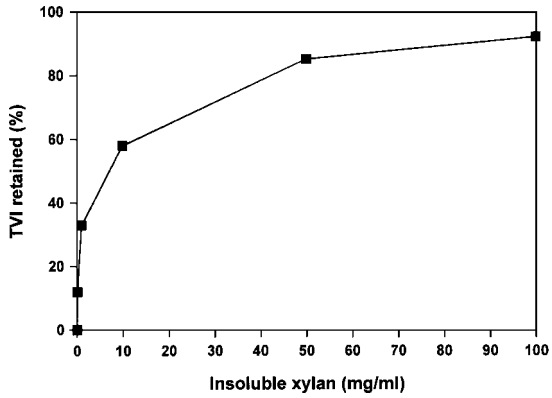

Figure 3 Binding of purified TVI to different concentrations of xylan

Identical amounts of TVI were incubated with various concentrations of insoluble xylan. The quantity of protein retained by the polysaccharide was evaluated by determining the protein concentration in the supernatant after recovering the polysaccharide by centrifugation.

lacking this module were unable to adhere significantly to the polymer. Additionally, it demonstrates that TVI can function independently of the other xylanase modules. The binding of the TVI polypeptide to xylan increased with increasing xylan-to-enzyme ratios (Figure 3). The relative affinity (K_r) was estimated to be 1.55 l/g, which is within the range of reported affinities of CBDs for cellulosic materials (0.9–15 l/g). The affinity of the TVI derivative for other polysaccharides was assessed. The results, presented in Table 2 and Figure 2(D), show that TVI also binds to Avicel and acid-swollen cellulose, but with a lower affinity than for xylan. These results indicate that TVIs from XylU and XylV have a high affinity for xylan, similar to the corresponding domain from *Clostridium stercorarium* xylanase A [14], and should therefore be classified as XBDs.

Effect of TVI on the catalytic activity of XylV

To evaluate the effect of TVI on the catalytic activity of XylV, the molar activities and kinetic properties of purified FL, CD and CDCBD for soluble and insoluble xylan were determined. The results show that all the enzymes have similar activities towards soluble xylan, suggesting that deletion of the TVI and NodB modules from XylV did not affect the function of the xylanase towards soluble substrates (Table 2). In contrast, CD showed a decreased capacity to hydrolyse insoluble xylan compared with FL and CDCBD, demonstrating that the TVI from XylV enhances the catalytic properties of the xylanase catalytic domain. We have investigated the possibility of the binding domain disrupting the structural integrity of insoluble xylan, by monitoring the capacity of CD to hydrolyse the polymer when mixed with equimolar concentrations of TVI. The results in Table 2 show that TVI does not enhance the catalytic properties of CD *in trans*, suggesting that TVIs potentiate the catalytic activity of xylanases by promoting tight interactions between the enzyme and the substrate. Interestingly, purified XylV hydrolyses β-glucan (Table 2) and lichenan (results not shown), which is unusual because most family 11 xylanases have been found to be devoid of endoglucanase activity.

As expected, when soluble xylan was used as substrate the K_m and V_{max} values of the three xylanases were very similar (Table

Table 2 Molar activities of XylV and its truncated derivatives for soluble, insoluble and oat spelt xylan and β-glucan

The final concentrations of substrates were 5, 10 and 20 g/l for soluble, insoluble and oat spelt xylan respectively. Results are means ± S.E.M. ($n = 3$). Abbreviation: n.d., not determined.

Enzyme	Enzyme activity (kat/mol)			
	Soluble xylan	Insoluble xylan	Oat spelt xylan	β-Glucan
FL	1771.4 ± 101.1	49.2 ± 1.2	532.4 ± 36.4	15 ± 3.4
CDCBD	1722.3 ± 131.1	51.5 ± 1.6	493.8 ± 15.4	n.d.
CD	1846.7 ± 67.8	20.5 ± 2.3	485.6 ± 12.9	n.d.
CD + TVI	n.d.‡	21.3 ± 1.9	n.d.	n.d.

Table 3 Kinetic properties of XylV and its truncated derivatives during the hydrolysis of soluble and insoluble xylan

The K_m values were determined with the direct method developed by Eisenthal and Cornish-Bowden [18]. The concentrations of soluble and insoluble xylan employed ranged from 0.1 to 10 g/l and from 30 to 600 g/l respectively. Abbreviation: n.d., not determined.

Enzyme	Soluble xylan		Insoluble xylan	
	K_m (g/l)	V_{max} (kat/mol)	K_m (g/l)	V_{max} (kat/mol)
FL	2.49	2027.8	n.d.	n.d.
CDCBD	2.50	1974.5	101.6	2018.4
CD	2.47	2169.9	169.0	2208.5

3). Determination of the kinetic parameters of xylanases acting on insoluble xylan was not straightforward. At low concentrations of insoluble xylan (0.1–5 g/l) there was a significant difference in the slope of the Lineweaver–Burk curves for CD and CDCBD, suggesting a higher activity for the xylanase derivative containing the XBD (Figure 4A). However, under these experimental conditions a determination of K_m and V_{max} was impossible because the curve did not follow that of a typical Michaelis–Menten equation. Kinetic parameters were calculated with accuracy only when substrate concentrations were raised significantly (30–600 g/l), by using the method of Eisenthal and Cornish-Bowden [18]. Surprisingly, at higher concentrations of insoluble xylan the apparent values of K_m and V_{max} for CD and CDCBD were similar (Table 3 and Figure 4B) and the V_{max} values for soluble and insoluble substrates were identical. Taken together, these results indicate that XBDs enhance the function of adjacent catalytic domains in xylanase, but this effect is revealed only at low and medium concentrations of insoluble xylan. It is possible that xylan structure is not homogeneous, with some regions of the insoluble fraction being more susceptible to hydrolysis. At very high substrate concentrations (more than 30 g/l) the predominance of these regions would increase and therefore the kinetic properties of the xylanase would be similar to those of soluble xylan.

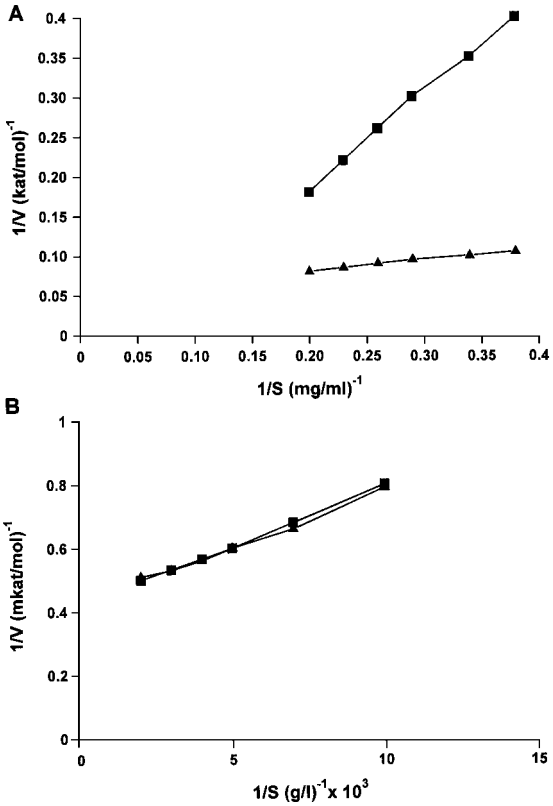

Figure 4 Lineweaver–Burk plots of CD (▲) and CDCBD (■) xylanase activities on insoluble xylan, at low (A) and high (B) substrate concentrations

Xylanase activities were determined at 60 °C with various concentrations of insoluble xylan in 50 mM sodium phosphate buffer, pH 7.0.

Function of XylV NodB domain

To assess the capacity of the C-terminal domain of XylV to remove acetyl groups from acetylated birchwood xylan, the activities of FL, Nod, CDCBD, CD and NodCBD towards acetylated substrate were determined. The results show that all XylV derivatives containing Nod removed acetyl groups from acetylated xylan, whereas the truncated derivatives of XylV lacking this domain (CD and CDCBD) displayed no apparent deacetylase activity (Table 4). The activity of Nod towards the insoluble material was lower than that of NodCBD or FL, suggesting that TVI, and possibly CD, increase the deacetylation mediated by Nod against insoluble xylan. The effect of Nod on the release of reducing sugars from acetylated xylan by various truncated derivatives of XylV was evaluated. The results (Table 4) show that pretreatment of the substrate with Nod increases the xylanase activity of the CD, probably by increasing the amount of substrate available. Additionally, the molar activity of CD towards the acetylated substrate increased at higher concentrations of Nod. Finally, the results suggest that full-length XylV exhibits a higher molar activity than CDCBD, which lacks the

Table 4 Xylanase and deacetylase activities of different XylV derivatives towards acetylated insoluble and birchwood xylan

The final concentrations of substrates were 4 mg/ml. Insoluble xylan was prepared from acetylated birchwood xylan by the same method as for oat spelt xylan. For the results designated 'Nod → CD', the substrate was pretreated for 20 min with Nod before xylanase activity was measured. Results are means ± S.E.M. ($n = 3$). Abbreviation: n.d., not determined.

	Xylanase activity (kat/mol)		Deacetylase activity (kat/mol)	
Enzyme	Birchwood xylan	Insoluble xylan	Birchwood xylan	Insoluble xylan
FL	411.1 ± 15	338.7 ± 6	15.1 ± 2.3	8.7 ± 0.4
CDCBD	392.6 ± 13	198.9 ± 9	0.0	0.0
CD/BSA (1:1)	389.9 ± 18	102.1 ± 4	0.0	0.0
CD/Nod (1:1)	415.4 ± 21	128.3 ± 7	11.9 ± 1.1	6.8 ± 0.9
CD/Nod (1:4)	496.6 ± 23	148.6 ± 12	n.d.	n.d.
Nod → CD	696.7 ± 28	185.5 ± 11	n.d.	n.d.
Nod	n.d.	n.d.	11.8 ± 2.5	5.9 ± 1.10
NodCBD	n.d.	n.d.	13.9 ± 1.4	8.3 ± 1.2

NodB domain, for the insoluble acetylated substrate. Taken together, these results show that Nod is functional and, in conjunction with the family 11 xylanase domain, has an important role in the hydrolysis of acetylated birchwood xylan by the enzyme.

DISCUSSION

Polysaccharidase aggregation, a characteristic of *C. thermocellum* and other anaerobic micro-organisms, might contribute both to increasing the overall stability of the enzymes and to potentiating the synergistic interactions between the cellulosomal catalytic components. Enzymic synergism is important in the hydrolysis of complex substrates such as the plant cell wall and might be the reason for the presence of xylanases in a predominantly cellulolytic multi-enzyme complex. Results presented here and previously [6] identify two further xylanases that are components of the cellulosome of *C. thermocellum* [6], increasing the number of cellulosomal xylanases to six. Given the sequence identity of XylU and XylV, it is likely that they are the products of a DNA duplication event, although it is unclear whether this event occurred in *C. thermocellum* or before the transfer of this DNA sequence into the bacterium. It would be interesting to know the evolutionary pressure(s) that gave rise to XylU and XylV. Organisms use a wide variety of polysaccharides and need to adapt the type of enzymes secreted to the available substrates. XylU and XylV are two identical xylanases except that XylV has the potential to hydrolyse acetylxylans more efficiently. Consequently, the organism can, potentially, modulate the type of xylanase secreted to suit the available substrate, expressing XylV when the substrate available is acetylated xylan, or XylU for less-substituted xylose polymers. Although two promoter-like sequences were found upstream of *xynU* and *xynV* (results not shown), suggesting that both genes are not organized in an operon, only a comprehensive study of the molecular mechanisms that regulate the expression of the repertoire of *C. thermocellum* xylanases can verify this possibility.

Results presented here support the findings of Sun et al. [14] suggesting that TVIs have a high affinity for xylan. Like the XBD from *Thermomonospora fusca* xylanase A, TVIs also have affinity for insoluble cellulose. Indeed, we have recently reported the presence of such a domain in a bacterial cellulase [19], suggesting that TVIs might also be important in cellulase function. The results described here show that TVI increases the activity of the xylanase catalytic domain against insoluble substrates, although it is unable to affect the rate of hydrolysis of soluble substrates. Our results suggest that the XBDs from *C. thermocellum* xylanases do not disrupt the structure of xylan but probably function by increasing the concentration of the catalytic domain on the substrates, promoting a more intimate contact between the enzyme and xylan and thereby enhancing the rate of catalysis of insoluble polymers. Therefore, *in vivo*, enzymes containing XBDs might compete for the available substrates much better than xylanases displaying low substrate affinities, especially at lower xylan concentrations.

This report provides the first evidence that cellulosomal enzymes contain discrete XBDs. Although the CBD from CipA anchors the complex to the plant cell wall and actively participates in cellulose hydrolysis [20], other polysaccharide-binding domains in the catalytic subunits might potentiate enzyme catalysis by mediating local interactions between the polysaccharidases and the plant cell wall polymers. Moreover, it is known that cellulosomal polysaccharidases can be bound individually to the bacterium through the interaction of their dockerin domains with cohesin domains found in envelope proteins [21]. Under these circumstances the presence of polysaccharide-binding domains might be important in the function of individually cell-bound clostridial polysaccharidases. It is now apparent that the repertoire of xylanases from *C. thermocellum* is varied and consists of at least six enzymes. All of them have remarkably complex molecular architectures containing various non-catalytic domains. For an organism that does not use xylose as a carbon source the expression of such a large array of xylan degrading enzymes seems to be a waste of energy. It is possible that the *C. thermocellum* xylanases, by removing the matrix polysaccharides that encase the cellulose microfibrils, could make the cellulose more accessible to the organism's cellulases. Although TVIs potentiate xylanase activity by binding to xylan, the domain does have a weak affinity for cellulose. Under some circumstances the binding of xylanases to cellulose might be disadvantageous for the bacterium. After releasing the xylan components of the plant material, the xylanases might be retained on the cellulosic material and thus limit the access of the endoglucanases for cellulose. TVIs have high affinities for oligosaccharides [14]; this affinity for soluble xylose polymers might provide a mechanism for releasing enzymes from recalcitrant substrates in particular circumstances.

Here we provide the first example of a bi-functional cellulosomal enzyme that also binds to xylan. XylV has both a β-1,4-xylanase activity, owing to its CD, and deacetylase activity, vested in the C-terminal Nod. Our results demonstrate that the presence of both catalytic domains contributes to the efficient

hydrolysis of acetylated xylans by the enzyme, suggesting a co-operative interaction of both catalytic modules. Therefore the removal of acetyl substituents from xylan renders the substrate more susceptible to depolymerization by the xylanase domain. This type of architecture has also been described for other xylanases [8], although only the results in this report demonstrate synergy between both domains.

In summary, we have shown that the consortium of xylanases produced by *C. thermocellum* is extensive and that similar xylanases with different molecular architectures have evolved to deal with different types of xylanase. Finally, we have shown that XBDs and accessory xylan esterase modules are important in the hydrolysis of the xylose polymer.

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