Specific Adsorption of Clostridium stercorarium Xylanase to Amorphous Cellulose and Its Desorption by Cellulobiose

Goro Takada, Shiichi Karita, Asako Takeuchi, Md. Mainul Ahsan, Tetsuya Kimura, Kazuo Sakka, and Kunio Ohmiya

Department of Bioscience, Faculty of Bioresources and *Center for Molecular Biology and Genetics, Mie University, Tsu 514, Japan

Received January 10, 1996

Clostridium stercorarium xylanase (XynA) composed of a family 11 catalytic domain of glycosyl hydrolases and family VI CBDs bound to amorphous cellulose, i.e., acid-swollen cellulose (ASC), but not highly crystalline cellulose, and it was released from the cellulose protein complex by wash with a cellulobiose solution. The $K_a$ and [PC]$_{max}$ values of ASC were 0.25 liter/mmol and 26 mmol/g.

Key words: Clostridium stercorarium; xylanase; cellulose-binding domain

Many cellulases comprise two or more discrete domains that can work independently in a polypeptide chain. A common arrangement is a catalytic domain connected to a cellulose-binding domain (CBD) via a linker segment enriched in Pro, Thr, and/or Ser residues, and the binding ability of a cellulase is ascribed to CBD. Recently, the catalytic domain and CBD derived from an endoglucanase CenA of Cellulomonas fimi have been shown to work synergistically in the disruption and hydrolysis of cellulose. CBDs, which have been found not only in cellulases but also in xylanases, can be grouped into 9 families, families I to IX, on the basis of amino acid sequence homology. Among them, well-characterized are the CBDs of families I, II, and III from Trichoderma reesei CBH1, C. fimi endo- and exo-glucanases, and Clostridium cellulovorans cellulose-binding protein CbpA, respectively. The CBDs of family I are exclusively found in fungal cellulases and all the other CBDs are found in a variety of bacterial enzymes.

Clostridium stercorarium, a thermophilic anaerobe, ferments xylan well and several genes related to xylan hydrolysis were cloned from this bacterium and characterized along with their gene products. In our previous papers, XynA encoded by the xynA gene of C. stercorarium F-9 was shown to be the predominant xylanase species in this organism and to consist of a catalytic domain belonging to family 11 at the N-terminus and two direct repeats of about 90 amino acids with a short spacing at the C-terminus. Preliminary deletion analysis indicated that the repeated sequences of XynA were not essential for catalytic activity and were responsible for the binding of this enzyme to Avicel. Recently, the repeated sequences of XynA and the homologous sequences found in other enzymes have been compiled in family VI of DBD families.

We now report that XynA specifically binds to amorphous cellulose but not highly crystalline cellulose and XynA is released from the XynA-cellulose complex by washing with a cellulobiose solution.

XynA was purified from the periplasmic fraction of E. coli JM109 harboring pXYN as described previously. The purified enzyme preparation which gave a single band on SDS-polyacrylamide gel electrophoresis (PAGE) was used for all cellulose-binding assays. A cellulose-binding assay mixture contained either 10 mg of dewaxed cotton or 0.2 mg of acid-swollen cellulose (ACS) and an appropriate amount of the enzyme in 200 µl of PC buffer (50 mM sodium phosphate-12 mM sodium citrate, pH 6.3). The dewaxed cotton with a crystallinity index of 1 was obtained from Kanda and was prepared from Avicel (Merck) as described previously. The mixture was incubated on ice for 30 min with occasional strings. After centrifugation to remove cellulase protein complex, the free protein concentration in the supernatant ([P], µg) was measured by Lowry's method. The bound polypeptide concentration ([PC], µmol/g cellulose) was calculated from the difference between the total polypeptide concentration and [P]. All assays were done in triplicate. Adsorption parameters were estimated from the following equation according to Langmuir's adsorption isotherm:

$$\frac{1}{[PC]} = \frac{1}{K_a[PC]_{max}} \times \frac{1}{[P]} + \frac{1}{[PC]_{max}}$$

where $K_a$ (liters/µmol) and [PC]$_{max}$ are the equilibrium adsorption constant and the maximum amount of bound enzyme.

Figure 1 shows a double reciprocal plot of the adsorption of XynA to ASC. The plots were linear within experimental error, indicating that the adsorption of XynA to cellulose proceeds in accordance with a Langmuir-type isotherm. The $K_a$ and [PC]$_{max}$ values for ASC were estimated to be 0.25 liter/µmol and 26 µmol/g. This [PC]$_{max}$ value corresponds to approximately 1.38 g of XynA per g of ASC. On the other hand, a very small amount of XynA bound to dewaxed cotton, i.e., the [PC]$_{max}$ value for this cellulose allomorph was 0.15 µmol/g. There was little or no apparent

---

* Present address: University of Osaka Prefecture, Sakai 593, Japan.
** Corresponding author. Phone: 81-592-31-9621. Fax: 81-592-31-9634. E-mail address: sakka@bio.mie-u.ac.jp

Abbreviations: ASC, acid-swollen cellulose; CBD, cellulose-binding domain; PAGE, polyacrylamide gel electrophoresis.
Fig. 2. Effects of Cellulose, SDS, and Urea on the Adsorption of XynA to ASC.

An appropriate amount of XynA was incubated with ASC in PC buffer containing various concentrations of cellulose (■) or glucose (□) and the bound polypeptide concentrations were measured. The result was expressed as the ratio in percentage of the value obtained with the saccharides to that without the saccharides.

adsorption of XynA to other polysaccharides such as xylan, chitin, β-1,3-glucan and Sephadex. These results indicated that XynA had a high affinity exclusively for amorphous cellulose while all of the CBDs so far reported except for the CBDs of C. inos CenC17 showed the highest affinity for crystalline cellulose allomorphs, e.g., dewaxed cotton and bacterial crystalline cellulose, and some of them were reported to have an affinity for chitin or Sephadex.8,16) The Ke (0.25 liter/μmol) of XynA for ASC was comparable with the values of CenA,18,19 and Irpex lacteus exoglucanase En-1 and endoglucanase En-1(4) for crystalline cellulose (0.401, 0.901, and 0.551 liter/μmol), and the values of En-1 and En-1 for ASC (0.44 and 0.41). On the other hand, the [PC]max value of XynA (26 μmol/g) for ASC was greater than the values of CenA, En-1, and En-1 for crystalline cellulose (8.1, 1.6, and 1.78 μmol/g), and those of En-1 and En-1 for ASC (3.51 and 3.78 μmol/g).

The effects of incubation conditions on the adsorption of XynA to ASC were examined. The adsorption of XynA to ASC occurred immediately after their mixing. The adsorption was moderately dependent on the concentration of incubation buffer, i.e., when a cellulose-binding assay was done in various concentrations of sodium phosphate buffer, the amount of XynA bound to ASC increased with increasing concentrations of phosphate buffer (pH 7.0) and nearly reached a plateau at 50 mM as to phosphate ion. The adsorption of XynA to ASC was not severely affected by incubation temperature and pH. These observations were consistent with the results obtained with the well-characterized CBDs. On the other hand, the low concentration (0.1%) of cellulose abolished the adsorption of XynA to ASC while the same concentration of glucose showed little or no effect (Fig. 2). This was in a striking contrast to the finding that soluble carbohydrates such as cellulose had no effect on the adsorption of the CBD from C. cellulovorans CbpA to Avicel.6) The presence of SDS (0.1%) and urea (6 M) in the assay mixture also prohibited XynA from binding to ASC to about 50% and 30%, respectively, of the maximum attainable value. Since hydrophobic interaction, i.e., stacking of aromatic residues against the faces of sugar rings, has been observed in most protein carbohydrate interactions,19) the tyrosine and/or phenylalanine residues present in the CBDs of XynA should be important in its adsorption to cellulose.

Application of the CBD of C. inos CenA was reported for affinity purification on the basis of the specific interaction between a fusion protein connected with the CBD and crystalline cellulose.20) For evaluating the usability of the CBDs of XynA as a tag for purification with cellulose, we investigated whether only XynA bound to ASC in the periplasmic proteins of an E. coli recombinant expressing the xynA gene and it was released from the complex by washing with a cellulose solution. As shown in Fig. 3, it was apparent that XynA was efficiently recovered from the crude enzyme solution. Western blotting analysis using an antiserum raised against XynA indicated that the minor bands recovered from the complex along with XynA were derivatives of it due to proteolysis (data not shown). The intriguing property of the CBDs from XynA that adsorption and desorption can be controlled by cellulose solution would provide an efficient affinity purification system by constructing a fusion protein comprising a target polypeptide and the CBDs.

Acknowledgments. This research was supported in part by a Grant-in-Aid for Scientific Research (No. 04203219) from the Ministry of Education, Science, and Culture of Japan. We thank Professor T. Kanda of Shinshu University for his generous gift of the dewaxed cotton.

References
Adsorption of *C. stercorarium* Xylanase to Amorphous Cellulose


