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Structural insight into potential cold adaptation mechanism through a psychrophilic glycoside hydrolase family 10 endo-β-1,4-xylanase

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ABSTRACT

The cold-adapted xylanases can catalyze at low temperature and hold great potential in food industry applications. Here we describe the first crystal structure of a cold-adapted glycoside hydrolase (GH) family 10 xylanase XynGR40 and its complex with xylobiose at 2.15 and 2.50 Å resolution. The enzyme folds into a typical GH10 $(\beta/\alpha)_8$ TIM-barrel, with E132 and E243 serving as the catalytic residues. The xylobiose was observed to occupy the −1 and −2 subsites. Structural comparison with a thermophilic GH10 xylanase highlighting various parameters that may explain the cold adaptation features were analyzed. Synergistic effects of the increased exposure of hydrophobic residues, the higher flexibility of substrate-binding residues, more flexible loops, and the ratios of special amino acid residues, may result in the cold adaptation of XynGR40.

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1. Introduction

Xylan is one of the most abundant renewable resources in nature. Xylan is composed of a β-1,4-linked xylopyranose backbone, which is frequently decorated by acetylated glucuronic acids and α-L-arabinofuranoses. Complete xylan decomposition requires concerted action of an array of xylanolytic enzymes, including xylanase (endo-1,4-β-xylanase, E.C.3.2.1.8), β-xylosidase (1,4-β-xylosidase, E.C.3.2.1.37), α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72), ferulic acid esterase (EC 3.1.1.73), and *p*-coumaric acid esterase (EC 3.1.1.x) (Juturu and Wu, 2012). As a crucial member among the xylan degrading enzymes, xylanase is capable of cleaving the xylopyranose backbone by hydrolyzing β-1,4-D-glycosidic bonds to yield xylooligosaccharides (Polizeli et al., 2005).

Xylanases have been widely used in various industrial applications, including biofuel production, juice clarification, brewing industry, dough processing, textile treatment, animal feed preparation, and pulp bleaching (Wong et al., 1988). Xylanases with various catalytic properties are required to meet the industrial demands. For instance, pulp bleaching which is performed at high temperature and alkaline pH conditions requires thermophilic and alkaline-stable enzymes. On the other hand, animal feed production and application that involves transient heat treatment and gastric acid environment demands thermostable and acidophilic xylanases (Chen et al., 2015). The cold-adapted xylanases, which can operate efficiently at low and intermediate temperatures are required in many of the low or moderate temperature processes, especially in juice extraction and food industry (Collins et al., 2005).

Xylanases are mainly classified into glycoside hydrolase (GH) families 5, 7, 8, 10, 11 and 43 based on the sequence similarities of the catalytic domain. A majority of xylanases belong to GH 10 and 11 which also possess superior potential in industrial applications (Collins et al., 2005). A number of crystal structures of GH10 (Harris et al., 1996; Manikandan et al., 2006; White et al., 1996) and GH11 (Ay et al., 1998; Sabini et al., 1999; Wakarchuk et al., 1994) xylanases have been solved. Xylanases from GH10 adopt the $(\beta/\alpha)_8$ TIM-barrel fold, and generally have a higher molecular mass and lower pI. On the other hand, GH11 xylanases that fold into β-jelly rolls are featured by a lower molecular weight and a

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higher pI (Meng et al., 2015). As heat stability is one highly valued properties in many commercial usages, crystal structures of thermophilic and thermostable xylanases were analyzed in a number of studies. Structural features including more intra- and intermolecular interactions (i.e., disulfide bond and hydrogen bond), a more compact overall fold, stabilized N- and C-terminal end, fusion with carbohydrate-binding motif, and lower B-factor have been proposed to endow enzyme higher heat stability (Cheng et al., 2014; Kumar et al., 2000; Querol et al., 1996). In contrast to the extensively studied thermostable xylanases, limited information is available for the psychrophilic xylanases. To our knowledge, only seven cold-adapted xylanases from GH10 and four from GH11 have been characterized and no structure has been reported so far. The only psychrophilic xylanase with its structure solved belongs to GH8 and adopts a different fold from those of GH10 xylanases (Van Petegem et al., 2003).

In our previous work, we cloned and expressed a cold-adapted GH10 family xylanase XynGR40 from the environmental DNA of goat rumen contents, which exhibited high activity at low temperatures and remained active even at 0 °C (Wang et al., 2011). These properties endow XynGR40 with a great potential for both basic research and food industrial application. To further investigate its cold adaptation mechanisms, we solved the structures of XynGR40 and its xylobiose complex. By structural analysis and comparison with other GH10 enzymes, some important features for catalysis, substrate binding, and, especially, cold adaptation are presented.

2. Materials and methods

2.1. Protein preparation

The full length sequence of *xynGR40* ORF (GenBank: HM156498) without signal peptide had been cloned and constructed into the recombinant plasmid pET-*xynGR40* in our previous work (Wang et al., 2011). The truncated sequence without the C-terminal esterase domain was amplified by polymerase chain reaction (PCR) with the forward primer 5′-GACGACGACAA GATGGAGAATCTTTATTTTCAGGGCGCTGGTGCTGGTGCTGCGCAGGG TCTGAAGGATGCCTACAAG-3′ and the reverse primer 5′-GAGGA GAAGCCCGGTTAGATCACGGCGTTGTCCATCTTGGGATC-3′. The PCR product was then cloned into the pET-46 Ek/LIC vector (Novagen, Madison, Wisconsin, USA). The recombinant plasmids were verified by sequencing and transformed into *Escherichia coli* BL21 (DE3). The recombinant *E. coli* cells were grown at 37 °C to an OD_{600} of about 0.6. The protein expression was induced at 18 °C for 20 h by adding 1 mM IPTG. The cells were harvest by centrifugation at 5000*g*, 4 °C for 20 min. Cell pellets were then resuspended in a lysis buffer consisting of 25 mM Tris, pH 7.5, 150 mM NaCl, and 20 mM imidazole, and disrupted with a French press (GuangZhou JuNeng Biology and Technology Co. Ltd., Guangzhou, China). After removing the cell debris, the supernatant was loaded onto a Ni–NTA column equilibrated with the lysis buffer. The recombinant protein was eluted using an imidazole gradient of 20–500 mM.

The collected fraction was then dialyzed against an ion-exchange buffer consisting of 25 mM Tris, pH 7.5 and loaded onto a DEAE column (GE Healthcare, Uppsala, Sweden). The XynGR40 protein was eluted at about 0.22 M NaCl when using a 0–0.5 M NaCl gradient and then dialyzed against a buffer containing 25 mM Tris, pH 7.5, 150 mM NaCl, and 10 mM DTT. The XynGR40 protein was concentrated to 13 mg/ml by using Centriprep (Millipore, Darmstadt, Germany) for storage. The purified recombinant XynGR40 has 355 amino acid residues in which 15 residues (MAHHHHHHVDDDDKM) at the N-terminus is from the pET46 vector. The molecular weight of recombinant XynGR40 is 41.1 kDa. All purification procedures were performed at 4 °C. The purity was checked by using SDS–PAGE.

2.2. Crystallization and data collection

Initial crystallization screening was performed manually using 1152 reservoir conditions from Hampton Research kit (Aliso Viejo, California, USA) and Wizard classic crystallization screen series (Rigaku, Washington, USA). All crystallization experiments were performed at 25 °C with the sitting-drop diffusion method. In general, 1 μl XynGR40 solution was mixed with 1 μl reservoir solution and equilibrated against 300 μl reservoir solution in 24-well Cryschem Plates (Hampton Research). Within 30 d, initial crystals grew in the condition of Wizard III random sparse matrix crystallization screen No. 42 [0.1 M Tris, pH 8.5, 0.2 M Li_2SO_4, 30% (w/v) PEG 4000]. The crystallization condition was optimized by adding 10 mM additive ATP-Na_2. The crystals reached dimension of about 0.8 × 0.5 × 0.1 mm within 15 d.

Prior to data collection, the crystal was mounted in a cryoloop and flash-cooled in liquid nitrogen with a cryoprotectant consisting of 0.1 M Tris, pH 8.5, 0.3 M Li_2SO_4, 35% (w/v) PEG 4000. Xylobiose complex crystal was obtained by soaking the XynGR40 crystals in cryoprotectant containing 10 mM xylobiose (Sigma) for 2 h. X-ray diffraction data sets were collected at beamline BL15A1 of the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan and the diffraction images were processed using HKL-2000 (Otwinowski and Minor, 1997).

2.3. Refinement and structural analysis

The XynGR40 in apo-form was solved by using the molecular replacement (MR) method with the *Phaser* program from the CCP4 suite (McCoy et al., 2007; Winn et al., 2011), using the xylanase IXT6 (PDB code: 2Q8X) from *Geobacillus stearothermophilus* as a search model. The $2F_o{-}F_c$ difference Fourier map showed clear electron densities for most amino acid residues. Further refinements by incorporating water molecules and ligands were carried out using the programs of Coot (Emsley and Cowtan, 2004), Phenix (Adams et al., 2002) and Refmac5 (Collaborative Computational Project, 1994). The xylobiose complex structure was solved by MR using the refined XynGR40 structure as a searching model and refined as described above.

Hydrogen bonds and salt bridges were calculated by using DSSP program (Kabsch and Sander, 1983) and ESBRI tools (http://bioinformatica.isa.cnr.it/ESBRI/). Surface features were analyzed with POPS (Cavallo et al., 2003). All figures were prepared by using Pymol (http://pymol.sourceforge.net/).

3. Results and discussion

3.1. Overall structure

XynGR40 in apo-form and in complex with xylobiose were solved at the resolution of 2.15 Å and 2.5 Å ($P2_1$), respectively. The data collection and refinement statistics are listed in Table 1. Each asymmetric unit of the crystal contains two protein molecules, denoted A and B. Consistent to the native-PAGE analysis, the crystal structure indicates that the XynGR40 exists as a monomer. The conformations of A and B are almost identical since superposition of the two molecules gave a root-mean-square deviations (RMSD) of 0.262 Å. In the refined apo-form structure of XynGR40, the two molecules A and B contain amino acid residues 3–332 and 1–332, respectively. The electron density map of residues 250–264 in molecule A and 258–264 in molecule B are

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