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Crystal structure of an acetyl esterase complexed with acetate ion provides insights into the catalytic mechanism

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ABSTRACT

We previously reported the crystal structure of an acetyl esterase (TcAE206) belonging to carbohydrate esterase family 3 from *Talaromyces cellulolyticus*. In this study, we solved the crystal structure of an S10A mutant of TcAE206 complexed with an acetate ion. The acetate ion was stabilized by three hydrogen bonds in the oxyanion hole instead of a water molecule as in the structure of wild-type TcAE206. Furthermore, the catalytic triad residue His182 moved 0.8 Å toward the acetate ion upon substrate entering the active site, suggesting that this movement is necessary for completion of the catalytic reaction.

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1. Introduction

The lignocellulose component of the plant cell wall is composed of cellulose, hemicellulose, and lignin [1,2]. Microbial degradation of lignocellulosic biomass in animal feed and food production, and softwood pulp bleaching in paper production is of increasing industrial significance [3–6]. Hemicelluloses are either highly or partially acetylated, which strongly inhibits degradation by polysaccharide-hydrolyzing enzymes [6]. However, the activity of major hemicellulose-hydrolyzing enzymes such as xylanases and mannanases can be enhanced by the enzymes acetylxylan esterase and acetyl esterase, which remove acetyl groups from hemicellulose [7,8]. Carbohydrate esterases (CE), which includes acetylxylan and acetyl esterase, are categorized into 16 families (CE1–16) within the CAZy database (http://www.cazy.org/Carbohydrate-Esterases.html) [9]. With the exception of CE4, most CE family enzymes contain an α/β-hydrolase fold and function as serine esterases via a catalytic triad [10–13].

The crystal structures of bacterial CE3 enzymes, which are acetylxylan esterases, have been reported for *Clostridium thermocellum* (CtCes3-1; PDB code 2vpt) [14] and *Sinorhizobium meliloti* (Sm23; PDB code 4tx1) [15]. We recently reported the crystal structure of a fungal CE3 acetyl esterase with a catalytic domain from *Talaromyces cellulolyticus* (TcAE206; PDB code 5b5s, previous PDB code 3x0h) [16]. Even though the structure of TcAE206 is similar to that of other CE3 enzymes, its substrate specificity differs considerably. TcAE206 exhibits enzymatic activity against acetylated oligosaccharides but not acetylated xylans [16]. In contrast, CtCes3-1 shows enzymatic activity against acetylated xylans and oligosaccharides [14] The substrate specificity of Sm23 was omitted from this comparison because acetylated xylans and oligosaccharides were not used as substrates [15]. The manner in which the acetylated carbohydrate moiety binds has yet to be fully elucidated, however. Here, we report for the first time the substrate/product complex structure of a CE3 family enzyme and discuss the substrate-induced conformational change in the generally basic residue His182.

Abbreviation: TcAE206, the catalytic domain of acetylesterase from *Talaromyces cellulolyticus*; S10A, mutant of TcAE206 substituted serine 10 with alanine; CE, carbohydrate esterase; CtCes3-1, acetylxylan esterase from *Clostridium thermocellum*; Sm23, acetylxylan esterase from *Sinorhizobium meliloti*; RMSD, root mean square deviation.

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2. Materials and methods

2.1. Protein expression and purification

An expression vector (pS10A) for substituting TcAE206 Ser10 with an Ala residue was constructed by site-directed mutagenesis as described previously [16]. *Escherichia coli* BL21 (DE3) harboring pS10A was cultivated and induced as described previously [16]. The cells were then harvested by centrifugation, dissolved in 20 mM Tris-HCl (pH 8.0) containing 50 mM NaCl, and stored at −80 °C. The cells were lysed by three cycles of freezing-thawing, and the resulting lysate was centrifuged (35,870 × g for 20 min at 4 °C); the resulting supernatant including mutant of TcAE206 (S10A) was loaded onto a HiTrap Q column (GE Healthcare, Buckinghamshire, UK) equilibrated with 20 mM Tris-HCl (pH 8.0) and eluted using a linear gradient of 0.0–1.0 M NaCl in the same buffer. The collected active fractions were saturated with 1.0 M $(NH_4)_2SO_4$ and then loaded onto a HiTrap PHE column (GE Healthcare) previously equilibrated with the same buffer containing 1.0 M $(NH_4)_2SO_4$. The enzyme was eluted with a linear 1.0–0.0 M $(NH_4)_2SO_4$ gradient. The active fractions were pooled, concentrated, and loaded onto a Superdex 200 pg gel filtration column (GE Healthcare) equilibrated with 20 mM Tris-HCl (pH 8.0) containing 50 mM NaCl. The purity of the target protein was confirmed by SDS-PAGE. The protein concentration was determined by measuring the absorption at 280 nm. The protein was then concentrated to 12 mg mL^{-1} in 20 mM Tris-HCl buffer (pH 8.0).

2.2. Crystallization and X-ray diffraction analysis

The initial crystallization screening of S10A was performed using Crystal Screen HT (Hampton Research) at 20 °C by the sitting-drop vapor diffusion method, in which a 0.5-μL volume of protein solution was mixed with an equal volume of reservoir solution. Small crystals were grown for 1 day in reagent No. G3, 0.1 M 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.5), 25% (v/v) polyethylene glycol monomethyl ester (PEGME) 550, and 10 mM $ZnSO_4$. The crystallization conditions were optimized using the hanging-drop vapor diffusion method, in which a 2.0-μL volume of protein solution was mixed with an equal volume of reservoir solution. S10A, with 10 mM D-glucose pentaacetate (Sigma-Aldrich, St. Louis, MO, USA), was crystallized in 0.1 M MES buffer (pH 6.5), 15–20% (v/v) PEGME 550, and 10 mM $ZnSO_4$ for 3 days at 20 °C. The resulting crystals were then soaked in reservoir solution containing 25% (v/v) polyethylene glycol 400 as a cryoprotectant. X-ray diffraction data for the S10A–acetate ion crystals were collected at the beamline BL44XU at SPring-8 (Hyogo, Japan) using 0.9-Å wavelength radiation. A total 180° of data were collected using a Rayonix MX300-HE CCD detector. All data were processed to 1.40-Å resolution using HKL2000 (DENZO and SCALEPACK) [17].

2.3. Phasing, refinement, and structural analysis

General data handling was carried out using the CCP4 package [18]. The initial model of the S10A mutant complexed with acetate ion was determined by molecular replacement using PHASER [19] with a search model of TcAE206 (PDB code 5b5s). The final model was built using COOT [20], and refinements were carried out using REFMAC5 [21]. Water molecules were added using COOT, and model geometry was analyzed using RAMPAGE [22]. Molecular graphics figures were created using PyMOL (http://pymol.sourceforge.net/) [23].

3. Results and discussion

3.1. Overall structure of TcAE206_S10A complexed with acetate ion

The complex structure of S10A–acetate ion was solved at 1.4-Å resolution. The prepared crystals belonged to space group $P3_221$, with unit cell a = b = 64.6 Å and c = 89.6 Å. The asymmetric unit consisted of one molecule with a solvent content of 49.4%, which corresponded to a Matthews coefficient of 2.43 $Å^3$ Da^{-1} [24]. After refinement, R_{work} was estimated at 12.2%, and R_{free} was estimated at 17.4%. Data collection and refinement statistics are shown in Table 1. The overall structure of S10A was constructed from Met1 to Ser207, with an acetate ion, solvent molecules, and metal ions. There was no major difference in overall structure between S10A and wild-type TcAE206 [16], with the root mean square deviation (RMSD) of 0.4 Å over 207 Cα atoms. Briefly, the structure of S10A formed an α/β-hydrolase fold and canonical SGNH-hydrolase superfamily fold comprising five central parallel β-strands and flanked by six α-helices, with a classic serine esterase catalytic triad (Ser-Asp-His).

The catalytic domain structure of TcAE206 is similar to the structures of CtCes3-1 (Z-score of 25.5 and RMSD of 2.0 Å over 192 Cα atoms) and Sm23 (Z-score of 19.8 and RMSD of 1.9 Å over 168 Cα atoms) [14–16]. In addition, the major residues constituting the catalytic triad (Ser10, Asp180, and His182) and oxyanion hole (Ser10, Gly62, and Asn92) in the active site of TcAE206 are completely conserved in CtCes3-1 (Ser44, Asp205, His208, Gly95, and Asn124) and Sm23 (Ser10, Asp187, His190, Gly50, and Asn90) (Supplementary Fig. S1). The coordinates and structural factors for TcAE206_S10A were deposited in the Protein Data Bank under the accession code 5B5L.

Table 1
Data collection and refinement statistics.

Data collection	
Wavelength (Å)	0.9
Space group	$P3_221$
Unit cell (a, b, c (Å))	64.6, 64.6, 89.7
(α, β, γ (°))	90, 90, 120
Resolution range (Å)	50.0–1.40 (1.42–1.40)[a]
Total No. of reflections	245,260
No. of unique reflections	42,027
Redundancy	5.8 (3.5)[a]
Completeness (%)	97.4 (94.2)[a]
R_{merge} (%)[b]	8.3 (37.3)[a]
$< I/\sigma(I) >$	43.8 (4.4)[a]
Refinement	
Resolution range (Å)	30.0–1.40
R_{work} (%)[c]/R_{free} (%)[d]	12.1/17.4
No. of protein atoms	1578
No. of ligands	10
No. of water molecules	202
RMSD	
Bond lengths (Å)	0.034
Bond angles (°)	2.3
Average *B* factor (Å^2)	17.1
Ramachandran statistics (%)	
Favored region	95.6
Allowed region	4.4
PDB accession number	5B5L

[a] Outer shell (1.42–1.40 Å).
[b] $R_{merge} = \sum_{hkl}\sum_i|I_i(hkl)-\langle I(hkl)\rangle|/\sum_{hkl}\sum_i Ii(hkl)$, where I_i(hkl) is the scaled intensity of the *i*th observation of reflection *hkl*. ⟨I(*hkl*)⟩is the mean value and the summation is over all measurements.
[c] $R_{work} = \sum_h\sum_i||F_o|-|F_c||/\sum|F_o|$.
[d] R_{free} is R_{work} for approximately 5% of the reflections that were excluded from the refinement.

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