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Crystal structure of β-1,3-xylanase catalytic module (XYL4-CM) from *Vibrio* sp. AX-4 in an atomic resolution

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Introduction

β-1,3-Xylanase (EC 3.2.1.32) is an enzyme capable of hydrolyzing the β-1,3-xylosidic linkages of β-1,3-xylan to produce β-1,3-xylooligosaccharides with a different xylose unit. β-1,3-xylanase from *Vibrio* sp. AX-4, termed XYL4, is a modular enzyme composed of a catalytic module (XYL4-CM) belonging to glycoside family 26 (GH-26) and tandem xylan-binding mocules (XYL4-XBMs) [1].

Recently, we reported that the crystallization and preliminary X-ray analysis of XYL4-CM [2]. The crystal of XYL4-CM belonged to space group $P2_12_12_1$, with unit-cell parameters a=51.6, b=75.8, c=82.0 Å. X-ray diffraction data was collected to 1.44 Å resolution [2].

Here, we report the atomic resolution X-ray crystal structure of XYL4-CM. This is the first analysis of β-1,3-xylanase. Investigating XYL4-CM structure and comparing it with structures of other GH-26 enzymes would be facilitate the structural and functional studies of GH-26 and β-1,3-xylanase.

Experimental Procedures

As described elsewhere, Se-Met crystals of XYL4-CM was obtained by hanging drop vapor diffusion method at 293 K, and the structure determination of XYL4-CM was carried out by multiple-anomalous diffraction method.

Furthermore, 0.900 Å wavelength was selected to X-ray measurement under 100 K on the PF NW12 station of Spring-8. Data set was integrated with HKL2000 [3], and scaled with SCALEPACK Structure of XYL4-CM was determined using the molecular replacement method. Manual model fitting using the program XtalView/Xfit [4], and the structure refinement was carried out using REFMAC [5]. The stereochemical quality of the final refined models was analyzed by the program PROCHECK [6]. Final coordinates and the structure factors of XYL4-CM were deposited in the protein Data Bank with code 2DDX.

Results

The crystal structure of XYL4-CM was determined at an atomic resolution of 0.86 Å. The final model of XYL4-CM exhibited an R-factor of 0.140 (R-free=0.148) includes 324 out of 355 residues with no break. However, the C- and N-terminal regions of the model, 1-22 and 344-355 respectively, could not be built due to poor electron density.

The overall fold of XYL4-CM revealed a classic $(\beta/\alpha)_8$ barrel (TIM-barrel), the same as other members of GH-26. The two glutamate residues of Glu-116 and Glu-212, which were previously demonstrated as an acid/base catalyst and a nucleophile by sequence and mutation analyses [1], located close to the C-termini of the forth and seventh β strands, respectively. These features of XYL4-CM are typical for enzymes classified in clan GH-A (also called the 4/7 superfamily), which includes GH-26.

A DALI [5] search revealed that the three dimensional structure of XYL4-CM shows a significant similarity with those of other GH-26 enzymes as follows; β-mannanase A from *Cellvibrio japonicus* (CjMan26A, Z score 19.7 with a root mean square deviation [rmsd] of 3.5 Å), β-mannanase from *Cellulomonas fimi* (CfMan26A-50K, Z score 17.0 with a rmsd of 3.4 Å), lichenase from *Clostridium thermocellum* (CtLic26A, Z score of 16.7 with a rmsd of 3.3 Å).

To characterize function of the active site region in more detail, crystallization and structure determination of XYL4-CM in complex with the substrate (β-1,3-xylooligosacchrides) has been conducted extensively.

References

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