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X-ray structures of *Thermoactinomyces vulgaris* R-47 α-Amylase 2 with Acarbose and β-Cyclodextrin

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Introduction

α-Amylase (α-(1,4)-D-glucan-4-glucanohydrolase; EC 3.2.1.1) belonging to glycoside hydrolase family 13 (1), catalyzes the hydrolysis of α-(1,4)-D-glucosidic linkages in starch to release α-anomer products. Cyclomaltooligosaccharides (cyclodextrins, CDs) are cyclic oligosaccharides with six (α-), seven (β-), or eight (γ-) glucose units, having a rigid conical structure. Usually, α-amylases can not hydrolyze CDs, but *Thermoactinomyces vulgaris* R-47 α-amylase 2 (TVAII, 585 amino acids, 67,500 Da) can efficiently hydrolyze CDs by what is called cyclodextrinase activity (2).

To obtain new insights into the CD-hydrolyzing mechanism of TVAII, we reported here the X-ray structures of TVAII/acarbose complex, and complexe of inactive mutant TVAII (Asp325-Asn325, Asp421-Asn421; D325N·D421N) with β-CD, at 2.9 Å and 2.8 Å, respectively.

Materials and Methods

A crystal of TVAII/acarbose complex was obtained by a soaking method using the reservoir solution containing 1 mM acarbose; soaking time was 1 hour. Crystals of D325N·D421N/β-CD were also obtained by a soaking method using reservoir solution containing 20 mM of β-CD. X-ray diffraction data for TVAII/acarbose and D325N·D421N/β-CD were collected at 100K using an ADSC/CCD detector system on the BL6A beam line in the Photon Factory (Tukuba, Japan), using a reservoir solution containing 20 %(w/v) of 2-methyl-2,4-pentadiol as a cryoprotected solution. Diffraction data were processed using the program MOSFLM and SCALA in the CCP4 program suite. Initial phases were determined by a molecular replacement method using the structure of TVAII as a probe model with the program CNS. Refinement statistics are listed in Table 1.

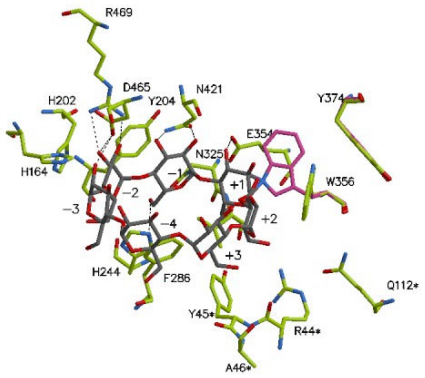
Results and Discussion

In both complexes, the interactions between ligands and enzymes at subsites -1, -2 and -3 were almost the same, but striking differences in the catalytic site structure were found at subsites +1 and +2, where Trp356 and Tyr374 changed the conformation of the side chain depending on the structure and size of the ligands. Trp356 and Tyr374 are thought to be responsible for the multiple substrate-recognition mechanism of TVAII, providing the unique substrate specificity. In the β-CD complex, the β-CD maintains a regular conical structure, making it difficult for Glu354 to protonate the O4 atom at the hydrolyzing site as a previously proposed hydrolyzing mechanism of α-amylase, as shown in Figure 1. From the X-ray structures, it is suggested that the protonation of the O4 atom is possibly carried out via a hydrogen atom of the inter-glucose hydrogen bond at the hydrolyzing site.

Table 1. Refinement statistics.

Data set (Complex with)	Acarbose	β-CD
Space group	$P2_12_12_1$	
a (Å)=	114.6	115.1
b (Å) =	119.3	118.8
c (Å) =	113.0	114.3
No. of refs.	31,277	38,561
Completeness (%)	99.3	98.2
$R_{crystal}$	0.179	0.200
R_{free}	0.243	0.256

Figure 1. The catalytic site structure of D325N·D421N/β-CD



References

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