The X-ray Structures of *Thermoactinomyces vulgaris* R-47 Mutant α-Amylases

Shigehiro KAMITORI*[1], Akashi OHTAKI[1], Akemi ABE[1],
Takehiro YOKOTA[2], Takashi TONOZUKA[2], Yoshiyuki SAKANO[2]
[1]Dept. of Biotechnology & Life Science, Tokyo Univ. of Agriculture & Tech., Naka-cho, Koganei, Tokyo 184-8588, Japan, [2]Dept. of Applied Biological Science, Tokyo Univ. of Agriculture & Tech., Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Introduction

Thermoactinomyces vulgaris R-47 produces two α-amylases, TVAI (637 amino acids) and TVAII (585 amino acids). In addition to hydrolyzing 1,4-α-D-glucosidic linkages of starch mainly to produce maltose, TVAI and TVAII can also efficiently hydrolyze cyclic oligosaccharides (cyclodextrins, CDs) to produce maltose. We reported the crystal structure of TVAII at 2.6 Å, showing that the active site of TVAII is wider and shallower than other structure solved-α-amylases, and that Phe286 possibly play a role to recognize CDs. In order to elucidate the substrate binding mode of TVAII and to confirm the role of Phe286, we carried out the X-ray structure analyses of the inactive mutant TVAII (D325N) complexed with maltohexaose (G6), and Phe286-replaced mutant TVAIIs (F286A and F286Y).

Experimental Section

Cystal data and refinement statistics are listed in Table 1. The structures were solved by a molecular replacement method using the structure of wild-type TVAII, and structures were refined using program CNS.

Table 1. Crystal Data and Refinement Statistics

	D325N/G6	F286A	F286Y
SR Facility	Photon Factory		
Beam Line	6A	18B	6A
Temperature (K)		100	
Resolution (Å)	3.3	3.2	3.2
No. of unique refs.	22,115	25,420	23,309
Completeness (%)	97.8	99.8	91.7
R-merge	0.137	0.122	0.121
Space group		$P2_12_12_1$	
Cell dimensions (Å)			
a	111.9	112.6	112.3
b	117.3	118.1	117.9
c	112.6	113.1	113.3
R-factor	0.224	0.213	0.204

Results and Discussion

The active site structure of D325N with G6 is illustrated in Fig. 1. Only four glucose units can be located in the electron density map. The torsion angles of glucosidic bonds at the hydrolyzing site of G6 are considerably deviated from those in a regular helical structure of amylose. The torsion angles of O5-C1-O4'-C4' and C1-O4'-C4'-C3' at the hydrolyzing site are observed to be 50 ° and 90 °, respectively. This binding mode of G6 as a substrate is very similar to those observed in the other α-amylases, suggesting that the TVAII-hydrolysing mechanism for amylose is equivalent to that of other α-amylases [1].

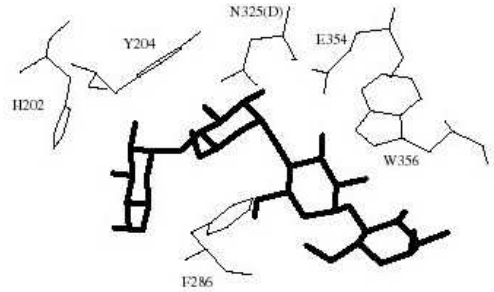

Fig. 1. Active site structure of D325N with four glucose units (solid lines) of G6.

Fig.2 shows the annealed omit map for the exchanged residues in F286A and F286Y. The active site structures of F286A and F286Y are almost identical to that of wild-type TVAII, except the exchanged residues, showing that the replacement of Phe286 does not lead to drastic structural changes in TVAII. From these structures and the kinetic parameters for CDs of F286A and F286Y, the 286th amino acid residue is thought to predominate the hydrolyzing activities for CDs, suggesting that Phe286 is an essential residue for TVAII to recognize CDs [2].

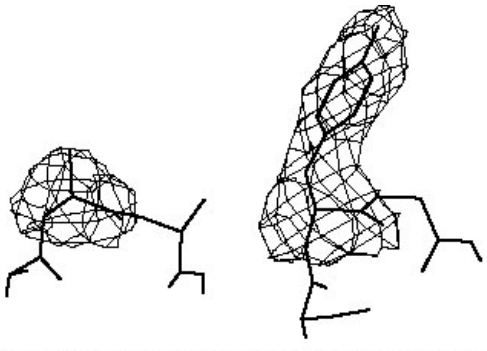

Fig. 2. Annealed omit map for Ala286 of F286A (left) and Tyr286 of F286Y (right).

References

[1] T. Yokota *et al.*, *Biosci. Biotechnol. Biochem.* **63**(3), 619-626 (2001).
[2] A. Ohtaki *et al.*, *Carbohydrate Res.* (2001) (*in press*)

* kamitori@cc.tuat.ac.jp