Crystal Structure Analysis of Glycosyltrehalose-hydrolyzing α-amylase from the Hyperthermophilic Archaeum Sulfolobus Solfataricus KM1

Taro TAMADA(0005007) and Ryota KUROKI*(0005031)

Pharmaceutical Research Laboratories, Kirin Brewery Co., Ltd.

α-amylase from *Sulfolobus solfataricus* KM1 (KM1-amylase) acts an exo-amylase to release trehalose from maltooligosyl trehalosides. We already determined wild-type structure of KM1-amylase (1) and complex structure of inactive mutant (E283Q) and substrate (maltotreosyl trehalose: MTT) [2000B0289-NL-np]. On the basis of these structural data, several mutants were designed to change enzymatic reaction mechanism. Here we report the X-ray diffraction study of D252S mutant and substrate complex.

Diffraction data of this complex were collected in cold nitrogen gas stream at 100K and recorded on a Mar CCD camera with a total oscillation range of 180°. Intensity data was processed with DENZO and SCALEPACK suite up to 2.3Å resolution. The number of observed reflections was 293,858, which were merged into 43,725 unique reflections with an R_{sym} of 0.071 (R_{sym} in the highest resolution shell of 0.158), a completeness of 95.1 % and multiplicity of around 6.

A clear density of substrate was apparent in the F_o - F_c omit map (Figure 1). There are two significant differences between both densities: (i) deletion of trehalose region in D252S complex; (ii) direction of O1atom in Glc3. The former means D252S mutant keeps amlylase activity. The latter shows O1 atom of Glc3 is β anomer in D252S complex whereas that is α anomer in E283Q complex. It is suggested that we succeed in chainging enzymatic reaction mechanism by mutagenesis (D252S). The crystallographic refinement is now in progress.

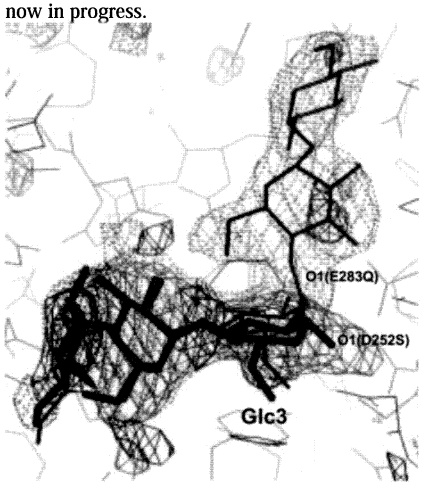

Figure 1. Sigma-A weighted Fo-Fc omit map (3σ) of substrate in D252S (line) and E283Q (dot) complex.

REFERENCE

(1) Feese, M. D. *et al.* (2000) *J. Mol. Biol.* **301**, 451-464.

Crystal Structure of Enzyme-Substrate Intermediate of T26E Mutant T4 Phage Lysozyme Determined at 1.35Å Resolution

Teikichi IKURA(0006112), Taro TAMADA(0005007), Ryota KUROKI (0005031)*

Pharmaceutical Research Laboratories, Kirin Brewery Co., Ltd.

Recent progress of structural determination utilizing synchrotoron radiation enables us to investigate the accurate mechanism of enzymatic reaction from a structural point of view. It was already reported that the mutation threonine 26 to glutamic acid (T26E) in the active site cleft of T4 phage lysozyme (T4L), produced an enzyme that cleaved the cell wall of *Escherichia coli* but left the product covalently bound to the enzyme. This mutant T4L was crystallized non-isomorphously to wild-type T4L and the tertiary structure was determined at 1.9 Å resolution by X-ray crystallography (Kuroki *et al.*, 1993).

In order to obtain more accurate structural information of this covalent intermediate, the purification and crystallization conditions were improved and crystals suitable for diffraction study were prepared. The diffraction data of this crystal were collected in cold nitrogen gas stream at 100K and recorded on a Mar CCD camera at SPring-8 (BL41XU). The crystal belongs to a space group $P2_12_12$ (a = 50.18 Å, b = 66.69 Å, and c = 48.83 Å). Intensity data were processed with programs DENZO and SCALEPACK suite up to 1.35 Å resolution. Crystallographic refinement was performed using CNX. The refined structure has an R-factor of 0.176 (R_{free} = 0.178 for 10% subset) between 30.0 and 1.35 Å resolution. This structure was seen to be a complex between T4-lysozyme and muramyl peptide consisting of N-acetyl glucosaminyl N-acetyl muramic acid (NAG-NAM) and the peptide, L-Ala - D-Glu - diamino pimelic acid - D-Ala. The electron density corresponding to a covalent linkage was clearly seen between the C1 atom of the muramic acid and the carboxyl oxygen of glutamate-26 introduced into T4-lysozyme (Figure 1). The high-resolution structural information of enzyme-substrate intermediate will show new, detailed insights into the catalytic reaction of this enzyme.

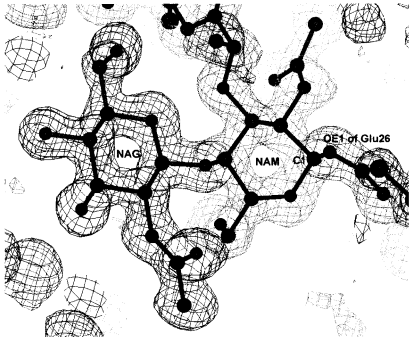

Figure 1. Sigma-A weighted Fo-Fc omit map at 2 sigma.

Reference

Kuroki, R. Weaver L. H. and Matthews, B. W. (1993) *Science* **262**, 2030-2033.