Crystal Structure Analysis of glycosyltrehalose-syntase (GTase) from hyperthermophilic archaea in Sulfolobales

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A pathway for production of treharose from soluble starch comprises two glycosidases: intramolecular glycosyl transferase (GTase) and α-amylase. GTase converts glycosidic bond between the last two glucose residues of amylose from an α-1,4 bond to an α-1,1 bond, making a non-reducing glycosyl trehaloside. α-amylase then cleaves the α-1,4 bond adjacent to the α-1,1 bond to release trehalose. We already determined the complex structure of α-amylase from *Sulfolobus solfataricus* KM1 with substrate (maltotreosyl trehalose) [2000B0289-NL-np]. Here we report the X-ray diffraction study of GTase from same source.

Crystals of KM1-GTase were grown by hanging drop method using PEG8000 as a precipitant, and belong to the monoclinic space group $P2_1$ with unit cell dimensions a = 71.1 Å, b = 84.4 Å, c = 128.2 Å and β = 103.5°. Assuming that the asymmetric unit contains two GTase molecules with a molecular mass of 85 kDa, the V_m value is calculated as 2.17 Å^3/Da, resulting in a solvent content of 43%. Diffraction data were collected in cold nitrogen gas stream at 100K and recorded on a Mar CCD camera with a total oscillation range of 160° at BL41XU. The oscillation angle and exposure time per flame were 1.0° and 2 sec, respectively. Intensity data was processed with DENZO and SCALEPACK suite up to 2.5Å resolution. Data collection and processing statistics are shown in Table 1. Initial phase was determined by molecular replacement method using the program AMORE in the CCP4 program suite. The crystallographic refinement is now in progress.

Table 1 Summary of the data collection

	total (last shell)
wavelength (Å)	1.0000
detector distance (mm)	150
resolution (Å)	2.5
total reflections	81,587
unique reflections	43,141
R_{merge}	0.095 (0.321)
completeness (%)	85.2 (85.1)

Molecular Conversion of Ovalbumin to Allow a Serpin Conformational Change by Mutation R339T at P14 Site

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The serpins are a family of serine proteinase inhibitors, which controls serine proteinases involved in diverse physiological reactions. They undergo a unique conformational change upon exertion of inhibition; after receiving the proteolytic cleavage at P1-P1' site, the reactive center loop is inserted into the central β-sheetA, thereby driving the trapped proteinase to the far opposite site.

Ovalbumin, a major component of egg white proteins, is grouped into the serpin super family because of the close similarity in the primary and tertiary structures. But ovalbumin is non-inhibitory and lacks the loop insertion mechanism due to unknown structural implications. There are three possible structural reasons for the defect. First, the reactive loop structure is significantly different. The reactive center assumes extended strand or distorted helical conformation in the inhibitory serpins, but in ovalbumin P9-P1 segment assumes regular α-helix with less flexible nature. Second, ovalbumin has a higher inherent stability than inhibitory serpin and refold in vitro into the native stability state. Third, the side chain of the P14 site, which works as a hinge for the loop-insertion, is Thr in most inhibitory serpins, but in ovalbumin it is a bulkier Arg339.

In the present beam time, we have examined the third possibility by X-ray crystallography using an ovalbumin mutant R339T in which Arg339 is replaced by Thr residue. The P1-P1' cleaved form of R339T was crystallized by hanging-drop vapor diffusion method using ammonium sulfate as a precipitant. The crystal belongs to a space group of P1 with cell dimensions of a = 60.18, b = 64.44, c = 67.04 Å, α =89.19, β = 63.25 and γ = 61.86°. Two molecules were estimated in an asymmetric unit. The diffraction data was collected with λ = 0.9 Å and a Mar Research CCD detector adjusted the detector-crystal distance of 154 mm under a cold nitrogen gas stream at BL41XU beamline. The collected images were processed with a program of HKL2000. Total of 145,697 reflections containing 71,164 unique data were collected with 95.0% completeness and R_{merge} of 0.057 up to 1.80 Å resolution. The structure has determined by molecular replacement with a search model already built by multiple isomorphous replacement at 3.2 Å using Au and Hg derivatives for unfreezed crystals. This model gave clear solutions in both rotation and translation search, and the structure has refined with CNS program. The final model contained 762 protein residues and 114 water molecules for two molecules with R = 0.262 and R_{free} = 0.310 for the data of 15-2.0 Å resolution. The rms deviations from standard geometry are 0.09 Å in bond length and 1.40° in bond angles. The final model of ovalbumin variant R339T shows fully loop-inserted conformation, like other inhibitory serpins. The conserved Thr residue is therefore considered to be essential for the loop insertion mechanism.