

CRYSTAL STRUCTURE ANALYSIS OF MALTOOLIGOSYL TREHALOSE SYNTHASE

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Maltooligosyl trehalose synthase (MTSase) is an enzyme which is produced from *Arthrobacter sp.* Q36. The enzymatic synthesis of trehalose involves two kinds of enzymes, MTSase and MTHase (maltooligosyl trehalose trehalohydrolase).

The crystallization was performed with using 20 % PEG2000 in the presence of 0.2 M MgCl₂, and protein concentration of 30 mg/ml buffered with 0.1 M tris-HCl at pH 8.5. Crystals grew to typical size of 0.2x0.2x1 mm within two weeks. This crystal diffracted up to 2.8 Å resolution by using rotating anode X-ray generator. The crystals belong to the space group $P2_12_12_1$, with unit cell dimensions of $a = 56.70$, $b = 140.1$, $c = 205.2$ Å with two molecules in the asymmetric unit with calculated solvent content of 0.54. The intensity data were collected by using the SPring-8 facility (BL41XU) for native crystal. The crystals were transferred to the solution containing 20 % glycerol as cryo-protectant, and flush frozen by thrusting into liquid nitrogen for cryo-diffraction experiments under 100 K.

The data collection were performed by using Rigaku RAXIS IV with a wavelength of 0.708 Å. Oscillation conditions were range of 1.0°, speed of 0.5°/min, 15 times repeating in 1 minute, and total range of 180°. Significant reflection was observed up to 2.3 Å. Data reduction and scaling were performed by using the program DENZO and SCALEPACK within 2.5 Å resolution. The total reflections measured were 273,123 and the number of independent reflections were 53,320. The completeness of data at 2.5 Å resolution was 92 % and the merging R-factor 0.052. The data is kept for future refinement of the structure. The heavy atom derivative searches are now underway.