High resolution X-ray crystallography of maltooligosyltrehalose synthase

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Introduction

Trehalose (α, α - trehalose) ,a non-reducing disaccharide containing α, α – 1,1 – glucosidic linkage, is known to be widely distributed in yeast, fungi, and plants It has about 45% sweetness of sucrose, and is gaining significant attention as a food additive and ingredient. In 1996, noble system of trehalose biosynthesis has been found from tewo different bacterial sources, the mesophilic *Arthrobacter* and an archaebacterium the thermophilic *Sulfolobus acidocaldarius* [1,2]. This system consists of two enzymes, maltooligosyl trehalose synthase (MTSase) and maltooligosyl trehalose trehalohydrolase (MTHase) which catalyse the following reactions in a coupled manner:

maltooligosaccharide ⇔ maltooligosyl trehalose
MTSase
⇒ trehalose +maltooligosaccharide
MTHase

The first reaction is an interesting intermolecular transglucosylation to convert the α- 1,4 to an α, α - 1,1 – glucosidic linkage of the reducing end of maltooligosaccharides, and the second is a hydrolytic reaction at the penultimate α – 1,4 linkage of the reducing end resulting in the release of trehalose.

We have studied X-ray crystallography of MTSase to determine its three-dimensional structure and make clear the mechanism of its reaction.

Experiment

crystallization

The purified intact MTSase was methylated[3] and crystallized by the hanging drop vapour-diffusion method at 25 ºC using a protein concentrration of 30 mg/ml in 0.1 M Tris-HCl (pH 8.5) with 0.2 M $MgCl_2$ and 20%(w/v) PEG 2000 as precipitant solutions. Within two weeks, typical size of crystals which have the cell dimensions of a = 56.7, b = 140.1, c = 205.2 Å, with the orthorhombic space group $P2_12_12_1$. This crystal diffracted to 2.4 Å resolution at cryo-temperature at SPring-8 synchrotron radiation facility.

But it was difficult to make Heavy-atom derivative because this crystal is apt to crack by soaking to the solution different from mother liquid.

So we tried again to detect a new crystallizing condition, and more stable crystal were obtained by increasing the consentration of PEG 2000 slowly from 11 to 13 %(w/v). This crystal have the cell dimensions of a = 56.5, b = 68.5, c = 93.8 Å, and β = 101.5º, with the monoclinic space group $P2_1$.

collection of intensity data set

Using this noble crystals, the intensity data set for the native crystals were carried out at the BL18B station at the Photon Factory using wave length 1.0 Å at room temperature, and for the two kinds of derivative crystals ($K_3UO_2F_5$ and HgAc) intensity data were collected on a Rigaku imagingplate diffractometer R-AXIS IIc with Cu-Kα radiation from rotating anode at room temperature.

Results

Native crystal was diffracted to 1.9 Å resolution, and two kind of derivative crystals were difracted to 2.4 Å (K3UO2F5) and 2.7 Å (HgAc) resolution.

Phase determination was performed by MIR method using program SHARP and SOLOMON, and three-dimentional structure of MTSase was obtained at 1.9 Å resolution.

Refinement of the structure was performed by slow-cooling protocol using program XPLOR. Finally R-factor and free-R were reducing to 0.197 and 0.256.

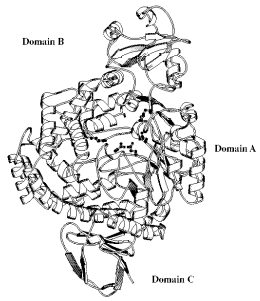

Fig. 1 Three-dimensional structure of MTSase

References

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