Acta Crystallographica Section F
**Structural Biology and Crystallization Communications**
ISSN 1744-3091

# Crystallization and preliminary X-ray analysis of isomaltase from *Saccharomyces cerevisiae*

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Received 8 July 2008
Accepted 26 September 2008

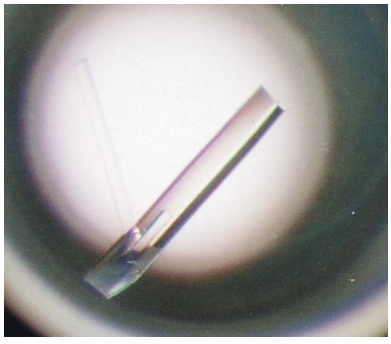
Isomaltase from *Saccharomyces cerevisiae* is an oligo-1,6-glucosidase that preferentially hydrolyzes isomaltose, with little activity towards isomaltotriose or longer oligosaccharides. An amino-acid sequence analysis of the isomaltase revealed that it belongs to glucoside hydrolase family 13. Recombinant isomaltase was purified and crystallized by the hanging-drop vapour-diffusion method with PEG 3350 as the precipitant. The crystals belonged to space group $C2$, with unit-cell parameters $a = 95.67$, $b = 115.42$, $c = 61.77$ Å, $\beta = 91.17^\circ$. X-ray diffraction data were collected to 1.35 Å resolution from a single crystal on a synchrotron-radiation source.

## 1. Introduction

$\alpha$-Glucosidases hydrolyze the terminal nonreducing $\alpha$-D-glucosidic linkage of disaccharides, oligosaccharides and polysaccharides, with the release of $\alpha$-glucose. The substrate-specificity of $\alpha$-glucosidases differs greatly depending on the source of the enzyme. Most $\alpha$-glucosidases (EC 3.2.1.20; $\alpha$-D-glucoside glucohydrolases) preferentially hydrolyze maltose (Needleman *et al.*, 1978), whereas another class of $\alpha$-glucosidases, oligo-1,6-glucosidases (EC 3.2.1.10; oligosaccharide oligo-1,6-glucohydrolases), act on the $\alpha$-1,6-glucosidic linkage of isomaltooligosaccharides and dextran (Linder & Sund, 1981; Suzuki *et al.*, 1982).

$\alpha$-Glucosidase is classified into glucoside hydrolase (GH) family 13 on the basis of its amino-acid sequence relationship to other glycosyl hydrolases. Many primary structures of members of GH family 13 from various origins are now available and have been compared with each other. The existence of four highly conserved regions (regions I–IV) and three acidic residues located in the conserved regions as catalytic residues have been reported (Matsuura *et al.*, 1984; Nakajima *et al.*, 1986; Svensson, 1988). Furthermore, all GH family 13 enzymes for which three-dimensional structures have been solved have a common multidomain structure composed of three domains: A, B and C (MacGregor *et al.*, 2001). Domain A is a catalytic domain containing a $(\beta/\alpha)_8$-barrel. Domain B is found inserted between the third $\beta$-sheet and the helix of the $(\beta/\alpha)_8$-barrel and may play a role in both enzyme stability and substrate binding. Domain C has a $\beta$-barrel structure of eight antiparallel $\beta$-strands in a double Greek-key motif. The catalytic nucleophile in the conserved region II is located in the loop extending from $\beta$-strand 4 and the general acid–base catalyst in the conserved region III is located at the C-terminus of $\beta$-strand 5.

*Saccharomyces cerevisiae* contains two $\alpha$-glucosidases, namely $\alpha$-1,4-glucosidase (maltase) and oligo-1,6-glucosidase (isomaltase), which preferentially hydrolyze maltose or isomaltose and methyl $\alpha$-D-glucopyroside ($\alpha$-mg), respectively. The expression of these enzymes is independently controlled by different polymeric genes,



namely *MAL* and *MGL* (Carlson, 1987; Vanoni *et al.*, 1989; Johnson & Carlson, 1992). Maltase (the *MAL*6 product of *S. cerevisiae*) preferentially hydrolyzes maltose but not isomaltose or α-mg, whereas isomaltase hydrolyzes isomaltose and α-mg but not maltose (Khan & Eaton, 1967; Needleman *et al.*, 1978).

Isomaltase from *S. cerevisiae* shows a notable substrate-specificity. Generally, oligo-1,6-glucosidases preferentially hydrolyze isomaltotriose and show high activity toward isomaltooligosaccharides or dextran (Linder & Sund, 1981; Suzuki *et al.*, 1982; Russell & Ferretti, 1990; Saburi *et al.*, 2006). However, isomaltase from *S. cerevisiae* shows highest activity toward isomaltose and little activity toward isomaltotriose and isomaltotetraose.

In 1997, the crystal structure of oligo-1,6-glucosidase from *Bacillus cereus* was solved at 2.0 Å resolution (Watanabe *et al.*, 1997). Recently, the structures of dextran glucosidase in an uncomplexed form and of its mutant in complex with isomaltotriose at 2.2 Å resolution have been determined (Hondoh *et al.*, 2008). However, in order to obtain a better understanding of the structure–function relationship of oligo-1,6-glucosidases, more precise structures are required. In the present work, we report the crystallization of *S. cerevisiae* isomaltase and present a preliminary crystallographic analysis of the crystals obtained.

## 2. Materials and methods

### 2.1. Enzyme production and purification

The cloning and expression of the isomaltase gene in *Escherichia coli* have been described previously (Yamamoto *et al.*, 2004). A transformant of *E. coli* containing the isomaltase expression plasmid was cultured in 150 ml Luria–Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl) containing 50 μg ml$^{-1}$ ampicillin at 310 K. Isopropyl β-D-1-thiogalactopyranoside was added (to a final concentration of 0.02 m*M*) when the optical density at 660 nm of the culture reached 0.6 and the culture was incubated for a further 12 h. *E. coli* cells were resuspended in 10 m*M* sodium phosphate buffer pH 6.8 and sonicated. The cell-free extract was applied onto a QAE-Toyopearl 650M column (Toyo Soda, Japan) equilibrated with 20 m*M* sodium phosphate buffer pH 6.8 and washed with the same buffer. The enzyme was eluted with a linear gradient of 0–150 m*M* NaCl in the same buffer. Active fractions were pooled and dialyzed against the same buffer and then subjected to chromatography on hydroxyapatite (Micro-Prep Ceramic Hydroxyapatite Type I; BioRad). The purified enzyme was eluted with a linear gradient of 20–200 m*M* sodium phosphate buffer pH 6.8.

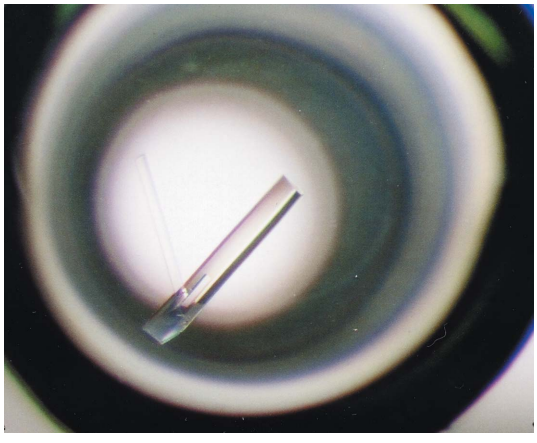


**Figure 1**
A single crystal of isomaltase. The longest dimension is approximately 1.5 mm.

**Table 1**
Data-collection statistics for *S. cerevisiae* isomaltase.

Values in parentheses are for the highest resolution shell (1.40–1.35 Å).

| | |
|---|---|
| No. of crystals used | 1 |
| Wavelength (Å) | 0.9000 |
| Resolution range (Å) | 50–1.35 (1.40–1.35) |
| No. of reflections | 615260 |
| No. of unique reflections | 146713 |
| Space group | *C*2 |
| Unit-cell parameters | |
| *a* (Å) | 95.67 |
| *b* (Å) | 115.42 |
| *c* (Å) | 61.77 |
| β (°) | 91.17 |
| Completeness (%) | 99.8 (99.5) |
| Redundancy | 4.2 (4.1) |
| $R_{\rm merge}$† (%) | 6.6 (12.2) |
| Average $I/\sigma(I)$ | 52.8 (15.7) |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl)\rangle$ is the average intensity of reflection *hkl*.

### 2.2. Crystallization

Prior to crystallization, the isomaltase was dialyzed against 5 m*M* HEPES buffer pH 7.3 and concentrated to 4.5 mg ml$^{-1}$ by ultrafiltration with a Centricon YM-10 filter (Millipore, USA). Hampton Research kits were used to determine the initial crystallization conditions. Drops consisting of 3 μl protein solution and 3 μl reservoir solution were equilibrated over 0.5 ml reservoir solution using the hanging-drop vapour-diffusion method. Crystals were obtained using solution No. 24 [0.2 *M* lithium acetate dehydrate, 20%(*w*/*v*) polyethylene glycol (PEG) 3350] of the PEG/Ion Screen at 293 K. The pH of the buffer solution and the concentration of PEG were optimized and the temperature was varied. After optimization, rod-shaped crystals grew to maximum dimensions of 0.2 × 0.2 × 1.5 mm in two weeks (Fig. 1). The reservoir solution consisted of 50 m*M* HEPES pH 7.3, 0.2 *M* lithium acetate and 19%(*w*/*v*) PEG 3350. The crystallization temperature was 288 K.

### 2.3. Data collection and processing

A single crystal was transferred from the crystallization drop to a cryoprotectant solution containing 50 m*M* HEPES pH 7.3, 0.2 *M* lithium acetate, 19%(*w*/*v*) PEG 3350 and 10%(*v*/*v*) glycerol for a few seconds. The crystal was then flash-cooled to 100 K in a nitrogen-gas stream in order to prevent radiation damage during data collection. A data set was collected from a single crystal using synchrotron radiation of wavelength 0.9000 Å on beamline BL44XU of SPring-8 (Hyogo, Japan) with an imaging-plate X-ray detector system (DIP6040). A total of 200 images were collected with 1° oscillation and an exposure time of 5 s per image. Data processing was performed with *HKL*-2000 (Otwinowski & Minor, 1997).

## 3. Results and discussion

The isomaltase crystals belonged to the monoclinic space group *C*2, with unit-cell parameters $a = 95.67$, $b = 115.42$, $c = 61.77$ Å, $\beta = 91.17°$. Detailed data-collection statistics are summarized in Table 1. The total of 615 250 measured reflections were reduced to 146 713 unique reflections with an overall $R_{\rm merge}$ of 6.6%. This represents 99.8% completeness at 1.35 Å resolution. The outermost shell of resolution between 1.40 and 1.35 Å was 99.5% complete with an $R_{\rm merge}$ of 12.2%.

Assuming the presence of one molecule in the asymmetric unit, the Matthews parameter ($V_M$; Matthews, 1968) of 2.5 Å$^3$ Da$^{-1}$ (50.1% solvent content by volume) is well within the allowed range for crystalline proteins.

Structural determination is in progress using molecular replacement with the reported structure of the oligo-1,6-glucosidase from *B. cereus* (PDB code 1uok; Watanabe *et al.*, 1997) as a search model.

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