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Crystallization and preliminary X-ray crystallographic studies on maltosyltransferase from *Thermotoga maritima*

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Thermotoga maritima maltosyltransferase (MTase) is a 73.7 kDa molecular weight amylolytic enzyme which catalyzes the transfer of maltosyl units from maltodextrins or starch to suitable acceptors. Crystals of recombinant MTase have been obtained by the hanging-drop vapour-diffusion method using ammonium phosphate as a precipitating agent. The crystals belong to space group $P4_122$ or its enantiomorph $P4_322$, with unit-cell parameters $a = b = 148.7$, $c = 106.7$ Å. The asymmetric unit appears to contain one subunit, corresponding to a very low packing density of 4.0 Å^3 Da^{-1}. The crystals diffract X-rays to at least 2.4 Å resolution on a synchrotron-radiation source.

1. Introduction

Maltosyltransferase (MTase) isolated from the hyperthermophilic bacterium *T. maritima* represents a novel type of maltodextrin glycosyltransferase acting on starch and malto-oligosaccharides. This enzyme has a unique transfer specificity for maltosyl units, transferring them from α-1,4-linked glucans or malto-oligosaccharides to other α-1,4-linked glucans, malto-oligosaccharides or glucose (Meissner & Liebl, 1998). MTase is extremely thermostable, with half-lives of about 2.5 h, 17 h and 21 d recorded at 363, 358 and 343 K (pH 6.5), respectively. The enzyme displays maximum activity at pH 6.5 and 358–363 K (Meissner & Liebl, 1998).

Analysis of the MTase primary structure (Meissner & Liebl, 1998) revealed low-level local similarity between MTase and the glycosyl hydrolase family 13 (also called the α-amylase family), the largest family of glycosyl hydrolases, which covers about 20 different enzyme types classified as hydrolases and transferases (for reviews, see Janeček, 1997; Kuruki & Imanaka, 1999). The common feature of the members of this family is the presence of a $(\alpha/\beta)_8$-barrel catalytic core domain which in all cases has a long loop or domain (termed domain B) inserted between the third β-strand and the third α-helix. Domain B was noted to be the least similar region of the enzymes from this family, greatly varying in both size and sequence (Machius *et al.*, 1995; Janeček *et al.*, 1997). Structure-based analysis of sequence similarities between MTase and α-amylases and related enzymes has permitted the tentative location of some secondary-structure elements of the $(\alpha/\beta)_8$-barrel core domain and the construction of a domain model for MTase (Meissner & Liebl, 1998). In particular, this analysis revealed that the size of domain B of MTase is larger than most other B domains.

The reaction chemistry of enzymes from the α-amylase family is based on a two-step acid-catalysis mechanism that requires two critical carboxylates, one acting as a general acid/base and the other as a nucleophile. In the first reaction step, one carboxylic group provides general acid catalysed leaving-group departure while a simultaneous nucleophilic attack of the anomeric centre of the sugar by the second carboxylate takes place to form a covalent glycosyl-enzyme intermediate (Svensson & Søgaard, 1993; Svensson, 1994; Strokopytov *et al.*, 1995). In the second step, the intermediate undergoes transglycosylation or hydrolysis with general base assistance to facilitate attack by an incoming group. In MTase, Glu414 and Asp385 were postulated to play the roles of the general acid/base and catalytic nucleophile, respectively, analogous to the roles of the equivalent residues in the other family 13 glycosyl hydrolases (Meissner & Liebl, 1998).

In order to elucidate the structural factors which determine the unique catalytic activity and high thermostability of MTase, we have initiated crystallographic studies on this enzyme. In this paper, we report the crystallization of recombinant MTase and preliminary X-ray analysis of the enzyme.

2. Materials and methods

T. maritima MTase was purified from an overproducing *Escherichia coli* strain JM83 as described previously (Meissner & Liebl, 1998). After purification, the enzyme was concentrated to 15 mg ml^{-1} (based on the Bradford assay; Bradford, 1976) in 5 m*M* MOPS buffer

pH 7.0 containing 0.02% sodium azide and was frozen and stored at 253 K. Analytical gel-permeation chromatography was performed on a Superdex 200 HiLoad 16/60 Column (Pharmacia) equilibrated with either 50 m*M* Tris–HCl buffer pH 7.0, 150 m*M* NaCl or McIlvaine buffer (prepared by titration of 0.1 *M* citric acid and 0.2 *M* Na_2HPO_4) pH 4.8, 150 m*M* NaCl. Prior to crystallization, the stored protein samples were concentrated to 18 mg ml^{-1} protein and centrifuged for 10 min at 25 000*g* to clarify the solution. Crystals were grown at 290 K using the standard hanging-drop vapour-diffusion technique. A 3 µl aliquot of the protein solution was mixed with an equivalent aliquot of a reservoir solution consisting of 0.35–0.40 *M* $NH_4H_2PO_4$ adjusted to pH 4.7–4.8 with an equimolar $(NH_4)_2HPO_4$ solution. Rod-like crystals of dimensions up to 0.25 × 0.25 × 1.5 mm appeared within 2–3 d.

The crystals of MTase grew reproducibly but proved to be moderately sensitive to radiation at room temperature. To perform data collection at cryogenic temperatures, the droplet containing crystals was gradually saturated with glycerol to 35%(*v*/*v*) in 5% increments, bathing the crystals in each solution for approximately 30 s. The crystal was then flash-frozen in a stream of nitrogen gas at 100 K using an Oxford Cryosystems Cryostream device. X-ray diffraction data from a cryo-cooled crystal were collected to 2.4 Å as 1° rotation frames on a MAR image-plate detector on station 7.2 at the SRS Daresbury Laboratory. The data were processed using the *DENZO*/*SCALEPACK* package (Otwinowski & Minor, 1997).

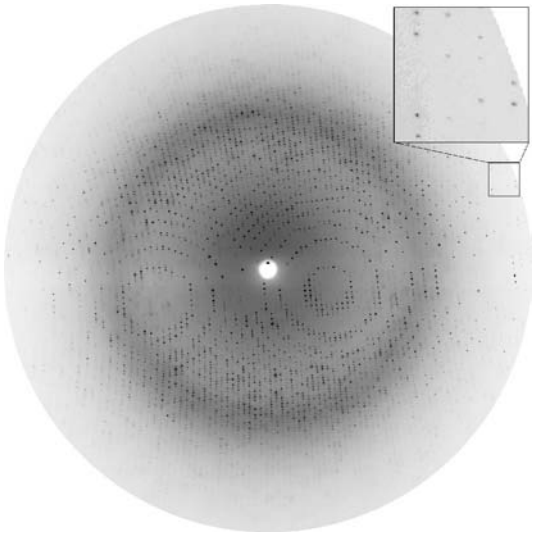

Figure 1
A representative 1° rotation frame of data collected from a frozen MTase crystal on a 30 cm MAR image-plate system on station 7.2 at the SRS Daresbury Laboratory. Enlargement of the edge of the diffraction image shows reflections to a resolution of 2.4 Å.

3. Results and discussion

Analysis of the X-ray diffraction data by the autoindexing routine in *DENZO* is consistent with a primitive tetragonal crystal system, class 422, with unit-cell parameters $a = b = 148.7$, $c = 106.7$ Å. Systematic absences were observed along the c^* axis with only the reflections with $l = 4n$ appearing to be present, indicating that the crystals belong to one of the enantiomorphic space groups $P4_122$ or $P4_322$. Significant reflections were observed up to the edge of the image-plate detector (Fig. 1), with the average $I/\sigma(I)$ value being ∼10 in the highest resolution shell (2.42–2.38 Å). A total of 198 549 measurements were made of 47 952 independent reflections. Data processing gave an $R_{\rm merge}$ of 0.047 for intensities (0.138 in the resolution shell 2.42–2.38 Å) and this data set is 99% complete (98% completeness in the highest resolution shell).

Previous studies (Meissner & Liebl, 1998) indicated that native MTase assembles to give a complex in excess of 450 kDa. However, our more recent gel-filtration studies on highly purified enzyme indicate a dimeric quaternary structure at both neutral (7.0) and acidic (4.8) pH. A self-rotation function calculated with the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994) in the resolution range 8–5 Å with an integration radius of 25 Å did not reveal any significant peaks besides the crystallographic symmetry peaks. The possibility of the existance of a non-crystallographic rotation axis lying parallel to but not coincident with a crystallographic axis was precluded by analysis of the native Patterson map using data between 15 and 5 Å resolution, which did not contain any significant peaks besides the origin peak. Hence, the asymmetric unit is very likely to contain one molecule. If this is the case, then given a molecular weight of 73.7 kDa, the V_m value of 4.0 Å^3 Da^{-1} corresponds to a rather high solvent content in the unit cell (about 66%; Matthews, 1968). However, this is not unusual for proteins isolated from hyperthermophiles (Yip *et al.*, 1995; Tahirov *et al.*, 1998). Our efforts are currently being directed towards a search for heavy-atom derivatives and the solution of the structure using multiple isomorphous replacement.

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