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Crystallization and preliminary X-ray crystallographic studies on 4-α-glucanotransferase from *Thermotoga maritima*

Anna Roujeinikova,[a] Carsten Raasch,[b] Svetlana Sedelnikova,[a] Wolfgang Liebl[b] and David W. Rice[a]*

[a]Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield S10 2TN, England, and [b]Institut für Mikrobiologie und Genetik, Georg-August-Universität, Göttingen, Germany

Correspondence e-mail: d.rice@sheffield.ac.uk

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Thermotoga maritima 4-α-glucanotransferase (GTase), a 52 kDa molecular-weight amylolytic enzyme, has been crystallized by the hanging-drop vapour-diffusion method using PEG monomethylether 5000 as a precipitating agent. A complete data set has been collected to 2.6 Å resolution using cryocooling conditions and synchrotron radiation. The crystals belong to space group $I222$ or $I2_12_12_1$, with unit-cell parameters a = 92.6, b = 180.3, c = 199.2 Å.

1. Introduction

4-α-Glucanotransferase (GTase, amylomaltase, disproportionating enzyme; E.C. 2.4.1.25), found in various bacterial and plant species, catalyzes a disproportionation reaction in which a segment of a 1,4-α-D-glucan is transferred to the 4-hydroxyl group of an acceptor sugar. In plants, this enzyme is assumed to be involved in starch metabolism (Takaha & Smith, 1999), whereas bacterial amylomaltases carry different physiological functions. In *Escherichia coli*, *Thermococcus litoralis* and *Clostridium butyricum*, amylomaltase is involved in exogenous maltooligosaccharide utilization and is thought to play a role in converting short maltooligosaccharides into longer chains upon which glucan phosphorylase can act, with release of glucose in the process (Boos & Shuman, 1998; Takaha & Smith, 1999; Xavier *et al.*, 1999; Goda *et al.*, 1997). In *Haemophilus influenzae* and *Aquifex aeolicus*, the genes for GTase are part of the glycogen operon, indicating that the enzyme might be involved in glycogen metabolism (Takaha & Smith, 1999). Amino-acid sequence comparisons indicate that except for two GTases from the hyperthermophilic archaea *Thermococcus litoralis* and *Pyrococcus* sp., which belong to the glycosyl hydrolase family 57 (Jeon *et al.*, 1997; Tachibana *et al.*, 1997), all known GTases are members of the glycosyl hydrolase family 13 (also called the α-amylase family; for a review, see Janecek, 1997).

GTase from the hyperthermophilic bacterium *T. maritima* is a 52 kDa molecular-weight enzyme that can convert starch, amylopectin and amylose by transferring maltosyl and longer dextrinyl residues to suitable acceptors (Liebl *et al.*, 1992). The primary structure of *T. maritima* GTase reveals high similarity to α-amylases, α-glucosidases and oligo-1,6-glucosidases with, for example, 37% identity with the sequences of α-amylases from *Dictyoglomus thermophilum* (AmyC) and *Bacillum megaterium* (Heinrich *et al.*, 1994). This clearly distinguishes the *T. maritima* enzyme from other known GTases which show only weak similarity with α-amylases and appear to be the most distantly related members of the α-amylase family (Janecek, 1997). It is also interesting to note that *T. maritima* GTase is Ca^{2+} dependent (Liebl *et al.*, 1992) like typical α-amylases and CGTases, whereas a calcium requirement has not been reported for any other GTase described to date. In addition, the hydrolytic activity of this enzyme is barely detectable (W. Liebl, unpublished data), while some other GTases display significant hydrolytic or cyclization activity (Takaha & Smith, 1999). *T. maritima* GTase thus represents a new type of enzyme within the large glycosyl hydrolase family 13. The sequence and biochemical properties of GTase from *T. neopolitana* have been reported recently (Berezina *et al.*, 1999). This enzyme shares 83% sequence identity with *T. maritima* GTase and may represent a second enzyme of this type.

Unlike GTase, which can transfer both maltosyl and longer dextrinyl residues, *T. maritima* has a second maltodextrin glucanotransferase, maltosyltransferase (MTase), which has a strict transfer specificity to maltosyl residues alone (Meissner & Liebl, 1998) and which shows only 18% sequence identity to GTase. We have recently reported crystallization of MTase (Burke *et al.*, 2000) as part of a study to identify the structural features that determine its substrate specificity. In this paper, we report the crystallization and preliminary X-ray analysis of recombinant

T. maritima GTase. Analysis of the crystal structure of this enzyme and its comparison with the structures of MTase and other family 13 glycosyl hydrolases and in particular with that of *Thermus aquaticus* amylomaltase (Przylas *et al.*, 2000) would be an important step towards our understanding of how the structural variations within this class of enzymes are related to substrate and reaction specificity.

2. Materials and methods

T. maritima GTase was expressed and purified as described previously (Heinrich *et al.*, 1994). After purification, the enzyme was concentrated to 32 mg ml^{-1} (concentration estimated using the Bradford assay; Bradford, 1976) in 20 m*M* Tris–HCl buffer pH 8.0, frozen and stored at 253 K. Analytical gel-permeation chromatography was performed on a Superdex 200 HiLoad 16/60 Column (Pharmacia) equilibrated with 50 m*M* MES buffer pH 6.5, 40 m*M* ammonium sulfate and 3 m*M* $CaCl_2$. Prior to crystallization, the stored protein samples were centrifuged for 10 min at 25 000*g* to clarify the solution. Preliminary crystallization trials were conducted at 290 K using Crystal Screens I and II and PEG/Ion Screen (Hampton Research). Typically, a 2 µl aliquot of the protein solution was mixed with an equivalent aliquot of a reservoir solution and allowed to equilibrate against 0.5 ml of the reservoir solution. The best crystals, which had a rod-like morphology and dimensions of about 0.05 × 0.05 × 0.3 mm, were obtained using 26–29%(*w*/*v*) PEG monomethylether 5000, 200–300 m*M* $(NH_4)_2SO_4$, 3 m*M* $CaCl_2$ and 80 m*M* MES pH 6.5 and grew after 1–2 weeks.

Test data collected from these crystals on station 14.2 at the SRS Daresbury Laboratory showed diffraction beyond 2.5 Å (Fig. 1). To perform data collection at cryogenic temperatures, the crystal was loop-mounted in a cryoprotecting solution containing 5% glycerol, 32%(*w*/*v*) PEG monomethylether 5000, 320 m*M* $(NH_4)_2SO_4$, 3 m*M* $CaCl_2$ and 80 m*M* MES pH 6.5 and then flash-frozen in a stream of nitrogen gas at 100 K using an Oxford Cryosystems Cryostream device. X-ray diffraction data from a cryocooled crystal were collected to 2.6 Å as 0.5° rotation frames on a CCD detector on station 9.6 at the SRS Daresbury Laboratory. The data were processed using the *DENZO*/*SCALEPACK* package (Otwinowski & Minor, 1997). Calculation of the self-rotation function was performed using the *POLARRFN* program (Collaborative Computational Project, Number 4, 1994).

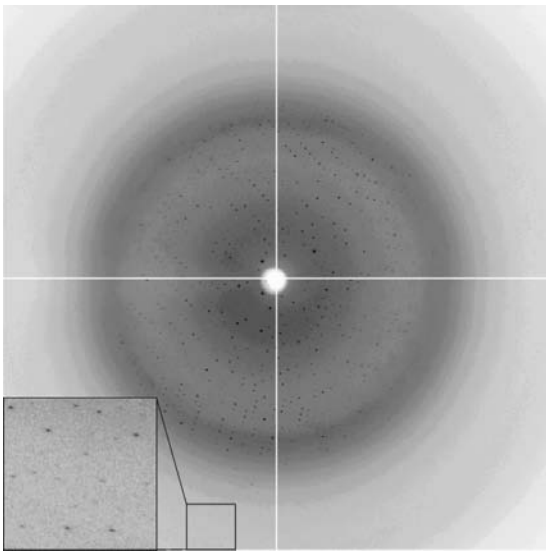

Figure 1
A representative 0.5° rotation frame of data collected from a GTase crystal using a CCD detector on station 14.2 at the SRS Daresbury Laboratory. A magnified rectangle shows diffraction spots beyond 2.5 Å resolution.

3. Results and discussion

Analysis of the X-ray diffraction data by the autoindexing routine in *DENZO* is consistent with a body-centered orthorhombic crystal system (*I*222 or $I2_12_12_1$), with unit-cell parameters a = 92.6, b = 180.3, c = 199.2 Å. The average $I/\sigma(I)$ value is 22.5 for all reflections (resolution range 15.00–2.60 Å) and 2.3 in the highest resolution shell (2.66–2.60 Å). A total of 191 062 measurements were made of 45 945 independent reflections. Data processing gave an $R_{\rm merge}$ of 0.053 for intensities (0.357 in the resolution shell 2.66–2.60 Å) and these data were 89% complete (64% completeness in the highest resolution shell).

When subjected to gel filtration, more than 90% of the protein eluted as a single peak with a molecular weight of about 60 kDa, indicating that GTase is mostly monomeric in solution, with only a small fraction of molecules forming higher oligomers. Calculations of the Matthews coefficient for two, three or four molecules in the asymmetric unit give values of 4.0, 2.7 and 2.0 Å^3 Da^{-1}, respectively, all of which lie in the range observed for protein crystals (Matthews, 1977). For the self-rotation function calculated using data in the resolution range 10–6 Å with an integration radius of 20 Å, no dominant features that can be confidently assigned to non-crystallographic axes were found in the κ = 90, κ = 120 or κ = 180° sections. Thus, we are currently unable to determine the protein contents of the asymmetric unit. 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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