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Purification, crystallization and preliminary X-ray diffraction studies of *N*-acetylglucosamine-phosphate mutase from *Candida albicans*

N-acetylglucosamine-phosphate mutase (AGM1) is an essential enzyme in the synthesis of UDP-*N*-acetylglucosamine (UDP-GlcNAc) in eukaryotes and belongs to the α -D-phosphohexomutase superfamily. AGM1 from *Candida albicans* (CaAGM1) was purified and crystallized by the sitting-drop vapour-diffusion method. The crystals obtained belong to the primitive monoclinic space group $P2_1$, with unit-cell parameters $a = 60.2$, $b = 130.2$, $c = 78.0$ Å, $\beta = 106.7^\circ$. The crystals diffract X-rays to beyond 1.8 Å resolution using synchrotron radiation.

1. Introduction

UDP-*N*-acetylglucosamine (UDP-GlcNAc) is a UDP sugar and is synthesized from fructose-6-phosphate supplied by the glycolytic pathway (Watzel & Tanner, 1989; Mio *et al.*, 1999; Hofmann *et al.*, 1994; Mio *et al.*, 1998). UDP-GlcNAc is used as a precursor in the synthesis of cell-wall chitin and glycoproteins. Although UDP-GlcNAc is also utilized in mammalian cells, fungi require a larger amount of UDP-GlcNAc owing to the synthesis of the cell wall.

N-Acetylglucosamine-phosphate mutase (AGM1; EC 5.4.2.3) is an essential enzyme in the synthesis of UDP-GlcNAc. AGM1 catalyzes the intramolecular transfer of a phosphoryl group. This synthetic step consists of the interconversion of *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) and *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P). The process of UDP-GlcNAc biosynthesis differs in prokaryotes and eukaryotes and AGM1 has only been identified in eukaryotes (Mio *et al.*, 2000). It is well known that AGM1 requires Mg^{2+} ions to exhibit maximum activity; complete inhibition has also been achieved with Zn^{2+} ions (Cheng & Carlson, 1979; Fernandez-Sorensen & Carlson, 1971). AGM1 is a member of the α -D-phosphohexomutase superfamily, as it catalyzes a reversible intramolecular phosphoryl transfer on its sugar substrate (Shackelford *et al.*, 2004; Whitehouse *et al.*, 1998). Other superfamily members exist as various oligomeric states (monomers, dimers and trimers) in solution (Jolly *et al.*, 1999; Maino & Young, 1974). Phosphomannomutase/phosphoglucomutase from *Pseudomonas aeruginosa* (PaPMM/PGM; PDB code 1k2y), which catalyzes the reversible conversion of mannose-6-phosphate to mannose-1-phosphate and glucose-6-phosphate to glucose-1-phosphate, shares 20.7% sequence identity with AGM1 from *Candida albicans* (CaAGM1) as calculated by the program FASTA (Lipman & Pearson, 1985; Pearson & Lipman, 1988) and exists as a monomer in solution (Regni *et al.*, 2002, 2004). Several structures of enzymes in this superfamily have been reported to date. They are similar to each other, being composed of four domains arranged in a 'heart shape' (Liu, 1997; Regni *et al.*, 2002, 2004). However, for AGM1 only the atomic coordinates of the C-terminal domain from *Mus musculus* (112 amino-acid residues of 542 amino-acid residues) has been deposited (PDB code 1wjw) and overall structures have not been determined. Multiple sequence alignment in the superfamily shows that AGM1 has significant sequence similarity to other members with regard to the presumed active-site loops, but that AGM1 has a different relative location of several loops; these loops were not identified as affecting the activity by previous mutation analysis (Shackelford *et al.*, 2004; Mio *et al.*, 2000). Also, little is known about the substrate specificity. Determination of the three-dimensional structure of this enzyme is thus necessary in order to gain a better

Table 1

Data-collection statistics for CaAGM1.

Values for the highest resolution shell are in parentheses.

| | |
|--|--|
| Space group | $P2_1$ |
| Unit-cell parameters (\AA , $^\circ$) | $a = 60.17$, $b = 130.22$, $c = 77.96$, $\beta = 106.68$ |
| No. of measured reflections | 328097 |
| No. of independent reflections | 81775 |
| Resolution range (\AA) | 50–1.93 (2.00–1.93) |
| R_{merge}^\dagger (%) | 4.1 (25.4) |
| Completeness ($I > 1\sigma$) (%) | 94.9 (85.0) |
| $I/\sigma(I)$ | 31.3 (4.8) |

$^\dagger R_{\text{merge}} = \sum |I_i - \langle I_i \rangle| / \sum \langle I_i \rangle$, where I_i is the observed intensity and $\langle I_i \rangle$ is the average intensity over symmetry-equivalent measurements.

understanding not only of its catalytic mechanism, but also of the additional important differences between the enzymes included in this superfamily.

Here, we describe the purification, crystallization and preliminary X-ray data for CaAGM1. *C. albicans* is a disease-causing fungus that leads to a life-threatening systemic infection (referred to as ‘candidiasis’) in immunocompromized hosts (Rogers & Balish, 1980). It is impossible for fungi to live without biosynthesis of the cell wall and the chitin of the cell wall is synthesized from UDP-GlcNAc. A specific inhibitor of AGM1 from pathogenic fungi could therefore be a new candidate antifungal reagent that would inhibit cell-wall synthesis.

2. Purification

CaAGM1 is composed of 544 amino-acid residues and its molecular weight is about 60 kDa. For the crystallization experiments, the ORF of CaAGM1 was cloned between the *Bam*HI and *Eco*RI sites of pGEX-2T to express CaAGM1 as a glutathione *S*-transferase (GST) fusion. CaAGM1 was overproduced and sonicated by previously described methods (Mio *et al.*, 2000), except that after the addition of isopropyl 1-thio-galactopyranoside the cells were grown for an additional 22 h at 295 K. The soluble fraction was applied onto a Glutathione Sepharose 4 FastFlow column (Amersham Biosciences, Piscataway, NJ, USA) and was washed with PBS at 277 K. The purified protein was eluted with a GST elution buffer (20 mM Tris–HCl, 0.15 M NaCl, 10 mM glutathione pH 8.5). The GST was then removed by a 16 h treatment with thrombin at 277 K. Finally, this solution was applied onto a DEAE-Toyopearl 650 column (Tosoh, Tokyo, Japan) and eluted using a gradient of 0–0.5 M NaCl contained

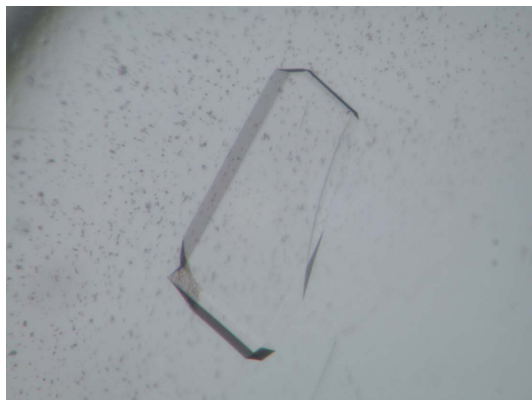


Figure 1
Crystal of CaAGM1.

in elution buffer [20 mM Tris–HCl, 1 mM EDTA, 5% (v/v) glycerol pH 7.5] at 277 K. The purity of the fraction used for crystallization was tested by SDS–PAGE. Gel-filtration chromatography analysis (data not shown) suggested that the purified protein exists as a monomer in solution. The final yield of CaAGM1 per litre of culture is approximately 2.5 mg. It was desalted and concentrated to a volume of 10 mg ml^{−1}.

3. Crystallization

Crystallization attempts were carried out using Crystal Screens 1 and 2, Crystal Screen Cryo and PEG/Ion Screen (Hampton Research, Aliso Viejo, CA, USA). All attempts at crystallization were performed using the sitting-drop vapour-diffusion method at 293 K. Mixtures of 1.25 μ l protein solution (10 mg ml^{−1} in 50 mM Tris–HCl pH 7.6 and 1 mM dithiothreitol) and an equal volume of the reservoir solution were equilibrated against 100 μ l reservoir solution. Crystals were obtained using reservoir solution containing 200 mM NH₄H₂PO₄ and 14–20% (w/v) polyethylene glycol 3350 within a week with approximate dimensions of 0.5 × 0.2 × 0.03 mm (Fig. 1).

4. X-ray data collection

The crystals were soaked briefly in a cryoprotectant solution prepared by the addition of 15% (v/v) glycerol to the reservoir solution and the crystals were mounted in a loop and flash-cooled in an N₂ gas stream at 95 K. X-ray diffraction studies were performed at SPring-8 (BL44B2) and the Photon Factory (BL5). The diffraction data used for the refinement were collected at BL44B2 with a MAR CCD detector using an oscillation angle of 0.5°, 15 s exposure per frame and a wavelength of 1.000 \AA . The crystal-to-detector distance was set at 115 mm. Diffraction was observed to a resolution exceeding 1.8 \AA . The data set from a single crystal was processed and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL2000* package (Otwinowski & Minor, 1997).

The crystals were found to belong to the primitive monoclinic system and systematic absences of reflections ($k = 2n + 1$ at $0k0$) indicated the space group of the data set to be $P2_1$. The statistics for the intensity data are shown in Table 1. Assuming the presence of two CaAGM1 molecules per asymmetric unit, the calculated Matthews coefficient V_M was 2.42 $\text{\AA}^3 \text{Da}^{-1}$ (Matthews, 1968), which corresponds to a solvent content of 49.2%. Phasing by the multiple isomorphous replacement method and the structure refinement were successful and the structure will be published soon.

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