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Purification, crystallization and preliminary X-ray analysis of the glucosamine-6-phosphate *N*-acetyltransferase from human liver

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Glucosamine-6-phosphate *N*-acetyltransferase from human liver, which catalyzes the transfer of an acetyl group from acetyl coenzyme A (AcCoA) to the primary amine of D-glucosamine 6-phosphate to form *N*-acetyl-D-glucosamine 6-phosphate, was expressed in a soluble form from *Escherichia coli* strain BL21 (DE3). The protein was purified to homogeneity using Ni^{2+}-chelating chromatography followed by size-exclusion chromatography. Crystals of the protein were obtained by the hanging-drop vapour-diffusion method and diffracted to 2.6 Å resolution. The crystals belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 50.08$, $c = 142.88$ Å.

1. Introduction

The nucleotide sugar UDP-*N*-acetyl-glucosamine (UDP-GlcNAc) is an important metabolite in eukaryotic cells. It holds a key position in the N-linked glycosylation of protein, which impacts on diverse physiological and pathological processes, and in the generation of glycosylphosphatidylinositol linkers, which anchor the membrane protein to the cell. The *de novo* biosynthesis of UDP-GlcNAc includes the acetylation of D-glucosamine 6-phosphate (Glc6P) and uridylylation of *N*-acetyl-glucosamine 1-phosphate after an intramolecular phosphate transfer (Schachter, 1978).

Glucosamine-6-phosphate *N*-acetyltransferase (GNA1; EC 2.3.1.4) is a novel amino-sugar *N*-acetyltransferase member of the GCN5-related *N*-acetyltransferase (GNAT) superfamily (Mio *et al.*, 1999; Peneff *et al.*, 2001; Dyda *et al.*, 2000; Vetting *et al.*, 2005). GNA1 catalyzes the transfer of an acetyl group from acetyl coenzyme A (AcCoA) to the primary amine of Glc6P to form *N*-acetyl-D-glucosamine 6-phosphate (GlcNac6P), which is the key step in the *de novo* biosynthesis of UDP-GlcNAc. Genes encoding GNA1 have been characterized in various eukaryotic organisms such as the murine gene *EMeg32* and the *Saccharomyces cerevisiae* gene *YFL017C* (Mio *et al.*, 1999; Boehmelt, Fialka *et al.*, 2000). It has been noted that GNA1 controls multiple cell-cycle steps in *S. cerevisiae* and the inactivation of *YFL017C* was lethal (Lin *et al.*, 1996) and that *EMeg32* was essential for embryonic development, while EMeg32-dependent UDP-GlcNAc levels influence cell-cycle progression and susceptibility to apoptotic stimuli (Boehmelt, Wakeman *et al.*, 2000).

To date, only structures of GNA1 from *S. cerevisiae* have been determined: apo GNA1 and the GNA1–AcCoA and GNA1–CoAGlcNAc6P complexes (Peneff *et al.*, 2001). *S. cerevisiae* GNA1 shows a dimeric structure in which each monomer exhibits a classic GNAT fold and the GlcNac6P-binding site is located at the dimer interface. The catalytic and the substrate-specificity mechanisms of the enzyme were also illustrated from the structures. The peptide lengths of human and murine GNA1 are longer than that of yeast at the N- and C-termini (human and murine GNA1, 184 amino acids; yeast GNA1, 149 amino acids) and the sequence identity between human and yeast GNA1 is 32%. Since GNA1 is a potential target for the development of antifungal agents (Mio *et al.*, 2000), it is important to determine the human GNA1 structure and compare it with those from other organisms. Here, we present the expression, purification, crystallization and preliminary crystallographic studies of GNA1 from human liver to form the basis of the structure determination of human GNA1.

Table 1
Data-collection statistics for human GNA1.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	50–2.6 (2.74–2.6)
Completeness (%)	87.4 (63.8)
R_{sym}† (%)	6.7 (30.0)
Mean $I/\sigma(I)$	9.5 (1.81)
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 50.08$, $c = 142.88$
No. of observed reflections	62499
No. of unique reflections	5369
Molecules per ASU	1
Solvent content (%)	51

† $R_{sym} = \sum_h[\sum_i |I(h)_i - \langle I(h)\rangle|/\sum I(h)_i]$, where $I(h)_i$ is the ith observation of reflection h and $\langle I(h)\rangle$ is the mean intensity of all observations of h.

2. Materials and methods

2.1. Cloning and expression

The GNA1-encoding gene was amplified by polymerase chain reaction (PCR) from a human liver cDNA library. The primers used were 5′-CGCGGATCCATGAAACCTGATGAAACTCCTAT-3′ and 5′-CCGCTCGAGTTACTTTAGAAACCTCCGACACA-3′, which contain sequences for *Bam*HI and *Xho*I restriction sites, respectively. The PCR product was treated with the enzymes and ligated into the *Bam*HI/*Xho*I-linearized vector pET28a (Novagen), which contains an N-terminal His_6 tag with the sequence MGSS**HHHHHH**SSGL-VPRGSHMASMTGGQQMGRGS. The final vector was verified by DNA sequencing. The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3). Cells were cultured in 20 ml Luria–Bertani (LB) medium containing 50 µg ml^{-1} kanamycin at 310 K overnight; the overnight culture was then added to 1 l fresh LB medium containing 50 µg ml^{-1} kanamycin and grown until an OD_{600} of 0.6–0.8 was reached. The cells were then induced with 0.5 m*M* (final concentration) isopropyl β-D-thiogalactopyranoside and grown for a further 10 h at 303 K. The cells were harvested by centrifugation and resuspended in buffer *A* (20 m*M* Tris–HCl, 0.5 *M* NaCl pH 7.5).

2.2. Protein purification

The resuspended cells were disrupted by sonication on ice, the cell debris was removed by centrifugation for 30 min at 18 000 rev min^{-1} twice and the supernatant was loaded onto a 5 ml HiTrap Ni^{2+}-chelating column (GE, USA) equilibrated with buffer *A*. The bound target protein was eluted by applying a linear gradient of 50–500 m*M* imidazole in buffer *A*. The protein was further purified on a HiLoad Superdex-75 column (GE, USA) using 200 m*M* NaCl in 20 m*M* Tris–HCl pH 7.5 as eluent. The purity of the protein was checked by SDS–PAGE in each step.

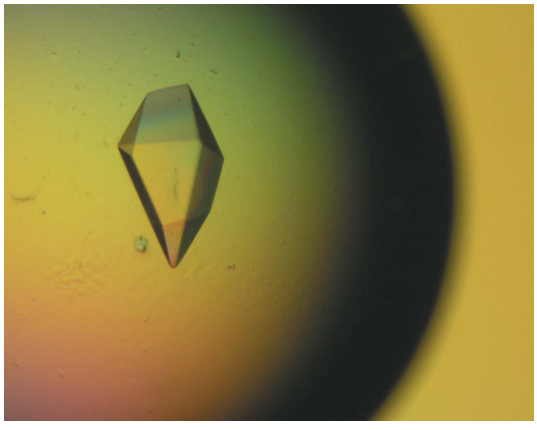

Figure 1
Crystals of human GNA1. The dimensions of the largest crystal are approximately 0.1 × 0.1 × 0.3 mm.

2.3. Crystallization and X-ray data collection

The purified sample was concentrated to 12 mg ml^{-1} by ultrafiltration (Millipore Amicon). The concentrated protein solution contained 150 m*M* NaCl and 20 m*M* Tris–HCl pH 7.5. Crystallization experiments were performed by the hanging-drop vapour-diffusion method at 289 K. Crystal Screen, Crystal Screen 2 and Index kits (Hampton Research, USA) were used as initial screening conditions. 1 µl protein solution was mixed with an equal volume of reservoir solution and equilibrated against 500 µl reservoir solution.

X-ray diffraction data were collected on a Bruker SMART 6000 CCD detector mounted on a Bruker–Nonius FR591 rotating-anode generator with Cu $K\alpha$ radiation. A total of 1000 frames were collected with an exposure of 60 s per 0.2° oscillation image. The crystal-to-detector distance was set to 6 cm and the 2θ angle was set to 0°. During data collection, the crystal was flash-cooled and maintained at 100 K using nitrogen gas. The data were processed using the Bruker online *PROTEUM* software suite.

3. Results

Human GNA1 was expressed in *E. coli* in a soluble form and purified to homogeneity by a two-step chromatography procedure. SDS–PAGE showed the molecular weight of the sample to be about 25.0 kDa, which is consistent with the theoretical molecular weight of GNA1 plus about 4.0 kDa for the hexahistidyl tag fusion peptide. The peak position on gel-filtration chromatography indicates the sample exists in a dimeric form in solution; the acetylating activity of the sample towards Glc6P was also determined (data not shown). Crystals appeared after 2 d in more than 30 conditions. After optimizing around condition No. 82 from the Index kit, single crystals suitable for diffraction (Fig. 1) were obtained in buffer containing 0.2 *M* $MgCl_2$, 0.1 *M* Bis-Tris pH 5.5, 25%(*w*/*v*) PEG 3350 with a protein concentration of 9 mg ml^{-1}. Prior to data collection, the crystal was soaked in cryoprotectant [crystallization buffer with an extra 27%(*w*/*v*) PEG 600] for 30 s. The crystal diffracted to better than 2.6 Å resolution and belonged to space group $P4_12_12$ or $P4_32_12$, as indicated by the systematic absences. The unit-cell parameters are $a = b = 50.08$, $c = 142.88$ Å; assuming one molecule per asymmetric unit, the Matthews coefficient is 2.54 Å^3 Da^{-1} (Kantardjieff & Rupp, 2003), corresponding to a solvent content of 51%. The crystallographic parameters and data-collection statistics are listed in Table 1.

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