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# Purification, crystallization and preliminary X-ray data for *Escherichia coli* GlmU: a bifunctional acetyltransferase/uridyltransferase

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Crystals of *Escherichia coli* GlmU, a bifunctional enzyme catalyzing the acetylation of glucosamine-1-phosphate and uridylylation of *N*-acetylglucosamine-1-phosphate to produce UDP-GlcNAc, have been prepared in complex with coenzyme A and UDP-GlcNAc. These crystals belong to space group *R*32, with unit-cell parameters $a = 104.5$, $c = 648.2$ Å, diffract to at least 2.1 Å resolution and may contain two subunits of the trimeric enzyme per asymmetric unit.

## 1. Introduction

The intermediary metabolite UDP-*N*-acetylglucosamine (UDP-GlcNAc) is of central importance to all organisms and is an essential precursor for bacterial biosynthesis of peptidoglycan, lipopolysaccharide, enterobacterial common antigen and teichoic acids (Varon *et al.*, 1993). The bacterial *glmU* gene encodes the protein *N*-acetylglucosamine-1-phosphate pyrophosphorylase, a bifunctional enzyme that catalyzes the final two biosynthetic reactions of UDP-GlcNAc (Mengin-Lecreulx & van Heijenoort, 1993). The first reaction catalyzed by GlmU is the acetyl-CoA-dependent acylation of glucosamine-1-phosphate (Gehring *et al.*, 1996). The second reaction transfers the uridylyl group from UTP to *N*-acetylglucosamine-1-phosphate to produce the products pyrophosphate and UDP-GlcNAc.

The GlmU from *E. coli* is a homotrimer of 456 residues per subunit. Its two active sites are segregated into independent domains and appear to function independently (Gehring *et al.*, 1996; Brown *et al.*, 1999). The N-terminal domain catalyzes uridylyltransfer and displays amino-acid sequence homology and structural similarity with a variety of nucleotide diphosphate sugar pyrophosphorylases from bacteria as well as higher organisms. The C-terminal domain catalyzes acetyltransfer and shares sequence similarity with a number of bacterial and plant acetyltransferases as imperfect tandem hexapeptide repeats of the form (LIV)-(GAED)-$X_2$-(STAV)-$X$, termed hexapeptide repeats (Dicker & Seetharam, 1992; Bairoch, 1993; Vuorio *et al.*, 1994). Structurally, these hexapeptide-repeat sequences give rise to a characteristic polypeptide chain fold termed a left-handed parallel $\beta$-helix (L$\beta$H) (Raetz & Roderick, 1995).

X-ray structural data are available for a number of acyltransferases containing L$\beta$H structural domains, including UDP-*N*-acetylglucosamine acyltransferase (Raetz & Roderick, 1995), tetrahydrodipicolinate *N*-succinyltransferase (Beaman *et al.*, 1997) and a xenobiotic acetyltransferase (Beaman *et al.*, 1998). Recently, the X-ray crystal structure of GlmU-Tr, a truncated form of GlmU consisting of its N-terminal 331 residues, has been determined (Brown *et al.*, 1999). This structure confirmed earlier biochemical studies regarding the organization of the domains and described the polypeptide-chain fold of the pyrophosphorylase domain and its interaction with the product UDP-GlcNAc. However, GlmU-Tr lacks an intact acetyltransferase catalytic domain as well as the essential metal ion(s) required for pyrophosphorylase activity. We report here the crystallization of full-length bifunctional *E. coli* GlmU in the presence of activating metal ions, UDP-GlcNAc and coenzyme A.

## 2. Results and discussion

### 2.1. Overexpression and preparation

The *E. coli glmU* gene was cloned from genomic DNA by the polymerase chain reaction and inserted into a pET3a expression vector. Following transformation into competent BL21(DE3) cells, the enzyme was expressed by first transferring a single colony to 50 ml of LB media containing 50 µg ml$^{-1}$ ampicillin in a 250 ml flask. This culture was incubated for 6 h at 310 K, after which 5 ml aliquots of culture were withdrawn for each liter of fresh LB media to be inoculated. These 1 l cultures were grown in 2 l baffled culture flasks for 17 h at 310 K in LB media supplemented with 50 µg ml$^{-1}$ ampicillin. Constitutive expression was sufficiently leaky that IPTG induction was unnecessary. Cells were collected by centrifugation, washed with storage buffer (50 m*M* Tris–HCl pH 8.0 containing 3 m*M* $MgCl_2$) and frozen at 203 K.

The protein was prepared by suspending 10 g of thawed cells in lysis buffer (50 m*M* Tris–HCl pH 8.0, 3 m*M* $MgCl_2$, 1 m*M* DTT,

0.2 mg ml$^{-1}$ lysozyme) in a final volume of 100 ml. After a 30 min incubation at room temperature, the extract was sonicated and the solution clarified by centrifugation. The supernatant was slowly brought to 2%(*w*/*v*) in streptomycin sulfate, stirred for 30 min at 277 K and again clarified by centrifugation. This extract was applied to a 2.5 × 13 cm Poros HQ50 anion-exchange column (PE Biosystems) equilibrated in buffer *A* (50 m*M* Tris–HCl pH 8.0, 3.0 m*M* $MgCl_2$, 1 m*M* DTT) at 277 K. The column was washed with 200 ml of buffer *A* and eluted with a linear gradient of 0–50% buffer *B* (buffer *A* plus 1.5 *M* NaCl) in a total volume of 400 ml. Peak fractions were pooled and concentrated.

Prior to use, the concentrated anion-exchange-purified protein (whether or not previously stored at 203 K) was applied to a 340 ml Superdex 200 prep-grade column equilibrated with 10 m*M* Tris–HCl pH 7.5, 1 m*M* DTT, 50 m*M* NaCl, 0.02% azide. The peak fractions were pooled and concentrated to greater than 10 mg ml$^{-1}$ using a stirred-cell membrane concentrator. This material was used either immediately for crystallization experiments or stored for up to two weeks at 277 K. The mass of purified GlmU was determined by electrospray injection mass spectrometry using a Voyager RP Biospectrometry Workstation (PerSeptive Biosystems) in the Laboratory for Macromolecular Analysis of the Albert Einstein College of Medicine. The observed mass of 49 193 ± 4 Da is in agreement with the predicted subunit mass of 49 190 Da for the full-length polypeptide.

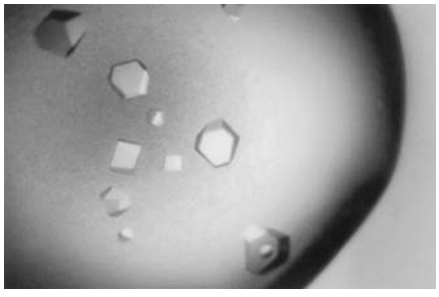

**Figure 1**
Crystals of *E. coli* GlmU. The size of the largest crystal shown is approximately 0.3 × 0.3 × 0.4 mm.

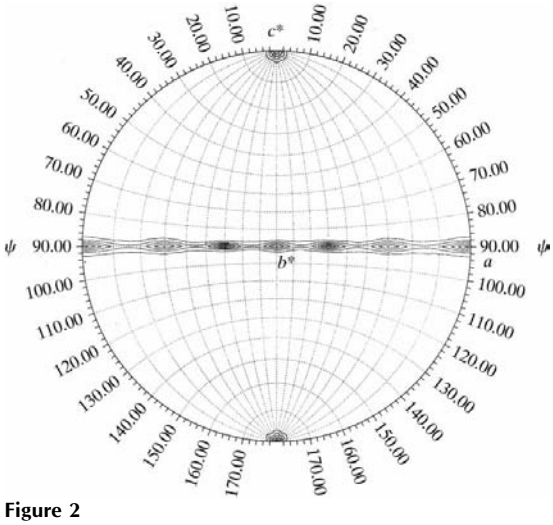


**Figure 2**
Self-rotation function ($\kappa = 180^\circ$) of *E. coli* GlmU. The circumference of the figure corresponds to $\varphi = 0^\circ$. Peaks corresponding to a molecular twofold axis are related to the twofold axis nearly parallel to *b**. Produced by *GLRF* (Tong & Rossmann, 1990).

### 2.2. Crystallization and data measurement

Crystals of GlmU were grown by the hanging-drop vapor-diffusion method at ambient temperature. Drops consisted of 4 µl of 8.3 mg ml$^{-1}$ protein, 14 m*M* UDP-GlcNAc, 28 m*M* $MgCl_2$ and 19 m*M* CoA mixed with 4 µl of reservoir solution containing 1.65 *M* ammonium sulfate, 50 m*M* MES (pH 5.4–6.4) and 2–10 m*M* $CoCl_2$. Crystals were visible in 48 h and grew to dimensions of 0.3 × 0.3 × 0.4 mm (Fig. 1). These crystals belong to the space group *R*32, with unit-cell parameters $a = b = 104.5$, $c = 648.2$ Å, $\alpha = \beta = 90.0$, $\gamma = 120^\circ$, and yield visible Bragg reflections to at least 2.1 Å resolution. A preliminary native X-ray diffraction data set was collected from two crystals at the Molecular Structure Corporation (The Woodlands, Texas) with an R-AXIS IV image-plate detector at ambient temperature using Cu *Kα* radiation produced by a Rigaku RU-H3R X-ray generator equipped with Osmic Blue optics and operating at 50 kV and 100 mA. These data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) (Table 1).

### 2.3. Rotation function and crystal packing

Since the crystal structure of the previously reported truncated form of *E. coli* GlmU is trimeric, a self-rotation function was calculated with 4 Å resolution data for both the $\kappa = 120$ and 180° rotation angles using the *GLRF* rotation-function program (Tong & Rossmann, 1990). These functions suggest the presence of a molecular twofold axis nearly perpendicular to the crystallographic threefold axis and in the direction of *b** (Fig. 2). Such an axis would be observed if two trimeric molecules of GlmU, both pierced by the crystallographic threefold axis, were arranged as a hexamer with 32 point symmetry. This type of packing arrangement could produce two subunits (one subunit from each trimer) per asymmetric unit, a molecular packing volume $V_M$ of 3.46 Å$^3$ Da$^{-1}$ and a reasonable estimated solvent content of 64% (Matthews, 1974). These crystals of full-length *E. coli* GlmU are suitable for a three-dimensional structure determination, which is in progress.

**Table 1**
X-ray data-collection statistics.

| | |
|---|---|
| Space group | *R*32 |
| Unit-cell parameters (Å) | $a = b = 104.5$, $c = 648.2$ |
| Data collection | |
| Resolution (Å) | 2.1 |
| No. of observed reflections | 391782 |
| No. of unique reflections | 77634 |
| $R_{merge}$† (%) | 10.7 |
| $R_{merge}$ (last shell)‡ (%) | 23.3 |
| Completeness (%) | 96.4 |
| Completeness (last shell) | 82.2 |

† $R_{merge}$ (%) = $(\sum|I_i - \langle I\rangle|\sum I_i) \times 100$, where $I_i$ is an individual intensity observation, $\langle I\rangle$ is the mean intensity for that reflection and the summation is over all reflections. ‡ The last shell is 2.18–2.10 Å.

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