Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of *N*-acetylglucosamine 6-phosphate deacetylase from *Escherichia coli*

F. M. Ferreira,[a] G. Mendoza-Hernández,[b] M. L. Calcagno,[b] F. Minauro,[b] L. F. Delboni[a] and G. Oliva[a]*

[a]Laboratório de Cristalografia de Proteínas e Biologia Estrutural, Instituto de Física de São Carlos, Universidade de São Paulo, Av. Dr Carlos Botelho 1465, Caixa Postal 369, 13560-970 São Carlos SP, Brazil, and [b]Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Apartado Postal 70-159, Cd. Universitaria, México, DF 04510, Mexico

Correspondence e-mail: oliva@if.sc.usp.br

Received 29 August 1999
Accepted 22 March 2000

N-Acetylglucosamine 6-phosphate deacetylase (E.C. 3.5.1.25), an enzyme from *Escherichia coli* involved in aminosugar catabolism, has been crystallized by the vapour-diffusion technique using phosphate as precipitant. X-ray diffraction experiments show the crystals to belong to the orthorhombic crystal system, with space group $P2_12_12$. The unit-cell parameters are a = 82.09 (2), b = 114.50 (1), c = 80.17 (1) Å. The crystals diffract to a maximum resolution of 1.8 Å and an initial data set was collected to 2.0 Å.

1. Introduction

N-Acetylglucosamine 6-phosphate deacetylase (E.C. 3.5.1.25) catalyses the conversion of *N*-acetylglucosamine 6-phosphate (GlcNAc6*P*) into glucosamine 6-phosphate (GlcN6*P*) and acetate and as such forms part of the pathway for aminosugar utilization in *E. coli*. The bacterium can use either glucosamine or *N*-acetylglucosamine from the environment for the synthesis of cell-wall and outer-membrane components, but both aminosugars can also be used as carbon and nitrogen sources (White, 1968). For this second purpose, *E. coli* possesses a series of enzymes which are involved in aminosugar catabolism and are encoded by a set of genes arranged in two divergent operons, *nagE* and *nagBACD*, located at 15.5 min on the bacterial chromosome (Plumbridge, 1990). Together, the operons constitute a regulon which becomes activated leading to gene expression when cells grow in the presence of aminosugars. The gene *nagE* encodes an *N*-acetylglucosamine-specific enzyme II of the phospho-*enol*-pyruvate-dependent phosphotransferase system, the product of which is GlcNAc6*P*. The genes *nagA* and *nagB* in the other operon encode the enzymes GlcNAc6*P* deacetylase and glucosamine 6-phosphate deaminase, respectively (Plumbridge, 1990, 1991), which are responsible for the catabolism of GlcNAc6*P* to produce Fru6*P*, which is the point of entry into the glycolytic pathway. The deaminase converts the product of the deacetylase reaction, GlcN6*P*, into Fru6*P* and ammonia. It is an allosteric enzyme presenting both homotropic cooperativity by the substrate GlcN6*P* and heterotropic activation by GlcNAc6*P* (Calcagno *et al.*, 1984; Plumbridge *et al.*, 1993) and its three-dimensional structure has been determined in both allosteric states (Oliva *et al.*, 1995; Horjales *et al.*, 1999). The gene *nagC* codes for a repressor protein, whose coinducer is also GlcNAc6*P*.

This multiple regulatory role of GlcNAc6*P* attracts attention to its specific deacetylase as a major factor controlling its intracellular concentration. The enzyme, a tetramer of identical 41 kDa subunits, has been purified from an overexpressing strain of *E.coli* and its kinetic mechanism has been described in detail (Souza *et al.*, 1997). In this paper, we report the crystallization and preliminary X-ray diffraction analysis of GlcNAc6*P* deacetylase, aiming towards its full three-dimensional structure determination and the elucidation of its catalytic mechanism.

2. Materials and methods

N-Acetylglucosamine 6-phosphate deacetylase was purified from an overexpressing strain of *E. coli* as reported previously (Souza *et al.*, 1997). Preliminary crystallization experiments were based on the sparse-matrix approach (Jancarik & Kim, 1991) using Crystal Screen I from Hampton Research. Small crystals were obtained from 8%(*w*/*v*) PEG 4000, 0.1 *M* sodium acetate pH 4.6 using the hanging-drop vapour-diffusion technique at 291 K (McPherson, 1982). Further experiments with varying the pH and the precipitant concentration resulted in large crystals of well defined hexagonal morphology. X-ray diffraction experiments, however, showed that this crystal form had very long unit-cell parameters (c = 490 Å) with limited resolution and strong anisotropy. New crystallization screening assays were carried out, with the best results being obtained with phosphate as precipitant. The droplets, 10 µl in total volume, were prepared by mixing 5 µl of enzyme solution at 10 mg ml^{-1} in 0.15 *M* phosphate-buffered saline pH 7.4 with 5 µl of reservoir solution,

Table 1
Data processing and merging statistics.

173 345 observed reflections, 50 702 unique reflections.

Resolution shell (Å)	Number of independent reflections	Redundancy	Reflections $I/\sigma(I) \geq 3$ (%)	Completeness (%)	R_{merge}†
30.00–4.93	3386	3.7	76.9	91.3	0.06
4.93–3.91	3296	3.7	79.1	93.5	0.05
3.91–3.42	3400	3.8	79.2	97.0	0.05
3.42–3.11	3430	3.6	76.3	99.0	0.06
3.11–2.88	3430	3.5	74.4	99.0	0.06
2.88–2.71	3429	3.4	72.1	99.0	0.08
2.71–2.58	3365	3.4	71.9	98.7	0.08
2.58–2.47	3403	3.3	71.9	98.5	0.09
2.47–2.37	3352	3.3	72.9	98.2	0.10
2.37–2.29	3346	3.3	72.5	98.4	0.10
2.29–2.22	3371	3.3	72.6	98.1	0.14
2.22–2.15	3359	3.3	73.2	98.6	0.13
2.15–2.10	3367	3.3	73.2	98.6	0.15
2.10–2.05	3378	3.2	72.3	99.3	0.18
2.05–2.00	3390	3.2	72.1	99.3	0.22
Overall	50702	3.4	74.6	97.7	0.06

† $R_{merge} = \sum_{hkl}|I - \langle I_{hkl}\rangle|/\sum_{hkl}\langle I_{hkl}\rangle$.

which contained 1 ml of 1.85 *M* sodium dihydrogen phosphate pH 4.0. The largest single crystal (Fig. 1), of dimensions 1.0 × 0.6 × 0.3 mm, grew in two weeks.

Crystals used for diffraction data collection were flash-frozen at cryogenic temperature (100 K). They were initially transferred for a few minutes to a conditioning solution consisting of the well solution with 20%(*v*/*v*) glycerol and were then directly mounted in loops on a goniometer head centered in a gaseous stream produced by a liquid-nitrogen boil-off (Garman & Schneider, 1997). Diffraction images at this stage systematically showed medium resolution, large mosaicity and streaked spot shapes. Dramatic improvement was achieved after annealing by removing the crystals from the cryostat and placing them in the same cryo-protective solution for at least 2 min and flash-cooling for a second time (Harp *et al.*, 1998).

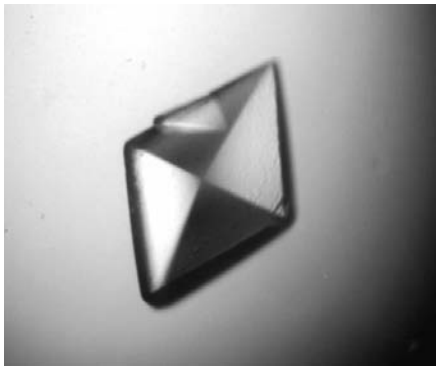

Figure 1
Large (1.0 × 0.6 × 0.3 mm) crystal of *N*-acetylglucosamine 6-phosphate deacetylase.

X-ray diffraction data were collected over 193 frames of 0.5° oscillation with a crystal-to-detector distance of 150 mm, using a MAR345 image-plate detector system mounted on the Protein Crystallography beamline at the Brazilian National Synchrotron Laboratory (LNLS; Polikarpov *et al.*, 1998) using monochromatic radiation of 1.378 Å wavelength. The choice of wavelength was determined by the balance between minimizing absorption effects and maximizing the flux in the spectrum available at this beamline (Polikarpov *et al.*, 1997; Rossmann & Blow, 1962).

The intensities were indexed and scaled using *DENZO* and *SCALEPACK*, respectively (Otwinowski & Minor, 1997). The self-rotation function was calculated using the programs *AMoRe* (Navaza, 1994), *ALMN* and *POLARRFN* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) and *GLRF* from the *REPLACE* package (Tong & Rossmann, 1997; Rossmann & Blow, 1962).

3. Results and discussion

X-ray data collected from a crystal measuring 0.9 × 0.6 × 0.4 mm showed orthorhombic Laue symmetry and systematic absences indicated the crystals to belong to the space group $P2_12_12$. The unit-cell parameters were determined to be a = 82.09 (2), b = 114.50 (1), c = 80.17 (1) Å with e.s.d.s estimated from the fitting of 2052 reflections in a 0.5° oscillation photograph and calculated with the program *DENZO* (Otwinowski & Minor, 1997). The calculated unit-cell volume is 7.535 (3) × 10^5 Å^3.

A total number of 173 345 reflections were integrated to a resolution of 2.0 Å and were then merged to obtain 50 702 unique reflections with an overall R_{merge} and completeness of 0.060 and 98.2%, respectively. In the last resolution shell (2.05–2.00 Å), the R_{merge} is 0.22 and the completeness is 99.3% (Table 1). Though observable reflections extend to a limit of 1.8 Å, the usual criteria of acceptable noise level [50% of the reflections with $I > 2\sigma(I)$] and accuracy ($R_{merge} < 0.25$) indicate that this initial data set is better described as extending to 2.0 Å resolution.

Assuming two monomers per asymmetric unit, the calculated Matthews coefficient (V_m; Matthews, 1968) is 2.30 Å^3 Da^{-1}, which corresponds to 46.1% solvent. If only one monomer is present, V_m is 4.60 Å^3 Da^{-1} and the solvent content is 73.1%.

N-Acetylglucosamine 6-phosphate deacetylase has been shown to be active as a homotetramer, following gel-filtration HPLC and sedimentation experiments (Souza *et al.*, 1997). If a dimer is contained in the asymmetric unit, then the tetrameric species could possibly be formed by the crystallographic twofold axis of the space group $P2_12_12$. Several self-rotation functions were calculated using different integration radii ranging from 15 to 40 Å and including data between 20.0 and 2.0, 2.5, 3.0 and 3.5 Å resolution. Conditions with the Patterson origin removed and the use of normalized structure factors were also tested. No consistent evidence for a non-crystallographic peak in the self-rotation function was observed. This result can be interpreted either as only one monomer in the asymmetric unit or, alternatively, as two independent monomers related by a non-crystallographic twofold axis that is approximately parallel to one of the cell axes, although no special features were observed in the self-Patterson map that support this latter hypothesis. To elucidate this question, the complete structure determination will be required.

Sequence alignment has shown no significant homology with other proteins of known three-dimensional structure, so a model for molecular replacement is not available. An extensive search for heavy-atom derivatives is currently under way. The expression of Se-Met-substituted enzyme is also being pursued, envisaging the use of MAD techniques.

We thank Dr Richard Garratt for manuscript revision and Dr Igor Polikarpov for access to the beamtime at the Brazilian National Synchrotron Laboratory (LNLS). GO is an International Scholar of the Howard Hughes Medical Institute. We also thank FAPESP, PRONEX and CNPq for financial support. GM-H, FM and MC acknowledge support from PAPIIT (DGAPA-UNAM) and CONACYT.

References

Calcagno, M., Campos, P. J., Mulliert, G. & Suástegui, J. (1984). *Biochim. Biophys. Acta*, **787**, 165–173.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Garman, E. F. & Schneider, T. R. (1997). *J. Appl. Cryst.* **30**, 211–273.
Harp, J. M., Timm, D. E. & Bunick, G. J. (1998). *Acta Cryst.* D**54**, 622–628.
Horjales, E., Altamirano, M. M., Calcagno, M. L., Garratt, R. C. & Oliva, G. (1999). *Structure*, **7**, 527–537.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
McPherson, A. Jr (1982). *Preparation and Analysis of Protein Crystals*, 1st ed., pp. 96–97. New York: John Wiley.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Oliva, G., Fontes, M. R. M., Garrat, R. C., Altamiarano, M. M. & Horjales, E. (1995). *Structrure*, **3**, 1323–1332.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Plumbridge, J. A. (1990). *J. Bacteriol.* **172**, 2728–2735.
Plumbridge, J. A. (1991). *Mol. Microbiol.* **5**, 2053–2062.
Plumbridge, J. A., Cochet, O., Souza, J. M., Altamirano, M. M., Calcagno, M. L. & Badet, B. (1993). *J. Bacteriol.* **175**, 4951–4956.
Polikarpov, I., Perles, L. A., de Oliveira, R. T., Oliva, G., Castellano, E. E., Garratt, R. C. & Craievich, A. (1998). *J. Synchrotron Rad.* **5**, 72–76.
Polikarpov, I., Teplyakov, A. & Oliva, G. (1997). *Acta Cryst.* D**53**, 734–737.
Rossmann, M. G. & Blow, D. M. (1962). *Acta Cryst.* **15**, 24–31.
Souza, J. M., Plumbridge, J. A. & Calcagno, M. L. (1997). *Arch. Biochem. Biophys.* **340**(2), 338–346.
Tong, L. & Rossmann, M. G. (1997). *Methods Enzymol.* **276**, 594–611.
White, R. J. (1968). *Biochem. J.* **106**, 847–858.