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Preliminary crystallographic analysis of ADP-glucose pyrophosphorylase from *Agrobacterium tumefaciens*

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ADP-glucose pyrophosphorylase catalyzes the conversion of glucose-1-phosphate and ATP to ADP-glucose and pyrophosphate, a key regulated step in both bacterial glycogen and plant starch biosynthesis. Crystals of ADP-glucose pyrophosphorylase from *Agrobacterium tumefaciens* (420 amino acids, 47 kDa) have been obtained by the sitting-drop vapor-diffusion method using lithium sulfate as a precipitant. A complete native X-ray diffraction data set was collected to a resolution of 2.0 Å from a single crystal at 100 K. The crystals belong to space group *I*222, with unit-cell parameters a = 92.03, b = 141.251, c = 423.64 Å. To solve the phase problem, a complete anomalous data set was collected from a selenomethionyl derivative. These crystals display one-fifth of the unit-cell volume of the wild-type crystals, with unit-cell parameters a = 85.38, b = 93.79, c = 140.29 Å and space group *I*222.

1. Introduction

Glycogen and starch serve as major energy-storage compounds for nearly all living organisms. ADP-glucose pyrophosphorylase (ADPGlc Ppase; EC 2.7.7.27) catalyzes the initial and rate-limiting step in bacterial glycogen and plant starch biosynthesis, the conversion of glucose-1-phosphate and ATP to ADP-glucose and pyrophosphate (Ballicora *et al.*, 2003, 2004). The ADP-glucose produced acts as the glucosyl donor for enzymatic glucose polymerization by various glycogen/starch synthases and branching/debranching enzymes.

ADPGlc Ppase enzymes are found in bacteria and plants, where they perform similar functions. The majority of bacterial enzymes appear to function as homotetramers with a subunit size of ~50 kDa (Ballicora *et al.*, 2003). The enzymes from plants, on the other hand, are active as heterotetramers consisting of catalytic and regulatory subunits with molecular weights of 50–60 kDa (Martin & Smith, 1995; Preiss & Sivak, 1998; Ballicora *et al.*, 2004). Despite the differences in oligomeric structure, alignments of the known sequences from bacterial and plant enzymes reveal high sequence identity in key regions, indicating a common origin (Smith-White & Preiss, 1992). In addition, hydrophobic cluster analysis of a number of ADPGlc Ppase sequences suggests that both bacterial and plant enzymes are of the α/β-structure class and share a common folding pattern despite possessing different quaternary structures and activator specificities (Ballicora *et al.*, 2003, 2004). The homology between bacterial and plant ADPGlc Ppases has allowed the use of the bacterial enzymes, which have simpler oligomeric structure and less complex molecular biology, as models for the plant systems.

As a key enzyme in a pathway generating renewable and biodegradable carbon, the activity of ADPGlc Ppase is highly regulated (Preiss & Romeo, 1994; Preiss & Sivak, 1998). The binding of allosteric effector molecules, the identity of which depends on the carbon-utilization pathway of the organism, modulates enzyme activity (Ballicora *et al.*, 2003, 2004). ADPGlc Ppases are differentially activated by glycolytic intermediates and inhibited by AMP, ADP and/or phosphate. These activators and inhibitors act together to provide a sensitive and powerful feed-forward and feedback regulation of an enzyme controlling the synthesis of an energy-storage compound.

To date, the bacterial ADPGlc Ppase enzymes have not been amenable to X-ray crystallography and a high-resolution atomic structure has not been reported. The recently solved structures of the distantly related sugar nucleotide pyrophosphorylases (Brown *et al.*, 1999; Olsen & Roderick, 2001; Blankenfeldt *et al.*, 2000; Sivaraman *et al.*, 2002) have provided evidence for a shared catalytic domain with ADPGlc Ppases. The sugar nucleotide pyrophosphorylases, however, do not display allosteric regulation and lack sequence similarity with the amino- and carboxy-terminal regions of ADPGlc Ppases that have been shown to be important in regulation. An atomic resolution structure of ADPGlc Ppase is necessary for complete understanding of the regulation of the biosynthetic pathway of glycogen and starch.

We have developed methods for high-level expression and purification of ADPGlc Ppase from the bacterium *Agrobacterium tumefaciens* (420 amino acids, 47 kDa; Gomez-Casati *et al.*, 2001). Mutagenesis studies on the recombinant *A. tumefaciens* ADPGlc Ppase have identified several key residues important for regulation (Gomez-Casati *et al.*, 2001) and recent analysis of chimeric proteins of the *A. tumefaciens* and *Escherichia coli* ADPGlc Ppase have identified regions responsible for regulation specificity (Ballicora *et al.*, 2002). To further investigate structural features of ADPGlc Ppase that affect catalysis, allosteric regulation and oligomerizaton, we have initiated efforts to determine the atomic structure of *A. tumefaciens* ADPGlc Ppase. Here, we describe the crystallization and preliminary X-ray diffraction analysis of *A. tumefaciens* ADPGlc Ppase.

2. Experimental results

To obtain sufficient quantities of highly purified enzyme for crystallization trials, wild-type *A. tumefaciens* ADPGlc Ppase was overexpressed in *E. coli* (Top10, Invitrogen) using the pSE420 expression vector with the Trc promoter (Invitrogen; Gomez-Casati *et al.*, 2001). The recombinant ADPGlc Ppase was purified as previously described using reverse-phase and ion-exchange chromatography (Gomez-Casati *et al.*, 2001). Prior to crystallization, the protein was concentrated to 5 mg ml^{-1} and stored in 50 m*M* glycine pH 7.5. Crystals of *A. tumefaciens* ADPGlc Ppase were prepared by sitting-drop vapor diffusion using 1.5 *M* lithium sulfate, 100 m*M* HEPES pH 7.5 as the precipitant (0.5 ml) and a droplet composed of protein (2 µl) and precipitant (2 µl) solutions. Crystals formed at a protein concentration of 5 mg ml^{-1} and grew in cubic shapes with average dimensions of 0.1 × 0.1 × 0.1 mm over a one-week period. Prior to immersion in liquid nitrogen for storage and data collection, the crystals were transferred briefly to mother liquor containing 20% glycerol for cryoprotection.

Diffraction data from the native *A. tumefaciens* ADPGlc Ppase crystals were collected at Stanford Synchrotron Radiation Laboratory (SSRL). A complete set of native data was obtained from a single crystal of the wild-type enzyme at 100 K on beamline 9-2. Diffraction data were reduced using *MOSFLM* (Leslie, 1998) and scaled with *SCALA* (Weiss, 2001) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). A summary of the crystallographic data is presented in Table 1. Crystals were found to be orthorhombic and to belong to space group *I*222, with unit-cell parameters $a = 92.03$, $b = 141.251$, $c = 423.64$ Å. The crystals diffracted to beyond 2 Å, but owing to the long unit-cell edge in the *c* dimension (423 Å) data were only collected to 2 Å.

To determine the number of molecules in the asymmetric unit, the Matthews coefficient (Matthews, 1968), self-rotation function and native Patterson were analyzed. The Matthews equation indicates that five or six molecules per asymmetric unit would yield a solvent content of 59 or 51%, respectively, while the self-rotation analysis gave a single large peak at $\kappa = 180°$, indicating twofold non-crystallographic symmetry. Since the self-rotation function did not account for the number of molecules predicted by the Matthews coefficient, the native Patterson was analyzed for off-origin peaks that would indicate translational symmetry along a crystallographic axis. Large peaks were detected on the *x* plane ($x = 0.5$) of the native Patterson at $y = 0$, $z = 0.2$ and $y = 0$, $z = 0.4$. These results indicate that at least three molecules are present in the asymmetric unit, with two molecules related by a translation of $z = 0.2$ (85 Å) and two molecules related by a translation of $z = 0.4$ (170 Å). In addition, the peak at $z = 0.2$ was 2.5 times as large as the peak at $z = 0.4$. Based on these results along with the Matthews equation and self-rotation function, a model consisting of five molecules in the asymmetric unit was developed. Three molecules, each separated by 85 Å, are stacked along the *c* axis of the unit cell. Molecules 1 and 2 and molecules 2 and 3 are separated by 85 Å, while molecules 1 and 3 are 170 Å apart. The additional two molecules are stacked similarly to the first three molecules but are rotated. This arrangement accounts for the location of the native Patterson peaks and the difference in peak height between the native Patterson peaks.

Table 1
Summary of crystallographic data.

Values in parentheses refer to the outermost shell (2.00–2.05 Å for the wild-type crystal and 2.10–2.15 Å for the selenomethionyl crystal).

	Native	Selenomethionyl
Beamline (SSRL)	9-2	9-1
Wavelength (Å)	1.0	0.97
Space group	*I*222	*I*222
Unit-cell parameters (Å)		
a	92.03	85.38
b	141.25	93.79
c	423.64	140.29
Resolution (Å)	40–2.0	47–2.1
Total No. of reflections	484222	462576
No. of unique reflections	180923	33214
Multiplicity	2.7 (1.7)	13.9 (14.3)
Completeness (%)	97.6 (88.6)	99.9 (100)
Anomalous completeness (%)	—	100 (100)
$I/\sigma(I)$	12.2 (3.2)	8.4 (2.3)
R_{sym} (%)	3.4 (17.7)	6.9 (30.8)

† $R_{sym} = 100\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the observed intensity and $\langle I\rangle$ is statistically weighted average intensity of multiple observations of symmetry-related reflections.

In an attempt to solve the phase problem, a complete anomalous data set was collected from selenomethionyl *A. tumefaciens* ADPGlc Ppase crystals. Selenomethionyl-labeled protein was produced by expression of *A. tumefaciens* ADPGlc Ppase in the *E. coli* methionine auxotroph strain DL41 (Doublié, 1997). Cells were initially cultured at 310 K in M63 minimal media supplemented with selenomethionine and were grown for an additional 48 h at 298 K following induction with IPTG. Selenomethionyl ADPGlc Ppase was purified using the same method as the wild-type enzyme but with the addition of 5 m*M* DTT to all buffers to prevent oxidation of the selenium. Crystals of selenomethionyl ADPGlc Ppase were obtained under the same conditions as the wild-type enzyme except that 5 m*M* DTT was added to the mother liquor during crystallization.

Several of the selenomethionyl ADPGlc Ppase crystals examined yielded similar results to the native crystals. However, a crystal form of the selenomethionyl ADPGlc Ppase was identified that displayed one-fifth of the unit-cell volume of the wild-type crystals: $a = 93.79$, $b = 140.29$, $c = 85.37$ Å. The Matthews coefficient (Matthews, 1968) indicates that this crystal form contains a single molecule in the asymmetric unit. The data were processed with *MOSFLM* (Leslie,

1998) and scaled using *SCALA* (Weiss, 2001) following the same methods used for the native crystals. A summary of the crystallographic data from the selenomethionyl ADPGlc Ppase is presented in Table 1.

Using the selenomethionyl *A. tumefaciens* ADPGlc Ppase crystal form containing a single molecule in the asymmetric unit should dramatically simplify the anomalous Patterson compared with the native crystal form. *A. tumefaciens* ADPGlc Ppase contains nine methionine residues in 420 amino acids. Initial analysis of the anomalous Patterson from the selenomethionyl *A. tumefaciens* ADPGlc Ppase crystal found 11 peaks above 6σ. The programs *SOLVE*/*RESOLVE* (Perrakis *et al.*, 1999), *Shake-n-Bake* (Weeks & Miller, 1999) and *CNS* (Brünger *et al.*, 1998) will be used to solve the anomalous Patterson and determine the phases.

Determination of the structure of *A. tumefaciens* ADPGlc Ppase will allow identification of the amino acids that affect catalysis, allosteric regulation and oligomerization. In addition, these studies will provide the foundation for X-ray crystallography experiments to examine the binding of substrate and allosteric effectors and examine how binding of these compounds affects the structure to alter the enzyme activity.

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References

Ballicora, M. A., Iglesias, A. A. & Preiss, J. (2003). *Microbiol. Mol. Biol. Rev.* **67**, 213–225.
Ballicora, M. A., Iglesias, A. A. & Preiss, J. (2004). *Photosynth. Res.* **79**, 1–24.
Ballicora, M. A., Sesma, J. I., Iglesias, A. A. & Preiss, J. (2002). *Biochemistry*, **41**, 9431–9437.
Blankenfeldt, W., Asuncion, M., Lam, J. S. & Naismith, J. H. (2000). *EMBO J.* **19**, 6652–6663.
Brown, K., Pompeo, F., Dixon, S., Mengin-Lecreulx, D., Cambillau, C. & Bourne, Y. (1999). *EMBO J.* **18**, 4096–4107.
Brünger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Gross-Kunstleve, R. W., Jiang, J. -S., Kuszewski, J., Nilges, N., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Doublié, S. (1997). *Methods Enzymol.* **276**, 523–530.
Gomez-Casati, D., Igarashi, R. Y., Berger, C., Brandt, M., Iglesias, A. A. & Meyer, C. R. (2001). *Biochemistry*, **40**, 10169–10178.
Leslie, A. G. W. (1998). *MOSFLM* v.6.0. MRC Laboratory of Molecular Biology, Cambridge.
Martin, C. & Smith, A. M. (1995). *Plant Cell*, **7**, 971–985.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Olsen, L. R. & Roderick, S. L. (2001). *Biochemistry*, **40**, 1913–1921.
Perrakis, A., Morris, R. J. H. & Lamzin, V. S. (1999). *Nature Struct. Biol.* **6**, 458–463.
Preiss, J. & Romeo, T. (1994). *Prog. Nucleic Acid Res.* **47**, 299–329.
Preiss, J. & Sivak, M. N. (1998). *Genet. Eng. (N.Y.)*, **20**, 177–223.
Sivaraman, J., Sauve, V., Matte, A. & Cygler, M. (2002). *J. Biol. Chem.* **277**, 44214–44219.
Smith-White, B. & Preiss, J. (1992). *J. Mol. Evol.* **34**, 449–464.
Weeks, C. M. & Miller, R. (1999). *J. Appl. Cryst.* **32**, 120–124.
Weiss, M. (2001). *J. Appl. Cryst.* **34**, 130–135.