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Crystallization and preliminary X-ray diffraction analysis of the catalytic subunit of ADP–glucose pyrophosphorylase from potato tuber

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ADP–glucose pyrophosphorylase is the key regulatory enzyme in the biosynthesis of starch in plants and glycogen in bacteria. The enzyme from potato tuber is comprised of a regulatory subunit and a catalytic subunit and is present as a heterotetramer ($\alpha_2\beta_2$). The catalytic subunit from potato tuber (50 kDa) was crystallized in four different forms, two of which are suitable for structural studies. A tetragonal crystal form obtained in the presence of the substrate analog Cr-ATP diffracted to 2.2 Å and belongs to space group $P4_1$ (or its enantiomorph), with unit-cell parameters $a = b = 110.57$, $c = 190.14$ Å. A second crystal form obtained diffracted to 2.8 Å and belongs to space group $P2$, with unit-cell parameters $a = 80.06$, $b = 138.84$, $c = 92.20$ Å, $\beta = 112.40^\circ$. As this protein displays no significant homology to any currently known protein structure, a search for heavy-atom derivatives has been initiated.

1. Introduction

ADP–glucose pyrophosphorylase (ADPGlc PPase; E.C. 2.7.7.27) catalyzes the synthesis of ADP–glucose from ATP and glucose-1-phosphate, forming pyrophosphate as a byproduct (Espada, 1962). This enzyme plays a key regulatory role in the biosynthesis of starch in plants (Preiss, 1991; Preiss & Sivak, 1998) and of glycogen in bacteria (Preiss, 1984). ADPGlc PPase from all sources is found to be allosterically regulated; however, the specificity toward the effector varies depending upon the source of enzyme. The enzyme from enteric bacteria is activated by fructose 1,6-bisphosphate and inhibited by adenosine monophosphate (Preiss *et al.*, 1966), whereas the enzyme from almost all higher plant sources is activated by 3-phosphoglyceric acid and inhibited by orthophosphate (Ghosh & Preiss, 1966).

ADPGlc PPase from all sources is found to exist as a tetramer in solution. The enzyme from enteric bacteria is homotetrameric (Haugen *et al.*, 1976), whereas the enzyme from higher plants is comprised of two different subunits, forming a $\alpha_2\beta_2$ structure (Morell *et al.*, 1987). The cDNAs of two potato (*Solanum tuberosum* L.) tuber ADPGlc PPase subunits have been cloned from separate genes (Nakata *et al.*, 1991). The subunits have dissimilar sizes of 50 and 51 kDa and display an overall protein sequence identity of 59%. The amino-acid sequence identity of ADPGlc PPases from different plant sources ranges between 85 and 90% for the small subunit and between 50 and 60% for the large subunit. The enzyme from potato tuber exists physiologically as a heterotetramer ($\alpha_2\beta_2$). However, the recombinant smaller subunit (50 kDa) forms an enzymatically active homotetramer (α_4) when expressed in the absence of the large subunit (Ballicora *et al.*, 1995). The small subunit expressed alone has a higher enzymatic activity than the heterotetramer, as well as a lower affinity for the activator 3-phosphoglyceric acid and a higher sensitivity to orthophosphate inhibition. In contrast, the large subunit by itself is not active. The above observations have led to the suggestion that the plant ADPGlc PPase small subunit functions as the catalytic subunit, whereas the large subunit modulates the regulatory properties of the small subunit (Ballicora *et al.*, 1995).

Despite the fact that nucleotide 5′-diphosphate-glucose pyrophosphorylases have been studied for decades, no three-dimensional structure is currently available. Earlier attempts to determine the structure of ADPGlc PPase from *Escherichia coli* were unsuccessful owing to the crystals' diffraction only to low resolution and their rapid decay (Mulichak *et al.*, 1988). Here, we present results of crystallization and preliminary X-ray diffraction analysis of the potato tuber ADPGlc PPase small subunit.

2. Materials and methods

2.1. Expression and purification

The small subunit of potato tuber ADPGlc PPase was overexpressed in *E. coli* strain AC701-504 and purified to homogeneity according to the method of Ballicora *et al.*

Table 1
Data-collection and processing statistics.

Space group	$P4_1$	$P2$
Unit-cell parameters (Å, °)	$a = b = 110.57$, $c = 190.14$	$a = 80.06$, $b = 138.84$, $c = 92.20$, $\beta = 112.40$
Maximum resolution (Å)	2.2	2.8
Resolution of data set (Å)	3.4	3.0
Number of unique reflections	24429	30342
$R_{\rm merge}$† (%)	12.1	9.5
Completeness (%)	79	82
V_M	2.9	2.4
Number of molecules per asymmetric unit	4	4

† $R_{\rm merge} = \sum\sum |I(h)i - \langle I(h)\rangle| / \sum\langle I(h)\rangle$; $I(h)$ is the observed intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean intensity of reflection h calculated after scaling.

(1995) with some modifications. Briefly, the cultures were grown at 310 K until OD_{600} reached 1.0; isopropyl-β-D-thiogalactopyranoside was then added to a final concentration of 0.5 μM. The incubation was continued at 298 K for 40 h and the cells were harvested by centrifugation at 6000*g* for 15 min. Cells were disrupted by sonication and the crude extracts were heated to 341 K for 5 min. The solution was centrifuged and the supernatant was precipitated with 60% ammonium sulfate. The additional steps were performed as described by Sowokinos & Preiss (1982). From 32 l of cell culture (approximately 150 g of wet cell paste), 8.4 mg of pure protein was obtained. The purified protein migrated as a single band on an SDS–PAGE gel and the specific activity (100 units mg^{-1}) pyrophosphorolysis assay (Ghosh & Preiss, 1966) indicated that the protein was pure and active. Cr-ATP was prepared according to the methods of Janson & Cleland (1974).

2.2. Crystallization

The purified enzyme was concentrated to 5 mg ml^{-1} in a solution containing 12% sucrose, 3 mM $MgCl_2$ and 3 mM HEPES pH 8.0. Crystallization trials were set up at 298 K by the sparse-matrix approach using the hanging-drop vapor-diffusion method (Jancarik & Kim, 1991). In the cases of crystal forms II and III, we included 0.3 mM Cr-ATP in the protein sample, whereas the protein solution contained 3 mM ADP–glucose for crystal form I.

2.3. X-ray diffraction analysis

Crystal forms I, II and III were harvested using the reservoir solution and mounted in thin-walled glass capillary tubes. X-rays were generated by a Rigaku RU-200 rotating-anode generator operating at 100 mA and 50 kV; monochromatic Cu $K\alpha$ radiation was obtained using MSC/Yale mirrors. Crystals were cooled to 277 K during the data collection. Diffraction intensities were measured using an R-AXIS II imaging-plate detector. Data were indexed with *DENZO* and were scaled and reduced using *SCALEPACK* (Otwinowski & Minor, 1997).

Crystal form IV was transferred to mother-liquor solution containing 30% glycerol and was flash-frozen. X-ray diffraction data were collected at the crystallographic beamline (Polikarpov *et al.*, 1998) at the Laboratório Nacional de Luz Sincrotron (LNLS, Campinas, Brazil). The synchrotron-radiation source at LNLS was set to a wavelength of 1.38 Å. Diffraction data were collected using a MAR 345 imaging-plate detector and were reduced and processed using the *HKL* suite of programs (Otwinowski & Minor, 1997).

3. Results and discussion

Initial crystallization experiments were carried out using the screening protocols of Jancarik & Kim (1991). In the presence of ADP–glucose, the protein crystallized immediately (4–5 h) from 11.9% PEG 8K, 0.2 M $MgCl_2$, 0.1 M sodium cacodylate pH 6.5 and grew to a maximum size of 0.2 × 0.2 × 0.4 mm in 2 d. These crystals (form I; Fig. 1*a*) belonged to the orthorhombic system and diffracted to a maximum resolution of 4.5 Å. The crystals were twinned and decayed rapidly in the X-ray beam, hence proving to be unsuitable for further analysis.

As the ADPGlc PPase demonstrates slow ATPase activity, we utilized the ATP analog Cr-ATP, which the enzyme is unable to hydrolyze. Form II crystals (Fig. 1*b*) were obtained in the presence of Cr-ATP (0.3 mM) under conditions similar to that of form I (18% PEG 8K, 0.2 M $MgCl_2$, 0.1 M sodium cacodylate pH 6.5). These crystals (0.1 × 0.1 × 0.1 mm) also belonged to the orthorhombic system, diffracted to 3.5 Å and decayed rapidly.

A third crystal form (form III, Fig. 1*c*) was grown from 30% PEG 4K, 0.1 M sodium citrate, 0.2 M ammonium acetate in the presence of 0.3 mM Cr-ATP. These crystals belong to the tetragonal system, space group $P4_1$ (or $P4_3$), with unit-cell parameters $a = b = 110.57$, $c = 190.14$ Å (Table 1). Crystal form III diffracted X-rays to a maximum resolution of 2.2 Å, but the complete data set was collected to 3.4 Å at 277 K owing to crystal decay. A fourth crystal form (form IV, Fig. 1*d*) was obtained from 20% PEG MME 2K, 0.15 M ammo-

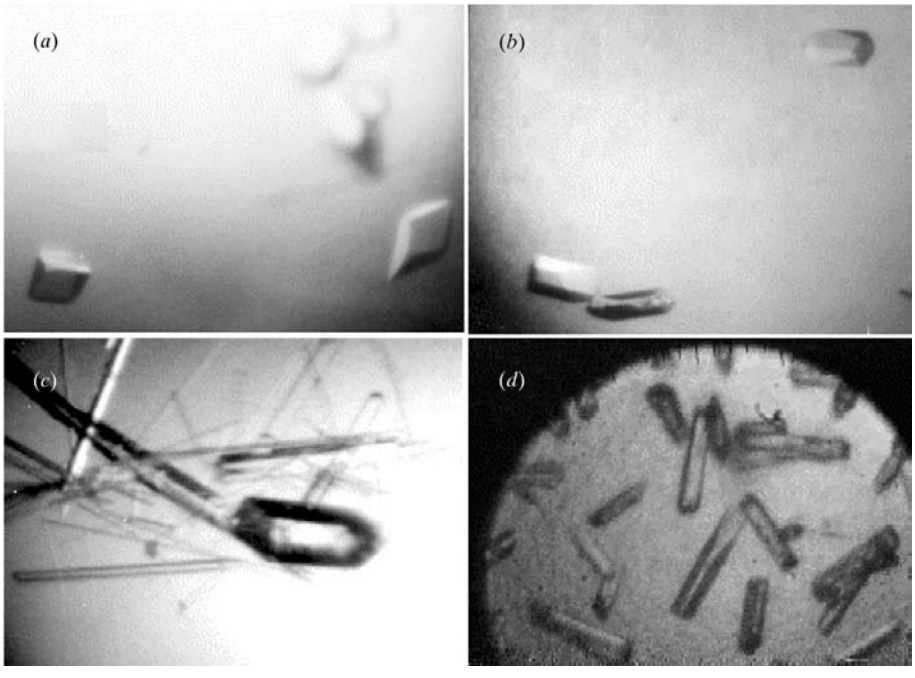

Figure 1
Photomicrographs of crystals of ADPGlc PPase. (*a*) Form I (orthorhombic system) ADPGlc PPase in complex with ADP–glucose. (*b*) Form II (orthorhombic system) ADPGlc PPase in complex with Cr-ATP. (*c*) Form III (space group $P4_1$) ADPGlc PPase in complex with Cr-ATP. (*d*) Form IV (space group $P2$) ADPGlc PPase uncomplexed.

nium sulfate, 0.1 *M* sodium acetate pH 4.6. These crystals belong to space group *P*2, with unit-cell parameters a = 80.06, b = 138.84, = 92.20 Å, β = 112.40°, and diffracted X-rays to 2.8 Å. A complete data set was collected to 3.0 Å at cryogenic temperature (Table 1). Consideration of the possible values of V_M indicates the presence of one tetramer in the asymmetric unit for both crystal forms III and IV (Matthews, 1968).

A database search using the primary structure of ADPGlc PPase did not reveal significant homology to any known protein structure, suggesting that this protein may have a unique fold (Smith-White & Preiss, 1992). Since ADPGlc PPase is the key regulatory enzyme in starch and bacterial glycogen biosynthesis, structural information would improve our understanding of the catalysis and the complex allosteric regulation of this enzyme.

We are currently trying to circumvent the crystal decay problems by establishing cryo-cooling conditions for forms II and III. Additionally, we are screening for heavy-atom derivatives of forms III and IV.

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