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Crystallization and preliminary X-ray crystallographic studies of phosphoenolpyruvate carboxykinase from *Corynebacterium glutamicum*

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Phosphoenolpyruvate carboxykinase (PCK) is a key enzyme involved in the regulation of gluconeogenesis. PCKs from higher animals require guanosine nucleotide for activity. PCK from *Corynebacterium glutamicum* is also GTP specific. X-ray diffraction data from a *C. glutamicum* PCK crystal were collected to 2.8 Å resolution. The crystals were monoclinic, belonging to space group $P2_1$, with unit-cell parameters $a = 71.7$, $b = 117.4$, $c = 161.3$ Å, $\beta = 92.9^\circ$. The presence of two molecules in the crystallographic asymmetric unit gives a V_M of 2.5 Å^3 Da^{-1} and a solvent content of 50.3%.

1. Introduction

Phosphoenolpyruvate carboxykinases [GTP/ATP; oxaloacetate carboxylase (transphosphorylating), EC 4.1.1.32/49] catalyze the reversible decarboxylation and mononucleotide-dependent phosphorylation of oxaloacetate (OAA) to phosphoenolpyruvate (PEP) and CO_2 in the presence of divalent cations (Mn^{2+} and Mg^{2+}),

$$\mathrm{OAA} + \mathrm{NTP} \longleftrightarrow \mathrm{PEP} + \mathrm{NDP} + \mathrm{CO_2}.$$

The primary function of this enzyme appears to be the catalysis of the committed step in gluconeogenesis, which helps to control blood sugar levels during fasting (Riedel *et al.*, 2001; Colombo *et al.*, 1978). There are two classes of PCKs depending on their nucleotide specificities: GTP-dependent PCKs, which are found in higher organisms and some bacteria (Hanson & Patel, 1994; Riedel *et al.*, 2001), and ATP-dependent PCKs, which are found in plants, yeast and some bacteria (Utter & Kolenbrander, 1972). There are significant amino-acid sequence identities within the two classes, but no significant similarity in amino-acid sequence between the ATP- and GTP-dependent PCKs, although the active-site residues are conserved in PCKs from all sources (Dunten *et al.*, 2002). This enzyme has been purified from a wide variety of animals, plants and bacteria (Bazaes *et al.*, 1997). However, crystal structures from only three different sources are currently available: the ATP-dependent PCKs from *Escherichia coli* (Matte *et al.*, 1996) and *Trypanosoma cruzi* (Trapani *et al.*, 2001) and the GTP-dependent human PCK (Dunten *et al.*, 2002). Structural analysis of *E. coli* PCK shows that bound ATP induces a 20° hinge-like rotation of the N- and C-terminal domains, which closes the active-site cleft in the presence of nucleotide compared with the open substrate-free structure (Tari *et al.*, 1996). Similar large hinge-like domain closures on substrate binding have been characterized crystallographically in adenylate kinase (Schulz *et al.*, 1990) and hexokinase (Bennett & Steitz, 1980). On the other hand, structural analysis of human PCK revealed no large domain movements in its bound-substrate form compared with the substrate-free structure (Dunten *et al.*, 2002). Human PCK binds GTP in the more common *anti* conformation (Dunten *et al.*, 2002), whereas *E. coli* PCK binds ATP in a *syn* conformation (Tari *et al.*, 1996). Not surprisingly, the adenine-binding site is unique in *E. coli* PCK. However, in human PCK there is also a unique guanine-binding pocket formed from three phenylalanines at residues 517, 525 and 530 and this seems to be conserved in all GTP-specific PCKs (Dunten *et al.*, 2002). Here, we report the crystallization and preliminary X-ray analysis of GTP-dependent *C. glutamicum* PCK. The sequence identity between GTP-dependent human (NCBI accession No. P35558) and *C. glutamicum* PCK (NCBI accession No. NP_602055) is about 45%. Structural information from this GTP-dependent PCK may provide insight into gluconeogenesis and also into the production of glutamine and lysine through the TCA-cycle intermediates in *C. glutamicum* (Riedel *et al.*, 2001).

2. Crystallization

Expression, characterization and purification of *C. glutamicum* PCK from *E. coli* using a GST tag has been described elsewhere (Aich *et al.*, 2003). The GST tag was cleaved by PreScission protease prior to crystallization. Initial screening for crystallization conditions was performed by the hanging-drop method using the Crystal Screen I matrix (Hampton

Research, Laguna Niguel, USA) at room temperature. Each drop consisted of 1 µl protein solution (10 mg ml^{-1}) and 1 µl reservoir solution (0.2 *M* ammonium acetate, 0.1 *M* sodium acetate pH 4.6, 30% PEG 4000) and was suspended over 500 µl of reservoir solution. Many fine needle-like crystals with amorphous precipitate were found the next day. With 25% PEG 4000 at pH 4.1–4.5, larger crystals were produced after 3–7 d. The dimensions of the largest crystal as shown in Fig. 1 are 0.25 × 0.1 × 0.5 mm. The presence of protein in these crystals was confirmed by crystal staining with a protein-specific dye (Izit Crystal Dye, Hampton Research) and also by running an SDS–PAGE gel after dissolving these crystals into the gel loading buffer. At higher pH (5.0), a thick cluster of crystals was formed without any amorphous precipitate (Fig. 2).

3. Data collection and processing

Crystals were frozen in liquid nitrogen after transfer to a cryoprotectant consisting of 25% glycerol, 30% PEG 4000, 0.1 *M* ammonium acetate pH 4.3. X-ray diffraction data of *C. glutamicum* PCK were collected at 100 K from a frozen crystal using a CCD detector (MAR CCD 165) on beamline 14-ID-B at BioCARS, APS (Chicago). The data collection was performed with a total oscillation range of 180°, using radiation of 0.9984 Å wavelength with an exposure time of 15 s for 1° frames. The crystal-to-detector distance was 200 mm. The crystal diffracted to 2.8 Å resolution with an overall completeness of 91%. A diffraction image is shown in Fig. 3. The data were indexed and scaled with the *HKL* package (Otwinowski & Minor, 1997). Symmetry and systematically absent reflections suggested that the *C. glutamicum* PCK crystals belonged to space group $P2_1$, with unit-cell parameters $a = 71.7$, $b = 117.4$, $c = 161.3$ Å, $\beta = 92.9°$. The calculated Matthews coefficient (V_M) of 2.5 Å^3 Da^{-1} indicated the presence of four molecules per unit cell (Matthews, 1968). The solvent content was estimated to be 50.3%. Data-collection statistics are shown in Table 1. The self-rotation function was calculated using the program *POLARRFN* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The data in the resolution range 20–3 Å did not show any identifiable peaks representing non-crystallographic symmetry. A preliminary model of the structure was found by molecular replacement using the program *AMoRe* (Navaza, 1994). Human PCK (PDB code 1khg; Dunten *et al.*, 2002) was used as the search model. Structural refinement of these crystal data is now in progress.

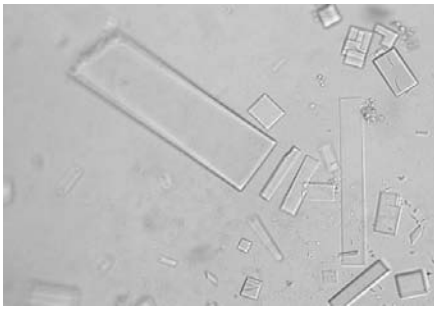

Figure 1
Crystals of *C. glutamicum* PCK at pH 4.3.

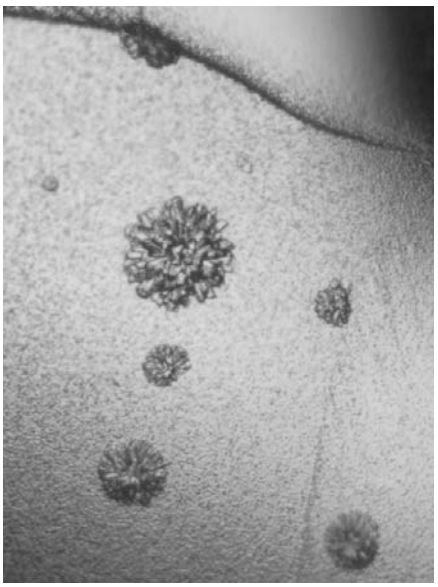

Figure 2
Cluster of *C. glutamicum* PCK crystals at pH 5.0.

Table 1
X-ray data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.91–2.8 Å).

X-ray wavelength (Å)	0.9984
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 71.7$, $b = 117.4$, $c = 161.3$, $\beta = 92.9$
Resolution range (Å)	50.0–2.8
Total No. of reflections collected	111706
Unique reflections	62873
Data completeness (%)	91 (76)
R_{merge}† (%)	14.2 (43.9)
Mean $I/\sigma(I)$	4.9 (0.76)

† $R_{merge} = \sum |I_i - \langle I \rangle| / \sum I_i$.

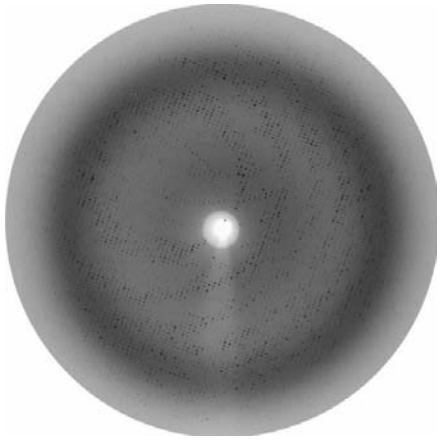

Figure 3
Diffraction image of *C. glutamicum* PCK crystal collected on beamline 14-ID-B at BioCARS, APS (Chicago).

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