Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of inorganic polyphosphate/ATP-glucomannokinase from *Arthrobacter* sp. strain KM

Takako Mukai,[a] Shigeyuki Kawai,[a] Shigetarou Mori,[a] Bunzo Mikami[b] and Kousaku Murata[a]*

[a]Department of Basic and Applied Molecular Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan, and [b]Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Correspondence e-mail: kmurata@kais.kyoto-u.ac.jp

Received 26 May 2003
Accepted 14 July 2003

Inorganic polyphosphate [poly(P)]/ATP-glucomannokinase from *Arthrobacter* sp. strain KM phosphorylates glucose and mannose, utilizing both ATP and poly(P) as phosphoryl donors. The enzyme was overexpressed in *Escherichia coli*, purified and crystallized by means of the hanging-drop vapour-diffusion method with ammonium sulfate as the precipitant. The crystals were orthorhombic and belonged to space group $P2_12_12_1$, with unit-cell parameters a = 66.2, b = 83.7, c = 103.8 Å. Assuming two molecules per asymmetric unit, V_{sol} is 0.49. X-ray diffraction data to 2.83 Å have been collected from a single crystal.

1. Introduction

Recent studies have shown that glucose is phosphorylated to glucose-6-phosphate (G-6-P) by several kinds of 'glucose-phosphorylating kinases'. These are ATP-dependent hexokinase (ATP:D-hexose 6-phosphotransferase; EC 2.7.1.1), ATP-dependent glucokinase (ATP:D-glucose 6-phosphotransferase; EC 2.7.1.2), inorganic polyphosphate [poly(P)]/ATP-dependent glucokinase [poly(P):D-glucose 6-phosphotransferase; EC 2.7.1.63], ADP-dependent glucokinase (EC 2.7.1.147) and bifunctional ADP-dependent glucokinase/phosphofructokinase (Cardenas *et al.*, 1998; Dorr *et al.*, 2003; Phillip *et al.*, 1999). ATP-dependent hexokinase and glucokinase convert glucose to G-6-P, utilizing ATP as a phosphoryl donor. The former accepts several hexoses and seems to occur in eukaryotes and some archaea, while the latter is specific for glucose and is mainly found in bacteria (Cardenas *et al.*, 1998). Poly(P)/ATP-dependent glucokinase is also specific for glucose. However, it can utilize not only ATP but also poly(P) and is found in certain bacteria such as *Propionibacterium shermanii* and *Mycobacterium tuberculosis* (Phillip *et al.*, 1999). ADP-dependent glucokinase and glucokinase/phosphofructokinase use ADP for phosphorylation, but not ATP or poly(P), and only occur in some archaea (Dorr *et al.*, 2003). Of these glucose-phosphorylating kinases, the three-dimensional structures of the hexokinases from yeast (Anderson *et al.*, 1978), human brain (Aleshin *et al.*, 1998), rat (Mulichak *et al.*, 1998) and the parasite *Schistosoma mansoni* (Mulichak *et al.*, 1998) and of the ADP-dependent glucokinases from *Thermococcus litoralis* (Ito *et al.*, 2001) and *Pyrococcus horikoshii* (Tsuge *et al.*, 2002) have been determined.

Although these glucose-phosphorylating kinases catalyze the same reaction through the use of various phosphoryl donors, the accumulated information on their three-dimensional and primary structures indicates that they can be divided into several families and subfamilies (Fig. 1) (Bork *et al.*, 1993; Dorr *et al.*, 2003). Of the glucose-phosphorylating kinases, ADP-dependent glucokinase and glucokinase/phosphofructokinase belong to the 'ribokinase family', while the others are classified into the 'hexokinase family' (Dorr *et al.*, 2003; Tsuge *et al.*, 2002). The kinases in the

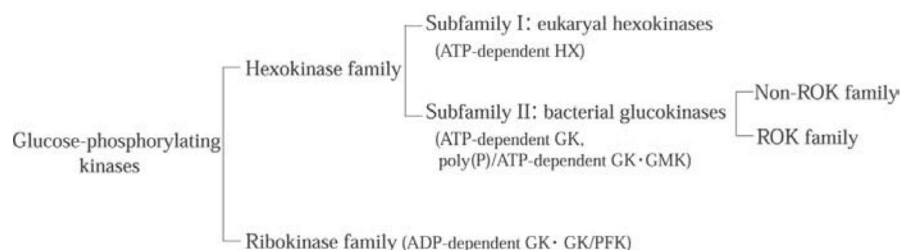

Figure 1
Classification of glucose-phosphorylating kinases. GK, glucokinase; GMK, glucomannokinase; HX, hexokinase; PFK, phosphofructokinase. Poly(P)/ATP-dependent glucokinase and glucomannokinase belong to the ROK family, while ATP-dependent glucokinases are found in both the ROK and non-ROK families.

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hexokinase family are further divided into two subfamilies, eukaryal hexokinases (ATP-dependent hexokinases) and bacterial glucokinases [ATP-dependent glucokinases and poly(P)/ATP-dependent glucokinases]. Although several characteristic motifs of the hexokinase family, including 'phosphate 1' and 'phosphate 2', are found in the primary structures of both eukaryal hexokinases and bacterial glucokinases, the primary structures, other than the motifs, are not conserved (Phillip *et al.*, 1999; Bork *et al.*, 1993). Therefore, whether or not the eukaryal hexokinases and bacterial glucokinases evolved from a common ancestor is currently a matter of debate. Furthermore, the bacterial glucokinases can also be classified into the so-called 'ROK' and 'non-ROK' protein families (Dorr *et al.*, 2003). The ROK (repressor, ORF, kinase) family includes bacterial proteins such as transcriptional repressors, sugar kinases and as yet uncharacterized open reading frames (Saier *et al.*, 1994). Some bacterial glucokinases in this family have been shown to be involved in catabolite repression (Dorr *et al.*, 2003).

We recently purified a novel enzyme, poly(P)/ATP-glucomannokinase, from a cell extract of *Arthrobacter* sp. strain KM and cloned the gene for the enzyme (Mukai, Kawai, Matsukawa *et al.*, 2003). Poly(P)/ATP-glucomannokinase phosphorylates mannose in addition to glucose by use of poly(P) and ATP, with a high affinity for glucose, and belongs to the bacterial glucokinase subfamily of the ROK protein family (Mukai, Kawai & Murata, 2003).

The three-dimensional structure of poly(P)/ATP-dependent glucomannokinase will contribute to the understanding of the unsolved poly(P)-utilization mechanism and may provide insights into the structures of other bacterial glucokinases and proteins in the ROK family, addressing the question regarding the 'ancestor protein' of the kinases in the hexokinase family. Therefore we attempted to determine the three-dimensional structure of poly(P)/ATP-dependent glucomannokinase. In this article, we present the preliminary X-ray data for a crystal of the enzyme.

2. Crystallization

The poly(P)/ATP-glucomannokinase gene, which was cloned from a genomic DNA library of *Arthrobacter* sp. strain KM (Mukai, Kawai, Matsukawa *et al.*, 2003), was expressed in *Escherichia coli* BL21 (DE3) (Novagen, Darmstadt, Germany) and the recombinant enzyme was purified as described in Mukai, Kawai & Murata (2003). The purified enzyme was dialyzed against 10 m*M* potassium phosphate pH 7.0 overnight at 277 K and then concentrated by ultrafiltration with a Centriprep (Amicon, Beverly, MA) to a final concentration of 14 mg ml^{-1}. 100 m*M* glucose was added to the protein solution to bring the final glucose concentration to 10 m*M*. Protein concentrations were determined by the method of Bradford (1976).

On SDS–PAGE (Laemmli, 1970) and Sephadex G-150 column chromatography, the enzyme was determined to be a monomer of 30 kDa, which was the same molecular weight as that of the native enzyme purified from *Arthrobacter* sp. strain KM (Mukai, Kawai, Matsukawa *et al.*, 2003).

The purified enzyme was crystallized by means of the hanging-drop vapour-diffusion method using 24-well Linbro tissue-culture plates at 293 K. Briefly, 3 µl of protein solution containing 10 m*M* glucose was mixed with 3 µl reservoir solution on a siliconized cover slip and the mixture was placed over 0.5 ml reservoir solution. Initial screening for crystallization conditions was performed with commercial crystallization kits from Hampton Research (Laguna Niguel, CA, USA). During the initial screening, small crystals of the protein were obtained when ammonium sulfate was used as the precipitant and then the conditions were optimized by changing the concentrations of the protein, precipitant and other components of the reservoir solution. The optimal crystallization conditions were determined to be a mixture consisting of 10 mg ml^{-1} enzyme, 10 m*M* glucose, 2.0 *M* ammonium sulfate, 2%(*v*/*v*) polyethylene glycol 400 and 0.1 *M* Na HEPES pH 8.0. Under these conditions, prismatic colourless crystals grew to a maximum size of 0.1 mm in two weeks at 293 K (Fig. 2).

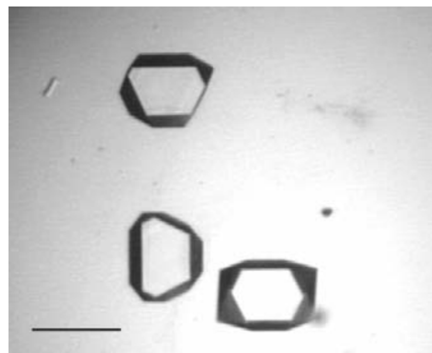

Figure 2
Crystal of poly(P)/ATP-glucomannokinase of *Arthrobacter* sp. strain KM. The bar indicates 0.1 mm.

Table 1
Data-collection statistics for a poly(P)/ATP-glucomannokinase crystal.

Values in parentheses are data for the highest resolution shell.

X-ray source	Cu *Kα*
Wavelength (Å)	1.54
Resolution (Å)	50.0–2.83 (2.97–2.83)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	*a* = 66.2, *b* = 83.7, *c* = 103.8
Total observations	38228 (4642)
Independent reflections	12458 (1842)
Completeness (%)	96.2 (96.2)
$\langle I/\sigma(I)\rangle$	9.7 (2.4)
R_{sym} (%)	9.9 (35.6)

3. X-ray analysis

A crystal was mounted in a thin-walled glass capillary for X-ray analysis. The ends of the capillary were filled with the reservoir solution and then sealed with wax. The diffraction data for a native crystal were collected over the resolution range 50.0–2.83 Å with a Bruker Hi-Star multiwire area detector at 293 K using Cu *Kα* radiation generated by a MacScience M18XHF rotating-anode generator and were processed using the *SADIE* and *SAINT* software packages (Bruker).

The crystal belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters *a* = 66.2, *b* = 83.7, *c* = 103.8 Å. From the 38 228 reflections observed, 12 458 independent reflections were obtained, with an R_{sym} value of 9.9%. The data exhibited a completeness of 96.2% at 2.83 Å resolution. The data-collection statistics for a crystal of the poly(P)/ATP-glucomannokinase are summarized in Table 1.

Based on the molecular weight of poly(P)/ATP-glucomannokinase (30 kDa) and space group $P2_12_12_1$, it was assumed that each crystal contains two molecules of protein per asymmetric unit. This assumption gives a V_M value (Matthews, 1968) of 2.4 Å^3 Da^{-1} and a solvent content of 49%. The V_M value and solvent content lie within the ranges usually found for protein crystals.

A search for heavy-atom derivatives for phasing by the multiple isomorphous replacement method is now in progress.

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