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Crystallization and preliminary crystal analysis of yeast hexokinase PI and PII

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Hexokinase is the prime enzyme of the Embden–Meyerhof pathway and is responsible for the first stage of energy conversion. It catalyzes the transfer of a phosphate to glucose to form glucose-6-phosphate. Yeast hexokinase PII is also known to play an important role in glucose signal transduction. Crystals of yeast hexokinase isoforms PI and PII were obtained by vapour-diffusion techniques using the hanging-drop method. Isoform PI crystals belong to the space group $P2_12_12_1$, with unit-cell parameters a = 62.12, b = 78.87, c = 144.74 Å. Unit-cell parameters for isoform PII crystals are a = b = 142.81, c = 58.46 Å and the space group is $I4$. Synchrotron diffraction data have been collected to 2.2 Å resolution from the isoform PII crystal, whereas isoform PI diffracted to 3.1 Å.

1. Introduction

Hexokinase (ATP:D-hexose 6-phosphotransferase, E.C. 2.7.1.1; HK) is a member of the kinase family of tissue-specific isoenzymes. The enzyme hexokinase catalyzes the first reaction in the catabolism of glucose: the formation of glucose-6-phosphate using ATP as a phosphoryl donor. It is the prime enzyme in the glycolytic pathway. Hexokinase exists in various forms within different organisms. It has been shown by cloning the hexokinase genes from *Saccharomyces cerevisiae* that there are two isoenzymes of hexokinase in yeast: PI and PII, which have an overall homology in their amino-acid sequences of about 76% (Kopetzki *et al.*, 1985; Frohlich *et al.*, 1985). Isoenzyme PII has a total of 486 residues, whereas PI has 485 amino-acid residues. Yeast hexokinase PII has two functionally different phosphorylation sites (Heidrich *et al.*, 1997) and can be a phosphoprotein *in vivo* (Vojtek & Fraenkel, 1990).

Crystallographic structures of yeast hexokinase PI and PII were deposited in the Protein Data Bank (PDB) in the 1980s, when the primary sequence was unknown. The enzymes' primary sequences were guessed from the electron density or labelled as unknown (Anderson *et al.*, 1978; Bennet & Steitz, 1980). The publication of the amino-acid sequence showed that many residues in the structure were not correct. We started to perform crystallization experiments in order to solve the structure of yeast hexokinase with the correct amino-acid sequence and to obtain more information about the mechanism of induced fit which hexokinase performs while binding to its substrate. An overall overview on hexokinase from yeast to mammals has been written by Wilson (1995). Crystallographic structures of human hexokinase (Aleshin *et al.*, 1998) and hexokinase from *Schistosoma mansoni* (Mulichak *et al.*, 1998) have recently been reported. Here, we report the crystallization conditions and preliminary data for isoforms PI and PII of yeast hexokinase and also their molecular-replacement solution.

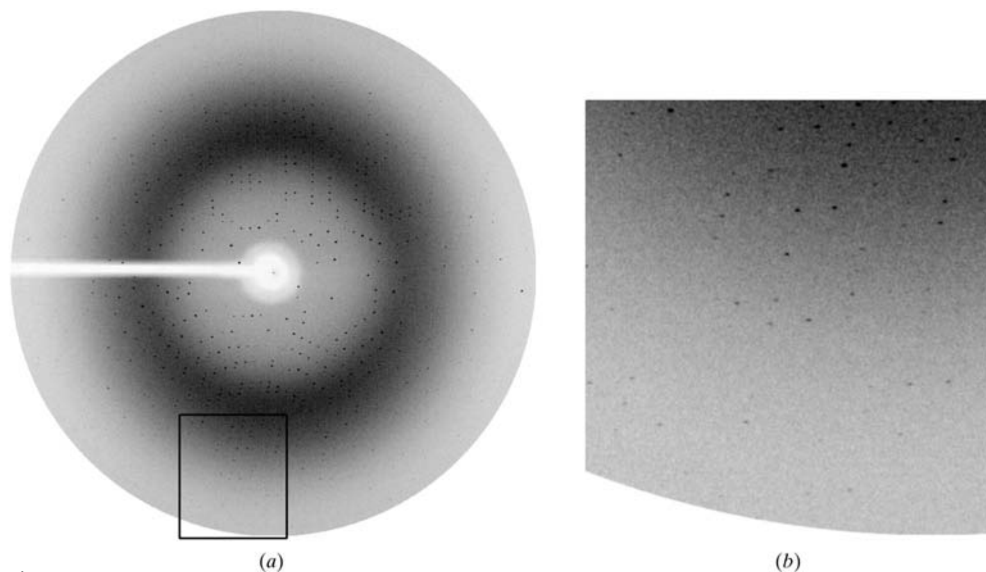

Figure 1
X-ray diffraction image of yeast hexokinase PII. The diffraction image was taken from a MAR Research detector, with a crystal-to-film distance of 200 mm. The oscillation range is 1°. The detector edge corresponds to 2.17 Å. (*a*) One diffraction image; (*b*) enlargement of the indicated portion of the image in (*a*).

2. Methods and results

Hexokinase PI and PII enzymes from baker's yeast were purchased from Sigma; samples were applied to an ion-exchange Resource

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Table 1
Data-collection and processing statistics.

Statistical values for the highest resolution shell are shown in parentheses, corresponding to 3.17–3.0 and 2.28–2.20 Å for yeast hexokinase PI and yeast hexokinase PII, respectively.

Crystal	PI	PII
Unit-cell parameters (Å)		
a	62.12	142.81
b	78.87	142.81
c	144.74	58.46
Space group	$P2_12_12_1$	$I4$
Resolution (Å)	12.0–3.10	13.0–2.2
$I/\sigma(I)$	8.1 (3.5)	6.7 (2.4)
R_{sym} (%)	0.114 (0.418)	0.113 (0.403)
Completeness (%)	93.4 (96.7)	95.1 (96.8)
Unique reflections	12353	2523
Multiplicity (%)	2.86 (2.78)	2.1 (1.85)

column (Pharmacia) and eluted with a 0.1–1 *M* linear NaCl gradient in 20 m*M* Tris–HCl pH 6.0. Protein was initially dialyzed against 1 l of phosphate buffer 100 m*M* pH 7.0 for 5 h; the buffer was then changed and a new dialysis took place overnight at 277 K. After dialysis, the protein was concentrated to about 10 mg ml^{-1}. The two isoforms are difficult to separate and a further purification was performed in order to attempt to separate the isoforms as much as possible. Initial screening of the crystallization conditions was performed using a protein concentration of 10 mg ml^{-1}, 0.1 *M* phosphate buffer in the pH range 5.5–6.5 and 25–35% PEG 4000 as precipitant. A second screening was performed substituting PEG with ammonium sulfate (2.0–3.2 *M*) as precipitant and 0.1 *M* phosphate buffer in the pH range 7.3–7.7. The hanging-drop vapour-diffusion technique was used for all conditions. All crystallization trials described here were performed in the absence of any ligands. Drops containing 3 µl of precipitant solution and 3 µl of protein solution were prepared on siliconized glass cover slips and suspended over reservoirs containing 1 ml of the same precipitant solution.

Needle-like crystals of yeast hexokinase PI grew within two to three weeks in various drops. These crystals were approximately 0.5 mm in length. Seven months later, crystals of hexokinase PII were observed under similar conditions. These crystals were larger and had a bipyramidal shape. Crystals were mounted in quartz capillaries and data collection was performed at the Protein Crystallography (PCr) beamline (Polikarpov *et al.*, 1998) at the Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil. The synchrotron-radiation wavelength was set to 1.38 Å and all diffraction images were collected on a MAR345 image plate. The image plate was operated in the 300 mm scanning mode. All data were collected at low temperature (277 K). The collected images were processed and scaled with *DENZO* and *SCALEPACK* (Otwinowski, 1993).

The hexokinase PI crystals belong to the orthorhombic space group $P2_12_12_1$. The crystals of hexokinase PII belong to the tetragonal space group $I4$ and diffract to 2.2 Å resolution. A summary of the diffraction data collected from crystals of yeast hexokinase PI and PII is provided in Table 1.

Molecular-replacement solutions were calculated with the program *AMoRe* (Collaborative Computational Project, Number 4, 1994; Navaza, 1994). We used as search models the coordinates of both yeast hexokinase PI (PDB code 1hkg; Bennet & Steitz, 1980) and yeast hexokinase PII (PDB code 2yhx; Anderson *et al.*, 1978) deposited in the PDB. In both models, the residues labelled 'unknown' were deleted before the models were used as search models for the molecular-replacement solution.

2.1. Yeast hexokinase PI

Hexokinase PI crystals are needle shaped and belong to space group $P2_12_12_1$, with unit-cell parameters a = 62.12, b = 78.87, c = 144.74 Å. Typical crystal dimensions are 0.5 × 0.05 × 0.05 mm. The number of molecules per asymmetric unit was estimated based on calculated values of V_m, the ratio of unit-cell volume to protein mass, and estimated values of the fractional volume occupied by solvent (Matthews, 1968). One protein molecule is present in the asymmetric unit and the solvent content is approximately 62.6%. An X-ray data set 93.4% complete to 3.1 Å was collected from a crystal and processed. The scaling of all data gave an $R_{\rm merge}$ of 11.4%.

In the molecular replacement, the best solution for the yeast hexokinase PI crystal was found using the 1hkg model; a correlation value of 61.5% and an R factor of 47.7% were obtained after ten cycles of rigid-body refinement. Starting with the 2yhx model, fitting yielded a solution with a correlation coefficient of 47.9% and an R factor of 53.2%.

2.2. Yeast hexokinase PII

The yeast hexokinase PII crystals have an elongated bipyramidal shape. One crystal was used to collect a data set which was 91.1% complete and was obtained after 50 images (Fig. 1). The crystal dimensions were 0.25 × 0.4 × 1.0 mm and the crystal diffracted to 2.17 Å resolution. The crystal belongs to space group $I4$, a new crystal form for yeast hexokinase crystals, and had unit-cell parameters $a = b$ = 142.81, c = 58.46 Å.

From the molecular weight of the protein (approximately 53 kDa) and the space group $I4$, it can be inferred that one protein molecule is present in the asymmetric unit. The assumption of one monomeric molecule yields a V_m of 2.77 Å^3 Da^{-1}. This corresponds to a solvent fractional volume of 55%.

For the yeast hexokinase PII crystal, the 2yhx structure gave the best molecular-replacement solution. After rigid-body refinement, the correlation value was 56.6% and the R factor was 38.9%. When the 1hkg model was used, the correlation coefficient was much lower, with a value of 25.7%; the R factor was 51.2%.

We plan to proceed with refinement of yeast hexokinase PII structure at 2.2 Å resolution and this refined structure will be used as a search model for the molecular-replacement structure solution of the PI isoform.

Refined yeast hexokinase PI and PII structures will provide a basis for their structural comparisons, which may shed more light on the changes in hexokinase conformation in the process of enzymatic catalysis.

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