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Crystallization and preliminary X-ray diffraction analysis of mouse prostaglandin F_{2α} synthase, AKR1B3

Aldo-keto reductase 1B3 (AKR1B3) catalyzes the NADPH-dependent reduction of prostaglandin H₂ (PGH₂), which is a common intermediate of various prostanoids, to form PGF_{2α}. AKR1B3 also reduces PGH₂ to PGD₂ in the absence of NADPH. AKR1B3 produced in *Escherichia coli* was crystallized in complex with NADPH by the sitting-drop vapour-diffusion method. The crystal was tetragonal, belonging to space group *P*₄₁₂₁₂ or *P*₄₃₂₁₂, with unit-cell parameters *a* = *b* = 107.62, *c* = 120.76 Å. X-ray diffraction data were collected to 2.4 Å resolution at 100 K using a synchrotron-radiation source.

1. Introduction

Aldo-keto reductases (AKRs) are soluble monomeric proteins with a molecular mass of 37 kDa and NADPH-dependent oxidoreductase activity. AKR proteins are widely distributed in prokaryotes and eukaryotes, fall into 15 families (Penning & Drury, 2007) and metabolize a number of substrates, including aldehydes, monosaccharides, steroids, polycyclic hydrocarbons, isoflavonoids and prostaglandins (PGs), in the presence of NADPH (Jez *et al.*, 1997). Aldose reductase (EC 1.1.1.21), named AKR1B1 in humans and AKR1B3 in mice, is considered to be the prototypical enzyme of the AKR superfamily. In addition to these canonical aldose reductases, a second group named aldose reductase-like proteins (ARLPs) have been characterized on the basis of sequence homology (at least 60–70% identity to aldose reductase). AKR1B7, initially characterized as a mouse vas deferens androgen-dependent protein, belongs to the ARLP group. X-ray crystallographic structures of members of the AKR superfamily have shown that these enzymes have a common three-dimensional fold known as the (α/β)₈-barrel fold, *e.g.* human AKR1B1 (Wilson *et al.*, 1992), human AKR1B10 (Gallego *et al.*, 2007), rat AKR1B14 (Sundaram *et al.*, 2011), human AKR1C2 (Jin *et al.*, 2001; Nahoum *et al.*, 2001), human AKR1C3 (Lovering *et al.*, 2004), rat AKR1C9 (Hoog *et al.*, 1994; Bennett *et al.*, 1996, 1997) and *Trypanosoma brucei* AKR5A2 (Kilunga *et al.*, 2005). The nucleotide cofactor binds in an extended conformation at the top of the α/β-barrel, with the nicotinamide ring projecting down towards the centre of the barrel and pyrophosphate straddling the barrel lip (Petrash, 2004). Kubiseski *et al.* (1992) have established that the enzyme follows a sequential ordered mechanism in which NADPH binds before the aldehyde substrate and NADP⁺ is released after the alcohol product is formed. When the first complete crystal structure of human AKR1B1 was solved in 1992, the conserved Tyr48 was shown to fulfil the role of a catalytic acid for NADPH-dependent reduction (Wilson *et al.*, 1992).

Recently, we reported that human AKR1B1, mouse AKR1B3 and mouse AKR1B7 are associated with PGF_{2α} synthase (PGFS; EC 1.1.1.188) activity, which catalyzes the reduction of PGH₂, a common intermediate of various prostanoids, to PGF_{2α} (Kabututu *et al.*, 2009). Moreover, we found that AKR1B1 and AKR1B3, but not AKR1B7 and AKR1C3, also catalyze the isomerization of PGH₂ to PGD₂ in the absence of NADPH. Although both AKR1B1 and AKR1B3 show similar pH-rate profiles for PGFS and PGD₂ synthase (PGDS) activities, the PGDS activities of AKR1B1 and AKR1B3 were found to be 89 and 24% when compared with their PGFS activities (Nagata

et al., 2011). In order to further reveal the structure–function relationship of these isozymes, the three-dimensional structure of AKR1B3 and its comparison with that of AKR1B1 are required. Here, we report the crystallization and preliminary X-ray diffraction study of AKR1B3 in complex with NADPH.

2. Experimental

2.1. Protein expression and purification

Recombinant AKR1B3 was expressed in *Escherichia coli* BL21 Star (DE3) (Novagen) as described previously (Nagata *et al.*, 2011). The *E. coli* cells were disrupted by sonication in buffer *A* (50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl, 10 mM imidazole) containing a protease-inhibitor cocktail (Roche). The sample was applied onto Ni-NTA agarose resin (Qiagen) and eluted with 250 mM imidazole in buffer *A*. The sample was treated with thrombin (GE Healthcare) at 293 K for 14 h and then applied onto a HisTrap HP column (GE Healthcare) equilibrated with buffer *A*. The flowthrough fraction containing AKR1B3 without the His tag was pooled and applied onto a HiLoad 16/60 Superdex 75 column (GE Healthcare), which was developed with buffer *B* (20 mM Tris–HCl pH 8.0, 150 mM NaCl). The purified protein was concentrated to 30 mg ml^{−1} using a Vivaspin (Sartorius) and stored at 193 K until the crystallization experiments. Up to 7.3 mg purified AKR1B3 was obtained from 1 l of *E. coli* cell culture.

2.2. Crystallization of AKR1B3

Initial crystallization screening of AKR1B3 in the presence of 1 mM NADPH was performed by the sitting-drop vapour-diffusion method on an Intelli-Plate 96 (Art Robbins Instruments). Needle-shaped crystals appeared in PEG/Ion Screen condition No. 48 [0.2 M ammonium citrate dibasic pH 5.1, 20% polyethylene glycol (PEG) 3350; Hampton Research] within 1 d. After optimizing the conditions in 24-well sitting-drop plates (Hampton Research), rod-shaped crystals with maximum dimensions of 0.25 × 0.25 × 1.25 mm appeared after one week at 293 K (Fig. 1). The drops consisted of 1 µl protein solution (15 mg ml^{−1} AKR1B3, 2 mM NADPH, 20 mM Tris–HCl pH 8.0, 150 mM NaCl) and 1 µl reservoir solution (0.2 M ammonium citrate dibasic, 22.7% PEG 3350). 500 µl reservoir solution was added to the deep well of the 24-well sitting-drop plates.

Figure 1
Crystals of AKR1B3. The crystals were obtained in a sitting drop.

3. Data collection and processing

X-ray diffraction data were measured on beamline BL44XU at SPring-8 (Harima, Japan). Prior to data collection, the crystal of AKR1B3 was soaked in cryoprotectant solution (0.2 M ammonium citrate dibasic, 25.5% PEG 3350, 1 mM NADPH, 10% 2-methyl-2,4-pentanediol), fished out with a standard nylon loop and flash-cooled in a nitrogen-gas stream at 100 K. The diffraction patterns were recorded on a MX225HE CCD detector (Rayonix). The wavelength, crystal-to-detector distance, crystal oscillation angle per image and beam-exposure time were set to 0.9 Å, 300.0 mm, 0.5° and 5 s, respectively (Fig. 2). A complete data set was collected from 360 images covering 120° in total.

Figure 2
X-ray diffraction image from an AKR1B3 crystal. The frame edge in the enlargement is 2.4 Å.

Table 1

Crystal parameters and X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution shell.

Crystal system	Tetragonal
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 107.62$, $c = 120.76$
Resolution range (Å)	50–2.4 (2.49–2.40)
No. of molecules in the asymmetric unit	2
V_M (Å ³ Da ^{−1})	2.45
V_{solv} (%)	50
No. of measured reflections	1214259
No. of unique reflections	28017
$R_{merge}^{\dagger}$ (%)	8.7 (22.7)
Completeness (%)	99.0 (95.7)
Average $\langle I/\sigma(I) \rangle$	24.5 (4.7)

$\dagger R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the value of the i th measurement of the intensity of a reflection, $\langle I(hkl) \rangle$ is the mean value of the intensity of that reflection and the summation is over all measurements.

The data set was processed using the *HKL-2000* program suite (Otwinowski & Minor, 1997). The crystal of AKR1B3 was tetragonal, belonging to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 107.62$, $c = 120.76$ Å. From the 1 214 259 accepted observations to 2.4 Å resolution, 28 017 unique reflections were obtained. Assuming the presence of two monomers of AKR1B3 in the asymmetric unit, the crystal volume per enzyme mass (V_M) and the solvent content were calculated to be 2.45 Å³ Da^{−1} and 50%, respectively. A summary of the data statistics is presented in Table 1.

Molecular-replacement calculations using the program *MOLREP* (Vagin & Teplyakov, 2010) from the *CCP4* program package (Winn *et al.*, 2011) with human placenta aldose reductase (PDB entry 1ads; Wilson *et al.*, 1992) as a search model and refinement of the structure are currently under way. The solved atomic structure should provide insights into the differences in PGDS activity between AKR1B1 and AKR1B3.

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