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

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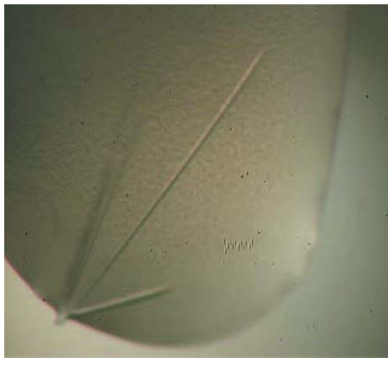

Aldo-keto reductase 1B3 (AKR1B3) catalyzes the NADPH-dependent reduction of prostaglandin H_2 (PGH_2), which is a common intermediate of various prostanoids, to form $PGF_{2\alpha}$. AKR1B3 also reduces PGH_2 to PGD_2 in the absence of NADPH. AKR1B3 produced in *Escherichia coli* was crystallized in complex with NADPH by the sitting-drop vapour-diffusion method. The crystal was tetragonal, belonging to space group $P4_12_12$ or $P4_32_12$, 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 $(\alpha/\beta)_8$-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 $PGF_{2\alpha}$ synthase (PGFS; EC 1.1.1.188) activity, which catalyzes the reduction of PGH_2, a common intermediate of various prostanoids, to $PGF_{2\alpha}$ (Kabututu *et al.*, 2009). Moreover, we found that AKR1B1 and AKR1B3, but not AKR1B7 and AKR1C3, also catalyze the isomerization of PGH_2 to PGD_2 in the absence of NADPH. Although both AKR1B1 and AKR1B3 show similar pH–rate profiles for PGFS and PGD_2 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 m*M* NaH_2PO_4 pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole) containing a protease-inhibitor cocktail (Roche). The sample was applied onto Ni–NTA agarose resin (Qiagen) and eluted with 250 m*M* 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 m*M* Tris–HCl pH 8.0, 150 m*M* 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 m*M* 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 m*M* NADPH, 20 m*M* Tris–HCl pH 8.0, 150 m*M* 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.

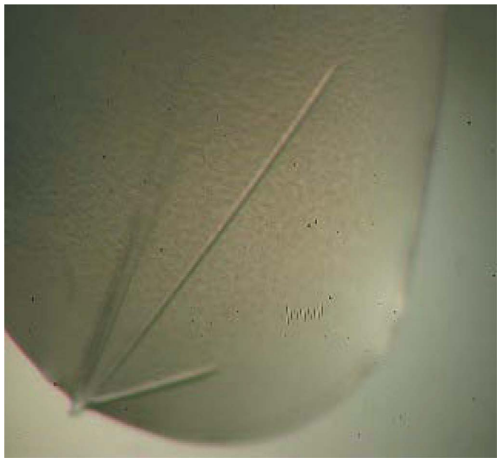

Figure 1
Crystals of AKR1B3. The crystals were obtained in a sitting drop.

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 m*M* 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.

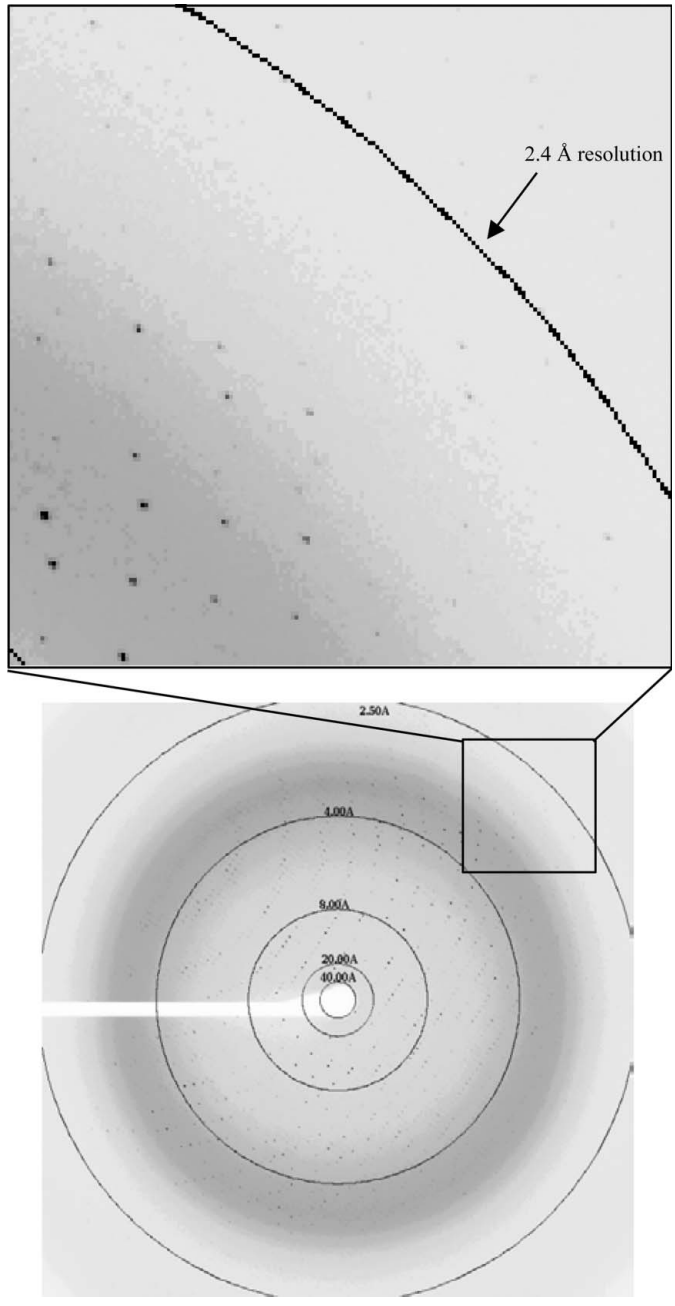

Figure 2
X-ray diffraction image from an AKR1B3 crystal. The frame edge in the enlargement is 2.4 Å.

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}† (%)	8.7 (22.7)
Completeness (%)	99.0 (95.7)
Average $\langle I/\sigma(I)\rangle$	24.5 (4.7)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the value of the ith 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 Å^3 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 *CCP*4 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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References

Bennett, M. J., Albert, R. H., Jez, J. M., Ma, H., Penning, T. M. & Lewis, M. (1997). *Structure*, **5**, 799–812.
Bennett, M. J., Schlegel, B. P., Jez, J. M., Penning, T. M. & Lewis, M. (1996). *Biochemistry*, **35**, 10702–10711.
Gallego, O., Ruiz, F. X., Ardèvol, A., Domínguez, M., Alvarez, R., de Lera, A. R., Rovira, C., Farrés, J., Fita, I. & Parés, X. (2007). *Proc. Natl Acad. Sci. USA*, **104**, 20764–20769.
Hoog, S. S., Pawlowski, J. E., Alzari, P. M., Penning, T. M. & Lewis, M. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 2517–2521.
Jez, J. M., Flynn, T. G. & Penning, T. M. (1997). *Biochem. Pharmacol.* **54**, 639–647.
Jin, Y., Stayrook, S. E., Albert, R. H., Palackal, N. T., Penning, T. M. & Lewis, M. (2001). *Biochemistry*, **40**, 10161–10168.
Kabututu, Z., Manin, M., Pointud, J. C., Maruyama, T., Nagata, N., Lambert, S., Lefrançois-Martinez, A. M., Martinez, A. & Urade, Y. (2009). *J. Biochem.* **145**, 161–168.
Kilunga, K. B., Inoue, T., Okano, Y., Kabututu, Z., Martin, S. K., Lazarus, M., Duszenko, M., Sumii, Y., Kusakari, Y., Matsumura, H., Kai, Y., Sugiyama, S., Inaka, K., Inui, T. & Urade, Y. (2005). *J. Biol. Chem.* **280**, 26371–26382.
Kubiseski, T. J., Hyndman, D. J., Morjana, N. A. & Flynn, T. G. (1992). *J. Biol. Chem.* **267**, 6510–6517.
Lovering, A. L., Ride, J. P., Bunce, C. M., Desmond, J. C., Cummings, S. M. & White, S. A. (2004). *Cancer Res.* **64**, 1802–1810.
Nagata, N., Kusakari, Y., Fukunishi, Y., Inoue, T. & Urade, Y. (2011). *FEBS J.* **278**, 1288–1298.
Nahoum, V., Gangloff, A., Legrand, P., Zhu, D.-W., Cantin, L., Zhorov, B. S., Luu-The, V., Labrie, F., Breton, R. & Lin, S.-X. (2001). *J. Biol. Chem.* **276**, 42091–42098.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Penning, T. M. & Drury, J. E. (2007). *Arch. Biochem. Biophys.* **464**, 241–250.
Petrash, J. M. (2004). *Cell. Mol. Life Sci.* **61**, 737–749.
Sundaram, K., Dhagat, U., Endo, S., Chung, R., Matsunaga, T., Hara, A. & El-Kabbani, O. (2011). *Bioorg. Med. Chem. Lett.* **21**, 801–804.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.
Wilson, D. K., Bohren, K. M., Gabbay, K. H. & Quiocho, F. A. (1992). *Science*, **257**, 81–84.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.