Crystallization and preliminary X-ray analysis of a rat aldose reductase-like protein (AKR1B14)

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Mouse vas deferens protein/aldo-keto reductase 1B7 (AKR1B7) is involved in the detoxification of isocaproaldehyde, a steroidogenesis byproduct, and of 4-hydroxynonenal formed by lipid peroxidation. The rat orthologue of AKR1B7 has recently been named AKR1B14 in the AKR superfamily. Recombinant AKR1B14 was expressed in a bacterial system and purified to homogeneity. The purified protein was crystallized from polyethylene glycol solutions using the hanging-drop vapour-diffusion method and an X-ray diffraction data set was collected to 1.86 Å resolution. The crystals belonged to space group $P2_1$, with unit-cell parameters $a = 50.66$, $b = 69.14$, $c = 72.27$ Å, $\beta = 96.4°$. This is the first crystallization report of a rodent AKR1B7 orthologue.

1. Introduction

Members of the aldo-keto reductase (AKR) superfamily are involved in the key steps of diverse metabolic pathways for prostaglandins, steroids, carbohydrates, endogenous aldehydes and ketones, and other exogenous substances (Oates, 2008; Jez *et al.*, 1997). Most of them are monomeric cytosolic proteins of about 320 amino-acid residues in size with related structures and evolutionary origins. Aldose reductase (AR), which belongs to the AKR1B subfamily, is an NADPH-dependent enzyme that catalyses the first step in the polyol pathway and has been implicated in the onset of secondary complications of diabetes in humans (Oates, 2008; Dunlop, 2000; Yabe-Nishimura, 1998). AR shows broad substrate specificity for various aldehydes and monosaccharides and is involved in various physiological roles, including those involving metabolism of retinoids, steroids and xenobiotics, and defensive mechanisms against oxidative stress (Conklin *et al.*, 2007; Petrash, 2007; Crosas *et al.*, 2003; Wermuth & Monder, 1983).

In addition to the ubiquitously expressed canonical AR, multiple AR-like proteins that show high sequence similarity (67–69%) to human and rodent ARs have been identified as tissue-specific in mice and rats. In mice, these AR-like proteins are androgen-dependent vas deferens protein (AKR1B7; Pailhoux *et al.*, 1990; Lau *et al.*, 1995) and fibroblast growth factor-inducible protein (AKR1B8; Donohue *et al.*, 1994; Lau *et al.*, 1995); they are expressed in the adrenal cortex, testis and ovary (Martinez *et al.*, 2001; Brockstedt *et al.*, 2000; Aigueperse *et al.*, 1999; Donohue *et al.*, 1994; Pailhoux *et al.*, 1990). These two AR-like proteins reduce aldehydes, but are clearly distinct from mouse AR by their poor efficiency in reducing glucose (Lefrancois-Martinez *et al.*, 1999; Srivastava *et al.*, 1998). AKR1B7 and AKR1B8 share high sequence identity (82%), but their properties, including their substrate specificity, kinetic properties for common substrates and inhibitor sensitivity, are different. AKR1B7 is suggested to be a major enzyme that is responsible for the elimination of isocaproaldehyde, which is derived from the side-chain cleavage of cholesterol during steroidogenesis (Lefrancois-Martinez *et al.*, 1999). The enzyme also reduces the cytotoxic 4-hydroxynonenal, a major product of lipid peroxidation (Martinez *et al.*, 2001), and reduces prostaglandin H_2 to prostaglandin $F_{2\alpha}$ (Kabututu *et al.*, 2009). AKR1B8 exhibits a high catalytic efficiency towards long-chain aliphatic aldehydes including 4-hydroxynonenal (Srivastava *et al.*, 1998), but has poor reactivity towards isocaproaldehyde (Martinez *et al.*, 2001) and prostaglandin

crystallization communications

Table 1
Summary of data-collection and refinement statistics for AKR1B14–NADPH.

Values in parentheses are for the highest resolution shell.

Data collection and processing	
X-ray source	Rigaku RU300 rotating-anode generator
Detector	MAR345
Wavelength (Å)	1.54179
Unit-cell parameters (Å, °)	$a = 50.66$, $b = 69.14$, $c = 72.27$, $\beta = 96.4$
Space group	$P2_1$
Diffraction data	
Resolution range (Å)	30–1.86 (1.93–1.86)
Measured reflections	167355 (12172)
Unique reflections	47073 (3640)
Completeness (%)	97.6 (90.6)
R_{merge}† (%)	4.7 (27.9)
$I/\sigma(I)$	13.5 (2.3)

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the average intensity over symmetry-related reflections and $I_i(hkl)$ is the observed intensity.

H_2 (Kabututu *et al.*, 2009). The two enzymes also differ in their coenzyme specificity and inhibitor sensitivity. AKR1B7 exhibits reductase activity using both NADPH and NADH as coenzymes and is insensitive to AR inhibitors such as sorbinil and tolrestat (Martinez *et al.*, 2001; Kabututu *et al.*, 2009), whereas AKR1B8 is strictly NADPH-dependent and is inhibited by AR inhibitors (Srivastava *et al.*, 1998).

Recent rat proteomic and genomic analyses have identified two AR-like proteins, AKR1B13 (Zeindl-Eberhart *et al.*, 2001) and AKR1B14 (Val *et al.*, 2002), which share high sequence identity to mouse AKR1B8 (95%) and AKR1B7 (87%), respectively. The rat enzymes are thought to be orthologues of the corresponding mouse enzymes. Indeed, AKR1B13 exhibits almost identical substrate specificity and inhibitor sensitivity to those of mouse AKR1B8 (Endo *et al.*, 2009) and the enzymatic properties of AKR1B14 are equivalent to those of mouse AKR1B7 (Martinez *et al.*, 2001). In humans, one AR-like protein, AKR1B10, has been characterized and is also known as small intestine reductase (Cao *et al.*, 1998; Hyndman & Flynn, 1998). AKR1B10 is NADPH-dependent and is inhibited by AR inhibitors. However, AKR1B10 differs from the above two types of rodent AR-like proteins in its efficient reductase activities towards retinals (Crosas *et al.*, 2003) and some drug ketones (Martin *et al.*, 2006) and shares lower sequence identity (76–82%) to the rodent AR-like proteins. Thus, based on the differences in substrate and coenzyme specificity, inhibitor sensitivity and amino-acid sequences, mammalian AR-like proteins can be divided into three types, namely AKR1B7-type, AKR1B8-type and AKR1B10.

Owing to the potential functional roles of the three types of AR-like proteins, it is important to compare the tertiary structures of these enzymes with AR in order to understand the structural reasons for their differences in enzymatic properties and the selectivity of AR inhibitors. Crystal structures of coenzyme–AR inhibitor complexes of AKR1B8 (Wilson *et al.*, 1995) and AKR1B10 (Gallego *et al.*, 2007) have been solved, but those of AKR1B7-type enzymes have yet to be determined. In order to elucidate the structure–function relationship of the AKR1B7-type enzymes, we have initiated a three-dimensional structure determination of recombinant rat AKR1B14. In this study, we report the first crystallization and preliminary X-ray analysis of AKR1B14.

2. Protein expression and purification

The cDNA for AKR1B14 (accession No. NM_053781) was obtained from a total RNA preparation of a female Wistar rat adrenal gland by reverse-transcription PCR using a pair of sense and antisense primers, 5′-GGGG**CATATG**ACAACCTTCG-3′ and 5′-TTTT**GGATCC**TCAGTATTCCTC-3′, which contained *Nde*I and *Bam*HI sites (in bold), respectively. The PCR products were ligated into pCold IV vectors (Takara) and the recombinant protein was expressed in *Escherichia coli* BL21 (DE3) pLysS cells (Invitrogen) using the protocol described previously for AKR1B13 (Endo *et al.*, 2009). The cells were harvested by centrifugation at 5000*g* for 5 min at 278 K, suspended in lysis buffer (0.1% Triton X-100, 5 m*M* 2-mercaptoethanol and 1 m*M* EDTA in 10 m*M* Tris–HCl pH 7.5) and sonicated at 150 W for 5 min using a UH-150 Ultrasonic homogenizer (SMT Co., Tokyo, Japan). The cell extract was obtained by centrifugation at 12 000*g* for 15 min and dialyzed against 10 m*M* Tris–HCl pH 8.0 containing 5 m*M* 2-mercaptoethanol, 0.5 m*M* EDTA and 20%(*v/v*) glycerol. The recombinant AKR1B14 was purified from the cell extract following the protocol used for the purification of AKR1B13 (Endo *et al.*, 2009), which included consecutive column-chromatographic steps on Q-Sepharose, Sephadex G-100 and Red-Sepharose (GE Healthcare Biosciences). SDS–PAGE of the purified enzyme revealed a single 36 kDa protein band on Coomassie Brilliant Blue staining. Finally, purified AKR1B14 was concentrated to 23 mg ml^{-1} by ultrafiltration for crystallization. The preparation of the *E. coli* cell extract and the purification of the enzyme were carried out at 278 K.

3. Crystallization and X-ray data collection

Crystals of the AKR1B14–NADPH binary complex were grown using the hanging-drop vapour-diffusion method (McPherson, 1985) at 295 K in crystallization buffer containing 0.1 *M* HEPES pH 7.5, 20% polyethylene glycol 4000 and 10% 2-propanol (Hampton Research Crystal Screen 1 condition No. 41). The final concentration of the protein in the binary complex was 22.6 mg ml^{-1}. Droplets were prepared by mixing 2 µl of the binary-complex solution (comprising AKR1B14 and NADPH in a 1:3 molar ratio) with an equal volume of crystallization buffer. The mixture was placed on siliconized cover slips and equilibrated at 295 K against 1 ml reservoir solution. Crystals were obtained within one week with a longest dimension of approximately 0.2 mm prior to X-ray diffraction analysis.

The crystal used for X-ray diffraction analysis was directly soaked in a cryoprotectant solution (20% ethylene glycol in the crystallization buffer) and flash-cooled at 100 K. Diffraction data were collected on a MAR345 image plate mounted on a Rigaku RU-300 rotating-anode X-ray generator operated at 50 kV and 90 mA. Each frame was recorded with 600 s exposure and 1.0° oscillation around φ. The crystal-to-detector distance was set to 150 mm so that the spots were well resolved. The data were processed and scaled using *HKL*-2000 and *SCALEPACK* (Otwinowski & Minor, 1997).

4. Results

AKR1B14 crystallized in space group $P2_1$, with unit-cell parameters $a = 50.66$, $b = 69.14$, $c = 72.27$ Å, $\beta = 96.4°$. The Matthews coefficient (V_M) was calculated to be 2.12 Å^3 Da^{-1}, assuming that two molecules (molecular weight 36 kDa) of AKR1B14 were present in the asymmetric unit, with an estimated 42% solvent content (Matthews, 1968). A near-complete set of data was collected from a single crystal to a resolution of 1.86 Å (data-collection statistics are given in Table 1).

Determination of the crystal structure of the rat AKR1B14 binary complex is currently being attempted by the molecular-replacement method using the coordinates of AR (PDB code 1pwl) as the starting model. If successful, this would be the first crystal structure deter-

mination of the AKR1B7-type AR-like protein, which has important functional roles (Kabututu *et al.*, 2009; Martinez *et al.*, 2001; Lefrancois-Martinez *et al.*, 1999). The structure will help in the elucidation of the catalytic mechanism of the enzyme and a comparison between this structure and those of AKR1B8, AKR1B10 and AR will reveal important information about their different structural features and their relationship to the functional roles of the enzymes. This information may also be useful in the development of selective AR inhibitors for the treatment of diabetic complications.

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