Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction analysis of mouse 3(17)α-hydroxysteroid dehydrogenase

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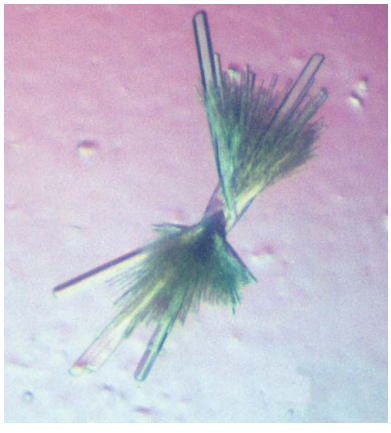
Received 26 May 2005
Accepted 10 June 2005
Online 23 June 2005



The 3(17)α-hydroxysteroid dehydrogenase from mouse is involved in the metabolism of oestrogens, androgens, neurosteroids and xenobiotic compounds. The enzyme was crystallized by the hanging-drop vapour-diffusion method in space group $P222_1$, with unit-cell parameters a = 84.91, b = 84.90, c = 95.83 Å. The Matthews coefficient (V_M) and the solvent content were 2.21 Å^3 Da^{-1} and 44.6%, respectively, assuming the presence of two molecules in the asymmetric unit. Diffraction data were collected to a resolution of 1.8 Å at the Swiss Light Source beamline X06SA using a MAR CCD area detector and gave a data set with an overall R_{merge} of 6.8% and a completeness of 91.1%.

1. Introduction

3(17)α-Hydroxysteroid dehydrogenase [3(17)α-HSD; EC 1.1.1.209] is a bifunctional enzyme that acts on 3α- and 17α-hydroxysteroids using NAD(P)$^+$ as the coenzyme. The enzyme has been purified from the kidneys of rabbit (Lau *et al.*, 1982) and mouse (Nakagawa *et al.*, 1989) and its suggested roles are the metabolism of oestrogens and androgens as well as the metabolism of xenobiotic carbonyl compounds and alicyclic alcohols. A recent study revealed that the mouse enzyme is expressed in the kidney and other tissues, including the brain, where it efficiently reduces 3-ketosteroids (5α-dihydrotestosterone, deoxycorticosterone and precursors of neuroactive progesterone derivatives) and 17-ketosteroids (dehydroepiandrosterone, dehydroepiandrosterone sulfate and oestrone; Ishikura *et al.*, 2004). Thus, 3(17)α-HSD plays a pivotal role in controlling the intracellular concentrations of various steroid hormones.

Mouse 3(17)α-HSD is a monomer with a molecular weight of 39 kDa and exists in two isoforms that show identical properties except for their isoelectric points (Nakagawa *et al.*, 1989). Cloning of cDNA for mouse 3(17)α-HSD revealed that the two isoforms of the enzyme belong to the aldo–keto reductase (AKR) family (Hyndman *et al.*, 2003) and differ by only five residues in their 323-amino-acid sequences (Ishikura *et al.*, 2004). The sequence of one isoform is identical to that of a protein (AKR1C21) predicted from a gene, Akr1C21 (Vergnes *et al.*, 2003), and the other isoform is believed to be a product from an unidentified gene. Mouse 3(17)α-HSD shares high sequence identity (69–76%) with 3α-HSDs of rat (AKR1C9), mouse (AKR1C14) and human (AKR1C2, AKR1C3 and AKR1C4), 20α-HSDs of rabbit (AKR1C5), rat (AKR1C8), mouse (AKR1C18) and human (AKR1C1), and mouse 17α-HSD type 5 (AKR1C6) in the AKR family. Most of the 3α-, 17α- and 20α-HSDs are bifunctional or multifunctional enzymes and exhibit low or moderate additional activities of 3α-, 3α-, 17α- and/or 20α-HSD (Deyashiki *et al.*, 1995; Penning *et al.*, 2000; Couture *et al.*, 2002; Steckelbroeck *et al.*, 2004; Ishikura, Nakajima, Kaneko *et al.*, 2005), but do not display the 17α-HSD activity of 3(17)α-HSD. The substrate specificity for alicyclic alcohols of mouse 3(17)α-HSD also differs from that of the other HSDs in the AKR family. 3(17)α-HSD specifically oxidizes the *R* forms of indan-1-ol and 1,2,3,4-tetrahydronaphthol (Nakagawa *et al.*, 1989; Ishikura, Nakajima, Carbone *et al.*, 2005), whereas the other HSDs show specificity for their *S* forms. A site-directed mutagenesis study suggested that Thr270 of mouse 3(17)α-HSD contributes to the dual coenzyme specificity as well as the stereospecificity for the *R* forms of the alicyclic alcohols (Ishikura, Nakajima, Carbone *et al.*,

Table 1
Data-collection statistics for AKR1C21.

Statistics for the highest resolution shell are given in parentheses.

X-ray source	SLS beamline X06SA
Detector	MAR CCD
Wavelength (Å)	1.00002
Space group	$P222_1$
Unit-cell parameters (Å)	a = 84.91, b = 84.90, c = 95.83
Resolution range (Å)	50.0–1.80 (2.0–1.8)
Measured reflections	195019 (48362)
Unique reflections	59131 (14752)
Completeness (%)	91.1 (84.8)
$R_{\rm merge}$† (%)	6.8 (47.5)

† $R_{\rm merge} = \sum I - \langle I \rangle | / \sum I$, where I is the intensity measurement for a given reflection and $\langle I \rangle$ is the average intensity for multiple measurements of this reflection.

2005). Crystallographic studies of 3α-HSDs [AKR1C2 (Jin *et al.*, 2001), AKR1C3 (Lovering *et al.*, 2004) and AKR1C9 (Bennett *et al.*, 1997)] and 20α-HSDs [AKR1C1 (Couture *et al.*, 2003) and AKR1C5 (Couture *et al.*, 2004)] have led to proposed reaction mechanisms and substrate-binding residues, but the crystal structure of 3(17)α-HSD has yet to be determined. To elucidate the unique substrate recognition of mouse 3(17)α-HSD based on its tertiary structure, we have initiated a crystallographic analysis of the recombinant isoform (AKR1C21).

2. Expression, purification and crystallization

The cDNA for AKR1C21 was obtained by reverse-transcription PCR from the total RNA of an ICR mouse kidney. The cDNA coding for the protein was inserted into pkk223-3 plasmids and expressed in *Escherichia coli* JM109 following published methodology (Ishikura *et al.*, 2004). The recombinant enzyme was then purified to homogeneity from the cell extract as described previously (Nakagawa *et al.*, 1989). SDS–PAGE indicated one band at around 39 kDa corresponding to the enzyme. The enzyme in buffer *A* (10 m*M* Tris–HCl pH 7.5 containing 2 m*M* 2-mercaptoethanol) was used to screen for crystallization conditions.

Crystallization conditions were determined by the hanging-drop vapour-diffusion method (McPherson, 1985) using Hampton Research Crystal Screen 2 (Hampton Research, Laguna Niguel, CA, USA). Crystals were obtained from condition No. 30 (0.1 *M* HEPES pH 7.5, 10% polyethylene glycol 6000, 5% 2-methyl-2,4-pentanediol). Droplets were prepared by mixing 3 µl of the protein (16 mg ml^{-1}) and NADPH in buffer *A* (the molar ratio of the enzyme to NADPH was 1:3) with 3 µl reservoir solution (0.1 *M* HEPES pH 7.5, 10% polyethylene glycol 6000, 5% 2-methyl-2,4-pentanediol). The mixture was placed on siliconized cover slips and equilibrated against 1 ml reservoir solution at a temperature of 295 K. Crystals grew within one week, with a longest dimension of approximately 0.3 mm (Fig. 1).

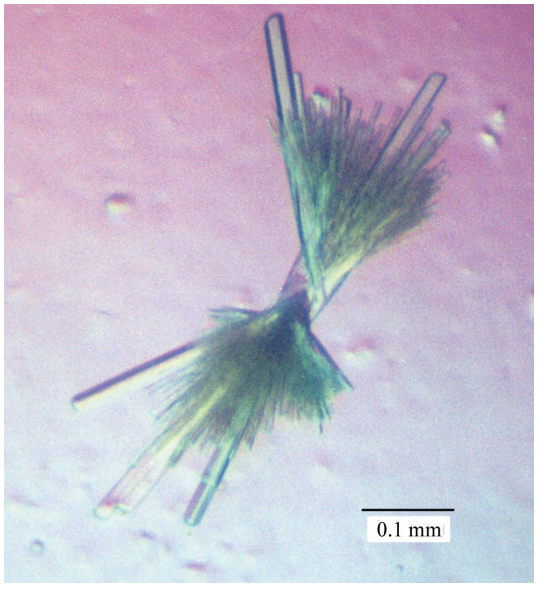


Figure 1
Crystals of AKR1C21.

3. Data collection

An AKR1C21 crystal was picked up from a droplet with a nylon loop (Hampton Research, Laguna Niguel, CA, USA), transferred to a cryoprotectant solution (0.1 *M* HEPES pH 7.5, 25% polyethylene glycol 6000, 10% 2-methyl-2,4-pentanediol) and then placed directly into a cold nitrogen-gas stream at 100 K. X-ray diffraction data collection was then carried out with a MAR CCD detector using synchrotron radiation of wavelength 1.00002 Å at beamline X06SA of the Swiss Light Source (SLS). Each frame was recorded with a 3 s exposure and steps of 0.4° oscillation around φ. The crystal-to-detector distance was set at 120 mm so that the spots were well resolved. The data was processed using *XDS* and scaled using *XSCALE* (Kabsch, 1993).

4. Results

A 91.1% complete data set was collected to a resolution of 1.8 Å (data-collection statistics are shown in Table 1). AKR1C21 crystallized in space group $P222_1$, with unit-cell parameters a = 84.91, b = 84.90, c = 95.83 Å (scaling of the data assuming a tetragonal space group resulted in a significant increase in the value of $R_{\rm merge}$). Assuming that two molecules (MW = 39000 Da) are present in the asymmetric unit and assuming space group $P222_1$, the Matthews coefficient ($V_{\rm M}$) was 2.21 Å^3 Da^{-1}, with an estimated solvent content of 44.6% (Matthews, 1968). The calculation of a self-rotation function (Brünger *et al.*, 1990) using data between 15 and 4 Å displayed a peak equal to 34% of the origin peak at ψ = 90, φ = 45 and κ = 180.0°, confirming the presence of two molecules in the asymmetric related by a non-crystallographic twofold axis.

AKR1C21 shares high sequence identity (69–73%) with 3α-HSDs (AKR1C2 and AKR1C9) and 20α-HSD (AKR1C1), the crystal structures of which have been determined (Jin *et al.*, 2001; Bennett *et al.*, 1997; Couture *et al.*, 2003). The molecular-replacement method using the crystal coordinates of these homologous proteins will be used in an attempt to determine the crystal structure of AKR1C21. The structure of AKR1C21 will be the first tertiary structure determined for this protein and will be used to elucidate the catalytic mechanism for this bifunctional enzyme that acts on both 3α- and 17α-hydroxysteroids and possesses a dual coenzyme NAD(P)$^+$ specificity.

This work was supported by an ARC Linkage International Award. We thank Vincenzo Carbone and Connie Darmanin for their assistance with the preparation of the protein sample for crystallization.

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