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# Crystallization and preliminary crystallographic analysis of human L-xylulose reductase

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Human L-xylulose reductase was crystallized from buffered polyethylene glycol solutions using the hanging-drop vapour-diffusion method. The crystals diffract to 2.1 Å resolution and belong to the orthorhombic *P*222 space group, with unit-cell parameters $a = 72.9$, $b = 74.1$, $c = 87.9$ Å. This is the first crystallization report of a xylulose reductase that is identical to diacetyl reductase.

## 1. Introduction

L-Xylulose reductase (EC 1.1.1.10) is an enzyme of the uronate cycle that accounts for about 5% of the total glucose metabolism per day in humans (Winegrad *et al.*, 1967). The enzyme has been partially purified from guinea-pig liver and is known to specifically catalyze the oxidoreduction between xylitol and L-xylulose using NADP(H) as coenzyme (Hickman & Ashwell, 1958; Arenis & Touster, 1969). Recently, a cDNA for hamster L-xylulose reductase has been cloned (Ishikura *et al.*, 2001) and the corresponding amino-acid sequence shows 67% identity with mouse lung carbonyl reductase (MLCR; Nakanishi *et al.*, 1995), which belongs to the short-chain dehydrogenase/reductase (SDR) superfamily (Jörnvall *et al.*, 1995; Tanaka *et al.*, 2001). The recombinant hamster L-xylulose reductase is a tetramer composed of 26 kDa subunits. The hamster enzyme exhibits broad substrate specificity for several pentoses, tetroses, trioses and $\alpha$-dicarbonyl compounds, such as diacetyl, methylglyoxal and 2,3-pentanedione, although L-xylulose and diacetyl are the best substrates for the enzyme. The $\alpha$-dicarbonyl compounds are formed in the tissues or ingested as components of foods and beverages (Thornalley, 1996; Otsuka *et al.*, 1996), exhibiting direct mutagenic activity in the Ames test against *Salmonella typhimurium* strains (Bjeldanes & Chew, 1979; Marnett *et al.*, 1985; Dorado *et al.*, 1992), and are involved in the formation of advanced glycation end products (Thornalley, 1996). L-Xylulose reductase also moderately reduces threoses, which generate superoxide anions (Ortwerth *et al.*, 1998). Thus, L-xylulose reductase may act as a detoxification enzyme towards the reactive $\alpha$-dicarbonyls and short-chain sugars.

The molecular weight and substrate specificity of hamster L-xylulose reductase for $\alpha$-dicarbonyl compounds are similar to those of mammalian diacetyl reductases (Díez *et al.*, 1974; Provecho *et al.*, 1984; Hara *et al.*, 1985) and chicken D-erythrulose reductase (Maeda *et al.*, 1998). The identity of L-xylulose reductase with diacetyl reductase (EC 1.1.1.5), which reduces diacetyl (2,3-butanedione) to acetoin (3-hydroxy-2-butanone), has been demonstrated by copurification of the two enzymes from hamster and guinea-pig livers (Ishikura *et al.*, 2001; Nakagawa *et al.*, 2002).

L-Xylulose reductases with almost the same properties as the hamster enzyme have been cloned and expressed from humans, guinea pigs, rats and mice (Nakagawa *et al.*, 2002). Tissue-distribution and immunohistochemical studies indicate that the enzyme is highly expressed in kidney and liver and is localized in the brush-border membranes of proximal tubular cells of mouse kidney. These findings suggest that L-xylulose reductase in renal tubules plays a role in the production of xylitol, which has been shown to act as an osmolyte in the airway surfaces of the lung (Zebner *et al.*, 2000). The local accumulation of the osmolyte xylitol in the brush-border membrane may be responsible for water absorption in the proximal tubules as well as cellular osmoregulation against osmolytic stress occurring in the tubules.

The amino-acid sequence of human L-xylulose reductase consists of 244 residues and was found to be identical to that of human sperm protein P34H (Légaré *et al.*, 1999), except for one amino-acid substitution (G239R). The replaced amino acid in P34H was outside the substrate-binding site in the modelled structure of human L-xylulose reductase (Nakagawa *et al.*, 2002) and should not affect their enzymatic functions involving the metabolism of dicarbonyl compounds and glucose.

Mammalian L-xylulose reductases share 62–67% sequence identity with MLCR (Nakanishi *et al.*, 1995), but the substrate specificity is distinct in the two enzymes. MLCR shows broader substrate specificity for various carbonyl compounds than L-xylulose reductase, but does not reduce sugars or oxidize xylitol. To elucidate the reaction

mechanism of L-xylulose reductase based on its tertiary structure, we have initiated a three-dimensional structure analysis of the recombinant enzyme from human. In this study, we present the first report of the crystallization and preliminary X-ray analysis of human L-xylulose reductase.

## 2. Experimental

### 2.1. Expression and purification

The cDNA for human L-xylulose reductase was obtained by reverse-transcription PCR from human kidney mRNA. The cDNA coding for the protein was inserted into pRset plasmids and expressed in *Escherichia coli* BL21 (DE3). The recombinant enzyme was then purified from the cell extract as described previously (Nakagawa *et al.*, 2002). SDS–PAGE indicated one band at around 30 kDa corresponding to the enzyme. The enzyme (18 mg ml$^{-1}$) in buffer *A* (10 m*M* Tris–HCl pH 7.5 containing 2 m*M* 2-mercaptoethanol and 20% glycerol) was subjected to buffer replacement with buffer *B* (10 m*M* Tris–HCl pH 7.5, 2 m*M* 2-mercaptoethanol) prior to use in crystallization. This was carried out in a 10 kDa Ultrafree-4 centrifugal filter unit (Millipore) by repeated cycles of centrifugal concentration [3200*g* with a 2704 rotor in a Megafuge 1.0R centrifuge (Heraeus)] and dilution with buffer *B*. On completion, the enzyme at a concentration of 17 mg ml$^{-1}$ was used in the crystallization.

### 2.2. Crystallization and X-ray data collection

Crystals of human L-xylulose reductase were grown at 295 K by vapour diffusion (McPherson, 1985). 158 µl of the enzyme (17 mg ml$^{-1}$) in buffer *B* was mixed with 16 µl of 12.9 m*M* NADPH. Each droplet consisted of 3 µl of the enzyme–NADPH mixture (the molar ratio of enzyme to NADPH was 1:8) mixed with a matching volume (3 µl) of solution from the well (15% PEG 8000, 0.05 *M* potassium phosphate and 0.1 *M* MES buffer pH 6.5). Crystals grew within one week to average dimensions of approximately 0.3 × 0.3 × 0.05 mm (Fig. 1). Diffraction data from one crystal was recorded at room temperature (291 K) 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 a 600 s exposure and 1° oscillation around $\varphi$. The crystal-to-detector distance was set at 190 mm so that the spots were well resolved. The data was processed and scaled using the *HKL* software package (Otwinowski & Minor, 1997).

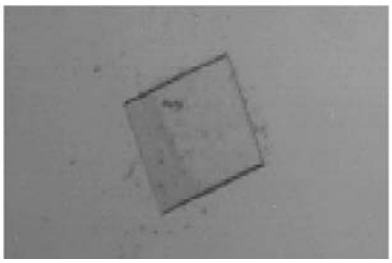

**Figure 1**
Crystal of human L-xylulose reductase. The dimensions of the crystal in this photograph are 0.3 × 0.3 × 0.05 mm.

**Table 1**
X-ray data-collection statistics.

Values in parentheses refer to the highest resolution shell, 2.17–2.10 Å.

| | |
|---|---|
| Space group (crystal system) | *P*222 (orthorhombic) |
| Unit-cell parameters | |
| *a* (Å) | 72.9 |
| *b* (Å) | 74.1 |
| *c* (Å) | 87.9 |
| Resolution† (Å) | 20.0–2.1 (2.17–2.1) |
| Observed reflections | 131758 |
| Unique reflections | 28300 (2688) |
| $R_{\rm merge}$† (%) | 8.1 (15.5) |
| Completeness (%) | 99.4 (96.2) |
| $I/\sigma(I)$ | 13.2 (5.5) |
| Averaged redundancy | 4.7 (2.5) |

† $R_{\rm merge} = (\sum |I_i - \langle I \rangle| / \sum I_i)/100$, where $I_i$ is an individual intensity observation, $\langle I \rangle$ is the mean intensity for that reflection and the summation is over all reflections.

## 3. Results

A near-complete data set was collected to a resolution of 2.1 Å (data-collection statistics are shown in Table 1). Human L-xylulose reductase crystallized in the *P*222 space group, with unit-cell parameters *a* = 72.9, *b* = 74.1, *c* = 87.9 Å. Assuming that two molecules (MW = 26500 Da) are present in the asymmetric unit and a space group of *P*222, the Matthews coefficient ($V_{\rm M}$) was calculated to be 2.24 Å$^3$ Da$^{-1}$, with an estimated solvent content of 45% (Matthews, 1968).

Human L-xylulose reductase (accession number AB013846) shares 67% sequence identity with MLCR (Nakanishi *et al.*, 1995), whose crystal structure has been published (Tanaka *et al.*, 1996) and which belongs to the SDR superfamily (Jörnvall *et al.*, 1995; Tanaka *et al.*, 2001). The molecular-replacement method using the coordinates of MLCR will be used in an attempt to determine the crystal structure of human L-xylulose reductase. The structure of human L-xylulose reductase will be the first tertiary structure determined for a xylulose reductase as well as a diacetyl reductase and will be used to elucidate the catalytic mechanism for the enzyme. Additionally, a comparison between the structure of human L-xylulose reductase and that of MLCR will reveal very important information regarding the different structural features between their active sites that account for their distinct substrate specificities.

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