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Crystallization and preliminary X-ray diffraction analysis of monkey dimeric dihydrodiol dehydrogenase

Ossama El-Kabbani,[a]* Roland P.-T. Chung,[a] Paul A. Ramsland,[a] Yukuhiko Asada,[b] Syuhei Ishikura,[b] Noriyuki Usami[b] and Akira Hara[b]

[a]Department of Medicinal Chemistry, Victorian College of Pharmacy, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia, and [b]Laboratory of Biochemistry, Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan

Correspondence e-mail: ossama.el-kabbani@vcp.monash.edu.au

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Dihydrodiol dehydrogenase catalyzes the NADP$^+$-linked oxidation of *trans*-dihydrodiols of aromatic hydrocarbons to corresponding catechols and exists in multiple forms in mammalian tissues. The dimeric form of mammalian dihydrodiol dehydrogenase has a primary structure distinct from the previously known mammalian enzymes and may constitute a novel protein family with the prokaryotic proteins. Monkey kidney dimeric dihydrodiol dehydrogenase was crystallized from buffered ammonium phosphate solution using the hanging-drop vapour-diffusion method. The crystals diffract to 2.65 Å resolution in the laboratory and belong to the hexagonal $P6_122$ or $P6_522$ space group, with unit-cell parameters $a = b = 122.8$, $c = 121.3$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$.

1. Introduction

Dihydrodiol dehydrogenase (DD; E.C. 1.3.1.20) oxidizes *trans*-dihydrodiols of aromatic hydrocarbons to the corresponding catechols in the presence of NADP$^+$ as coenzyme. The various forms of the enzyme have been classed into either monomeric or dimeric enzymes. The quantitatively predominant monomeric forms isolated from various mammalian tissues are found to be identical to 3α- and 17β-hydroxysteroid dehydrogenases as well as aldehyde and aldose reductases (Wörner & Oesch, 1984; Hara *et al.*, 1991, 1996; Deyashiki *et al.*, 1995), members of the aldo–keto reductase superfamily (Bohren *et al.*, 1989; Jez *et al.*, 1997). Thus, DD plays a role not only in the metabolism of xenobiotic hydrocarbons but also in the metabolism of endogenous steroids and prostaglandins. Dimeric DD composed of 36 kDa subunits has been isolated from rabbit lens, pig tissues, dog liver and monkey kidney; it differs from the monomeric DDs in its inability to oxidize hydroxysteroids and reduce aldoses and glucuronic acid (Hara *et al.*, 1987, 1991; Nakayama *et al.*, 1991; Sato *et al.*, 1994).

A recent study indicated that dimeric DD catalyzes the oxidation of several pentoses and is identical to NADP$^+$-dependent D-xylose dehydrogenase (Aoki *et al.*, 2001). Dimeric DD shows no significant sequence similarity with members of the aldo–keto reductase superfamily or the short-chain dehydrogenase/reductase family, although it reduces various carbonyl compounds similarly to carbonyl reductase, a member of the short-chain reductase/dehydrogenase family (Jörnvall *et al.*, 1995). Dimeric DD shares sequence similarities (14–25% identity) with putative gene products of microorganisms and *Zymomonas mobilis* glucose–fructose oxidoreductase. This finding suggests that dimeric DD is a member of a novel protein family which includes proteins from prokaryotic organisms (Arimitsu *et al.*, 1999).

While recombinant dimeric DD has been successfully expressed and two residues of the enzyme have been suggested to be involved in the catalytic function (Asada *et al.*, 2000), the crystal structure of the enzyme has yet to be determined. To elucidate the reaction mechanism of the dimeric DD based on its tertiary structure, we have initiated a three-dimensional structure analysis of the recombinant enzyme from Japanese monkey (*Macaca fuscata*) kidney. Moreover, a comparison of the crystal structure of dimeric DD from the monkey with the structure of glucose–fructose oxidoreductase from *Z. mobilis* will reveal very important information regarding the common structural features between mammalian and prokaryotic members of a novel protein family. In this study, we present the first report of the crystallization and preliminary X-ray analysis of a dimeric DD.

2. Experimental

2.1. Expression and purification

The recombinant dimeric DD of Japanese monkey kidney was expressed in an *Escherichia coli* system as described previously (Asada *et al.*, 2000) and purified from the cell

Figure 1
Crystals of monkey dimeric DD. The dimensions of the largest crystal in this photograph are 0.6 × 0.15 × 0.15 mm.

extract as described previously (Hara *et al.*, 1987; Arimitsu *et al.*, 1999). About 10 mg of the purified enzyme, which showed 98% purity based on SDS–PAGE analysis, was initially concentrated by ultrafiltration using an Amicon YM-10 membrane. The enzyme concentrate (at 9 mg ml^{-1}) in buffer *A* (10 m*M* Tris–HCl pH 8.5 containing 5 m*M* 2-mercaptoethanol, 0.5 m*M* EDTA and 20% glycerol) was subjected to buffer replacement with buffer *B* (10 m*M* Tris–HCl pH 8.5, 2 m*M* 2-mercaptoethanol) prior to use for crystallization. This was carried out in a 10 kDa Microsep concentrator (Pall Gelman) by repeated cycles of centrifugal concentration (7000*g* with a JA25.15 rotor in an Avanti J-25 centrifuge; Beckman) and dilution with buffer *B*. The enzyme was finally concentrated in a Microcon concentrator (Millipore) to a volume of 309 µl and a concentration of 18.5 mg ml^{-1}.

2.2. Crystallization and X-ray data collection

Crystals of dimeric DD were grown at 295 K by vapour diffusion (McPherson, 1985). The enzyme solution (309 µl) in buffer *B* was mixed with 41 µl of 7.15 m*M* NADPH. Each droplet consisted of 3 µl of the enzyme–NADPH mixture (the molar ratio of enzyme to NADPH was 1:4) mixed with a matching volume (3 µl) of solution from the well (1.5 *M* ammonium phosphate, 0.1 *M* sodium citrate buffer pH 5.6). Crystals grew within 2 d to average dimensions of approximately 0.6 × 0.15 × 0.15 mm (Fig. 1). The crystals were picked up with a nylon loop and flash-cooled at 105 K in a stream of gaseous nitrogen. Diffraction data from one flash-cooled crystal (105 K) were recorded 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 1800 s exposure and 1° oscillation around φ. The crystal-to-detector distance was set at 250 mm so that the spots were well resolved and room temperature was set to 291 K during data collection. The *HKL* software package (Otwinowski & Minor, 1997) was used for data processing and scaling.

3. Results

A near-complete data set was collected to a resolution of 2.65 Å (data-collection statistics are shown in Table 1). Dimeric DD crystallized in the $P6_122$ or $P6_522$ (reflection conditions $00l$: $l = 6n$) space group, with unit-cell parameters $a = b = 122.8$, $c = 121.3$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. The calculation of a self-rotation function using the *X-PLOR* package (Brünger *et al.*, 1990) suggests that the molecules of the dimer may be related by a twofold axis which is perfectly aligned with the crystallographic axis. Additionally, the scaling of the data assuming space group $P6_1$ or $P6_5$ did not result in any significant decrease in the value of $R_{\rm merge}$. Assuming that one molecule (MW = 36 000 Da) is present in the asymmetric unit and the space group is $P6_122$ or $P6_522$, the Matthews coefficient ($V_{\rm M}$) was calculated to be 3.50 Å^3 Da^{-1} and the estimated solvent content was 65%.

Monkey dimeric DD shares 20% sequence identity with glucose–fructose oxidoreductase from *Z. mobilis*, the crystal structure of which has been published (Kingston *et al.*, 1996). The molecular-replacement method using the coordinates of glucose–fructose oxidoreductase is currently being used in an attempt to determine the crystal structure of dimeric DD. However, because of the low sequence identity between the two enzymes, it is likely that a search for heavy-atom derivatives will be necessary to determine the structure of dimeric DD by the multiple isomorphous replacement method. The structure of monkey dimeric DD will be the first tertiary structure determined for a dimeric form of DD and will be used to elucidate the catalytic mechanism of the enzyme. Additionally, a comparison between the structure of monkey dimeric DD and the structure of glucose–fructose oxidoreductase from *Z. mobilis* will reveal very important information regarding the common structural features between mammalian and prokaryotic members of a novel protein family.

Table 1
X-ray data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P6_122$ or $P6_522$
Unit-cell parameters	
$a = b$ (Å)	122.8
c (Å)	121.3
$\alpha = \beta$ (°)	90.0
γ (°)	120.0
Resolution (Å)	20.0–2.65 (2.74–2.65)
Observed reflections	149086
Unique reflections	29882 (2736)
$R_{\rm merge}$† (%)	8.5 (29.4)
Completeness (%)	99.0 (90.9)
$I/\sigma(I)$	10.8 (3.9)
Averaged redundancy	5.0 (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.

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