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Crystallization and preliminary X-ray crystallographic studies of 7α-hydroxysteroid dehydrogenase from *Escherichia coli*

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Abstract

Crystals of 7α-hydroxysteroid dehydrogenase from *E. coli*, which is a tetramer in its active form, have been obtained by a hanging-drop vapor-diffusion method in the presence of the coenzyme NAD^+. Crystals as large as 0.25 × 0.25 × 0.75 mm could be grown within a month at pH 8.5 with polyethylene glycol as precipitating agent. Preliminary X-ray crystallographic analysis revealed that they belong to one of the enantiomorphic space groups $P4_12_12$ or $P4_32_12$ with dimensions $a = b = 81.66$ and $c = 214.6$ Å, having two subunits in an asymmetric unit. The crystals diffract to at least 2.3 Å resolution.

1. Introduction

7α-Hydroxysteroid dehydrogenase (7α-HSDH; E.C. 1.1.1.159), the most abundant hydroxysteroid dehydrogenase in human intestinal flora, has been found in numerous genera of bacteria and in mammalian liver as well. It is involved in the metabolism of the primary bile acids (cholic acid and chenodeoxycholic acid); it catalyzes dehydrogenation of the hydroxyl group at position 7 of the steroid skeleton and yields oxo bile acids. Two kinds of 7α-HSDH have been cloned, sequenced, and characterized. One is the NAD^+-dependent 7α-HSDH from *E. coli* HB101 (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991; Yoshimoto, Nagai, Ito & Tsuru, 1993) and the other is the $NADP^+$-dependent 7α-HSDH from *Eubacterium sp.* strain VPI12708 (Baron, Franklund & Hylemon, 1991). Each of them is a homo-tetrameric enzyme having a similar molecular weight and exhibits a similar substrate specificity. The deduced amino-acid sequences of the enzymes show that both of them belong to the short-chain dehydrogenase family (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991; Baron, Franklund & Hylemon, 1991). More than 50 known enzymes, most of which exhibit mutually different substrate specificities, belong to the short-chain family. The substrates are alcohol, glucose, 15-hydroxyprostaglandin, several hydroxysteroids and so forth. Like the medium-chain enzymes, each short-chain enzyme is a homo-dimer or a homo-tetramer and employs an NAD or an NADP molecule as a cofactor, but, unlike the medium-chain enzymes, each short-chain enzyme binds no Zn atoms. Each subunit, comprising about 250 amino-acid residues, is divided into the N-terminal coenzyme-binding domain and the C-terminal catalytic domain (Persson, Krook & Jörnvall, 1991).

In order to elucidate the common catalytic mechanism and the origin of differences among substrate specificities of the enzymes belonging to the short-chain dehydrogenase family, we have begun crystallographic studies on 7α-HSDH from *E. coli* (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991; Yoshimoto, Nagai, Ito & Tsuru, 1993). In this paper we report the crystallization and preliminary X-ray crystallographic studies on this enzyme.

2. Materials and methods

2.1. *Crystallization*

7α-Hydroxysteroid dehydrogenase from *E. coli* (7α-HSDH) was overexpressed, purified, and lyophilized as described before (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991). The 7α-HSDH enzyme, in its active form, is a tetramer of identical subunits, each of which comprises 255 amino-acid residues and has a molecular mass of about 27 kDa. A factorial approach for crystallization using the vapor-diffusion method (Jancarik & Kim, 1991) resulted in small crystals. We then optimized the crystallization conditions so as to obtain large crystals. Crystals suitable for crystallographic analysis were grown using a typical 'hanging-drop' method (McPherson, 1982) at 293 K. The droplet solutions were prepared by mixing the protein, reservoir and detergent solutions, bufferred by 100 m*M* Tris (pH 8.5), at the ratio of 4:3:1. The protein solution contained 30 mg ml^{-1} 7α-HSDH and 4 mg ml^{-1} NAD^+; the reservoir solution, 28%(*w*/*v*) polyethylene glycol with mean molecular weight of 6000 and 200 m*M* sodium acetate; the detergent solution, 0.8%(*w*/*v*) β-octylglucoside. NAD^+ and β-octylglucoside were purchased from Sigma Chemical Company (St Louis, MO, USA) and Dojindo Laboratories (Kumamoto, Japan), respectively. The other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. *X-ray analysis*

A crystal was mounted in a silanized thin-walled glass capillary following the usual method. X-ray data collection was performed by an oscillation method at 293 K using a Rigaku R-AXIS IIc area detector with Cu $K\alpha$ radiation, which was generated by a Rigaku RU200 rotating-anode generator operating at 45 kV and 110 mA and focused by a Supper double-focusing mirror. Laue group and unit-cell parameters were determined by data-processing software (*PROCESS*) attached to the R-AXIS system. The best diffraction data from a native crystal was collected up to 2.3 Å resolution with the longest crystal edge aligned nearly parallel to the spindle

axis. The data set was composed of 23 frames of diffraction images, each of which was recorded with an exposure time of 120 min and an oscillation range of 2.2°. The diffraction data was processed with the program *PROCESS*.

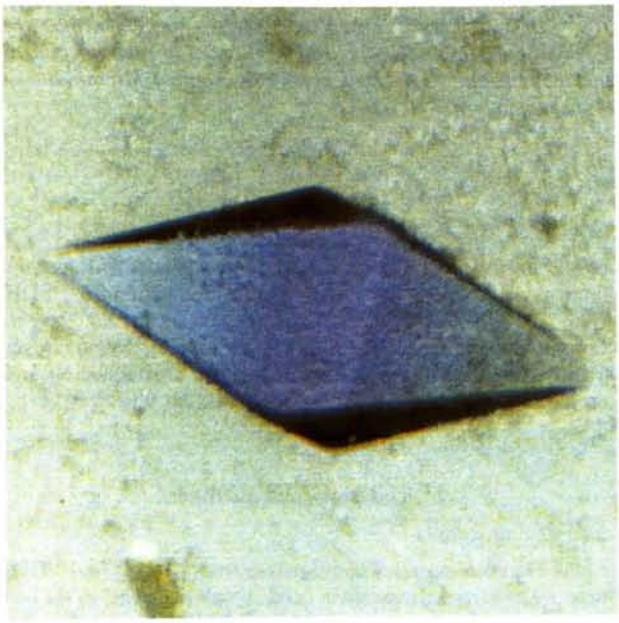

Fig. 1. A tetragonal crystal of 7α-hydroxysteroid dehydrogenase from *E. coli*. The size is *ca* 0.25 × 0.25 × 0.75 mm (*c* axis).

3. Results and discussion

Crystals of the apoenzyme originally obtained by ammonium sulfate precipitation during purification (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991) were found to be quite unsuitable for X-ray structure analysis because of their crystallographic disorder. Crystals grown under the optimized conditions as described above were found to be of good quality. The present crystal is most probably the holoenzyme like a number of other NAD^+-dependent dehydrogenases whose crystal structures have been solved. Thus, 3α, 20β-hydroxysteroid dehydrogenase (Ghosh, Wawrzak, Weeks, Duax & Erman, 1994) and dihydropteridine reductase (Varughese, Skinner, Whiteley, Matthews & Xuong, 1992), which are members of the short-chain dehydrogenase family, were each co-crystallized with NADH. The crystal structure analyses of these enzymes have revealed that they are in fact holo-enzymes. The present crystal is a tetragonal bipyramid and typically grew up to 0.25 × 0.25 × 0.75 mm within a month (Fig. 1). In order to obtain large crystals, it was essential to add β-octylglucoside to the droplet solutions, although no such detergents were used for the original isolation of the enzyme (Yoshimoto, Higashi, Kanatani, Xu, Nagai, Oyama, Kurazono & Tsuru, 1991).

The unit-cell dimensions determined with the program *PROCESS* were $a = b = 81.66$ and $c = 214.6$ Å. Systematic absences were observed along the a^* and c^* axes: only the reflections with $h = 2n$ and $l = 4n$ were observed along the $(h00)$ and $(00l)$ axes, respectively, indicating one of the enantiomorphic space groups $P4_12_12$ or $P4_32_12$. An assumption of two subunits per asymmetric unit leads

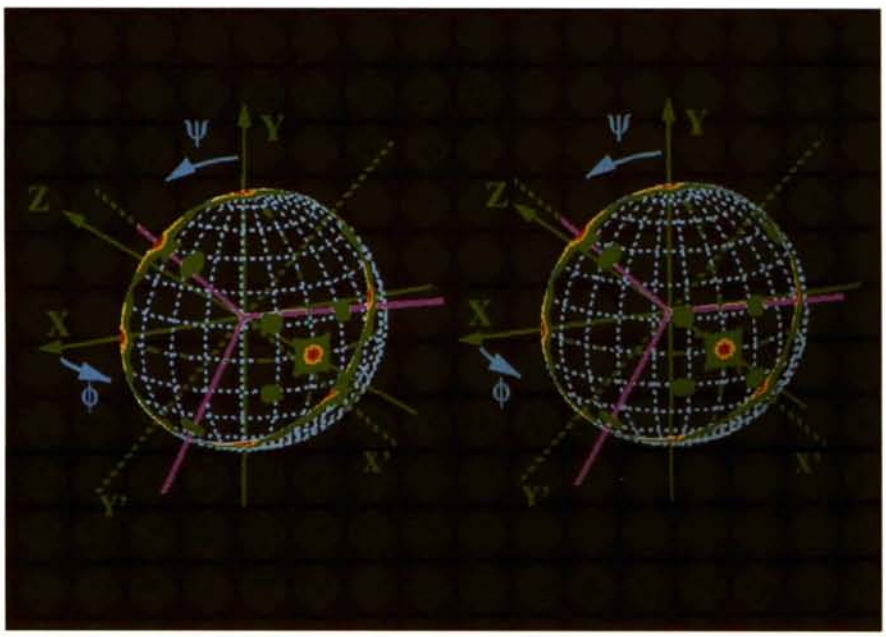

Fig. 2. Stereo pair of a spherical contour plot showing the result of the self-rotation function calculations for the 7α-hydroxysteroid dehydrogenase crystal. The calculations with the program *X-PLOR* employing the integration radii of 30.0–5.0 Å were carried out using the reflections within the range of 10.0–2.5 Å. The spherical polar angle κ was fixed at 180°; the φ and ψ angles were varied from 0 to 180° with the increment of 1°. The regions where the values are more than 2σ above the average are drawn in red. Yellow regions indicate the peaks between 1σ and 2σ above the average; green regions, between the average value and 1σ above the average. The latitude and longitude lines are shown at an interval of 15°. While each solid green line (labeled *X*, *Y*, *Z*) coincides with the direction of the crystallographic twofold screw axes, each dotted green line (labeled *X'*, *Y'*) coincides with the direction of the crystallographic twofold rotation, as well as, screw axes. A set of deduced non-crystallographic twofold rotation axes are shown in magenta.

Table 1. *Data-collection statistics for the 7α-HSDH crystal*

Resolution limit (Å)	2.30
Number of observed reflections	79500
Number of unique reflections collected	28146
Completeness (%)	84.9
Merging R factor based on intensity (%)	7.29

to an empirically acceptable V_m value of 3.34 $Å^3\,Da^{-1}$, corresponding to a solvent content of 63% (Matthews, 1968). If this were true, the tetrameric molecule having a 222 point-group symmetry would have one of its twofold rotation axes coincided with one of the crystallographic twofold rotation axes. As a result of careful examination of self-rotation functions calculated with *X-PLOR* (Brünger, Kuriyan & Karplus, 1987), the remaining two non-crystallographic twofold rotation axes were recognized (Fig. 2). The directions of the three twofold rotation axes comprising the molecular 222 point-group symmetry can be uniquely assigned to a set of three peaks found in the figure; the φ and ψ angles of the peaks are (0, 45°), (33, 130°) and (108, 73°), respectively. As expected, the direction (0, 45°) coincides with one of the crystallographic twofold rotation axes and the three directions are mutually perpendicular to each other.

The native crystal did not show any sign of decay during data collection up to 2.3 Å resolution. Statistical details of the data set is presented in Table 1. A search for heavy-atom derivatives intended for phasing by multiple isomorphous replacement is underway.

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