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Crystallization and preliminary X-ray analysis of the complex of NADH and 3α-hydroxysteroid dehydrogenase from *Pseudomonas* sp. B-0831

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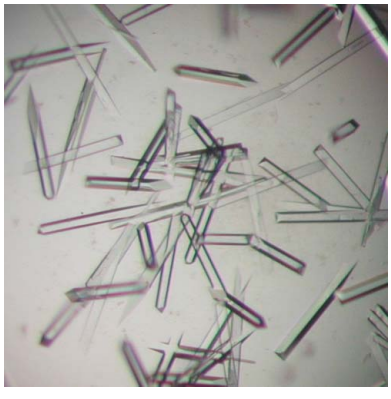


The $NAD(P)^+$-dependent enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD) catalyzes the reversible interconversion of hydroxyl and oxo groups at position 3 of the steroid nucleus. The complex of NADH and 3α-HSD from *Pseudomonas* sp. B-0831 was crystallized by the hanging-drop vapour-diffusion method. Refinement of crystallization conditions with microseeding improved the quality of the X-ray diffraction data to a resolution of 1.8 Å. The crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 62.46, b = 82.25, c = 86.57 Å, and contained two molecules, reflecting dimer formation of 3α-HSD, in the asymmetric unit.

1. Introduction

3α-Hydroxysteroid dehydrogenase (3α-HSD) oxidizes the hydroxyl group at position 3 of the steroid nucleus in the presence of $NAD(P)^+$ and conversely reduces the corresponding oxo group in the presence of NAD(P)H. 3α-HSDs have been found in many microorganisms. Prokaryotic 3α-HSDs belong to the short-chain dehydrogenase/reductase (SDR) superfamily (EC 1.1.1.50), which are dimeric or tetrameric enzymes containing a Rossmann nucleotide-binding fold (Bennett *et al.*, 1997). 3α-HSDs can also catalyze the carbonyl reduction of non-steroidal ketones and aldehydes (Möbus & Maser, 1998; Ueda *et al.*, 2004*a*) and are supposed to participate in steroid degradation by utilizing steroids as the sole carbon source. In clinical diagnostics, prokaryotic 3α-HSD has been used for the measurement of total bile acids in serum (Oda *et al.*, 1989) and has been developed as a highly sensitive and commercially available reagent on the basis of the unique enzyme-cycling method (Ueda *et al.*, 2004*b*).

We previously cloned 3α-HSD from *Pseudomonas* sp. B-0831 and expressed it in *Escherichia coli* (Suzuki *et al.*, 1993). The enzyme from *Pseudomonas* sp. B-0831 forms a homodimer comprised of two 26 kDa monomer proteins and its amino-acid sequence shares about 50% identity with that from *Comamonas testosteroni* ATCC11996, which has been studied extensively among bacterial 3α-HSDs (Maser *et al.*, 2000; Hwang *et al.*, 2005). The crystal structures of 3α-HSD from *C. testosteroni* and its binary complex with NAD^+ have been reported previously (Grimm *et al.*, 2000). In contrast to the NAD^+-dependent 3α-HSD from *C. testosteroni*, 3α-HSD from *Pseudomonas* sp. B-0831 has the ability to use not only NAD^+ but also $NADP^+$ (Ueda *et al.*, 2004*c*,*d*). This broad cofactor specificity has particular advantage in clinical diagnostics because nucleotide analogues such as thio-NAD^+ and thio-$NADP^+$ which have high molar extinction coefficients can be used as a cofactor, making it possible to determine the amount of 3α-hydroxysteroids with high sensitivity (Ueda *et al.*, 2004*b*). In addition, we recently characterized the binding kinetics of nucleotide cofactors to 3α-HSD from *Pseudomonas* sp. B-0831 and showed that it has different binding modes to different nucleotides (Ueda *et al.*, 2004*c*,*d*). Transient-phase kinetic studies have shown that NAD^+ binding to 3α-HSD from *Pseudomona* sp. B-0831 is a one-step mechanism and NADH binding is a two-step mechanism, which may be accompanied by a conformational change of 3α-HSD.

Here, we report the crystallization and preliminary crystallographic analysis of the complex of NADH and 3α-HSD from

Table 1
Data-collection statistics for the complex of NADH and 3α-HSD from *Pseudomonas* sp. B-0831.

Values in parentheses are for the last shell.

X-ray source	BL38B1
Detector	Jupiter210 CCD
Wavelength (Å)	1.000
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 62.46, b = 82.25, c = 86.57
Resolution range (Å)	50.00–1.80 (1.90–1.80)
Measured reflections	308293
Unique reflections	41605
Completeness	99.43 (98.70)
R_{merge} (%)	16.8 (24.9)

Pseudomonas sp. B-0831. This crystal structure will provide not only the NADH-binding mechanism but also the broad cofactor specificity of 3α-HSD from *Pseudomonas* sp. B-0831 and can be compared with that of the complex of NAD^+ and 3α-HSD from *C. testosteroni* (Grimm *et al.*, 2000).

2. Materials and methods

2.1. Materials

NADH was purchased from Calzyme Laboratories Inc. (USA). Recombinant 3α-HSD from *Pseudomonas* sp. B-0831 was expressed in *E. coli* and was purified as described previously (Suzuki *et al.*, 1993). The protein concentration was determined at an OD of 280 nm and was calculated using a molar absorption coefficient of $1.4 \times 10^4\ M^{-1}\ cm^{-1}$, which was determined by amino-acid composition analysis.

2.2. Crystallization

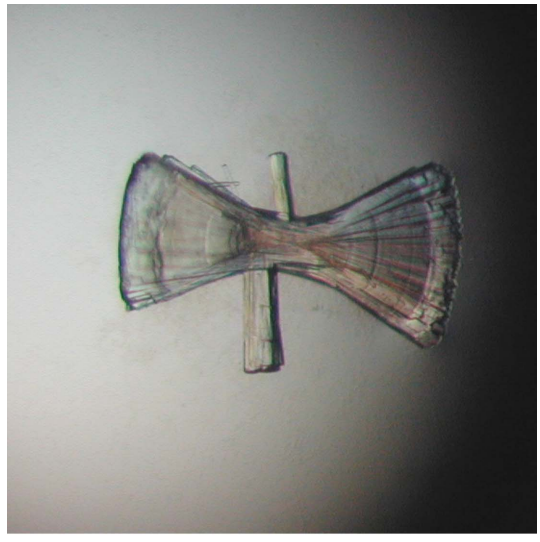
Purified recombinant protein and NADH stock solutions were prepared at concentrations of 31 mg ml^{-1} (1.2 m*M*) and 5 m*M* in 10 m*M* Tris–HCl pH 8.0, respectively. Crystallization conditions were identified by the hanging-drop vapour-diffusion method using Hampton Research Crystal Screens I and II (Hampton Research, USA) as the initial screen. All drops were prepared by mixing 2 µl sample solution (0.3 m*M* 3α-HSD and 0.4 m*M* NADH) with 2 µl reservoir solution. Droplets were placed on siliconized cover slips and were equilibrated against 400 µl reservoir solution at a temperature of 277 K. Condensed clusters of thin plate-shaped crystals were obtained from condition No. 32 of Hampton Research Crystal Screen II (0.1 *M* sodium chloride, 0.1 *M* HEPES pH 7.5, 1.6 *M* ammonium sulfate). To obtain single crystals, these crystals in the condensed form were used for microseeding. Subsequent refinement of the buffer conditions gave optimal crystallization conditions consisting of 0.1 *M* Tris–HCl pH 9.0 containing 0.14 *M* sodium chloride and 1.4 *M* ammonium sulfate.

2.3. Data collection

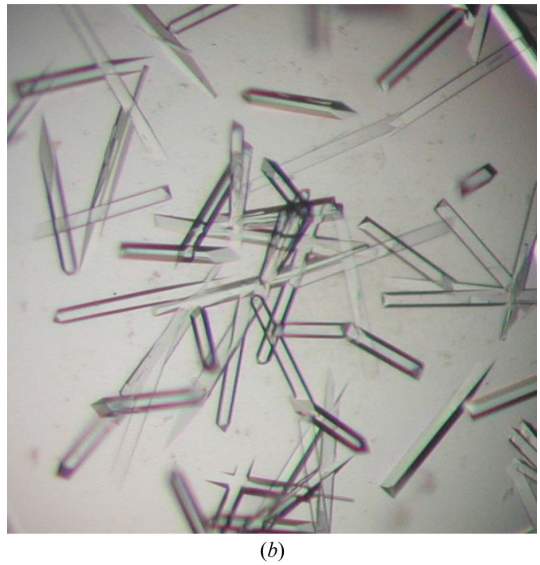
A crystal of the complex of NADH and 3α-HSD picked up from a droplet using a nylon loop (Hampton Research, USA) was transferred into a cryoprotectant solution (0.1 *M* Tris–HCl pH 9.0, 30% sucrose, 0.14 *M* sodium chloride, 1.4 *M* ammonium sulfate) and was placed directly into a cold nitrogen-gas stream at 100 K. Preliminary X-ray diffraction data were collected at 100 K under a nitrogen-gas stream with a Jupiter210 CCD detector using synchrotron radiation of wavelength 1.000 Å at BL38B1 of SPring-8 (Hyogo, Japan). Diffraction data were collected by the standard oscillation method in 1.0° increments using a crystal-to-detector distance of 130 mm and an exposure time of 20 s per image. Diffraction data for the crystal were obtained in the resolution range 50.0–1.8 Å and were processed using the *HKL*2000 program package (*DENZO* and *SCALEPACK*; Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

3. Results and discussion

For crystallization of the complex of NADH and 3α-HSD from *Pseudomonas* sp. B-0831, microseeding was found to be effective to obtain single crystals. Prior to microseeding, only crystals similar to condensed clusters of thin plates appeared under any of the crystallization conditions used in this study (Fig. 1*a*). The best diffracting crystals were obtained from a mixture of sample solution (0.3 m*M* 3α-HSD and 0.4 m*M* NADH) and reservoir solution (0.1 *M* Tris–HCl pH 9.0, 0.14 *M* sodium chloride, 1.4 *M* ammonium sulfate) in the presence of crystal solution for microseeding. Rod-shaped single



(*a*)



(*b*)

Figure 1
Crystals of 3α-HSD from *Pseudomonas* sp. B-0831 in complex with NADH. (*a*) Crystals similar to condensed clusters of thin plates. (*b*) Improved rod-shaped single crystals after microseeding.

crystals grew to dimensions about 0.4 × 0.05 × 0.05 mm within a week (Fig. 1*b*).

The crystals of the complex of NADH and 3α-HSD belonged to space group $P2_12_12_1$, with unit-cell parameters a = 62.46, b = 82.25, c = 86.57 Å. The V_M value (Matthews, 1968) was calculated to be 2.1 Å^3 Da^{-1}, assuming the presence of two molecules in the asymmetric unit, and the solvent content was 42.3%. The presence of two molecules in the asymmetric unit would correspond to dimer formation of 3α-HSD from *Pseudomonas* sp. B-0831 in solution (Ueda *et al.*, 2004*d*). The V_M value and solvent content are within the range usually observed in protein crystals. A total of 308 293 reflections containing 41 605 unique data were collected with 99.4% completeness and an R_{merge} of 16.8% to 1.80 Å resolution. The structure of 3α-HSD from *Pseudomonas* sp. B-0831 complexed with NADH will be determined by molecular replacement using the structure of 3α-HSD from *C. testosteroni* (PDB code 1fk8; Grimm *et al.*, 2000) as the search model.

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