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Crystallization and preliminary X-ray analysis of pyridoxal 4-dehydrogenase, the second enzyme in degradation pathway I of pyridoxine

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Pyridoxal 4-dehydrogenase (PLDH; EC 1.1.107) is the second enzyme in the bacterial degradation pathway I of vitamin B_6, which catalyzes the oxidation of pyridoxal to 4-pyridoxolactone using NAD^+. PLDH from *Microbacterium luteolum*, a dimeric protein with a subunit molecular weight of 38 kDa, was crystallized at 277 K in a drop solution comprising 15%(*w*/*v*) polyethylene glycol 4000, 0.15 *M* sodium acetate, 7.5 m*M* *n*-octyl-β-D-glucoside and 0.075 *M* Tris–HCl pH 7.5 by the sitting-drop vapour-diffusion method. The crystals were monoclinic and belonged to space group *C*2, with unit-cell parameters a = 107.0, b = 56.7, c = 130.2 Å, β = 103.6°. Diffraction data were collected from a single crystal to 2.0 Å.

1. Introduction

There are two different degradation pathways of pyridoxine, one of the free forms of vitamin B_6, in bacteria that use pyridoxine as a carbon and nitrogen source (Nelson & Snell, 1986). In pathway I, found in *Pseudomonas* MA and MA-1 and *Microbacterium luteolum*, pyridoxine is first oxidized to pyridoxal by pyridoxine 4-oxidase and then degraded *via* seven enzyme-catalyzed steps to succinic semialdehyde, ammonia and carbon dioxide (Nelson & Snell, 1986). Pyridoxal 4-dehydrogenase (PLDH; EC 1.1.1.107) catalyzes the oxidation of the hemiacetal form of pyridoxal to 4-pyridoxolactone using NAD^+ (Fig. 1) and is involved as the second enzyme in the pathway.

PLDH was partially and homogeneously purified from *Pseudomonas* MA (Burg & Snell, 1969) and *M. luteolum* (Trongpanich *et al.*, 2002), respectively. The enzyme is a dimeric protein with a subunit molecular weight of 38 kDa that belongs to the aldo–keto reductase (AKR) superfamily and forms a new family (AKR15) together with D-*threo*-aldose 1-dehydrogenase (Yokochi *et al.*, 2004). The enzyme shows several unique properties compared with previously studied AKRs.

The AKR superfamily is one of three enzyme superfamilies that encompass NAD(P)(H)-dependent oxidoreductases (Hyndman *et al.*, 2003), which catalyze the reduction of aldehydes, ketones, monosaccharides, ketosteroids and prostaglandins. AKRs also catalyze the oxidation of hydroxysteroids and *trans*-dehydrodiols of polycyclic aromatic hydrocarbons. They generally show much higher activity for the reduction of aldehydes than for the dehydrogenation (oxidation) of the corresponding alcohols (Kavanagh *et al.*, 2002). Their substrate specificities are broad: many synthetic aldehydes such as 2-nitrobenzaldehyde are good substrates. NADP(H) is generally a better substrate than NAD(H). In contrast, PLDH only catalyzes the oxidation of pyridoxal hemiacetal to form its lactone, shows no reactivity towards synthetic aldehydes and shows a higher affinity for NAD(H) than NADP(H) (Yokochi *et al.*, 2004). These properties suggest that PLDH is a fully diverged AKR with a unique active-site structure.

Here, we describe the crystallization and preliminary X-ray diffraction studies of PLDH from *M. luteolum*.

2. Methods and results

2.1. Protein expression and purification

The enzyme was purified from recombinant *Escherichia coli* JM109 cells harbouring plasmids pTRPLD and pKY206 (Yokochi *et al.*,

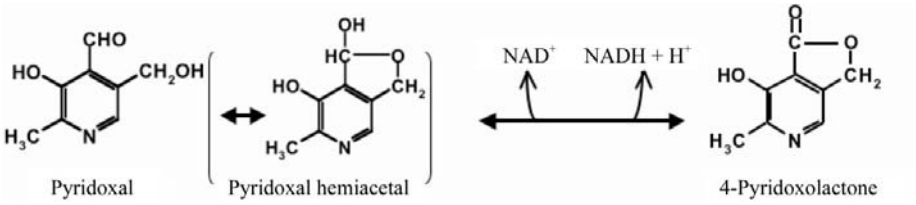

Figure 1
The enzyme reaction catalyzed by pyridoxal 4-dehydrogenase.

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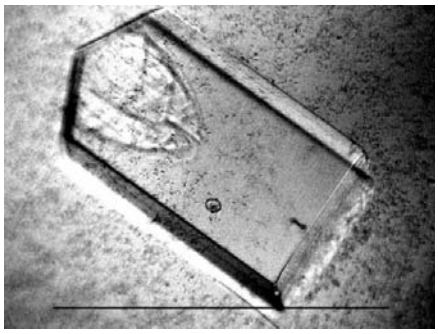

Figure 2
Crystal of PLDH (0.5 × 0.3 × 0.1 mm). The scale bar is 0.5 mm in length.

2004). The purification steps used were essentially the same as those described previously (Trongpanich *et al.*, 2002). The purified enzyme was dialyzed against 20 m*M* Tris–HCl buffer pH 8.0 containing 0.1% 2-mercaptoethanol, 0.9 m*M* NAD^+ and then concentrated by ultrafiltration with Ultra Free C3LGC (Millipore). In order to stabilize the enzyme, 2 m*M* DTT and 1 m*M* octyl β-D-glucoside were added to the clear concentrated solution.

2.2. Crystallization

The enzyme was crystallized at 277 K by the sitting-drop vapour-diffusion method using Crystal Clear Strips from Hampton Research (Laguna Niguel, CA, USA). Initially, low-quality crystals of the enzyme were found on sparse-matrix screening using commercial crystallization kits from Hampton Research. After improvement of the conditions, the solution most suitable for crystallization was determined to be a mixture consisting of 3 µl enzyme solution (20 mg ml^{-1}) and 4 µl 15%(*w*/*v*) polyethylene glycol 4000, 0.15 *M* sodium acetate, 7.5 m*M* *n*-octyl-β-D-glucoside and 0.075 *M* Tris–HCl pH 7.5. Crystals of the enzyme grew from the droplets in two weeks at 277 K (Fig. 2).

Table 1
Data-collection statistics for a crystal of pyridoxal 4-dehydrogenase (PLDH).

Values in parentheses are for data in the highest resolution shell.

Wavelength (Å)	0.72
Resolution (Å)	50–2.00 (2.07–2.00)
Crystal system	Monoclinic
Space group	*C*2
Unit-cell parameters (Å, °)	a = 107.0, b = 56.7, c = 130.2, β = 103.6
Total observations	225517 (4818)
Independent reflections	50473
Completeness (%)	97.7 (94.4)
$I/\sigma(I)$	12.1 (2.8)
R_{merge} (%)	6.4 (55.9)

2.3. X-ray analysis

A crystal of the enzyme picked up from a droplet was transferred to a cryoprotectant solution [30% glycerol, 0.1 *M* Tris–HCl pH 7.5, 0.2 *M* sodium acetate, 20%(*w*/*v*) polyethylene glycol 4000]. The crystal was mounted on a nylon loop (Hampton Research, Laguna Niguel, CA, USA) and placed directly in a cold nitrogen-gas stream at 100 K. X-ray diffraction images were collected from the crystal at 100 K in the nitrogen-gas stream with a Quantum 4R CCD detector using synchrotron radiation of wavelength 0.72 Å at station BL-38B1 of SPring-8 (Japan). The crystal-to-detector distance was set to 200 mm and 1.0° oscillation images were recorded with an exposure time of 6 s. Diffraction data were obtained from the crystal in the resolution range 50–2.0 Å and were processed using the *HKL*2000 program package (*DENZO* and *SCALEPACK*; Otwinowski & Minor, 1997). The space group of the crystal was determined to be *C*2 (monoclinic), with unit-cell parameters a = 107.0, b = 56.7, c = 130.2 Å, β = 103.6°. Of the 225 517 total reflections measured, 50 473 independent reflections were obtained with an R_{merge} value of 6.4%. The data set was 97.7% complete at the resolution limit of 2.0 Å.

The preliminary X-ray crystallographic properties of the enzyme are summarized in Table 1. The V_M value (Matthews, 1968), the crystal volume per unit protein molecular weight, was calculated to be 2.04 $Å^3$ Da^{-1} assuming two molecules of the enzyme to be present in an asymmetric unit; the solvent content was 51.0%. The V_M value and solvent content lie within the ranges usually found for protein crystals.

A selenomethionine derivative of the enzyme (PLDH) is now in preparation for further analysis.

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