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FOR THE RECORD

Crystallization and preliminary X-ray analysis of aldehyde dehydrogenase from *Vibrio harveyi*

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Abstract: Aldehyde dehydrogenase from *Vibrio harveyi* catalyzes the oxidation of long-chain aliphatic aldehydes to acids. The enzyme is unique among the family of aldehyde dehydrogenases in that it exhibits much higher specificity for the cofactor $NADP^+$ than for NAD^+. The sequence of this form of the enzyme varies significantly from the NAD^+ dependent forms, suggesting differences in the three-dimensional structure that may be correlated to cofactor specificity. Crystals of the enzyme have been grown both in the presence and absence of $NADP^+$ using the hanging drop vapor diffusion technique. In order to improve crystal size and quality, iterative seeding techniques were employed. The crystals belong to space group $P2_1$, with unit cell dimensions a = 79.4 Å, b = 131.1 Å, c = 92.2 Å, and β = 92.4°. Freezing the crystal to 100 K has enabled a complete set of data to be collected using a rotating anode source (λ = 1.5418 Å). The crystals diffract to a minimum d-spacing of 2.6 Å resolution. Based on density calculations, two homodimers of molecular weight 110 kDa are estimated to be present in the asymmetric unit. Self-rotation functions show the presence of 3 noncrystallographic twofold symmetry axes.

Keywords: aldehyde dehydrogenase; crystallization; X-ray diffraction $NADP^+$ specificity

Aldehyde dehydrogenases constitute a large family of $NAD(P)^+$-dependent enzymes found in both prokaryotes and eukaryotes. The enzyme catalyzes the dehydrogenation of aldehydes via the formation of a thiohemiacetal covalent intermediate with the aldehyde substrate. The substrate specificity of the enzyme is broad and is usually dependent on its subcellular localization.

In mammalian systems, three classes of aldehyde dehydrogenases have been identified on the basis of amino acid sequence and subcellular distribution: class 1, cytosolic; class 2, mitochondrial; and class 3, tumor-associated (Lindahl & Hempel, 1991). The class 1 and class 2 enzymes exist as homotetramers of 50–55 kDa subunits, whereas class 3 aldehyde dehydrogenases are homodimers (Jones et al., 1988). The three-dimensional structure of the enzyme has not yet been determined, although crystallization conditions have been reported for a member of each of the three classes (Rose et al., 1990; Hurley & Weiner, 1992; Baker et al., 1994).

In most cases, aldehyde dehydrogenases are NAD^+ specific. However, a few enzymes have been identified that usually exhibit a relatively weak interaction with the $NADP^+$ cofactor (Lindahl, 1992). Interestingly, an $NADP^+$ specific form of aldehyde dehydrogenase has been isolated from the bioluminescent bacterium, *Vibrio harveyi*. This form of the enzyme exhibits very tight binding to the cofactor (K_m = 1.4 μM for $NADP^+$ with saturating dodecanal), 40 times lower than that reported for other aldehyde dehydrogenases (Vedadi et al., 1995).

The enzyme from *V. harveyi* contains a cysteine residue in the active site, Cys 289, which corresponds to Cys 302 in mammalian class 1 and 2 aldehyde dehydrogenases (Hempel & Pietruszko, 1981; Abriola et al., 1987). This cysteine is a superreactive residue that forms a thiohemiacetal covalent intermediate with the aldehyde substrate. Mutagenesis of this residue in *V. harveyi* to a serine residue resulted in a significant decrease in activity, without preventing coenzyme binding (Vedadi et al., 1995). Analogous results have been found for the enzyme from rat liver mitochondria (Farrés et al., 1995), indicating that the cysteine residue of the bacterial and mammalian enzymes have similar catalytic functions. Other residues, including Glu 253, Gly 229, and Gly 234 (from *V. harveyi*) appear to be conserved between the two enzymes (Vedadi et al., 1995) and have been implicated in coenzyme binding (Loomes & Jörnvall, 1991; Hempel et al., 1993; Wang & Weiner, 1995).

Structural studies of the $NADP^+$ specific enzyme from *V. harveyi* will enable a detailed comparison with the NAD^+ specific enzymes to be performed. Such structural comparisons should reveal any structural changes that may account for the differences in specificity and identify residues involved directly in $NADP^+$ binding.

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Results: Aldehyde dehydrogenase from *V. harveyi* was cloned, expressed, and purified according to the procedure outlined by Meighen and coworkers (Vedadi et al., 1995). The protein was dialyzed into 25 mM Hepes, pH 7.0, 10 mM beta-mercaptoethanol, and concentrated to 10 mg/mL (as analyzed by the Bradford assay). Crystallization trials were conducted using the hanging drop vapor diffusion technique (McPherson, 1982). Initial trials using the sparse matrix conditions described by Jancarik and Kim (1991) resulted in very thin plate-like crystals from polyethylene glycol (PEG); average M_r 8,000; BDH), 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 6. Further screening around these conditions resulted in plates from a broad range of PEG (18–30%, w/v) and pH 5–7. No crystals appeared in the absence of sodium acetate. The crystals often appeared as plates with poorly defined edges and were unsuitable for diffraction analysis. Seeding experiments were employed to improve the size and quality of the crystals. Small crystals were crushed in a harvest solution containing 22% (w/v) PEG, 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 7. Serial dilutions of this seed solution were used to obtain the ideal concentration of seed crystals. To a fresh drop of 2 μL of protein (at a concentration of 10 mg/mL), 2 μL of the seed solution was added and equilibrated over a well solution containing 18% (w/v) PEG, 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 7. Single diamond-shaped crystals were obtained from this microseeding method; however, they were still too small to be useful for X-ray diffraction analysis. Typical dimensions of these crystals were 0.05 × 0.05 × 0.01 mm. In order to improve the crystal size, macroseeding techniques were employed. To a drop containing 2–5 μL of fresh protein was added an equal volume of 30% (w/v) PEG, 400 mM sodium acetate, and 200 mM sodium cacodylate, pH 7, containing 3–5 small crystals. The crystals used for the macroseeding were first washed in a solution containing 12% (w/v) PEG, 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 7. Within 7–10 days, the seed crystals grew to a typical size of 0.25 × 0.25 × 0.02 mm. Crystals of identical morphology were also obtained upon addition of 0.1 mM $NADP^+$ to the dialyzed protein prior to setting up the hanging drops.

Using a rotating anode source (Rigaku RU300, λ = 1.5418 Å), crystals of native aldehyde dehydrogenase diffract to better than 2.5 Å resolution. Because the crystals are thin, and in order to eliminate any problems with crystal decay during data collection, diffraction data were collected from a frozen crystal. The crystal was first transferred to a cryoprotectant solution containing 12.5% (w/v) glycerol, 22% (w/v) PEG, 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 7, for less than 5 min. A rayon CryoLoop (Hampton Research) was used to pick up the crystal, which was then immersed into liquid nitrogen. The frozen crystal was transferred into a stream of nitrogen gas maintained at 120 K (Oxford Cryosystems, Oxford, UK).

The unit cell dimensions were found to be a = 79.4 Å, b = 131.1 Å, c = 92.2 Å, β = 92.4°, and, because reflections of the type $0k0$ were systematically found absent for $k = 2n + 1$, the space group was determined to be $P2_1$. Using a molecular mass of 110 kDa (for the homodimer) and assuming the presence of two dimers per asymmetric unit, a V_M value of 2.18 was obtained. This value falls within the range observed by Matthews (1968) and corresponds to a solvent content of 30%.

A complete data set to 2.8-Å resolution was collected on a single native crystal frozen to 120 K. The data were processed, scaled, and merged using the DENZO software package (Otwinowski, 1993). Data collection and processing statistics are given in Table 1. Close inspection of the axial reflections along a and c reveal weak intensities for odd reflections, particularly at low resolution, suggesting that the monoclinic crystals may exhibit pseudo orthorhombic symmetry.

Table 1. *Data collection statistics for aldehyde dehydrogenase*

	Native	$K_2Hg(C_2H_3O_2)_2$
Soak concentration (mM)[a]	—	0.05
Soak time (h)	—	2.5
Resolution (Å)	2.8	2.8
Total reflections	188,944	279,548
Independent reflections	43,715	44,556
% Complete	94	93
Average I/σ	16.8	17.6
R_{merge} (%)[b]	6.9	7.0
R_{anom} (%)	—	4.6
R_{deriv} (%)[c]	—	20.3

[a]Soak carried out in 22% PEG 8K, 200 mM ammonium acetate, 100 mM sodium cacodylate, pH 7.

[b]$R_{merge} = \Sigma|I - \langle I\rangle|/\Sigma I$ (summed over all intensities).

[c]$R_{deriv} = \Sigma|F\,\text{deriv}_h - F\,\text{nat}_h|/\Sigma F\,\text{nat}_h$ (in the resolution range 40–2.8Å).

A self-rotation function (Crowther, 1972) was calculated using data in the resolution range 8–3.5 Å to determine the noncrystallographic symmetry elements present. POLARRFN in the CCP4 suite of programs was used for these calculations (CCP4, 1994). Figure 1 is the self-rotation for the κ = 180° section. Three noncrystallographic twofold axes are evident from this section and are marked with arrows. Two axes, labeled 1 and 2 in Figure 1, lie

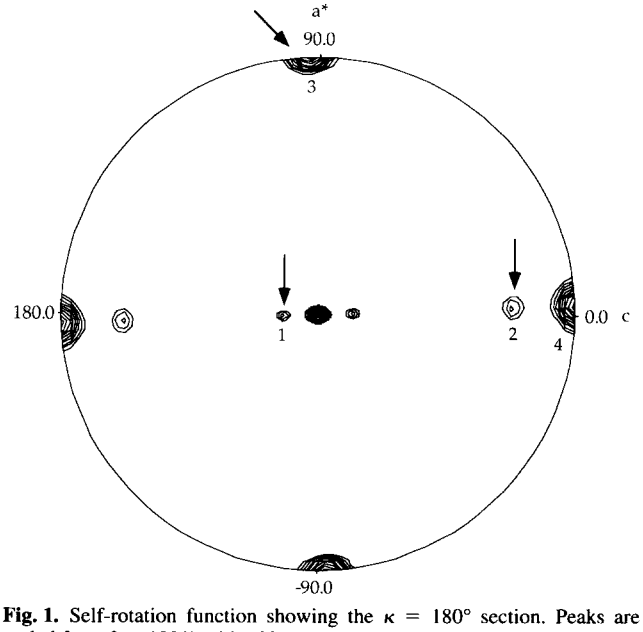

Fig. 1. Self-rotation function showing the κ = 180° section. Peaks are scaled from 0 to 100% with 100% representing the origin peak. Phi angles and the position of the axes are marked on the circumference. The twofold axes are marked with arrows. Contour lines are plotted for all peaks greater than 40% of the origin peak in intervals of 5%.

15.6° and 74.5° from the *b*-axis, respectively. Both of these peaks are ~52% of the origin peak. The third twofold axis, labeled 3 in Figure 1, lies 2.4° from the *a**- or *c*-axis. The peak 2.4° from the *c*-axis is equivalent to that found close to the *a**-axis and is generated as a result of the twofold noncrystallographic axis and the center of symmetry. This peak is 89% of the origin peak.

Because a cysteine residue (289) is involved in the catalytic activity of the enzyme (Vedadi et al., 1995), $K_2Hg(C_2H_3O_2)_2$ was used to derivatize the crystals. Diffraction data to 2.8 Å resolution were collected on a crystal soaked in 0.05 mM $K_2Hg(C_2H_3O_2)_2$ for 2.5 h (Table 1). Upon derivatization of a native crystal, a 2% change in the *c*-axis was observed. An isomorphous difference Patterson map showed four Harker peaks indicating that each active site cysteine residue present in the asymmetric unit had been derivatized by the heavy metal.

Chemical modification and site-directed mutagenesis studies of human liver mitochondrial aldehyde dehydrogenase have shown that Glu 268 is essential for catalytic activity, suggesting that it may function as a general base necessary for the activation of the essential cysteine residue (Abriola et al., 1987; Wang & Weiner, 1995). Sequence analysis among a number of aldehyde dehydrogenases, including that from *V. harveyi*, have shown this residue to be completely conserved (Vedadi et al., 1995). Further derivative screening will utilize K_2OsO_4 because this heavy metal compound is known to bind to glutamate and aspartate residues.

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