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Crystallization and preliminary diffraction analysis of porcine heart mitochondrial NADP$^+$-dependent isocitrate dehydrogenase

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Abstract

Isocitrate dehydrogenases [isocitrate:NAD(P)$^+$ oxidoreductase (decarboxylating), E.C. 1.1.1.42] are ubiquitous metabolic enzymes which occur in all living organisms. The NADP$^+$-dependent mitochondrial isocitrate dehydrogenase from pig heart has been crystallized from polyethylene glycol/sodium sulfate mixtures in the presence of Mg^{2+} and isocitrate. The crystals belong to space group $C2$ with $a = 137.0$, $b = 113.4$, $c = 65.0$ Å and $\beta = 98.5°$, and diffract to at least 2.4 Å resolution. There are two protein monomers per asymmetric unit which are related by non-crystallographic twofold symmetry.

1. Introduction

Isocitrate dehydrogenase [isocitrate:NADP$^+$ oxidoreductase (decarboxylating), E.C. 1.1.1.42] catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. Isocitrate dehydrogenase belongs to a larger functional class of metal-dependent decarboxylating (2*R*, 3*S*) 2-hydroxyacid dehydrogenases which also includes isopropylmalate dehydrogenase (IMDH) and tartarate dehydrogenase. The structure, regulation, and mechanism of these enzymes have been under investigation since their discovery more than half a century ago.

Isocitrate dehydrogenases (IDH's) are ubiquitous in living organisms because of their vital roles in both energy metabolism and biosynthesis. The metabolic roles of IDH isozymes are determined by their dependence on either NAD$^+$ or NADP$^+$, but not both, and by their cellular compartmentalization. IDH isozymes from various sources differ greatly in primary structure. The two IDH's which have been most intensively studied are the mitochondrial NADP$^+$-dependent IDH from porcine heart and the NADP$^+$-dependent IDH of *Escherichia coli*. These two enzymes have two of the least similar amino-acid sequences among the decarboxylating dehydrogenases. The two sequences are 14% identical in a triple sequence alignment with yeast mitochondrial IDH (Haselbeck, Colman & McAlister-Henn, 1992). A subsequent pair-wise alignment using the *Wisconsin* program suite (Devereux, Haeberli & Smithies, 1984) found 24% sequence identity with 16 gaps. This level of similarity is near the threshold of significance, and is exceptionally low for two enzymes which catalyze the same reaction.

A hallmark of the decarboxylating dehydrogenases is their dependence on magnesium or manganese. For the porcine NADP-dependent IDH, the pH dependence of the K_m for total isocitrate and total metal ions suggest that the metal–tribasic isocitrate complex is the actual substrate (Colman, 1972; Ehrlich & Colman, 1976) and a ^{13}C NMR study indicates that for isocitrate bound to the enzyme all three carboxyl groups remain ionized over the pH range 7.5–5.5 (Ehrlich & Colman, 1987). Since there are marked differences in the pH dependence of equilibrium binding of free manganous ion, free isocitrate and manganous–isocitrate complex, it was postulated that the metal ion binds to distinguishable but mutually exclusive sites in the absence and presence of isocitrate (Ehrlich & Colman, 1976). Affinity cleavage of the NADP-dependent IDH by Fe^{2+}–isocitrate or Fe^{2+} alone demonstrates distinct sites of cleavage by free metal ions and by metal–isocitrate (Soundar & Colman, 1993). Metal-catalyzed cleavage of the enzyme occurs at Asp253 and His309 in the enzyme–metal isocitrate, and at Asp273 in the binary enzyme–metal complex. The two aspartates either correspond to, or are in the vicinity of, the metal ligands expected on the basis of homology to *E. coli* IDH. ^{113}Cd NMR experiments also yield insights into the ligands of enzyme-bound metal ion (Ehrlich & Colman, 1989).

The binding of coenzymes and coenzyme fragments to the pig heart NADP-specific isocitrate dehydrogenase has been measured by coenzyme and protein fluorescence as well as by UV difference absorption titrations (Mas & Colman, 1985). ^{31}P NMR spectroscopy indicates that the 2′-phosphate of enzyme-bound coenzyme remains ionized over the pH range 5–8 whereas that of the free nucleotides changes with a pK of 6.13 (Mas & Colman, 1984). These results suggest that the pK of the 2′-phosphate of enzyme-bound nucleotide may be much lower, perhaps due to proximity of a positively charged group in the binding site. The ^1H NMR spectrum of porcine isocitrate dehydrogenase reveals a histidine peak which is perturbed by NADP and NADPH but not by coenzyme fragments lacking the nicotinamide, indicating that a histidine is in the region of the

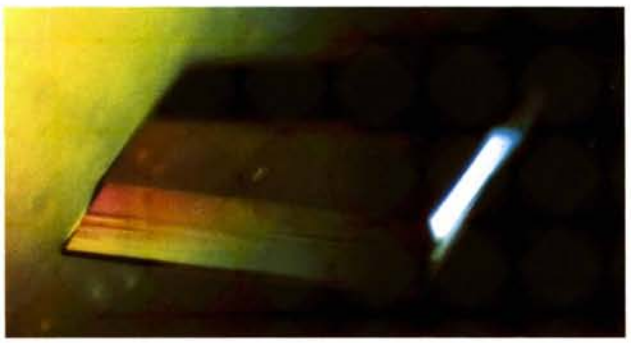

Fig. 1. Porcine heart mitochondrial NADP$^+$-dependent isocitrate dehydrogenase crystal. Actual size of the crystal is approximately 0.4 × 1.0 × 1.5 mm.

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nicotinamide binding site (Ehrlich & Colman, 1985). Thus, solution studies have resulted in predictions about the pig heart enzyme's environment at or near the coenzyme and substrate sites.

The only structure of any IDH currently available is that of the *E. coli* enzyme (Hurley *et al.*, 1989). Structures of *E. coli* IDH bound to substrate and cofactor identified key residues in the active site (Hurley, Dean, Koshland & Stroud, 1991). Many, but not all, of these residues are also present in the aligned sequence of porcine mitochondrial $NADP^+$-dependent IDH. The alignment of some isocitrate and Mg^{2+}-binding-site residues strongly suggests that the two enzymes share a common tertiary structure. In contrast, some of the key $NADP^+$-binding residues (*e.g.* Arg292′, Val351, Tyr391, Arg395) of *E. coli* IDH do not appear to be conserved. In order to provide a rigorous basis for understanding the mechanism of eukaryotic IDH's, and to understand better the evolutionary relationship between eukaryotic and prokaryotic IDH's, we have been pursuing three-dimensional structure determination of the best characterized eukaryotic isozyme, porcine mitochondrial $NADP^+$-dependent IDH.

2. Methods and results

Protein was purified as described by Bacon, Bednar & Colman (1981) and Smyth & Colman (1991) and modified by Soundar & Colman (1993). The enzyme was concentrated to 16 mg ml^{-1} in 0.1 *M* triethanolamine HCl pH 7.7 and either 0.1 *M* or 0.4 *M* Na_2SO_4. The enzyme solution was then adjusted to 4 m*M* isocitrate and 2 m*M* $MnSO_4$. 8 µl of protein was mixed with 8 µl of well solution and equilibrated through the vapor phase. Crystals (Fig. 1) were grown at 277 K in hanging or sitting drops by vapor diffusion against 16–20% PEG 6000, 0.1 *M* Na_2SO_4, 0.1 *M* triethanolamine acetate pH 7.7. Diffraction data were collected at room temperature on an R-AXIS II image-plate detector using double-mirror focused Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode generator operating at 50 kV and 100 mA. The crystals diffract to at least 2.4 Å (Fig. 2). Intensity data were indexed and processed with the *HKL* program package (Otwinowski, 1988). On the basis of auto-indexing, the crystals belong to space group *C*2. The unit-cell parameters for crystals at room temperature are $a = 137.0$, $b = 113.4$, $c = 65.0$ Å, $\beta = 98.5°$. No lattice of higher symmetry could be accommodated without unreasonable distortions of cell parameters, the smallest being 3.4% for *C*-centered orthorhombic. A native data set has been collected which is 86% complete to 2.5 Å resolution, with a merging *R* factor on intensity of 5.0%. Assuming two molecules are present per asymmetric unit, $V_m = 2.6$ Å^3 Da^{-1} (Matthews, 1968). Different numbers of molecules per asymmetric unit lead to improbable packing parameters.

The apparent presence of two IDH monomers per asymmetric unit led us to analyze the self-rotation function. The self-rotation function calculated in polar coordinates with the program *POLARRFN* (Fig. 3) using data from 15.0 to 4.0 Å and $R = 30.0$ Å (Collaborative Computational Project, Number 4, 1994) yielded a peak at $\theta = 54$, $\varphi = 55$, $\kappa = 180°$, where the polar axis is along *z*. This peak is 41% of the height of the origin, whereas the next-highest peak is 23% of the height of the origin. Cross-rotation and translation searches with several models derived from the *E. coli* IDH structure have not yielded a solution, and a search for heavy-atom derivatives is in progress.

Porcine heart IDH, and most other decarboxylating dehydrogenases, are functional homodimers (Kelly & Plaut, 1981*a*,*b*; Bailey & Colman, 1985). Crystal structures of dimeric decarboxylating dehydrogenases are available in two different space groups for IMDH (Imada *et al.*, 1991; Hurley & Dean, 1994) and in one for *E. coli* IDH (Hurley *et al.*, 1989). In all three of these space groups, the two monomers in the functional dimer are related by exact crystallographic twofold axes. None of these crystal forms contain non-crystallographic twofold

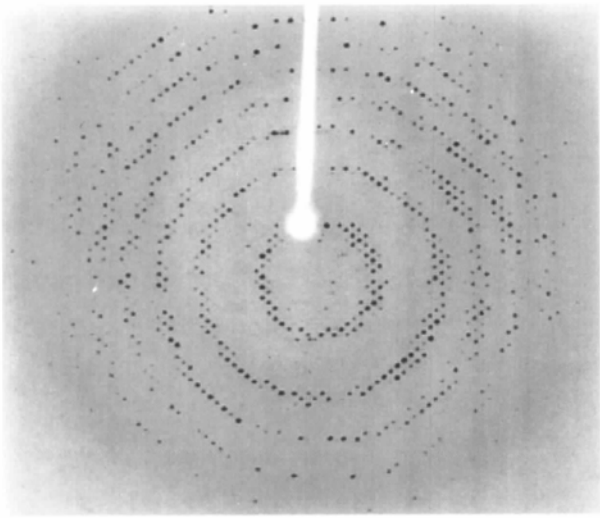

Fig. 2. 1.5° oscillation image of porcine IDH crystals measured at room temperature for 10 min with mirror-focused Cu $K\alpha$ radiation on an R-AXIS II imaging plate 150 mm from the crystal. The edges of the detector along the horizontal axis through the beam position correspond to 2.5 Å resolution.

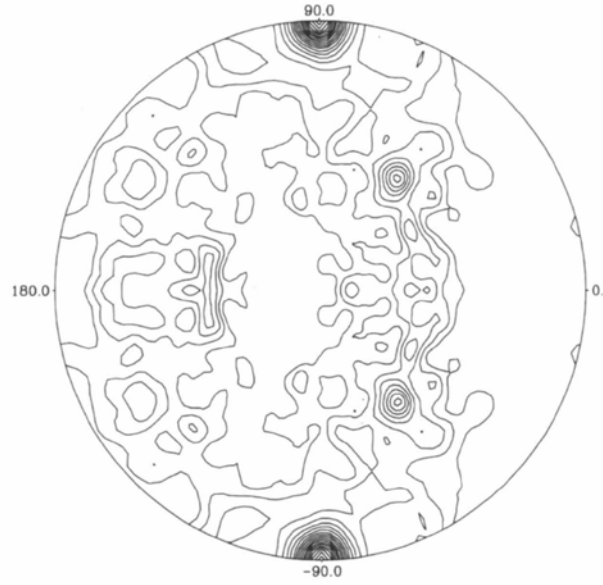

Fig. 3. Self-rotation function of porcine IDH crystal. $\kappa = 180°$ section. Contours are plotted at intervals of 5% of the height of the origin. The highest non-crystallographic peak is at $\theta = 54$, $\varphi = 55°$.

axes. In the present crystal form, it is possible either that the two molecules in the functional dimer are related by crystallographic symmetry and the dimers are related by local symmetry or that the subunits of the dimer are related to each other by local symmetry while the dimers are related by crystallographic symmetry. We are not aware of any biochemical data which would rule out either possibility.

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