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addenda and errata

Crystallization and preliminary X-ray crystallographic analysis of yeast NAD$^+$-specific isocitrate dehydrogenase. Corrigendum

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The article by Hu *et al.* [(2005), *Acta Cryst.* F**61**, 486–488] is corrected.

The report by Hu *et al.* (2005) described crystallization and preliminary diffraction data for yeast NAD$^+$-specific isocitrate dehydrogenase (IDH). The technical aspects of the crystallographic work are not compromised. However, the crystals were later found to be of the yeast nicotinimidase Pnc1p and not IDH. Three factors contributed to the misidentification of the protein in the crystals. First, the protein sample used for crystallization was ∼97% pure isocitrate dehydrogenase as estimated by denaturing gel electrophoresis. Second, the large unit cell with $a = b = 302.0$, $c = 112.1$ Å seemed consistent with the size expected for an octameric IDH molecule with subunits of molecular mass ∼38 kDa. Third, at the writing of report, the initial experimental electron-density maps revealed features expected of IDH, as IDH and nicotinamidase structures both contain multi-stranded β-sheets flanked by α-helices and a prominent loop containing a two-stranded antiparallel β-sheet.

As the chain-tracing exercise progressed, however, it became apparent that the protein was not IDH. The coordinates for the completed protein backbone were used in a *DALI* search (Holm & Sander, 1993) and a nicotinamidase from the bacterium *Pyrococcus horikoshii* (pdb code 1im5) (Du *et al.*, 2001) was returned as the highest scoring molecule. A search of the yeast genome with this bacterial nicotinamidase sequence returned the sequence of the 24 kDa nicotinimidase Pnc1p, an enzyme that functions in the NAD$^+$ salvage pathway (Ghislain *et al.*, 2002). That Pnc1p was the minor contaminant in the original protein sample was confirmed by gel electrophoresis followed by mass spectrometry. The Pnc1p amino-acid sequence was completely consistent with that observed in the experimental electron density. The large unit cell resulted from the presence of seven Pnc1p molecules in the asymmetric unit.

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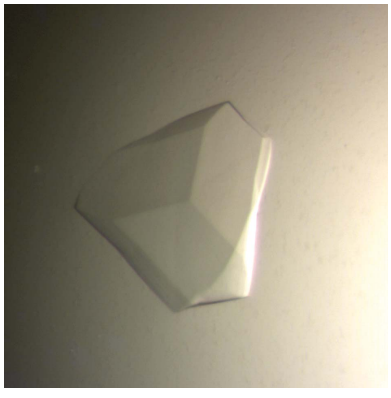

NAD$^+$-specific isocitrate dehydrogenase (IDH; EC 1.1.1.41) is a complex allosterically regulated enzyme in the tricarboxylic acid cycle. Yeast IDH is believed to be an octamer containing four catalytic IDH2 and four regulatory IDH1 subunits. Crystals of yeast IDH have been obtained and optimized using sodium citrate, a competitive inhibitor of the enzyme, as the precipitating agent. The crystals belong to space group $R3$, with unit-cell parameters a = 302.0, c = 112.1 Å. Diffraction data were collected to 2.9 Å from a native crystal and to 4.0 Å using multiwavelength anomalous diffraction (MAD) methods from an osmium derivative. Initial electron-density maps reveal large solvent channels and the molecular boundaries of the allosteric IDH multimer.

1. Introduction

The oxidative decarboxylation reaction catalyzed by mitochondrial NAD$^+$-specific isocitrate dehydrogenase (IDH, EC 1.1.1.41) is a rate-limiting step in the tricarboxylic acid cycle. *Saccharomyces cerevisiae* IDH has been described as responsive to energy needs owing to allosteric properties including activation by AMP and inhibition by ATP and NADH (Hathaway & Atkinson, 1963). Yeast IDH is thought to be an octamer composed of four IDH1 and four IDH2 subunits (Keys & McAlister-Henn, 1990). These subunits contain 349 and 354 amino-acid residues, respectively, and share 42% sequence identity (Cupp & McAlister-Henn, 1991, 1992). The yeast IDH1 and IDH2 subunits also share significant sequence identity (>30%) with non-allosteric homodimeric bacterial enzymes of known crystal structure, including *Escherichia coli* NADP$^+$-specific isocitrate dehydrogenase (Hurley *et al.*, 1989, 1991; EC 1.1.1.42) and *Thermus thermophilus* 3-isopropylmalate dehydrogenase (IMDH; Imada *et al.*, 1991; EC 1.1.1.85), a leucine-biosynthetic enzyme. These bacterial enzymes lack the $\beta\alpha\beta\alpha\beta$ motif characteristic of the nucleotide-binding Rossmann fold found in many dehydrogenases (Rossmann *et al.*, 1974), suggesting that the decarboxylating dehydrogenases represent a distinct structural family.

Mutagenesis and two-hybrid analyses of yeast IDH (Cupp & McAlister-Henn, 1993; Zhao & McAlister-Henn, 1997; Panisko & McAlister-Henn, 2001; Lin *et al.*, 2001; Lin & McAlister-Henn, 2002, 2003) suggest that the basic structural/functional unit within the octameric enzyme is an IDH1/IDH2 heterodimer, with catalytic isocitrate- and NAD$^+$-binding sites contained primarily in the IDH2 subunit and regulatory isocitrate- and AMP-binding sites contained primarily in the IDH1 subunit. It is unclear how four such heterodimers might be organized in the putative octameric holoenzyme. We have initiated crystallographic analyses of this enzyme in order to elucidate the structural relationship between the homologous catalytic and regulatory binding sites in the two subunits, to assess structural bases for allosteric regulatory properties and to determine the structural basis for previous observations based on kinetic and ligand-binding analyses (Lin & McAlister-Henn, 2002, 2003) that IDH contains only half the active ligand-binding sites predicted by comparisons with known bacterial enzyme structures.

Table 1
Data collection and statistics.

Values in parentheses are for the highest resolution shell.

	Native	Os peak	Inflection point	Remote
Beamline	APS 19BM	ALS 8.2.1		
Exposure time (s)	10	5	5	5
Distance (mm)	200	250		
Oscillation angle (°)	0.5	1.0		
Range of data (°)	140	120 + inverse beam		
Wavelength (Å)	1.0332	1.1401	1.1405	1.1390
Unit-cell parameters (Å)	a = 302.0, c = 112.1	a = 302.1, c = 112.7		
Space group	$R3$	$R3$		
Resolution (Å)	50.0–2.9 (2.95–2.90)	50.0–4.0 (4.14–4.00)		
Completeness (%)	100.0 (100.0)	99.7 (99.9)	99.0 (93.2)	99.2 (94.9)
Redundancy	4.4 (4.3)	3.7 (3.5)	3.7 (3.1)	3.7 (3.1)
Average $I/\sigma(I)$	21.0 (3.4)	11.6 (2.9)	12.1 (2.9)	12.1 (2.6)
$R_{\rm sym}$†	0.070 (0.559)	0.097 (0.434)	0.096 (0.432)	0.097 (0.456)

† $R_{\rm sym} = \sum I_{hkl} - \langle I \rangle / \sum I_{hkl}$.

2. Protein preparation and crystallization

A multicopy pRS426 plasmid carrying both *IDH1* and *IDH2* genes (Zhao & McAlister-Henn, 1997) was used for overexpression of IDH in yeast. Each gene is preceded by its authentic promoter and the *IDH1* gene contains codons for five histidine residues at the 3′ end of the coding region. Expression in a yeast strain lacking IDH and purification of the heterooctameric enzyme using Ni^{2+}–nitrilotriacetic acid (NTA) chromatography were conducted as described previously (Zhao & McAlister-Henn, 1997). Pooled elution fractions were concentrated and dialyzed in buffer *A* (10 m*M* Tris–HCl pH 7.4, 40 m*M* NaCl, 10 m*M* sodium citrate, 4 m*M* $MgCl_2$) with 5% glycerol at 277 K overnight. IDH was further purified using Affigel Blue column chromatography with elution using buffer *A* containing 1 *M* NaCl. Pooled elution fractions were concentrated and dialyzed in buffer *A* at 277 K overnight. Protein purity was assessed with sodium dodecyl sulfate/10% polyacrylamide gels. The purified form of IDH used for crystallization contained the 5× histidine tag on the IDH1 subunit. Crystallization trials with enzyme lacking this tag are now in progress.

Crystallization was conducted at 297 K using the hanging-drop vapor-diffusion method. Initial crystallization conditions were established using commercial screens from Hampton Research (Crystal Screen I and II) and Emerald BioSystems (Wizard I and II). Each hanging drop contained 1 µl IDH (35 mg ml^{-1}) and 1 µl reservoir solution and was equilibrated over 1 ml reservoir solution. Small crystals of IDH formed within 7 d in 0.1 *M* HEPES pH 7.5 containing 1.4 *M* sodium citrate. Citrate is known to be a competitive inhibitor of IDH (Atkinson *et al.*, 1965). Conditions were optimized and crystals grew to full size after 14 d using a drop containing 3 µl IDH and 3 µl 0.1 *M* HEPES pH 7.5 with 0.9 *M* sodium citrate over 1 ml reservoir solution (Fig. 1).

3. Data collection and preliminary X-ray analysis

Yeast IDH crystals diffract to 3.5 Å using our home laboratory Rigaku/MSC FR-D rotating-anode X-ray source equipped with HiRes^2 optics and R-AXIS HTC image-plate detectors. Synchrotron radiation typically improved the diffraction limit by ∼0.5 Å for these crystals and a 2.9 Å native data set was collected at beamline 19BM at the Advanced Photon Source (APS; Table 1). Crystals were cryo-protected prior to data collection with a 1 min soak in reservoir solution that was made 50% saturated with D-sorbitol. Considering that the low diffraction limit may arise from a high solvent content, we speculate that the asymmetric unit contains three or four heterodimers based on Matthews parameter estimates of 3.1 and 4.1 Å^3 Da^{-1}, respectively, for this *R*3 crystal form (Matthews, 1968). Molecular replacement using IDH or IMDH models available in the Protein Data Bank (Berman *et al.*, 2000) was unsuccessful; therefore, various heavy-atom compounds were screened for derivatization.

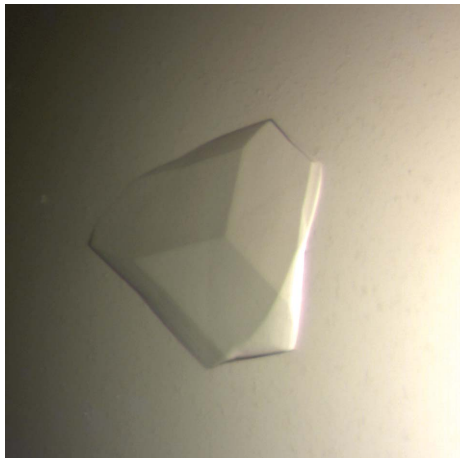

Figure 1
A crystal of pentahistidine-tagged yeast IDH. The longest dimension of the crystal is 300 µm.

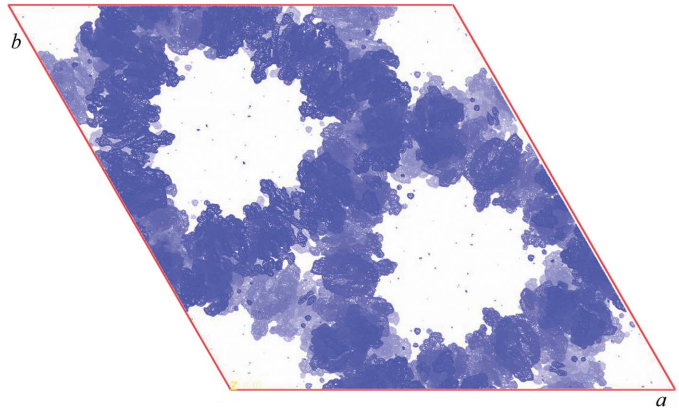

Figure 2
Electron density calculated using density modification with phases from a three-wavelength osmium MAD experiment. The unit cell is shown, indicating large solvent channels parallel to the *c* axis, which is perpendicular to the plane of the figure. The figure was prepared using *O* (Jones *et al.*, 1991).

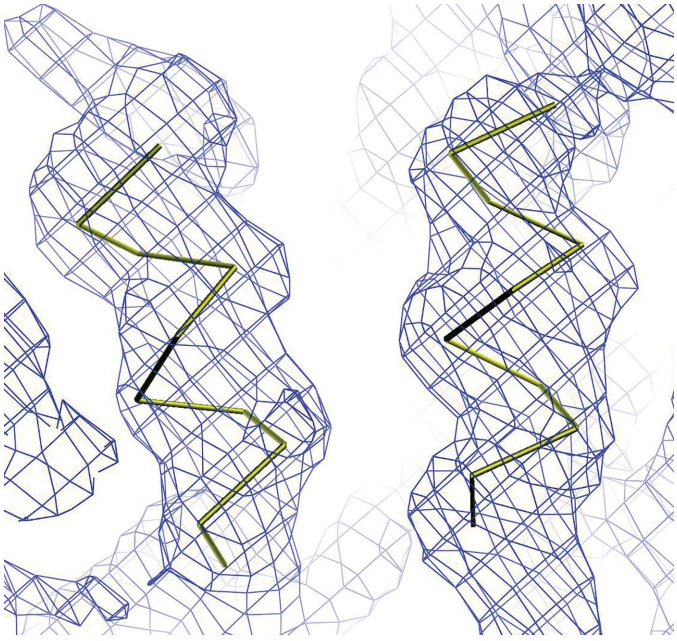

Figure 3
A pair of α-helices observed in the electron-density map shown in Fig. 2. The figure was prepared using *O* (Jones *et al.*, 1991).

Multiwavelength anomalous diffraction (MAD) data (Hendrickson, 1991) were collected to 4.0 Å at beamline 8.2.1 at the Advanced Light Source (ALS) for a yeast IDH osmium derivative. The data include a high-energy remote wavelength as a reference and peak and inflection-point wavelengths chosen according to a fluorescence spectrum acquired at the osmium *L* edge for the crystal (Table 1). All data sets were processed using *HKL*2000 (Otwinowski & Minor, 1997). *SOLVE* and *RESOLVE* routines were used to locate osmium positions, calculate phases and improve phases with density modification (Terwilliger & Berendzen, 1999; Terwilliger, 2000). The mean figure of merit for phases calculated to 4.3 Å is 0.46. Fig. 2 shows electron density for the yeast IDH crystal unit cell, indicating a distinct molecular boundary and large solvent channels parallel to the *c* axis. In addition, secondary structure is observed in the electron-density map (Fig. 3). The calculated solvent content for the crystal is 60% for four IDH heterodimers and 70% for three heterodimers in the asymmetric unit. The appearance of the map suggests the higher solvent content. Structure determination of yeast IDH using the osmium phases with homologous IDH and IMDH structures as templates for model building is in progress.

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