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Crystallization and preliminary X-ray diffraction analysis of the trigonal crystal form of *Saccharomyces cerevisiae* alcohol dehydrogenase I: evidence for the existence of Zn ions in the crystal

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Saccharomyces cerevisiae alcohol dehydrogenase I crystallized as trigonal plates using 20% 2-propanol and 20% PEG 4000 in 0.1 *M* sodium citrate buffer pH 5.6 in the presence of 1 m*M* NAD^+. The crystals diffract to 3.0 Å resolution and belong to the trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 146.3$, $c = 68.1$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. X-ray data were collected from frozen crystals at the 17-ID beamline of the Advanced Photon Source. A Zn fluorescence scan of the crystal produced a peak at 9671.6 eV, suggesting the existence of Zn ions in the crystal.

1. Introduction

In yeast, alcohol dehydrogenase I reduces acetaldehyde to ethanol in glycolysis (Young *et al.*, 1982). The enzyme is active as a tetramer of molecular weight 150 kDa (Branden *et al.*, 1975). The DNA sequence of the enzyme was determined (Wills & Jornvall, 1979; Bennetzen & Hall, 1982) and aligned with the three-dimensional structure of horse liver ADH, suggesting strong similarity in a subunit (Jornvall *et al.*, 1978). A schematic view of the active site of the enzyme has been presented (Plapp *et al.*, 1987). The enzyme binds one NAD molecule as a coenzyme and two Zn atoms per subunit (Hayes & Velick, 1954; Eklund *et al.*, 1976). One Zn atom has an essential role in binding substrates and participates in an acid–base system in the catalytic site of the enzyme. A second Zn atom is also located in the catalytic site; its function is unknown (Eklund *et al.*, 1976).

The crystal structure of horse alcohol dehydrogenase has been solved (Branden *et al.*, 1973; Eklund *et al.*, 1976, 1982) and binding of the substrate in a ternary complex of the enzyme has been studied (Eklund *et al.*, 1982). Site-directed mutagenesis and thermostability studies of native and chimeric yeast ADHs suggest that at least part of the subunit's contacts observed in dimeric horse liver ADH are located at homologous positions in the tetrameric form of yeast ADH and that inter-subunit electrostatic repulsion between Glu101 and Asp236 can cause yeast ADH to become unstable (DeBolle *et al.*, 1995, 1997). Hexagonal crystals of the *Saccharomyces cerevisiae* ADH were prepared (Ramaswamy *et al.*, 1994), but no structure has been reported to date.

2. Materials and methods

Purified *S. cerevisiae* alcohol dehydrogenase I (EC 1.1.1.1) was purchased from Sigma. SDS–PAGE analysis showed a single band at 38 kDa corresponding to a monomer of the enzyme. Powdered yeast ADH was dissolved in 0.1 *M* sodium citrate buffer pH 6.0, dialyzed against 50 times the volume of 0.1 *M* sodium citrate buffer pH 6.0 and concentrated to a final concentration of 15 mg ml^{-1}. Prior to crystallization, the protein solution was centrifuged for 5 min at 25 000*g* to clarify the solution.

Crystallization was performed at 295 K using the hanging-drop vapor-diffusion method. An initial crystallization screen was performed using Hampton Research Crystal Screens I and II. Further refinement of the crystallization condition and addition of NAD^+ yielded better crystals. The protein solution consisted of 15 mg ml^{-1} in 0.1 *M* sodium citrate buffer pH 5.6 containing 1 m*M* NAD^+ and the reservoir solution consisted of 20% 2-propanol, 20% PEG 4000 in 0.1 *M* sodium citrate buffer pH 5.6. 7 µl protein solution was mixed with 3 µl reservoir solution and equilibrated against 1 ml of reservoir solution. Plate-shaped crystals appeared overnight and grew over 2–3 d (Fig. 1).

A crystal was scooped into a nylon loop and cooled to cryogenic temperature for data collection. Data were collected at the Advanced Photon Source (APS, Argonne, IL, USA) beamline 17-ID (IMCA-CAT). Diffrac-

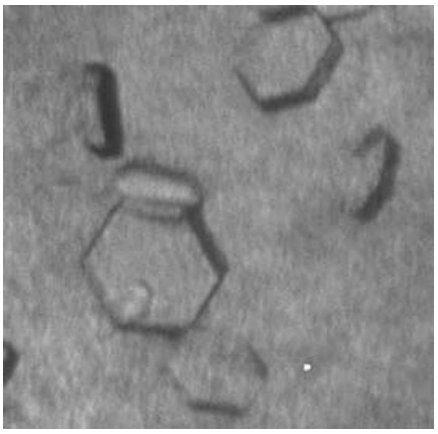

Figure 1
Crystals of *S. cerevisiae* alcohol dehydrogenase I. The largest crystal has dimensions 600 × 600 × 80 µm.

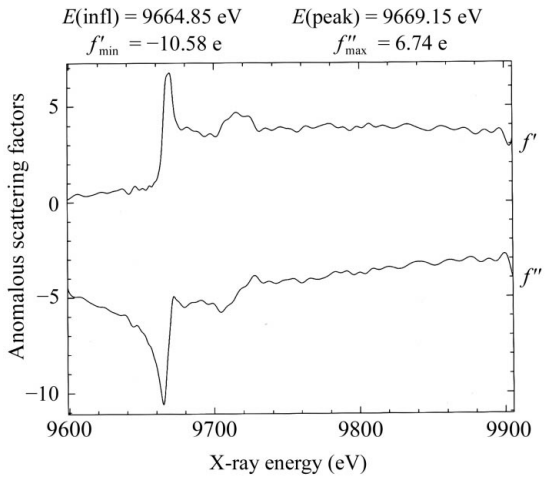

Figure 3
f' and f'' plot calculated from a Zn fluorescence scan on yeast ADH crystals at beamline 17-ID at the Advanced Photon Source.

Figure 2
Diffraction pattern of yeast ADH crystals at beamline 17-ID at the Advanced Photon Source.

tion images were recorded at a crystal-to-detector distance of 145 mm with 1° oscillation per image and an exposure time of 20 s per frame on a MAR165 CCD detector. A Zn fluorescence scan was taken from the same crystal using a Bicron Fluorescence Detector, increasing the monochromator energy in small steps. All data were processed using *XGEN* (Howard, 2000).

3. Results and discussion

The *S. cerevisiae* alcohol dehydrogenase I was crystallized using PEG 4000 as a precipitant. Initial crystallization trials displayed small plate-shaped crystals (50 × 50 × 10 µm), which were improved by the addition of 1 m*M* NAD to the protein solution. Eventually, 600 × 600 × 80 µm crystals were formed when the reservoir solution contained 20% 2-propanol and 20% PEG 4000 in 0.1 *M* sodium citrate buffer pH 5.6. The pH range 5.0–7.5 produced the same quality crystals. X-ray diffraction experiments were carried out on beamline 17-ID (IMCA-CAT) at the APS. The systematic absences indicated that the crystal belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 146.3$, $c = 68.1$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. A solvent content of 55% and an acceptable crystal packing density V_M of 2.8 Å^3 Da^{-1} were calculated using the method of Matthews (1968), assuming the ADH crystals to contain two monomers in the asymmetric unit. Diffraction data were collected to 3.0 Å (Fig. 2) with an overall R_{merge} of 11% in the 99–3.0 Å resolution shell and an overall completeness of 99.92% (Table 1). The unit-cell parameters determined are similar to those reported by Ramaswamy *et al.* (1994) for a hexagonal form. This may be a coincidence or it may be that they in fact had found the same form as this, but had misidentified the space group. If we scale our data assuming 622 symmetry we obtain an R_{merge} value of 47.6%; we are therefore confident that the crystals belong to point group 321 rather than 622.

Table 1
Summary of crystallographic data.

Synchrotron	APS, 17ID-B
Wavelength (Å)	1.2819
Crystal-to-detector distance (mm)	120
Crystal system	Trigonal
Space group	$P3_121$ or $P3_221$
Resolution (Å)	3.0
Monomers per a.u.	2
Total observations	183580
Unique reflections	16972
Completeness (%)	
Overall†	99.92
Outer shell‡	100
R_{merge} (%)	
Inner shell§	4.6
Overall†	11
Outer shell‡	44.6
$I/\sigma(I)$	
Inner shell§	56
Overall†	310.8
Outer shell‡	1.25

A Zn fluorescence scan from the ADH crystal showed a peak at 9671.6 eV (Fig. 3) indicating the presence of Zn atoms in the crystal. Since the protein solution used for crystallization had been dialyzed thoroughly against a Zn^{2+}-free buffer, we suggest that the Zn^{2+} ions are tightly bound in the ADH crystal. The amino-acid sequence of yeast ADH was aligned with human and horse ADH (data not shown), for which crystal structures are available. This analysis suggests that Cys44, His67 and Cys154 residues of yeast ADH are involved in binding Zn^{2+} ions in the active site of the enzyme.

Data were collected in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

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