Trigonal Crystal Form of Yeast Alcohol Dehydrogenase

K. J. Kim, A. J. Howard

Illinois Institute of Technology, Chicago, IL, U.S.A.

Introduction

In yeast, alcohol dehydrogenase I (ADH) has the function of reducing acetaldehyde to ethanol in glycolysis. The enzyme is active as a tetramer of molecular weight of 140,000 Da.[1] The enzyme binds one NAD molecule as a co-enzyme and two zinc atoms per subunit.[2] A zinc atom has an essential role in binding substrates and participating in an acid-base system in catalytic site of the enzyme, and a second zinc atom with unknown function is also located in the catalytic site.

Materials and Methods

Crystallization was performed at 295K using the hanging drop-vapor diffusion method. An initial crystallization screen was performed using Hampton Research Crystal Screen and Crystal Screen II. Further refinement of crystallization conditions and addition of NAD^+ yielded better crystals. The protein concentration was 15 mg/ml in 0.1 M sodium citrate, pH 5.6 containing 1 mM NAD^+. The reservoir contained 20% isopropanol and 20% PEG4000 in 0.1 M sodium citrate buffer pH 5.6. An x-ray data set has been collected at 100K at the 17-ID beamline of the Advanced Photon Source. All crystallographic data were processed with X-GEN.

Results and Discussion

The crystal diffracts to 7.8 Å. The crystals belong to space group $P3_121$, with unit parameters $a = b = 146.28$, $c = 68.13$ Å, $\alpha = \beta = 90.0$, $\gamma = 120°$. A solvent content of 55% and an acceptable crystal packing density *Vm* of 2.8 $Å^3\ Da^{-1}$ were calculated using the method of Matthews, assuming the ADH crystals to contain two molecules per asymmetric unit. A Zn fluorescence scan showed a peak at 9671.6 eV indicating the presence of Zn atoms that are tightly bound in the ADH crystals. Amino acid alignment of yeast ADH with structure-solved human and horse ADH showed that, probably, the Cys-44, His-67, and Cys-154 residues of yeast ADH are involved in binding with Zn atoms in the active site of the enzyme. We are now attempting structure determination by molecular replacement (using human ADH as a search model) and by Zn multiwavelength anomalous diffraction.

Acknowledgments

Data were collected at beamline 17-ID 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 is supported by the U.S. Department of Energy, Office of Science, Office of Basic Science, under Contract No. W-31-109-ENG-38.

References

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