Crystal structure analysis of glutathione-independent formaldehyde dehydrogenase from *Pseudomonas putida*

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Introduction

Formaldehyde dehydrogenase (EC 1.2.1.1) has been found in a wide variety of organisms. These enzymes belong to the zinc-containing medium-chain alcohol dehydrogenase family.

The FDH from *Pseudomonas putida* (PFDH; EC 1.2.1.46) is a unique enzyme that can catalyze the NAD^+-dependent but glutathione-independent oxidation of formaldehyde without the external addition of glutathione. The PFDH enzyme, in its active form, is a homo-tetramer of identical subunits, each of which comprises 398 amino acid residues and contains two zinc ions. The three-dimensional structure of PFDH will be useful to clarify the mechanism of glutathione-independent oxidation of formaldehyde by the enzyme.

Experimental and Results

Crystals of PFDH were obtained by the hanging-drop vapor diffusion method as described [1]. The crystals belong to a trigonal space group $P3_112$ with cell dimensions of $a = b = 85.74$ Å and $c = 190.9$ Å. Assuming two subunits per asymmetric unit, a V_M value of 2.41 $Å^3$/Da, corresponding to a solvent content of 49 % is obtained. For data collection under cryogenic conditions, crystals in a droplet were transferred through a series of harvesting solutions (2 mg/ml NAD^+ and 2.0 M ammonium sulfate in 0.1 M Tris buffer (pH 8.0)) in which the concentration of the glycerol was increased successively in three steps starting from 0 % (v/v) for more than 30 minutes, then to 10 % (v/v) for 1 minute, and to a final concentration of 20 % (v/v) for 1 minute. Crystals were mounted in nylon loops and flash-frozen in cold nitrogen gas stream at 100 K just before the data collection.

We tried to solve the structure of PFDH by the MAD method using intrinsic zinc atoms (2 zinc atoms / subunit). MAD data collection were performed at beamline 18B, Photon Factory. XAFS measurements were carried out around zinc K absorption edge using a PFDH crystal in cold nitrogen gas stream at 100 K. Then, three data sets were collected from a new single crystal of PFDH on and around the zinc K absorption edge (so-called 'edge' ($\lambda = 1.28301$ Å), 'peak' ($\lambda = 1.28255$ Å), and 'remote' ($\lambda = 1.20000$ Å) data sets) at 100 K using an ADSC Quantum-4R CCD detector. All data sets were integrated using the program MOSFLM. Scaling and processing were performed using the CCP4 program suite. Three data sets were almost complete (over 99 %) up to 1.8 Å resolution.

Phase calculations were done by the MAD (as a special case of MIR) method. The 'edge' data set was used as the 'native' data set. Difference anomalous Patterson maps (both Bijvoet and Dispersive Patterson maps) showed prominent peaks corresponding to four zinc atoms in the asymmetric unit. Program RSPS was used to determine the zinc sites. Single isomorphous replacement phases based on a single zinc site were used to locate remaining three zinc sites through difference Fourier syntheses. Heavy-atom parameters were refined with the program MLPHARE. The MAD phases at 1.8 Å resolution were improved by solvent-flattening and histogram-matching using the program DM (hereafter MAD-DM phases).

All the model building and correction procedures were carried out on a Silicon Graphics OCTANE, using the program XtalView version 4.0. The quality of the MAD-DM (defined above) electron density maps at 1.8 Å was so good that almost all the main chains and side chains could easily be traced with the aid of the programs Xskel and Xfit as implemented in the program XtalView. Thus, the polypeptide chain segment corresponding to residues 2-395, two zinc atoms and one NAD^+ molecule for each of the two subunits were built from the MAD-DM electron density map at 1.8 Å resolution without ambiguity. Crystallographic refinement was performed with maximum-likelihood procedure of the program REFMAC. At the latter stages of the refinement, bulk-solvent correction was applied using the program REFMAC and water molecules were automatically added using the program ARP. The stereochemistry of the model was verified using the program PROCHECK. The structural detail of PFDH will be published elsewhere (Tanaka, N. et al., in preparation).

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References

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