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Three-dimensional structure of a nicotinoprotein formaldehyde dehydrogenase

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Introduction

Formaldehyde dehydrogenases (FDH; EC 1.2.1.1) have been found in a wide variety of organisms. These enzymes belong to the zinc-containing medium-chain alcohol dehydrogenase (ADH) family. Most FDHs are known to require NAD^+ and glutathione for the oxidation of formaldehyde. Formaldehyde dehydrogenase from *Pseudomonas putida* (PFDH; EC 1.2.1.46) is a unique enzyme that can catalyze NAD^+-dependent oxidation of formaldehyde without the external addition of glutathione. Since formaldehyde is a toxic compound, it is natural that most organisms have developed several oxidation systems to counteract this compound. The PFDH enzyme, in its active form, is a homotetramer of identical subunits, each of which comprises 398 amino acid residues and two zinc ions (the catalytic zinc and the structural zinc), and has a molecular mass of about 42 kDa. A comparison of amino acid sequences shows that PFDH belongs to the zinc-containing medium-chain ADH family, although there are extensive variations in the amino acid sequence. When PFDH is incubated with NAD^+ and either formaldehyde or acetaldehyde, NADH production can be detected as an increase in A_{340}, whereas no increase in A_{340} is observed with propanal or longer chain aldehydes. However, PFDH catalyzes the efficient dismutation of a wide range of aldehydes where two molecules of the aldehydes are converted to one molecule each of the corresponding carboxylate and alcohol.

A remarkable feature of PFDH, as compared with the ADHs, is that the enzyme is a nicotinoprotein. Nicotinoproteins are proteins that contain NAD(P)(H) as a tightly bound redox active cofactor. The NAD(P)(H) in these enzymes is tightly but not covalently bound to the enzyme and does not exchange with the cytosolic NAD(P)(H) pool in contrast to typical ADHs that use NAD(P)(H) as an exchangeable coenzyme (co-substrate). It is an interesting question whether there are common structural features among the nicotinoproteins, and if so, which features are responsible for the tightly bound NAD(P)(H) cofactor. Thus, the three-dimensional structure of PFDH is essential not only to clarify the mechanism of the glutathione-independent oxidation of formaldehyde, but also to reveal the structural origin of the tightly bound cofactor among the nicotinoprotein dehydrogenases.

Results and Discussion

The crystal structure of PFDH was determined by the multiwavelength anomalous diffraction (MAD) method using intrinsic zinc ions and refined to an R-factor of 0.171 (free R-factor of 0.206) at a 1.65 Å resolution [1]. The present model includes one NAD^+ molecule, two zinc ions and all of the non-hydrogen atoms except for the N-terminal (Ser1) and C-terminal (Ala398) residues for each of the two crystallographically independent subunits. In addition, a total of 967 water molecules and two sulfate ions are included per asymmetric unit (ASU). The geometry of the current model was such that the root-mean-square deviations (RMSDs) from ideal values are 0.009 Å for bond lengths and 0.025 Å for bond distances. According to a Ramachandran plot for the current model, a total of 586 (89.6 %) non-glycine and non-proline residues have their ϕ, ψ angles in the most favored region, whereas the remaining 68 (10.4 %) non-glycine and non-proline residues are in the additional allowed region.

The 170-kDa-homotetrameric PFDH molecule shows 222-point group symmetry. Although the secondary structure arrangement and the binding mode of catalytic and structural zinc ions in PFDH are similar to those of typical ADHs, a number of loop structures that differ between PFDH and ADHs in their lengths and conformations are observed. A comparison of the present structure of PFDH with that of horse liver ADH, a typical example of an ADH, reveals that a long insertion loop of PFDH shields the adenine part of the bound NAD^+ molecule from the solvent, and a tight hydrogen-bond network exists between the insertion loop and the adenine part of the cofactor, which is unique to PFDH. This insertion loop is completely conserved among the nicotinoprotein formaldehyde dehydrogenases, whereas it is replaced by a short turn among typical ADHs. Thus, the insertion loop specifically found among the nicotinoprotein formaldehyde dehydrogenases is responsible for the tight cofactor binding of these enzymes and explain why PFDH can effectively catalyze alternate oxidation and reduction of aldehydes without the release of cofactor molecule from the enzyme.

References

[1] N. Tanaka et al., submitted.

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