

Structure and Reaction mechanism of Peroxiredoxin and Xanthine Oxidoreductase

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Introduction

Rat HBP23/Prx I belongs to the 2-Cys peroxiredoxin type I family, and exhibits peroxidase activity coupled with reduced thioredoxin (Trx) as an electron donor. We analyzed the dimer-oligomer interconversion of HBP23/Prx I by gel filtration. HBP23/Prx I existed the mixture of several oligomeric form, such as dimer, tetramer or decamer, in the liver homogenate. The several mutants of HBP23/Prx I existed as dimeric or decameric form under the gel-filtration condition. The Cys83Ser mutant was predominantly dimeric, and we previously determined the crystal structure of this mutant as dimer (PDB entry 1QQ2). On the other hand, the Cys52Ser mutant existed mostly as decameric form. To elucidate the structural differences between dimeric and decameric structure of HBP23/Prx I, we tried to crystallize and determine the structure of the Cys52Ser mutant.

Materials and methods

The Cys52Ser mutant of HBP23/Prx I was expressed in *E. coli* using pET3a vector. The purified protein was prepared as 5 mg/ml in 5 mM sodium acetate, pH 5.0, 2 mM DTT, 1 mM CHAPS. Crystallization was performed by the hanging drop vapor diffusion method. 2 μ l of the protein solution mixed with 2 μ l of reservoir solution (0.17 M ammonium acetate, 20% glycerol, 25% PEG4000, 84 mM sodium acetate, pH 5.0), and the plate was settled at 20°C in ~2 weeks. The crystals were soaked with a precipitant solution containing 30% glycerol. The data set was collected at the BL-5A beamline of the Photon Factory. The diffraction data were processed and scaled with HKL2000. Molecular replacement was performed with EPMR using the coordinates of the crystal structure of human 2-Cys Prx II purified from erythrocytes (PDB entry 1QMV). The model was refined with CNS. The final R-factor and free R-factor were 20.5% and 28.0%, respectively.

Result and Discussion

The diffraction data were collected to 2.9 Å resolution. The crystal belongs to the P2₁ space group with one molecule in the asymmetric unit. The Cys52Ser mutant was crystallized as a toroidal decamer. A decamer is composed of five dimers, forming an (a₂)₅ complex. We observed clear densities of the C-terminal loops (residues 176-198), whereas this was not observed in the Cys83Ser mutant (dimer structure), probably because of dislocation. The residues of Cys83 in decamer structure are located at the dimer interfaces. The Cys52Ser mutant has a Cys83-Cys83 disulfide bond at one dimer-dimer interface (S-S separation of ~2.3 Å), and van der Waals interaction at

the other four interfaces (mean S-S separation of 3.6 Å). This weak interaction through the Cys83 residue might control the interconversion between dimeric and decameric structure of HBP23/Prx I.

Xanthine oxidoreductase (XOR) catalyzes hydroxylation of hypoxanthine to xanthine and xanthine to urate at the molybdenum center with concomitant reduction of NAD⁺ to NADH at the FAD cofactor. Substrate specificity of XOR is very low; it catalyzes the hydroxylation of a wide variety of heterocyclic substrates such as purines, pyrimidines, and pterins, in addition to aldehydes. Points of current interest in the hydroxylation mechanism are the binding modes of hypoxanthine and xanthine substrates and the roles of other amino acids in the active site pocket. The active site is made up of a molybdopterin and highly conserved five amino acid residues (Glu802, Arg880, Phe914, Phe1009 and Glu1261 in bovine number). We determined crystal structures of inactive XOR (desulfo or demolybdo-enzyme) bound with hypoxanthine, xanthine and uric acid. Hypoxanthine binds with C2 carbon close to the active site molybdenum and makes a hydrogen bond with Glu802, whereas makes no interaction with Arg880. The findings suggest the hypoxanthine bound structure implies the binding mode of hypoxanthine during catalysis. This is consistent with site-directed study of human XOR. When the corresponding Glu in human XOR was replaced by a Val, the enzyme still displayed activity with xanthine, but almost no turnover was observed for hypoxanthine.

In the urate bound structure with demolybdo XOR, the oxygen atom of 8-OH is well superimposed with the oxygen atom of active enzyme, which will be incorporated into substrates, suggesting the structure implies xanthine binding mode. 2-OH has hydrogen bonds with Arg880, which is essential for xanthine hydroxylation. Spectroscopic study and HPLC analysis displayed that hydroxylation at the C-2 position occurs prior to that at the 8-position, suggesting that 2-position hydroxylation is crucial for 8-position hydroxylation. In crystallographic analyses of xanthine complexes with the bovine desulfo-enzyme, even at 1.9 Å resolution, the binding mode of xanthine was not delineated clearly. The electron density seems to represent a mixture of various orientations, presumably reflecting the very broad substrate specificity of this enzyme. Mixed binding modes may also explain the substrate inhibition at high concentrations of xanthine.

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