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Crystal Structure Analysis of L-Lactate Oxidase

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Introduction

The α-hydroxyacid oxidases are a group of flavoproteins which catalyze the flavin mononucleotide (FMN)-dependent oxidation of their respective substrates. These enzymes have been found to share remarkable similarities in catalytic properties with common structural motifs, making it likely that they constitute a family. L-lactate oxidase (LOX) from *Aerococcus viridans* catalyzes the oxidation of L-lactate using molecular oxygen and produces pyruvate and H_2O_2. We had made the site-directed mutant, LOX-R181M, where Arg181 has been replaced by Met to determine the effect of removing the positive charge at this position.

The LOX-R181M was crystallized well to good quality for structure analysis with a small amount of additive reagent(1). Here, we describe the crystal structure of the LOX wild obtained by the same crystallization method of the mutant, and discuss how the substrate recognition does.

Materials and Methods

Crystals were transferred to a drop of reservoir solution and soaked for about 10 min. Due to the presence of PEG as a precipitant in the crystallization protocol, the concentration of PEG in the drop is gradually increasing during this time. A crystal was mounted in a cryo-loop and flash-frozen in a nitrogen-gas stream at 100K. X-ray diffraction data were collected with a CCD detector using synchrotron radiation at beam-line BL5A and NW12 of PF/AR KEK, Japan. The wavelength was 1 Å, and the crystal-to- detector distance was maintained at either 200 or 130 mm. A total of 180 flames were collected with 1° oscillation and 5 sec exposures. The data were collected up to 1.8 Å resolution and were processed with the program *HKL 2000.*

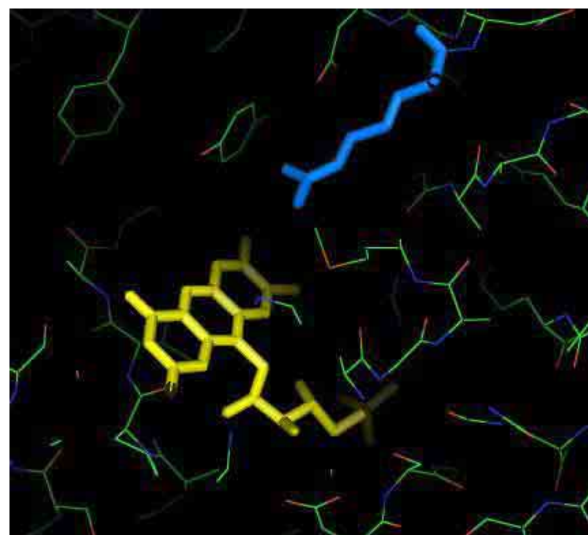
Crystals of the LOX belong to the tetragonal space group *I*422, with unit-cell parameters $a = b = 191.096$Å, and $c = 194.497$Å. With the molecular weight of the LOX (41,000Da), we assume that four LOX monomers exist in an asymmetric unit, resulting in a Matthews coefficient, V_M, of 2.71 $Å^3Da^{-1}$. This value is in the normal range for globular protein crystals. Solvent content of the crystal is 50.4%.

The structure was determined by the molecular replacement method using the program CNS. Self- and cross-rotation functions showed a tetramer formation of the enzyme, the initial search model (LOX-R181M monomer) was generated to a tetramer with non-crystallographic symmetry. All refinements were carried out using CNS and CCP4:REFMAC5. Refined model of the wild type LOX consisted of four molecules (Wild-A, Wild-B, Wild-C and Wild-D).

Results and Discussion

The overall fold of the LOX monomer was a typical $(\beta/\alpha)_8$-barrel first observed in a triose-phosphate isomerase. The r.m.s. deviations of the main chain among four monomers whose both terminals and flexible regions are excluded in the calculation are 0.701 Å (wild-A/B), 0.621 Å (wild-A/C), 0.635 Å (wild-A/D), 0.451 Å (wild-B/C), 0.513 Å (wild-B/D) and 0.421 Å (wild-C/D). Molecular exclusion chromatography of the enzyme by Sephadex G-100 provided the basis for the report that wild-type LOX was a tetramer in 50 mM potassium phosphate buffer, pH 7.0, in the presence of 0.2 M potassium chloride (2). Thus our LOX crystals have four monomers in the asymmetric unit, it suggests that the enzyme catalyses actively as a tetramer in the solution.

The LOX enzyme has a catalysis activity from L-lactate to pyruvate with an oxigenation by the FMN group. In the reaction, the proton donation from the Arg181 residue is important. We looked into the structure around acitive site, we've found some additional amino acids.



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