## Structures of Arg-181 Mutant and Wild Type of L-lactate Oxidase

from *A. Viridans* <u>Yasufumi Umena<sup>a,b</sup></u>, Kazuko Yorita<sup>c</sup>, Takeshi Matsuoka<sup>d</sup>, Makoto Abe<sup>a</sup>, Akiko Kita<sup>ae</sup>, Kiyoshi Fukui<sup>c</sup>, Tomitake Tukihara<sup>b</sup>, Yukio Morimoto<sup>a,e</sup>, <sup>a</sup>Research Reactor Inst., Kyoto Univ., Japan. <sup>b</sup>Inst. for Protein Res., Japan. Osaka Univ., Japan. <sup>c</sup>Inst. Enzyme Res., Univ. of Tokushima, Japan. <sup>d</sup>Asahi Kasei Pharma, <sup>e</sup>RIKEN Harima Inst., Japan. E-mail: de74yas@rri.kyoto-u.ac.jp

L-lactate oxidase (LOX) from Aerococcus viridans is a member of the a-hydroxyacid-oxidsase flavoenzyme family and catalyzes the oxidation of L-lactate by O2 with pyruvate and H2O2 as products. Arg-181 residue is conserved throughout the family and located around the FMN. Arg-181 has been replaced by Met to determine the effect of removing the positive charge on the residue. Effects of the replacement on kinetic properties have been reported previously. In this report, there are small effects on reactivity of the reduced flavin with O<sub>2</sub>. On the other hand, the efficiency of reduction of the oxidized flavin by L-lactate is greatly reduced. The result demonstrated the importance of Arg-181 residue in the binding substrate and its interaction with flavin. Both Wild type and R181M mutant were crystallized to belong to the tetragonal space group I422. We solved both structures about 2 Å resolution using molecular replacement method with glycolate oxidase as a search model, which is a member of the family and 35% identity with LOX. LOX structures formed the tetramer by four-fold symmetry in the asymmetric unit and packed densely in the unit cell. We demonstrate the comparison of structures with other families and the function of Arg-181 residue based on the crystallographic study of LOX structures of Arg-181 mutant and wild type.

Keywords: protein crystallography, flavoprotein, structureactivity relationships of enzymes