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X-ray structure of copper-zinc superoxide dismutase derived from *Citrus limon*

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1 Introduction

Superoxide dismutase (SOD) catalyzes disproportionation of superoxide (SO) to oxygen and peroxide. SOD plays an important role in the defence system of the body against oxidative stress through the elimination of reactive oxygen species (ROS). There are three types of SOD depending on metal ions in active site: Cu/Zn type binding both copper and zinc, Fe or Mn type binding iron or manganese, and Ni type binding nickel.

Copper–zinc superoxide dismutase (CuZnSOD) occurs in the cytosol (SOD1) or extracellular compartment (SOD3) of eukaryotic cells and the periplasm of a few Gram-negative bacteria. Recombinant SOD derived from *Citrus limon* (Cl_SOD) exists as a monomer and a dimer in a 70%:30% ratio, in which the activity of the dimer is 65% higher than that of the monomer [1].

Here, the first crystal structure of Cl_SOD is determined. The overall structure resembles those of CuZnSODs from fruits and vegetables. The enzyme crystallized as a tetramer (a dimer of dimers), in which the dimer represents the functional biological assembly [2].

2 Experiment

Crystals of Cl_SOD were obtained in a droplet containing a mixture of 0.8 µl protein solution (8.0-8.5 mg/ml in 50 mM Tris-HCl, pH 8.0, 20 mM NaN_3) and 0.8 µl reservoir solution (0.03 M Magnesium chloride, 0.03 M Calcium chloride, 0.05 M Imidazole, 0.05 M MES, pH 6.5, 20% (v/v) PEG 550 MME, 10% (w/v) PEG 20000) in a well containing 50 µl reservoir solution using the sitting-drop method at 293 K. X-ray diffraction data were collected on the PF BL-5A in the KEK, and processed using the programs XDS and the CCP4 suite. The structure was determined by molecular replacement with the program MOLREP using the structure of of SOD from *Potentilla atrosanguinea* (PDB ID: 2Q2L) as a search model. Further model building was performed in program Coot and the structure was refined using REFMAC5.

3 Results and Discussion

The structure of the Cl_SOD monomer comprises eight β-strands that are connected by seven loops to form a Greek key motif and that host the Zn and Cu atoms. This arrangement is a typical of CuZnSODs from eukaryotes. The hydrophobic residues are tightly packed in the center of the β-barrel structure and there are two surface loops that facilitate zinc binding and electron channelling for the reaction. From the crystal packing, there are two alternative Cl_SOD tetrameric assemblies (Fig. 1). The calculated contact surfaces based on PISA for the tetramer, alternative dimer 1 (Mol-A,B or Mol-C,D) and alternative dimer 2 (Mol-A,C or Mol-B,D) were 5260, 634.6 and 411.5 Å, respectively. These values suggest that the tetramer is likely to be composed of Mol-A,B and Mol-C,D pairs. The alternative dimer 1 organization is also found in CuZnSODs from spinach (PDB ID: 1SRD) and tomato (PDB ID: 3KM2). Therefore, alternative dimer 1 was considered to be the biological Cl_SOD dimer.

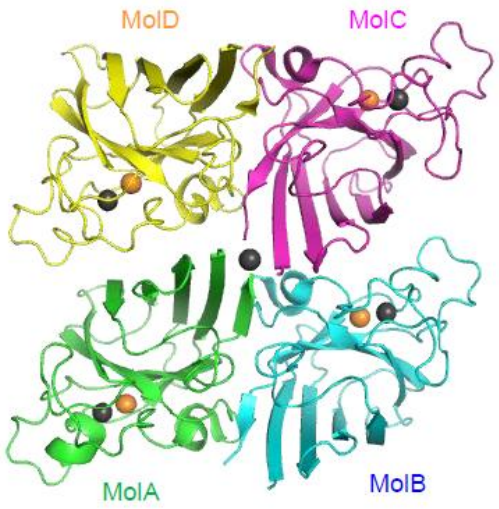

Fig. 1: The structure of Cl_SOD.
Zn and Cu are represented in black and orange spheres, respectively.

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References

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