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X-ray structure of super oxide dismutase from *Staphylococcus equorum* and the role of Ser126

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1 Introduction

Superoxide dismutase (SOD) catalyzes disproportionation of superoxide (SO) to oxygen and peroxide. SOD is regarded as the first-line defence of the human body against toxic SO, which causes inflammation and radiation damage. 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. Manganese superoxide dismutase (MnSOD) is widely found in bacteria and mitochondria. We have determined the X-ray structure of *Staphylococcus equorum* MnSOD (SeMnSOD) and showed that it forms homodimer. Dimer-interface engineering of SeMnSOD have been studied to improve the thermal stability of SeMnSOD for application. As a promising design to stabilize the dimer form of SeMnSOD, Ser126 was substituted for Cys for introducing disulfide bond at the dimer interface, however the constructed S126C mutant form of SeMnSOD did not show drastic increase in thermal stability than we expected, and showed lower enzymatic activity than wild type. In this study, we determined the X-ray structure of S126C mutant form of SeMnSOD and found a partly formed disulfide bond at the dimer interface [1].

2 Experiment

Crystals or SeMnSOD S126C were obtained in a droplet containing a mixture of 0.12 µl protein solution (32.5 mg/ml in 50 mM Tris-HCl, pH 8.0) and 0.12 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 SeMnSOD wild type (PDB ID 5X2J) .

3 Results and Discussion

There are six molecules in the asymmetric unit. S126C mutant showed a trimer of the dimer, A-B, C-D, and E-F. A dimer structure (A-B) of SeMnSOD S126C was shown in Fig.1. In the structure of S126C mutant, the electron occupancies for the S-S bonds at each dimer interface of A-B, C-D, and E-F were between 50 and 70%, suggesting that the S-S bond might not be fully formed or was broken. The breakage of an S-S bond, when tryptophan is present at a distance less than ~5 Å, has been reported [2]. The breakage is also observed as radiation damage upon X-ray diffraction analysis of proteins [3].

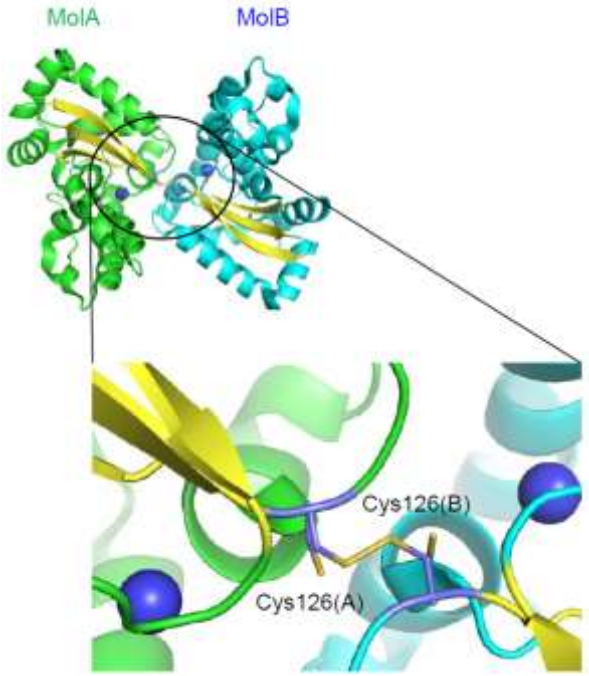

Fig. 1: Dimer structure of SeMnSOD S126C.

Fig. 2 showed the structures of dimer interface of wild type and S126C mutant. In the structure of S126C mutant, the S-S bond was sandwiched between Trp163(A) and Trp163(B) at ~3.9 Å. Trp163 is one of the key residues that are responsible for efficient enzymatic reaction. Alternative Cys126(A,B) Sγ that is not forming the S-S bond, was oriented and could interact with Oδ of Asn145 (A,B), respectively. The presence of Asn145 might alleviate the formation of S-S bond by Cys126 Sγ when an S-S bond breakage occurred. In the structure of wild type, Ser126 formed water-mediated hydrogen bonds with

Glu164 that could interact with His105 forming active site. Structure analysis of Ser126Cys mutant suggested that Cys126 formed imperfect disulfide bond at the dimer interface, and that Ser126 has a role in both enzymatic activity and stability in SeMnSOD.

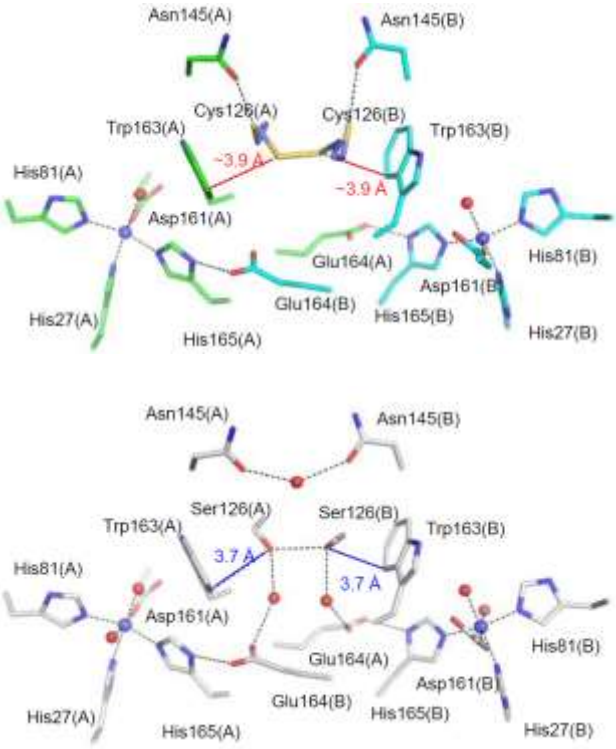

Fig. 2: Structures of dimer interface of S126C mutant (up) and wild type SeMnSOD (down).
Blue and red spheres represent metal ions and water molecules, respectively.

Acknowledgement

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References

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