Effect of (His)6- and (Cys)4- adducts on the electrochemical behavior of SOD.

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1. Intoroduction

Superoxide dismutases (SODs) provide protection against oxidative stress in the physiological system by specifically catalyzing the dismutation of the superoxide radical (O_2^-) to molecular oxygen and hydrogen peroxide via a catalytic cycle of alternating reduction and oxidation of the active-site metal in the protein. To date, the electrochemical properties of various types of SODs have been reported by the method so called self-assembled monolayer-modified electrodes. However, these results are not necessarily in good agreements. This is due, in part, to the difficulties of controlling the orientation of SOD and its interaction on the surface of electrode. Here, we generate recombinat SOD proteins with (His)6-tag or (Cys)4-tag. Using these recombinant proteins, the electrochemical behavior of (Cys)4-SOD and (His)6-SOD will be characterized. We expect that both (His)6- and (Cys)4-SOD orient towards the gold surface by virtue of (His)6- and (Cys)4-moieties, generating more effective redox potential (Fig.1).

2. Method

We performed PCR using human Cu,Zn-SOD as a template. The amplified DNA fragments of SOD and (Cys)4-SOD which contains the four cysteine residues fused to the N-terminal region of SOD were ligated pET30 vector. The pET30 expression plasmids were introduced *Escherichia coli* and the proteins were expressed and purified by (Ni^{2+})-beads. Proteins were fixed on gold disk electrodes and each electrochemical character was compared using the cyclic voltammetry (CV).

3. Results and Discussion

As shown in figure 2B, SOD, (Cys)4-SOD and (His)6-SOD proteins were expressed and purified. The apparent molecular mass of the expressed protein determined by SDS agrees with the theoretic subunit size calculated from the amino acid sequence of each recombinant SOD. The purified proteins were incubated with gold electrodes in 50 mM Bis-Tris(pH7.8) at 25℃ for 6 hrs. Using gold electrodes pre-treated with C2-NTA(Ni^{2+}), the electrochemical behaviors of SOD and (His)6-SOD were investigated (Fig.3). A quasi-reversible cyclic voltammogram containing 50 μM SOD was observed with a formal potential $E^{0'} = 85$ mV (Ag/AgCl). The electrochemical behavior of (His)6-SOD C2-NTA(Ni^{2+})-modified gold electrodes was investigated. A quasi-reversible cyclic voltammogram containing 5 μM SOD was observed with a formal potential $E^{0'} = 140$ mV (Ag/AgCl).

Since the (His)6-moiety enable (His)6-SOD to orient towards the gold electrode surface, it has more efficient redox potential than the non-tagged SOD. We are currently testing the effect of (Cys)4-moiety of SOD on the electrochemical behavior on a bear gold electrode.

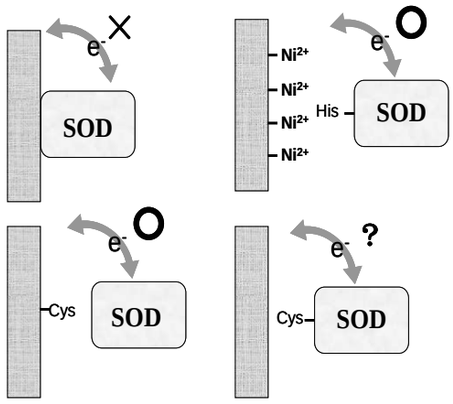

Fig.1 Effect of the His- or Cys- modification of SOD protein on the interaction with the gold electrode sufaces.

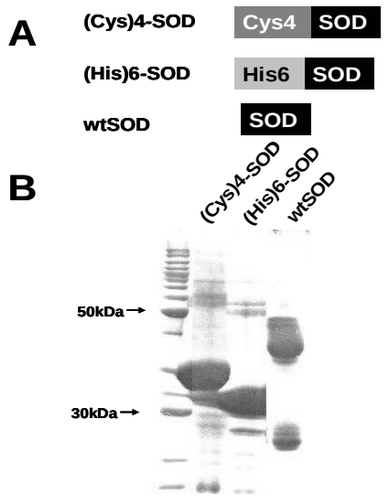

Fig.2 Production of the recombinat SOD proteins.(A) Schimatic representation of the recombinat Cys4-SOD , His-SOD and SOD. (B) Purification of (Cys)4-SOD , (His)6-SOD and SOD. Proteins were fractionated by SDS-PAGE and visualized by coomassie blue staining.

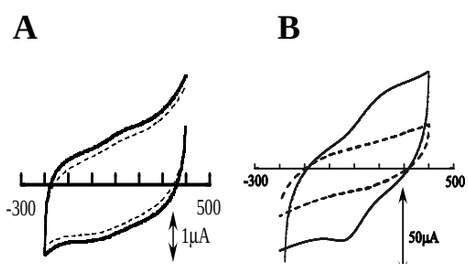

Fig.3 (A) CV of SOD on cysteine-modified electrode in Bis-Tris Buffer solution (pH7.8).
(B) CV of (His)6-SOD on C2-NTA(Ni^{2+})-modified electrode in Bis-Tris Buffer solution (pH7.8).