Cyclic Voltammetry of Cucumber Ascorbate Oxidase

Takeshi Sakurai
Institute for Molecular Science, Okazaki 444 and Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920-11

(Received March 13, 1996)

Direct electrochemistry of cucumber ascorbate oxidase at Au electrodes modified with various promoters gave redox waves originating from the type 1 copper.

Blue copper proteins, which have only the type 1 copper in relatively small sized protein molecules of ca. 10 kD to 20 kD, have been electrochemically studied in detail.[1-4] However, copper proteins belonging to the different groups of copper proteins have been the unexploited targets of electrochemistry. Here we report the cyclic voltammetry of cucumber ascorbate oxidase whose molecular weight is 140 kD per dimer. Ascorbate oxidase contains three types of coppers, the type 1 copper (blue copper), the type 2 copper (non-blue copper), and a pair of type 3 coppers (ESR non-detectable coppers) in its active site.

The cyclic voltammogram of ascorbate oxidase (Figure 1a) at a Au electrode modified with bis(4-pyridyl) disulfide[5] gave the cathodic peak at 338 mV and the anodic peak at 400 mV. The formal potential was 369 mV against standard hydrogen electrode

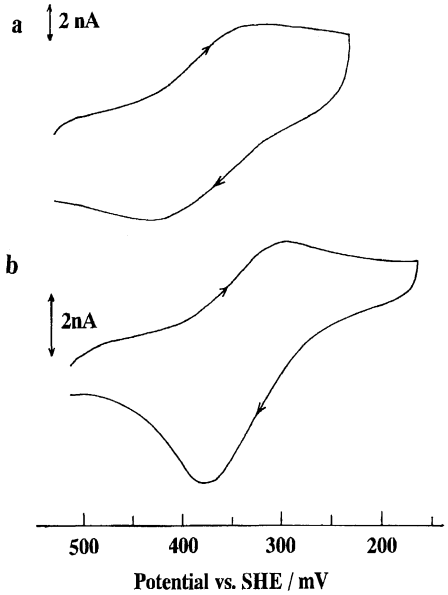
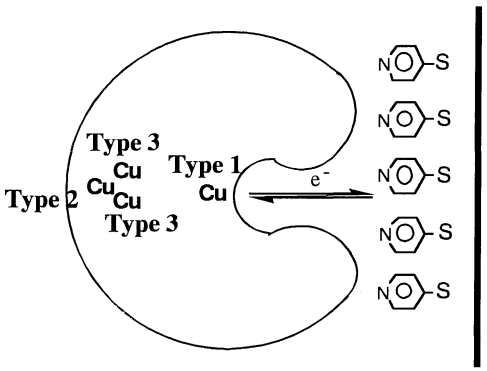


Figure 1. Cyclic voltammograms of ascorbate oxidase at Au electrodes modified with bis(4-pyridyl) disulfide (a) and 3,3'-dithiodipropionic acid (b). Measurement conditions: Protein concentration, 6.5 x 10^{-5} mol dm^{-3}; Buffer solution, 0.2 mol dm^{-3} potassium phosphate (pH 6.0); Sweep rate, 1 mV s^{-1}; Temperature, 25 °C.

(SHE), being identical with the redox potential of the type 1 copper determined by the potentiometric titration.[6] Therefore, although we don't have the direct evidence, it is most appropriate that the redox waves came from the type 1 copper. While the other types of coppers are supposed to have the similar redox potentials, they are much more deeply buried inside the protein molecule as compared to the type 1 copper according to the crystal structure.[7] It is apparent that the observed redox waves were not derived from a Cu ion eliminated from the active site because we obtained the same voltammogram even after treating the enzyme with a cation exchange resin, Chelex 100 (Bio Rad). Since one of imidazole ligands to the type 1 copper is at the bottom of the cavity to accommodate ascorbate,[4] the distance between the type 1 copper and the exterior of the protein molecule is considerably longer than that of blue copper proteins. The anodic wave is supposed to be produced by the back electron transfer from the trinuclear center to the type 1 copper site.



The heterogeneous rate for the one electron transfer process is estimated to be 8 x 10^{-3} cms^{-1} from the peak separation, ΔEp of 62 mV according to the Nicholson's method.[8] The rate is as rapid as to be comparable to that of the blue copper proteins.[9]

When a Au electrode was modified with cationic bis(2-aminoethyl) disulfide, anionic 3,3'-dithiodipropionic acid (Figure 1b) and hydrophobic diphenyl disulfide,[10] the formal potentials were 356 mV, 345 mV, and 323 mV, respectively. The decreases of the redox potential would have been brought about by the strong interaction between the protein molecule and promoters which led to the slight modification on the protein structure. Further, the anodic peak current was slightly stronger than the cathodic current as shown in Figure 1b. This might have taken place because the trinucelar center composed of the type 2 copper and a pair of type 3 coppers is partly reduced in the resting form.[11] However, since the similar voltammogaram was obtained in the second sweep, an unknown electron transfer process might take place together with the electron transfer between the type 1 copper and the Au electrode. Differing from the direct electrochemistry of blue copper proteins,[3,4] a glassy carbon electrode could not promote the redox waves.

The present results not only extend the limit of electrochemistry for macromolecules but also give a clue in studying the electron transfer process of multicopper oxidase.

This study was supported by Grants-in-Aid for Scientific

Research (#07215229 and #07640740).

References

1 F. A. Armstrong, in "*Structure and Bonding*" Springer-Verlag, Berlin (1990), No. 72, p. 137.
2 A. M. Bond and H. A. O. Hill, in "*Electron Transfer in Metalloproteins,*" ed by H. Sigel and A. Sigel, Dekker, New York (1991), p. 431.
3 T. Sakurai, O. Ikeda, and S. Suzuki, *Inorg. Chem.*, **29**, 4715 (1990).
4 O. Ikeda and T. Sakurai, *Eur. J. Biochem.*, **219**, 813 (1994).
5 I. Taniguchi, K. Toyosawa, H. Yamaguchi, and K. Yashukouchi, *J. Chem. Soc., Chem. Commun.*, **1982**, 1032.
6 K. Kawahara, T. Sakurai, S. Suzuki, and A. Nakahara, *Inorg. Chim. Acta*, **92**, L33 (1984).
7 A. Messerschmidt, R. Ladenstein, R. Huber, M. Bolognesi, L. Avigliano, R. Petruzzelli, A. Rossi, and A. Finazzi-Agro, *J. Mol. Biol.*, **224**, 179 (1992).
8 R. S. Nicholson and I. Shain, *Anal. Chem.*, **36**, 706 (1964).
9 T. Sakurai and F. Nose, submitted for publication.
10 T. Sakurai and F. Nose, *Chem. Lett.*, **1995**, 1075.
11 T. Sakurai and J. Takahashi, *Biochem. Biophys. Res. Commun*, **215**, 235 (1995).