Direct Bioelectrocatalytic Behavior and Kinetic Analysis of Wild Type and Mutant Bilirubin Oxidases

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Direct electron transfer (DET) between enzymes and electrodes has been attracting considerable attention for construction of the 3rd generation biosensors and biofuel cells[1] and it has been investigated extensively using several kinds of carbon electrodes, thiol-modified gold electrodes and so on. DET-based catalytic phenomena have been reported for limited species of enzymes. All these enzymes have more than one redox centers and it has been proposed that one of the redox centers can work as a build-in mediator (or a specific site) for electron transfer (ET) between enzymes and electrodes[1]. Bilirubin Oxidase (BOD, EC 1.3.3.5) from *Myrothecium verrucaria* catalyzes the four-electron reduction of dioxygen to water with concomitant oxidation of bilirubin to biliverdin. The enzyme is a family of multi-copper enzyme and contains four copper atoms that are spectroscopically classified as an EPR-active type 1, an EPR-active type 2, and a pair of EPR-inactive type 3 coppers. The type 1 copper site accepts electrons from electron donating substrates, and the type 2-3 cluster serves as electron donating site to reduce O_2 into water. The coordination sphere of type 1 copper is comprised of two nitrogen (two His) and two sulfur (Cys and Met) donors. The mutants of BOD, in which the Met467 residue coordinating to type 1 copper was replaced with another amino acid residue, have been expressed[2]. In this work, we attempted to realize a DET-type bioelectrocatalytic reduction of O_2 to H_2O with wild type and mutant BODs using glassy carbon electrode (GCE) and highly oriented pyrolytic graphite electrode (HOPGE, edge plane).

O_2-saturated solution gave a sigmoidal and steady-state cathodic wave around 400 mV in the presence of wBOD at HOPGE, as shown in Fig. 1. On the other hand, the cathodic wave of M467Q BOD appeared around 200 mV. This result is accord with the fact that the formal potential $E^{\circ\prime}$ of M467Q BOD (220 mV[2]) is more negative than that of wBOD (460 mV). One of the important approaches to understand the DET-type BOD catalysis is quantitative interpretation of the current-potential curves. Considering the DET-model given in Scheme 1, the enzymatic model for DET-type catalysis is written by

$$S + \Gamma_R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \Gamma S \xrightarrow{k_2} P + \Gamma_O \quad (1)$$

$$\Gamma_O + ne \underset{k_b}{\overset{k_f}{\rightleftarrows}} \Gamma_R \quad (2)$$

The *i*-*E* curve is expressed by

$$\frac{i}{nFA} = k_c \Gamma_t \frac{c_s}{c_s + K_S} \quad (3)$$

with

$$k_c = \frac{k_2 k_f}{k_2 + k_f} \quad (4)$$

$$K_S = \frac{(k_{-1} + k_2)(k_f + k_b)}{k_1 (k_2 + k_f)} \quad (5)$$

where n, F , A, Γ_t, c_s and K_S are the number of electron, the Faraday constant, the electrode surface area, the total surface concentration of the enzyme, the bulk concentration of substrate and the Michaelis constant for substrate, respectively. k_1, k_{-1}, k_2 and k_c are the rate constants of enzymatic reactions. k_2 involves the intramolecular electron transfer from type 1 to type 2-3 cluster. When c_s » K_s, the current density I can be expressed by

$$I = nF\Gamma_t \frac{k_2 k_f}{k_2 + k_f} \quad (6)$$

The surface ET rate constants k_f and k_b are expressed by the following Butler-Volmer-type equations:

$$k_f = k^\circ \exp[-\alpha(nF/RT)(E - E^{\circ\prime})] \quad (7)$$

$$k_b = k^\circ \exp[(1-\alpha)(nF/RT)(E - E^{\circ\prime})] \quad (8)$$

where k°, and α are the standard surface ET rate constant at $E^{\circ\prime}$, and the transfer coefficient, respectively. The experimental current-potential curves were fitted to equations (6)-(8) with k_2/k° and $k_2\Gamma_t$ as adjustable parameters using non-linear regression analysis program. As summarizing Table 1, M467Q BOD exhibited larger k_2 value than wBOD. These results suggest that the negative shift of $E^{\circ\prime}$ of type 1 copper enhances the intramolecular electron transfer.

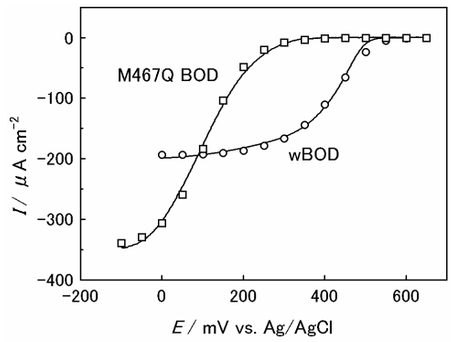
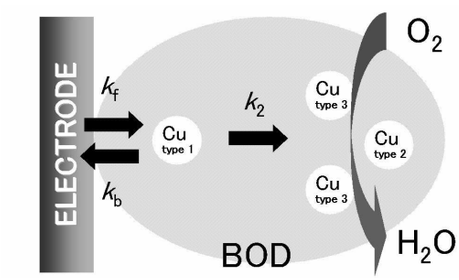


Fig. 1. Background current-corrected steady-state linear sweep voltammograms recorded with wBOD and M467Q BOD-adsorbed electrodes prepared with HOPGE (edge plane), at pH 7.0. The open square and circles represent the regression curves.



Scheme 1. Schematic representation of the DET-type bioelectrocatalytic reduction of O_2 to H_2O with wBOD and M467Q BOD adsorbed on electrodes.

Table 1. Evaluated parameters of DET-type wBOD and M467Q BOD catalysis at various carbon/graphite electrodes.

Enzyme	Electrode	k_2/k°	$k_2\Gamma_t$ / 10^{-9}mol cm^{-2} s^{-1}
wBOD	HOPGE	2.7	1.8
M467Q BOD	HOPGE	9.2	3.6
wBOD	GCE	4.0	0.1
M467Q BOD	GCE	12.0	0.1

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