

**Development of Direct Electron Transfer-Type Cascade
System by Alcohol and Aldehyde Dehydrogenases**

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Abstract

Oxidoreductases are biocatalysts with high activity and substrate specificity under mild conditions (room temperature, normal pressure, and neutral pH), which realize efficient energy conversions for a sustainable low-carbon society. Some redox enzymes can proceed with catalytic reactions on electrically-connected electrode materials, which is called direct electron transfer (DET)-type bioelectrocatalysis (Fig. 1). DET-type reactions are expected to be applied to bioelectrochemical devices such as biosensors, biofuel cells, and bioreactors.

In this study, we focused on membrane-bound alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AIDH) from *Gluconobacter oxydans*. They can catalyze two-step oxidation from ethanol to acetate via acetaldehyde in the respiratory electron transfer system called acetic acid fermentation. Both enzymes are heterotrimers with the catalytic centers of pyrroloquinoline quinone (PQQ) for ADH and molybdopterin (Moco) for AIDH, respectively. The electron transfer pathways are estimated to be from the catalytic centers where substrates are oxidized to the final natural electron acceptor, ubiquinone-10 (UQ₁₀), via the membrane-bound cytochrome *c* subunits composed of three hemes *c* (called hemes 1*c*, 2*c*, and 3*c* from the N-terminus in this order). However, since the structures of both enzymes are still unknown, the catalytic mechanism has not been elucidated.

On the other hand, the DET activity of ADH was confirmed in a previous report, but details such as the suitability of electrode materials and the electron transfer mechanism are not clear. In addition, no electrochemical studies on ALDH have been conducted. In this study, we attempted the basic evaluation of DET-type reaction of ADH and AIDH from the viewpoint of structural biology and bioelectrochemistry, and its application development.

In Chapter 1, the author attempted to investigate characteristics of ADH and AIDH from viewpoints of structural biology and bioelectrochemistry. First, three-dimensional (3D) structures of ADH and AIDH were elucidated using cryo-electron microscopy (cryo-EM) and single particle image analysis. DET-type bioelectrocatalysis by the two enzymes was then characterized based on their 3D structures. Particularly, the author focused on the cyanide (CN⁻) effects on their bioelectrocatalytic properties, because CN⁻ coordination to hemes *c* was expected to interfere with the heme *c*-related electron transfer pathways of ADH and AIDH. Their bioelectrochemical characteristics were compared based on the CN⁻ effects from viewpoints of kinetics and thermodynamics.

In Chapter 2, the author attempted to develop bienzymatic DET-type cascade system with ADH and AIDH. First, DET-type bioelectrocatalysis by ADH and AIDH was improved at pyrene-derivative-functionalized multi-walled carbon nanotubes. The optimized electrode platform

exhibited strong DET-type activities of ADH and AIDH. A mediatorless bienzymatic cascade system was then constructed. The theoretical model explaining the cascade reaction was discussed with assumptions concerning the enzyme adsorption and the concentration of the intermediate product, which was consistent with the experimental results. An ethanol biofuel cell constructed with the bienzymatic bioanode showed the highest power density in the present ethanol biofuel cells, and the Faraday efficiency for acetate production by the electrolysis of the cell reached 100%. This is the first report to experimentally and theoretically discuss a mediatorless bienzymatic cascade system.

This study will lead to an efficient use of biomass ethanol. In addition, the new findings with structural biology and bioelectrochemistry will pioneer the enzymatic utilization in next-generation biomimetic devices and a multi-catalytic cascade system.

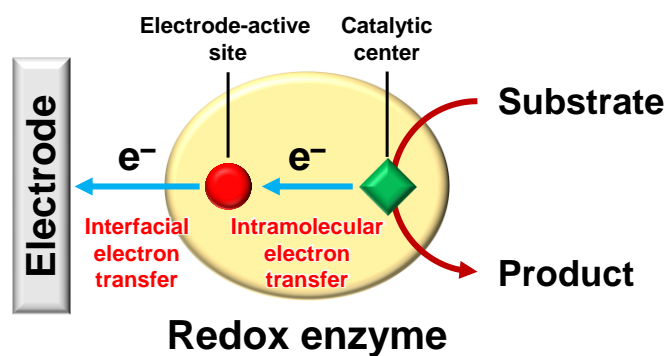


Figure 1. Schematic view of DET-type bioelectrocatalysis for oxidation