Electrocatalytic Oxidation Of Ethanol With Alcohol Dehydrogenase Through A Complex Containing Phenanthroline-Quinone As A Mediator

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INTRODUCTION

Nicotinamide adenine dinucleotide (NADH, reduced from) is a coenzyme, which is widely seen in many redox enzymes. The electrocatalytic oxidation of NADH has been of interest in connection with the development of biodevices. However, it is well known that the direct oxidation of NADH requires a large overpotential on conventional electrodes whereas reported reversible formal potential is about -0.32 V (vs. NHE). To reduce the overpotential, various quinone compounds were used as mediators so far. Abruna and coworkers investigated a variety of transition metal complexes containing phenanthroline-quinone (PQ) for electrocatalytic oxidation of NADH[1,2].

Our final goal is construction of bio-anode for the biofuel cell based on alcohol dehydrogenase (ADH, NAD^+ dependent) (scheme 1) and a novel electrode modified with the redox polymer containing PQ. We present here the electrochemical properties of an iron complex containing PQ cast on glassy carbon (GC) electrode.

EXPERIMENTAL

Synthesis of $[Fe^{II}(PQ)_3](PF_6)_2$ (**1**)

$FeCl_2$ was added to a suspension of PQ in water under inert atmosphere. After the mixture was stirred for 2h, an excess of NH_4PF_6 was added and the precipitate which appeared was collected. The complex was washed with water and dried in vacuo for 24h.

Electrochemistry

NADH and ADH (EC 1.1.1.1) from *Saccharomyces cerevisiae* were obtained from SIGMA. ALS electrochemical analyzer was used for electrochemical measurements. A three electrode cell with a complex **1** modified working electrode, a Pt wire counter electrode, and a Ag/AgCl reference electrode was used. The complex **1** was dissolved in acetonitrile, and then the solution was cast onto the GC electrode. After drying, the modified working electrode was used in acetate or phosphate buffer at room temperature.

RESULTS AND DISCUSSION

Figure 1 shows pH dependence of cathodic half-wave potential of **1**. The complex **1**-modified electrode was stable over the pH range of 3~9. We obtained values of -60 mV/pH unit for this electrode, which is close to the theoretical value of -59 mV/pH unit.

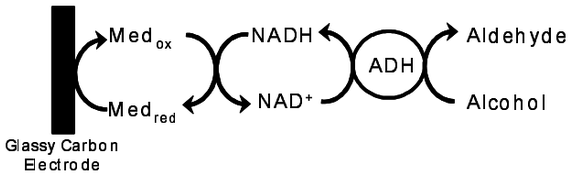
The cyclic voltammogram of the complex **1**-modified electrode in phosphate buffer at pH 8.8 was shown in Figure 2a. The clear redox response associated with PQ-based redox reaction was observed at -0.06 V. With NADH, an electrocatalytic current was observed at the oxidation potential of PQ implying the effective electrocatalyzed oxidation of NADH by this modified electrode (Figure 2b). The large potential shifts were obtained when compared to the NADH oxidation at a bare GC electrode which takes place at +0.56 V (Figure 2d). It has been indicated that the complex **1** is effective in decreasing overpotential and re-oxidizing NADH at the optimum pH value of ADH.

ADH can oxidize ethanol to acetaldehyde in the presence of NAD^+. This cofactor acts an acceptor of electrons generated in the enzymatic reaction and is transformed to its reduced form, NADH. After loading ADH and ethanol in the NADH solution (pH 8.8), the anodic current was larger than that of NADH oxidation (Figure 2c). This result indicates that the NADH generated by the enzymatic reaction, is subsequently oxidized to NAD^+ by a complex **1**.

In conclusion, we have shown here the effectiveness of the complex **1** modified electrode as a bio-anode for biofuel cell. Further studies on the stable immobilization of this complex on the electrode are under way.

REFERENCES

1) Goss, C; Abruna, H. D., *Inorg. Chem*., 1985, 24, 4263.
2) Abruna, H. D. et al., *Anal. Chem*., 1996, 68, 3688.



Scheme 1**.** Enzymatic and electrochemical reaction at electrode surface.

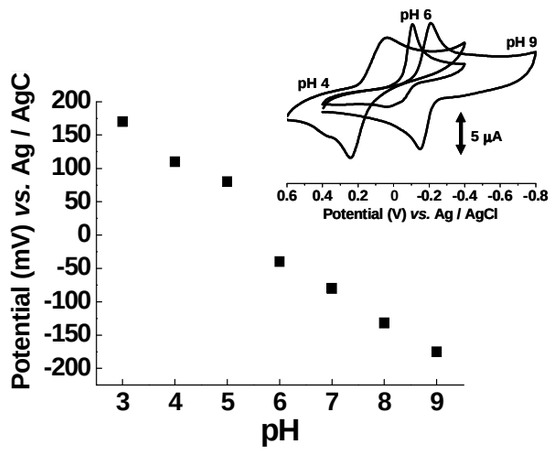


Figure 1**.** Cathodic half-wave potential for **1** as a function of pH. Scan rate:100 mV/s. Inset: The cyclic voltammograms of **1** at pH4, pH8, and pH9.

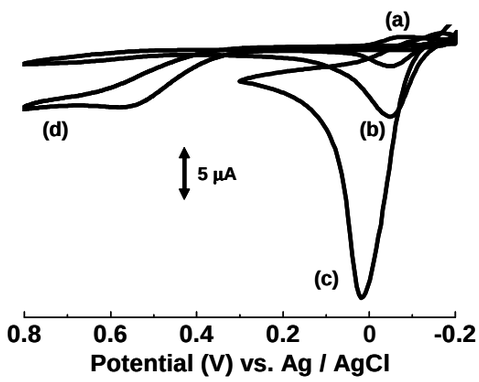


Figure 2. Cyclic voltammograms at a scan rate of 2 mV/s for a GC electrode modified with the complex **1**, (a), (b), and (c) and for a bare electrode (d) in phosphate buffer (pH 8.8). (a) 0 mM NADH. (b) 2.0 mM NADH. (c) 2.0 mM NADH + 10 µM ADH + 10 mM EtOH. (d) 2.0 mM NADH.