Electrocatalytic Oxidation of Alcohol at a Polypyrrole Diaphragm Electrode Coated with Diaphorase and Alcohol Dehydrogenase

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1 INTRODUCTION

Enzyme sensors have been recognized as useful analytical tools for biologically important compounds. Among various types of enzyme sensors, much attention has recently been focused on amperometric sensors based on mediated electron-transfer. A conventional sensor of this type needs an electron mediator which should be added to an analyte solution before the measurement. Immobilization of mediators in the enzymes films eliminates this handling complexity but, in general, reduces the amperometric responses.

We here report a new type of mediated enzyme reaction systems where mediators and analytes are separated by diaphragm electrodes. Figure 1 shows the system investigated in the present study. Ethanol (an analyte) in benzene phase permeates through the polypyrrole (PPy) diaphragm. The permeated analyte is catalytically oxidized at the diaphragm electrode coated with diaphorase (Dp) and alcohol dehydrogenase (ADH) in the aqueous phase containing NADH and an electron mediator which are impermeable to the membrane. Miniaturization of this system will serve as an amperometric enzyme sensor without addition of mediator or NADH to the sample solutions.

2 EXPERIMENTAL

Dp (2030 U / mg) (Unichika), ADH (340 U / mg), NADH (SIGMA) and glutaraldehyde, ethanol (Wako Chemical Co.) were used as received. Pyrrole (Kanto Chemical Co.) was used immediately after being purified by distillation under a reduced pressure.

A PPy micro diaphragm was fabricated in a microhole (ca. 80 μm in diameter) in a thin glass plate (ca. 80 μm thick) by interfacial electropolymerization. Before the polymerization, one side of the plate was sputtered with Pt and then coated with a photoresist (OFPR 5000, TOK Co.) so as to expose Pt only in the hole.

The above plate was set in an electrolysis cell separating a 1.0 M (M = mol x dm⁻³) KCl and pyrrole / benzene (1 / 1 in volume) solutions. A counter electrode (Pt wire) and an SCE were immersed in the aqueous phase. Upon electropolymerization at 0.9 V vs. SCE, the microhole was completely covered with PPy. Recently, Lee and coworker reported that the lateral growth of PPy was promoted along the heterogeneous liquid–liquid interface. We terminated the polymerization when ca. 90 μC was passed. The thickness of the PPy film was ca. 20 μm.

Next, the PPy diaphragm was coated with a Dp / ADH membrane. The enzyme membrane was prepared from an aqueous mixture composed of equal volumes of 5 wt % Dp, 23 wt % ADH and 10 wt % glutaraldehyde. The final activity of the enzyme membrane was 20 mU / mm² for Dp and 20 mU / mm² for ADH.

The characteristics of the diaphragm electrode was
investigated in a cell consisted of two compartments (Fig. 2). Sol. 1 was an 1 M KCl / 5 mM phosphate buffer aqueous solution (pH 7) and Sol. 2 was a benzene solution.

3 RESULTS AND DISCUSSION

The PPy film served as a diaphragm as well as an electrode in this system. However, the electrical conductivity of PPy decreases when the electrode potential exceeds 0.3 V vs. SCE at pH 7\(^9\). Figure 3 shows background-subtracted voltammograms of 0.5 mM Co(phen)_3^{2+} at the PPy diaphragm electrode. Co(phen)_3^{2+} gave a broad oxidation peak around 0.2 V vs. SCE. The addition of NADH into the aqueous phase (Sol. 1) increases the oxidation current, clearly indicating that Dp immobilized at the PPy diaphragm electrode acts as a catalyst for the electron transfer from NADH to Co(phen)_3^{3+}. A further increase in the oxidation current was observed upon adding ethanol into the benzene solution (Sol. 2). Ethanol permeated through the PPy diaphragm and was oxidized catalytically at the Dp / ADH coated PPy diaphragm electrode in the aqueous phase as shown in Fig. 1. Under the present conditions, no obvious permeation of NADH and Co(phen)_3^{2+} through the PPy diaphragm was observed. The above results demonstrate the possibility to separate an analyte from a mediator and NADH in NADH-based amperometric enzyme sensors.

REFERENCES