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Immobilization of alcohol dehydrogenase on poly[1-(2-carboxyethyl)pyrrole] film for fabrication of ethanol-responding electrode

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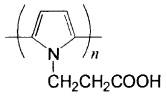
Abstract

Alcohol dehydrogenase (ADH) was immobilized on the film of poly[1-(2-carboxyethyl)pyrrole], which was prepared by electrochemical polymerization of 1-(2-carboxyethyl)pyrrole. The film exhibited a stable conductivity of 10^{-4} - 10^{-3} S/cm over the temperature range 0 - 200 °C. A significant amount of ADH (50-100 g/cm^2) was immobilized on the polymer film by use of a condensing reagent. Although activity of ADH was decreased by the immobilization, the ADH-immobilized polymer film showed amperometric response to ethanol at +0.85 V vs. SCE in the presence of nicotinamide adenine dinucleotide (a redox mediator). These results demonstrate that the polymer film acts as both an ADH-supporting layer and a conductive medium in the alcohol-driven amperometric device.

Keywords: Electrochemical polymerization, Detectors

1. Introduction

Immobilization of redox enzymes on electrode is a key technique in fabrication of amperometric biosensors, and conductive polymer films are promising candidates for the electrodes in these devices. Physical adsorption and entrapment of enzymes to the conductive polymers by pioneer chemists opened this area [1]. We have been interested in design of conducting polymers that can be modified chemically and in covalent binding of enzymes on it. In a previous study, we prepared poly[1-(2-carboxyethyl)pyrrole] (PPy-COOH) having a conductive polypyrrole backbone and carboxyl side-chains for covalent binding of enzymes, and applied to a glucose sensor combined with glucose oxidase [2, 3]. The amperometric detection of such biochemical compounds includes an interest for design of biofuel cells.



PPy-COOH

In this study, modification of the PPy-COOH film by covalent binding of alcohol dehydrogenase (ADH) was carried out for ethanol detection. ADH belongs to the largest group of proteins that need nicotinamide adenine dinucleotide (NAD^+/NADH) as cofactor. Amperometric study for the NAD^+/NADH depending enzyme allows designing a broad range of dehydrogenase-based devices.

This sensing system contains the enzyme reaction (eq (1)) and electrochemical oxidation of NADH (eq (2)), which should serve as a quantitative detection of ethanol.

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + NADH + H^+ \quad (1)$$

$$NADH \longrightarrow NAD^+ + H^+ + 2e^- \quad (2)$$

2. Experimental

2.1 Materials

Yeast Alcohol dehydrogenase (ADH) [EC 1.1.1] and nicotinamide adenine dinucleotide (NAD^+) were

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purchased from Roche Diagonostics GmbH. 1-Cyclohexyl-3-(2-morpholinethyl)carbodiimide metho-*p*-toluenesulfonate (CMC) was purchased from Aldrich Chem. Co.

1-(2-Carboxyethyl)pyrrole was synthesized by base catalyzed hydrolysis of 1-(2-cyanoethyl)pyrrole (Aldrich Chem. Co.) and isolated as light brown crystals in a 78% yield. It was identified by IR and ^{1}H NMR spectra.

2.2 Preparation of PPy-COOH film

The PPy-COOH film was obtained on a gold electrode by electrochemical polymerization: a potential of +1.2 V vs. saturated calomel electrode (SCE) was applied to the gold electrode (geometric area 0.25 cm^2) for 100 mC in a propylene carbonate solution containing 0.1 M 1-(2-carboxyethyl)pyrrole and 0.2 M $Bu_4N \cdot BF_4$. Fig. 1 and 2 represent the working (gold) electrode and the apparatus for electorchemical reaction, respectively. The prepared film of PPy-COOH was assigned by IR spectra [3].

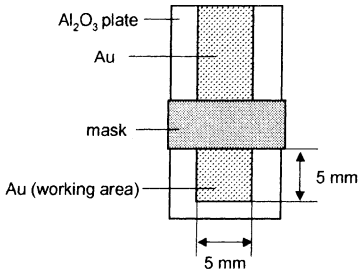

Fig. 1. Working electrode

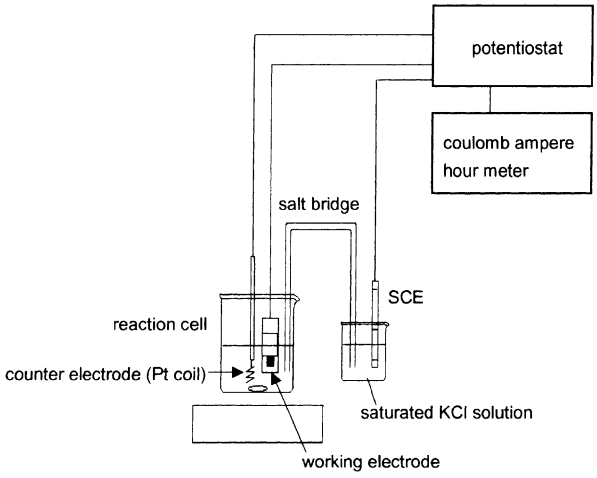

Fig. 2. Apparatus for electrochemical reactions

2.3 Immobilization of ADH

The PPy-COOH film was soaked for 10-72 h in a solution of 14 mg/mL ADH (2 mL) containing 35 mM CMC as a condensing reagent, and then washed with water. Amount of the immobilized ADH was determined by Lowry's protein assay [4].

2.4 Determination of enzyme activity and kinetic parameters for immobilized ADH

The PPy-COOH electrode supporting ADH was soaked in a carbonate buffered solution (0.05 M, pH 9.0, 30°C) containing 1 mM NAD^+ and given amount of ethanol. The production of NADH was monitored as absorbance change at 340 nm. Initial rate of the NADH production was analyzed with Micaelis-Menten equation and Lineweaver-Burk plot [5].

2.5 Amperometric detection of ethanol with ADH-immobilized polymer film

The PPy-COOH electrode supporting ADH was placed in a carbonate buffered solution (0.05 M, pH 9.0, 10 mL, 30°C) containing 10 mM NAD^+ (Fig. 2), and constant potential of +0.85 V vs. SCE was applied. After the background current was allowed to be constant, amperometric response to ethanol was recorded.

3. Results and Discussion

3.1 Characteristics of immobilized ADH

The kinetics of ADH is descried by an ordered bi-bi mechanism, which involves two substrates (NAD^+ and alcohol), a ternary complex (enzyme-NAD^+-alcohol), and two products (NADH and aldehyde) [6-8]. In this study, for convenience, the kinetics at constant concentration of NAD^+ were approximated with Micaelis-Menten equation (eq (3)):

$$V = \frac{V_{max}}{1 + K_m/[S]} \quad (3)$$

where V is reaction rate, [S] is concentration of substrate, K_m is the Michaelis constant, V_{max} is the maximum rate that corresponds to product of the total concentration of enzyme ($[E]_0$) and the rate constant of catalytic reaction in forward direction (k_{cat}). Lineweaver-Burk plot, based on initial rate, gave straight lines, and the kinetic parameters were estimated as shown in Table 1. V_{max} was significantly decreased by the immobilization, but K_m value, that indicates affinity of the enzyme to the substrate, was little affected.