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Determination of formal potential of NADH/NAD$^+$ redox couple and catalytic oxidation of NADH using poly(phenosafranin)-modified carbon electrodes

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ABSTRACT

The electrochemical regeneration of NADH/NAD$^+$ redox couple has been studied using poly(phenosafranin) (PPS)-modified carbon electrodes to evaluate the formal potential and catalytic rate constant for the oxidation of NADH. The PPS-modified electrodes were prepared by electropolymerization of phenosafranin onto different carbon substrates (glassy carbon (GC) and basal-plane pyrolytic graphite (BPPG)) in different electrolytic solutions. The formal potential was estimated to be -0.365 ± 0.002 V vs. SHE at pH 7.0. As for the bare carbon electrodes, the oxidation of NADH at the BPPG electrode was found to be enhanced compared with the GC electrode. For the PPS-modified electrodes, it was found that the electrocatalysis of PPS-modified electrodes for the oxidation of NADH largely depends on the carbon substrate and electrolyte solution employed for their preparation, i.e., the PPS-modified BPPG electrode prepared in 0.2 M $NaClO_4$/acetonitrile solution exhibits an excellent and persistent electrocatalytic property toward NADH oxidation in phosphate buffer solution (pH 7.0) with a diminution of the overpotential of about 740 and 670 mV compared with those at the bare GC electrode and the PPS-modified GC electrode prepared in 0.2 M H_2SO_4 solution, respectively. A quantitative analysis of the electrocatalytic reaction based on rotating disk voltammetry gave the electrocatalytic reaction rate constants of the order of 10^3–10^4 M^{-1} s^{-1} depending on the preparation conditions of the PPS-modified electrodes.

1. Introduction

The adenine derivatives, nicotinamide adenine dinucleotide (NAD$^+$) and its reduced form NADH are the key central charge carriers in living cells. NAD$^+$-dependent dehydrogenases, the vast majority of redox enzymes, require NADH/NAD$^+$-coenzymes for their operation [1,2]. NADH/NAD$^+$-dependent dehydrogenases have been frequently utilized in analytical chemistry [3] and organic chemistry [4]. In electrochemistry, coupling of NAD-dependent enzyme reactions with electrode processes has become of interest in designing and developing amperometric biosensors and biofuel cells [5,6]. In such systems, an efficient electrochemical regeneration of NADH/NAD$^+$ redox couple [7] is essential. Thus, the reversible and efficient electrochemical oxidation of NADH to its enzymatically active form is regarded as the key in the application of any of NAD$^+$-dependent dehydrogenases as receptor compounds in amperometric biosensors. In view of this great importance, electrocatalysts for the oxidation and reduction of the NADH/NAD$^+$ couple have been developed.

In aqueous solution at pH 7.0, the thermodynamic redox potential ($E^{\circ\prime}$) for the NADH/NAD$^+$ redox couple was estimated based on equilibrium measurements for some enzyme catalyzed reactions to be -0.315 V vs. NHE (i.e., -0.557 V vs SCE) [8]. Electrochemistry of the NADH/NAD$^+$ couple has been extensively studied at different electrodes (e.g. gold, platinum and glassy carbon) and it has been demonstrated that the electrochemical oxidation/reduction process is highly irreversible and the direct oxidation of NADH often requires high overpotentials [9–12], which are increased further by the presence of enzyme [13]. The oxidation of NADH at bare electrodes appears to occur via two successive one-electron transfer steps involving radical intermediates, which often causes side reactions, electrode fouling, and interfering background currents in real samples [14–17] and sometimes leads to the formation of enzymatically inactive forms of NAD$^+$ [18]. In addition, the process of electrochemical oxidation of NADH is not very reproducible since it has been shown to depend on the history and pretreatment of the electrode. Similarly, the electroreduction of NAD$^+$ requires high overvoltages and additionally produces radical intermediates followed by the dimer formation [19]. An appropriate selection of electrode material along with redox mediators (non-biological or NAD$^+$-dependent enzymes) can significantly decrease the overpotential for the oxidation process bringing closer to the thermodynamic value as well as can greatly enhance the process efficiency avoiding the formation of enzymatically inactive byproducts.

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In an effort to accelerate the interfacial electron transfer kinetics at low overpotential, various types of electrocatalysts (mediators) have been employed for the electrochemical regeneration of NAD^+ by bioelectrochemists. Organic compounds that undergo two-electron reduction–oxidation processes such as quinones, phenylenediamines and aminophenols have been found to be ideal for the mediation of NADH oxidation, although single-electron-transfer mediators (e.g. ferrocene derivatives) are also capable of oxidizing NADH [20,21]. The use of electrode-immobilized mediators (adsorbed or covalently coupled) [22–24] (such as ortho-quinones [25], para-quinones [26], phenazine, phenoxazine and phenothiazine derivatives [27,28], Os-complexes [29]) has also been applied for the oxidation of NADH. Complexes of Ru have been reported to be active for the electrocatalytic reduction of NAD^+ to NADH [30]. The oxidation of NADH with the regeneration of NAD^+ can also be obtained by enzymatic methods [31]. Many enzymes also have been used to provide the bioelectrocatalytic reduction of NAD^+ [32]. However, all these systems are suffered from the relatively large overpotentials required for the oxidation/reduction of the NADH/NAD^+ couple. The electrocatalytic rates of the enzymatic systems are generally too slow to produce an observable catalytic current on the cyclic voltammetric time scale [33]. Moreover, a high cost and low reproducibility and repeatability are other disadvantages of enzyme electrodes. Several reports have demonstrated that redox conducting polymers such as poly(methylene blue), poly(neutral red) (PNR), poly(phenothiazine) and poly(thionine) are favorable mediators possessing efficient electrocatalysis for NADH oxidation at lower potential and the advantages of using polyazines as electrocatalysts for the regeneration of NADH/NAD^+ [34–37], which provided the application of these polymers in bioelectroanalysis, namely for the construction of improved dehydrogenase-based biosensors [38,39]. Among the polyazine mediators used so far, PNR film was found by Karyakin et al. to electrocatalyze both the oxidation of NADH and the reduction of NAD^+ [34,40] as well as to regenerate the NADH/NAD^+ couple effectively, allowing the determination of the formal potential (ca. −0.59 V vs. SCE at pH 6.0) [41]. They believed that upon its electropolymerization the structure that reminds the molecular structure of flavins (which mimic the NADH dehydrogenase activity) would be synthesized. Phenosafranin (PS) is an N-substituted phenazine with a half-wave potential of −0.458 V at pH 7.0. Poly(phenosafranin) (PPS) forms an electroactive polymer like PNR, being composed of PS units linked via secondary amino nitrogen with keeping the essential structure of monomers [42]. PPS which follows a $2e–2H^+$ redox process per PS moiety efficiently electrocatalyzes the oxidation of NADH [43]. The catalytic activity of PPS originates from the phenazinium ring which is considered as the electroactive center of the polyazine group. So, it can be assumed that the electropolymerization of PS may possibly generate flavin-type moiety which can effectively perform as a biological catalyst for the purpose of NADH/NAD^+ regeneration.

In this study, we have explored the regeneration of the NADH/NAD^+ redox couple on PPS-modified carbon (GC and BPPG) electrodes owing to the importance in bioelectrochemistry. We have also examined the potential utility of the PPS-modified electrodes, prepared from different experimental conditions, toward the electrocatalytic oxidation of NADH. In addition, the kinetics of the mediated electrooxidation of NADH has been investigated using a rotating disk electrode (RDE) with the aim of finding the capabilities of the modified electrodes as electron transfer mediator.

2. Experimental

2.1. Chemicals and reagents

Phenosafranin (PS; Acros Organics, USA), reduced and oxidized forms of β-nicotinamide adenine dinucleotide (NADH and NAD^+, Oriental Yeast Co. Ltd., Japan), H_2SO_4, $HClO_4$ and acetonitrile (Kanto Chemicals, Japan) were used as received. Phosphate buffer solution (PBS; 0.2 M (M = mol/dm^3), pH 7.0), prepared by $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ (Kanto Chemicals, Japan), was used as the supporting electrolyte for electrochemical experiments. The solutions throughout this work were always prepared using deionized water from a Milli-Q water system (Millipore, Japan). Solutions of NADH and NAD^+ were freshly prepared using PBS (pH 7.0) before each experiment. A basal-plane pyrolytic graphite (BPPG; Union Carbide Co.; Φ = 5 mm in diameter) and glassy carbon (GC; Bioanalytical Systems Inc. (BAS); Φ = 3 mm) disks were used as the working electrodes. All of the other chemicals were of analytical grade and were used without further purification.

2.2. Apparatus and electrochemical measurements

Electrochemical experiments were carried out using a conventional two-compartment three-electrode electrochemical cell equipped with the above-mentioned working electrodes, a spiral Pt wire as counter electrode and a $Ag|AgCl|KCl_{(sat)}$ as reference electrode. Cyclic voltammetric measurements were performed with a computer-controlled ALS CHI 750Cz electrochemical analyzer (BAS) driven with a general-purpose electrochemical system software (BAS). Steady-state voltammograms were obtained by rotating disk electrode (RDE) voltammetry using a computer-controlled ALS CHI 750Cz electrochemical analyzer combined with an electrode rotation speed controller from Nikko Keisoku Co., Japan. A personal computer was used for data storage and processing. Electrolyte solutions were deaerated by bubbling Ar gas (99.99%) for at least 30 min prior to electrochemical measurements. All the measurements were carried out at room temperature (25 ± 1 °C).

2.3. Construction of modified electrodes

The GC electrode surface was first carefully polished with alumina (1.0 and 0.06 μm) on a polishing cloth. The electrode was placed in a Milli-Q water container, and a bath ultrasonic cleaner was used to remove the adsorbed particles. The electrode was then cleaned by cycling the electrode potential between −0.2 and +1.5 V versus $Ag|AgCl|KCl_{(sat)}$ in 0.1 M H_2SO_4 at a scan rate of 100 mV s^{-1} until reproducible voltammograms were recorded. The electrooxidative polymerization of PS was carried out following previous reports [42,43] on two different substrates (GC and BPPG). Typically, PPS films were electrolytically deposited on GC (abbreviated as PPS/GC[1]) in 0.2 M H_2SO_4 solution containing 1 mM phenosafranin. The potential of the working electrode was repetitively cycled for 10 times between −0.65 and 1.4 V versus $Ag|AgCl|KCl_{(sat)}$ at a scan rate of 50 mV s^{-1}. The electropolymerization of PS onto GC electrode (PPS/GC[2]) was also performed in 0.1 M $NaClO_4$ aqueous solution (pH 1.0) containing 1 mM PS by potential-sweep electrolysis (10 cycles) at 50 mV s^{-1} in the potential range of −0.65 to 1.4 V versus $Ag|AgCl|KCl_{(sat)}$. By keeping the same experimental condition, a PPS film was formed at a BPPG electrode (PPS/BPPG[2]). Another PPS-modified BPPG electrode (PPS/BPPG[3]) was prepared from 0.2 M $NaClO_4$ acetonitrile solution containing 1 mM PS by potential-sweep electrolysis (10 cycles) at 50 mV s^{-1} in the potential range of −0.2 to 1.4 V versus $Ag|AgCl|KCl_{(sat)}$. The electrodes thus prepared were soaked in boiling water for a few minutes to dissolve any unreacted phenosafranin monomer, adsorbed on the electrode surface or trapped in the polymer matrix. After rinsing the modified electrodes with water, they were used for electrochemical experiments. The redox activity of the PPS-modified GC electrode (PPS/GC[2]) is shown in the inset of Fig. 4. Similar redox responses were observed for the other PPS-modified electrodes. The surface concentration of PPS-modified electrode (Γ/mol cm^{-2}) was obtained using the following equation [44]

$$\Gamma = Q / nFA \quad (1)$$

where Q is the integrated charge of the anodic peak obtained at the PPS-modified electrodes (corrected for the base line), n is the number