An Excellent Electrocatalysis of Poly(phenosafranine)-modified Electrode for Oxidation of Reduced β-Nicotinamide Adenine Dinucleotide

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A poly(phenosafranine)-modified electrode, which can be prepared by a potential-sweep oxidative electrolysis of the corresponding monomer in both aqueous and acetonitrile media, shows excellent electrocatalytic activity for NADH oxidation in phosphate buffer solutions (pH 7), with an activation overpotential which is more than 1 V lower than that of the bare electrode.

There has been considerable interest in the electrocatalytic oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to enzymatically active NAD^+ because of the large number (over 250) of dehydrogenase enzymes that use this coenzyme or the closely related β-nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH).[1,2]

Recently, we have reported[3] an electrocatalysis of poly(thionine)-modified electrode for oxidation of NADH, and have demonstrated that such polymer-modified electrodes with a three-dimensional distribution of mediators and of the so-called coated-wire type are preferable, compared with the mediators that have been employed either adsorbed on the electrode surface as a monolayer or as free-diffusing catalysts,[1,2,4–12] because of the much larger catalytic activity of polymer coatings than monolayers owing to volume and the possibility of reducing the size of *in vivo* sensor devices. In this case, the modification of the electrode surface by poly(thionine) film allowed us to lower the activation overpotential by about 400 mV compared with that of the bare, unmediated electrode, *i.e.* the electrocatalytic oxidation of NADH could occur at *ca.* 0.1 V *vs.* Ag/AgCl† at pH 7. However, the overpotential is still high as can be seen from comparison with the formal potential ($E^{\circ\prime} = -0.515$ V for the NAD^+/NADH redox couple).[13] In this communication, we

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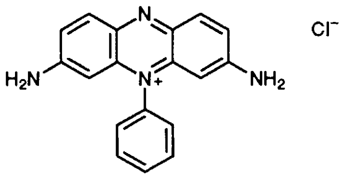
† Potentials were measured and are quoted with respect to an Ag/AgCl (KCl-saturated) electrode.



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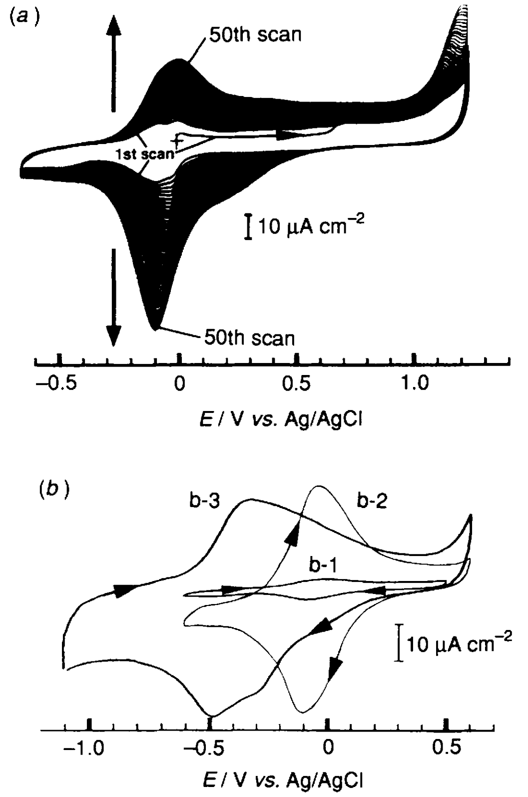

Fig. 1 (*a*) Cyclic voltammograms recorded continuously during the electropolymerization of **1** at a BPG electrode (0.20 cm^2) in an acidic aqueous solution (pH 1.0) containing $NaClO_4$ (0.1 mol dm^{-3}), $HClO_4$ and **1** (1 mmol dm^{-3}) under an N_2 atmosphere. Potential scan rate: 50 mV s^{-1}. (*b*) Cyclic voltammograms of the PPS-modified BPG electrodes in $NaClO_4$ (1.0 mol dm^{-3}) solutions containing (b-1, 2) HCl + KCl buffer (50 mmol dm^{-3}, pH 0.92) and (b-3) citric acid and phosphate buffer (50 mmol dm^{-3}, pH 7.0). Potential scan rate: 5 mV s^{-1}. The PPS-modified electrodes were prepared (b-1) by repeating potential scan 50 times as in a and (b-2,3) by repeating potential scan 10 times at 50 mV s^{-1} between −0.2 and 1.4 V *vs.* Ag/AgCl in acetonitrile containing $NaClO_4$ (0.2 mol dm^{-3}) and **1** (1 mmol dm^{-3}). Γ: (b-1) 4.6 × 10^{-10} mol cm^{-}; (b-2) 7.7 × 10^{-9} mol cm^{-2}; (b-3) 9.4 × 10^{-9} mol cm^{-2}.

report the increased lowering (more than 1 V compared with the bare electrode) of the overpotential for the oxidation of NADH by the poly(phenosafranine)-modified electrode, prepared by electropolymerization of phenosafranine (PS) **1**, as well as preparation and electrochemical behaviour.

The poly(phenosafranine) (PPS) film-modified electrodes were prepared by electrooxidative polymerization of **1** (Wako Pure Chemical Industries Ltd.) using a three-electrode, two-compartment cell.[3] Typically, the film formation was achieved at a basal-plane pyrolytic graphite (BPG, Union Carbide Co) electrode in 0.1 mol dm^{-3} $NaClO_4$ aqueous solution (pH 1.0) (or 0.2 mol dm^{-3} $NaClO_4$ acetonitrile solution) containing 1 mmol dm^{-3} **1** by a potential-sweep electrolysis at 50 mV s^{-1} [Fig. 1(*a*)], where a platinum wire was used as the counter electrode and an Ag/AgCl (KCl-saturated) electrode as the reference electrode. **1** electrooxidized irreversibily and the anodic peak corresponding to the oxidation of NH_2 groups of **1** (probably to the cation radicals[3]) was observed at around 1.2 V [Fig. 1(*a*)], suggesting that the NH_2 groups substantially take part in the polymerization as usually expected for most aromatic compounds containing NH_2 groups.[14] The reversible redox response observed in the potential region between *ca.* −0.3 and +0.3 V increased continuously with successive potential scans, indicating the build-up of polymeric products (PPS) on the electrode surface.‡ As the potential scan was continued, the electrode was covered with a purple–red thin film with a fairly smooth surface. For example, the film of Γ (surface coverage of the electroactive sites) = 9.3 × 10^{-10} mol cm^{-2}, prepared by 50 potential scans at 50 mV s^{-1} between −0.65 and 1.25 V. In addition, we found that the electrolysis in 0.2 mol dm^{-3} $NaClO_4$ acetonitrile, where the oxidation of the NH_2 groups of **1** was observed in the region 1.0 to 1.4 V, was more effective in preparing the thicker films with larger Γ values, *e.g.* the film with Γ = 9.4 × 10^{-9} mol cm^{-2} was prepared by 10 potential scans at 50 mV s^{-1} between −0.2 and 1.4 V. The Γ values of the films to be prepared could be arbitrarily controlled in the range 10^{-10} to 10^{-8} mol cm^{-2} by the number and rate of potential scan, the concentration of **1** and/or the solvent.

The reversible redox response of the PPS film deposited on the electrode surface was observed, for example, at *ca.* −0.1 and *ca.* −0.4 V at pH 1 and 7 respectively [Fig. 1(*b*)]. After the electrolysis the electrode was soaked in boiling water for several minutes to dissolve the unconverted monomers of **1**, adsorbed on the electrode surface and/or trapped in the polymer matrix and then transferred into aqueous solutions. After the first few potential scans in the base electrolyte solution, typically at a scan rate of 50 mV s^{-1} between −0.6 and 0.7 V, an almost steady-state cyclic voltammogram was obtained and a further successive potential scanning for 1 h resulted in a small percentage decrease in the current response.§ The pH dependence of the redox response in the pH range 1–7 suggested that protons and electrons take part in the overall electrode reaction in a ratio of 1 : 1 to 1 : 2. However, it seemed not to be as simple as suggested by the broad shape of the voltammogram with two reduction peaks at pH 7.¶ The reaction mechanism remains to be clarified.

Fig. 2 shows typical cyclic voltammograms demonstrating the electrocatalytic activity of the PPS-modified BPG electrodes for the oxidation of NADH in 0.2 mol dm^{-3} phosphate buffer solution (pH 7.0). The anodic peak potential for the direct oxidation of NADH at the bare BPG electrode is *ca.* 0.7 V, being close to the values so far reported for carbon electrodes.[1–4] As can be readily seen from the comparison of this peak potential with the $E^{\circ\prime}$ (−0.515 V at pH 7.0) of the NAD$^+$/NADH redox couple,[13] the overpotential for NADH oxidation is very high as is well known for the conventional, unmodified electrodes. This is the primary reason for developing the electrocatalytic materials that enable the oxidation of NADH at much lower overpotentials.[1,2] The voltammograms ii and v in Fig. 2 observed in the presence of NADH show a greatly enhanced oxidation current compared with those (voltammograms i and iv) for the PPS film itself in the range of much more negative potential than the anodic peak potential

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‡ A similar formation of the PPS film was also observed on glassy carbon and platinum electrodes as the electrode substrate.

§ The irreversible current observed at more than *ca.* 0.6 V is considered to correspond to the oxidation of the PPS film itself, which results in its degradation, because the reversible redox response gradually decreases upon the continuous potential scanning where the upper potential limit is over *ca.* 0.7 V.

¶ The shape of redox wave varies significantly with the film thickness, the potential scan rate, the kind and concentration of the supporting electrolyte as well as the pH.

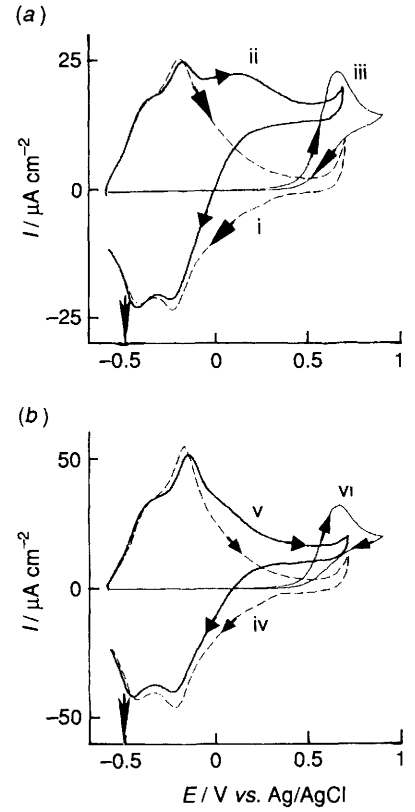

Fig. 2 Cyclic voltammograms of (i, ii, iv, v) PPS-modified BPG electrodes in deaerated 0.2 mol dm^{-3} phosphate buffer solution (pH 7.0) in the absence (i, iv) and in the presence (ii, iii, v, vi) of 0.5 mmol dm^{-3} NADH. Voltammograms iii and vi were obtained at a bare BPG electrode. Γ: 1.1×10^{-8} mol cm^{-2}. Potential scan rate: (*a*) 5 mV s^{-1}, (*b*) 10 mV s^{-1}. The arrow on the abscissa represents the $E^{\circ\prime}$ (-0.515 V) of the NAD$^+$/NADH redox couple.

at the bare electrode.‖ Hydrodynamic voltammetry under flow injection conditions,[15] which possesses a much higher current-sensitivity than cyclic voltammetry, was used to determine the lower limit of the potential at which it is possible to detect the current for the electrocatalytic oxidation of NADH at the PPS-modified glassy carbon electrode. Consequently, it was found that using the PPS-modified electrode the electrocatalytic current for NADH oxidation could be satisfactorily detected even at around -0.40 V (Fig. 3). These facts demonstrate the excellent electrocatalytic oxidation of NADH by the PPS film.

Torstensson and Gorton[6] have reported the catalytic oxidation of NADH using graphite electrodes modified with *N*-methylphenazinium sulfate ($E^{\circ\prime} = -0.115$ V at pH 7) and *N*-ethylphenazinium sulfate ($E^{\circ\prime} = -0.165$ V) with phenazinium rings (as electroactive moieties) similar to those of PPS. In this case, the overpotential has been reduced by 550 mV compared with the naked graphite electrode. Albery *et al.*[11,16] have also reported that the conducting organic salt *N*-methylphenaziniumtetracyanoquinodimethanide can be used as electrode for the electrocatalytic oxidation of NADH between -0.055 and 0.345 V. Thus, the phenazinium ring may be one of the most effective catalytic basic structures for NADH oxidation.[2,4,5,7–11] Among the mediators so far reported for NADH oxidation, to our knowledge, the carbon electrodes with electrodeposited PPS film electrocatalyse at the most negative potential (-0.40 V). Recently, Persson and Gorton[7] have examined the electrocatalytic oxidation of NADH at modified electrodes based on a series of heterocyclic aromatic structures and have found that the $E^{\circ\prime}$ (-0.500 V at pH 7) of 3-β-naphthoyl-neutral red (3-NNR), which contains a phenazine ring as an electroactive moiety, is very close to the $E^{\circ\prime}$ (-0.515 V) of the NAD$^+$/NADH couple, but no electrocatalytic activity can be observed with the 3-NNR modified electrode. This can be explained by the small thermodynamic driving force needed for the electrocatalytic oxidation of NADH as well as the poor activity of the imino structure.[7]

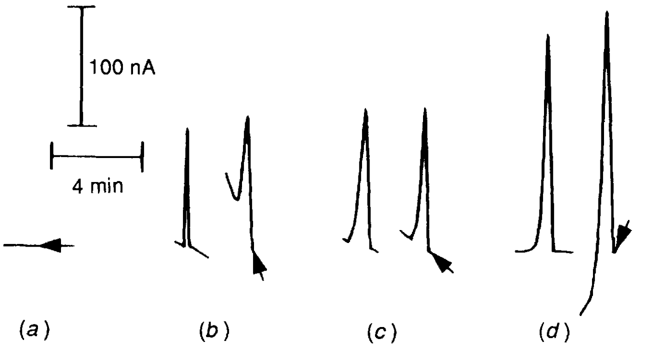

Fig. 3 Flow injection response for the oxidation of NADH obtained at the PPS-modified glassy carbon electrode. Flow rate: 1.0 ml min^{-1}, injected volume of NADH solution: 20 μl, electrolyte and carrier: 0.2 mol dm^{-3} phosphate buffer solution (pH 7.0), applied potential: (*a*) -0.50, (*b*) -0.40, (*c*) -0.35, (*d*) -0.30 V *vs.* Ag/AgCl.

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‖ The complexity of the cyclic voltammograms in the presence of NADH with varying potential scan rate and film thickness (not shown) infers that the electrocatalytic reaction is also complex.

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