Electrocatalysis of Poly(thionine)-modified Electrodes for Oxidation of Reduced Nicotinamide Adenine Dinucleotide

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A poly(thionine)-modified basal-plane pyrolytic graphite electrode, which can be prepared by a potential-sweep electrolysis of the corresponding monomer in acetonitrile media, shows an excellent electrocatalytic activity for NADH oxidation in phosphate buffer solutions (pH 6.9), with an activation overpotential which is *ca.* 400 mV lower than that of the bare electrode.

The electrocatalytic oxidation of dihydronicotinamide adenine dinucleotide (NADH) to the corresponding oxidized form (NAD$^+$) in aqueous solution has received a great deal of interest in respect of developing amperometric biosensors for NAD$^+$-dependent dehydrogenases.[1]

Redox mediators for accelerating the highly irreversible oxidation of NADH at the conventional electrodes such as platinum and carbon have been studied, including catechols,[2] quinones,[3] redox dyes,[4–7] metal complexes[8] and organic conducting salts.[9] In most cases, these mediators have been employed either adsorbed on electrode surfaces on a monolayer level or as free-diffusing catalysts.[1–10] Polymer-modified electrodes with a three-dimensional distribution of mediators and of the so-called coated-wire type should be more preferable for the present purpose because of the much larger catalytic response of polymer coatings than monolayers owing to the volume effect[11]. Miniaturization for *in vivo* sensor devices should also be possible.[1]

However, there have been only few reports of the electrocatalytic oxidation of NADH using electrodes modified with polymer films with redox-mediating functions, *e.g.* the polymer-bound dopamine,[3] the cross-linking polymer network chemically immobilizing thionine as a redox mediator,[12] the conducting polymer poly(3-methylthiophene)[13] and the electropolymerized poly(mercaptoquinone).[14] In this communication, we report electrocatalysis of the poly(thionine)-modified electrode, prepared by electropolymerization of thionine in acetonitrile media, for the oxidation of NADH in neutral aqueous solutions.

The poly(thionine)-modified electrodes were prepared typically by potential-sweep electrolysis at a potential scan rate of $50\,\mathrm{mV\,s^{-1}}$ in the potential range of −0.2 to 1.4 V *vs.* Ag/AgCl (KCl-saturated) electrode in $0.2\,\mathrm{mol\,dm^{-3}}$ $NaClO_4$ acetonitrile solution containing $10\,\mathrm{mmol\,dm^{-3}}$ thionine. The basal-plane pyrolytic graphite area ($0.20\,\mathrm{cm^2}$) (BPG, Union Carbide Co) was used as the electrode substrate and a platinum wire as the counter electrode. The electrodes modified in this manner had a purple, fairly even film. The surface coverage (Γ) of the electroactive sites in the films prepared could be controlled in the range *ca.* 10^{-10} to $10^{-8}\,\mathrm{mol\,cm^{-2}}$ by the number of scans, the potential scan rate and/or the concentration of thionine. In the latter case, we found that the potential-sweep range, especially the upper potential limit, is the most important factor for preparing the poly(thionine)-modified electrode. That is to say, in order to achieve the formation of the polymer films of thionine one must sweep the electrode potential over a potential at which the oxidation of NH_2 groups of thionine molecule (probably to the cation radicals[15]) occurs. The corresponding anodic peak was observed at *ca.* 1.3 V. Thus, the potential-sweep electrolysis in the range of, for example, −0.2 to 0.7 V resulted in no substantial film formation and in this case the Γ value was less than the monolayer coverage of *ca.* $10^{-10}\,\mathrm{mol\,cm^{-2}}$. These facts may suggest that the cation radicals (produced electrolytically) of the NH_2 groups substantially take part in the polymerization as usually accepted for most NH_2-group-containing aromatic compounds.[16] In addition, it is found that the potential-sweep electrolysis is more effective in preparing the smooth film than the constant-potential electrolysis and that the addition of acids such as CF_3CO_2H to the electrolyte medium markedly facilitates the film formation. Further study concerning the preparation and structure of poly(thionine) is under way.

The electrodes thus prepared were soaked in boiling water for 1 min to dissolve the unconverted thionine monomer, adsorbed on the electrode surface and/or trapped in the polymer matrix. For further electrochemical measurements, the electrode was then transferred into the deaerated $0.2\,\mathrm{mol\,dm^{-3}}$ phosphate buffer solutions (pH 6.9) with or without NADH. After the first few potential scans in the base electrolyte solution, typically at a scan rate of $50\,\mathrm{mV\,s^{-1}}$ between −0.6 and 0.7 V *vs.* Ag/AgCl, the almost steady-state cyclic voltammogram was obtained and a further successive potential scanning for 1 h resulted in at most a 10% decrease in the current response. This point and the aforementioned facts should be compared with the results previously obtained for thionine-modifed electrodes.[5,17] To prepare the thionine-modified electrodes mainly for photogalvanic cells, Albery *et al.*[17] originally conducted the modification of paltinum and tin oxide electrodes with thionine by holding the electrode at 1.1–1.5 V *vs.* SCE (saturated calomel electrode) for several minutes in $0.05\,\mathrm{mol\,dm^{-3}}$ H_2SO_4 solution containing thionine and prepared modified electrodes with a coverage of the order of up to 20 monolayers. Their results were then essentially confirmed by Torstensson and Gorton[5] who also used a graphite electrode. However, they have reported that if the pH was increased from the value of the electrolyte solution used ($0.05\,\mathrm{mol\,dm^{-3}}$ H_2SO_4) to pH 4, the layer was immediately desorbed and that the electrochemical activity was lost and did not reappear when the electrode was examined again in acid solution.[5]

Fig. 1 shows typical cyclic voltammograms demonstrating the electrocatalytic activity of the poly(thionine)-modified BPG electrodes with the different coverages for the oxidation of NADH in $0.2\,\mathrm{mol\,dm^{-3}}$ phosphate buffer solution (pH 6.9). The anodic peak potential for the oxidation of NADH at the bare BPG electrode is *ca.* 0.7 V, being close to the values previously reported for carbon electrodes.[1–3,18] As seen from the comparison with the reported, reversible formal potential ($E^{\circ\prime}$) of −0.560 V *vs.* SCE[18] at pH 7,[19] the overpotential for NADH oxidation is considerably high as is well known for the conventional unmodified electrodes, being the primary reason for developing the electrocatalytic materials that enable the oxidation of NADH at much lower overpotentials.[1] The reversible redox response of the poly(thionine) film itself can be observed over a wide range of potentials *ca.* −0.3 to 0.3 V and its centre is located *ca.* 0 V (assumed to be close to the $E^{\circ\prime}$ value), although the response for the thinner film ($\Gamma = 1.4 \times 10^{-10}\,\mathrm{mol\,cm^{-2}}$) is not well-defined.† Such a wide-range redox response, which has been very often observed for electroactive electropolymerized films,[11] may reflect any molecular interactions among the redox sites in the films. The voltammograms Fig. 1(*b*) and (*d*) observed in the presence of NADH show a greatly enhanced oxidation current and a large negative shift in the anodic peak potential of about 400 mV in comparison with that [Fig. 1(*e*)] obtained at the bare BPG electrode. This fact demonstrates the electrocatalytic oxidation of NADH by the poly(thionine) film. The second anodic peak current observed at almost the same potential as that at the bare BPG electrode corresponds to the direct, unmediated oxidation of NADH that must penetrate the film to reach the electrode surface.

The fact that the relative amplitude of the peak currents mediated and unmediated at *ca.* 0.3 and 0.7 V, respectively, depends on the film coverage [compare Fig. 1(*b*) and (*d*)] and the potential scan rate for a given film (not shown here) indicates that the overall electrocatalytic NADH oxidation under consideration might be controlled by some characteristic processes (*e.g.* the cross-exchange reaction between NADH and the thionine site, the diffusion of NADH and the diffusion-like charge propagation *via* an electron hopping

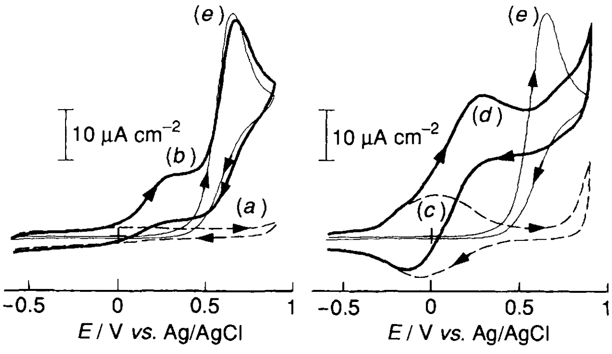

Fig. 1 Cyclic voltammograms of (*a*–*d*) poly(thionine)-modified BPG electrodes in deaerated $0.2\,\mathrm{mol\,dm^{-3}}$ phosphate buffer solutions (pH 6.9) in the absence (*a*, *c*) and in the presence (*b*, *d*, *e*) of $1\,\mathrm{mmol\,dm^{-3}}$ NADH. Voltammogram *e* was obtained at a bare BPG electrode. Surface coverage (Γ): (*a*, *b*) $1.4 \times 10^{-10}\,\mathrm{mol\,cm^{-2}}$, (*c*, *d*) $3.5 \times 10^{-9}\,\mathrm{mol\,cm^{-2}}$. Potential scan rate; $5\,\mathrm{mV\,s^{-1}}$.

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† The irreversible current observed at more than *ca.* 0.7 V is considered to correspond to the oxidation of the poly(thionine) film itself, which results in its degradation, because the reversible redox response around 0 V gradually decreases upon the continuous potential scanning where the upper potential limit is over *ca.* 0.7 V.

between the thionine sites in the film) and thus the mechanism should be complicated.[11] As would be expected from the theoretical treatment concerning the mediated electron-transfer kinetics at film-modified electrodes,[11] in the case of monolayer coatings, the overall mechanism can be simplified; the limiting current (i_{lim}) corresponding to the mediated reaction, obtained with the rotating disc electrode voltammetry, is a function of the current (i_k) corresponding to the electron cross-exchange between NADH and the thionine site and the Levich current (i_L) representing the mass transfer of NADH in the solution; $i_{\text{lim}}^{-1} = i_L^{-1} + i_k^{-1}$. The conventional Koutecky–Levich plot for the present case yielded a value of 2.1×10^3 dm^3 mol^{-1} s^{-1} for the rate constant for the catalysed oxidation of NADH. This value is almost comparable with those previously obtained for the electrocatalytic oxidation of NADH at the electrodes modified with the mediators such as 1,2-benzophenoxazine-7-one (8×10^2 dm^3 mol^{-1} s^{-1}),[6] 5-methylphenazinium- or tetrathiafulvalene–tetracyanoquinodimethane radical salts (1.5×10^3 to 1.3×10^4 dm^3 mol^{-1} s^{-1}),[9] 4-methyl catechol (3.3×10^3 dm^3 mol^{-1} s^{-1})[3] and *ortho*-quinone (1×10^3 dm^3 mol^{-1} s^{-1}).[2]

Flow-injection analysis of NADH with a constant potential amperometric detector (*e.g.* at 0.1 V *vs.* Ag/AgCl) using the poly(thionine)-modified glassy carbon electrode proved to be a sensitive detection means with stable response and a wide dynamic range of *ca.* 1 µmol dm^{-3} to 1 mmol dm^{-3}.

In conclusion, the present study demonstrates that the oxidative electropolymerization of thionine in acetonitrile media leads to the formation of an electroactive (electrochemically reversible), chemically stable, water-insoluble, thin films of poly(thionine) on the pyrolytic graphite electrodes, and the resulting poly(thionine)-modified electrode exhibits an excellent electrocatalytic activity for NADH oxidation in neutral aqueous solutions with an activation overpotential *ca.* 400 mV lower than that at the bare, unmediated electrode. A quantitative analysis of the mechanism of the electrocatalysed reaction based on the rotating disc voltammetry is currently being studied.

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