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Immobilisation of enzymes on poly(aniline)–poly(anion) composite films. Preparation of bioanodes for biofuel cell applications

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Abstract

Immobilisation of enzymes is important for applications such as biosensors or biofuel cells. A poly(histidine) tag had been introduced on the C terminus of a lactate dehydrogenase enzyme. This mutant enzyme was then immobilised onto poly(aniline) (PANi)–poly(anion) composite films, PANi–poly(vinylsulfonate) (PVS) or PANi–poly(acrylate) (PAA). The NADH produced by the immobilised enzyme in the presence of β-nicotinamide adenine dinucleotide (NAD^+) and lactate is oxidised at the poly(aniline)-coated electrode at 0.05 to 0.1 V vs. saturated calomel electrode (SCE) at 35 °C. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Enzyme immobilisation; Poly(aniline) composite films; NADH; Biofuel cell

1. Introduction

Fuel cells convert chemical energy into electrical energy as long as fuel is supplied and in the presence of a catalyst. Biocatalysts (microorganism, enzymes) can be an alternative to the use of transition metal catalysts in fuel cells. They are very efficient and can be developed for a wide range of fuels; they are also renewable and operate at moderate temperatures. We are investigating the design of bianodes for biofuel cell applications based on lactate dehydrogenase enzymes. The current of the anodic compartment of the fuel cell will be generated by the oxidation of NADH enzymatically produced in the presence of NAD^+ and substrate.

However, the direct oxidation of NADH at pH 7 requires a large overpotential at bare electrodes, at pH 7. Therefore, the use of a mediator is necessary. In our laboratory, we have shown that poly(aniline) (PANi) films doped with poly(anions), i.e. poly(acrylate) (PAA), poly(vinylsulfonate) (PVS) or poly(styrenesulfonate) (PSS), catalyse NADH oxidation at pH 7 [1–3].

LDH (E.C.1.1.1.27, from rabbit muscle) have been previously immobilised by adsorption onto poly(aniline) films [1,2], and via a cross-linking technique onto poly (pyrrole)–poly(vinylsulfonate) films [3], to prepare lactate or pyruvate biosensors. We have chosen to use mutant lactate dehydrogenase enzymes that have been designed for immobilisation onto the electrode surface.

In the present work, we described some results on the immobilisation of a tagged lactate dehydrogenase on PANi–PAA or PANi–PVS composite films. These enzymes, immobilised on the poly(aniline) composite films, produce NADH in the presence of NAD^+ and lactate.

2. Experimental

β-Nicotinamide adenine dinucleotide (NAD^+, ~98%, Sigma), lactate, poly(acrylic acid, sodium salt) and poly (vinylsulfonic acid, sodium salt) were purchased from Aldrich and used without further purification. Mutagenesis and protein expression were performed as described elsewhere [4].

Electropolymerisation of aniline was performed as described previously [5,6]. The poly(aniline)–poly(anion) electrode was soaked in enzyme solution (0.91 mg/ml) for 2 h at 4 °C. Chronoamperometry experiments were recorded

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using an EG&G model 263A potentiostat coupled with a thermostated cell (35 °C), containing 10 ml of 1.0 M Tris–HCl buffer pH 7.1 and 30 mM NAD^+. The working electrode was a glassy carbon rotating disc (0.38 cm^2), the counter-electrode was a platinum foil and the reference electrode a saturated calomel electrode (SCE).

3. Results and discussion

Lacate dehydrogenase (LDH) enzymes were immobilised onto poly(aniline)–poly(anion) composite films in order to generate NADH and pyruvate in the presence of NAD^+ and lactate (Fig. 1). The enzymatically produced NADH is oxidised at the poly(aniline) film, and the reduced film (leucoemeraldine state) is electrochemically reoxidised (to the emeraldine state). Poly(aniline)–poly(anion) composites such as poly(aniline)–poly(acrylate) [PANi–PAA] and poly(aniline)–poly(vinylsulfonate) [PANi–PVS] catalyse NADH oxidation at pH 7 at 0.05 and 0.1 V vs. SCE, respectively [5,6]. A poly(histidine) tagged LDH (LDH-Chis) was designed to immobilise this enzyme on the poly(aniline)–poly(anion) composite films. Poly(histidine) tagged alkaline phosphatase has been immobilised on poly(pyrrole) films doped with Ni^{2+}, complexed by the histidine tag [7]. In our experiments, Ni^{2+} was found to be unnecessary for the immobilisation of the enzyme on PANi–PAA or PANi–PVS. Thus, all experiments were performed without added nickel ions.

Fig. 2 shows the catalytic current observed for NADH oxidation against the concentration of lactate in the electrochemical cell. The currents increase with the concentration of lactate. The same experiment was performed for PANi–PAA and PANi–PVS composite films (Fig. 2a). Higher catalytic currents are obtained with PANi–PVS. It has been shown that PANi–PVS gives higher amperometric responses towards NADH oxidation at concentrations above 0.2 mM [5,6]. Therefore, the difference observed for

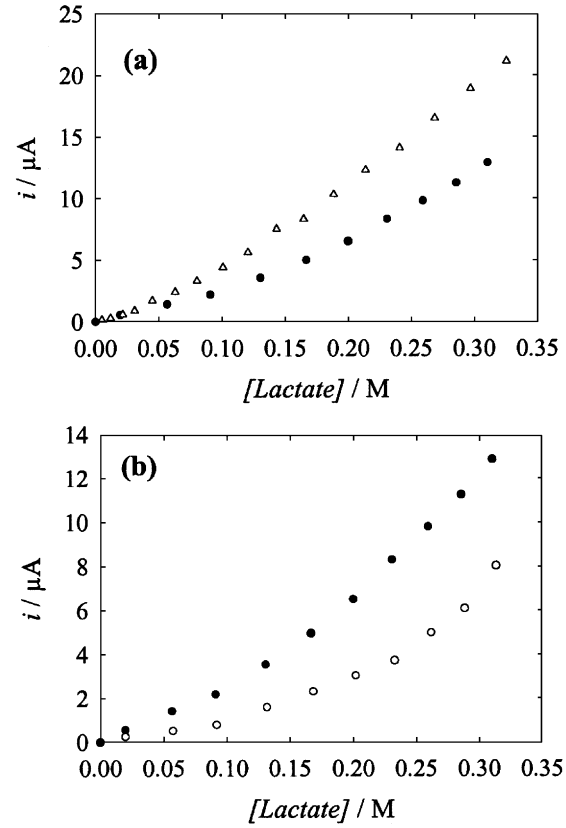

Fig. 2. Current recorded at +0.05 and +0.1 V vs. SCE for PANI–PAA and PANI–PVS, respectively, coated glassy carbon electrodes (deposition charge = ~200 mC, A = 0.38 cm^2, thickness = ~10 μm) at 9 Hz in 1 M Tris–HCl buffer solution, pH 7.1 at 35 °C. (a) LDH-Chis immobilised on △: PANI–PVS, ●: PANI–PAA; PANI–PAA composite filmas and ○: wild-type LDH, ●: LDH-Chis.

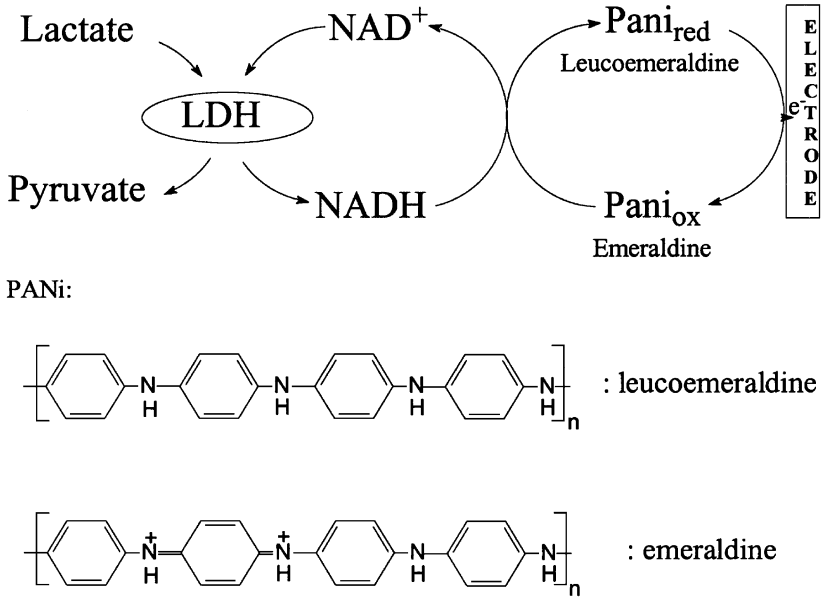

Fig. 1. Proposed scheme of the enzymatically produced NADH at poly(aniline)–poly(anions)-coated electrodes.

the two films could be due either to the difference in the electrocatalytic response of PANi–PVS and PANi–PAA towards NADH oxidation, or due to more enzyme being immobilised on PANi–PVS.

The same experiments were performed with wild-type LDH (*Bacillius stearothermophilus*) (on PANi–PAA films, Fig. 2b). Although the LDH-Chis mutant is less active than the LDH-WT [4], it gave a higher amperometric response, probably due to a better immobilisation of LDH-Chis on the composite film. The LDH-Chis may attach to the PANi composite films via a strong electrostatic or covalent bonding. The histidine groups may attack the poly(aniline) backbone, as it is known that PANi can undergo nucleophilic attack [8]. New mutants have been prepared and QCM experiments are being carried out to better understand the immobilisation of the mutant LDH-Chis onto poly(aniline)–poly(anion) composite films.

4. Conclusion

A poly(histidine) mutant lactate dehydrogenase had been successfully immobilised on poly(aniline)–poly(anion) composite films. The immobilised enzyme produces NADH in the presence of lactate and NAD^+. The enzymatically produced NADH is oxidised at the poly(aniline) composite films which act as a catalyst. The mutant enzyme gives better results than the wild-type. The catalytic currents also depend on the type of poly(anion) used in the composite film. These results provide the basis for the development of a novel bioanode for use in a biofuel cell.

Acknowledgements

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