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A novel electro-optical sensor format with generic applicability for exploitation with NAD(P) dependent enzymes

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Abstract

This paper describes the development of a novel optically interrogated enzyme electrode with generic applicability for NAD(P) dependent enzymes. The example reported here employs a multi-enzyme pathway comprising the enzymes pyruvate kinase, hexokinase, glucose-6-phosphate dehydrogenase and diaphorase. The final substrate of this pathway, dichlorophenol indophenol (DCPIP), was immobilised within an ultra-thin polymer film of *o*-phenylenediamine, itself electrochemically polymerised onto a conductive gold coating on the surface of a support polyethylene sheet. Dichlorophenol indophenol (DCPIP) absorbs within the visible region of the spectrum with a $\lambda_{max} \approx 600$ nm. When reduced, the molar absorption coefficient at this wavelength decreases significantly and DCPIP effectively becomes colourless ($DCPIPH_3$). Ultra-thin layers of gold (< 10 nm thickness) exhibit an optical absorption minimum at wavelengths of approximately 520 nm and therefore light within this region of the spectrum may be transmitted with relative ease through the polymer/gold/polyethylene optrode. Results presented within this paper show how this electro-optical sensor may be used to determine concentrations of adenosine triphosphate (ATP) within a sample. In the presence of ATP a colour change from blue to colourless was observed for DCPIP when the assay was performed in solution. However, when DCPIP was immobilised within a polymeric film onto the surface of gold coated electrodes, a colour change from blue to red was observed corresponding to a third redox state of DCPIP (DCPIPH).

Keywords: NAD(P); Optical; Inert polymers; DCPIP; Reversible

1. Introduction

Since the late 1980s many workers have described a variety of approaches for immobilising enzymes and other biomolecules within electrochemically polymerised films at an electrode surface (Foulds and Lowe, 1986; Umana and Waller, 1986). Approaches such as these offer benefits over some other immobilisation techniques, since they allow for the controlled deposition of these polymer films. By chronoamperometrically monitoring the charge passed during the deposition stage of these polymeric films, the thickness of the polymer (Myler et al., 1997) and hence the quantity of enzyme and/or other biomolecule immobilised may be determined.

Despite the vast interest in membrane-based enzyme electrodes, little commercial success has been achieved with biosensors of this type, mainly due to problems associated with reliability and/or robustness (Kress-Rogers, 1997). If these problems can be overcome, then these biosensors offer a means of increasing the selectivity and sensitivity of the overall system.

The largest group of redox enzymes is the dehydrogenases that rely on either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP) as soluble cofactors. Despite in excess of 250 dehydrogenases relying on NAD and 150 on NADP (Cass, 1990), relatively few of these enzymes, to date, have been exploited within biosensors. Problems associated with electrode fouling have so far limited the use of this redox couple within commercial instruments

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(NCIUB, 1986; Warrington, 2001), however, the level of interest in NAD(P) remains high due to the number of enzyme pathways that could be exploited within sensors (Sobolov et al., 1996; Stevenson et al., 1995).

Within this paper, an ultra-thin polymeric film of poly(*o*-phenylenediamine) has been used to immobilise dichlorophenol indophenol (DCPIP) onto the surface of gold sputtered-coated polyethylene supports. DCPIP is a substrate for the final enzyme (diaphorase) of this multi-enzyme pathway. Polymers that possess a delocalised π-electron system along the chain have been used previously for the immobilisation of enzymes (Palmisano et al., 2000). In this case, the monomer *o*-phenylenediamine was anodically polymerised onto a conductive surface to eventually form an insulating film on the surface of an electrode (Losito et al., 2001; Dong-Hun et al., 1995; Sasso et al., 1990; Chai and Krantz, 1994). The thickness of the poly(*o*-phenylenediamine) film may easily be controlled since the process is self-limiting (Losito et al., 2001; Dong-Hun et al., 1995; Sasso et al., 1990; Chai and Krantz, 1994) and hence the amount of DCPIP immobilised may also be controlled.

The multi-enzyme pathway used within this research, adapted from Hansen et al. (1993) incorporates the enzyme pyruvate kinase (PK), hexokinase (HK), glucose-6-phosphate dehydrogenase (G-6-PDH) and diaphorase (D) (Fig. 1). This multi-enzyme pathway is sensitive to ATP and indeed when ATP is present within a sample, DCPIP the substrate for the final enzyme within this pathway is reduced and a decrease in absorbance is observed at 600 nm, when the assay is performed in solution. When DCPIP is immobilised with a polymeric film of *o*-phenylenediamine, a colour change from blue to red is observed with an increase in absorbance in the blue-green region of the spectrum.

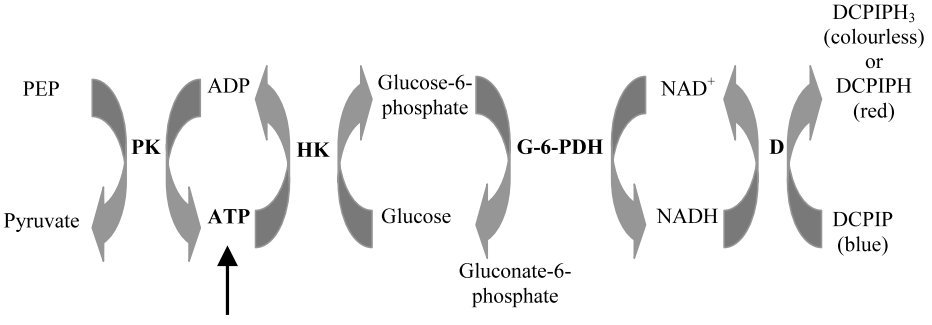
2. Experimental

2.1. Enzymes and reagents

The following enzymes were used within the multi-enzyme pathway: PK (EC 2.7.1.40) type III from rabbit muscle (350–600 units mg^{-1} of protein), hexokinase (EC 2.7.1.1) type F-300 from bakers yeast (130–250 units mg^{-1} of protein), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) type VII from bakers yeast (300–400 units mg^{-1} of protein) and diaphorase (EC 1.8.1.4) from Clostridium kluyveri (5–20 units mg^{-1} of protein), all purchased from Sigma Chemical Company (Poole, Dorset, UK). Phosphoenolpyruvate and dichlorophenol indophenol were also purchased from Sigma Chemical Company (Poole, Dorset, UK). ATP, D-glucose, NAD, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, sodium chloride, magnesium acetate and *o*-phenylenediamine dihydrochloride (all AnalaR grade) were purchased from VWR International Ltd (Poole, Dorset, UK). Glutaraldehyde (50% solution, Grade II) was purchased from Sigma Chemical Company (Poole, Dorset, UK). Electrolube Silver Conductive Paint was obtained from Maplin Electronics (Manchester, England, UK).

2.2. Buffers and solutions

A phosphate buffer (pH 7.4) comprising 5.3×10^{-2} M disodium hydrogen orthophosphate, 1.3×10^{-2} M



Key:

PEP	Phosphoenol pyruvate	**HK**	Hexokinase
ADP	Adenosine diphosphate	**G-6-PDH**	Glucose-6-phosphate dehydrogenase
ATP	Adenosine triphosphate	**D**	Diaphorase
NAD	Nicotinamide adenine dinucleotide	**DCPIP**	Dichlorophenol Indophenol
PK	Pyruvate kinase		

Fig. 1. Multi-enzyme pathway.