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A comparison of redox polymer and enzyme co-immobilization on carbon electrodes to provide membrane-less glucose/O_2 enzymatic fuel cells with improved power output and stability

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ABSTRACT

Glassy carbon and graphite electrodes modified with films of enzyme and osmium redox polymer, cross linked with poly (ethylene glycol) diglycidyl ether, were used for elaboration of a glucose/O_2 enzymatic fuel cell. The redox polymers [Os(4,4′-dimethoxy-2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^+$ and [Os(4,4′-dichloro-2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^+$ were selected to facilitate transfer of electrons from the glucose oxidase (GO*x*) active site to the T1 Cu site of multicopper oxygenases of *Trametes hirsuta* laccase (*Th*Lacc) and *Myrothecium verrucaria* bilirubin oxidase (*Mv*BOD). Maximum power density at pH 5.5 of 3.5 μW cm^{-2} at a cell voltage of 0.35 V was obtained for an assembled membrane-less fuel cell based on *Th*Lacc on glassy carbon as cathode, in the presence of 0.1 M glucose, 37 °C, with lower power observed at pH 7.4 and 4.5. Replacement of the *Th*Lacc cathode with that of *Mv*BOD produced 10 μW cm^{-2} at 0.25 V under pseudo-physiological conditions. Replacement of glassy carbon with graphite as base electrode material resulted in increased redox polymer loading, leading to an increase in power output to 43 μW cm^{-2} at 0.25 V under similar conditions. Improved stabilization of biofilms was achieved through covalent anchoring of enzyme and redox polymer on graphite electrodes, derivatized via electrochemical reduction of the diazonium cation generated *in situ* from *p*-phenylenediamine. Enzymatic fuel cells using this approach retained 70% power at 24 h, whereas fuel cells prepared without chemical anchoring to graphite retained only 10% of power over the same interval.

1. Introduction

An enzymatic fuel cell (EFC) uses enzymes, in place of traditional metal catalysts such as platinum, to convert chemical energy to electrical energy. Because of enzyme diversity, fuels such as hydrogen, alcohols and sugars, with oxygen as oxidant, can be employed in research-based EFC prototypes (Palmore et al., 1998; Palmore and Kim, 1999; Heller, 2004; Barrière et al., 2006). Of these, the glucose/O_2 EFC has received most attention due to the relatively high concentration of glucose in blood (5–8 mM), leading to potential application of EFCs to *in vivo* power production for low energy (~10 μW) demanding biomedical devices (Heller, 2004). Although an EFC can theoretically meet the power demands of some biomedical devices, stability of power output of EFCs remains an issue. For example, an implanted EFC in a rat, using enzymes and mediators contained in solution, generated intermittent power for 10 days (Cinquin et al., 2010). In that report EFC enzymes and mediators were encased within membranes to prevent reactant crossover and catalyst leakage. Immobilization of EFC catalytic components at the electrode surface allows miniaturization, and has led to development of μm dimension EFCs (Mano et al., 2003). However, leaching of enzyme and/or mediator from the electrode surface can occur, leading to short term instability of bioelectrocatalytic films (Gregg and Heller, 1991a,b; Boland et al., 2009a). A realistic goal may be the development of miniature semi-implantable glucose/O_2 systems to provide power for the lifetime of an implanted glucose sensor (typically <1 week) and may be discarded after their first and only use, thereby eliminating the need for longer term stability (Chen et al., 2001; Kim et al., 2003).

In recent years, progress in development of prototype EFCs capable of meeting power and operational lifetime requirements for such applications has been made (Heller, 2004; Mano et al., 2003; Minteer et al., 2007), although comparison of power output and performance between reports is rendered difficult due to different electrode materials, modifications and approaches used. We initially focused on developing oxygen-reducing cathodes based on co-adsorption of multicopper oxygenases and redox polymer mediators on glassy carbon and graphite disk electrodes, enabling

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comparison of half-cell performance (Barrière et al., 2004, 2006; Kavanagh et al., 2008, 2009; Jenkins et al., 2009). Combining cathodes with glucose oxidase (GO*x*) anodes yielded EFCs producing power densities of 16 μW/cm^2 (Barrière et al., 2006) and 17 μW/cm^2 (Kavanagh et al., 2009) under pseudo-physiological conditions using a *Trametes versicolor* or *Melanocarpus albomyces* laccase cathode, respectively.

In our present investigation, we compare *Myrothecium verrucaria* bilirubin oxidase (*Mv*BOD) and *Trametes hirsuta* laccase (*Th*Lacc), co-immobilized with a common [Os(4,4′-dichloro-2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^+$ redox polymer ($E^{\circ\prime}$ = +0.35 V *vs.* Ag/AgCl, Med$_2$), as O_2 cathodes. We combine cathodes with glucose oxidizing GO*x* anodes, mediated by [Os(4,4′-dimethoxy-2,2′-bipyridine)$_2$(polyvinylimidazole)$_{10}$Cl]$^+$, ($E^{\circ\prime}$ = −0.05 V *vs.* Ag/AgCl, Med$_1$), and compare power output for these membrane-less EFCs under pseudo-physiological conditions assembled on glassy carbon (GC) and graphite disk electrodes. Recent efforts have focused on increasing the stability of the biocatalytic films through coupling to surface modified electrodes. For example, diazonium salt chemistry can introduce functional groups to electrode surfaces for covalently anchoring of enzymes (Pellissier et al., 2008), mediator (Boland et al., 2008a, 2009a) or co-immobilized redox polymer and enzyme (Boland et al., 2008b, 2009b; Jenkins et al., 2009) or DNA (Hajdukiewicz et al., 2010). In this report we compare covalently anchored enzyme and redox polymer films on surface derivatized graphite electrodes to those prepared with underivatized graphite, to evaluate EFC performance with respect to stability.

2. Experimental

2.1. Materials

Synthesis of the redox polymers was achieved by adapting literature procedures (Kober et al., 1988; Forster and Vos, 1990), using $(NH_4)_2OsCl_6$ (Aldrich) as starting material to prepare the *cis*-Os(4,4′-dimethoxy-2,2′-bipyridine)$_2$Cl$_2$ and *cis*-Os(4,4′-dichloro-2,2′-bipyridine)$_2$Cl$_2$ complexes, which were then complexed, via ligand substitution reaction in ethanol/water solvent, to a previously pre-synthesized polyvinylimidazole (PVI) polymer. Poly(ethylene glycol)diglycidyl ether (average Mn ~ 526) was purchased from Sigma–Aldrich. GO*x* and *Mv*BOD were obtained from Sigma–Aldrich and *Th*Lacc was donated by VTT Technology, Finland (K. Kruus). Unless otherwise stated all other chemicals were obtained from Sigma–Aldrich. All buffers were prepared from solutions of the selected base then adjusted to the desired pH using solutions of the acid.

2.2. Apparatus

Teflon-shrouded GC electrodes (3 mm diameter, IJ Cambria), and graphite disc electrodes (3 mm diameter), formed by shrouding graphite rods (Goodfellow) in glass tubes using heat-shrinkable tubing and establishing an electrical connection to copper rods (Farnell) at the rear with silver epoxy resin (Farnell), were used as working electrodes. Cyclic voltammetry was carried out with a CHI 650 potentiostat, using a GC or graphite electrode, Ag/AgCl (3 M KCl) and platinum wire as working, reference and counter electrodes, respectively (IJ Cambria). Membrane-less EFCs were assembled by insertion of anode and cathode into a compartmentless electrochemical cell containing 5 mL of electrolyte solution. The anode and cathode were externally connected through a resistance box (IET Labs) over a resistance range of 5 MΩ to 1 kΩ, and the voltage between the electrodes measured with a multimeter (Keithley) for each load.

2.3. Methods

Electrodes were prepared, based on previously reported ratios of redox polymer to enzyme (Boland et al., 2009b; Kavanagh et al., 2009) by depositing a drop (12 μL) containing the enzyme (6 μL of either GO*x* of 10 mg/mL (1500 U/mL), *Th*Lacc 390 U/mL or *Mv*BOD 95 U/mL), the redox polymer (3 μL of a 8–10 mg/mL solution/suspension in water) and poly(ethyleneglycol) diglycidyl ether (3 μL of a 15 mg/mL solution in water) as a crosslinker onto 3 mm diameter GC or graphite electrodes, followed by at least 24 h drying of the film. Unless otherwise stated, current and power densities were measured at 37 °C in buffered solutions containing 0.15 M NaCl, 0.1 M glucose and saturated O_2. Modification of the graphite electrodes by introduction of amine functional groups was achieved by electrochemical reduction of the diazonium cation generated *in situ* from *p*-phenylenediamine (Baranton and Bélanger, 2005; Boland et al., 2009b). Briefly, 8 mM of $NaNO_2$ was added into a 10 mM acidic solution (HCl) of *p*-phenylenediamine to generate *in situ* the diazonium cation. The solution was kept in complete darkness and in an ice bath (approximately 4 °C) and allowed to react for 5 min under argon and stirring. Surface derivatization was carried out by electrochemical reduction, in the diazonium cation-generating solution, by scanning from 0.4 V to −0.4 V *vs.* Ag/AgCl at 20 mV s^{-1} for four cycles. The resulting modified electrodes were removed and rinsed with acetonitrile and then water, followed by ultrasonication for 1 min to remove any loosely bound species. Electrodes were then rinsed again with copious amounts of water and dried under a stream of argon.

3. Results and discussion

3.1. Enzymatic fuel cell design

A simplified model of the enzymatic fuel cell design is shown in Fig. 1A. At the anode, glucose is oxidized by GO*x* to yield gluconolactone with an osmium redox polymer, Med$_1$, to shuttle electrons from the otherwise insulated FAD/FADH$_2$ active site to the electrode surface. The electrons then pass through the circuit where they are transferred, via Med$_2$, to the multicopper oxygenase active site (*Th*Lacc or *Mv*BOD) where they are recombined with protons in the presence of O_2 to form H_2O.

In order for current to flow a potential difference (ΔE) must be established between the anode and cathode, primarily influenced by the redox potential of anode and cathode electron transfer mediators, Med$_1$ and Med$_2$. Modification of 2,2′-bipyridine (bpy) ligands of a redox polymer [Os(bpy)$_2$(polyvinylimidazole)$_{10}$Cl]$^+$ (Forster and Vos, 1990) with electron donating/withdrawing groups in the 4,4′-positions allows manipulation of the redox potential of the Os(II/III) redox transition to address electron transfer to/from enzymes. The redox polymers Med$_1$ and Med$_2$ (Fig. 1B) are selected to facilitate thermodynamically favourable transfer of electrons from the GO*x* active site ($E^{\circ\prime}$ = −0.35 V *vs.* Ag/AgCl) (Swoboda and Massey, 1965, 1966; Gregg and Heller, 1991a,b; Heller, 1992) to the T1 Cu of multicopper oxygenases ($E^{\circ\prime}$ = +0.57 V *vs.* Ag/AgCl for *Th*Lacc (Shleev et al., 2005) +0.4 V *vs.* Ag/AgCl for *Mv*BOD (Xu et al., 1996)) whilst maintaining a potential difference between the anode and cathode. The redox polymer, Med$_1$, has been previously reported on as a mediator for oxidation of glucose by GO*x* (Taylor et al., 1995) and, more recently, by pyranose dehydrogenase (Zafar et al., 2010). It is selected as mediator at the anode, as its redox potential is 0.3 V more positive than that of the bound FAD cofactor providing a favourable thermodynamic driving force for electron transfer from the GO*x* active site. We initially compare mediated *Th*Lacc and *Mv*BOD cathode performance in a membrane-less EFC assembly using the common GO*x*/Med$_1$-based bioanode. Cyclic