Electrochimica Acta 182 (2015) 320–326

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Glucose oxidation by osmium redox polymer mediated enzyme electrodes operating at low potential and in oxygen, for application to enzymatic fuel cells

Isioma Osadebe, Peter Ó Conghaile, Paul Kavanagh, Dónal Leech*

School of Chemistry & Ryan Institute, National University of Ireland Galway, University Road, Galway, Ireland

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form 15 September 2015
Accepted 15 September 2015
Available online 21 September 2015

Keywords:
Redox polymer
enzymatic fuel cell
glucose
glucose dehydrogenase

ABSTRACT

Graphite electrodes modified with a redox polymer, [Os(4,4′- dimethoxy-2,2′-bipyridine)$_2$(polyvinyl imidazole)$_{10}$Cl]Cl ($E^{\circ\prime} = -0.02$ V vs Ag/AgCl), crosslinked with glucose oxidising enzymes and various amounts of multi-walled carbon nanotubes are investigated for current generation in the presence of glucose in physiological buffer solutions. Enzyme electrodes based on glucose oxidase and FAD-dependent glucose dehydrogenase are compared in the presence and absence of oxygen. The highest glucose oxidation currents are produced from enzyme electrodes containing 68% w/w multi-walled carbon nanotubes in the deposition matrix. The FAD-dependent glucose dehydrogenase and glucose oxidase enzyme electrodes provide similar current density of ~0.8 mA cm^{-2} in de-oxygenated 50 mM phosphate-buffered saline at 37 °C containing 5 mM glucose concentration. Current densities under the same conditions, but in the presence of oxygen are 0.50 mA cm^{-2} and 0.27 mA cm^{-2}, for glucose dehydrogenase and glucose oxidase enzyme electrodes, respectively, with decreased currents a result of oxygen reduction by the redox polymer in both cases, and oxygen acting as a co-substrate for the glucose oxidase-based electrodes. Application of the anodes in membrane-less enzymatic fuel cells is demonstrated by connection to cathodes prepared by co-immobilisation of [Os(2,2′-bipyridine)$_2$(polyvinyl imidazole)$_{10}$Cl]Cl redox polymer, *Myrothecium verrucaria* bilirubin oxidase and multi-walled carbon nanotubes on graphite electrodes. Power densities of up to 270 μW cm^{-2} are achieved, showing promise for *in vivo* or *ex vivo* power generation under these conditions.

1. Introduction

Enzymatic biofuel cells (EFCs) generate electrical energy through enzymatic reactions oxidising fuel at the anode and reducing oxidant at the cathode [1,2]. The oxidoreductase enzymes used in EFCs provide specificity at fuel cell electrodes that open the possibility for miniaturisation of the system by eliminating the need for casings and separating membranes [3–5]. In addition, enzyme catalysts can oxidise complex fuels, for example glucose at the anode, under mild conditions (20– 40 °C, neutral pH) compared to metal-based fuel cell catalysts. Research on EFCs is of increasing interest as such miniaturised membrane-less EFCs could generate power from sugar and oxygen present in physiological fluids or easily transportable packs, thus providing potential for application to power portable electronic devices, biosensors and implantable devices [6–8].

In most EFC electrodes mediators are incorporated to allow for electron shuttling from the enzyme active site to electrodes as active sites are buried within a proteinaceous insulating envelope too distant from the electrode surface to permit rapid electron transfer to occur. Mediators possessing a redox potential suitable for rapid electron transfer between the enzyme active site and the electrode surface can improve current capture at an electrode surface. In redox mediation, to have an effective electron exchange, the thermodynamic redox potentials of the enzyme and the mediator should be tuned. The tuning of these potentials is a compromise between the need to have a high cell voltage and a high mediated current. Mediators can be sub-classified into organic or metal-based mediators [9], with the focus here on metal based osmium complexes as mediators. The osmium complexes are selected due to the fact that they are stable in the reduced/oxidised Os(II/III) states [10] within biologically

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* Corresponding author.
E-mail address: Donal.leech@nuigalway.ie (D. Leech).

http://dx.doi.org/10.1016/j.electacta.2015.09.088
0013-4686/

relevant potential ranges (−0.4 to 0.4 V vs. Ag/AgCl). Also, the redox potential of the Os(II/III) metal centre transition can be tuned by selection of appropriate electron-donating/withdrawing coordinating ligands.

Integration of nanomaterials as components of enzyme electrodes provides increased current capture as demonstrated by inclusion of gold nanoparticles, platinum nanoparticles, carbon nanoparticles etc. [11–15]. The use of multi-walled carbon nanotubes (MWCNTs) has been shown to improve glucose oxidation currents for enzyme electrodes prepared by crosslinking the nanomaterial with enzymes and osmium-based redox polymers [16–19], attributed to improved retention of enzyme activity [20]. We previously reported that enzyme electrodes prepared by crosslinking glucose-oxidising enzymes and an [Os(4,4′-dimethyl-2,2′-bipyridine)$_2$(polyvinyl imidazole)$_{10}$Cl]Cl (Os(dmbpy)$_2$PVI) redox polymer with increasing amounts of MWCNTs maximum current density is obtained for enzyme electrodes containing 68% w/w MWCNTs, beyond which the retained enzyme activity decreases [20]. Glucose oxidase, GOx, is a widely used glucose oxidising enzyme due to its commercial availability and high turnover rate, thus becoming a benchmark for comparison of glucose oxidising enzyme electrodes. The main disadvantage however is its sensitivity to oxygen [21,22], which can reduce the power output of EFCs [22,23] through oxygen competition for electrons [10]. As a result, oxygen-insensitive glucose oxidising enzymes, such as the glucose dehydrogenases, may be utilised to alleviate this issue.

Here we report on the use of [Os(4,4′-dimethoxy2,2′-bipyridine)$_2$(polyvinyl imidazole)$_{10}$Cl]Cl (Os(dmobpy)$_2$PVI) co-immobilised with either GOx, or FADGDH on graphite electrodes with varying amounts of MWCNTs, in an attempt to improve the current and cell voltage of glucose|oxygen EFCs. The Os(dmobpy)$_2$PVI is selected as it has a lower reported redox potential, −0.02 V vs Ag/AgCl, compared to Os(dmbpy)$_2$PVI previously used, 0.12 V vs Ag/AgCl [21,24,25]. This lower anode mediator redox potential can contribute to an overall increased difference in potential between anode and cathode of an assembled EFC leading to improved power output of the fuel cell. As before [20] MWCNT addition to Os(dmobpy)$_2$PVI enzyme electrodes is optimised for current output. The electrodes are then used as anodes in membrane-less EFCs by combining with a cathode consisting of bilirubin oxidase (BOd) crosslinked with MWCNTs and [Os(2,2′-bipyridine)$_2$(polyvinyl imidazole)$_{10}$Cl]Cl (Os(bpy)$_2$PVI) redox polymer on graphite electrodes [26].

2. Experimental

2.1. Materials

All chemicals and biochemicals were purchased from Sigma-Aldrich, unless otherwise stated. The glucose oxidase is from *Aspergillus niger* (GOx EC 1.1.3.4.) and the FAD-dependent glucose dehydrogenase is from *Aspergillus sp.* (FADGDH 1.1.99.10, Sekisui, Cambridge, USA; product GLDE-70-1192). The *Myrothecium verrucaria* bilirubin oxidase (*Mv*BOd) is provided by Amano Enzyme Inc. (Nagoya, Japan). The MWCNTs (product 659,258; Sigma-Aldrich) were pre-treated under reflux in concentrated nitric acid for 6 h and isolated by filtration. Polyethylene glycol diglycidyl ether (PEGDGE) was purchased from Sigma-Aldrich (average *Mn* ~ 526). All aqueous solutions unless otherwise stated were prepared in Milli-Q water (18 MΩ cm). Redox polymers Os(dmobpy)$_2$PVI, Os(dmbpy)$_2$PVI and Os(bpy)$_2$PVI were synthesised according to literature procedures [27,28].

2.2. Anode enzyme electrode preparation

Electrodes were prepared from graphite rods (Graphite store, USA, 3.0 mm diameter, NC001295) insulated with heat shrink tubing and the exposed disk polished on fine grit paper to create a geometric working surface area of 0.0707 cm^2. Enzyme electrodes assembly was achieved by depositing 9.6 μL of a 5 mg mL^{-1} redox polymer aqueous solution, 4.8 μL of a 10 mg mL^{-1} enzyme aqueous solution, 2 μL of a 15 mg mL^{-1} PEGDGE aqueous solution and different volumes of a 46 mg mL^{-1} aqueous dispersion of acid-treated MWCNTs on the surface of the graphite working electrode and allowing the deposition to dry for 24 h.

2.3. Electrochemical measurements

Electrochemical tests were conducted using a CH Instrument 1030 multichannel potentiostat in a three electrode cell containing 50 mM phosphate buffer saline (PBS, 150 mM NaCl) pH 7.4, at 37 °C as electrolyte and a Ag/AgCl (3 M KCl) reference electrode, a graphite working electrode and a platinum mesh counter electrode (Goodfellow).

2.4. Enzyme activity assays

The GOx activity was determined using an o-dianisidine, horseradish peroxidase coupled spectrophotometric assay by monitoring absorbance change (Agilent 8453 UV-visible spectrophotometer) at 460 nm [29]. The average activity obtained was 234 ± 82 U mg^{-1} which correlates well with the reported activity from Sigma-Aldrich (100–250 U mg^{-1}).

The FADGDH activity was determined using a nitrotetrazolium blue (NTB) and phenazine methosulfate (PMS) coupled spectrophotometric assay by monitoring absorbance change at 570 nm [30]. The average activity obtained was 266 ± 58 U mg^{-1} which is lower than that reported from Sekisui (1180 U mg^{-1}). These assays were also utilised to estimate the activity of the enzyme when immobilised on the electrode surface, assuming that the enzyme activity remains the same at the surface as it was for the solution-phase assay.

2.5. Fuel cell assembly and testing

The EFCs were constructed by combining anode enzyme electrodes with a previously described enzymatic cathode [26]. These cathode enzyme electrodes were prepared as described for anode enzyme electrodes except using *Mv*BOd as enzyme, Os(bpy)$_2$PVI as redox polymer and a volume of the 46 mg mL^{-1} aqueous dispersion of acid-treated MWCNTs to provide 78% w/w MWCNTs in the coating procedure. The EFC current and power densities were estimated from linear sweep voltammetry (LSV) obtained at 1 mV s^{-1} and normalised to the geometric area of the current-limiting electrode. All electrodes were operated in phosphate buffered saline (PBS, 50 mM phosphate buffer containing 150 mM NaCl, pH 7.4) solutions at 37 °C unless otherwise stated.

3. Results and discussion

A substantial increase in current density due to added MWCNTs was previously reported for enzyme electrodes prepared using co-immobilisation of Os(dmbpy)$_2$PVI redox polymer and glucose-oxidising enzymes on graphite electrodes, thereby showing promise for generation of higher current and application as EFC anodes [20]. The voltage, and hence power output, of an assembled EFC can be increased if an anode can provide current density at