Biosensors and Bioelectronics 54 (2014) 455–461

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Self-powered biosensor for ascorbic acid with a Prussian blue electrochromic display

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ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form
30 October 2013
Accepted 8 November 2013
Available online 21 November 2013

Keywords:
Biofuel cell
Self-powered sensor
Ascorbic acid
Prussian blue
Electrochromism
Carbon nanotubes

ABSTRACT

We report on the development of a nanocarbon based anode for sensing of ascorbic acid (AA). The oxidation of AA on this anode occurs at a quite low overpotential which enables the anode to be connected to a biocathode to form an ascorbic acid/O_2 biofuel cell that functions as a self-powered biosensor. In conjunction with a Prussian blue electrochromic display the anode can also work as a truly self-powered sensor. The oxidation of ascorbic acid at the anode leads to a reduction of the Prussian blue in the display. The reduced form of Prussian blue, called Prussian white, is transparent. The rate of change from blue to colourless is dependent on the concentration of ascorbic acid. The display can easily be regenerated by connecting it to the biocathode which returns the Prussian blue to its oxidized form. In this way we have created the first self-powered electrochromic sensor that gives quantitative information about the analyte concentration. This is demonstrated by measuring the concentration of ascorbic acid in orange juice. The reported quantitative read-out electrochromic display can serve as a template for the creation of cheap, miniturizable sensors for other relevant analytes.

1. Introduction

Interest in biofuel cells (BFCs) has mainly been focused on future application as implantable micro-power sources. Recently they gained a new role in the form of self-powered sensors (Arechederra and Minteer, 2011). The first example of such a device was described by Katz et al. (2001). The authors presented a new concept of a self-powered biosensor comprising a BFC generating different open circuit potential (OCP) dependent on the concentration of the fuel. Depending on the enzyme immobilized on the surface of the bioanode (glucose oxidase or lactate dehydrogenase) either glucose or lactate was used as a fuel/analyte. In both examples the same biocathode was used with immobilized cytochrome *c*/cytochrome oxidase responsible for dioxygen reduction. In the absence of the fuel the BFC did not generate any voltage. The OCP increased logarithmically with the increase of the fuel concentration showing the expected Nernstian dependence. Both described BFCs produced very little power to drive a device able to show the change of OCP. So the term "self-powered" was rather assigned to the fact that the cell was able to give a different output related to the change of the fuel concentration. Although in principle able to power a small read-out device, it was not demonstrated to give any signal noticeable without an externally powered device such as a potentiostat or a multimeter.

Following this definition several other self-powered biosensors were described. The Minteer group presented an example of mitochondrial bioelectrocatalysis harnessed for detecting the presence of explosives (Germain et al., 2008). As the power output was not related to the concentration of explosives, the sensor acted like a binary device detecting the presence of e.g. nitrobenzene even in 1 fM solution.

Deng et al. (2010) introduced a new approach to self-powered biosensing. They used inhibition of a BFC for sensing cyanide. In this case the decrease of the power output of a glucose/oxygen BFC was proportional to the concentration of the analyte. This effect was caused by the inhibition of laccase reducing O_2 by CN^- ions.

An interesting variant of a self-powered biosensor was developed by Hanashi et al. (2009). They used a capacitor as a transducer coupled to a light-emitting diode. The charging rate of the capacitor was proportional to the fuel concentration. When charged the capacitor was discharged through the LED, making it blink with a frequency indicative of the fuel concentration. Called the BioCapacitor the sensor was used to measure the concentration of glucose in a sample. A few more examples of devices working on the same principle were presented by Miyake et al.

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http://dx.doi.org/10.1016/j.bios.2013.11.033

(2011a,b) for glucose and fructose sensing. Since the readout from these type of sensors can be done with the naked eye this makes it a self-powered sensor in a more intuitive way than the Katz and Willner definition, being completely independent of external power sources.

In this paper we present a novel concept for a self-powered sensor for ascorbic acid (AA, vitamin C) based on electrochromism, i.e. the ability of some compounds to change colour when their oxidation state is changed. At the heart of the device is an AA/O_2 BFC acting as a power source. The BFC comprises an enzyme catalyzed air-breathing cathode (Zloczewska and Jönsson-Niedziolka, 2013) and a carbon based non-enzymatic anode for AA oxidation. The power output of the BFC is proportional to the concentration of AA in the electrolyte solution. Thus the BFC is in itself a self-powered sensor of the Katz-Willner type. To the best of our knowledge, this is the first example of a AA/O_2 biofuel cell demonstrated being used as a self-powered sensor. However, using the BFC to reduce or oxidize an electrochromic display we can create a reusable, truly self-powered biosensor. The device is a development of a concept presented by Möller et al. (2010) where a self-powered display was demonstrated using a viologen-based pigment. Our display is instead based on Prussian blue (PB), a dye made popular among painters in the 18th century Bartoll (2008). PB is deep blue in its oxidized state, but the reduced form is transparent. By connecting the anode of the BFC to the PB-display the dye turns transparent. The rate of the decolourization is proportional to the amount of analyte. The display can then be revived and turned into its original state by connecting it to the BFC cathode. Similarly to the BioCapacitor this kind of device is therefore a truly self-powered sensor with no external power sources needed.

A similar self-powered sensing device was very recently reported by Liu and Crooks (2012). It also contained a layer of PB which was changing the colour in the presence of the analyte, in this case glucose and hydrogen peroxide. Crooks's device has a binary function that reacts in the presence of the analyte, but gives no quantitative information. Thus, to the best of our knowledge, we present the first truly self-powered electrochromic sensor giving quantitative information of analyte concentration. We show that an AA/O_2 BFC can function as a Katz-Willner-type self-powered sensor for AA in the range from ca. 20 μM to 6 mM, covering the physiological concentrations (Procházková et al., 1998) up to those found in vitamin C rich citrus fruit (Vermeir et al., 2007). The electrochromic display is a simple, reusable sensor that can give quantitative information without any additional equipment if the display colour is compared with reference colours, like a pH strip. Used with a smart phone camera and a dedicated app, as presented by Delaney et al. (2011) for chemiluminescence, it could allow high-precision electrochromic readout of quantitative information without specialised equipment. Vitamin C is a compound essential for healthy living, and since the human body is not able to synthesize AA it needs to be provided with food. Thus a cheap sensor for AA might be a useful device. The electrochromic device was tested in the range from 1 to 6 mM AA. Its function was shown by measuring the AA-concentration in orange juice.

2. Experimental section

2.1. Materials

Pyrene-1,3,6,8-tetrasulfonic acid tetrasodium salt hydrate (PTSA), $K_3[Fe(CN)_6]$ and multiwalled carbon nanotubes (MWCNTs; OD = 20–30 nm, ID = 5–10 nm, length = 0.5–200 μm) were purchased from Sigma–Aldrich. Single walled carbon nanotubes (SWCNTs) were bought from Shenzhen Nano-tech Port Co. Ltd. Bilirubin oxidase (BOD) from Myrothecium sp. (EC 1.3.3.5) with an activity of 2.60 U/mg was donated by Amano Enzyme Inc. Toray Teflon Treated Carbon Paper TGP-H-090 was purchased from the Fuel Cell Store, CO. Methyltrimethoxysilane (MTMOS) and N-trimethoxysilylpropyl-N,N, N-trimethylammonium chloride, 50% in methanol (TMASiCl) were from ABCR and hexadecyl-trimethyl-ammonium bromide (CTAB) and tetramethoxysilane (TMOS) from Sigma–Aldrich. Tin-doped indium oxide coated glass (ITO electrodes) was obtained from Delta Technologies, Limited, USA (resistivity 8–12 Ω per square). The epoxy was prepared from an Epoxy Embedding Medium Kit (EEMK) from Fluka. Nafion Membrane N115 was from Du Pont. McIlvaine's buffer was prepared from Na_2HPO_4 (POCH S.A.) and citric acid (Chempur). Phosphate buffer was prepared from H_3PO_4 and NaOH and was used for making the solutions used in the sensor. Methanol (MeOH) and HCl were purchased from Chempur, L(+)-ascorbic acid from Riedel-de Haël and $FeCl_3 \cdot 6H_2O$ from Alfa Aesar. Negatively charged carbon nanoparticles (CNPs, ca. 7.8 nm mean diameter, Emperor 2000) were from Cabot Corporation (Dukinfield, United Kingdom). Deionised water (> 15 MΩ cm) obtained from an ELIX system (Millipore) was used for the preparation of all solutions.

2.2. Instrumentation and electrochemical measurements

All electrochemical measurements were performed on the potentiostats Autolab or μ Autolab III (Metrohm Autolab). When a standard three-electrode cell was used, $Ag|AgCl|KCl_{3M}$ electrode and platinum wire were used as a reference (RE) and counter electrode, respectively. All measurements were performed under air.

2.3. Preparation of the biofuel cell

We prepared two sets of BFCs, both with the same air-breathing biocathode, but with different anodes. The anodes were made up of ITO-electrodes modified with carbon nanomaterials. The first type of anode was modified with vertically aligned carbon nanotubes (VACNTs) and the second type with carbon nanoparticles (CNPs). The first type was prepared by gluing the carbon nanotubes to ITO electrodes according to a previously described procedure (Zloczewska et al., 2011). Briefly, VACNTs were grown in a tube furnace by thermal CVD on Si wafers with 10 nm Al_2O_3 and 1 nm layer of Fe as catalyst (700 °C, 10 sccm. acetylene, 30 min). The as-grown forests of tubes were then transferred onto the ITO electrodes and glued with a home-made conductive adhesive prepared from the EEMK mixed with MWCNTs. The active electrode surface was masked with insulating tape before being used, with the area of the projected surface from 0.015 to 0.03 cm^2. The electrodes were stored in a dry place at room temperature when not in use. The length of the VACNT was ca. 380 μm (see Fig. S4 in supplementary materials). The VACNT forests are superhydrophobic, so to enable wetting of the forest interior by the aqueous solution we briefly immersed the samples in isopropanol and then washed them with a copious amount of water. The wetted electrodes were further stored in water. The second type of anode modified with CNPs was prepared by a layer-by-layer method of silica particles and CNPs. First silicate beads (TMA-beads) were prepared via a modified Stöber method (Lesniewski et al., 2009; Stöber et al., 1968). 105.5 μl of TMASiCl and 537 μl of TMOS were mixed with 1 ml MeOH. Then 10 ml of aqueous solution of CTAB, 3 ml $NH_{3,aq}$ and 9 ml MeOH were added and constantly stirred for the next 2 h. The obtained white precipitate was filtered, washed with EtOH and left to dry in room temperature. White powder was further refluxed for 24 h with 1 mM HCl in EtOH to remove the remains of CTAB. Then it was again filtered, washed with EtOH and water and left to dry.

Suspensions of 5 mg/ml TMA-beads and CNPs were obtained by the dispersion in MeOH and acetonitrile, respectively. The ITO