Biosensors and Bioelectronics 26 (2011) 2890–2896

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

ATP microelectrode biosensor for stable long-term *in vitro* monitoring from gastrointestinal tissue

Bhavik Anil Patel[a,b,*], Michelle Rogers[a], Talia Wieder[a], Danny O'Hare[a], Martyn G. Boutelle[a]

[a] Department of Bioengineering, Imperial College London, London SW7 2AZ, UK
[b] Centre for Biomedical and Health Sciences Research, University of Brighton, Brighton BN2 4GJ, UK

ARTICLE INFO

Article history:
Received 14 August 2010
Received in revised form
22 November 2010
Accepted 23 November 2010
Available online 1 December 2010

Keywords:
Adenosine tri-phosphate
Serotonin
Ileum
Colon
Hexokinase
Biosensor

ABSTRACT

We have developed a stable and selective ATP biosensor for long-term *in vitro* tissue monitoring. The electrode was fabricated by entrapping glucose oxidase (GOx) and hexokinase (HEX) in a poly-phenol film on a Pt microelectrode. The biosensor was stable to a fixed concentration of glucose for over 20 min and had a limit of detection of 9.9 ± 3.2 nM, with a sensitivity of 45.8 ± 1.22 pA μM^{-1}. Most significantly of all, the response on the ATP biosensor did not alter in the presence of 1 mM ascorbic acid, 5 μM dopamine, 5 μM serotonin, 5 μM ADP and 5 μM AMP. The ATP biosensor was also shown to have excellent stability over 7 days, and showed only a 23.92 ± 3.55% loss in sensitivity. The ATP biosensor was utilised for the *in vitro* detection of ATP from gastrointestinal tissue. The ATP biosensor response was stable for 5 h during *in vitro* recordings from ileum tissue. ATP release was shown to be greater from the mucosal surface in the ileum compared to the colon.

1. Introduction

Adenosine triphosphate (ATP) is an important biological molecule with a variety of biological functions. It is well known to play a key role in metabolism, but ATP also influences vascular tone (Burnstock, 2008a; Ralevic, 2009) and functions as a neurotransmitter (Evans et al., 1992). In addition, ATP has an important function within the immune system where, again, it is active as a signalling molecule in the cascade which controls clotting (Bours et al., 2006). ATP is thought to have a significant influence in gastrointestinal motility by regulating serotonin (5-HT) release (Burnstock, 2008b; Christofi et al., 2004; Cooke et al., 2003). ATP is also known to be released from glial cells and may mediate signalling of neurons (Fields and Burnstock, 2006; Abbracchio et al., 2009). There is a clear need for highly stable and selective measurements of ATP that have good temporal and spatial resolution for measurements in complex biological matrixes.

There are various techniques that can provide direct measurements of ATP at physiological concentrations. These include liquid chromatography (Manfredi et al., 2002; Sudo et al., 2000; Deng et al., 2003), fluorescence (Foy and Pacey, 1996; Hou et al., 2005; Zhang et al., 2009), chemiluminescence (Wang et al., 2005; Pérez-Ruiz et al., 2003), bioluminescence (Qiu et al., 2009; Kamidate et al., 2005) and amperometric techniques (Kueng et al., 2004; Llaudet et al., 2005; Masson et al., 2008; Soldatkin et al., 2009). Of all these methods, only microelectrode sensors using constant-potential amperometry have the adequate spatial and temporal resolution and robustness for *in vitro* or *in vivo* measurements.

As ATP is not electroactive, amperometric detection of ATP is carried out using enzymatic reactions on the electrode surface. Two such coupled enzyme schemes are glycerol kinase with glycerol-3-phosphate (Llaudet et al., 2005) and GOx with HEX. In this scheme, ATP is sensed by HEX which then competes with GOx for supplied glucose (Scheller and Pfeiffer, 1980; Kueng et al., 2004; Soldatkin et al., 2009). These enzymes are used to sense ATP, and generate hydrogen peroxide, which is detected at the electrode. The current generated this way can be directly related to the ATP concentration present in the sample as long as the concentration of glycerol/glucose is known.

Many amperometric ATP sensors have been developed, but limited information is known about how these electrodes respond in the presence of key biological interferences. Some single electrode ATP biosensors developed have shown to be selective against ADP (Katsu and Yamanaka, 1993; Soldatkin et al., 2009), but they have shown to be prone to biological interferences (Soldatkin et al., 2009). In some they have overcome this issue by utilising a 'null' electrode to remove interferences (Llaudet et al., 2005; Masson et al., 2008), although this a good strategy the size of the inter-

* Corresponding author at: Centre for Biomedical and Health Sciences Research, University of Brighton, Huxley Building, Brighton BN2 4GJ, UK.
Tel.: +44 0 1273 642148; fax: +44 0 1273 679333.
E-mail address: B.A.Patel@brighton.ac.uk (B.A. Patel).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.11.033

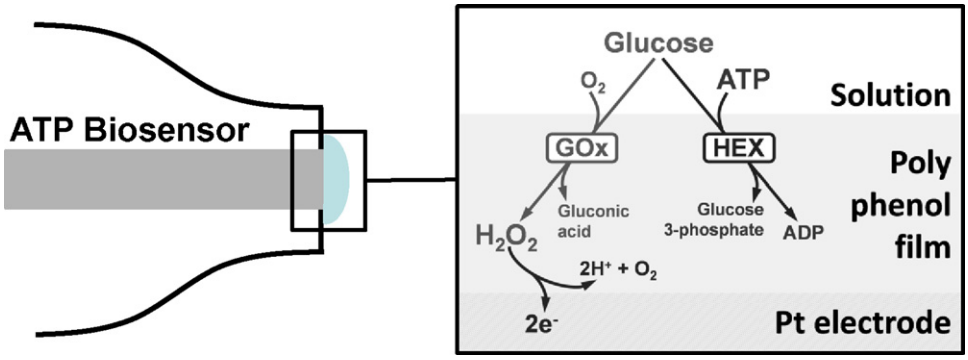

Fig. 1. Schematic diagram showing the operation of the ATP biosensor. The ATP electrode is fabricated using a combination of HEX and GOx entrapped in a poly-phenol film.

ference signal must be small compared to the signal measured for the analyte of interest, and the stability of both the sensor and 'null' electrode must be good during biological recordings. The stability of current electrodes that have excellent biological characteristic is also varied. ATP biosensors vary, with devices shown to lose 70% response after 1 week (Compagnone and Guilbault, 1997) to biosensors loosing 35–60% after 2–3 weeks (Kueng et al., 2004; Liu and Sun, 2007). However no studies using ATP biosensors have looked into the stability of ATP electrodes for long-term biological monitoring.

We have developed a new selective ATP biosensor capable of long-term biological monitoring. The ATP biosensor was composed of GOx and HEX trapped in an electropolymerised poly-phenol film (Fig. 1). The ATP sensor was assessed and characterised to study its sensitivity, selectivity and reproducibility and stability. Following characterisation of the ATP biosensor, the device was assessed for long-term *in vitro* measurement of ATP from ileum and colon tissue.

2. Materials and methods

2.1. Materials and reagents

All experiments were carried out in oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4 (composition: 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose). For preparation of the biosensor: phenol, glucose oxidase (GOx, 100 U/mg) and hexokinase (HEX, 250 U/mg) were purchased from Sigma–Aldrich. 5-Hydroxytryptamine (5-HT), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), ascorbic acid (AA) and dopamine (DA) were purchased from Sigma–Aldrich and solutions were prepared fresh each day.

2.2. ATP biosensor fabrication

To make the biosensor, a 50 μm Teflon insulated platinum wire (A-M Systems Inc., USA) was threaded through a 27G hypodermic needle. Epoxy resin (Robnor resins, CY1301 & HY1300) was used to fill the internal volume of the needle to secure the wires in place. Once the epoxy had cured, the sharp end of the needle was cut in a 45° angle using a diamond saw (Buehler) to expose the platinum microelectrodes. Alumina slurries (1-, 0.3-, and 0.05 μm) were used sequentially to polish the electrode surface. A schematic diagram of the fabricated ATP biosensor is shown in Fig. 1. The Pt needle electrode was placed in a solution of PBS buffer pH 7.2 containing 50 mM phenol, 40 mg/ml HEX and 10 mg/ml GOx. The pH of the solution was maintained at pH 7.2. The microelectrode along with a stainless steel wire and "no leak" Ag|AgCl (3 M KCl, model EE009, Cypress Systems Inc., USA) was placed in the enzyme solution for 10 min stabilisation time. The electrode was held at 0 V for 20 s, polarised to +0.9 V for 10 min for electropolymerisation of the phenol film and then held at 0 V for 20 s. Following electropolymerisation, the fabricated ATP biosensor was rinsed with deionised water and stored overnight at 4 °C in PBS buffer before use.

2.3. Sensor characterisation studies

The ATP biosensor was assessed in an electrochemical cell with a Ag|AgCl reference and Pt wire auxiliary electrode. Experiments were carried out using a CHI 1030 potentiostat. All measurements were performed at +750 mV vs. Ag|AgCl in Krebs buffer (pH 7.4). For calibrations, aliquots of substrate were added under stirred conditions. Experiments were carried out over 7 days to study the stability of the enzymes in response to varying concentrations of ATP and glucose.

For selectivity measurements, flow injection analysis (FIA) was carried out. An in-house flow cell was produced, using silicone elastomer, as described previously (Anastassiou et al., 2006). The flow cell was connected to a quaternary HPLC pump (HP1050, Aligent), where the flow rate was set at 1 ml min^{-1}. Pump A was used to maintain a constant flow of Krebs buffer pH 7.4 containing 11 mM glucose. The cell was switched to pump B, which contained various interferents, for the duration of 20 s. The responses of an unmodified Pt, ATP and SENT electrode were assessed for selectivity in the presence of 1 mM ascorbic acid, 10 μM serotonin, 10 μM dopamine, 5 μM AMP and 5 μM ADP.

For some studies of selectivity, a sentinel (SENT) or 'null' electrode was used, as others have shown this to be an effective means of achieving selectivity (Llaudet et al., 2005; Masson et al., 2008). The SENT electrode was fabricated in a similar fashion as the ATP biosensor; however the active enzymes were replaced with denatured GOx. We chose thermal denaturing of GOx as this provides a more compact structure than chemical denaturing methods (Zoldák et al., 2004). 200 mg/ml of GOx was heated to 70 °C for 5 min, before being left to cool to ambient temperature. Following this a solution containing 50 mg/ml denatured GOx and 50 mM phenol was prepared in 0.1 M phosphate buffered saline (PBS) pH 7.2 and used to make the SENT electrode.

2.4. Biological tissue preparation

All animal experiments were carried out in compliance with the relevant laws (UK Home Office) and institution guidelines. Male guinea-pigs weighing 300–400 g were euthanized using CO_2 gas. A segment of ileum was removed 15–20 cm proximal to the ileocecal junction and placed in oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4. A 1 cm long segment of ileum was then transferred to a flow bath containing the same oxygenated Krebs'