Biosensors and Bioelectronics 41 (2013) 96–102

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Development of a rapid low cost fluorescent biosensor for the detection of food contaminants

Terry F. McGrath [a,*], Karl Andersson [b,c], Katrina Campbell [a], Terry L. Fodey [d], Christopher T. Elliott [a]

[a] ASSET Technology Centre, Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University, Stranmillis Road, Belfast BT9 5AG, Northern Ireland
[b] Ridgeview Instruments AB, Ulleråkersvägen 62, Uppsala SE75643, Sweden
[c] Deptartment of Oncology, Radiology, and Radiation Sciences, Uppsala University, Sweden
[d] Agri-food and Biosciences Institute, Stoney Road, Belfast BT4 3SD, Northern Ireland

ARTICLE INFO

Article history:
Received 29 May 2012
Received in revised form
19 July 2012
Accepted 25 July 2012
Available online 16 August 2012

Keywords:
Optical biosensor
Domoic acid
Food contaminants
Prototype fluorescence biosensor
Low cost

ABSTRACT

A prototype fluorescent based biosensor has been developed for the antibody based detection of food related contaminants. Its performance was characterised and showed a typical antibody binding signal of 200–2000 mV, a short term noise of 9.1 mV, and baseline slope of −0.016 mV/s over 4 h. Bulk signal detection repeatability ($n=23$) and reproducibility ($n=3$) were less than 2.4%CV. The biosensor detection unit was evaluated using two food related model systems proving its ability to monitor both binding using commercial products and inhibition through the development of an assay. This assay development potential was evaluated by observing the biosensor's performance whilst appraising several labelled antibody and glass slide configurations. The molecular interaction between biotin and an anti-biotin antibody was shown to be inhibited by 41% due to the presence of biotin in a sample. A food toxin (domoic acid) calibration curve was produced, with %CVs ranging from 2.7 to 7.8%, and a midpoint of approximately 17 ng/ml with further optimisation possible. The ultimate aim of this study was to demonstrate the working principles of this innovative biosensor as a potential portable tool with the opportunity of interchangeable assays. The biosensor design is applicable for the requirements of routine food contaminant analysis, with respect to performance, functionality and cost.

1. Introduction

Safe food is an essential requirement of modern society, and governmental authorities throughout the world are constantly monitoring the food supply chain in an attempt to ensure an adequate safety level. One well recognised threat is the chemical contamination of food, where compounds such as drug residues, pesticides and natural toxins are unintentionally present in food stuffs.

Biosensor methods can be applied, as rapid screening tools, to detect such contaminants. They can be developed using several types of transducer (Reder-Christ and Bendas, 2011) one of which is based on fluorescence either through quenching (Wang et al., 2011) or labelling of the biological recognition elements, e.g., fluorescently labelled antibody (Taitt et al., 2008). Whilst using labelled antibodies to detect low molecular weight compounds (<1000 Da), such as natural toxins or chemical food contaminants, an inhibition based assay is generally needed to reach the required sensitivity (Yu et al., 2005).

There are a limited number of publications showing fluorescence biosensors being used for the detection of low molecular weight food contaminants. Ngundi et al. (2006) and Sapsford et al. (2006) both demonstrate the use of the Naval Research Laboratory (NRL) array biosensor for the detection of mycotoxins. Schultz et al. (2007) show proof-of principle for a portable fluorescence biosensor for the detection of aflatoxin B1 based on quenching. Sun et al. (2011) and Wang et al. (2011) show the use of fluorescence based biosensors for the detection of pesticide residues. Both methods are based on the action of pesticide residues on the activity of acetylcholinesterase and use forms of quenching to indicate enzyme activity thus pesticide concentration.

Commercial biosensors have advanced greatly over the years with improvements in sensitivity, and increased throughput by array format (Malic et al., 2011), multiple simultaneous channels (Roh et al., 2011) or both (Abdiche et al., 2011). These instruments tend to be outside the budgetary restraints of most food contaminant laboratories. A few commercial biosensors have been developed specifically for food analysis. One such example is the surface plasmon resonance (SPR)-based Biacore Q (Ferguson et al., 2005) with others being various adaptations of the NRL array biosensor (Ligler et al., 2007). These biosensors have many attributes highly sought after for routine food contaminant analysis

* Corresponding author. Tel +44 2890 976543.
E-mail address: t.mcgrath@qub.ac.uk (T.F. McGrath).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.07.081

such as ease-of-use, fast assay times, sensitivity, good repeatability and reproducibility (GE Healthcare, 2011) with the prototype NRL array biosensor showing multiplexing capabilities (Taitt et al., 2008). However the substantial cost of the Biacore Q limits its customer base to the larger food companies and government facilities and the applications on the NRL array biosensor have mainly been focused on the detection of bacterial contamination and plant toxins that pose a potential terrorism risk (Constellation Technology, 2009, MBio, 2011, ThomasNet News, 2007).

The aim of the present research was to develop, from first principles, a low cost, potentially portable, fluorescence biosensor capable of detecting low levels of contaminants and compounds of interest in food analysis. We demonstrate that a simple and inexpensive fluorescence biosensor can perform to the detection capabilities required in food monitoring laboratories, and hence prove it is possible to engineer an analytical biosensor which also matches their budgetary requirements. The research presented outlines the performance characterisation of the biosensor, the signal inhibition obtained using two model compounds (biotin and domoic acid, Fig. 1) as well as the biosensor detection unit's performance during assay development leading to the construction of calibration curves.

2. Material and methods

2.1. Reagents

HBS-EP buffer was obtained from GE Healthcare, UK. Domoic acid was obtained from Fluorochem, UK. 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), 2-(*N*-morpholino) ethanesulfonic acid (MES), biotin, bovine serum albumin (BSA), ethanolamine, ethylene diamine, fluorescein sodium and *N*-hydroxysulfosuccinimide (NHS) were obtained from Sigma-Aldrich, UK. The acid surface slide, biotin glass slide and DyLight 488 labelled anti-biotin monoclonal antibody (1.8 mg/ml) were obtained from Stratech Scientific Ltd., UK. DyLight 488 antibody labelling kit, fluorescein isothiocyanate (FITC) antibody labelling kit, goat anti-mouse DyLight 488 labelled antibody (1 mg/ml) and goat anti-rabbit DyLight 488 labelled antibody (1 mg/ml) were obtained from Thermo Scientific, UK. Amino functionalised slides, carboxymethyldextran (CMD) functionalised slides, HC polycarboxylate hydrogel amino derivatised (AHC) slides, HC polycarboxylate hydrogel (HC) slides, HC polycarboxylate hydrogel NHS activated (HCX) slides were obtained from XanTec Bioanalytics GmbH, Germany.

2.2. Instrumentation

From first principles, a prototype biosensor detection unit, *L* 32 cm × *W* 24 cm × *H* 16 cm, was constructed that was composed of an illumination component, a sensor unit, a flow cartridge and a peristaltic pump, as illustrated in Fig. 1. The illumination component contained a blue LED, a collecting lens and a 488 nm bandpass (20 nm) optical filter. The sensor unit was based on a fluorescence detector from a LigandTracer Green prototype (Ridgeview Instruments AB, Uppsala, Sweden) containing a Hamamatsu photodiode, a collecting lens and a 535 nm bandpass (20 nm) optical filter. The sensor unit was connected to a PC using a general-purpose LabJack U12 data acquisition device (LabJack Corp, Lakewood, Colorado) which measured the detector output voltage 1–3 times per second. The integrated flow cartridge, see Fig. 1, was made of a steel bracket designed to clamp immunofunctionalised glass slides (25 mm × 75 mm × 1 mm) onto a flow cell made of epoxy plastic. The flow cell diameter was approximately 8 mm with a volume of approximately 30 µl and was sealed to the glass slide through an o-ring. The flow cell had one inlet peek tubing and one outlet peek tubing. On the outlet tubing, an aspirating peristaltic pump (Pharmacia Biotech Pump P-1) was attached and set to aspirate at approximately 150 µl/min. The inlet tubing was manually moved from one vial to another to change the aspirating liquid from running buffer, to sample or regeneration solution. When changing liquids, the tubing was kept in air for 20 s to make an air bubble. The signal data points, created by the binding of a fluorescently labelled antibody to the immunospecific

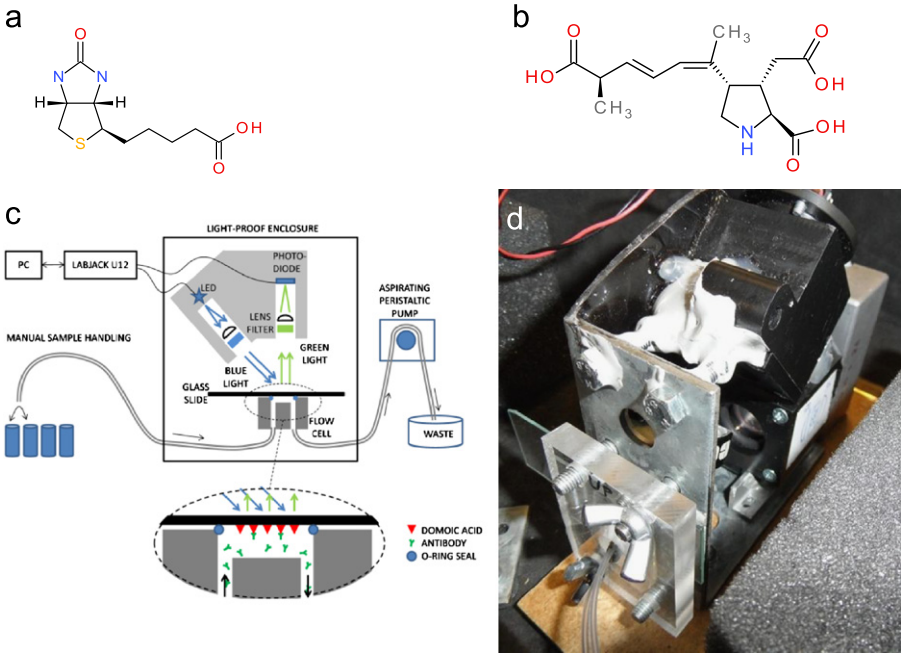

Fig. 1. (a) Chemical structure of biotin. (b) chemical structure of domoic acid. (c) diagram of the biosensor detection unit showing light source, sensor unit, flow cartridge and glass slide integrated within a light-proof enclosure (*L* 32 cm × *W* 24 cm × *H* 16 cm) and connected to a PC via a labjack U12 data acquisition device. (d) Photograph showing clamping of glass slide within the biosensor.