A flexible modular bio-instrument for amperometric detection of bioenergetic material: a RC-based measurement of antioxidant power

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ABSTRACT

A flexible modular biosensor for amperometric detection suitable for bioenergetic material has been designed, fabricated and tested. The system is made up by two flow cells in series provided of screen printed electrodes to measure the output generated current and of a flow system for electrolytic solution. The bio-instrument could be used for biosensors using several biological materials (e.g. DNA, RNA and proteins). In this work RC-complexes are used: photosynthetic electron transfer is activated by two LEDs (430 and 630nm) in order to enabling more excitations for different biological materials. RC-complexes with different sensitivity and selectivity to oxidants have been extracted from cyanobacteria (*Synecococcus elongatus*), algae (*Chlamydomonas reinhardtii*) and higher plants (*Spinacea oleracea* and *Medicago sativa*). Targeted applications belong to the agro-food, pharmaceutical and biomedical fields; in particular in this paper antioxidant power analysis has been explored.

Keywords: bio-instrument, amperometric detection, bioenergetics, photosynthesis, reaction centre, antioxidant.

1 INTRODUCTION

Various types of transduction methods are used in biosensors to convert the biochemical signal into an electric signal: amperometric and potentiometric transducers are among the most commonly adopted [1]. The former have been widely used since the introduction of the Clark's oxygen electrode in 1953 [2], which is the base of the simplest amperometric biosensors in use. Coupling enzyme-catalyzed electrochemical reactions to electrodes has been an attractive approach to developing sensors since the end of 1960s [3]. The first considerable commercial success was registered for glucose sensors with a market well past $\$10^8$/year for electrochemical sensors and $\$10^9$/year for all sensors in 1997 [3].

The amperometric miniaturized system described here is designed for general bioenergetic materials. The application of this work employs photosynthetic complex as biomediators. Photosynthetic proteins exhibit the ability of binding and specifically recognizing certain chemicals in the environment which produce changes in their physiological activities, such as a variation in the light-induced electron transfer across lipid membranes occurring during the photosynthesis process [4]. In nature, many chromophore molecules, including bacteriochlorophylls, bacterio-pheophytins and quinones, are arranged in Reaction Centres (RCs) (Fig.1) which maintain the same properties exhibited by the whole photosynthetic photosystem (PS), like the rapid, unidirectional electron transfer. They are able to generate supramolecular and self-assembling structures and, hence, are natural nanostructures. Therefore, even an individual protein molecule in the RC is a sophisticated molecular device.

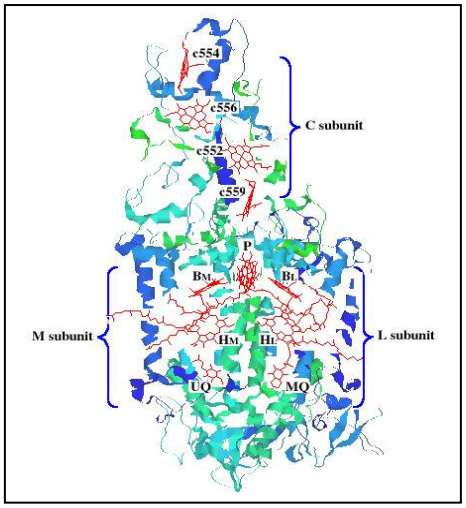

Figure 1: Reaction Centre structure.

The possibility of changing and monitoring the photosynthetic activity of photosystems and their RC subcomponents promotes the concept of innovative biosensors based on amperometric transduction by coupling the biological material with electrodes. Previous works from some of this paper authors report the fabrication of biosensors employing mutants of Photosystem II extracted from higher plants, deposited on screen-printed electrodes for pesticides detection in agriculture and environmental applications [5,6]. Tamura et al. in 1991 explored the feasibility of photosynthetic electrochemical cells based on

the above mentioned electron transfer occurring in chromatophores extracted from *Rhodopseudomonas viridis* and deposited onto SnO_2 electrodes [7]. For the same application, Lam et al. in 2006 developed a technique for immobilizing thylakoids membranes onto a functionalized gold electrode with the objective of improving the electron transfer efficiency [8].

The goal of the presented work is to exploit this emerging RC-biotechnology for the development of specific and sensitive biodevices able to operate with a wide range of biomediators thanks to the flexibility and modularity of the final instrument. An analysis of the most suitable immobilization procedures for the production of stable RC-biomediators is also performed. The targeted applications belong to the agro-food, pharmaceutical and biomedical fields. In particular in this work the possibility of measuring anti-oxidant power, by exposing RC-complexes to a solution containing both oxidizing (e.g. H_2O_2) and anti-oxidizing agents, is explored.

2 DESIGN AND FABRICATION

The miniaturized system realized is innovative: it is based on bioactive materials (e.g. photosynthetic microorganisms), designed to be flexible, modular and small, featuring two sensing cells in series equipped with optical excitation, current measurement system and flow service pipes.

2.1 Sensing Mechanism

In the Reaction Centre, the absorption of a quantum of light by the primary donor chlorophyll dimer P680 raises an electron from the ground state to the excited state, from which it can pass to the primary acceptor Q_A. This electron transfer is very fast and directive. This phenomenon can be transduced externally into an output current, which can be amplified or converted into a voltage signal for easier processing and data analysis.

This signal is generated if the RC-complexes are deposited onto an electrode biased at a positive voltage and an electrolytic solution closes the circuit by circulating ions that compensate the electrons charge transfers. The sensing mechanism is based on the total or partial inhibition of the electron transfer due to the presence of a chemical or physi-chemical environmental condition reacting either by direct binding to the RC-complexes [3] or by changing the equilibrium of the local environmental chemistry. In particular, when oxidant agents are added to the solution and come into contact with the photosystem, they scavenge part of the electrons flowing to the primary acceptor along the photosynthetic chain and the fully electron transfer cannot take place. Consequently, the current output level decreases according to the oxidants concentration.

2.2 Biosensor Description

The biosensor is made up by an innovative measurement cell where lodging for the biological material, excitation light source, electrodes and flow are integrated in a compact miniaturized sensor (30mm external diameter, 20mm height). Fig.2 represents a schematic cross section of the biosensor which helps to explain the features addressing the above mentioned specifications. The optical module at the bottom is necessary for the electron transfer excitation, considering the photosynthetic nature of the biosamples. Two LEDs with emission wavelengths of 430 and 630 nm are used in order to provide a flexible excitation for different RCs and to target the maximum photosynthesis rates corresponding to the light absorption peaks in the spectra of the antenna pigments (chlorophyll *a*, *b* and carotenoids) (Fig.3) [9].

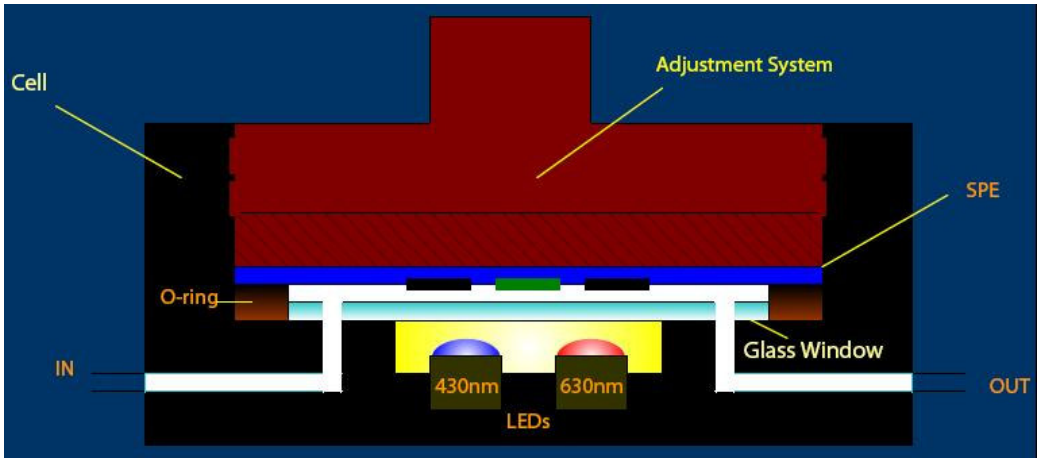

Figure 2: Schematic cross section of the biosensor equipped with two excitation LEDs at the bottom, screen-printed electrodes

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(SPE) deposited with the photosynthetic biomediator and a flow system.

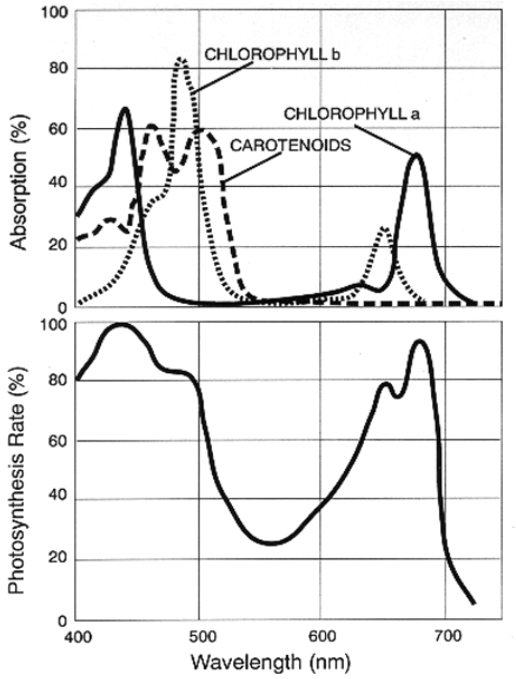

Figure 3: Top: light absorption spectrum of the most common antenna pigments of the photosystem; Bottom: Photosynthesis rate spectrum [9].

The intensity of the LEDs in the two cells of the instrument are shown in Table 1. They have been measured at room temperature by the LI-COR Biosciences LI-250A Light Meter, which uses a high stability silicon photovoltaic detector (blue enhanced) with 0.4% accuracy. A glass window separates the optical compartment from the biological chamber. The electrolytic solution flows into this chamber not directly but through three small holes in the glass window in order to preserve the biomaterial from physical damage or leaking.

	Red LED [µmol/s.m^2]	**Blue LED [µmol/s.m^2]**
Cell 1	4.45	1.16
Cell 2	4.48	1.12

Table 1: Measured LED Light Intensity.

The biological chamber dimension is given by the o-ring thickness, resulting in a volume of 4-6µl. Such a small volume makes the flow exchange very fast and consequently the biochemical reactions are improved thus increasing the response time of the sensor. The biosample is deposited onto the central working electrode of disposable screen printed Ag/AgCl and graphite electrodes (SPE). This is held in place by a screw-type adjustment system. Fig. 4 shows a picture of the open cell before inserting the SPE. Fig.5 shows a picture of the complete bio-instrument, made up by two cells in series with a rotary peristaltic pump circulating the electrolytic solution at a 0.2ml/min.

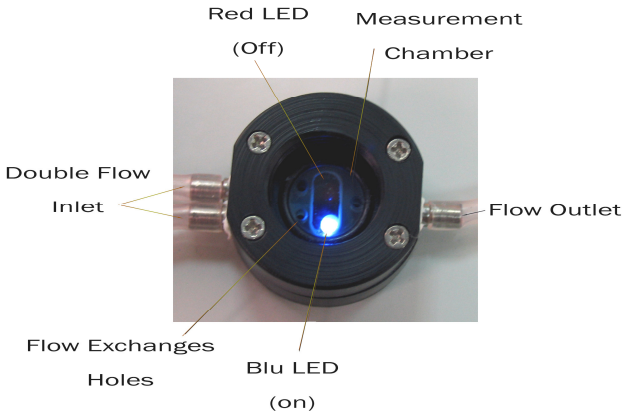

Figure 4: Image of the biosensor cell with one of the two LEDs on. Two flow inlets are provided for mixing solutions or administrating stimuli.

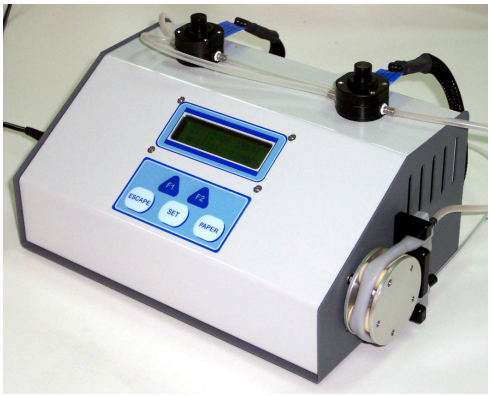

Figure 5: Photograph of the fabricated biosensor with two flow cells for biomediators in series.

3 APPLICATIONS: ANTIOXIDANT POWER ANALYSIS

The biosensor described can be applied in the field of food safety analysis for measuring antioxidant powers by directly measuring the concentration of oxidant agents in the solution. By adding both H_2O_2 and an antioxidant agent, the photosynthesis electrons scavenging of H_2O_2 will be decreased by the reaction with antioxidants and consequently the output current will increase with respect to the calibration current observed in the presence of H_2O_2 alone. The amount of the registered current increase can be directly correlated to the antioxidant concentration and power: the bigger the variation the higher the power at a constant concentration.

3.1 Biosamples preparation

RC-complexes with different sensitivity and selectivity to oxidants have been extracted from cyanobacteria (*Synecococcus elongatus*), algae (*Chlamydomonas reinhardtii*) and higher plants (*Spinacea oleracea* and *Medicago sativa*). This diverse approach is suggested by the fact that although the RC of green plants, algae and cyanobacteria appear to use the same fundamental mechanisms of energy transfer, primary charge separation, electron transfer and charge stabilization, the resistance to oxidants can be partially different. One purpose of this study is to increase both stability and selectivity of the biomediator towards oxidants through the use of various immobilization procedures. The production of RC hybrids with organic and inorganic compounds has been implemented by oriented immobilizations of RCs onto solid surfaces and some results are reported in Table 2. Thylakoids extracted from *Spinacea oleracea* were immobilized and the Photosystem II (PSII) oxygen generation was measured with a Clark's electrode immediately after immobilization and after 15 days storage in the dark. The best techniques which allow the preservation of most of the PSII activity involve the use of magnetic beads with polymers and BSA with glutaraldehyde. After keeping the hybrid components for 360h in the dark at 5°C, the PSII activity strongly decreases till becoming absent in 50% of the reported cases. An effective storage can only be achieved through freezing.

Immobilization procedure	**% PSII activity after immobilization**	**% PSII activity after storage**
BSA-glutaraldehyde	68±5	16.3±2
Urethane polymer -BSA	50±6	15±3
Gelatine-glutaraldehyde	45±3	Nr
Alginate gel	22±4	Nr
Magnetic beads-polymers	70±3	14.6±4
Photo-crosslinkable resin	19±3	Nr

Table 2: PSII activity measured as oxygen production in the Clark's electrode after immobilization and after 360 h of storage in the dark at 5°C (Nr: not revealed).

Experimental measurements aiming at calibrating the system with known concentrations of antioxidants will be performed shortly. The calibration procedure will measure the ratio of the current signals in the presence and absence of different oxidant (H_2O_2) concentrations in the sample. Sensitivity and detection limit will be calculated for the various biomediators employed, with the possibility of regenerating the electrode by washing it out using the measuring buffer.

The first antioxidant activity experiments will be performed with standards of xanthophyll pigments (zeaxanthine, astaxantine, antaraxantine), also at 37ºC under a continuos light exposure to study any antioxidant power modification in stress conditions, expected to be elicitors of the production of xanthophylls photoprotective pigments.

4 CONCLUSIONS

There is an increasing demand worldwide for low cost, fast and reliable methods for monitoring of chemical species. Biosensors offer all these advantages since they can be easily used both in laboratory and field applications. The objective of this work was to build a biosensor which can be easily adopted for any multi-biomediator analysis using an electrochemical miniaturized compact flexible system. It could be used for biosensors using several biological materials (e.g. DNA, RNA and proteins). The emerging RC-biotechnology has been adopted for the development of specific and sensitive biodevices able to operate with a wide range of biomediators extracted from different organisms. The first envisioned application is about food safety analysis with measurements of the anti-oxidant power of xanthophyll pigments (zeaxanthine, astaxantine, antaraxantine).

Acknowledgements

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