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Short communication

Functionalization of gold screen printed electrodes with bacterial photosynthetic reaction centers by laser printing technology for mediatorless herbicide biosensing

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ABSTRACT

The development of an amperometric biosensor for herbicide detection, using bacterial reaction centers (RC) as biorecognition element, is presented. RC immobilization on gold screen printed electrodes was achieved by LIFT, a powerful physisorption-based immobilization technique that enhances the intimate contact between the protein and the electrode surface. As a result, stable photocurrents driven by direct electron transfer at the donor side were observed, both in the presence and in the absence of a quinone substrate in solution. The addition of quinone UQ_0 increased the photocurrents, while the UQ_0-free system showed higher sensitivity to the herbicide terbutryn, a model inhibitor, acting as photocurrent attenuator. In spite of its simple design, the performances achieved by our mediatorless device are comparable or superior to those reported for analogous RC-based photoelectrochemical cells, in terms of both terbutryn sensing and photocurrent generation.

1. Introduction

The fabrication of low-cost, stable, sensitive and selective biosensors for herbicides has gained considerable attention since modern agriculture makes massive use of pesticides harmful for human health [1]. The use of photosynthetic proteins, among the natural herbicide targets, is a straightforward strategy for this aim [2,3]. Herbicide analyses are typically performed by gas chromatography (GC), high performance liquid chromatography (HPLC) [4] and more recently by capillary electrophoresis (CE) [5]. Nevertheless, biosensor devices based on amperometric detection appear one of the most successful strategies for overcoming the typical disadvantages of the above-mentioned techniques, such as the employment of expensive equipment and the requirement of complex time-consuming procedures. In particular, in inhibition-type amperometric biosensors based on photosynthetic proteins, photocurrents are progressively attenuated as the amount of analyte increases. One of the main challenges when dealing with protein/electrode interfaces is to achieve good electronic wiring between the two counterparts, often requiring mediators [6]. The laser induced forward transfer (LIFT) is an advanced tool for the direct immobilization of biosystems. Due to the high impact pressure of the transferred droplets at the receiver substrate the physical adsorption of biomaterials onto the surface is enhanced [7], improving the electrochemical communication with the electrode.

The reaction center (RC) is the pivotal protein in the photosynthetic process, promoting the photoreduction of ubiquinone by drawing electrons from cytochrome c_2. This reaction takes place in specialized compartments of the bacterial membrane and, coupled with a cascade of other reactions occurring in the dark, leads to the conversion of radiant energy in chemical energy available to fuel the bacterial metabolism [8]. The RC photochemical activity starts with the absorption of a photon, which promotes the primary donor D to its singlet excited state (D^*). Subsequently, one electron is transferred from D^* to the electron acceptor quinone Q_A and finally to quinone Q_B. The reduction of the resulting D^+ by cytochrome c_2 allows the photoinduced transfer of a second electron to the quinones. The final acceptor quinone Q_B, upon double reduction and protonation, is released and substituted by a new quinone molecule from an exogenous pool. While Q_A is firmly bound to the protein, Q_B is loosely bound, acting as a substrate. This latter feature makes the quinone Q_B site the target for herbicide that competitively bind to this pocket, blocking the electron transfer and thereby inhibiting the whole protein activity [3,9]. Integration of this system in a photoelectrochemical cell enables the generation of photocurrents upon illumination in the presence of suitable mediators,

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required for wiring the $D^+ Q_B^-$ pair to the external circuit. Cathodic or anodic photocurrents can be generated depending on the potential applied to the working electrode: in particular, cathodic photocurrents can be detected under reducing potentials due to electron injection at the donor side and anodic ones under oxidizing potentials due to electron withdrawal from the acceptor side.

In the present work we show for the first time the functionalization of gold screen-printed electrodes (SPEs) with LIFT-immobilized RCs from the bacterium *Rhodobacter* (*R.*) *sphaeroides*, in order to fabricate a bio-hybrid device for energy conversion and/or biosensing applications. Thanks to the optimal contact between RCs and electrode, we could detect mediatorless cathodic photocurrents driven by direct electron transfer (DET) at the donor side. The role of exogenous quinone, often used as a substrate and/or mediator at the acceptor side [10,11], was assessed for optimizing not only the electrical response but also the sensing performances toward RC inhibitors. In this work we tested the herbicide terbutryn, showing high affinity toward the RC [9,12] and therefore suitable to evaluate the biosensor potentialities. Though terbutryn is no longer permitted as an herbicide in the EU (Legislation 2002/2076), screening the environment for its presence is still relevant, due to its persistence, its illegal application in agriculture, and several legal uses of products containing it [13].

2. Material and methods

2.1. Chemicals

Terbutryn and 2,3-dimethoxy-5-methyl-p-benzoquinone (UQ_0) were purchased from Sigma-Aldrich. Any buffer components, including N,N-dimethyldodecylamine N-oxide (LDAO), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and (ethylenedinitrilo)tetraacetic acid (EDTA) were biotech-grade and purchased from Sigma-Aldrich.

2.2. RC preparation

R. sphaeroides R26.1 cells were grown photo-heterotrophically in Sistrom medium and harvested at the early stationary phase. Reaction centers were extracted and purified according to Isaacson [14] and stored in Tris 10 mM, LDAO 0.025% (*v*/*v*) and EDTA 1 mM pH 8.0 (TLE buffer) at −20 °C. Reaction center stocks were 80 μM, calculated using an extinction coefficient of $2.88 \times 10^5\ M^{-1}\ cm^{-1}$ at 802 nm [15].

2.3. Preparation of LIFT-modified electrodes

The RC immobilization onto the screen printed working electrode (WE) was carried out by means of an experimental setup described elsewhere [16]. 10 μL of 40 μM RC in TLE buffer was drop-cast on a donor substrate resulting in a thin film that was selectively deposited on the receiver substrate (the WE of the SPE), positioned parallel, in close proximity (300 μm) to the donor substrate. Two-dimensional patterns of RC droplets were printed on the gold WE. Adjusting the separation distance of the droplets a continuous film of the biomaterial was created, covering entirely the WE. Each droplet was deposited by a single laser pulse. The laser pulse fluence was 470 mJ/cm^2 with a 60 μm diameter spot size on the donor substrate, resulting in 150 μm diameter droplets. For comparison, RC layers were immobilized also by a simple drop-casting/evaporation procedure using a micropipette. The same RC solution and equivalent volumes were employed, depositing in both cases about 32 μg/cm^2 of protein.

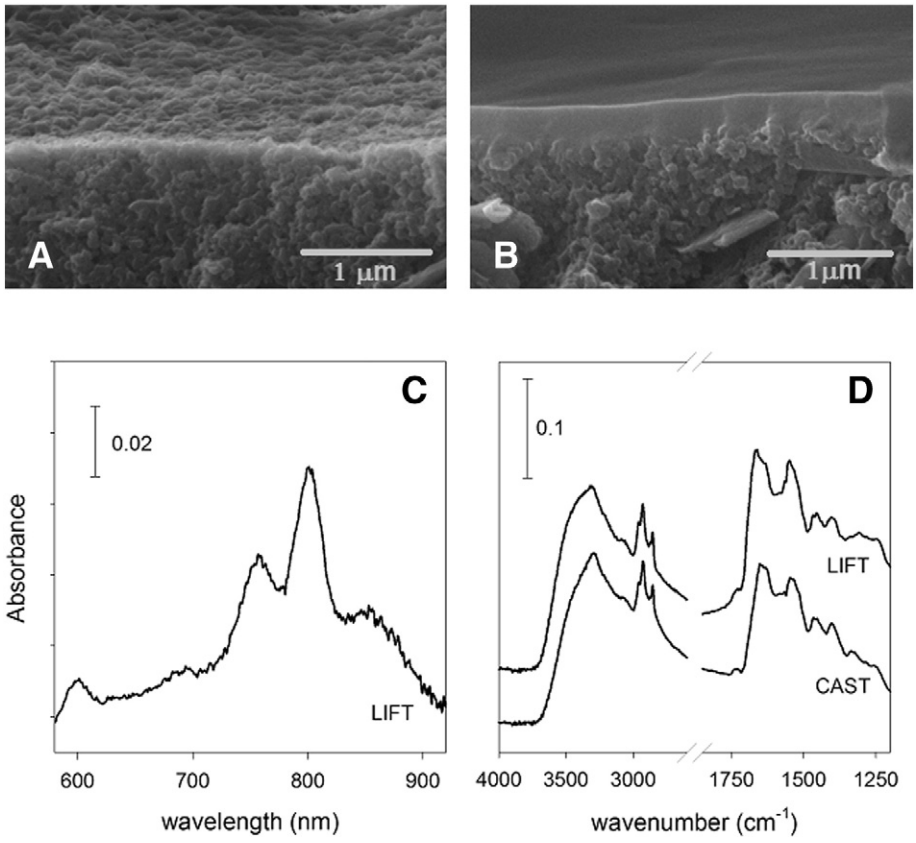

Fig. 1. (A and B) Cross-sectional SEM images of the gold WE before and after RC printing by LIFT. (C) Vis-NIR spectrum of RCs deposited by LIFT onto the gold WE. (D) Mid-infrared spectra of RCs deposited by LIFT and by drop-casting onto the gold WE.