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An impedimetric immunosensor based on interdigitated microelectrodes (IDμE) for the determination of atrazine residues in food samples

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Abstract

A novel impedimetric immunosensor for atrazine detection has been developed. The immunosensor is based on an array of interdigitated μ-electrodes (IDμE) and immunoreagents specifically developed to detect this pesticide. Immunochemical determination of atrazine is possible without the use of any label. An atrazine-haptenized protein was covalently immobilized on the surface of the interdigitated μ-electrodes area (interdigits space) previously activated with (3-glycidoxypropyl)trimethoxysilane. Before, the gold electrodes were blocked using *N*-acetylcysteamine to prevent non-specific adsorptions. All biofunctionalization steps were characterized by chemical affinity methods and impedance spectroscopy. Immunosensors measures are made by exposing the sensor to solutions containing a mixture of the analyte and the specific antibody. With this configuration, the immunosensor detects atrazine with a limit of detection of 0.04 $\mu g\,L^{-1}$ without the use of any label. The potential of the immunosensor to analyze pesticide residues in complex sample matrices, such as red wine, has been evaluated. The results shown that after solid-phase extraction atrazine can be determined in this type of sample with a limit of detection of 0.19 $\mu g\,L^{-1}$, far below the Maximum Residue Level (MRL) established by EC for residues of this herbicide in wine.

Keywords: Immunosensor; Antibodies; Interdigitated μ-electrodes; Impedance spectroscopy; Atrazine; Food safety

1. Introduction

It is well known that binding of biomolecules to solid supports produce changes in their physical properties such as the refractive index or the interface potential. This fact has been exploited to develop a variety of sensing systems based on optical (surfaces plasmon resonance, ellipsometry, grating coupler, etc.), acoustic (i.e. quartz crystal microbalances) or electrochemical (impedance spectroscopy, cyclic voltammetry, etc.) properties. Electrochemical biosensors have revolutionized modern chemical analysis because of their technical simplicity and fast response (Murphy, 2006; Patel, 2006; Pumera et al., 2007; Rodriguez-Mozaz et al., 2006; Tudorache and Bala, 2007; Wang, 2006). Mass fabrication, low cost and decentralized infield analysis are some of their important advantages. Within them, electrochemical impedance spectroscopy (EIS) is being rapidly developing because the possibility to record directly information on biorecognition events, occurring at the electrode surfaces, inducing capacitance and resistance changes (Guan et al., 2004; Katz and Willner, 2003). Thus, when a biological receptor binds to its counterpart, there is a change in the impedance of the system that enables direct measurement of an electrical signal, allowing the development of label-free biosensing devices. A major drawback of early sensors was that impedance changes due to biorecognition are very small. However, interdigitated microelectrodes (IDμEs) have drawn recently great attention since their sensitivity is higher than conventional electrodes (Berggren et al., 2001; Laschi and Mascini,

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2006; Moreno-Hagelsieb et al., 2007; Navratilova and Skladal, 2004). Different sensor approaches have accomplished to avoid the use of labels when the target analyte is a biomacromolecule, a bacteria or a virus (i.e. Haddour et al., 2006; Kerman et al., 2006), but in few occasions performance of these transduction principles has been demonstrated to detect small organic molecules at low concentration levels (i.e. Kreuzer et al., 2006; Hu et al., 2003; Hleli et al., 2006). Thus, impedimetric label-free devices have been described to detect DNA hybridization (Ma et al., 2006) or biomolecules such as interferon-gamma (Bart et al., 2005), but direct detection of contaminants and residues in complex matrices is still a challenge.

The objective of this work has been the development of a label-free immunosensor device for pesticide residue analysis in food products and atrazine has been selected as model substance. Recently, we have reported the potential of impedance spectroscopy as transducing principle, using immunoreagents passively absorbed on the surface of the device (Valera et al., 2007). This type of immobilization method produced invalid and erratic results due to desorption of the biomolecule from the surface during measurement. Moreover, non-specific absorption phenomena on the gold surfaces provided prevented performing reliable measurements with this device. In this contribution we report a much more robust label-free impedimetric immunosensor, based on the use IDμEs arrays conveniently derivatized through covalent procedures to ensure reliability and to prevent these non-specific absorption. These modifications have allowed performing measurements on a real complex matrix, such as red wine.

2. Experimental

2.1. Instrumentation

Electrochemical and conductive measurements were carried out at room temperature in a probe station (Faraday cage) KARL SUSS. Impedance analyses were performed using an Agilent 4294A Precision Impedance Analyzer and conductive measurements were performed using an Agilent 4156C Semiconductor Parameter Analyzer. The pH and the conductivity of all buffers and solutions were measured with a 540 GLP pH meter and a LF 340 conductimeter, respectively (WTW, Weilheim, Germany). Absorbances were read on a SpectramaxPlus microplate reader (Molecular Devices, Sunnyvale, CA). The competitive curves were analyzed with a four-parameter logistic equation using the software SoftmaxPro v2.6 (Molecular Devices) and GraphPad Prism version 4.00 for Windows (GraphPad Sofware, San Diego California USA). Data shown correspond to the average of at least three replicates per concentration of atrazine.

2.2. Chemicals and immunochemicals

Chemical and immunochemical reagents were purchased from Aldrich (Milwaukee, WI). Atrazine was obtained as a gift from Ciba-Geigy (Barcelona, Spain). Anti-IgG-HRP was obtained from Sigma Chemical Co. (St. Louis, MO). The preparation of antiserum As11 and related immunochemicals for atrazine analysis, such as 2d-BSA, has been described previously (Gascón et al., 1997). Non-specific antibodies were obtained from antisera of non-treated White New Zealand Rabbits.

2.3. Purification of the antibody

The antisera (As11 and As pre, 1.8 mL) were purified by $(NH_2)_2SO_4$ precipitation as described (Manson, 1992). The precipitate was reconstituted in 20 mM PBS (1 mL) and the solution filtered through a 0.45 μm PVDF filter (Millex-HV). Following an affinity purified IgG fraction was obtained using a HiTrap™ Protein A HP column. The eluted antibody fraction was then dialyzed and lyophilized to obtain around 10 mg of pure IgG fraction (Ab11 and Ab pre, respectively).

2.4. Buffers

Unless otherwise indicated PBS is 0.01 M phosphate buffer, 0.8% saline solution and the pH is 7.5. PBST is PBS with 0.05% Tween 20. Coating buffer is 0.05 M carbonate–bicarbonate buffer, pH 9.6. The buffer to measure is PBS diluted with Milli-Q water to obtain a conductivity of 1.6 μS cm^{-1}.

2.5. Fabrication of the arrays of interdigitated μ-electrodes

Thin Au/Cr (∼200 nm thickness) interdigitated μ-electrodes 3.85 μm thick and with an inter-electrode gap of 6.8 μm were patterned on a Pyrex 7740 glass substrate (purchased from Präzisions Glas & Optik GmbH, 0.7 mm (±0.05) thickness). For the immunosensor measurements, electrode arrays were constructed consisting on six IDμE's organized on a 0.99 cm^2 area (see Fig. 1). The Pyrex substrate was first cleaned using absolute ethanol. Following metal deposition was performed by sputtering and the interdigitated μ-electrodes were patterned by a photolithographic metal etching process. The chromium layer, much thinner than the gold layer, was deposited prior the gold to improve adhesion to the Pyrex substrate. Every IDμE has two electrodes that were used to connect them to the impedance analyzer.

2.6. Biofunctionalization of the sensor surfaces

Biofunctionalization with 2d-BSA was done selectively on the surfaces of the gold electrodes as shown in Fig. 2. For this purpose the IDμEs were treated as follows.

2.6.1. Surface cleaning

Before functionalization, the IDμE samples were first cleaned with ethanol:Mili-Q water 70:30 and absolute ethanol absolute in an ultrasonic bath for 10 min and dried under N_2 flow. Following the samples were immersed in a freshly prepared piranha solution (7:3, v/v, H_2SO_4:H_2O_2) for 1 min and subsequently rinsed thoroughly with absolute ethanol.