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Inhibition-based biosensor for atrazine detection

Cristina Tortolini [a,b], Paolo Bollella [a], Riccarda Antiochia [a], Gabriele Favero [a], Franco Mazzei [a,*]

[a] Department of Chemistry and Drug Technologies, Sapienza University of Rome, Italy
[b] Department of Chemistry, Sapienza University of Rome, Italy

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ABSTRACT

This work presents an inhibition-based biosensor for the fast, simple and inexpensive determination of atrazine. The method is based on the inhibition of the enzyme tyrosinase from mushrooms (Tyr), immobilized on screen printed electrodes (SPEs). To optimize the biosensor performances several carbon based SPEs: graphite (G), graphene (GP), and multiwalled carbon nanotubes (MWCNTs) and immobilization techniques (physical and chemical) have been tested. Tyrosinase was immobilized on the electrode surface by either polyvinyl alcohol with styrylpyridinium groups (PVA-SbQ) as cross-linking agent or Nafion membranes as physical entrapment or bovine serum albumin with glutaraldehyde as chemical immobilization. In the presence of catechol as substrate, atrazine can be determined thanks to its inhibition activity towards the enzyme which catalyzes the oxidation of catechol to o-quinone. Under optimum experimental conditions, the best performance in terms of catalytic efficiency has been demonstrated by MWCNTs screen printed electrode with PVA-SbQ as immobilization method. The developed inhibition biosensor displays a linearity range towards atrazine within 0.5–20 ppm, a LOD of 0.3 ppm and acceptable repeatability and stability. This analysis method was applied to spiked drinking water samples with recoveries close to 95% respect to measurements carried out in PBS buffer. The low cost of this device and its good analytical performances suggest its application for the screening and monitoring of atrazine in real matrices.

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1. Introduction

The wide use of pesticides and herbicides in agriculture to preserve crops from pests, their intrinsic toxicity, associated, in several cases, with a remarkable persistence in the environment, represents a potential danger for the health of ecosystems. The availability of analytical methods for the monitoring of their concentration, in different matrices (mainly waters and soil), is a key component of any strategy of environmental management and control.

In particular chloro-triazine compounds, belonging to organic pollutants which are highly toxic, carcinogenic and allergenic have been widely used in the environment causing several issues to the ecosystems. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATZ) is one of the most broadly used pesticides in agriculture. It was introduced in 1958 as herbicide for the control of dicotyledons and mainly used in the cultivation of corn, sorghum and sugar cane [1,2]. This herbicide was absorbed by radical and leaves, to a lesser extent and it was transported by xylem at level of photosystem II, inhibiting electronic transport.

Atrazine produces different damages to people, animal and plants and for this reason it is important to have rapid, sensitive and efficient analysis methods. This herbicide is most widely used in USA and Australia whereas it was banned in Europe because of persistent groundwater contamination [3–5].

As a consequence of the ecotoxicological implications of atrazine presence in environmental and food matrices, a growing number of methods have been developed; in particular, the chromatographic ones coupled with mass spectrometry show the best analytical performances and are used as reference methods in qualified laboratories [6–9]. Those methods are expensive, time-consuming, sometimes require pre-concentration or extraction steps, and are not suitable for in situ monitoring applications. For these reasons, the research has been focused on the development of on the spot, sensitive and cheap devices. In this field an important role can be played by enzymatic and antibody-based biosensors [10–14], in particular employing optical [15–17] and electrochemical transduction [18–24]. In this respect, amperometric biosensors are the most suitable for determination of atrazine

* Corresponding author.
E-mail address: franco.mazzei@uniroma1.it (F. Mazzei).

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because of good sensitivity, low cost, potential for miniaturization and automation [25–28].

In this work is reported the realization and characterization of a tyrosinase based biosensor for the determination of atrazine. The developed biosensor is based on the inhibition effect of atrazine towards the catalytic activity of the blue copper protein tyrosinase.

Tyrosinase was immobilized onto three different carbon based screen printed electrodes: graphite (G), graphene (GP) and multiwalled carbon nanotubes (MWCNTs) by means of photocrosslinkable poly(vinyl alcohol) with styrylpyridinium groups (PVA-SbQ), Nafion membranes as physical entrapment or bovine serum albumin (BSA) with glutaraldehyde (GA). Catechol was used as substrate for measuring the enzymatic activity of the immobilized tyrosinase by means of the amperometric monitoring (at −200 mV vs. Ag/AgCl) of the reduction of biocatalytically liberated quinone species produced from the oxidation of catechol in the presence of molecular oxygen.

The biosensor was characterized in its main bioelectroanalytical properties and then applied to the determination of atrazine in fortified natural water samples.

2. Experimental

2.1. Materials

Tyrosinase (Tyr) from mushroom (polyphenol oxidase, PPO-EC 1.14.18.1) lyophilized powder (5771 U/mg of solid), Nafion, glutaraldehyde, BSA and catechol were purchased from Sigma–Aldrich (USA).

Atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine] (Pestanal®) was purchased from Fluka (USA).

The polymeric film employed for enzymatic entrapping was a photocrosslinkable poly(vinyl alcohol) with styrylpyridinium groups (PVA-SbQ) obtained from Polysciences, Inc. (USA). All other chemicals were of analytical grade.

Stocks solutions of catechol were prepared in phosphate buffer 0.1 M pH 6.6 with KCl 0.1 M. High purity deionized water (Resistance: 18.2 MΩ cm at 25 °C; TOC < 10 μg L^{-1}) obtained from a system MilliQ-UV, Millipore (France) has been used to prepare all the solutions.

2.2. Tyrosinase immobilization procedures

For the tyrosinase immobilization three different methods were used, in this way a comparison between different immobilization techniques was carried out. The methods used were performed as the following way:

Method 1 – PVA-SbQ: SPEs/PVA-SbQ/Tyr were prepared by mixing 4 μL of a Tyr enzymatic solution with 8 μL of PVA-SbQ solution (50 mg of PVA-SbQ solubilized in 400 μL distilled water). The resulting Tyr-PVA-SbQ solution was homogenized by vortex-mixing for 30 s, and 4 μL of this solution was spread uniformly onto the working electrode surface characterized by a final Tyr activity of 288 U. The modified SPEs were then exposed for 20 min under a UV lamp (λ = 365 nm) at room temperature to allow the physical immobilization of the enzyme by photopolymerization.

Method 2 – Nafion: a Nafion 117 solution (5%, w/v) was diluted with ethanol and NaOH solution to give a stock solution of 1%, pH 5.0. SPEs/Naf/Tyr were prepared by spreading uniformly 4 μL of this solution containing 288 U of Tyr to the surface of the SPEs working electrode, and then let to dry overnight at room temperature.

Method 3 – GA-BSA: 100 μL of GA (2.5%, w/w) and 300 μL of a BSA solution (49 mg/mL in PBS 0.1 M with KCl 0.1 M, pH 6.5) were mixed together and 4 μL of the resulting solution containing 288 U of tyrosinase were spread onto the SPE working surface. The obtaining biosensor was let to dry at room temperature for about 2 h.

Ready to use biosensors obtained with all the three methods were stored at 4 °C.

2.3. Electrochemical measurements

Amperometric experiments were carried out by using a μ-Autolab type III potentiostat (Eco Chemie, The Netherlands) controlled by means of the GPES Manager program (Eco Chemie, The Netherlands) with a conventional three-electrodes configuration. Screen-Printed Electrodes (SPEs) (DropSens, Oviedo, Spain) constituted by different carbon based working electrode (G, GP and MWCNTs) with a surface diameter of 4 mm, a glassy carbon rod as auxiliary electrode (Cat. 6.1241.020, Metrohm, Switzerland) and $Ag/AgCl/KCl_{sat}$ (Cat. 6.0726.100, Metrohm, Herisau, Switzerland), 198 mV vs. NHE as reference electrode, were used. Chronoamperometric experiments were carried out in batch conditions by using a thermostatted glass cell, model 6.1415.150, Metrohm (Herisau, Switzerland) connected to a circulation bath for temperature control. The calibration plot was obtained by adding several aliquots of catechol standard solution at different concentrations into the buffer media. The linear regression was calculated by using the software GraphPad Prism 5 from GraphPad Software Inc. (USA).

2.4. Preparation of water samples

Natural water samples were collected from three different sampling places in a lake near Rome. The same aliquot of atrazine standard solution was added in the calibration bottles to obtain a final atrazine concentration of 10 ppm. All solutions were used without any pretreatment and were freshly prepared just before the measurements.

3. Results and discussion

3.1. Tyrosinase-based biosensors

The influence of pH on the cathodic peak current of the Tyr/SPE biosensor was evaluated by cyclic voltammetry, in the presence of 20 μM catechol solution in a 0.1 M phosphate buffer in the pH range: 4.5–8.0; with 0.1 M KCl solution at scan rate 5 mV/s.

The cathodic peak current increased with increase in pH up to 6.5, reaching a maximum value, after that, the signal decreased (Fig. 1). Therefore, phosphate buffer solution at pH 6.5 was selected as the supporting electrolyte throughout all measurements. The characterization of the influence of temperature on the biosensor performances was evaluated in the same conditions previously

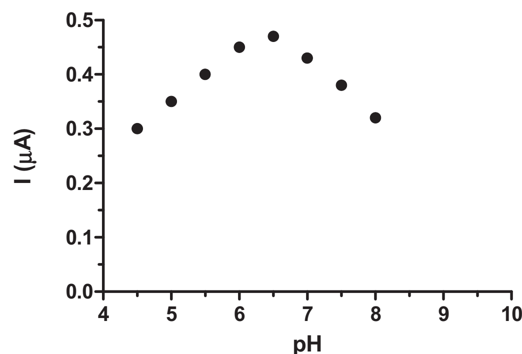

Fig. 1. The effect of pH on the biosensor response PBS buffer 0.1 M with KCl 0.1 M. Catechol concentration used as substrate in the experiments was 20 μM, and *T* = 37 °C, applied potential: −200 mV vs. Ag/AgCl.

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