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Biosensor based on inhibition of monoamine oxidases A and B for detection of β-carbolines

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ABSTRACT

β-Carbolines are inhibitors of monoamine oxidases (MAO-A and MAO-B) and can be found in foods, hallucinogenic plant or various drugs. We have developed a fast analysis method for β-carbolines based on the inhibition of MAO. The enzymes were immobilized on screen-printed electrodes modified with a stabilized film of Prussian blue that contain also copper. We have used benzylamine as substrate for the enzymatic reaction and the hydrogen peroxide was measured amperometrically at −50 mV. The detection limits obtained were 5.0 μM for harmane and 2.5 μM for both harmaline and norharmane. The MAO-A is inhibited by all three tested β-carbolines (harmane, norharmane, and harmaline) while MAO-B is inhibited only by norharmane. The presence of norharmane in mixtures of β-carbolines can be identified based on the difference between the cumulative inhibition of MAO-A by all β-carbolines and MAO-B inhibition. The developed biosensors were used for food analysis.

1. Introduction

β-Carbolines such as harmane, norharmane, harmaline, etc. are a group of alkaloids possessing a tricyclic pyrido[3,4-b]indole ring with various substituents. They are naturally present in plants, animals and humans, but also significant quantities are found in tobacco smoke [1] or foods produced during cooking at high temperature, processing or smoking [2]. Rapid increase in plasma levels and high bioavailability of norharman and harman was found after consumption of β-carboline-containing foods or tobacco smoking [3]. The β-carbolines are inhibitors of monoamine oxidases (MAO) [4] and are psychoactive compounds found in "legal highs" [5] or hallucinogenic plant preparations such as ayahuasca [6]. In pharmacology, MAO-A selective inhibitor drugs are useful as antidepressants [7] and MAO-B selective inhibitor drugs are employed for treatment of Parkinson's disease [8], but dangerous interactions may appear, for example the foods rich in tyramine are contraindicated for the patients under MAO inhibitors medication: the "cheese effect" [9]. Consumption of β-carbolines rich foods (including drinking coffee) and smoking were associated with a protection against Parkinson's disease based on MAO inhibition [10], but excess consumption from various sources may lead to unpredictable neurochemical dysfunctions [11].

β-Carbolines are currently analyzed by various chromatographic or electrophoresis methods that are based on expensive equipments and require long and complicated extraction sample pretreatments [12]. The β-carbolines are electroactive compounds, but the carbon nanotubes based electrodes were used as detectors in chromatography [13] rather than standalone analytical devices. To the best of our knowledge, there are no reported alternative analytical methods-such as biosensors-for the selective, fast and low cost detection of β-carbolines in complex real samples. This paper presents the first developed electrochemical biosensor for the analysis of β-carbolines from food samples based on the inhibition of monoamine oxidase (MAO).

The physiological role of MAO is the oxidative deamination of biogenic monoamines (tyramine and neurotransmitters like dopamine or adrenaline, etc.) or potentially toxic exogenous amines [14]. The amines oxidation by MAO in the presence of oxygen leads to the production of hydrogen peroxide and the corresponding aldehyde. We have used an artificial substrate (benzylamine) for MAO. The enzymatic activity was determined by quantification of the production of hydrogen peroxide using screen-printed electrodes modified with Prussian blue with copper (PB–Cu). There are two isoforms of monoamine oxidase (MAO-A and MAO-B), distinguished by their differences in substrate and inhibitor selectivities. Numerous β-carbolines are reversible competitive inhibitors of MAO-A [4], but only norharmane is a specific inhibitor of MAO-B [15]. We have used the inhibitor selectivity of MAO-A/B for different β-carbolines to identify the presence of

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norharmane in a mixture of β-carbolines without separation of the analytes.

2. Materials and methods

2.1. Reagents

Monoamine oxidase (MAO, EC 1.4.3.4) from human recombinant type A (MAO-A; 120 UI/mg solid) and monoamine oxidase from human recombinant type B (MAO-B; 48 UI/mg) were provided by Sigma (Germany), stored at −20 °C in refrigerator and used as received. Analytical grade iron(III) chloride, potassium chloride, hydrochloric acid 37%, sodium phosphate dibasic, potassium phosphate monobasic, hydrogen peroxide 30%, citric acid, sodium hydroxide, sodium carbonate and boric acid were purchased from Sigma-Aldrich. Copper(II) sulfate anhydrous (98%) and potassium hexacyanoferrate(III) were obtained from Alfa Aesar and from Merck, respectively. Methyltrimethoxysilane (MTMOS) (Aldrich), tetramethoxysilane (TMOS) (Aldrich), HCl 37% (Merck), polyethylene glycol 600 (PEG 600) (Sigma) were used for the enzyme immobilization. 500 mM standard stock solution of benzylamine hydrochloride (Sigma, Germany) was prepared daily in distilled water. Working solutions of benzylamine were prepared in the electrochemical cell containing supporting electrolyte after baseline stabilization. Stock solutions of 5 mM harmane, norharmane and harmaline (Sigma, Germany) were prepared by dissolving the corresponding amount of each inhibitor in methanol. The phosphate buffer solution 0.05 M (PBS) with pH 7.38 supplemented with KCl 0.1 M was prepared with Milli-Q ultrapure water (Millipore).

2.2. Apparatus

The amperometric measurements were performed with a galvanostat/potentiostat Autolab PGSTAT302N (Metrohm-Autolab) controlled by a PC with the software Nova 1.8. The screen-printed electrodes were produced by DropSens (Spain) on a ceramic substrate (producer code DS-110). The screen-printed electrodes have a round working electrode with a diameter of 4 mm, an auxiliary electrode (both WE and AE are made with carbon based ink) and a Ag/AgCl pseudorefecence electrode. The electromagnetic noise produced by magnetic stirring was reduced with the filter from the ECD module set to 1 s.

2.3. Prussian blue–copper mixture (PB–Cu) deposition

The screen-printed carbon electrode was introduced in 6 mL cell with a freshly prepared solution containing 0.5 mM $FeCl_3$, 0.25 mM $CuSO_4$ and 0.5 mM $K_3Fe(CN)_6$ in 10 mM HCl and 100 mM KCl. The PB–Cu film was formed on the electrode surface by cyclic voltammetry (20 cycles between −0.1 V and 0.5 V at a scan rate of 50 mV/s). The modified electrodes were dried at room temperature for at least 1 h before use. For comparison, electrodes with PB without Cu were produced using a similar protocol from a solution without 0.25 mM $CuSO_4$.

2.4. Enzyme immobilization

The immobilization of MAO on the PB–Cu screen-printed electrodes was carried out according to a procedure previously described [16], the entrapment in sol–gel being a biocompatible method that allows the co-immobilization of stabilizers and optimization of the matrix properties aiming both an appropriate environment for enzyme and good mass transport of substrate or inhibitors [17]. The sol–gel precursor solution (solution A) was prepared from 44 μL of H_2O, 40 μL of 1 mM HCl, 10 μL of TMOS, 5 μL of MTMOS and 5 μL of PEG 600 and let to hydrolyze overnight at 4 °C. A 3% (m/v) solution of hydroxyethyl-cellulose (HEC) medium viscosity was prepared in 3 mL water by magnetically stirring the mixture for few hours and then 0.45 g of graphite was added (solution B). 10 μL of MAO-A enzyme solution or 7 μL of MAO-B enzyme solution diluted with 3 μL PBS were mixed with 5 μL of solution B and kept under ice for 10 min. Then, 10 μL of solution A were added on the mixture. 3.5 μL from the final solution were rapidly spread onto screen-printed carbon electrodes modified with PB–Cu film. Final enzymatic activity was of 0.17 UI on each MAO-A biosensor, respectively of 0.21 UI for MAO-B biosensor. The electrodes were dried about 1 h at room temperature and then kept in a desiccator at 4 °C.

2.5. β-Carbolines analysis

The amperometric measurements of the immobilized enzymatic activity were made at −50 mV in 6 mL magnetically stirred PBS. The current intensity was recorded and, after current stabilization, 5 mM benzylamine (final concentration in the cell) was injected. The time necessary to reach the plateau was less than 1 min. The difference of the current intensity between the baseline and the plateau was measured and is correlated with the activity of the immobilized enzyme. The cell was washed with distilled water between measurements. In the first step, the initial response of the electrodes was measured (I_0), then electrodes were incubated for 10 min in 6 mL stirred standard or sample solutions and the remaining enzymatic activity (I_1) was measured by injecting the substrate in the sample. The percentage of inhibition ($I\%$) was determined according to the following formula: $I\% = ((I_0 - I_1)/I_0) \times 100$ and correlated with the concentration of β-carbolines. The biosensors were regenerated by incubation in PBS solution for 5 min and finally, the enzymatic activity was measured again and should be equal with the initial activity (I_0). One biosensor can be used for up to 7 inhibition measurements.

2.5.1. Real sample analysis

β-Carbolines were determined in various food samples from a local store: unroasted green coffee, smoked fish (sprat), chicken ham and green tea. First, the food samples were crushed in a mortar and ~0.5 g were weighted in 2 mL Eppendorf tubes. Then, 1.5 mL of 10 mM NaOH was added over of each sample and the solution was vigorously mixed. The resulted solution was centrifuged and the liquid was discarded. Subsequently, the solid residue was mixed with 1 mL of borate buffer pH 10.59 (boric acid–sodium carbonate) followed by centrifugation and liquid discarding. Both these extraction steps were used to remove the biogenic amines that may be substrate for MAOs. The β-carbolines were extracted with 1 mL of ethyl acetate and the solvent was evaporated [13]. Finally, the β-carbolines were recuperated in 1 mL methanol. The same β-carbolines were analyzed by injecting between 20 and 150 μL from the resulted methanol solution in the amperometric cell following the optimized procedure. The samples were analyzed directly or spiked with known concentrations of β-carbolines (before extraction).

3. Results and discussions

3.1. PB–Cu film formation onto screen-printed carbon electrodes

The Prussian blue mediator is very sensitive towards hydrogen peroxide, but suffers from low stability at neutral and weakly basic pH [18]. Various strategies have been proposed for the stabilization of Prussian blue such as deposition in the presence of surfactants [19], creation of analog compounds with other metals