Biosensors and Bioelectronics 83 (2016) 45–53

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Electrochemical sensing platform amplified with a nanobiocomposite of L-phenylalanine ammonia-lyase enzyme for the detection of capsaicin

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ARTICLE INFO

Article history:
Received 11 January 2016
Received in revised form
12 April 2016
Accepted 13 April 2016
Available online 14 April 2016

Keywords:
Capsaicin
Phenylalanine ammonia lyase
Differential pulse voltammetry
Electrochemical biosensor
Food samples
Molecular docking

ABSTRACT

The present study involves the development of a sensitive electrochemical biosensor for the determination of capsaicin extracted from chilli fruits, based on a novel signal amplification strategy using enzyme technology. For the first time, platinum electrode modified with multiwalled carbon nanotubes where phenylalanine ammonia-lyase enzyme was immobilized using nafion was characterized by attenuated total reflectance infrared spectroscopy, transmittance electron microscopy and thermo-gravimetric analysis supported by computational methods. Cyclic and differential pulse voltammetry measurements were performed to better understand the redox mechanism of capsaicin. The performance of the developed electrochemical biosensor was tested using spiked samples with recoveries ranging from 98.9 to 99.6%. The comparison of the results obtained from bare and modified platinum electrodes revealed the sensitivity of the developed biosensor, having a detection limit (S/N=3) of 0.1863 μg mL^{-1} and electron transfer rate constant (*ks*) of 3.02 s^{-1}. Furthermore, adsorption and ligand-enzyme docking studies were carried out to better understand the redox mechanisms supported by density functional theory calculations. These results revealed that capsaicin forms hydrogen bonds with GLU355, GLU541, GLU586, ARG and other amino acids of the hydrophobic channel of the binding sites thereby facilitating the redox reaction for the detection of capsaicin.

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1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide), is an alkaloid compound found mainly in hot chilli peppers and fruits (capsicum annum and capsicum frutescence) (Reilly et al., 2001; Srinivasan, 2015). Together with its derivative, dihydrocapsaicin, they have a strongest burning effects that is believed to be evolved as a plant protection against herbivores (Supalkova et al., 2007). Capsaicinoids have been reported to have high antioxidant activity (Henderson et al., 1999), anti-tumoral (Sanchez et al., 2006), anti-bacterial (Satyanarayana, 2006) and anti-carcinogenic properties (Huynh and Teel, 2005). Capsaicin has been used for different applications in the past; for example: manufacturing of spices, chilli sauces, pain-inducing defensive pepper sprays (Pershing et al., 2006) and creams for the treatment of painful conditions such as psoriasis, rheumatoid arthritis, diabetic neuropathy, cluster headache and reflex sympathetic dystrophy (Hautkappe et al., 1998). Sanchez and co-workers have reported capsaicin as a promising anti-tumor agent in hormone^{-}refractory prostate cancer (Sanchez et al., 2006). It has also been reported that the metabolism of capsaicinoids by enzymes such as P450 can produce reactive electrophiles capable of modifying biological macromolecules (Reilly and Yost, 2006).

Literature studies revealed that carbon nanotubes (CNTs), due to their unique sensing properties have received considerable attention in the field of electrochemical sensing. Some of their unique properties includes excellent conductivity, large surface area and good biocompatibility (Bathinapatla et al., 2015, 2016; Dresselhaus et al., 1988; Hu and Hu, 2009; Wang and Dai, 2015). For this purpose, they are widely used in electronic, biomedical, pharmaceutical, catalytic, analytical and material fields. Additionally, their special nanostructural properties are attributed to their overwhelming advantages in fabricating electrochemical sensors.

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http://dx.doi.org/10.1016/j.bios.2016.04.037
0956-5663/© 2016 Published by Elsevier B.V.

The CNT-based electrochemical transducers offer substantial improvements in the performance of amperometric enzyme electrodes, immunosensors and biosensors (Kachoosangi et al., 2008; Lyons and Keeley, 2008; Wang, 2005). Specifically, phenylalanine ammonia-lyase received much attention for studies involving the regulation of phenolic biosynthesis. The phenolic portion of capsaicinoids is formed from phenylalanine as a product in the phenylpropanoid pathway (Reyes-Escogido et al., 2011; Sutoh et al., 2006). This enzyme possesses primary features of an electron mediator, because of its stability and good catalytic activity. Electron transfer in biological systems is one of the key reasons for considerable interest in the direct electron transfer between redox enzyme and electrode surfaces (Kuznetsov and Ulstrup, 1999).

In the past, several methods have been reported for the determination of capsaicin, namely: Scoville organoleptic test (Kachoosangi et al., 2008), high performance liquid chromatography (Othman et al., 2011; Supalkova et al., 2007), thin layer chromatography (Spanyar and Blazovich, 1969), UV–vis spectroscopy (Othman et al., 2011), optical biosensor (Mohammad et al., 2014) and electrochemistry (Kachoosangi et al., 2008; Manaia et al., 2012; Mohammad et al., 2013; Randviir et al., 2013; Ya et al., 2012; Yardım and Şentürk, 2013).

However the classical method, 'Scoville Organoleptic Test' suffers from few drawbacks such as cost factor, lack of proximity and poor sensitivity. On the other hand, HPLC methods have some limitations like elaborate sample preparation, high cost of instrument, and sample preparation for the detection of capsaicinoids. Recently, Xue and co-workers reported an electrochemical sensor for the detection of capsaicin using mesoporous cellular foams (Xue et al., 2015). To the best of our knowledge, this is the first electrochemical biosensor using PAL/Nafion/MWCNTs/Pt-E reported for the detection of capsaicin. The developed biosensor offers advantages such as reproducibility, repeatability, precision, accuracy and objectivity over the classical Scoville and HPLC methods. The PAL/Nafion/MWCNTs/Pt-E biosensor is simple, cost effective and sensitive compared to existing chromatographic methods.

Capsaicin is a hydrophobic molecule, characterized into 3 functional groups; the aromatic head group with hydrogen bond potentiality, the dipolar amide bond region and the hydrophobic tail (see supplementary data, Fig. S1). The approach used in this work took advantage of the characteristic feature of this molecule probing its ability to undergo redox process through enzymatic reactions. Accordingly, this work reports on the performance of a platinum electrode (Pt-E) modified with multiwalled carbon nanotubes (MWCNTs) coated with phenylalanine ammonia-lyase (PAL) enzyme infused with nafion. The PAL enzyme was chosen for the electrochemical redox reaction of capsaicin and its related compounds due to its good catalytic activity. In addition, adsorption of the enzyme on the MWCNTs along with the interaction between the enzyme and capsaicin molecule were performed using molecular docking studies. Moreover, the electrochemical methods employed in this study demonstrated the proof-of-concept that this approach can easily be incorporated into a biosensing device that is relatively simple and less expensive, in contrast to the existing Scoville test and HPLC methods used in the food industry.

2. Experimental

2.1. Instrumentation

Voltammetric measurements were carried out using a three electrode system in an electrochemical cell (Metrohm, Herisau, Switzerland) consisting of a 3 mm diameter disc-working electrode (Pt-E); Ag/AgCl as a reference (saturated AgCl, 3 M KCl) electrode, and the platinum wire as a counter electrode using a 797 VA Computrace instrument. A 781 pH/ion meter coupled with an 801 stirrer (Metrohm, Herisau, Switzerland) was used to adjust the pH of the buffer solutions at room temperature. All working solutions including the buffer were prepared with deionized water from a water purification system, Aqua MaxTM Basic 360 (Trilab, South Africa (SA)). Since MWCNTs are insoluble in most solvents, sonication in DMF was employed using an Ultra-sonic 50,194 (Labcon, SA) for effective dispersion, prior to immobilization on Pt-E. The Scientific oven Series 2000 was used to evaporate DMF. The attenuated total reflectance (ATR) spectra were obtained using Perkin-Elmer FTIR, Midrand, South Africa. Thermal analysis studies were carried out on the TGA/DSC, 1 SF/1346 model operating on a STAR^e Software version 9.20 supplied by Mettler Toledo (Johannesburg, SA). The samples were placed in a 10 μL alumina sample holder for thermal analysis at a heating rate of 10 °C min^{-1}.

2.2. Reagents and chemicals

All chemicals were of analytical grade and used as received without any further purification. Capsaicin-360376-IG (cas no. 404–86–4) and 20–30% MWCNT basis, O.D. × L 7–12 nm × 0.5–10 μm (cas number 308,068–56–6) were purchased from Sigma Aldrich (Durban, SA). *N,N*-dimethylformamide (DMF) (cas no. 68–12–2), glacial acetic acid (cas no. 64–19–7) and sodium acetate anhydrous were supplied by Associated Chemical Enterprises (Johannesburg, SA). Nitrogen gas (99.9% purity) was obtained from AFROX (Durban, SA). Ethanol (absolute, 99.9%) used for extraction of samples and nafion (cas no. 31,175–20–9), were supplied by Capital Lab Supplies (Durban, SA). Phenylalanine ammonia lyase-*Rhodotorula glutanis* (PAL) (101M8617) was purchased from Sigma-Aldrich, USA.

2.3. Preparation of working solutions

Sodium acetate buffer solution of pH 4.0 was prepared by mixing sodium acetate and acetic acid (0.1 M each) in a ratio of 15:85 respectively. This solution was then stored at 4 °C until used. The solution of PAL enzyme was prepared by adding approximately 3.0 mg into 1 mL of 67 mM phosphate buffer solution (pH 7.4). The standard solution of capsaicin was prepared by dissolving approximately 10 mg capsaicin standard powder in 100 mL of absolute ethanol (99.9% purity) to produce 100 mg L^{-1} standard solution and refrigerated at 4 °C.

2.4. Preparation of PAL/Nafion/MWCNTs/Pt-E

The bare Pt-E was prepared manually by polishing on a mirror like surface with an alumina slurry (≤ 3 μm) and then rinsed with distilled water. Then, the MWCNTs previously dispersed in DMF (5 mg in 1 mL) and sonicated for 5 min were immobilized onto the surface of the Pt-E by dropping 10 μL aliquot and oven-dried at 50 °C. Later, ~ 10 μL of 5% nafion solution was casted to form a film on MWCNTs/Pt-E. The PAL enzyme was physically adsorbed on the Nafion/MWCNTs/Pt-E surface by dropping 10 μL and allowing the solvent to dry at room temperature for 2 h. The modified PAL/Nafion/MWCNTs/Pt-E electrode was then rinsed with the buffer solution prior to the electrochemical determinations. The optimized electrochemical detection conditions were as follows: 10 mL of 0.1 M acetate buffer solution pH 4.0 used as an electrolyte, 0.2 mL chilli extract and scan rate of 0.01 V s^{-1}.

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