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Tyramine detection using PEDOT:PSS/AuNPs/1-methyl-4-mercaptopyridine modified screen-printed carbon electrode with molecularly imprinted polymer solid phase extraction

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ABSTRACT

Tyramine (4-hydroxyphenethylamine), which is a monoamine metabolized by monoamine oxidase (MAO), exists widely in plants, animals, fermented foods, and salted foods. The incidence of hypertension, or "cheese effect", which is associated with a large dietary intake of tyramine while taking MAO inhibitors has been reported; therefore, the measurement of tyramine is an urgent concern. Herein, an efficient approach that integrates a molecular imprinting polymer for solid phase extraction (MISPE) technique with a sensitive electrochemical sensing platform (SPCE/PEDOT: PSS/AuNP/1-m-4-MP) for the quantification of tyramine is presented. Enhanced electrode conductivity was achieved sequentially by constructing a conductive polymer (PEDOT: PSS) on a screen-printed carbon electrode (SPCE), followed by electrodeposition with gold nanoparticles (AuNPs) and, finally, by modification with positively charged 1-methyl-4-mercaptopyridine (1-m-4-MP) using an Au–S bond. Tyramine was isolated selectively and pre-concentrated by the MISPE technique; electroanalysis that used differential pulse voltammetry (DPV) in NaOH (0.1 M, pH 13) was conducted successively. Experimental parameters (such as modes of electrode modification, ratio of PEDOT: PSS, pH of electrolyte, time required for AuNP deposition, and 1-m-4-MP concentrations) that were associated with optimal detection conditions were evaluated also. We obtained a linear concentration range (5–100 nM, R^2=0.9939) with LOD and sensitivity at 2.31 nM, and 3.11 μA nM^{-1} cm^{-2}, respectively. The applicability of our technique was demonstrated by analyzing tyramine in spiked serum and milk. The feature of our newly developed analytical methods that coupled sample pre-treatment (sample clean-up and pre-concentration) with sensitive detection makes it a promising tool for quantifying of tyramine.

1. Introduction

Tyramine (4-hydroxyphenethylamine), which is a monoamine derived from the decarboxylation of tyrosine that was catabolized by tyrosine decarboxylase (Connil et al., 2002) and metabolized by monoamine oxidase (MAO), is found mainly in foods that are fermented, aged, or spoiled. Tyramine stimulates the release of catecholamines through the displacement of stored monoamines such as epinephrine, norepinephrine, and dopamine, from neuronal storage (pre-synaptic) vesicles (Kaludercic et al., 2011). Therefore, an acute tyramine ingestion or intake of tyramine while using MAO inhibitors (MAOI) can lead to tyramine pressor response or "cheese reaction" with symptoms of vasoconstriction, increased heart rate, and high blood pressure (Cantarini et al., 2004; Martini et al., 1981). MAO inhibitors are effective in treating anxiety, depression (atypical and anergic bipolar depression), and Parkinson disease through the promotion of circulating dopamine levels (Thase, 2012; McCabe-Sellers, 2004). These inhibitors may reduce the metabolism of tyramine that leads to pressor effect. Moreover, a hypotensive crisis may also be caused through repetitive exposure to tyramine, whose metabolized molecule, octopamine, dominate proportionally in synaptic vesicles, which results in restrained neural activation due to a reduced amount of norepinephrine (Hauptmann et al., 1996). A high level of tyramine, octopamine, and *syn*ephrine may be involved in migraine and cluster headaches, due to increased circulation of these neuromodulators in the amygdala, hypothalamus, and dopaminergic system (Chen et al., 2007). To better understand the biological

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functions of tyramine, a fast, easy, and sensitive analytical method is required.

Various efforts have been made to analyze the content of tyramine, such as high performance liquid chromatography (HPLC), high performance capillary electrophoresis (HPCE), and mass spectrometry analysis (Calbiani et al., 2005;Gianotti et al., 2008;Xu et al., 2009). Though these approaches offer excellent selectivity and detection sensitivity, one has to take into consideration the high cost of instruments, intensive labor and time required for sample pretreatment, and analysis time. Electrochemical analysis that possesses the advantages of high sensitivity, low-cost, and short analysis time has gained increasing attention. Cooper and Venton (2009) reported an electrochemical detection of tyramine with a LOD of 18 nM (Cooper and Venton, 2009). Although several tyramine analytical strategies have been developed, there remains a need for simple, rapid, and sensitive analytical methods that are coupled with the necessary pretreatment of samples.

Molecularly imprinted polymer (MIP), which is one of the common techniques for sample pre-treatment, is characterized as a tailor-made polymer with specific binding sites for distinct template molecules that have a definite size, shape, and particular functional groups. The intermolecular interactions between an analyte (template) and a functional monomer (e.g., methacrylic acid) generally include hydrogen bonds, ionic interactions, and dipole-dipole interactions that drive the molecular recognition phenomena to form a three-dimensional polymer network with excessive cross-linking agents (Vasapollo et al., 2011). MIP is cost-effective, easy to prepare, robust to harsh chemical/physical conditions, and reusable (Chen et al., 2011), and it has been applied broadly in several fields like chemical sensors (Alizadeh et al., 2010; Chuang et al., 2009; Hu et al., 2006; Lakshmi et al., 2009; Reimhult et al., 2008), chromatographic applications (Benito-Pena et al., 2009), and selective adsorbents for solid-phase extraction (SPE) (Long et al., 2009; Rezaei et al., 2009). Lately, MIP for solid-phase extraction (MISPE) has been adapted for on-line and off-line analytical procedures (Tamayo et al., 2007; Turiel and Martin-Esteban, 2010). The on-line MISPE features simple manipulation of samples and low sample loss and contamination (He et al., 2007; Masque et al., 2000). However, the off-line approach is used commonly because of its simplicity (Vasapollo et al., 2011).

Chemically modified electrodes (CME) have been used widely in electroanalysis. Screen-printed carbon electrodes (SPCE) are used frequently for CME because they are disposable, easily modified, and cost-effective. Attempts to improve the sensitivity of SPCE through chemical modification have been reported elsewhere (Jia et al., 2013). Modification with conductive polymers such as PEDOT:PSS (Poly(3,4-ethylenedioxythiophene):Polystyrene sulfonate) (Matsushita et al., 2013) and deposition with gold nanoparticles (AuNPs) (Liao and Ho, 2009) for enhancement of conductivity and electron transfer have been reported. The utilization of CME detects heavy metals through the modification of probing molecules. A SERS-active substrate, 4-mercaptopyridine (4-MP) with groups of sulfhydryl and pyridyl that are used commonly for the formation of a self-assembled monolayer, can interact with metal through a lone pair of electrons of N or S atoms or π electrons (Zheng et al., 2014). The positively charged 4-MP demonstrates excellent sensitivity, stability, and reproducibility for detecting chromium (Boiadjiev et al., 2005), through the interaction with negatively charged analytes under neutral and acid conditions (Ozoemena and Nyokong, 2005). Intracellular pH of live cells can be detected by 4-MP functionalized nanoparticles (Nowak-Lovato et al., 2010).

Here, we developed a sensitive analytical approach for the measurement of tyramine by coupling MISPE with CME SPCE (SPCE/PEDOT: PSS/AuNP/1-m-4-MP) using differential pulse voltammetry (DPV). The peak current of tyramine phenolate was measured under alkaline conditions due to elevated electronic density (de Castro et al., 2008). We achieved increased current by the modification of 1-methyl-4-mercaptopyridine (1-m-4-MP), a derivative of 4-MP with a methyl group attached to the nitrogen atom of pyridine, onto AuNPs by an Au-S bond. In addition, we analyzed serum and milk samples spiked with tyramine to investigate the matrix effect and to validate the performance of the sensing platform.

2. Experimental

2.1. Reagents and materials

4-MP, iodomethane (methyl iodide), EDOT, PSS, gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), tyramine, tyrosine, levodopa, methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM), azobisisobutyronitrile (AIBN), ferrocyanide ($K_4Fe(CN)_6$), phosphate buffered saline (PBS, 0.5 M NaH_2PO_4 and 0.5 M Na_2HPO_4), background buffer (BGE, borax buffer, 70 mM, pH 9.5, $Na_2B_4O_7$), acetonitrile, diethyl ether, acetic acid, methanol, acetone, hydrogen peroxide (H_2O_2), potassium chloride (KCl), lithium perchlorate ($LiClO_4$), sodium hydroxide (NaOH), and all other chemicals were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). The reagents used were of analytical grade or of the highest purity that was available commercially, and they were used as received. All solutions were prepared with deionized water at a resistivity not less than 18 Ω cm (Milli-Q, Bedford, MA).

2.2. Apparatus

Electrochemical experiments were carried out with a model 624c electrochemical workstation (CH Instruments, Austin, TX). Conventional triple-electrode, SPCE (area: 0.071 cm^2, Zensor, Taichung, Taiwan) were purchased from CH Instruments. The electrode surface morphologies were studied using a scanning electron microscopy (SEM) (model JSM 5200, JEOL). Analysis of electrode elements was performed by ESCA (electron spectroscopy for chemical analysis, PHI 5000, Versaprobe, ULVAC-PHI). Analysis chemical structure was confirmed by NMR spectroscopy (Bruker AM-300). The eluents of molecularly imprinted polymers (MIP) were analyzed by capillary electrophoresis (CE) (Agilent Technologies, Santa Clara, CA). The deposition AuNPs on electrodes was confirmed by energy-dispersive X-ray spectroscopy (EDS) (Oxford 6111, JEOL). The modification of 1-m-4-mp was confirmed by X-ray photoelectron spectroscopy (XPS) (JPS9200, JEOL).

2.3. Synthesis of 1-methyl-4-mercaptopyridine

For the synthesis of 1-m-4MP, iodomethane was attacked by the lone pair of ring nitrogen of 4-MP, iodine was served as leaving group. Iodomethane (2.8 mM) was added to a solution containing 4-MP (2.8 mM) in acetonitrile (120 mL). The mixture was stirred for 30 min at room temperature and then heated at 50 °C for 6 h. After the mixture turned brown, it was dried by a rotary evaporator to reduce volume. Cold diethyl ether (100 mL) was then added to dissolve excessive 4-MP, and to precipitate 1-m-4MP. The structure of 1-m-4MP and 4-MP were confirmed by NMR spectroscopy. The 1H NMR spectroscopy for 1-m-4MP is [δ2.590 (s, 3H, N-CH_3), δ7.559–7.682 (d, 2H, 3, 5-H), δ8.305–8.478 (d, 2H, 2, 6-H)], for 4-MP [δ7.317–7.481 (d, 2H, 3, 5-H), δ7.707–8.350 (d, 2H, 2, 6-H)] (Fig. S1).

2.4. Modification of SPCE/PEDOT:PSS/AuNP/1-m-4MP

Fig. 1 exhibits the scheme for SPCE modification and tyramine