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Novel impedimetric dopamine biosensor based on boronic acid functional polythiophene modified electrodes

Muamer Dervisevic [a], Mehmet Senel [a,*], Emre Cevik [b]

[a] Institute of Biomedical Engineering, Fatih University, B. Cekmece, Istanbul 34500, Turkey
[b] Department of Genetics and Bioengineering, Faculty of Engineering, Fatih University, B. Cekmece, Istanbul 34500, Turkey

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ABSTRACT

In this study we report a new, simple and first impedimetric biosensor based on 3-Thienyl boronic acid for dopamine detection. Biosensor electrode preparation is 1 min long by simple electro-polymerization of 3-Thienyl boronic acid and copolymer Thiophene P($TBA_{0.50}Th_{0.50}$). Strong interaction between dopamine and thin layer of boronic acid has provided bio-sensing electrode high selectivity and stability, linear range of 7.8 to 125 μM, and detection limit of 0.3 μM. Characterization and optimization studies were conducted using electrochemical impedance spectroscopy (EIS) and cyclic voltammogram (CV). In order to test reliability of proposed biosensor real sample application study has been conducted using non-diluted human urine and it has been found that biosensor selectivity and recovery is excellent. As well P($TBA_{0.50}Th_{0.50}$) based electrode and dopamine interaction has been proven by single frequency impedance measurements. Biosensors acquired good reproducibility, stability, selectivity and very low interference.

1. Introduction

Dopamine (DA) is involved in the reward and pleasure centers of the brain as one of the most important catecholamine neurotransmitter in mammalian central nervous system [1,2,3], which also plays a role in the regulation of movement [4], having a stimulating effect on the heart and regulates a flow of information to different areas of brain [5]. Playing role as neuromodulator in the brain and influencing a variety of motivated behaviors [6], abnormal high or low levels of DA can cause several neurological diseases such as Alzheimer's disease for which DA concentration in urine is approximately 20.14 ng/ml (Mean $n = 14$) [7], for Parkinson's disease DA concentration 0.43 μg/g tissue [8] and in urine 0.139 μmol/mol creatinine [9], for Schizophrenia its found to be approximately 208.9 μg/3.5 h ($n = 11$) [10], for Huntington's disease ranges from 0.75 to 5.6 ng/mg tissue which depends on brain region [11], etc. Therefore there is strong need for selective and reliable method which can be used for precise determination of DA concentration in vitro and vivo. Various approaches such as spectrophotometry [12], mass spectrometry [13], high performance liquid chromatography (HPLC) [14], colorimetric detection [15], ion chromatography [16], fluorescent detection [17] have been developed and applied in DA detection. However these methods have some disadvantages such as requiring skilled personnel and highly specialized equipment, time consuming, high cost, complex sample preparations and pretreatments, etc. Where's detection of DA with electrochemical biosensor is straight forward method which does not requires highly skilled personal, takes short time for analysis and most important no need for sample pretreatment. In comparison to other techniques, EIS sensors have received considerable attention due to their remarkably strong operability, high sensitivity, and good selectivity [18].

Detection of DA by different types of electrochemical methods such as differential pulse voltammetry (DPV), cyclic voltammogram (CV) and very small amount of reports based on electrochemical impedance spectroscopy (EIS) has been shown in Table 2. All of them are concentrated on decreasing interference of ascorbic acid (AA) or detection of AA [19,20] which in human body can be as high as several millimolars and its overwhelming oxidation peak covers DA peak [21]. Various strategies based on fact that AA and DA are oppositely-charged at physical pH [22] has been developed to suppress or separate AA oxidation peak or by increasing selectivity towards DA, for example by incorporating Ion exchange membrane Nafion [23] or self-assembled monolayers (SAMs) [24]. In order to overcome the complex producers and try to develop EIS biosensor which will be able to apply in real sample without pretreatments and dilutions we employed 3-Thienyl boronic acid as and polymer surface on electrode. As already known boronic acids can covalently interact with cis-diol- containing molecules to form boronate esters [25,26] and on the other side ortho-quinols such as dopamine has strong interaction with boronic acids [27]. It is well documented that over the pH range of 6–8 two neighboring hydroxyl groups on dopamine undergo relatively fast reversible reaction with boronic acid [26,28]. It was found that dopamine combine with both boronic acid

* Corresponding author.
E-mail address: msenel81@gmail.com (M. Senel).

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functionally attached to polymer back bone [28], and free phenylboronic acid in solution [26]. When dopamine binds to boronic acid moiety, resistance charge transfer (Rct) increases on the other hand the oxidation potential of dopamine shifts to anodic direction, thus avoiding the mask of redox peak of ascorbic acid in excess amount [29]. To the best of our knowledge, electrochemical polymerized film based on 3-thineyl boronic acid and dopamine interaction has not been reported yet. Which are sufficient evidence and reasons to select boronic acid based polymers for impedimetric DA biosensors. There are few works reported using boronic acids such as reported by Plesu et al., impedimetric DA biosensor based poly(aniline boronic acid) [30] and electrochemical DA biosensor based on molecularly imprinted poly(acrylamidophenylboronic acid) film reported by Hong et al. [29].

Herein we report impedimetric DA biosensors based on 3-Thienyl boronic acid. Impedimetric sensors was prepared by one minute single-step electropolymerization of 3-Thienyl boronic acid and copolymer Thiophene (Th) which is first DA biosensor reported based on this polymer. Strong interaction between DA and thin layer of boronic acid provided bio-sensing electrode high selectivity towards DA, which is also supported in literature [27]. Thin layer of P($TBA_{0.50}Th_{0.50}$) has provided ability to detect DA in non-diluted healthy human urine sample with excellent recovery values as well reliability. Interference studies has been conceded with very high concentrations of AA and uric acid (UA) and showed very low interference values and single frequency impedance clearly demonstrated successful interaction between DA and electrode surface. P($TBA_{0.50}Th_{0.50}$) electrode showed excellent selectivity and sensitivity towards DA as well high reliability.

2. Experimental

2.1. Materials

Dopamine hydrochloride, Thiophene 99% and 3-Thienyl boronic acid were obtained from Sigma-Aldrich, Natrium Fluoride (NaF) and Hydrochloric acid 37% (HCl) purchased from Merck. All other chemicals were of analytical grade and were used without further purification.

2.2. Preparation of bio-sensing electrode

Pencil graphite electrode (PGE) with dimensions of 10 mm, 2 mm, and 0.5 mm (length, thickness and height) which makes surface area of 51 mm^2 was cleaned by sonication in ethanol to remove adsorbed organic materials or any other impurity from the electrode surface. After cleaning, electrochemical polymerization of 3-Thienyl boronic acid was carried out in a solution of different monomer concentrations (Table 1) and 0.2 M NaF in 0.5 M HCl. Electro-polymerization was carried out in potential range of −1.0 V to 2.0 V in an unstirred solution with the scan rate of 100 mV s^{-1}. In the end electrode was washed with dH_20 and was ready for use in dopamine detection analysis.

2.3. Electrochemical analysis

Electrochemical impedance spectroscopy (EIS) and cyclic voltammogram (CV) measurements were performed via a three electrode system set composed of Ag/AgCl as reference, Platinum (Pt) wire as auxiliary, and electro-polymerized TBA on PGE as working electrode, all immersed in an electrochemical cell filled with 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl which served as redox probe. For EIS studies impedance spectra was collected in the frequency range of 100 Hz to 1000 kHz with applied alternating wave of 10 mV amplitude. Where CV is used for characterization of electro-polymerized electrode surface and was recorded in the range of −0.2 V to 0.7 V with scan rate of 100 mV s^{-1}. Once EIS measurements were obtained Rct values were calculated by means of designed electrical equivalent circuit model. Results were analyzed and fit by CHI instruments and Ivium Technologies software's.

Table 1
Monomer concentration for electro-polymerized polymers.

Electrode	3-Thienyl boronic acid (mM)	Thiophene (mM)
TBA	50.0	0
P($TBA_{0.75}Th_{0.25}$)	37.5	12.5
P($TBA_{0.50}Th_{0.50}$)	25.0	25.0
P($TBA_{0.25}Th_{0.75}$)	12.5	37.5

Table 2
Comparison of P($TBA_{0.50}Th_{0.50}$) electrode based DA biosensor with previous reported works.

Electrode	DM	Linear range	DL	Real sample	Ref.
Fullerene-C_{60}Au	CV	1 nM–5 μM	0.26 nM	Urine, serum	[5]
Au-DT/MOA	AM	0.01–5 μM	20 nM	Urine	[32]
TCPP/CCG	DPV, CV	0.01–70 μM	0.01 μM	Urine, serum	[1]
MIP/GCE	DPV	50 nM–10 μM	33 nM	DHI	[25]
MWCNT/polv(AABA)	DPV	50 nM–2 μM	20 nM	No	[29]
PA-MWCNT/GCE	EIS	10 μM–1 mM	14.1 μM	No	[33]
EPPGE	CV	0.2–25 μM	90 nM	Horse blood serum	[34]
MWCNT/Nafion-GC	DPV	10 nM–10 μM	2.5 nM	No	[35]
Poly-Tiron/GCE	DPV	0.2–45.8 μM	0.07 μM	DHI	[36]
P($TBA_{0.50}Th_{0.50}$)	EIS	7.8–125 μM	0.3 μM	Urine	This work

Detection method (DM); detection limit (DL); cyclic voltammogram (CV); electrochemical impedance spectroscopy (EIS); amperometric (AM), differential pulse voltammetry (DPV), dopamine hydrochloride injection (DHI).

2.4. Dopamine detection

After electro-polymerized electrode was assembled it was immersed into the dopamine solution with certain concentrations (Fig. 1). After which it was carefully removed from dopamine solution and gently rinsed with dH_2O. When electrode was ready for measurement it was immersed in electrochemical cell containing 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution from which the electrochemical impedance spectroscopy response value was recorded after 45 min incubation in dopamine solution. In the end, dopamine calibration curves were plot using impedance variations ($\Delta Rct = Rct_{(P(TBA0.50Th0.50)/DA)} - Rct_{(P(TBA0.50Th0.50))}$) with each measurement repeated 3 times ($n = 3$).

2.5. Instrumentations

Electrochemical measurements were performed using CompactSoft portable electrochemical interface and impedance analyzer (*Ivium Technologies*) for cyclic voltammogram recodes whereas for electrochemical impedance measurements were carried out using a CHI Model 6005 electrochemical analyzer. Measurements were recorded via a three electrode system set which was composed of Ag/AgCl as reference, Platinum (Pt) wire as auxiliary, and electro-polymerized TBA on PGE as working electrode, all immersed in an electrochemical cell filled with 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl which served as redox probe. Conventional three-electrode electrochemical cell was purchased from CH Instruments. A scanning electron microscope (SEM) was employed to observe the surface of the samples. For SEM, pieces of the samples were mounted on stubs and coated with gold using a sputter coater. SEM micrographs of the samples were taken using a JEOL NeoScope scanning electron microscope.