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A novel approach for the selective determination of tryptophan in blood serum in the presence of tyrosine based on the electrochemical reduction of oxidation product of tryptophan formed in situ on graphite electrode

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ABSTRACT

In this study, a novel method was proposed for the selective determination of tryptophan (TRP) in blood serum in the presence of tyrosine. This method is based on the electrochemical reduction of 2-amino-3-(5-oxo-3,5-dihydro-2H-indol-3-yl)-propionic acid (5-O-3,5DH-TRP) formed by the oxidation of TRP on the electrochemically treated pencil graphite (ETPG) electrode surface at a suitable potential value. The parameters affecting the TRP determination were deeply investigated. The optimal pH value was determined as 3. The highest reduction current intensity was obtained at the accumulation potential and time values of +0.95 V and 120 s, respectively. The reduction peak current values of 5-O-3,5DH-TRP versus TRP concentration at the ETPG electrode showed linearity in the range from 0.5 μM to 50.0 μM (R^2 = 0.9962) with a detection limit of 0.05 μM (S/N = 3). The reduction peak intensity of 5-O-3,5DH-TRP on the ETPG electrode showed no significant change in the presence of different interfering substances. The analytical application of the proposed novel method was successfully tested by using human blood serum samples.

1. Introduction

Tryptophan (TRP) is an essential amino acid for human body. It has to be taken by foods in daily diet to maintain the nitrogen balance. It also serves as a precursor for serotonin and melatonin which improve the sleep, mood and mental health. TRP supplements have been often used as antidepressants, sleep aids and weight-loss aids to increase the serotonin and melatonin level in body fluids. Moreover, it has been implicated as a possible cause of schizophrenia (Safavi and Momeni, 2010) in people who cannot metabolize it properly. When it is improperly metabolized, it creates a waste product in the brain that is toxic, causing hallucinations and delusions (Huang et al., 2009). Therefore, simple, sensitive and low-cost determination of TRP is of great significance to people's health. Moreover, TRP coexists with different amino acids in body fluids. Therefore, its selective determination is also very important in body fluids especially in blood serum.

Because of their simplicity, ease of miniaturization, high sensitivity and relatively low cost as compared to conventional colorimetric and spectrophotometric methodologies (Strochkova et al., 1997), the use of electrochemical procedures have been extensively investigated in the determination of biologically important molecules in recent years. In this manner, some studies have been devoted to electrochemical determination of TRP lately because of its electroactivity. These studies are mainly based on the electrochemical oxidation of TRP at different unmodified and modified electrodes (Agüi et al., 1999; Jin and Lin, 2004; Yu et al., 2008; Huang et al., 2009; Tang et al., 2010). According to these studies, the oxidation potential value of TRP was almost same with that of tyrosine (TYR) which is found together with TRP in blood serum. As a result, to determine TRP in the presence of TYR selectively in blood serum based on its electroactivity, different determination strategies should be developed.

In the case of unmodified and modified electrodes, the surface regeneration of the electrode is very crucial in the subsequent analysis because in many cases the oxidized molecules can polymerize on the electrode surface and can change the surface properties of the electrode. Moreover, the electrode modifications usually involve laborious preparation procedures and the modified electrodes can exhibit long-term instability and loss of sensitivity.

The single-use disposable electrodes may overcome the regeneration drawback of the modified electrodes. In this manner, pencil lead can be seen an important electrode material for the electroanalytical applications due to its low cost and commercial availability. This electrode has already been used in many electroanalytical applications (Wang and Kawde, 2001; Wang et al., 2003; Özcan et al., 2007, 2008). It was shown that the electrochemical treatment can change the surface and redox characteristics of carbon based

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electrodes (Engstrom, 1982; Engstrom and Strasser, 1984; Shi and Shiu, 2001; Li et al., 2009; Thiagarajan et al., 2009). We have lately investigated the preparation of electrochemically treated pencil graphite (ETPG) electrodes and their electroanalytical application in the determination of low levels of dopamine (Özcan and Şahin, 2009), uric acid (Özcan and Şahin, 2010) and paracetamol (Özcan and Şahin, 2011) in blood serum.

In this study, we have focused on the application of the ETPG electrode for the determination of TRP in the presence of TYR in blood serum samples. For this purpose, we have first investigated the electrochemical behavior of TRP and TYR by ETPG electrode. The obtained results showed that TRP and TYR oxidized almost same potential values in our experimental conditions as was reported previously (Agüi et al., 1999; Jin and Lin, 2004; Yu et al., 2008; Huang et al., 2009; Tang et al., 2010). This means that the using of the oxidation peak of TRP is not suitable for the determination of TRP in blood serum samples because of the interference of TYR. To overcome this limitation and determine the TRP in blood serum samples, we have used a different approach which was firstly proposed in our previous study (Özcan and Şahin, 2011). The proposed novel method is based on the electrochemical reduction of electrochemical oxidation product of TRP 2-amino-3-(5-oxo-3,5-dihydro-2H-indol-3-yl)-propionic acid (5-O-3,5DH-TRP) which is formed in situ on the ETPG electrode. The selectivity of the reduction peak for TRP prevents the interference of TYR. The effects of different parameters were deeply investigated. The analytical application of the proposed method was successfully tested by the determination of TRP in blood serum samples.

2. Experimental

2.1. Chemicals

Tryptophan (99%) and tyrosine (98+%) were obtained from Sigma. Other chemicals were of analytical reagent grade and used without further purification. Aqueous solutions were prepared with ultra pure deionized water (Sartorius). The voltammetric measurements were performed in 0.1 KH_2PO_4 buffer solutions (PBS). The human blood serum samples were obtained from Hospital of Anadolu University.

2.2. Apparatus

Electrochemical experiments were carried out by a conventional three-electrode system. Pencil graphite (PG) and electrochemically treated pencil graphite were used as working electrodes. Pt wire and saturated calomel electrode (SCE) were used as an auxiliary and reference electrode, respectively. Electrochemical treatments of PG electrodes were achieved by Voltalab PGZ402 Potentiostat/Galvanostat. Differential pulse voltammetry (DPV) measurements were performed by Autolab PGSTAT 100 Potentiostat/Galvanostat with GPES 4.9 version conversion software (EcoChemie, The Netherlands).

PG electrodes were Tombo leads with a diameter of 0.5 mm. The PG was prepared by cutting the leads into 3 cm long sticks and 1.2 cm was treated. A Noki pencil model 2000 (Japan) was used as a holder for PG electrode. Electrical contact with the PG electrode was obtained by soldering a metallic wire to the metallic part of the holder.

2.3. Electrochemical treatment of PG electrode

The electrochemical treatment of PG was performed in 0.1 M H_3PO_4 solution by potential cycling between −0.3 V and +2.0 V with a scan rate of 50 mV s^{-1} for 3 scans (Özcan and Şahin, 2009, 2011). The prepared electrodes (ETPG) were stored at room temperature in a desiccator until their use.

2.4. Adsorptive stripping differential pulse voltammetry (ASDPV) measurements

The voltammetric determination of TRP was performed by ASDPV using ETPG electrode. In order to accumulate 5-O-3,5DH-TRP on the electrode surface, the ETPG was waited at +0.95 V for 120 s in the measurement solution without stirring containing appropriate amount of TRP. Then, the electrode was waited for 2 s without stirring in the same medium. Finally, the stripping of the adsorbed 5-O-3,5DH-TRP was performed by potential scanning from +0.5 V to +0.2 V. Each measurement was performed with a fresh electrode and repeated three times.

2.5. High performance liquid chromatography analysis (HPLC)

The TRP and TYR content of the blood serum samples were also determined by HPLC analysis according to literatures (Neckers et al., 1980; Wedner et al., 1997). HPLC analysis was performed by Agilent 1100 system equipped with a fluorescence detector. A reversed phase Inertsil ODS-3 (5-μm, 4.6 × 250-mm) column which was thermostated at 40 °C was used. The column was eluted with 0.015 M KH_2PO_4 buffer solution (pH 6) containing 40 mL acetonitrile per litter of solution with a flow rate of 0.8 mL min^{-1}. The wavelengths used for the detection of TRP and TYR are 285 nm (excitation) and 365 nm (emission).

To precipitate the blood serum proteins, 30% $HClO_4$ solution was added into the blood serum samples and the samples were diluted 3.5 times by the equal amounts of standard TRP and TYR solutions. After that, the samples were vortexed for 10 s and centrifuged at 12,000 × g for 10 min. 10 μL of the supernatant was injected onto the column.

3. Results and discussion

3.1. Principle of the selective TRP determination

It is well known that TRP generally coexists with TYR in biological fluids and they show similar electrochemical behaviors at untreated carbon based electrodes. Therefore, it is important to develop a rapid and simple method for the selective determination of each molecule in the presence of the other. In this manner, we have first investigated the electrochemical behavior of TRP and TYR in PBS (pH 3) using ETPG electrode. Since the oxidation and reduction peak current intensities of organic molecules on the ETPG depend on the contact time of ETPG electrode with the analysis solution (Özcan and Şahin, 2009, 2010, 2011), the ETPG electrode was immersed into the analysis solution for a definite time period (120 s) before the measurement. The obtained cyclic voltammograms are shown in Fig. 1a. As can be seen, there was no electrochemical activity of the ETPG electrode in blank PBS (Fig. 1a-A) in the potential interval of +0.2 V and +1.2 V. On the other hand, a well-defined oxidation peak was observed at +0.83 V (versus SCE) in individual solutions of both 0.1 mM TRP and TYR (Fig. 1a-B and C, respectively). While TRP and TYR showed almost same behavior in the forward (anodic) scan, a small reduction peak was obtained in the reverse (cathodic) scan at +0.29 V (versus SCE) only in TRP solution (Fig. 1a-B). It can be easily seen from Fig. 1a, the individual determination of TRP and TYR cannot be achieved based on the electrochemical oxidation of these molecules when the analysis solution contains both of them. In this manner, the obtained reduction peak can be an alternative route for the selective electrochemical determination of TRP in the presence of TYR.