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Abstract

Amperometric Biosensing of L-glutamate Using Reduced Graphene Oxide and Glutamate Oxidase†

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Abstract: Determination of L-glutamate in biological media is very important since it is the most common excitatory neurotransmitter related to some neurological diseases such as Parkinson's, communication dysfunction, stroke, epilepsy or schizophrenia. Aiming to study pathways of these diseases as well as for evaluation of medical treatment, it is very important to have rapid and reliable methods for the determination of L-glutamate. This study presents a new approach of enzyme-based biosensor operating at -0.1V, what ensures its good sensitivity and selectivity. Reduced graphene oxide applied in the biosensor, allowed monitoring of L-glutamate via electro oxidation of NH_3 released during the reaction catalyzed by Glutamate oxidase.

Keywords: amperometric biosensor; L-glutamate; RGO; Glutamate oxidase; oxidation of ammonia

1. Introduction

Neurological diseases such as Parkinson's, communication dysfunction, stroke, epilepsy or schizophrenia can be caused by abnormal transmission of L-glutamate since it is the most common excitatory neurotransmitter. Aiming to control these diseases as well as for research purposes, it is very important to have reliable, simple methods for the determination of glutamate. Recently, the most commonly used glutamate methods are long-term, requiring complex equipment and special training of service personnel [1,2]. It is known, methods based on electrochemical biosensors are recognized as the most optimal in terms of price, simplicity and reliability. Almost all of them are based on the oxidation reaction of hydrogen peroxide at Pt electrodes, which is carried out at high electrode potentials ca.+ 0.6 V vs. Ag/AgCl. In this study is proposed an amperometric enzyme-based biosensor operating at -0.1V vs. Ag/AgCl, what ensures its good sensitivity and selectivity. After applying thermally reduced graphene oxide (RGO) for the design of biosensor, the concentration of L-glutamate was monitored according to the electro oxidation of ammonia (NH_3) released during the reaction catalyzed by Glutamate oxidase (GluOx). The main characteristics and advantages of proposed biosensor have been compared with a conventional Pt-based amperometric biosensor.

2. Materials and Methods

2.1. Materials

GluOx from *Streptomyces sp.* (EC 1.4.3.11) (), Merck KGaA, Germany. Other chemical reagents were obtained from Sigma–Aldrich and were of analytical grade. RGO was synthesized according to the protocol reported by Ieva Sakinyte doctoral dissertation (Vilnius University).

2.2. Construction of L-Glutamate Biosensor

The biosensor consists of semipermeable membrane with immobilized GluOx, working graphite electrode as a contact zone and isolating corps. Aiming to design enzymatic membranes the mixture containing GluOx, bovine serum albumin in PBS, glutaraldehyde and RGO paste, which was

prepared by mixing of RGO powder with the pasting liquid consisting of 10% polyvinyl dichloride in acetone, was deposited on the inner surface of the ring-fixed membrane (working area Ø 2.4 mm) and then left at 4 °C overnight. After that membrane was mechanically attached to the surface of the graphite electrode. In a control Pt-based version GluOx was immobilized in to the semipermeable membrane and the Pt at +0.6 V, served as a working electrode.

3. Discussion

After applying of RGO and GluOx for the biosensor design the concentration of L-glutamate could be monitored according to the release of NH_3 producing in the reaction catalyzed by GluOx (Figure 1).

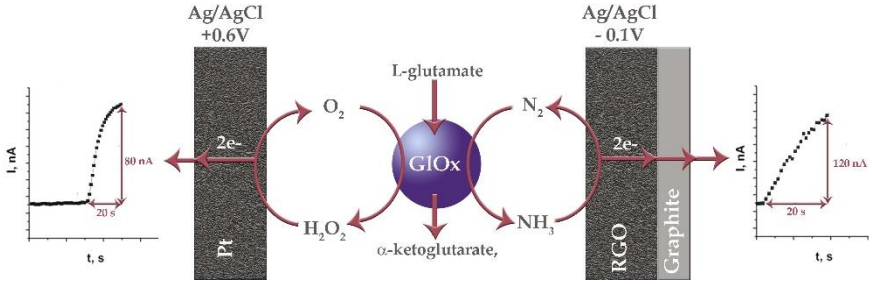

Figure 1. Principal scheme of two different pathways of L-glutamate amperometric biosensing when Pt and RGO utilizing as electrode surfaces. Responses of biosensors to 0.5 mM of L-glutamate obtained at potentials of +0.6 V (left) and -0.1 V (right).

Dependence of the biosensor response to L-glutamate vs. applied electrode potential allowed to determine the most optimal working potential of -0.1 V *vs.* Ag/AgCl. The conducted studies confirmed that at -0.1 V the biosensor registers the NH_3 oxidation current. The influence of oxygen reduction at this potential on the response of the biosensor was also evaluated. Finally, the reliability of the biosensor was confirmed in real brain extract samples, and its performance was compared with a traditional biosensor based on GluOx acting on a Pt electrode at +0.6 V.

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