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The design, development and application of electrochemical glutamate biosensors

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ABSTRACT

The development of biosensors for the determination of glutamate has been of great research interest for the past 25 years due to its importance in biomedical and food studies. This review focusses on the various strategies used to fabricate glutamate biosensors as well as their performance characteristics. A brief comparison of the enzyme immobilisation method employed and the performance characteristics of a range of glutamate biosensors are described in tabular form and then described in detail throughout the review: some selected examples have been included to demonstrate the various applications of these biosensors to real samples.

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1. Introduction

Glutamate is considered the primary neurotransmitter in the mammalian brain that facilitates normal brain function [1]. At high concentrations, glutamate can induce neurotoxicity, which causes damage to brain tissue, thus linking it to a number of neurodegenerative disorders such as Parkinson's disease, multiple sclerosis [2] and Alzheimer's disease [3]. In cellular metabolism, glutamate also contributes to the urea cycle and tricarboxylic acid (TCA)/Krebs cycle. It plays a vital role in the assimilation of NH_4^+ [4]. The intracellular glutamate levels outside of the brain are typically 2–5 mM/L, whilst the extracellular concentrations are ~0.05 mM/L [5]. It is also present in high concentrations in the liver, kidney and skeletal muscle [6].

Glutamate is also found in many food products in the form of monosodium glutamate (MSG), which is added to reduce salt intake and enhance flavour [7]. The use of MSG has been controversial. Although the European Union (EU) limits the use of MSG in foods to 10 g/kg of the product, it is typically found in high concentrations in food products that claim to contain no added MSG [8]. It can also be used to mask ingredients of poor freshness. The concentration of MSG in foods can vary significantly. The presence of MSG in wastewater is also a concern due to its inhibitory effects on wheat seed germination and root elongation [9].

Electrochemical biosensors for the detection of glutamate offer a faster, more user-friendly and cheaper method of analysis than classical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS).

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This review discusses electrochemical biosensors fabricated based on glutamate oxidase or glutamate dehydrogenase (GLDH), the method of enzyme immobilisation and, where applicable, the application of glutamate biosensors to biological and food samples.

1.1. Biosensors based on glutamate oxidase

In this section, the fabrication methods are subdivided according to the technique of enzyme immobilisation. The electrochemical response can generally be described by the following equations:

$$\text{Glutamate} + O_2 \xrightarrow{\text{GluOx}} H_2O_2 + \alpha\text{-ketoglutarate} + NH_3 \quad (1)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (2)$$

Equation 1 represents the enzymatic oxidation of glutamate to form α-ketoglutarate and H_2O_2. Equation 2 describes the electrochemical detection of hydrogen peroxide at the base transducer, which generates the analytical response.

1.2. Entrapment

Entrapment is defined as the integration of an enzyme within the lattice of a polymer matrix or a membrane, whilst retaining the protein structure of the enzyme [10]. In addition to the immobilisation of enzymes, membranes can also eliminate potential interfering species that may be present in complex media such as serum and food.

A selective biosensor for the determination of glutamate in food seasoning was developed by incorporating glutamate oxidase into a poly(carbamoyl) sulfonate (PCS) hydrogel. The glutamate oxidase (GluOx_–PCS mixture was then drop-coated onto the surface of a thick-film platinum electrode [11]. Liquid samples (1, 10 and 100 µL) were diluted to 10 mL with phosphate buffer. The biosensor was then used to determine the glutamate recovery from different concentrations of the sample. The results generated correlated favourably with an L-glutamate colorimetric test kit.

A recent application of a micro-glutamate biosensor for investigating artificial cerebrospinal fluid (CSF) under hypoxic conditions was described [12]. The fabrication method is a complex, multistep process whereby glutamate oxidase is incorporated with chitosan (CHIT) and ceria–titania nanoparticles (Fig. 1).

The nanoparticles are able to store and release oxygen in its crystalline structure; it can supply O_2 to GluOx to generate H_2O_2 in the absence of environmental oxygen. The biosensor was evaluated with artificial CSF, which had been fortified with glutamate over the physiological range; the device was found to operate over the concentration of interest under anaerobic conditions.

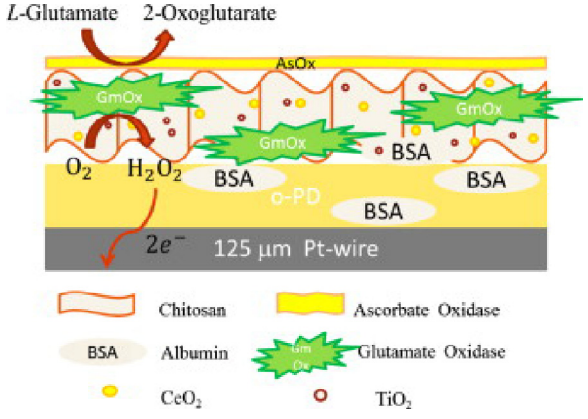

Fig. 1. Schematic illustration of the biosensor design and the GluA detection principle. (Reprinted with permission from Ref. [12], Elsevier).

A device for measuring glutamate levels in brain extracellular fluid using a relatively simple fabrication procedure was reported [13]. The procedure involved dipping a Teflon-coated platinum wire of 60-µm radius into a buffered solution containing GluOx and *o*-phenylenediamine (PPD), followed by a solution containing phosphatidylethanolamine (PEA) and bovine serum albumin (BSA). The GluOx was entrapped by the electropolymerisation of PPD on the surface of the electrode. The PPD and PEA was used to block out interferences.

An interesting entrapment approach using polymers to encapsulate GluOx within a gold electrode was reported [14]. The first step involved the immersion of a gold disc electrode in 3-mercaptopropionic acid (MPA) solution, followed by drop-coating layers of poly-L-lysine and poly(4-styrenesulfonate). Once dry, a mixture of GluOx and glutaraldehyde was drop-coated onto the surface to form a bilayer. The authors suggested that MPA increases the adhesion of the polyion complex to the gold surface by the electrostatic interaction between the carboxyl groups present on the MPA and the amino groups present on the poly-L-lysine. A response time of only 3 s was achieved after 20 nM glutamic acid was added, yielding a current of 0.037 nA (1.85 nA/µM). A linear response was observed between 20 and 200 µM. Both the response time and limit of detection are superior to previously discussed biosensors. It was suggested that the rapid response was due to the close proximity of the enzymatic reaction to the surface of the electrode. For this method of fabrication of GluOx-based biosensors, the latter approach leads to the lowest limit of detection.

The increased interest in glutamate measurement has led to the commercial development of an in vivo glutamate biosensor by Pinnacle Technology Inc. [15]; this has been successfully used to monitor real-time changes of glutamate concentrations in rodent brain. The biosensor employs an enzyme layer consisting of GluOx and an 'inner-selective' membrane, composed of an undisclosed material that eliminates interferences. The enzymatically generated hydrogen peroxide is monitored using a platinum–iridium electrode. The biosensor possesses a linear range up to 50 µM. The manufacturers indicate that the miniaturised biosensor requires calibration upon completion of an experiment to ensure the selectivity and integrity of the sensors.

1.3. Covalent bonding

The application of a GluOx-based biosensor for the measurement of glutamate in the serum of healthy and epileptic patients has been described [16]. The fabrication method consists of electrodepositing CHIT, gold nanoparticles (AuNPs) and multiwalled carbon nanotubes (MWCNTs) on the surface of a gold electrode.

The serum sample was diluted with phosphate buffer solution (PBS) before analysis. The concentration of glutamate in the sample was determined using a standard calibration curve constructed from the amperometric responses obtained with glutamate in PBS. The results compared favourably with a colorimetric test kit. A low operating potential of +0.135 V versus Ag/AgCl for measuring the enzymatically generated H_2O_2 was significantly lower than those measured by other biosensors based on GluOx [13,17]. The time taken to reach 95% of the maximum steady state response was 2 s after the initial injection. This method of fabrication, although complex, possesses a significantly lower operating potential than other biosensors fabricated using entrapment techniques.

1.4. Cross-linking

Enzyme immobilisation can be achieved by intermolecular cross-linking of the protein structure of the enzyme to other protein molecules or within an insoluble support matrix. Jamal et al. [17] described a complex entrapment method that involved drop-coating