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A bio-imprinted urease biosensor: Improved thermal and operational stabilities

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Abstract

Despite the increasing number of applications of biosensors in many fields, the construction of a steady biosensor remains still challenging. The high stability of molecularly bio-imprinted enzymes for its substrate can make them ideal alternatives as recognition elements for sensors. Urease (urea aminohydrolase, EC 3.5.1.5), which catalysis the hydrolysis of urea to ammonia and carbon dioxide, has been used in immobilized form in artificial kidney for blood detoxification. According to one report approximately half a million patients worldwide are being supported by haemodialysis.

In this study, the enzyme of urease was first complexed by using a substrate analogue, thiourea, in aqueous medium and then this enzyme was immobilized on gelatin by crosslinking with glutaraldehyde on a glass electrode surface. Similarly, urease noncomplexed with thiourea was also immobilized on a glass electrode in the same conditions. The aim of the study was to compare the two biosensors in terms of their repeatability, pH stability and thermal stability, and also, linear ranges of two biosensors were compared with each other.

Keywords: Bio-imprinted biosensors; Urease biosensor; Biosensor stability; Molecular imprinting technology

1. Introduction

In the past "Molecular Imprinting" was mainly confined to the separation of organic molecules and to nonenzymatic catalysis. With advancement in this technology, its potential and utility in field of biotechnology was also realized [1]. In order to improve it applicability, new variations in the conventional imprinting technique have emerged, such as "bioimprinting", metal chelation imprinting, affinity imprinting and a combination of immobilization and "bioimprinting" [2–8]. Several application areas have been envisaged for imprinted matrices the use of molecularly imprinted polymers in separation and isolation, the use of molecularly imprinted polymers as antibody and receptor mimics in immunoassay-type analyses, the use of molecularly imprinted polymers as enzyme mimics in catalytic applications, and the use of molecularly imprinted polymers in biosensor-like devices [9–13]. The memory of effect of enzymes caused by bio-imprinting without a further immobilization step was found to depend on the water content of medium and was completely lost when the reaction was carried out in the presence of a certain amount of water [14–18]. However, water is an indispensable milieu for most enzymatic reactions. Here we demonstrate that the combinatorial crosslinked imprinting approach. Keyes et al. employed a different kind of methodology to alter the catalytic properties of enzymes [19,20]. In their strategy, the enzyme complexed with ligands was crosslinked by using glutaraldehyde. This whole process was carried out in aqueous buffer, probably because the present imprinting concept in organic solvents was not known to that time.

In our contribution, we discussed potential application of the technique of molecular imprinting with emphasis on an enzyme with its competitive inhibitor and its using for the construction of a biosensor.

Below, schematic illustration of imprinting methodology is showed. The enzyme of urease was first complexed by using thiourea (Scheme 1).

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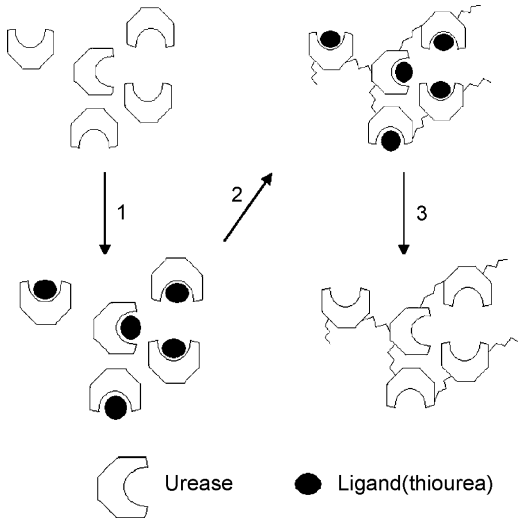

Scheme 1. Schematic diagram of the imprinted process (1, modification of the enzyme with thiourea; 2, immobilization and crosslinking with glutaraldehyde; 3, removal of thiourea from the active sites of the enzymes).

And then the enzyme–thiourea complex was immobilized with gelatin by the help of glutaraldehyde on a pH electrode tip. The influence of the imprinting of urease by its competitive inhibitor on the analytical performance such as pH and temperature and operational stabilities, of the resulting biosensor is discussed in this paper.

2. Experimental

2.1. Chemicals

Urease (E.C. 3.5.1.5, Type III: from Jack Beans 29,500 units/g solid), gelatin (Type 3, 225 Bloom), glutaraldehyde (Grade II, 25% aqueous solution) were purchased from Sigma (USA), Tris–HCl, urea, thiourea, were purchased from Merck (Germany).

2.2. Apparatus

pH electrode and pH meter were used for the potentiometric pH measurements (Hanna Instruments, Portugal). A water bath was used for preparation of bioactive material (Stuart scientific Linear Shaker bath SBS 35)(UK). All the measurements were carried out of constant temperature using a thermostat (Haake JF, Germany) Magnetic stirrer (IKA-Combimag, RCO) and pH meter with electrode (WTW pH 538, Germany) for preparing buffer solutions were used. The temperature was maintained at constant in the reaction cell by circulating water at appropriate temperature around the cell compartment during the experiment.

2.3. Procedure

2.3.1. Bio-imprinting of urease with thiourea

In the preparation of bio-imprinting biosensor, firstly, the enzyme urease (30 U/50 μL) and its competitive inhibitor thiourea (1.5 mg/50 μL) were incubated together for 1 h. If urease is added to a mixture of urea and thiourea the activity of the enzyme is strongly diminished. Like urea thiourea is bound to the active centre of the enzyme. Thus the active site of the enzyme is temporarily blocked by the 'false substrate'. The inhibition is called competitive because urea and thiourea compete on equal terms for the binding site of urease. Characteristically increasing the concentration of substrate reduces the effect of the inhibitor. Inhibition can be overcome. Consequently, in our system, by the help of thiourea, the active site of urease can be protected from the negative effects of the immobilization procedure during this process.

2.3.2. Preparation of the imprinted biosensor

Two milligrams per 50 μL of gelatin was added to urease–thiourea mixture mentioned above. This mixture was spread over the glass pH electrode. The electrode was allowed to wait for 30 min for drying. At the end of the preparation step, the bioactive layer of the biosensor was crosslinked by glutaraldehyde (2.5%). Finally, thiourea in the bioactive layer was removed for 1 h by waiting in the distilled water. The other biosensor was prepared in the same way. But there was no pretreatment with thiourea before the immobilization process. The enzyme and gelatin amount, and glutaraldehyde percentage were same as the bio-imprinted biosensor.

2.3.3. Measurement procedure

The bio-imprinted biosensor based on urease was put in to the thermostatic reaction cell containing working buffer (pH 6, 50 mM Tris–HCl buffer) and the magnetic stirrer was fixed at a constant speed. A few minutes later, the pH was equilibrated because of the diffusion between working buffer and pH probe. At this time, urea was injected into the thermostatic reaction cell. pH started to increase and a few minutes later it reached constant value due to the enzymatic reaction equilibration below:

$$\text{urea} + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

Measurements were carried out by the change of pH related to urea concentration added to the reaction cell. For all urea concentrations 10 min reaction period was chosen. The enzymatic reaction above was equilibrated within 10 min at all urea concentrations. So, response time was kept constant as 10 min.

3. Results and discussion

The bio-imprinted urease biosensor was compared with urease biosensor in their stability characteristics because the aim of this work was to improve the stabilities of urease biosensor by imprinting technique. For this purpose, for both biosensors optimum temperature and pH, thermal, pH and operational stability investigations were carried out. And all data were compared with each other for each parameter tested.

3.1. Optimum temperatures of the biosensors

The results obtained with two biosensors showed that the best activities were monitored at the same temperature, 30 °C. So,