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A novel enzyme biosensor for steroidal glycoalkaloids detection based on pH-sensitive field effect transistors

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Abstract

For the design of a biosensor sensitive to steroidal glycoalkaloids, pH-Sensitive Field Effect Transistors as transducers and immobilised butyrylcholinesterase as a biorecognition element have been used. The total potato glycoalcaloids can be measured by this biosensor in the concentration range 0.5–100 μM with detection limits of 0.5 μM for α-chaconine and of 2.0 μM for α-solanine and solanidine, respectively. The responses of the developed biosensors were reproducible with a relative standard deviation of about 1.5% and 5% for intra- and inter-sensor responses (both cases, $n = 10$, for an alkaloid concentration of 5 μM), respectively. Moreover, due to the reversibility of the enzyme inhibition, the same sensor chip with immobilised butyrylcholinesterase can be used several times (for at least 100 measurements) after a simple washing by a buffer solution and can be stored at 4 °C for at least 3 months without any significant loss of the enzymatic activity.

Keywords: Electrochemical biosensor; pH-SFET; ENFET; Enzyme inhibition; Steroidal glycoalkaloids

1. Introduction

Every year, consumption of potatoes containing higher than normal levels of steroidal glycoalkaloids (α-solanine, α-chaconine and solanidine) is associated with human deaths and poisonings and a lot of livestock deaths [1]. Such potatoes and individual alkaloids have been shown to be teratogenic and embryotoxic. High concentration of alkaloids may cause acute poisoning, including gastrointestinal and neurological disturbances, in man, with death being caused by central nervous system depression. Other studies have also suggested that there is an increased risk for cancers of the brain, breast, endometrium, lung and thyroid associated with the consumption of large quantities of potatoes [1].

These considerations are sufficient to demonstrate the strong necessity for glycoalkaloids control in the fields of agriculture, food analysis and health care. Routinely used methods for glycoalkaloids analysis involve calorimetric detection [2], high performance liquid chromatography [3] and, during the last time, ELISA methods [4]; however, each of these methods has disadvantages, such as high price of analysis and their time-consuming nature. Biosensors seem to be a very promising tool to overcome the problems described above. Up to now, there have been no commercially available biosensors for glycoalkaloids detection, nor has there been any research proposed. We therefore propose the development of enzyme sensor for potato steroidal glycoalkaloids detection.

2. Experimental

Butyrilcholinesterase (BuChE) (EC 3.1.18) from horse serum, 13 U/mg; bovine serum albumin; α-chaconine, α-solanine and solanidine from potato sprouts were purchased from Sigma. Glutaraldehyde (GA) was obtained from Serva. Other chemicals were of analytical grade.

pH-Sensitive Field Effect Transistors (pH-SFETs) were fabricated at the Research Institute of Microdevices (Kiev, Ukraine). The chips contain two identical Si_3N_4-pH-SFETs, the design and operation mode of which have

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been previously described [5]. Biologically active membranes on the transducer surface were formed by a method of protein cross-linking in saturated GA vapour [6]. Measurements were conducted in daylight at room temperature. The differential output signal between the measuring and reference sensitive elements was registered with the laboratory devices manufactured in-house, and the kinetic (dU/dt) maximum response of the biosensor was plotted. The evaluation of the enzyme activity inhibition by glycoalkaloids was carried out in a 10 mM K,Na-phosphate buffer, pH 7.5, and at an excess concentration of butyrylcholine chloride. The level of inhibition due to the action of a definite concentration of alkaloids was evaluated by comparison of the biosensor response levels with and without inhibitor.

3. Results and discussion

Recently [7], it was shown that potato glycoalkaloids inhibit reversibly the activity of butyrylcholinesterase. Since BuChE hydrolyses butyrylcholine according to the reaction:

$$\text{Butyrylcholine} + H_2O \xrightarrow{\text{BuChe}} \text{Choline} + \text{Butyrate} + H^+,$$

the products of this reaction can be detected by pH-SFET-transducers. The decrease in the electrochemical biosensor output signals caused by enzyme inhibition are proportional to the steroidal alkaloid concentration in the tested sample.

Measurements of butyrilcholinesterase inhibition were performed for various substrate concentrations and reproducible inhibition effects were obtained at BuChCl concentrations above 10 mM. From these results, a fixed substrate concentration of 10 mM was chosen for inhibitory analysis.

An example of the enzyme inhibition effect of glycoalkaloids concentration (Fig. 1) was investigated. As can be seen, α-chaconine, α-solanine and solanidine can be detected within the range of 0.5–100 μM depending on the type of steroidal alkaloid. The detection limits were estimated to be 0.5 μM for α-chaconine and 2.0 μM for α-solanine/solanidine. The dynamic ranges for the compounds examined show that such biosensors are suitable for a quantitative detection of glycoalkaloids in foodstuff samples as these analytical devices are sensitive in a broader range than the one needed. The total glycoalkaloid concentrations in potato tubers destined for human consumption can reach 20–200 mg/kg (about 25–250 μM) [8].

The responses of the glycoalkaloid sensitive sensors created are reproducible. The relative standard deviations are about 1%, and 5% for intra- and inter-sensor responses (both cases, $n = 10$, for an alkaloid concentration of 5 μM), respectively.

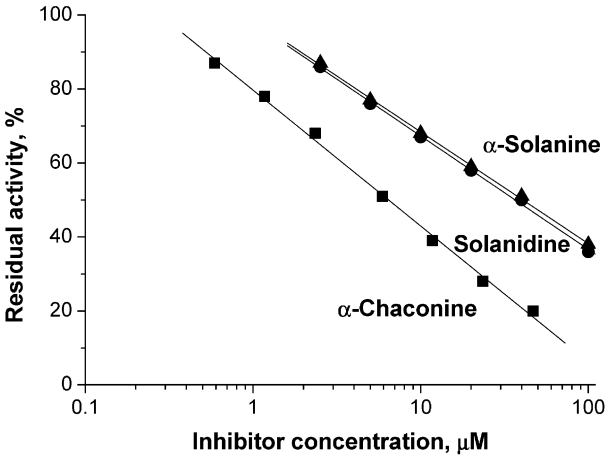

Fig. 1. Calibration curves for the detection of glycoalkaloids by pH-SFET-based biosensor. Measurement conditions: 10 mM K,Na-phosphate buffer, pH 7.5, room temperature.

Moreover, the same sensor with immobilised enzyme can be used repeatedly (for at least 100 measurements) after a simple washing by buffer and can be stored at 4 °C without substantial loss in activity of the biologically active material not less than 3 months.

4. Conclusions

An innovative process for the detection of steroidal glycoalkaloids based on the use of pH-sensitive field effect transistors coupled to butyryl cholinesterase have been developed. In comparison to the methods routinely used, the biosensor method proposed is simple, inexpensive, fast (the overall time for one analysis is less than 10 min) and reliable. The biosensor developed could find successful application fields, especially in agriculture, food quality control and health care.

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