Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Talanta 66 (2005) 28–33
Talanta
www.elsevier.com/locate/talanta

Analysis of the potato glycoalkaloids by using of enzyme biosensor based on pH-ISFETs☆

Alexey P. Soldatkin[a,*], Valentyna N. Arkhypova[a,b], Sergei V. Dzyadevych[a,b], Anna V. El'skaya[a], Jean-Michel Gravoueille[c], Nicole Jaffrezic-Renault[b], Claude Martelet[b,**]

[a] *Laboratory of Biomolecular Electronics, Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, 150 Zabolotnogo St., Kiev 03143, Ukraine*
[b] *Ecole Centrale de Lyon, CEGELY, UMR CNRS 5005, 36 Avenue Guy de Collongue, 69134 Ecully Cedex, France*
[c] *ARVALIS-Institut du Végétal, Station Expérimentale, Halle Technologique Pomme de Terre, 91720 Boigneville, France*

Received 26 January 2004; received in revised form 5 April 2004; accepted 10 November 2004
Available online 18 December 2004

Abstract

The applicability of an enzyme biosensor based on pH-ISFETs for direct determination of total glycoalkaloids content in real potato samples, without any pre-treatment, is shown. The results of determination of the total glycoalkaloids concentrations in potato samples from different experimental varieties obtained by the biosensor are well correlated with the analogous data obtained by the HPLC method with standard complex sample pre-treatment procedure. The detection of total glycoalkaloids content by biosensors is reproducible, the relative standard deviation was around 3%. The dependence of total glycoalkaloids content on various parts of the potato tuber and their size, different growing area has been shown using the biosensor developed.

The method based on biosensors is cheap, easy to operate and requires a shorter analysis time than the one needed using traditional methods for glycoalkaloids determination. The biosensor can operate directly on potato juice, or even directly on a suspension of potato or plant material. It can provide a way to save time and costs, with a possibility of taking rapid assessment of total glycoalkaloids content in a wide variety of potato cultivars. Furthermore the operational and storage stability of this biosensor are quite good with a drift lower than 1% per day and response being stable for more than 3 months.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Biosensor; ISFET; Butyryl cholinesterase; Glycoalkaloids; Potatoes

1. Introduction

One of the world's major agricultural crops, the cultivated potato (*Solanum tuberosum* L.) is consumed daily by millions of people from diverse cultural background. Potatoes are grown in the almost 80% of all countries, and worldwide production stands in excess of 350 million tonnes per annum, a figure exceeded only by wheat, maize and rice [1]. Despite its status as a food of first importance, the potato tuber contains toxic glycoalkaloids that cause sporadic out-breaks of poisoning in humans [2]. Glycoalkaloid poisoning elicits a wide variety of symptoms – ranging from gastrointestinal disorders, through confusion, hallucination and partial paralysis to convulsion, coma and death – but is thought to stem from one or both of two quite distinct modes of action. The first is inhibition of the enzyme acetylcholinesterase, which allows hydrolysis of the neurotransmitter acetylcholine, a key process in nerve impulse conduction across cholinergic synapses [3]. Neurological symptoms such as weakness, confusion and depression, which have been noted in patients suffer-

☆ Presented at the Third Bi-National France–Israel Workshop on Biosensors, Biochips and Nanobiotechnology, Beer-Sheva, Eilat (Israel), November 30–December 4, 2003.

* Corresponding author.
** Co-corresponding author. Tel.: +33 4 72186248; fax: +33 4 78331140.
E-mail addresses: a_soldatkin@yahoo.com (A.P. Soldatkin), claude.martelet@ec-lyon.fr (C. Martelet).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.11.006

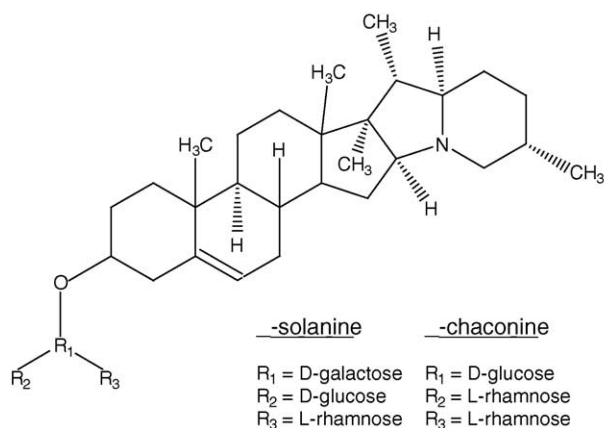

Fig. 1. Molecular structures of α-solanine and α-chaconine. Solanidine consists of a steroidal backbone without attached sugar moieties.

ing from glycoalkaloid poisoning, are likely manifestations of this anti-acetylcholinesterase activity [4]. The other major biological action of glycoalkaloids is their ability to disrupt sterol-containing membranes [5]. This action is thought to be responsible for damaging cells in the gastrointestinal tract and also in other tissues organs into which glycoalkaloids are transported (e.g. blood, liver) following absorption.

Glycoalkaloids were first identified in potatoes by Baup early in the 19th century [6], a wider information on their chemistry, biochemistry, distribution, physiology and toxicology became available, and reviewed later [7,8]. Main glycoalkaloids in potatoes are α-solanine and α-chaconine, both triglycosides of solanidine, a steroidal alkaloid derived from cholesterol. Their molecular structures are shown in Fig. 1. Solanidine is a steroidal backbone without attached sugar moieties.

In general, analysis of the potato glycoalkaloids is not a simple task. It needs three complex steps: (1) extraction of all the compounds of interest, (2) elimination, if necessary, of all interfering species, and finally (3) determination of the specific analyte concentration [8,9]. Further methods were proposed including more steps, such as derivatisation or hydrolysis.

Extraction solvents can be non-aqueous, acidic or combined. The literature describes over 20 different types of the solvents used for the glycoalkaloids extraction, for example, ethanol [10], 5% acetic acid [11], methanol–acetic acid–water (94:1:6) [12], methanol–chloroform (2:1) [13], etc.

Glycoalkaloids are commonly purified through one of the following paths: (1) precipitation with ammonium hydroxide [14]; (2) extraction with either aqueous Na_2SO_4 solutions [13] or water-saturated butanol [15]; (3) use of a C_{18} ion-pair chromatography cartridge [16]. A combination of these techniques can also be employed.

Current methodologies for the analysis of the potato glycoalkaloids and related compounds include: (1) colorimetry [17]; (2) thin layer chromatography [18]; (3) gas chromatography [19]; (4) high-performance liquid chromatography (HPLC) [12,15,16,27]; (5) immunoassays (ELISA) [20]. All these methods are complex. They need an expensive and bulky instrumentation with high power consumption and well-trained operators.

Recently biosensors based on pH-sensitive field effect transistor as a transducer and enzyme butyrylcholinesterase as a recognition element have been developed, characterised and optimised for sensitive detection of the glycoalkaloids in model solutions [21,22]. This paper describes the analysis of glycoalkaloids in real potatoes samples by using such an enzyme-based system.

2. Materials and methods

2.1. Materials

Butyrylcholinesterase (BuChE) (EC 3.1.1.8, from Horse Serum) with a specific activity of 13 U/mg solid; bovine albumin (fraction V, 98% purity), butyryl choline chloride (BuChCl) (98% purity), α-chaconine (95% purity), α-solanine (95% purity) from potato sprouts, and glutaraldehyde (grade II, 25% aqueous solution) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other reagents were of analytical grade and were used without any further treatment.

2.2. Sensor design and measurements

Ion sensitive field effect transistors (ISFETs) were fabricated at the Research Institute of Microdevices (Kiev, Ukraine). The potentiometric sensor chip contains two identical Si_3N_4-ISFETs, their design and operation mode have been previously described [23,24]. ISFETs were operated at a constant source current and drain-source voltage mode ($I_s = 200\ \mu A$, $V_{ds} = 1\ V$). Their pH-sensitivity was linear for pH values ranging from 2 to 12 with a slope of about 40 mV/pH.

All measurements were performed in daylight at room temperature in an open glass vessel filled with a vigorously stirred 5 mM phosphate buffer solution, pH 7.2. A 200 mM stock solution of BuChCl in deionised H_2O, and 2 mM stock solution of the glycoalkaloids in 5 mM acetic acid were prepared. The concentrations of substrates and inhibitors were adjusted by adding defined volumes of the stock solution of proper concentration. The differential output signal between the measuring and reference ISFETs was registered using laboratory ISFET-meter from Institute of Microtechnology (Neuchatel, Switzerland). After the estimation of the level of the enzyme inhibition, the initial enzyme activity was restored by washing out the biosensor enzymatic membrane in the working buffer solution for 5 min.

Download English Version:

https://daneshyari.com/en/article/10562906

Download Persian Version:

https://daneshyari.com/article/10562906

Daneshyari.com