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Development of an integrated electrochemical biosensor for sucrose and its implementation in a continuous flow system for the simultaneous monitoring of sucrose, fructose and glucose

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ABSTRACT

An integrated amperometric sucrose biosensor involving a 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM)-modified gold disk electrode (AuE) and coimmobilization of the enzymes invertase (INV) and fructose dehydrogenase (FDH) as well as the redox mediator tetrathiafulvalene (TTF) by means of a dialysis membrane is reported. Amperometry in stirred solutions at a detection potential of +0.10 V provided a linear calibration plot for sucrose over the 1.2×10^{-6}–3.0×10^{-3} mol L^{-1} concentration range, with a limit of detection of 3.6×10^{-7} mol L^{-1}. The practical usefulness of the biosensor was demonstrated by determining sucrose in condensed milk and in an infant food reference material with good results. Additionally, the biosensor was implemented together with commercial fructose and glucose amperometric biosensors in a continuous flow system to perform the multiplexed quantification of sucrose, fructose and glucose in a single experiment. The operational characteristics of the biosensors in this novel flow system were evaluated and their applicability was demonstrated through the simultaneous determination of the three sugars in the above-mentioned reference material.

1. Introduction

Sucrose is the organic compound commonly known as table sugar and sometimes called saccharose [1]. Since sucrose is a component of foodstuffs and beverages, precise information on the sucrose presence and content is very important for assessment of food quality [2]. The determination of sucrose can be carried out by a wide variety of analytical methods such as polarimetry, isotope dilution, chromatography and optical methods, which exhibit some practical disadvantages including expensive equipment and quite complex sample pretreatment [3]. Other available methods based on determination of density or refractory index, though simpler and faster, are less precise and sensitive to the presence of interfering components. Therefore, there is a demand to develop fast, inexpensive, selective and sensitive methodologies for sucrose determination.

In this context, enzyme-based electrochemical biosensors have been found to constitute versatile analytical devices with high selectivity and meeting the above-mentioned requirements for sucrose determination [4–7]. Most of the enzymatic cascade reactions reported for this purpose are based on the INV/mutarotase/glucose oxidase multienzyme system coupled with electrochemical detection of the produced H_2O_2 or consumed oxygen [8,9]. Alternatively, the INV/mutarotase/glucose dehydrogenase system has been also used with the spectrophotometric or electrochemical detection of generated NADH. Oxygen consumption was also shown to be suitable for the detection of sucrose based on a microbial cell-based biosensor [10].

The knowledge of the qualitative and quantitative distributions of sugars in fruits, vegetables, honey and other different natural foods is essential because these compounds are the major constituents of these products, and are involved in very important characteristics, such as flavor, maturity, quality, authenticity, storage conditions (sugars content diminishes rapidly during storage at ambient temperature), etc. Therefore, the determination of sugars is highly relevant in the food industry [11]. Simultaneous multidetection of sugars is being traditionally approached by means of chromatographic separation coupled to different detection techniques [11]. Among these, the refractive index-based measurement remains the most commonly used due to the lack of more selective physical/chemical properties of

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carbohydrates [12]. Attempts to increase the sensitivity have been successfully achieved by pulsed amperometry [13,14] and mass spectrometry [15]. Irrespective of the sensitivity of the detection mode, the efficient separation of closely related carbohydrates is compromised by their co-elution, thereby needing sample derivatization [16]. The selective detection of carbohydrates has been mainly achieved through catalytic reactions that produce new species that can be easily measured [17,18].

Attempts for the parallel determination of different sugars using coupled enzymes in specifically adapted flow-injection systems were in principle successful either using enzymatically catalyzed consumption of the monosaccharide prior to the determination of the respective disaccharide or by subtraction of the monosaccharide concentration from the sum of the total sugar signal [19].

In should be noted that most of the sucrose biosensors developed so far were not suitable for the simultaneous determination of different sugars. Due to the enzyme reactions involved in the functioning of these biosensors, samples containing both sucrose and glucose are difficult to be quantified selectively because glucose present in the sample will interfere in the determination of sucrose. Therefore, glucose has either to be removed from the sample solution or the corresponding glucose response has to be subtracted from the total response [6]. To eliminate these problems, a multienzyme electrode system using a time lag arising from reaction and diffusion has been developed [9].

In this work an integrated biosensor for sucrose determination based on the system INV/FDH has been developed for the first time. Both enzymes and the redox mediator TTF were co-immobilized by physical entrapment using a semipermeable dialysis membrane on a gold disk electrode modified with a MPA-SAM. The analytical performance of this biosensor has been evaluated under batch conditions. Additionally, a simple flow analysis system was successfully developed to perform the simultaneous determinations of sucrose, fructose and glucose in a single experiment by the appropriate integration of this sucrose biosensor with commercial fructose and glucose biosensors developed previously by our group. The performance of the new INV/FDH/TTF biosensor for the analysis of real samples was demonstrated by analyzing sucrose in condensed milk and in a reference material by batch amperometry. Moreover, the simultaneous determinations of sucrose, fructose and glucose in a reference material were accomplished by amperometric detection at the corresponding biosensors under continuous flow conditions.

2. Materials and methods

2.1. Apparatus and electrodes

Amperometric measurements were carried with single and bi-channel amperometric detectors purchased from InBea Biosensores S.L. (Madrid, Spain). A P-Selecta ultrasonic bath and a P-Selecta Agimatic magnetic stirrer were also used. Flow experiments were carried out using a Spetec Perimax-12 peristaltic pump.

XBAS-NS-AU gold disk electrodes (Ø~3 mm) were used as electrode substrates to be modified. A BAS MF-2052 Ag/AgCl/KCl (3 M) reference electrode and a Pt wire counter electrode were also employed. Fructose and glucose commercial biosensors developed previously by our research group and commercialized by InBea Biosensores S.L. were employed for the analysis of fructose and glucose, respectively. A 10 mL glass electrochemical cell was used for batch experiments, while a homemade methacrylate wall-jet cell (10 mL) was employed for flow injection measurements.

2.2. Reagents and solutions

Stock 0.1 mol L^{-1} D(+)-sucrose (Fluka) and D(+)-glucose (Panreac) solutions were prepared in 0.05 mol L^{-1} phosphate buffer of pH 6.0, while stock 0.1 mol L^{-1} D(−)-fructose (Sigma) solutions were prepared in 0.05 mol L^{-1} phosphate buffer of pH 4.5. More dilute standards were prepared by suitable dilution with the same phosphate buffer solution, which was also used as the supporting electrolyte. A 40 mmol L^{-1} MPA (Aldrich) solution, prepared in a 75/25% v/v EtOH/H_2O mixture, was employed for the SAMs formation. A 15 U μL^{-1} INV solution (Sigma, EC 3.2.1.26 from *Saccharomyces cerevisae*, 332.8 U mg^{-1}) prepared in phosphate buffer solution of pH 6.0 and a 5.15 U μL^{-1} FDH solution (MP Biomedical EC 1.1.99.11 from *Gluconobacter sp.*, 169 U mg^{-1}) prepared in phosphate buffer solution of pH 4.5 were used for the preparation of the INV–FDH–TTF–MPA–AuE biosensor. Moreover, a 0.5 mol L^{-1} TTF (Aldrich) solution in acetone was prepared. Dialysis membranes (10 K MWCO) were purchased from Cultek®. Muva-KI-1102 infant food reference material containing sucrose (4.01 ± 0.10)%, fructose (1.06 ± 0.03)%, glucose (2.44 ± 0.04)%, starch (24.78 ± 1.59)% and vitamin C (0.08291 ± 0.00935)% was purchased from Muva Kempten®.

Other solutions employed were: 2 mol L^{-1} KOH (Panreac) in water, 0.1 mol L^{-1} stock solutions of lactose, D-fructose, L-arabinose (Sigma), D-glucose (Panreac), D-galactose, lactulose (Fluka), citric acid (Merck), malic acid (Merck) and ascorbic acid (Fluka), prepared in 0.05 mol L^{-1} phosphate buffer of pH 6.0.

All chemicals used were of analytical-reagent grade and water was obtained from a Millipore Milli-Q purification system.

2.3. Procedures

2.3.1. Sucrose biosensor construction

Before the SAM deposition, the gold disk electrode was pretreated as described previously [20]. MPA-SAMs were formed by immersion of the treated AuE in a 40 mmol L^{-1} MPA solution in EtOH/H_2O (75/25, v/v) for at least 15 h. The monolayer modified electrode was rinsed with deionized water to remove physically adsorbed thiols and dried with a nitrogen stream.

Coimmobilization of the enzymes and the mediator was carried out as follows: a 3-μL aliquot of the 0.5 mol L^{-1} TTF solution was deposited on the MPA-modified electrode surface. Once the surface dried at room temperature, a 4-μL aliquot of the 5.15 U μL^{-1} FDH solution was deposited and allowed to dry again. Then, a 3-μL aliquot of the 15 U μL^{-1} INV solution was dropped on the modified electrode waiting to dry at room temperature. Finally, a 1.5 cm^2 piece of the dialysis membrane was fixed on top of the electrode surface and secured with an appropriate O-ring. The use of the dialysis membrane gave rise to a more stable coimmobilization of the enzymes and the mediator on the modified AuE than that obtained using other immobilization approaches such as crosslinking with glutaraldehyde. In this latter case, a significant loss of enzymes was observed after immersion of the biosensor in the solutions.

2.3.2. Electrochemical detection

Amperometry in stirred solutions with the sucrose biosensor was performed by applying a potential of +0.10 V (vs Ag/AgCl). Flow measurements were made using the system depicted in Scheme 1. The flow system was designed to allow the simultaneous detection of glucose, sucrose and fructose and consists of two channels, one for monitoring glucose and sucrose and a second channel for monitoring fructose. The corresponding flow cells were connected to peristaltic pumps and three-way connector valves were placed at the beginning of each flow channel