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Thermally stable improved first-generation glucose biosensors based on Nafion/glucose-oxidase modified heated electrodes

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ABSTRACT

We illustrate how the use of heated electrodes enhances the performance of glucose biosensors based on amperometric detection of the glucose-oxidase generated hydrogen peroxide. Nafion is shown to be an excellent matrix to protect glucose-oxidase from thermal inactivation during the heating pulses. The influence of the electrode temperature upon the amperometric response is examined. Temperature pulse amperometry (TPA) has been used to obtain convenient peak-shaped analytical signals. Surprisingly, up to 67.5 °C, the activity of Nafion-entrapped glucose-oxidase is greatly enhanced (24-fold) by accelerated kinetics rather than decreased by thermal inactivation. Amperometric signals even at elevated temperatures are stable upon prolonged operation involving repetitive measurements. The linear calibration range is significantly extended.

1. Introduction

The challenge of providing such reliable management of diabetes through the design of effective glucose biosensors remains the subject of considerable amount of research [1,2]. Amperometric enzyme electrodes, based on glucose-oxidase (GOx) bound to electrode transducers, play a leading role in this direction [2,3]. Both first and second generation amperometric devices, based on oxygen or artificial electron acceptors, respectively, have been widely used for the management of diabetes, with the former being more attractive for on-body continuous monitoring applications. Such monitoring offers a tighter management of diabetes by paying proper attention to the challenges of rejection of the sensor by the body, long-term stability of the enzyme and transducer, oxygen deficit, in vivo calibration, short stabilization times, and baseline drift [3].

The goal of this work was to combine the advantages of electrically-heated electrodes with first-generation glucose biosensors based on Nafion/GOx-modified electrodes. Temperature-dependent electrochemical processes have received a considerable recent attention in connection to hot-wire electrochemistry [4–6]. This technique involves the use of electrically-heated electrodes for enhanced electrochemical measurements through increased rates of mass transport and/or accelerated kinetics of redox processes. Recent efforts have illustrated the utility of electrically-heated electrodes for enhanced biosensing protocols ranging from the bioelectronic detection of DNA hybridization [7] to biocatalytic detection of metabolites [8,9]. Recently, an electrically heated copper oxide electrode was used for the detection of glucose and shikimic acid [10]. Lau et al. [11] applied indirectly heated electrodes for GOx-based glucose sensor for discriminating between glucose and ascorbic acid. Using indirectly heated electrodes modified with GOx in a polymer layer, it was possible to utilize the different thermal characteristics of glucose and ascorbic acid. They also observed a 1.8-fold enhancement of glucose response, upon using 40 °C electrode temperature. Later, Lau et al. reported on the use of heated electrodes for improving the specificity of PQQ-GDH glucose biosensors [12]. An anodic electrodeposition paint was used for immobilizing the enzyme.

In the present work we combined the advantages of electrically-heated electrodes with first-generation GOx-based glucose biosensors. We demonstrate that temperature pulse amperometry (TPA) [13,14] can be used to obtain highly stable peak-shaped analyti-

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cally-useful glucose signals that may be advantageous in the presence of a drifting background. The high-temperatures offer acceleration of the enzymatic and redox-transduction reactions. Furthermore, the entrapment of glucose-oxidase within a Nafion polymeric coating imparts good protection against thermal deactivation at the elevated temperatures.

2. Experimental

2.1. Materials

Analytical grade chemicals were used in all experiments. D(+)-glucose and potassium chloride (KCl) were purchased from Sigma. Glucose-Oxidase (GOx, EC 1.1.3.4) from *Aspergillus niger* and hydrogen peroxide from Sigma–Aldrich were properly stored in refrigerator before use. All the solutions were prepared with deionized water from Milli-Q system (>18 MΩ cm). Electrochemical measurements were carried out in a 0.1 M phosphate buffer saline, PBS (pH 7.0) solution. A mixture of 5 mM each, potassium ferrocyanide (Fisher Scientific) and potassium ferricyanide (Aldrich) were used for temperature calibration of the electrodes. Nafion® (5 wt.%) from Aldrich was utilized as a protective layer for the enzyme electrode. Glucose stock solutions were equilibrated for at least 24 h prior to use.

2.2. Instrumentation

Electrochemical measurements were performed using a μAutolab Electrochemical Analyser (Type II, Eco Chemie BV, Netherlands) with software GPES 4.9. Three-electrode cell setup was used for all the measurements. An in situ heated LTCC (low temperature co-fired ceramics) gold electrode (as described in [8,12]) was used as working electrode, Ag/AgCl (3 M KCl) and platinum wire were used as reference and counter electrode, respectively. An Agilent DC power supply (E 3645A, USA) served as the heating equipment to allow variations of the working-electrode temperature.

2.3. Temperature calibration

The stationary temperature of the heated bare LTCC gold electrode at the end of a 30 s. heat pulse was measured by using open circuit potentiometry in 0.1 M KCl solution containing 5 mM ferro-/ferricyanide. The temperature coefficient (-1.56 mV K^{-1}) was used to calculate the temperature difference. The calibration curve of temperature was obtained by regulating various heating currents and plotting the temperature difference (not shown).

2.4. Sensor fabrication

About 0.5 μL of a 25 mg mL^{-1} Glucose-Oxidase (GOx) solution in 0.1 M PBS was cast onto the gold electrode surface. The electrode was dried at room temperature (22 °C) for 10 min. Finally, about 2 μL of 1% Nafion (in 95% alcohol) was dropped onto the electrode surface and dried. The electrode was stored in the refrigerator when not in use.

3. Results

3.1. Effect of heating upon the oxidation of H_2O_2

Fig. 1 depicts cyclic voltammograms (CVs) for 1 mM H_2O_2 measured in phosphate buffer using heating (a) and non-heating (b) conditions at the (A) bare and (B) Nafion–GOx-modified LTCC gold electrodes. The applied heating current was 300 mA corresponding to 36.8 °C. In both cases, the oxidation current is significantly increased at the elevated temperature. No major difference between the bare and the Nafion–GOx-modified electrode is visible, except for the smaller signal associated with diffusion barrier through the Nafion coating. Hydrogen peroxide oxidation seems to be slightly hindered under both cold and hot conditions. Note also the small potential shift towards less positive values, indicating that the oxidation of hydrogen peroxide is slightly accelerated at the 'hot' conditions.

3.2. Temperature effect upon amperometric signals

Fig. 2 displays the effect of 30 s temperature pulses upon the amperometric signals of (A) 1 mM glucose and (B) 1 mM hydrogen peroxide at the glucose sensor. According to the CVs depicted in Fig. 1, we chose a potential of +0.6 V. The buffer solution temperature was kept constant at 21.5 °C during the entire experiment. The maximal electrode temperature during the pulses ranged between 29.4 and 67.5 °C. Temperature pulse amperometric signals were recorded as differences between the amperometric response before the beginning and at the end of each temperature pulse. The effect of electrode temperature was dramatic for 1 mM glucose as the signal increased from 41 to 967 nA (i.e., 24-fold) upon stepping the temperature between 29.4 and 67.5 °C. We checked, whether this enhancement can be attributed to an accelerated enzymatic activity of surface-confined glucose. For this we analyzed a 1 mM hydrogen peroxide sample (Fig. 2B). In this case the signal increased from 1.6 to 12.4 μA (only 8-fold) upon stepping the temperature between 29.4 and 67.5 °C. The substantially greater temperature effect upon the glucose response versus the peroxide signal (24-fold vs. 8-fold; A vs. B) clearly reflects that both

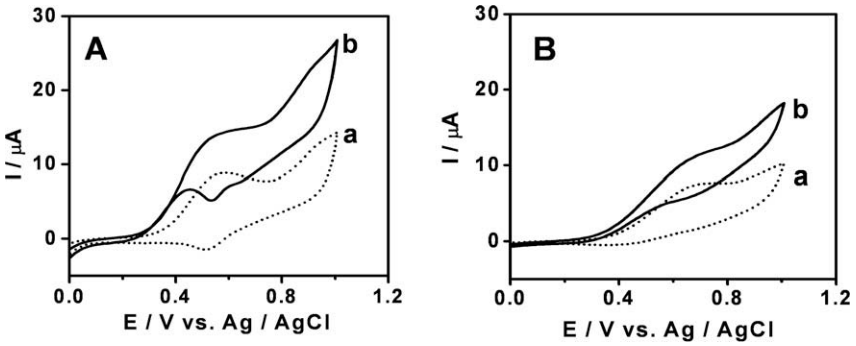

Fig. 1. Effect of heating upon the oxidation of 1 mM H_2O_2: (a) non-heating, (b) heating, 36.8 °C, at LTCC gold electrode (A) and at gold bioelectrode (B) in 0.1 M PBS (pH 7.0); scan rate, 50 mV s^{-1}.