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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.

Keywords

Glucose; Hot-wire electrochemistry; Temperature pulse amperometry (TPA); Nafion; Glucose oxidase

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

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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 analytically-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 MΩcm). 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 hours prior to use.

2.2. Instrumentation

Electrochemical measurements were performed using a μAutolab Electrochemical Analyser (Type II, Eco Chemie BV, Netherlands) with a 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 sec. 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 $0.5 \mu\text{L}$ 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 $2 \mu\text{L}$ 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

Figure 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

Figure 2 displays the effect of 30 sec 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 $+0.6 \text{ V}$. 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 to 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 $12.4 \mu\text{A}$ (only 8-fold) upon stepping the temperature between 29.4 to 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** the biocatalytic and the peroxide-oxidation reactions are enhanced by the temperature pulse. Furthermore, glucose oxidase seems to be unaffected by the relative high temperature, reflecting the protective role of its host Nafion layer. Lau et al. reported on a small (1.8-fold) enhancement when applying 40°C electrode temperature having glucose oxidase entrapped within an anodic electrodeposition paint [11]. Applying even higher temperatures did not yield further enhancement. Lau et al. were able to improve their glucose sensor using a glucose dehydrogenase coupled with Os-complexes as a surface-confined redox-mediator [12]. The improved design yielded an 8-fold signal enhancement upon increasing the temperature pulse from 28 to 48°C . Higher temperatures caused decreasing amperometric baselines of the amperometric signal which was attributed to loss of osmium mediator or

enzyme activity. Our experiments reveal that Nafion is an excellent matrix for protecting GOx from thermal inactivation during repetitive heating pulses.

3.3 Calibration of glucose concentration

Figure 3 depicts TPA signals obtained for various glucose concentrations ranging between 0 and 12 mM, in connection to 30 sec temperature pulses of 50 °C. The observed peak-shaped signals not only lead to increased sensitivity, but also to a more favorable peak-shaped response. Such signals are useful for monitoring glucose levels over prolonged operations, even in the presence of a drifting baseline. Also shown (inset) are calibration plots for glucose at different temperatures [30 (a), 40 (b) and 50 (c) °C]. These profiles illustrate that increasing the pulse temperature from 30 to 50 °C not only leads to higher sensitivity but also extends the linear range significantly from 3 to 12 mM. The increased enzyme activity at elevated electrode temperature allows oxidation of more glucose, leading to a higher hydrogen peroxide production and to increased oxidation currents. This way, higher glucose concentrations in the sample further increase the amperometric signals rather than causing saturation of the enzyme layer and a concentration independent signal.

3.4 Stability of the Amperometric Signals

Figure 4 demonstrates the high stability of the amperometric response recorded at a GOx-Nafion-modified gold electrode at +0.6 V, during 20 repetitive measurements of 1 mM glucose. The corresponding stability curves at different temperatures, shown in the inset, indicate a stable glucose response despite the elevated temperature and prolonged operation. Such high stability over 20 repetitive high-temperature pulses (51 °C) reflects the protective action of the Nafion immobilization matrix.

3.5 conclusions

We have shown that the use of elevated temperatures enhances the performance of first-generation glucose biosensors based on Nafion/GOx-modified electrodes. Confinement of GOx within the Nafion coating provided a protective action against thermal inactivation. Temperature pulses up to 67.5 °C led to a remarkable increase in the amperometric glucose signals. The linear calibration range is quadrupled. Numerous repetitive temperature pulses do not affect the enhanced enzymatic activity. The peak signals of the TPA operation offers a more favorable peak-shaped signal and should facilitate convenient measurements even in the presence of a drifting baseline.

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References

1. Ohara TJ, Rajagopalan R, Heller A. *Anal Chem* 1994;66:2451. [PubMed: 8092486]
2. Maidan R, Heller A. *Anal Chem* 1992;64:2889. [PubMed: 1463213]
3. Wang J. *Chem Rev* 2008;108:814. [PubMed: 18154363]
4. Wildgoose GG, Giovanelli D, Lawrence NS, Compton RG. *Electroanalysis* 2004;16:421.
5. Gründler P, Flechsig GU. *Microchim Acta* 2006;154:175.
6. Gründler P. *Curr Anal Chem* 2008;4:263.
7. Flechsig GU, Peter J, Hartwich G, Gründler J, Wangand P. *Langmuir* 2005;21:7848. [PubMed: 16089391]
8. Lau C, Flechsig GU, Gründler P, Wang J. *Anal Chim Acta* 2005;554:74.

9. Duwensee H, Vázquez-Alvarez T, Flechsig GU, Wang J. *Talanta* 2008;77:1757. [PubMed: 19159794]
10. Wei H, Sun JJ, Guo L, Li X, Chen GN. *Chem Commun* 2009:2842.
11. Lau C, Reiter S, Schuhmann W, Gründler P. *Anal Bioanal Chem* 2004;379:255. [PubMed: 15042273]
12. Lau C, Borgmann S, Maciejewska M, Ngounou B, Gründler P, Schuhmann W. *Biosens Bioelectron* 2007;22:3014. [PubMed: 17291745]
13. Wang J, Jasinski M, Flechsig GU, Gründler P, Tian B. *Talanta* 2000;50:1205. [PubMed: 18967816]
14. Wachholz F, Biala K, Piekarz M, Flechsig GU. *Electrochem Commun* 2007;9:2346.

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}

Fig. 2.

Amperometric response recorded at gold bioelectrode for various applied temperature pulses: (A) 29.4 °C, (B) 36.8 °C, (C) 45.8 °C, (D) 56.0 °C, (E) 67.5 °C in 0.1 M PBS (pH= 7.0), (A) [Glucose] = 1 mM and (B) [H_2O_2] = 1 mM. Inset shows the effect of applied heating current for (a) blank and (b) 1 mM glucose/1 mM H_2O_2 . Working potential, +0.6 V.

Fig. 3. Amperometric response recorded at +0.6 V using the heated glucose sensor (at 50 °C) for various glucose concentrations: (A) blank, (B) 3 mM (C) 6 mM (D) 9 mM and (E) 12 mM in 0.1 M PBS (pH= 7.0). Inset shows the calibration curves at (a) 30 °C, (b) 40 °C, and (c) 50 °C.

Fig. 4. Stability of the amperometric response for 1 mM glucose recorded at heated GOx-Nafion-gold electrode (37 °C) in 0.1 M PBS (pH= 7.0). Inset shows 20 repetitive measurements at two different temperatures.