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An interference-free glucose biosensor based on a novel low potential redox polymer mediator

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ABSTRACT

A highly sensitive and interference-free glucose biosensor based on a novel ruthenium complex-tethered redox polymer (Ru-RP) mediated enzymatic oxidation of glucose at −0.15 V (vs. Ag/AgCl) is described in this report. Through the co-immobilization of the Ru-RP and glucose oxidase (GOx) on a glassy carbon electrode (GCE) via a simple one-step chemical crosslinking process with glutaraldehyde, the crosslinked membrane displays excellent catalytic activity toward the oxidation of glucose with a current sensitivity of 24.3 $\mu A\,mM^{-1}\,cm^{-2}$ and a linear correlation between the oxidation current and glucose concentration up to 10 mM. More importantly, owing to the low operating potential of −0.15 V (vs. Ag/AgCl), potential interferences from naturally occurring interfering species in blood such as ascorbic acid, dopamine, uric acid, and common drug acetaminophen are effectively alleviated. Furthermore, the hydrophilic nature of the crosslinked membrane also effectively retards the diffusion of molecular oxygen. This glucose biosensor could be an attractive candidate in the development of miniaturized glucose biosensors.

1. Introduction

Diabetes mellitus, a worldwide public health problem, has claimed many lives and caused disability to many others in the world. As a result of insulin deficiency and hyperglycemia, patients suffering from diabetes mellitus experience blood glucose concentrations higher than the normal range of 80–120 $mg\,dL^{-1}$ (4.4–6.6 mM) and risk serious complications from heart disease, kidney failure, or blindness. The World Health Organization has estimated that the number of people suffering from diabetes mellitus will reach more than 300 million by the year 2025. Through a tight monitoring of blood glucose levels, diabetes mellitus can be effectively managed.

Chemically modified electrodes have been employed for the determination of various organic molecules [1–3]. Since Clark and Lyons pioneered the development of oxygen electrode-based enzyme electrodes in 1962 [4], enormous efforts have been devoted over the last 50 years to develop cost effective glucose biosensors with good sensitivity and stability [5–8]. Up to date, amperometric [9,10], potentiometric [11,12], coulometric [8], and impedimetric [13,14] glucose biosensors have been developed. Amongst them, the amperometric glucose biosensors are poised to play a leading role in blood glucose monitoring owing to its simplicity and easy-to-use methodology. Two generations of amperometric glucose biosensors have been developed. The first generation glucose biosensors rely on the biocatalytic reaction involving the reduction of the flavin group (flavin adenine dinucleotide, FAD) of glucose oxidase (GOx) by glucose. The reduced form of the flavin group ($FADH_2$) then reacts with molecular oxygen present in blood to regenerate the oxidized form of GOx (FAD). And subsequent measurements of hydrogen peroxide provide an indirect means for the quantification of blood glucose concentration. Two major drawbacks have limited their widespread use as the glucose biosensors of choice. The first limitation, known as the "oxygen deficit", stems from the fact that normal oxygen concentration in blood is about one order of magnitude lower than that of glucose. In order to alleviate this problem, common strategies include the use of a two-dimensional electrode which allows oxygen diffusion into the electrode from two directions while limiting glucose diffusion to only one direction [15] and by designing a high solubility oxygen-rich carbon paste electrode which serves as an internal source of oxygen for the electrode [16]. However, there exists a second and bigger challenge as a relatively high potential of +0.6 V (vs. Ag/AgCl) has to be engaged in the hydrogen peroxide measurements and such a high applied potential leads to interferences from easily oxidizable species such as ascorbic acid (AA), dopamine (DA), and uric acid (UA) as well as common drugs such as acetaminophen (AMP) in blood [17,18]. The current contribution from these undesirable interferants severely compromises the selectivity and accuracy of glucose monitoring.

Against this backdrop, second generation glucose biosensors are developed to mitigate the interferences. For example, by employing

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a synthetic electron acceptor (redox polymer mediator), the FAD center of GOx is electrically "wired" to the electrode surface [19]. Electrons are rapidly shuttled through a nondiffusional route between GOx and the electrode surface and subsequent reoxidation of the mediator at the electrode surface will generate a current signal which is proportional to the glucose concentration. Unfortunately, molecular oxygen can still lead to the natural enzymatic oxidation of glucose, a reaction which is now considered as a side reaction. Furthermore, although the measurements now take place at a relatively low applied potential, it can only slightly minimize the interferences from the commonly encountered naturally occurring interferants in blood.

In order to overcome this intricate problem of interferences, a number of strategies evolving around the regulation of solution species to the glucose biosensor have been proposed. For instance, permselective coating with transport properties based on charge, size, or polarity exclusion principle has been used [5,20–25]. Wilson and co-workers showed that alternate deposition of Nafion and cellulose acetate could eliminate interferences from AA, UA, and AMP, although the detection sensitivity was compromised as a result of the Nafion membrane which also impeded glucose diffusion [26]. In 2000, Heller's group demonstrated that micromembrane can be utilized to eliminate interferences from AA, UA, and AMP [27]. Another strategy to eliminate interferences from the mediated oxidation of glucose is to operate the glucose biosensor at a low enough applied potential so as not to provoke interfering reactions from the interferants [28]. Using this strategy, Nam and co-workers showed that when $[Ru(NH_3)_6]^{3+}$ was used as the mediator, it allowed the use of a relatively low applied potential of 0.0 V (vs. Ag/AgCl) and could effectively eliminate interferences from AA, DA, UA, and AMP [29]. Following this argument, if one could design a mediator-based glucose biosensor which operates at even lower applied potential, the contribution to the oxidation current from undesirable interferants can be further reduced or may even be completely eliminated. In this report, the feasibility of developing an interference-free amperometric glucose biosensor which can mediate enzymatic oxidation of glucose at a considerably low potential was investigated. A novel water-soluble and crosslinkable ruthenium complex-tethered redox polymer (Ru-RP) was first successfully synthesized. Excellent electron mediating power between GOx and substrate electrode at −0.15 V (vs. Ag/AgCl) was observed when the Ru-RP was crosslinked with GOx on a glassy carbon electrode (GCE). In addition to its high sensitivity and fast response time, the biosensor was practically immune to all potential interferants commonly encountered in glucose monitoring. This superior interference-free property was achieved through the use of an ultralow applied potential which would not provoke interferences from other easily oxidizable species. Moreover, the excellent mediating power of the Ru-RP and the hydrophilic nature of the membrane also effectively retarded the interference from molecular oxygen.

2. Experimental

2.1. Reagents

GOx (EC 1.1.3.4, from *Aspergilus niger*, 191 units mg^{-1}) was purchased from Fluka (CH-9407 Buchs, Switzerland). Hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$) was from Strem Chemicals (Newburyport, MA, USA). All other chemicals of certified analytical grade were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Glucose stock solutions were allowed to mutarotate for 24 h before use. Freshly prepared AA, DA, UA, and AMP solutions were used in the interference study. All electrochemical studies were carried out in pH 7.4 phosphate buffered saline (PBS) (20 mM phosphate + 0.15 M NaCl). All aqueous solutions were prepared with ultrapure deionized water (18.3 MΩ).

2.2. Apparatus

Electrochemical measurements were performed with a CHI650D electrochemical workstation (CH Instruments, Austin, TX, USA). A conventional three-electrode system comprising a GCE (3 mm in diameter) working electrode, an Ag/AgCl reference electrode, and a platinum wire counter electrode, was employed in all electrochemical experiments. All potentials reported in this work were referred to the Ag/AgCl reference electrode. Electrochemical impedance experiments were conducted with an IM6 electrochemical workstation (Zahner Elektrik, Germany). UV–vis spectra were recorded on an Agilent Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). Molecular weight was determined through high performance gel permeation chromatography (GPC) in water and standard poly (ethylene oxide) was used for calibration.

2.3. Preparation of the redox polymer

The copolymer backbone [poly (vinylpyridine-co-acrylamide), PVPAA] was synthesized according to a published procedure [30]. The Ru-RP was prepared according to the following procedure: 50 mg of PVPAA and 50 mg of $Ru(NH_3)_6Cl_3$ were dissolved in 4 mL of mixture solvent (ethylene glycol:water 1:1, v/v). The mixture was refluxed for 3 h at 80–90 °C. Upon cooling, the reaction mixture was centrifuged. To a rapidly stirred acetone, the supernatant was added drop-wise to precipitate the redox polymer. Further purification was carried out by treating the precipitated redox polymer with multiple water-dissolving acetone-precipitating cycles. The purified product was then dried under vacuum at 50 °C. Typical yields were between 55 and 90%.

2.4. Preparation of the biosensor

A GCE was polished on a microcloth with 0.3 and 0.05 μm alumina slurry sequentially and sonicated for 5 min in water and ethanol between each polishing step. Upon drying, 5 μL of a mixed solution containing 3 μL Ru-RP, 1 μL GOx, and 1 μL glutaraldehyde (GA) was applied onto the cleaned electrode. The electrode was kept under ambient conditions for at least 12 h. During this period of time, an irreversible crosslinking reaction took place and formed the desired glucose sensing membrane on the GCE. The glucose biosensor was stored at 4 °C when not in use.

3. Results and discussion

3.1. Synthesis and characterization of the Ru-RP

As depicted in Fig. 1A, vinylpyridine in the copolymer affords the availability of the pyridine ring which allows subsequent N-coordination with the redox active ruthenium complex; while acrylamide offers good chemical and mechanical stability and resistance to microbial degradation and serves as a support matrix [31]. The amide group present in acrylamide also allows chemical crosslinking with GA to enhance the stability of the enzyme in the glucose sensing membrane. Fig. 1B shows the synthesis route for the Ru-RP. Results from GPC showed a monomodal elution peak for the Ru-RP. This provided the first hint of a successful synthesis and that Ru-RP was a copolymer rather than blends of two homopolymers. The weight-average (M_w) molecular weight of the redox polymer was found to be 53,200 and the polydispersity index (PDI) was 1.56. Ruthenium loading in the Ru-RP, determined from elemental analysis, was found to be ~14%. To further confirm the