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Oxygen reduction on redox mediators may affect glucose biosensors based on "wired" enzymes

Antonin Prévoteau, Nicolas Mano*

Université de Bordeaux, Centre de Recherche Paul Pascal CRPP-UPR 8641, Univ. Bordeaux, F-33600, Pessac, France

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ABSTRACT

In glucose oxidase (GOx) based biosensors, O_2 has been reported to compete with the artificial redox mediator for GOx electrons, decreasing the current density of the devices. Here, we investigate the effect of O_2 on the redox mediator itself. To do so, a range of osmium-based redox polymers of different redox potentials has been investigated in non-physiological relevant conditions, *i.e.* under forced convection and under 1 atm O_2, to maximize the effect of the O_2. We show that molecular O_2 could be reduced on osmium complexes producing H_2O_2, a fraction of which could be further reduced to H_2O on the same Os complexes. This reduction occurs on polymers with apparent redox potential $E^{\circ\prime} \leq +0.07$ V *vs.* Ag/AgCl and the kinetic increases exponentially when $E^{\circ\prime}$ decrease. In addition to the consequent loss of sensitivity and selectivity for a biosensor, or of power density and faradaic efficiency in the case of a biofuel cell, the H_2O_2 produced during the reduction of O_2 on the Os complex may be deleterious for the enzyme. Our results suggest that the effect of O_2 on the mediator itself may also be a parameter to be taken into account for the design of efficient redox mediators.

1. Introduction

There is a particular need for the treatment of diabetes. According to the World Health Organization, in 2011, around 350 million people worldwide (3 million in France) have diabetes. This number is likely to more than double by 2030. In 2010, an estimated 4 million people died from diabetes [1]. In 2010, the healthcare expenditures on diabetes has accounted for ~11.6% of the total global healthcare expenditures in the world [2]. These are the reasons why so many papers are published every year on this subject. Coulometric and amperomeric glucose biosensors are the most widespread sensors for blood glucose monitoring. Current research particularly focus on two groups of electrochemical glucose biosensors: a first group that employs mediated electron transfer (MET), where electrons are shuttled from the enzyme to the electrode *via* a third chemical species, called a redox mediator, such as Os and Ru complexes, or ferrocenes [3,4] and a second group based on mediator-free direct electron transfer (DET), where enzymes are able to communicate directly with the electrode [5].

Two families of enzyme remain widely used for the elaboration of glucose sensors, each of them with their advantages and their disadvantages. Soluble quinoprotein–glucose dehydrogenase (PQQ–GDH) is one of the major enzymes used in biosensors systems for self-monitoring of blood glucose because this enzyme, unlike glucose oxidase (GOx), is independent of O_2. On the other side, GOx is more stable and selective for glucose over other blood sugars [3]. Recently, a third family of enzyme, FAD–glucose dehydrogenase, has also attracted some attention [6,7].

Numerous parameters may affect the performance of glucose sensors including but not restricted to: electrochemical interferences, patient misuse, hematocrit, O_2 concentration in blood, *etc.* High O_2 values, found in arterial samples or in patient treated by O_2, or low values of O_2, found in venous sample or in patient with chronic obstructive pulmonary disease, may lead to erroneous measurements of glucose concentration [8–11].

GOx catalyzes the 2 electron-glucose oxidation according to a Ping–Pong mechanism *via* its redox prosthetic group FAD/$FADH_2$ [12]. Oxidation of glucose and reduction of O_2 occurs in two sequential reductive (Eq. (1)) and oxidative half reactions (Eq. (2)).

$$\text{GOx-FAD} + \text{glucose} \rightarrow \text{GOx-FADH}_2 + \text{gluconolactone} \quad (1)$$

$$\text{GOx-FADH}_2 + \text{O}_2 \rightarrow \text{GOx-FAD} + \text{H}_2\text{O}_2 \quad (2)$$

O_2, the natural substrate for the oxidative half-reaction is reduced to H_2O_2. For this reason, it was reported earlier that O_2 (Eq. (2)) could compete with the artificial redox mediator (M) for GOx electrons (Eq. (3)), explaining the decrease of the current attributed to glucose oxidation current with the O_2 concentration [13].

$$\text{GOx-FADH}_2 + (2/x)\,\text{M}_{(\text{OX})} \rightarrow \text{GOx-FAD} + (2/x)\,\text{M}_{(\text{RED})} + 2\,\text{H}^+ \quad (3)$$

* Corresponding author. Tel.: +33 5 56 84 30 22l; fax: +33 5 56 84 56 00.
E-mail address: mano@crpp-bordeaux.cnrs.fr (N. Mano).

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Table 1
Osmium-based redox polymers and their determined apparent standard potentials.

No.	Formula	$E^{\circ\prime}$ *vs.* (Ag/AgCl)/V	Synthesis
I	PVP-[Os(dm-bim)$_3$]$^{2+/3+}$	−0.170	[44]
II	PVI-[Os(da-bpy)$_2$Cl]$^{+/2+}$	−0.155	[45]
III	PVP-[Os(dm-bim)$_2$(mpd-bim)]$^{2+/3+}$	−0.028	[a]
IV	PVI-[Os(dmo-bpy)$_2$Cl]$^{+/2+}$	+0.005	[46]
V	PVP-[Os(dm-bim)$_2$]$^{2+/3+}$	+0.070	Suppl. info.
VI	PVP-[Os(mpd-im)$_3$]$^{+/2+}$	+0.254	[a]
VII	PAA-PVI-[Os(tpy)(dm-bim)]$^{2+/3+}$	+0.320	Suppl. info.
VIII	PAA-PVI-[Os(dcl-bpy)$_2$Cl]$^{+/2+}$	+0.360	[45]

Polymer backbone: PVP, polyvinylpyridine; PVI, polyvinylimidazole; PAA, polyacrylamide.
Ligands: dm, dimethyl; bim, biimidazole; da, diamino; bpy, bipyridine; dmo, dimethoxy; m, methyl; mpd, méthylpyridyl; im, imidazole; tpy, terpyridine; dcl, dichloro.
[a] Abbott Diabetes Care.

where $x = 1$ or 2 is the number of electrons collected from GOx-$FADH_2$ by one oxidized form of the mediator, $M_{(OX)}$.

However, the effect of O_2 on the mediator itself has not been systematically investigated even though it has been reported by Tsujimura et al. that some redox mediators, such as hexaammineruthenium, thionine or a variety of quinones, could be oxidized by O_2 [14]. Salimi et al. recently showed that an electrode modified with carbon nanotubes and Os complex with a formal potential of −0.07 V *vs.* Ag/AgCl could reduce O_2 [15]. Abe et al. also reported the reduction of O_2 on Os complex immobilized in Nafion with a standard redox potential $E^{\circ\prime}$ of −0.1 V *vs.* Ag/AgCl [16]. Finally, Zakeerudin and co-workers reported that osmium complexes with formal potentials more negative than +0.05 V *vs.* Ag/AgCl could reduce dissolved O_2 and compromise the performance of a sensor [17]. All these studies tend to point out that O_2 may also react with the redox mediator (M) and not only with GOx (Eq. (4)):

$$O_2 + (2/x)\,M_{(RED)} + 2\,H^+ \rightarrow H_2O_2 + (2/x)\,M_{(OX)} \quad (4)$$

Despite these observations, little has been said about this effect and the interference of O_2 has always been attributed to the competition between O_2 and the redox mediator for the electrons of GOx (which is true *only* if the redox potential of the mediator is finely tuned, *vide infra*) [18–24].

We have decided in a series of two papers to explore reaction 4 and its consequences using a range of osmium-based redox polymers of different redox potentials as a proof of concept; even thought osmium complexes are now widely used in commercial implanted and non-implanted glucose monitoring system [3,25,26]. The ingenuity of these complexes relies on their design which can be tailored to choose their redox potential that make them selective to any endogenous species.

All experiments were performed in non-physiological relevant conditions, *i.e.* under forced convection and under 1 atm O_2, to maximize the effect of the O_2. In this first paper, we study the effect of O_2 on the Os-based redox mediators while in the second paper we will study the effect of O_2 on the whole biosensors; *i.e.* in presence of co-immobilized GOx or PQQ-GDH.

2. Experimental

2.1. Chemicals

Pyrroloquinoline quinone glucose dehydrogenase (PQQ-GDH) from *Acinetobacter calcoaceticus* was expressed in *Escherichia coli* (Invitrogen) and produced as described earlier [27]. Osmium-based redox polymers are listed in Table 1. All solutions were made with deionized water passed through an AQ 10 Milli-Q purification system from Millipore (Molsheim, France). Ultra-pure O_2 and argon were purchased from Air Liquide (Paris, France). Polyethylene glycol (400) diglycidyl ether (PEGDGE) was purchased from Polysciences Inc. (Warrington, PA) All other chemicals were from Fluka and used as received without further purification.

2.2. Electrode preparation

5 mm, 3 mm diameter glassy carbon (GC) electrodes (Pine Instrument, Raleigh, NC) or rotating Pt ring-GC disk electrode (RRDE, ref. E7R9G, theoretical collection efficiency of 37%) with 5.61 mm diameter GC disk and platinum ring (6.25 mm inner diameter and 7.92 mm outer diameter) were used as working electrodes. They were polished with 0.05 μm alumina slurry (Buehler, MicroPolish II), sonicated for 5 min and rinsed with ultrapure water. The electrode surfaces were made hydrophilic by exposure to low pressure O_2 plasma for 10 min. Electrodes in absence of enzyme were prepared at constant dry loading of 63.8 μg cm^{-2} with 10 wt% PEGDGE. In presence of PQQ-GDH enzyme, the hydrogel consisted of 60 wt% redox polymer, 30 wt% PQQ-GDH and 10 wt% PEGDGE for a total loading of 100 μg cm^{-2}. The electrodes were cured for at least 18 h at constant room temperature before they were used.

2.3. Electrochemical measurements

The measurements were performed using a bipotentiostat (CH Instruments, model CHI 842B, Austin, TX, USA) with a dedicated computer. A platinum spiral wire was used as counter electrode and all potentials were referred to a Ag/AgCl (3 M NaCl) electrode (BAS, West Lafayette, IN). All electrochemical measurements were performed in a water-jacketed electrochemical cell in 100 mM sodium phosphate buffer (PB) at pH 7.2. The electrodes were rotated using a Pine Instruments Rotator and the temperature was controlled by an isothermal circulator (Lab Companion, FR). Determination of the experimental RRDE collection efficiency was performed in a 0.2 mM ferricyanide solution under Ar, at 37 °C and 500 rpm. The disk potential was scanned from +0.4 V to −0.3 V to reduce ferricyanide and the corresponding ferrocyanide oxidation current was measured on the Pt ring at +0.6 V. The collection efficiency was 37.2 ± 0.4%. Electrochemical measurements of modified electrodes were only performed after 3 scans under Ar-saturated buffer until a good response in stability was reached. The apparent standard potentials ($E^{\circ\prime}$) of the redox polymers were estimated (±5 mV) by averaging the positions of the reduction and the oxidation peak potentials. The active Os complex surface concentrations were determined by integrating the oxidation and reduction peaks obtained by cyclic voltammetry at 1 mV s^{-1} under Ar. Constant potential chronoamperometry was performed to evaluate the effect of O_2 on electrodes modified with PQQ-GDH. The constant potentials were choose to reach the positive plateau current values whatever the glucose concentration, *i.e.* +0.1 V when using polymer I and +0.55 V for polymer VIII. O_2 and Ar were successively bubbled in the electrochemical cell. The solutions were previously deoxygenated before the beginning of the measurement (t = 0). Because PQQ-GDH is not stable at high temperature, all calibrations curves were performed at 25 °C to minimize the enzyme deactivation during the experiment time scale.

3. Results and discussion

3.1. Evidence of the O_2 catalytic reduction on an Os-based redox polymer

Fig. 1(A) shows the cyclic voltammogram (CV) of a bare GC electrode under Ar (thin line) and O_2 (thick line) at 1 mV s^{-1} in a PB buffer. While only the background current is observed under Ar,