The role of surface availability in membrane-induced selectivity for amperometric enzyme-based biosensors

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ABSTRACT

Amperometric enzyme-based biosensors, increasingly used for in vivo brain biomonitoring, typically suffer from electrochemical interference. At the potential (≥ 500 mV) necessary to oxidize the target analyte, often hydrogen peroxide (H_2O_2), non-specific electroactive species are easily oxidizable, resulting in poor biosensor selectivity. The use of permselective membranes, alone or in combination, is an efficient method to improve biosensor selectivity. These membranes are thin-film polymers (nm to μm thick), able to reduce electrochemical interference. However, the exact mechanism by which these membranes reduce interference is not entirely understood. As membrane assembly is a surface dependent process, we explored the putative role of surface availability in membrane-induced selectivity. We modified the surface of microelectrodes with the most effective permselective membrane configurations available. Microelectrodes surface was characterized by electrochemical methods and scanning electron microscopy. All membranes reduced non-specific oxidation for all non-specific electroactive species. However, only PmPD (poly-m-phenylenediamine) (alone and combined with Nafion) and OPPy (overoxidized polypyrrole) were selective against both cations and anions. Besides reducing electrochemical interference, all membranes also reduced the sensitivity for the target analyte, H_2O_2. The use of membrane combinations resulted in an additional decrease in non-specific oxidation without an increase in selectivity. This additional decrease was highly correlated with a loss in H_2O_2 sensitivity, suggesting a reduction of active electrode surface. Additionally, microscopic evaluation indicated an intriguing "inner polymerization" process in microelectrodes coated with membrane combinations. Our results point to a significant role of surface availability in the mechanisms underlying membrane-induced selectivity, crucial for the design and performance of enzyme-based amperometric biosensors.

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1. Introduction

Amperometric enzyme-based biosensors are powerful bioanalytical tools increasingly employed in several fields, ranging from food technology and environmental biomonitoring to biomedical applications [1–3]. Within the biomedical field, these biosensors are used for biomonitoring in a wide variety of physiological matrices [4]. In recent years, they have been successfully employed in in vivo monitoring of neurotransmitters in the living brain [5–12]. However, as stated by John Lowry in the 1990s measuring analytes in the living brain with biosensors remains a supreme technical challenge.

Amperometric enzyme-based biosensors typically convert the target analyte into an electroactive product, often H_2O_2, by an enzymatic reaction [13,14], and can be classified into 1st, 2nd or 3rd generation (Castillo [1]). First generation biosensors rely on direct electron transfer at the electrode surface. Changes in current at the electrode surface are related to changes in concentration of the target analyte. Unfortunately, at high applied potentials, necessary to oxidize H_2O_2 (≥ 500 mV), these biosensors are prone to suffer from electrochemical interference from the oxidation of non-specific electroactive species present within physiological matrices. Electrochemical interference results in low biosensor selectivity [15]. In the case of experimental neuroscience the major interferants are dopamine (DA), ascorbic acid (AA), uric acid (UA) and 3,4-dihydroxyphenylacetic acid (DOPAC) [16]. While AA exhibits the highest absolute oxidation currents, DA is the most difficult to eliminate and has the higher oxidation current per μM [5,16–18].

An elegant method to reduce interference and increase selectivity in 1st generation biosensors is the application of permselective membranes. These membranes are able to effectively reduce the

current generated by oxidation/reduction of the non-specific electroactive species.

Permselective membranes are typically polymeric thin films (nm to μM thick) assembled by self-assembly (as self-assembled monolayers, SAM) and/or by electropolymerization [5,11,15,16,19–22]. These membranes are thought to reduce interference by either size exclusion and/or by charge exclusion. However, the exact mechanism of action is not entirely understood [5,16]. The most effective and commonly used permselective membranes in in vivo biomonitoring are Nafion [5,20,23], poly(phenylenediamine) (PPD) [10,21,24,25], and overoxidized polypyrrole (OPPy) [9,22,26].

Nafion is often employed in the construction of modified 1st generation amperometric enzyme-based biosensors, in particular for in vivo applications. It is a negatively charged perfluorosulfonated derivative of Teflon, an ion-exchange polymer that is presumed to selectively block anions, but not cations (e.g. monoamines) [17]. Nafion thin films (nm to μm thick) are self-assembled and highly biocompatible. However, recent studies show that Nafion is less selective than other membranes such as PPD and OPPy [16].

Unlike Nafion, both PPD and OPPy are assembled by electropolymerization and thought to reduce interference by a mechanism of size exclusion. Electropolymerization is a self-limiting process, dependent on the active electrode surface area. PPD membranes can be assembled based on each of its 3 arene substitution patterns (o-, m-, and p-). However, PoPD and PmPD membranes are more selective than PpPD [21]. Electropolymerization of PPy results in a conductive polymer that requires an additional overoxidation step to produce non-conductive OPPy. It has been described that OPPy membranes are able to prevent electrochemical interference from most non-specific electroactive species, including DA [16,22].

Although the use of a single membrane is the most common application, a few in vivo studies report the successful use of Nafion combined with electropolymerized membranes, based on a layer-by-layer assembly [16,27]. Whilst the use of membrane combinations resulted in less electrochemical interference, it did not significantly improve selectivity.

Analyte sensitivity has been suggested by Lowry et al. as a reliable approximation of the available surface in thin films [15]. Several studies indicate that the assembly of permselective membranes may result in a decrease the analyte sensitivity of H_2O_2 of membrane coated electrodes [15,19]. However, it is still unclear if and how the assembly of permselective membranes influences analyte sensitivity and therefore electrode surface availability.

Since effective in vivo application of amperometric enzyme-based biosensors requires the use of a permselective membrane [28], it is our belief that a better understanding of the mechanisms of permselective membranes and its impact on surface availability is fundamental to develop highly sensitive biosensors with superior spatial resolution for in vivo application. The goal of this study is to understand the role of surface availability on membrane-induced selectivity and it implications for future amperometric enzyme-based biosensor design for in vivo applications.

Therefore, we explored the putative role of surface availability in membrane selectivity. We modified the surface of platinum needle type microelectrodes by coating them with a series of permselective membranes configurations. We have tested microelectrodes coated with Nafion, PmPD, PoPD and OPPy (alone or in combination) and compared them with bare microelectrodes. These microelectrodes were evaluated both electrochemically (by in vitro calibration and cyclic voltammetry) and visually by scanning electron microscopy.

2. Materials and methods

2.1. Materials

Platinum, silver, and stainless steel wires were obtained from Advent Research Materials. Silica tube (275 μm ID, 350 μm OD), was purchased from Avantes (Apeldoorn, The Netherlands). Nafion (5% wt in aliphatic alcohols), glutaraldehyde, o-phenylenediamine (oPD) m-phenylenediamine (mPD), pyrrole, DA, AA, UA, DOPAC, H_2O_2 (35% wt) and $K_3Fe(CN)_6$ were purchased from Sigma (St. Louis, Missouri, USA). A phosphate buffer solution (PBS) was used containing 145 mM Na^+, 1.2 mM Ca^{2+}, 2.7 mm K^+, 1.0 mM Mg^{2+}, 152 mm Cl^-, and 2.0 mM PO_4^- in ultrapurified water, brought to pH 7.4 with sodium hydroxide and degassed before use.

2.2. Biosensor manufacturing

Needle type platinum microelectrodes (200 μm ∅ × 1 mm long) were prepared in a similar fashion as described in Wahono et al. [16] and Cordeiro et al. [42]. The surface of all microelectrodes (excluding the bare ones) was modified by membrane assembly and allowed to cure for 48 h prior to electrochemical evaluation or microscopy evaluation (Scanning electron microscopy).

2.3. Membrane assembly

Microelectrodes were coated with either Nafion, PoPD, PmPD, OPPy, or combinations of Nafion-PoPD, Nafion-PmPD or Nafion/OPPy. Assembly procedures used for each of the membrane can be found in Supplementary Information (Section 6.1).

2.4. Microelectrode evaluation

To evaluate the role of surface availability on membrane selectivity, we have characterized the surface of bare and modified platinum microelectrodes. Evaluation was performed electrochemically using amperometric methods, and visually by scanning electron microscopy.

2.4.1. Electrochemical evaluation

Microelectrode calibrations were carried out in PBS of pH 7.4 at 700 mV vs. Ag/AgCl using a potentiostat (Pinnacle, model 3104 Pinnacle Tech. Inc., USA). Sensors were placed in PBS and steady state parameters (noise and baseline) were assessed after an initial equilibration period (approximately 45 min) when a stable current was reached. All interfering compounds (DA 2 μM; DOPAC 20 μM; UA 50 μM; and AA 200 μM) were added sequentially to a constantly stirred solution, prior to consecutive additions of H_2O_2 (50, 100 and 200 μM) [5,16,22]. We monitored changes in oxidation currents and calculated limit of detection (LOD) and Linear Range Sensitivity (LRS) based upon linear regression analysis.

Selectivity coefficient (SC) (Eq. (1)) and rejection coefficients (RC) (Eq. (2)) were calculated using previously described models [15,29]:

$$SC = \frac{I(\text{Analyte}), nA/[\text{Analyte}], \mu M}{H_2O_2 \text{ sensitivity, } nA/\mu M} \times 100 \qquad (1)$$

$$RC = \frac{I(\text{Analyte,bare}), nA/[\text{Analyte bare}], \mu M}{I(\text{Analyte,coated}), nA/[\text{Analyte coated}], \mu M} \times 100 \qquad (2)$$

Cyclic voltammetry experiments were performed in presence of either $Fe(CN)_6^{3-}$ (50 mM in 1 M KCl) or H_2O_2 (100 μM in PBS, pH 7.4) and carried out at different scan rates (10–300 mV/s).