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Polymer films as permselective coatings for H_2O_2-sensing electrodes

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Abstract

The performance of amperometric biosensors based on oxidation of the H_2O_2 generated by an oxidase suffers frequently from interference due to electrooxidizable species in a sample. For example, an effective biosensor of this type for the neurotransmitter, glutamate, in brain extracellular fluid (ECF) should show little or no amperometric response to readily oxidizable dopamine (DA) and ascorbate (AA), which are common constituents of ECF. In this work, platinum electrodes treated with four different polymeric, permselective films are compared quantitatively in terms of sensitivity to H_2O_2, DA and AA; and selectivity for H_2O_2 relative to either interferent. Here, the selectivity is defined as the ratio of the H_2O_2 sensitivity to the interferent sensitivity. Platinum electrodes coated with electrodeposited, overoxidized polypyrrole (OPP) and polyphenylenediamine films display H_2O_2 selectivities relative to AA of $\geqslant 130$ and $\geqslant 240$, respectively, and relative to DA of $\geqslant 100$ and 80, respectively. The impressive rejection of DA by OPP films observed in this study contrasts with published results showing enhanced DA permeability of polypyrrole electrode coatings. Nafion- and polyaniline-coated electrodes give inferior selectivity performance. The OPP-treated electrodes are judged superior overall given the best combination of H_2O_2 sensitivity (390 nA μM^{-1} cm^{-2}), detection limit (0.5 μM), response time (~5 s), and stability (>2 weeks), in addition to high H_2O_2 selectivities.

Keywords: Permselective films; Hydrogen peroxide sensing; Overoxidized polypyrrole

1. Introduction

Highly sensitive H_2O_2 electrodes have been combined with H_2O_2-producing oxidases to construct useful biosensors for various analytes [1–5]. The H_2O_2 generated as a result of enzyme-catalyzed oxidation of substrate (i.e., analyte) and transfer of electrons to oxygen subsequently is oxidized or reduced itself at the electrode surface to give a measurable current that can be correlated to the analyte concentration. Such biosensor constructs based on glutamate oxidase, for example, are applied increasingly for measurements of the neurotransmitter, L-glutamate, in living brain tissue [6,7]. But, the relatively high potential required to oxidize H_2O_2 efficiently (i.e., ~0.7–0.8 V vs. Ag/AgCl) at a platinum electrode [8] also results in the nonspecific oxidation of endogenous interferents. Electroactive species such as ascorbate (AA), which is present at ~0.2–0.5 mM in brain extracellular fluid (ECF) [5,9]; dopamine (DA) [10]; and several other monoamine neurotransmitters such as norepinephrine (NE) and serotonin [11] are oxidized readily at bare electrodes giving rise to false amperometric signals and electrode fouling. In biological environments (pH 7.4), DA ($pK_a = 8.87$) exists primarily as a cation, whereas AA ($pK_a = 4.10$) is mostly deprotonated [12]. Interferent rejection poses a particularly challenging problem, therefore, since both cations and anions must be contended with as well as compounds with some hydrophobic character, such as DA.

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A potentially effective approach to overcoming the interference problem involves the modification of the electrode surface with a self-assembled monolayer (SAM) or a polymeric film, which may be permeable to H_2O_2 and relatively impermeable to interfering species. These electrode coatings reject interferents by unfavorable chemical, electrostatic, or steric interactions [5,13–16]. For example, self-assembled alkanethiolate monolayers on gold present a significant steric barrier to all species as well as a hydrophobic resistance to polar species. However, the literature reports of reduced SAM-modified-electrode sensitivity to H_2O_2 suggest that SAMs may not be the best permselective electrode coatings for oxidase-based biosensors dependent on H_2O_2 measurement [17,18]. Negatively charged polymeric films, such as Nafion (a polytetrafluoroethylene-based ionomer), repel negatively charged interferents such as AA and urate [6,19–23]. Other polymeric coatings, such as cellulose acetate [11], appear to act primarily as a diffusional barrier against large interferents [11,24,25]. With all permselective electrode coatings, a balance must be struck between the selectivity conferred and the increase in sensor response time attributed to introduction of a H_2O_2 mass transfer resistance.

The use of electrochemically deposited polymers such as polypyrrole, polyphenylenediamine, polyaniline and polyphenol [26] as permselective layers in the construction of biosensors has been discussed in several reviews [27–29]. Electropolymerization techniques afford control over the properties of the deposited polymers and hence, the selectivity properties of the resultant biosensors. Polypyrrole (PP) and its derivatives are frequently used conducting polymers, due to their versatility and ability to covalently bind a variety of redox species [28,30]. PP can be generated electrochemically by the oxidation of aqueous pyrrole at potentials which avoid O_2 evolution [31]. Many amperometric sensors also have been constructed using polyphenylenediamine (PPD) or polyphenols as the permselective coating [2,26,32–35]. The PPD film appears to confer selectivity by limiting access of larger molecules such as AA to the electrode surface [13], while H_2O_2 is small enough to pass through. Among the electrochemically deposited polymers, polyaniline coatings exhibit great variety both in preparation procedure and in mode of application [36–38]. The morphological properties of polyaniline, polyphenol and polypyrrole films and their permeability can be controlled through polymerization conditions (time, potential, concentration and doping anion) [39]. Although many of these electrodeposited polymers can exist in an electrically conducting state, the best permselective barrier properties frequently are attained with nonconducting films, such as overoxidized polypyrrole (OPP).

This study will facilitate the design of selective biosensors based on the use of oxidases, such as glutamate oxidase, and the electrooxidation of the H_2O_2 generated by the enzyme-catalyzed reaction. Our application goal is a sensitive and selective biosensor for the important neurotransmitter, L-glutamate, based on L-glutamate oxidase. As such, this investigation focused on electrode coatings potentially capable of rejecting the common ECF interferents, AA and DA, while still enabling the sensitive and rapid electrochemical measurement of H_2O_2 at oxidizing potentials. Unlike many recent reports that described substantial, or even increased, DA sensitivity of electrodes coated with OPP [40–44], our OPP-coated electrodes exhibited dramatically reduced sensitivity to both AA and DA. In this report, linear-range sensitivities of coated and uncoated electrodes to interferents and to H_2O_2 are compared systematically to give quantitative information on selectivities of nonconducting polymer films.

2. Experimental

2.1. Materials

Glycine, L-ascorbic acid, resorcinol, 1,3-phenylenediamine, 3-hydroxytyramine (dopamine), aniline (99+%), pyrrole (98%), and Nafion® were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Glutaraldehyde (25% in water), anhydrous sodium phosphate dibasic and anhydrous sodium phosphate monobasic were obtained from Fisher Scientific (Tustin, CA, USA). Hydrogen peroxide (30% solution) was purchased from EM Science (Gibbstown, NJ, USA). All other chemicals were of analytical-reagent grade or better. Ultrapure water generated from a Millipore Milli-Q water system was used for preparation of all solutions used in this work. The phosphate buffered saline (PBS) used was composed of 50 mM Na_2HPO_4 with 100 mM NaCl (pH 7.2) unless specified otherwise.

2.2. Instrumentation

Electrochemical measurements were carried out using an EG&G Instruments Potentiostat/Galvanostat model 283 (Perkin–Elmer Instrument, Atlanta, TX, USA). Platinum or gold disk electrodes (~1.6 mm diameter), Ag/AgCl (3 M NaCl) electrodes and platinum wire were used as working, reference and counter electrodes, respectively (Bioanalytical Systems, West Lafayette, IN, USA). All potentials are reported versus the Ag/AgCl reference electrode.

2.3. Electrode preparation

Before each biosensor construction, the bare gold or platinum electrodes were polished with 1 μm diamond