Efficient Glucose Detection in Anaerobic Solutions Using an Enzyme-modified Electrode Designed to Detect H_2O_2: Implications for Biomedical Applications

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The finding that a 'first generation' glucose oxidase modified poly(*o*-phenylenediamine) coated Pt electrode, designed to detect H_2O_2, responded to glucose in N_2-saturated solutions with a sensitivity similar to that of air-saturated media is of considerable significance for the application of biosensors in biological systems where O_2 availability is severely restricted.

Ortho-Phenylenediamine (PD, 1,2-diaminobenzene) forms a self-sealing, highly insulating thin (*ca.* 10 nm) membrane containing trapped enzyme molecules when electropolymerised onto a Pt anode in enzyme–electrolyte solution.[1,2] When glucose oxidase (GOx) is used, the GOx-modified polyphenylenediamine (PPD) coated Pt (Pt/PPD/GOx) electrodes are considered 'first generation' sensors as they detect glucose by oxidising H_2O_2 formed in the presence of the natural co-substrate for GOx, dioxygen [reactions. (1)–(3)].

$$\beta\text{-D-glucose} + \text{GOx/FAD} \rightarrow \text{D-glucono-}\delta\text{-lactone} + \text{GOx/FADH}_2 \quad (1)$$

$$\text{GOx/FADH}_2 + O_2 \rightarrow \text{GOx/FAD} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (3)$$

A number of different laboratories have demonstrated that Pt/PPD/GOx electrodes possess a variety of properties indicating potential suitability for monitoring glucose levels in biomedical applications.[1–10] These properties include fast response time, linearity over the relevant range of concentration, effective elimination of interference by reducing agents such as ascorbic acid, freedom from protein and lipid fouling, stability *in vivo*, and ease of miniaturisation. However, since the mechanism of electrochemical signal generation involves oxidation of H_2O_2 [reaction (3)] formed from the reaction of O_2 with reduced enzyme [reaction (2)], changes in ambient oxygen tension may mimic changes in glucose concentration, undermining the reliability of the sensor to monitor glucose unambiguously.

We therefore investigated the sensitivity of Pt/PPD/GOx electrodes to glucose for different concentrations of O_2 in solution.

GOx was immobilised in poly(*o*-phenylenediamine) films by potentiostatic electropolymerisation of the monomer on the bare disk end of a freshly cut Teflon-insulated Pt wire (125–250 μm diameter) as described in detail recently.[8] Briefly, a deoxygenated solution of the monomer (300 mmol dm^{-3}) and GOx (5 mg cm^{-3}) was prepared in phosphate buffered saline (PBS, pH 7.4). The working electrode potential was maintained at +0.65 V *vs.* SCE during the electropolymerisation for 15 min using a large Pt wire as auxiliary electrode. All experiments using these Pt/PPD/GOx electrodes were performed in a standard three-electrode glass electrochemical cell containing 20 ml PBS thermostated at 25 ± 1 °C. To attain effective anaerobic conditions, all solutions were vigorously purged with O_2-free N_2 (average O_2 content 2 ppm, maximum O_2 content 5 ppm) for at least 30 min before recording began and a N_2 atmosphere maintained over the cell thereafter. In experiments involving solution O_2, either atmospheric air or pure O_2 from a gas cylinder was bubbled through the PBS. The mean ± standard error is reported with n = number of electrodes or number of electrodes times determinations. Background current recorded in PBS with Pt/PPD/GOx electrodes in the absence of glucose was small (2 ± 1 μA cm^{-2}, n = 19) and was subtracted from the total current to obtain the glucose response.

As reported previously,[1,3,8] glucose calibrations carried out amperometrically at +700 mV *vs.* SCE gave rapid response times ($t_{95\%}$ < 10 s) and followed Michaelis–Menten kinetics (goodness of fit, $r^2 > 0.999$) when performed in air-saturated solutions (n = 3): K_m = 20 ± 4 mmol dm^{-3}; and V_{max} = 249 ± 59 μA cm^{-2} (see Fig. 1). Since it is assumed that the current observed under these conditions is due to the oxidation of H_2O_2 [reaction (3)] produced by reaction (2), it was surprising that when the same electrodes were used under N_2-saturated conditions the overall response (n = 3 × 2) was not very different compared with air saturation: rapid response time; $r^2 > 0.998$; K_m = 20 ± 5 mmol dm^{-3}; and V_{max} = 206 ± 31 μA cm^{-2}, indicating only a small loss of sensitivity compared with air present (see Fig. 1). To ensure that the response in N_2-saturated PBS was not due to O_2 trapped in the polymer from previous experiments, glucose calibrations were performed with Pt/PPD/GOx electrodes that had never been exposed to solutions containing O_2; equally high sensitivity was observed under anaerobic conditions with these 'O_2 naive' sensors, V_{max} = 216 ± 33 μA cm^{-2}, n = 3. To confirm that the glucose current was enzyme-mediated, we determined the response of 100 mmol dm^{-3} glucose at bare Pt and Pt/PPD electrodes containing no GOx; the current was minimal in both cases and averaged 0.8 ± 0.1 μA cm^{-2}, n = 4.

To determine the sensitivity of Pt/PPD/GOx electrodes over a wider O_2 concentration range, either pure N_2, pure O_2 or air was passed through PBS containing 100 mmol dm^{-3} glucose (enzyme saturation). To monitor the concentration of O_2 in solution we developed a double potential pulse technique with carbon paste electrodes (CPEs) similar to differential pulse amperometry (DPA) applied to dopamine detection *in vivo*.[11] In our case two equally sized pulses are

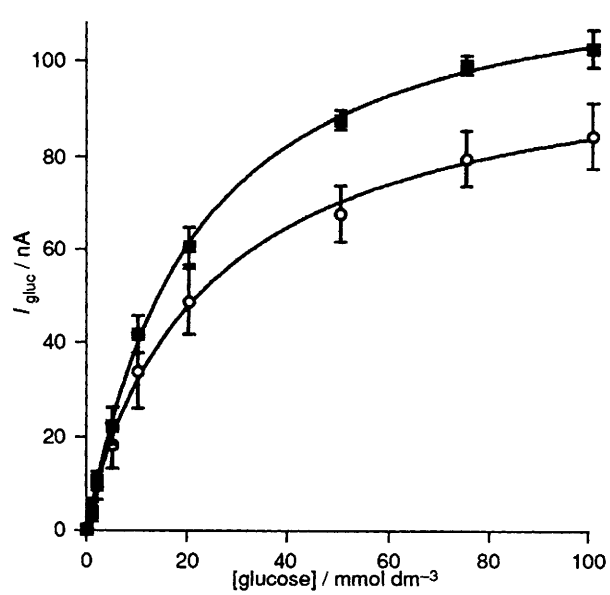

Fig. 1 Amperometric steady-state calibrations for glucose at 250-μm diameter Pt/PPD/GOx electrodes at 700 mV *vs.* SCE. Average values from experiments performed with the same set of three electrodes in either air-saturated (■) (V_{max} = 249 ± 59 μA cm^{-2}, n = 3) or N_2-saturated (○) (V_{max} = 206 ± 31 μA cm^{-2}, n = 3 × 2) PBS. The PBS current in the absence of glucose has been subtracted in each case.

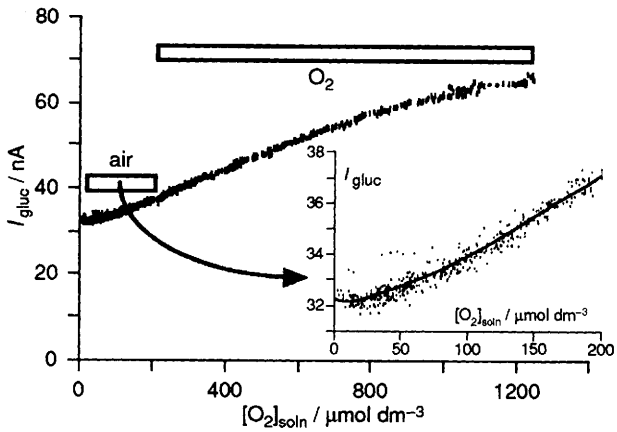

Fig. 2 Amperometric current for 100 mmol dm^{-3} glucose recorded at a 125 μm diameter Pt/PPD/GOx electrode in PBS at 700 mV *vs.* SCE during bubbling with either N_2 (minimum value = 260 μA cm^{-2}), air (maximum value = 300 μA cm^{-2}) or pure O_2 (maximum value = 530 μA cm^{-2}) plotted as a function of solution O_2 concentration. The inset shows data for the air region in greater detail.

applied, the first from a resting potential at −150 mV to −350 mV that corresponds to the foot of the reduction wave for O_2 at CPEs, and the second from −350 mV to −550 mV that reaches well up the reduction wave. The difference in the current sampled during these pulse pairs corresponds mainly to faradaic O_2 reduction with little contribution from capacitance effects. Using O_2 concentration data for both air- and O_2-saturated buffers,[12,13] the DPA current was converted to O_2 concentration using a linear ($r^2 > 0.998$) plot with slope 122 ± 5 nA mmol^{-1} dm^{-3}. Fig. 2 shows the dependence of glucose current on solution O_2 concentration. The most surprising aspect is confirmation of the finding that a significant part of the current recorded with these sensors is independent of O_2 in solution (see Fig. 1) since there is a significant glucose signal in N_2-saturated solutions and increasing solution O_2 from 0 to 200 μmol dm^{-3} (air saturation) increased the current by only 20%. Indeed, a closer inspection (inset, Fig. 2) shows that the glucose signal is effectively independent of the concentration of O_2 in solution in the range 0–50 μmol dm^{-3}. Thus in biological applications such as glucose detection in brain or subcutaneous tissue where the range of O_2 tension is 5–50 μmol dm^{-3},[13,14] glucose monitoring would be effectively free of interference by O_2. This finding may explain why Pt/PPD/GOx currents recorded in brain tissue *in vivo*[8] were similar to those recorded under air saturation conditions *in vitro*, despite the poor O_2 levels in the tissue.

The results suggest that mechanisms other than reaction of solution O_2 with reduced enzyme are responsible for the electrochemical signal generated with Pt/PPD/GOx biosensors. We suspect that O_2 formed on the Pt surface due to electrolysis of water even at a slow rate [together with the recycling reactions (2) and (3)] may be sufficient to mimic solution O_2 of 200 μmol dm^{-3} in the context of reaction (2) (see Fig. 2, inset); however, a full investigation of this phenomenon is underway and will be submitted for publication. It remains to be seen, for example, whether this behaviour is unique to Pt/PPD/GOx electrodes or applies to other H_2O_2-detecting sensors. Irrespective of the mechanism involved, the findings are of considerable significance for the application of these, and possibly other, enzyme-based sensors in biological systems where O_2 availability is severely restricted.

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