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PII: S0032-3861(97)00228-0

Polymer Vol. 39 No. 1, pp. 123–127, 1998

0032-3861/97/$17.00+0.00

Enzymatically prepared poly(hydroquinone) as a mediator for amperometric glucose sensors

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(Received 28 October 1996; revised 24 February 1997)

Poly(hydroquinone) (PHQ), synthesized from glucose-β-D-hydroquinone by peroxidase-catalyzed polymerization in aqueous solution and placed on glassy carbon electrodes, behaves as a redox mediator for glucose sensing. The highly selective nature of enzymatic catalysis leads to PHQ with a unique structure which is more soluble in organic solvents and more electrochemically active, as compared to that prepared via electrochemical methods. A glucose sensor is constructed in a pellet form with PHQ, glucose oxidase (GOD) and graphite powder. PHQ retains its redox activity and reversibility in the solid state and effectively mediates the electron transfer between the electrode and GOD. Resulting glucose biosensors possess sub-minute response times over a dynamic range from 1 to 30 mM. The PHQ mediator permits sensor operation at 100 mV (versus SCE), thereby reducing susceptibility toward common endogenous, easily oxidizable interferences. © 1997 Elsevier Science Ltd.

(Keywords: poly(hydroquinone); enzymatic polymerization; glucose sensor)

INTRODUCTION

Various redox-active polymers, such as polyaniline, polyacetylene, polypyrrole and polyphenylene, have been investigated for applications in rechargeable batteries, sensors, electrocatalysts and as semiconductors in electronic microdevices[1–3]. Quinone-containing redox polymers are of particular interest due to their unique two-electron redox behaviour and reversibility[4–9].

Applications of redox polymers in enzyme based biosensors, especially those for the determination of glucose, have attracted considerable attention in recent years. The glucose oxidase (GOD) based glucose sensor is the most extensively investigated enzyme-electrode sensor [10] and is based on the reaction shown in equation (1).

$$\text{glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \delta\text{-}gluconolactone + H_2O_2 \quad (1)$$

Either the consumption of oxygen or production of hydrogen peroxide is measured at the electrode surface and related to sample glucose concentrations through a previously prepared calibration curve. Sensors based on O_2 detection are affected by changes in endogenous O_2 levels which mandates a concomitant O_2 measurement. For this reason, H_2O_2 detection is preferred, but problems of interferences by easily oxidizable endogenous species frequently limit this approach in biological matrices. A number of organic compounds, such as ascorbic and uric acids, represent positive interference because they are oxidized at potentials lower than those required for the oxidative detection of H_2O_2. Another drawback of such conventional glucose sensors is that the H_2O_2 generated is harmful to GOD and thus limits sensor performance.

Many of these limitations can be overcome through the use of various redox active mediators to replace oxygen. As depicted in equation (2), GOD, while highly selective for glucose, is much less selective toward the electron acceptor and has the capability to replace O_2 with other electron acceptors.

$$\text{glucose} + M_0 \xrightarrow{\text{GOD}} \delta\text{-gluconolactone} + M_r \quad (2)$$

The reduced mediator (M_r) can be electrochemically oxidized back to M_0. Benzoquinone (BQ) and quinone derivatives[11–15], ferrocene[16–18], hexacyanoferrate[19] and osmium containing compounds[20] are among the most commonly used mediators. BQ, in particular, has been found to be an effective mediator for GOD[11–15]. Since BQ is water-soluble, earlier investigations have been performed by using semipermeable membranes[14,15] to reduce the dissolution of BQ into working solutions. More recently, quinones have been incorporated into polysiloxane[11] and amine-quinone copolymers[12,13] for use in glucose sensors. However, while the quinones are effectively immobilized, the quinone moiety in these polymers exhibits decreased electrochemical reversibility[11] and decreased mediation efficiency[13].

We have successfully synthesized a unique poly(hydroquinone) (PHQ) by enzymatic catalysis[21]. PHQ shows considerable electrochemical activity, stability and reversibility on glassy carbon electrodes. The present work reports our preliminary investigation of PHQ as a mediator for glucose sensors.

EXPERIMENTAL

Synthesis of poly(hydroquinone)

PHQ was synthesized as reported previously[21]. Briefly, a

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20 ml solution of glucose-β-D-hydroquinone (arbutin, from Sigma) and a 10 ml solution of H_2O_2 were pumped separately over a period of 3 h into a reactor containing a 20 ml solution of soybean peroxidase (SBP, Enzymol International, Columbus, OH). All solutions consisted of 0.1 M pH 6.5 phosphate buffer. The final substrate and enzyme concentrations in the reactor were 0.1 M, 0.2 M and 20 purpurogallin units/ml for arbutin, H_2O_2, and SBP, respectively. The reaction mixture was stirred at 200 rpm for 12 h to allow complete polymerization, then dialyzed to remove unreacted monomer and other small molecules. The resulting poly(arbutin) was highly water-soluble due to the hydrophilic nature of the sugar moiety. A typical polymerization gave a yield of 75%, with M_n = 3200 and M_w = 3700 for poly(arbutin)—representing a degree of polymerization of 12—as determined by GPC using Ultrahydrogel 500 and 250 columns (Waters, MA), and PEG (1450, 5000, 9000, 15 000, from Polysciences, PA) as molecular weight standards. This molecular weight determination provided the polymer size generated enzymatically. Poly(arbutin) was then heated at 60°C for 24 h in a 5 M HCl solution to remove the sugar moieties, and PHQ was obtained as a dark brown precipitate, which was washed extensively with deionized water to remove water-soluble components including glucose and SBP, and dried under vacuum for at least 24 h.

Construction of glucose sensors and electrochemical experiments

Glucose oxidase (GOD, Type VII-s from *Aspergillus niger*, 119 units per mg solid, from Sigma), PHQ and graphite powder were mixed in a specific weight ratio (see below), and pressed into cylindrical pellets with a cross-sectional area of 0.3 cm^2, and a thickness of *ca.* 1 mm. The pellets were attached to a glassy carbon electrode by wetting the electrode surface with a small amount of DMSO and pressing the electrode against the pellet. The sensor was dried in air for at least 24 h, and then rinsed with water prior to testing. Cyclic voltammetry was performed on a computer-controlled bioanalytical system (BAS 100W) potentiostat. A platinum mesh was used as the auxiliary electrode and SCE as the reference electrode. Unless specified, all electrochemical measurements were performed in 0.1 M phosphate buffer (pH 7) with 0.1 M KCl. All solutions were purged with N_2 for at least 15 min prior to testing.

RESULTS AND DISCUSSION

PHQ is known to be difficult to prepare, and only a few reports in the literature confirm its preparation, primarily via electrochemical methods[7,22]. The enzymatic technique applied in the present work leads to a PHQ that is structurally different from that prepared electrochemically (*Scheme 1*). In particular, the enzymatic route provides a PHQ with a symmetrical structure with all aryl–aryl linkages in the same location on the hydroquinone repeat units. Conversely, the electrochemical route provides a random linkage of the hydroquinone repeat units. As a result, higher chain flexibility is expected for the enzymatically prepared PHQ, and this hypothesis is consistent with its higher solubility in organic solvents despite its higher molecular weight (DP = 12, as compared to 6 for electrochemically prepared PHQ[7]). Specifically, the enzymatically prepared PHQ can be dissolved (> 2%, w/v) in a few common solvents such as DMSO, THF, DMF, methanol and acetone, thereby ensuring suitable polymer processibility. This observation is particularly interesting,

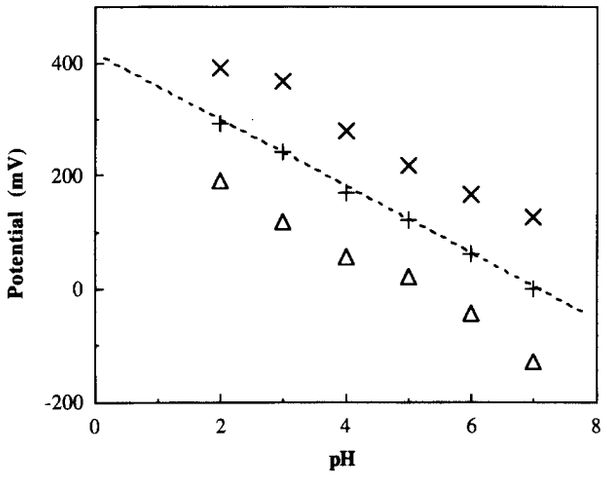


Figure 1 Effect of pH on the redox potentials of poly(hydroquinone). The potentials are determined from cyclic voltammograms of a PHQ coating on a glassy carbon electrode. The sweep rate is 100 mV/s. (X) oxidation potential; (Δ) reduction potential; (+) midpoint potential ($E_{1/2}$).

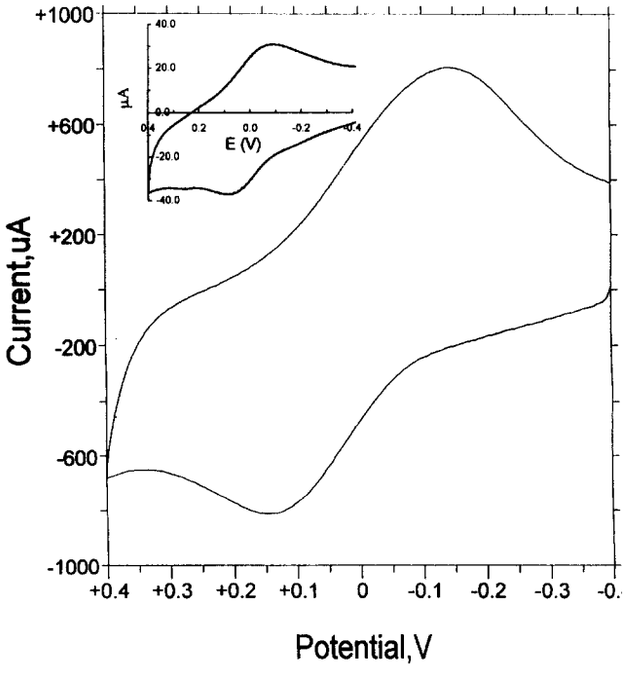
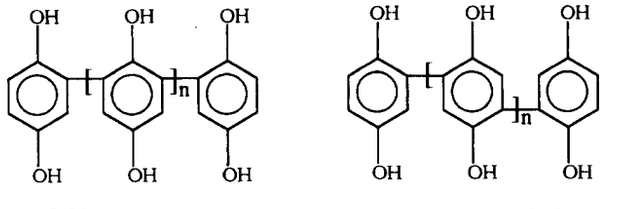


Figure 2 Cyclic voltammogram (CV) of a poly(hydroquinone) and graphite powder composite pellet attached to a glassy carbon electrode in pH 7 phosphate buffer. The pellet contains 50% (w) of PHQ. The inset is a CV of a thin PHQ coating without graphite on the same electrode as measured in the same buffer solution.



Scheme 1 Chemical structure of poly(hydroquinone).

because it is generally known that most of the quinone-type polymers have poor solubility in common organic solvents and, as a result, their applications have been largely limited[23].

Electrochemical properties of PHQ

The electrochemical properties of PHQ were examined by casting a film from a DMSO solution containing the polymer (2% w/v) onto the surface of a glassy carbon electrode, followed by air drying at room temperature with air purging. The oxidation and reduction potentials of PHQ in aqueous solutions (*Figure 1*) become more positive as the solution pH decreases, such that extrapolation to pH 0 yields a midpoint potential ($E_{1/2}$) of 410 mV versus SCE, which corresponds to the redox potential of hydroquinone in acidic solutions.

The construction of the glucose sensor utilized graphite powder to enhance the electrical conductivity of the PHQ mediator. A typical cyclic voltammetric response of a PHQ pellet containing graphite powder is shown in *Figure 2*. The redox potentials are nearly the same as those of thin PHQ coatings without graphite (*Figure 2*, inset), but the peak current is more than 15 times higher due to the improved conductivity and the increased electrode area provided by the graphite. A high mediation efficiency at a low working potential is a critical consideration for the performance of redox mediators used in glucose sensors. Such sensing performance depends on high electrochemical reversibility and activity of the mediators, two features that PHQ possesses, and this gives PHQ an advantage over other quinone-based sensors. Specifically, the oxidation and reduction peaks of the PHQ are separated by about 250 mV (*Figure 2*), much lower than that of benzoquinone attached to polysiloxane (770 mV)[11], thus demonstrating better electrochemical reversibility for PHQ. The $E_{1/2}$ of PHQ (~0 mV) is also lower than that of amine-quinone copolymers (> 90 mV)[13], suggesting a higher electrochemical activity for PHQ.

PHQ as a mediator for glucose sensing

Because the oxidized state of the mediator is required to interact with the reduced GOD to mediate the electron transfer between the electrode and enzyme active centre, the PHQ was first oxidized to the poly(benzoquinone) form by placing the PHQ sensor into a pH 7 phosphate buffer solution and maintaining its potential at 400 mV versus SCE. The oxidation was performed for up to 2 h until nearly no anodic current was observed. The electrode was then maintained under a specific potential to measure its response to changes in glucose concentration. The solution was purged with nitrogen for at least 15 min and maintained under N_2 to prevent interference from exogenous oxygen. As expected, the oxidation current increased almost immediately with an increase in glucose concentration, and reached a steady value within about 1 min (*Figure 3*). To confirm the observed signals come from the expected mediation reaction, an electrode was constructed with the same amounts of PHQ and graphite powder, but without GOD. This electrode did not show any response with the addition of glucose to the testing solution. Moreover, the same electrode placed in a 20 mM glucose solution and kept under the same potential (400 mV versus SCE) with the addition of GOD in the solution in an amount 50 times greater than that used in the aforementioned PHQ pellet sensor led to a slight change in anodic current (< 10% of that observed with a PHQ pellet sensor for a 1 mM change in glucose concentration). This result clearly indicates that signals observed from the sensor are not due to enzyme leaching from the electrode surface and catalyzing the reaction in bulk solution.

Compared to other quinone-containing copolymers, PHQ possesses a higher density of redox-active functional

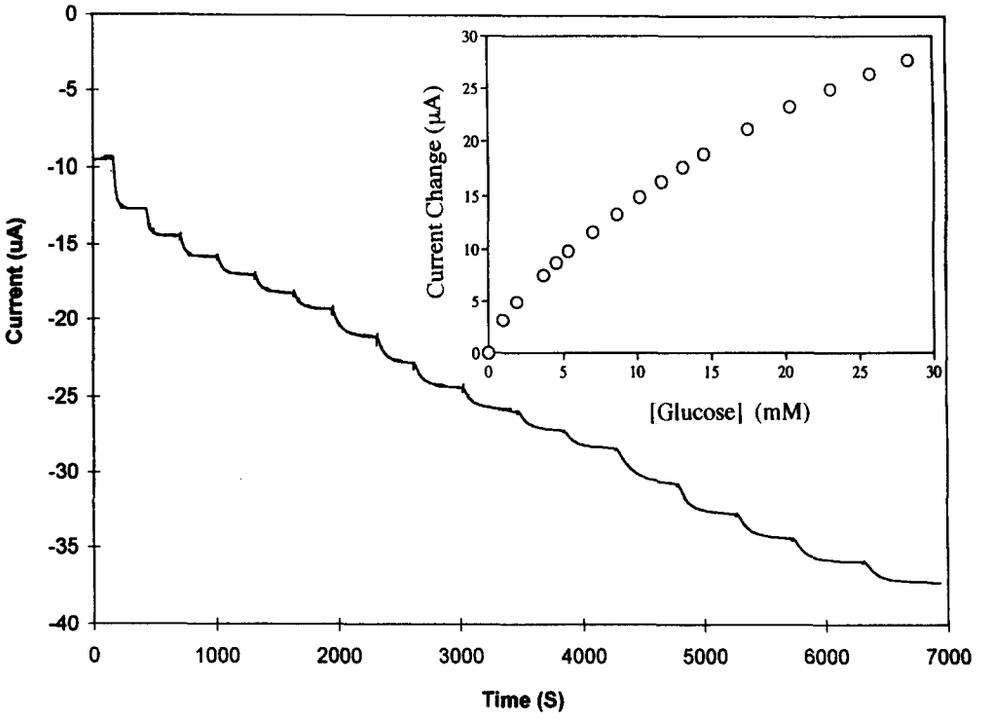


Figure 3 Response of the poly(hydroquinone) mediated sensor to changes in glucose concentration. Each step change in current corresponds to an addition of a glucose standard. The inset shows the plot of the change in anodic current versus the accumulated glucose concentration in the testing solution. The sensor was constructed with a weight ratio of graphite: PHQ: GOD = 1: 1: 0.5. The working potential is 400 mV versus SCE.

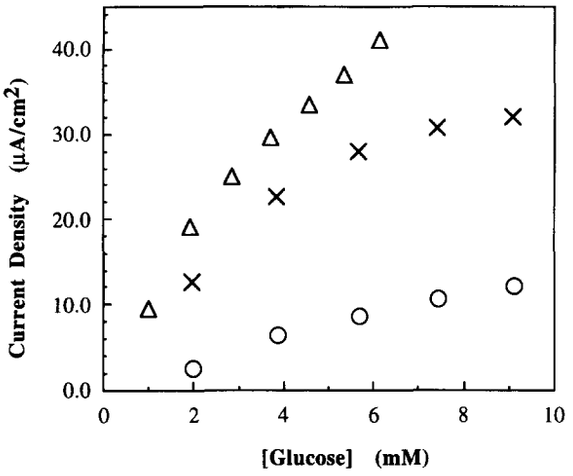


Figure 4 Calibration curves for poly(hydroquinone) glucose sensors. The weight ratio in the sensors are graphite: PHQ: GOD = (Δ) 1: 1: 0. 5; (X) 1: 0. 5: 0.25; (o) 1: 0. 5: 0. 1. The working potential is 400 mV versus SCE.

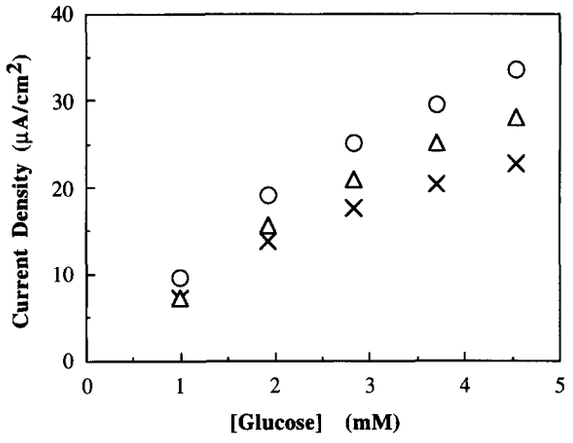


Figure 5 Calibration curves for poly(hydroquinone) glucose sensor at different working potentials. Sensor composition is graphite: PHQ: GOD = 1: 1: 0.5. (o) 400 mV; (Δ) 200 mV; and (x) 100 mV.

groups, thus allowing construction of glucose sensors with senstivities not limited by the loading of the mediator. In a study using free quinone as a mediator, stronger signals are obtained with higher amounts of quinone until its concentration reaches *ca.* 20% (w/w) in the sensor[15]. While the 20% quinone-loading is hardly possible for other quinone polymers (limited by the theoretical quinone content in the copolymers), it can be easily achieved with PHQ. Glucose calibration curves (in the form of current density versus glucose concentration) are shown in *Figure 4* for sensors containing PHQ with different compositions. For the purposes of this comparison, current densities are estimated based on a cylindrical cross-section area of the PHQ pellet. Higher exposed surface areas are actually present due to the graphite component. In comparison between these electrodes, while PHQ loadings (31%, 29% and 40%, w/w) are kept above 20%, the increase in signal strength is approximately proportional to the enzyme loading (6.2, 14 and 20% w/w), which suggests the sensing signal is not limited by the quinone content. Another advantage of PHQ is the lack of spacer groups between the hydroquinone units. It has been observed that a spacer between hydroquinone units can decrease mediation efficiency of quinone-amine copolymers[13].

The operating potential of the sensor could be as low as 0 mV in pH 7 solutions as indicated by the cyclic voltammogram of PHQ (*Figure 2*). While the lower potential is favoured to increase tolerance of the sensor to interference by other substances such as uric and ascorbic acids, the corresponding sensor sensitivity decreases dramatically. The current density of the glucose sensor based on quinone-amine copolymers[13] decreases by 80% when the potential shifts from 400 mV to 100 mV. Similar phenomena were observed for the PHQ sensor (*Figure 5*), but with a lower decrease in current density (< 40%). This observation, consistent with the low $E_{1/2}$, indicates again the better electrochemical activity for PHQ at low potentials, thus reducing susceptibility to interference.

CONCLUSIONS

Enzymatically prepared PHQ is a highly active and stable redox polymer that can effectively mediate the electron transfer between a sensor electrode and glucose oxidase at potentials lower than traditional glucose sensors. Compared to other investigated quinone-containing polymers, PHQ has several advantages including higher electrochemical activity and reversibility, higher density of the redox-active functional group and greater processability, for use in glucose sensors. Covalent attachment, not performed in this work, may be needed to enhance useful sensor lifetime. For example, the GOD sensor pellet suspended in aqueous buffer for two weeks lost *ca.* 38% in its current density capacity when measured using a 10 mM glucose solution. Although immobilization of GOD may be needed, the results presented herein demonstrate that glucose biosensors with high sensitivity, good selectivity and rapid response times can be constructed using enzymatically synthesized poly(hydroquinone).

ACKNOWLEDGEMENTS

We thank Tricia Fairbanks for help in preparing poly(hydroquinone) samples. This work was supported by a grant from the U.S. Army (DAAK60-93-K-0008) and USDA (Byproducts from Biotechnology Consortium).

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