

Full Paper

ORMOSIL Encapsulated Pyrroloquinoline Quinone-Modified Electrochemical Sensor for Thiols

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Abstract

An organically modified sol-gel glass (ORMOSIL) encapsulating pyrroloquinoline quinone (PQQ)-modified electrode for the rapid, sensitive and simple determination of thiol-containing compounds such as cysteine and glutathione is reported. The effect of applied potential, nature of thiol compound and pH on the response of the sensor was examined and optimum conditions were determined. The electrochemical responses and detection limits were found to be sensitive to the nature of thiols and pH. The electrochemical responses for cysteine and glutathione at an applied potential of -0.2 V (vs. Ag/AgCl) were found to be linear with detection limits of 18 nM for cysteine and 36 nM for glutathione at pH 3.5, whereas the detection limits at pH 8.5 were 0.5 μ M for cysteine and 1 μ M for glutathione. The electrode retained 95% of the original response for 7 days when stored at 4 °C. The ORMOSIL-encapsulated PQQ was also characterized by spectrophotometry. The absorbance measurement using 5,5'-dithiobis(2-nitrobenzoic acid) at 412 nm justify the PQQ-mediated oxidation of glutathione whereas fluorescence measurements (excitation wavelength = 380 nm; emission wavelength = 480 nm) justify the successful encapsulation of PQQ in ORMOSIL matrix.

Keywords: Thiol, PQQ, ORMOSIL, Amperometry, Cysteine, Glutathione

1. Introduction

The analytical devices for sensing sulfhydryl thiols are of great significance, specifically in biomedical applications. Electrochemistry has played central role in developing sensor technology for real-time analysis and accordingly various electrochemical approaches have been adopted for the determination of thiols. A recent review [1] summarizes the various electrochemical approaches for thiol analysis based on direct oxidation [2–4], substrate oxidation [5], pulsed electrochemical detection [6–8], mercury preconcentration [9–11], mediated mechanism [12–14], derivatization detection [15–17] and miscellaneous protocols [18–20]. Coupling of liquid chromatography with capillary electrophoresis has also been employed for such detection [21–23]. Although extensive efforts on the electrochemical detection of thiols have been made there still exists further need to resolve the problems of poor sensitivity and selectivity.

Pyrroloquinoline quinone (PQQ) is a redox cofactor present in a number of dehydrogenases occurring in a variety of gram-negative bacteria. Free PQQ has been shown to catalyze non-enzymatic reactions, including the oxidation of thiols to disulfide [24, 25]. Taking advantage of the latter together with its redox property, PQQ has been applied for electrochemical detection of thiols [26, 27]. The electrode in these investigations was prepared by entrapping PQQ in polypyrrole during pyrrole electropolymeriza-

tion. This approach has the limitation of PQQ leaching from the electrode surface leading to a decrease in the peak current [26, 28, 29].

Sol-gel derived materials have been extensively used for encapsulating proteins. A recent review summarizes the properties and applications of proteins encapsulated within sol-gel derived materials [30]. Organically modified sol-gel glass (ORMOSIL) has been used for the preparation of modified electrode [31]. Such materials are biocompatible and have easy protocol for modified electrode fabrication. In this communication, we report an electrochemical sensor that incorporates PQQ in ORMOSIL for the mediated detection of thiols. The electrochemical performances and detection of thiols i.e. cysteine and glutathione are reported. The characterization of ORMOSIL-encapsulated PQQ and PQQ-mediated oxidation of thiol is also performed by fluorescence spectroscopy and absorbance spectroscopy respectively.

2. Experimental

2.1. Materials

PQQ (approximately 98%), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and L-cysteine, were purchased from Sigma (St. Louis, MO). 3-Aminopropyltrimethoxysilane (97%), and glutathione (reduced 98%) were purchased from

Aldrich (Milwaukee, WI). 2-(3,4-Epoxy-cyclohexyl) ethyl-trimethoxysilane was obtained from Fluka (Steinheim, Switzerland). Cysteine containing dietary supplement (L-cysteine 500) tablets was purchased from General Nutrition Corp. (Pittsburgh, PA). All other chemicals used were of analytical reagent grade. The solutions were prepared in double distilled deionized water unless otherwise stated.

2.2. Preparation of PQQ- Encapsulated ORMOSIL-Modified Electrode

To 0.15 mg PQQ dissolved in 18 μL of 3-aminopropyltrimethoxysilane 5 μL of 2-(3,4-Epoxy-cyclohexyl) ethyl-trimethoxysilane was added, followed by 75 μL distilled deionized water and finally 2 μL HCl (0.1 N). The reaction mixture was homogenized and 1 μL of the mixture was spread-over the surface of Pt working electrode (BAS, MF 2013). Gelation was allowed to take place overnight at room temperature. The electrode was allowed to equilibrate in either 50 mM phthalate buffer (pH 3.5) or 50 mM citrate phosphate buffer (pH 8.5), unless otherwise stated, for 3 hours with mild stirring before use.

2.3. Fluorescence Spectroscopy of ORMOSIL-Encapsulated PQQ

Control ORMOSIL film without PQQ and PQQ-encapsulated sol-gel film were cast on a glass slide and studied using a fluorescence microscope (Olympus BX 51 TRF).

2.4. Absorbance Spectroscopy of PQQ-Encapsulated ORMOSIL

Both control and PQQ-encapsulated ORMOSIL were prepared in a micro-centrifuge tube. The ORMOSIL was washed with 50mM citrate phosphate buffer (pH 8.5) for 3 hours. Two mM glutathione was added to the microcentrifuge tube and aliquots withdrawn at different time intervals from the incubation mixture were allowed to react for 2 min with DTNB (1 mM) and absorbance noted at 412 nm.

2.5. Electrochemical Measurements

Amperometric measurements were performed using a voltammetric analyzer (Bioanalytical Systems, Model LC-4C) coupled to a chart recorder (Model BD 112, Kipp and Zonen, Holland). All experiments were conducted in a three electrode electrochemical cell with a working volume of 2 mL with PQQ-modified working electrode, Ag/AgCl reference electrode (BAS, MF 2063) and platinum wire auxiliary electrode. The working electrode was operated at desired potential and the background current was allowed to decay to a steady-state value. 100 μL of varying concentration of thiols were injected into the electrochemical cell

and new steady-state current was recorded. Unmodified Pt-electrode was used for control experiments. Experiments were conducted at several different applied potentials with varying solution pH to investigate the effect of these parameters on the sensor response.

A dietary supplement was analyzed for cysteine content. A tablet was weighed, ground to fine powder and dissolved in a known volume of 50 mM phthalate buffer (pH 3.5), filtered through a 0.2 μm filter to remove the insoluble cellulose and analyzed with the PQQ modified electrode.

3. Results and Discussion

Before determining the application of ORMOSIL entrapped PQQ modified electrode as a thiol sensor, it is necessary to establish that PQQ can be entrapped in ORMOSIL and retains the catalytic activity for thiol oxidation. To this end, fluorescence and absorbance spectroscopy studies were performed. It has been reported that PQQ shows a characteristic emission band centered at 480 nm when excited at 380 nm [25]. Fluorescence studies were performed to confirm the presence of PQQ in the ORMOSIL (Fig. 1). No fluorescence was observed with the control ORMOSIL not containing PQQ, whereas the ORMOSIL containing PQQ showed fluorescence. This observation confirmed the successful entrapment of PQQ in ORMOSIL. Subsequently, PQQ-mediated oxidation of thiol based on absorbance spectroscopy was monitored using DTNB (the reaction with thiol generates a yellow anion) as reported earlier [25]. There was decrease in absorbance at 412 nm with time which is characteristic of PQQ-mediated conversion of thiol to disulfide. No such decrease was observed with the ORMOSIL without PQQ. These results provided a confirmation of the PQQ functionality and mediated oxidation of thiol within the ORMOSIL matrix.

3.1. Electrochemical Performance of PQQ Encapsulated ORMOSIL-Modified Electrode

The response of the PQQ-modified electrode was optimized with respect to solution pH, applied potential and the nature of thiols as discussed below.

3.1.1. Effect of Applied Potential

One of the potential applications of the present sensor is in the construction of organophosphorus hydrolase (OPH)-based biosensor for monitoring of P-S linkage organophosphate pesticides such as malathion, s-demeton, acephate, azinophos-ethyl, and phosalone or acetylcholinesterase (AChE)-based biosensor for monitoring organophosphates and carbamate pesticides. Since OPH and AChE catalyze hydrolysis better in basic pH (8.5), we focused the characterization at pH 8.5.

Figure 2 shows the response of the PQQ-modified electrode as a function of the applied potential between

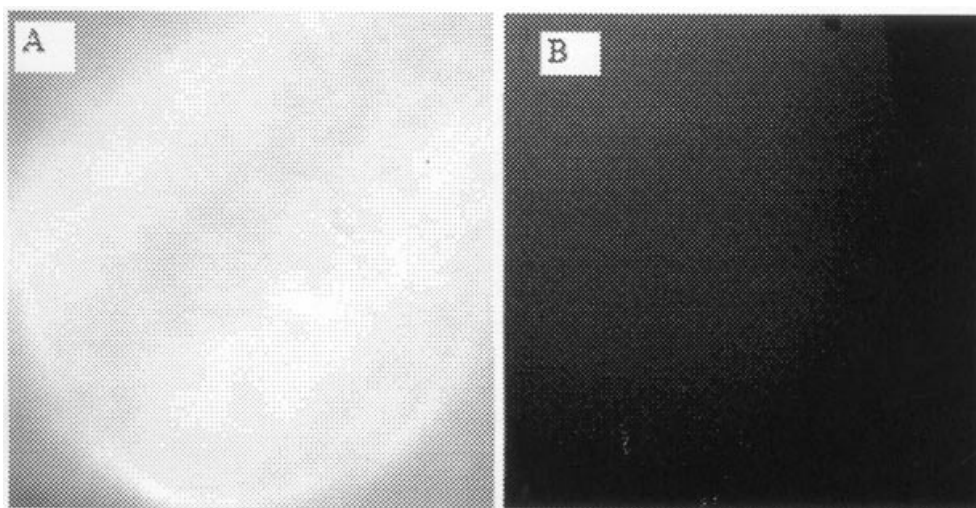


Fig. 1. Fluorescence microscopy images of ORMOsil containing PQQ (A) and control ORMOsil without PQQ (B).

+0.2 V to -0.3 V (vs. Ag/AgCl) at pH 8.5. As shown, the output current response of the sensor increased as the applied potential was made more negative. This trend is in agreement with the reported electrochemistry of PQQ and the redox potential of -150 mV [28, 32]. An operating potential of -0.2 V (vs. Ag/AgCl) was selected for amperometric studies to eliminate the need of deoxygenation. This operating potential is significantly lower than the +0.5 V employed for the polypyrrole entrapped PQQ [26] and should provide a high selectivity for thiol detection in presence of ascorbic acid, uric acid and dopamine.

3.1.2. Effect of pH

The effect of pH on the response of the sensor was studied by varying the solution pH from 3.5 to 8.5 (Fig. 3). Both, cysteine and glutathione showed increased response in acidic pH. According to some researchers use of acidic conditions is a better choice for electrochemical detection of cysteine [33]. This may be attributed to the availability of more sulfhydryl groups in acidic pH than in basic, as in acidic pH the oxidation of sulfhydryl to disulfide is kinetically slow [34].

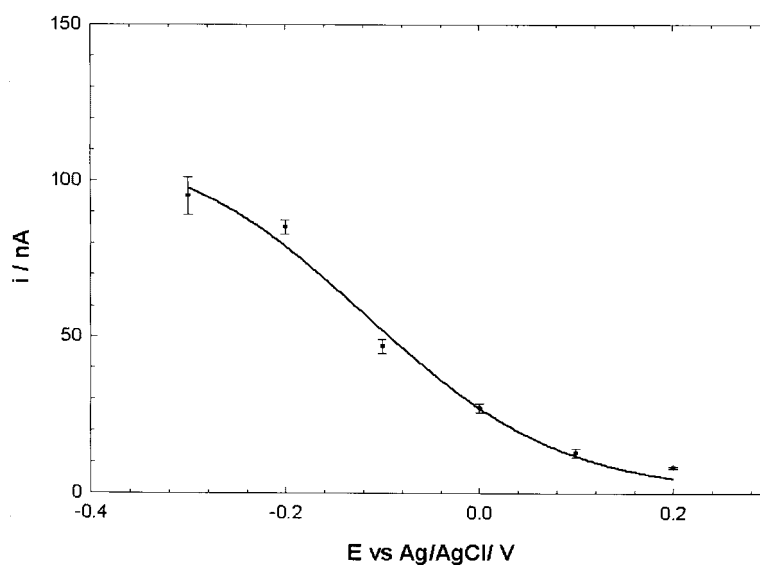


Fig. 2. Hydrodynamic voltammogram for glutathione in 50 mM citrate phosphate buffer pH 8.5. The glutathione concentration was 47.6 μ M. The data are given as mean \pm 1 SD for three sets of experiments.

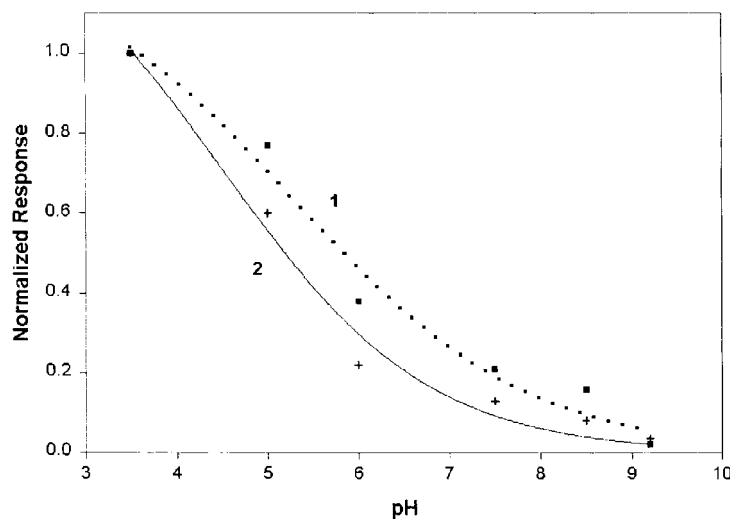


Fig. 3. Effect of pH on the response of the sensor for glutathione (1) and for cysteine (2). The applied potential was -0.2 V (vs. Ag/AgCl). The concentration of both substrates was 47.6 μ M. The response was normalized with respect to the response recorded in 50 mM phthalate buffer pH 3.5.

3.1.3. Effect of Nature of Thiol

Both glutathione and cysteine showed a similar behavior in terms of effect of pH and applied potential (data not shown), with the response for cysteine higher in magnitude than that for glutathione. This is in agreement with previous report [26] and may be attributed to the difference in structure of the two thiols which causes easier oxidation of $-SH$ in cysteine than glutathione.

3.1.4. Effect of PQQ Loading on the Performance of the Electrode

It was observed that the amount of PQQ affected the viscosity of the sol used for gelation. Higher PQQ loading resulted in a rapid film formation that subsequently peeled off from the electrode surface. Electrode modified with 0.15 mg of PQQ was used for future studies.

3.2. Analytical Characteristics

3.2.1. Calibration, Sensitivity and Selectivity

The calibration plots and the extracted analytical features of the PQQ-modified electrode for cysteine and glutathione are shown in Figure 4 and Table 1, respectively. As expected and in accordance with literature [26], the sensor had higher sensitivity and lower limit of detection for cysteine than glutathione. Figure 4A shows the dependence of the response of PQQ-modified electrode on the concentration of cysteine at pH 3.5 and pH 8.5.

The dependence of the PQQ-modified electrode response on glutathione concentration at pH 3.5 and pH 8.5,

Table 1. Characteristics of the calibration plots for the PQQ modified electrode.

Characteristic	Cysteine		Glutathione	
	pH 3.5	pH 8.5	pH 3.5	pH 8.5
Limit of detection	18 nM	0.5 μ M	36 nM	1 μ M
Slope (nA/ μ M)	259.63	10.96	170.43	5.96
Correlation coefficient	0.9904	0.9942	0.9903	0.9944

respectively, are shown in Figure 4B. The calibration plots are linear ($r^2 = 0.9903$) up to 1 μ M at pH 3.5 with a sensitivity of 170.4 nA/ μ M and up to 5 μ M at pH 8.5 with a sensitivity of 5.96 nA/ μ M. The lower limit of detection ($S/N = 3$) for glutathione analysis were determined to be 36 nM at pH 3.5 and 1 μ M at pH 8.5.

The low operating potential used in this work provides an additional benefit in terms of the sensor selectivity. Ascorbic acid, a notorious interferer in electrochemical analyses of biological samples showed no interference even at 30 μ M at both pH 3.5 and 8.5. The excellent selectivity can be attributed to the low working potential of -0.2 V (vs. Ag/AgCl) used in the present study

3.2.2. Response Time, Stability and Precision

The salient features of the present electrode are simple preparation protocol, rapid response (95% of maximum in < 2.5 minutes), and high precision (residual standard deviation of 3%, $n = 6$). The electrode retained 95% of the response for 7 days of continuous use (Fig. 5) when stored in dry condition at 4 $^{\circ}$ C.

3.2.3. Analysis of Cysteine in a Dietary Supplement

The PQQ encapsulated ORMOSIL modified electrode was used for quantitative detection of L-cysteine in an over the counter dietary supplement tablet. The amount of cysteine was determined to be 470 ± 20 mg ($n = 5$) and was in excellent agreement with the reported amount of 500 mg per tablet. The result demonstrates the practical usability of the present sensor for thiol analysis.

4. Conclusions

We reported herein an electrochemical sensor for thiol analysis based on the encapsulation of pyrroloquinoline quinone (PQQ) within organically modified sol-gel glass. The ORMOSIL-encapsulated PQQ was first characterized by fluorescence and absorbance measurements. The fluorescence studies proved the presence of PQQ in the ORMOSIL matrix. The PQQ mediated oxidation of glutathione was demonstrated by absorbance measurements. The resulting electrochemical sensor responded to

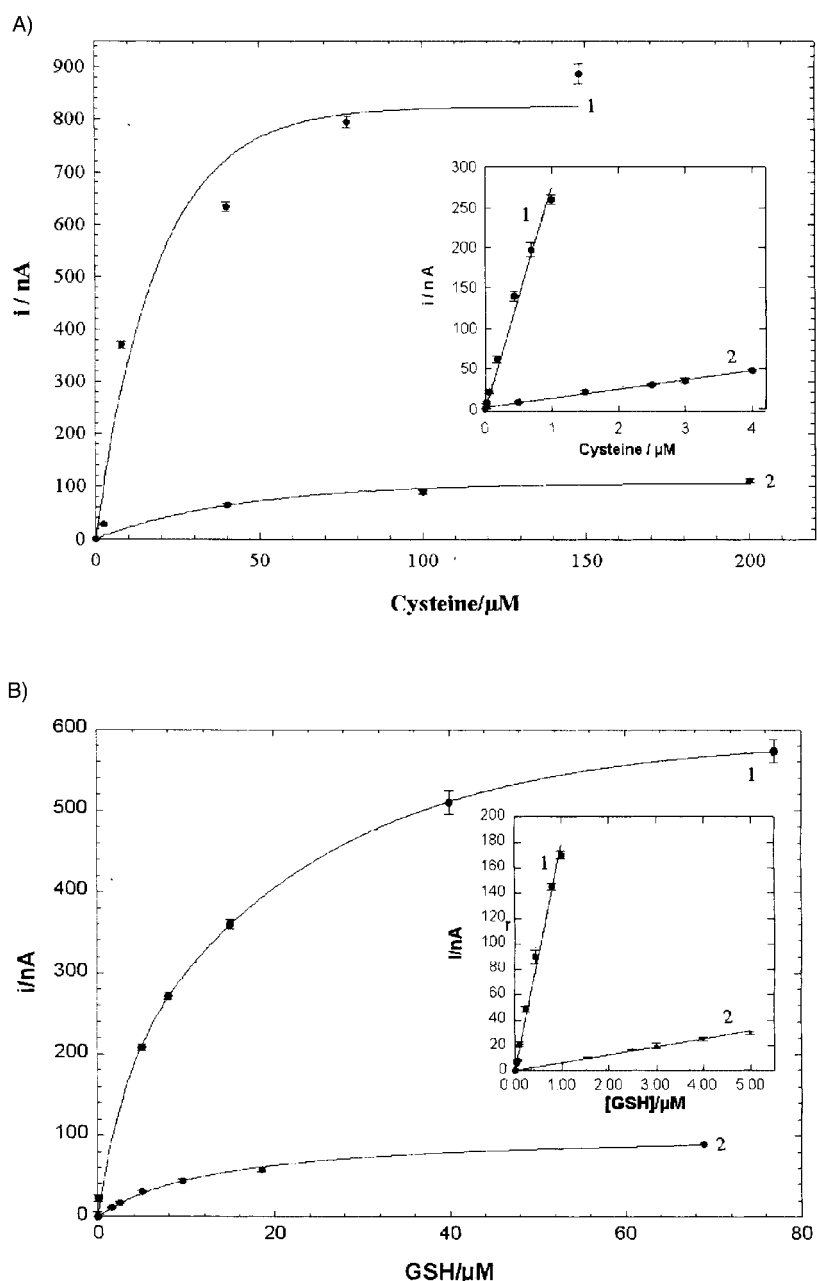


Fig. 4. A) Calibration plots for cysteine in 50 mM phthalate buffer pH 3.5 (1) and 50 mM citrate phosphate buffer pH 8.5 (2). The applied potential was -0.2 V (vs. Ag/AgCl). Inset shows the linear region for the calibration plots. Data are given as mean \pm 1 SD for three sets of experiments. B) Calibration plots for glutathione in 50 mM phthalate buffer pH 3.5 (1) and in 50 mM citrate phosphate buffer pH 8.5 (2). The applied potential was -0.2 V (vs. Ag/AgCl). Inset shows the linear region for the calibration plots. Data are given as mean \pm 1 SD for three sets of experiments.

thiols (cysteine and glutathione) nicely with excellent electrochemical performance and reproducibility. The various parameters for electrochemical sensing have been studied. The sensor showed excellent sensitivity to thiols for practical applications.

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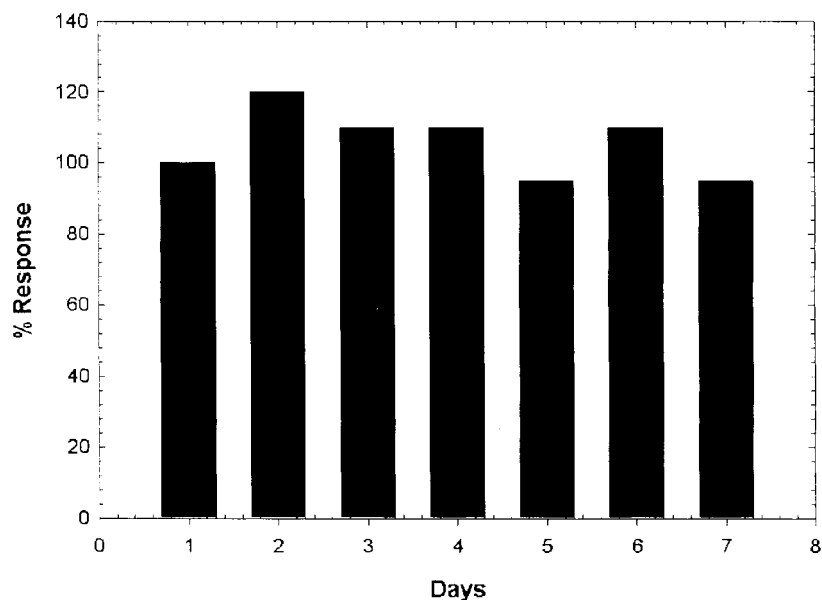


Fig. 5. The stability of PQQ encapsulated ORMOSIL-modified electrode when stored at 4°C between measurements.

6. References

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