

Determination of Hydrogen Peroxide on Modified Glassy Carbon Electrode by Polytrakis(2-aminophenyl)porphyrin Nanowire

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Nanowires of polytrakis(*o*-aminophenyl)porphyrin (PTAPPNW) were fabricated by electrochemical polymerization with the cyclic voltammetric method in anodic aluminum oxide (AAO) membranes. The glassy carbon electrode (GCE) modified by PTAPPNW, single-walled carbon nanotubes (SWNT) and Nafion as a binder was investigated with voltammetric methods in a phosphate buffer saline (PBS) solution at pH 7.4. The PTAPPNW + SWNT + Nafion/GCE exhibited strongly enhanced voltammetric and amperometric sensitivity towards hydrogen peroxide (H₂O₂), which shortened the response time and enhanced the sensitivity for H₂O₂ determination at an applied potential of 0.0 V by amperometric method. The PTAPPNW + SWNT + Nafion/GCE can be used to monitor H₂O₂ at very low concentrations in biological pH as an efficient electrochemical H₂O₂ sensor.

Key Words: Porphyrin nanowire, Single-walled carbon nanotubes, Hydrogen peroxide, Electrochemical sensor, Amperometry

Introduction

Hydrogen peroxide (H₂O₂) is a pale-blue, covalent liquid that is freely miscible with water and apparently able to cross cell membranes readily, *via* unknown pathways.¹ Numerous papers have described high (usually $\geq 50 \mu\text{M}$) levels of H₂O₂ as being cytotoxic to a wide range of animal, plant and bacterial cells in culture.¹⁻⁵ It is therefore widely thought that H₂O₂ is very toxic *in vivo* and must be rapidly eliminated, employing enzymes such as catalases, peroxidases (especially glutathione peroxidases) and thioredoxin-linked systems.^{1,6-9} H₂O₂ is an increasingly recognized, small molecule mediator of physiology, aging, and disease in living organisms.¹⁰⁻¹⁵ In this regard, aberrant production or accumulation of H₂O₂ within cellular mitochondria over time due to environmental stress and/or genetic mutation is connected to serious diseases where age is a risk factor, including cancer and neurodegenerative Alzheimer's, Parkinson's, and Huntington's diseases.¹⁶⁻¹⁸

Recently, many researchers have used the measurement of H₂O₂ concentration for the analysis of glucose in living systems. Titanium-porphyrins and cobalt-porphyrins as H₂O₂ catalysts have been used for the determination of glucose and H₂O₂ levels.¹⁹⁻²¹ After the discovery of carbon nanotubes (CNTs), they were used for the determination of dopamine,²²⁻³⁰ serotonin,^{29,30} norepinephrine,³¹ dihydronicotinamide adenine dinucleotide (NADH),³²⁻³⁴ and ascorbic acid (AA).³⁵ because CNTs have excellent biocompatibility and electron transfer ability. Meanwhile, many papers have used CNTs modified with molecular derivatives or enzymes to measure glucose and H₂O₂.³⁶⁻⁴¹ The gold electrode modified with SOD and polypyrrole was used to determine the H₂O₂ generated from the decomposition of active oxygen.⁴²

Their modified electrodes, with supporting molecules as a binder or ion-exchanger, have been applied to the determination

of many biomolecules. Since the pioneering work of Martin's group, nanomaterials of polyaniline, polypyrrole and polythiophene have been prepared using template synthesis.⁴³⁻⁴⁶ Several types of nanowires or nanotubes have been prepared using multiporous anodic aluminum oxide (AAO) membrane, in order to increase their tensile strength and size uniformity.⁴⁷⁻⁵⁴ Voltammetry is very useful for the preparation of high quality nanowires in the multiporous AAO substrate, due to the control of electrochemical values.⁵⁵⁻⁵⁹

In this study, the nanowires of polytrakis(*o*-aminophenyl) porphyrin (PTAPPNW) were prepared by electrochemical polymerization with cyclic voltammetric method in AAO membranes. We studied the electrochemical properties of PTAPPNW and its application to the determination of H₂O₂ by electrocatalytic reduction. The modified GCE prepared with single-walled CNTs (SWNT), Nafion and polyporphyrin nanowire was investigated for its H₂O₂ voltammetric and amperometric response.

Experimental Section

Chemicals and electrochemical apparatus. Tetrakis(*o*-aminophenyl) porphyrin (TAPP) was purchased from Midcentury. SWNT (1.2 - 1.5 nm in diameter produced by arc method) were purchased from Aldrich. AAO films (d = 200 nm, anodisc) for use as a template for the nanowires were purchased from Whatman. H₂O₂, and all other reagents used were of analytical grade. The pH of phosphate buffer saline (PBS) solution was adjusted with 0.1 M H₃PO₄ and 0.1 M NaOH. High purity argon was used for deaeration. All experiments were carried out at room temperature. Doubly distilled water with resistibility over 18 M Ω cm⁻¹ in a quartz apparatus was used to prepare all aqueous electrolyte solutions.

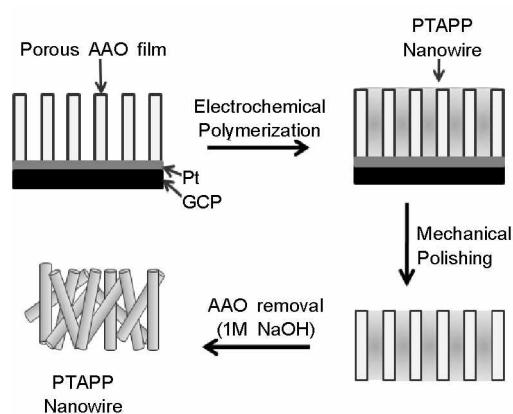


Figure 1. Schematic of PTAPP nanowire fabrication by electrochemical polymerization.

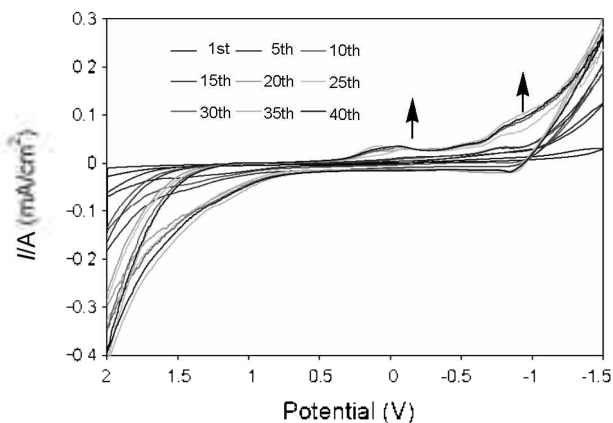


Figure 2. CVs of electrochemical polymerization of TAPP in PBS at the AAO membrane. Scan rate: 0.02 V/s, 40 cycles.

The voltammetric measurements were accomplished with a three-electrode potentiostat [Bioanalytical Systems (BAS) 100 B/W and CHI 700 C] in a ground Faraday cage. A platinum-wire electrode was used as an auxiliary electrode. A Ag/AgCl electrode (3.0 M NaCl) supplied by BAS was used as the reference electrode. A 3.0 mm-diameter GCE and glassy carbon plate (GCP: 25 × 25 mm) were used as a working electrode, the surface of which was highly polished with alumina paste prior to each experiment. All reported potentials were measured with respect to a Ag/AgCl electrode at room temperature under argon atmosphere. The pH measurements were performed by a pH glass electrode with a JENCO meter. A field emission scanning electron microscope (FE-SEM) image of the modified electrode was obtained on a JSM-7500F FE-SEM microanalyzer (JEOL).

Preparation of PTAPPNW. The TAPP (1 mg) solution of CH₃CN:CH₃Cl (1:1, 1 mL) sank into the pores of the Pt-coated (ab. 30 nm) AAO membrane slowly, after which PTAPPNW was prepared under cyclic voltammetry conditions by sweeping the potential from 2.0 to -1.5 V versus Ag/AgCl with a scan rate of 0.02 V/s, for 40 cycles at room temperature in PBS solution.⁶² After the preparation of PTAPPNW, the AAO membrane was treated with 1.0 M NaOH solution to dissolve the AAO template, and then washed several times

with distilled water carefully and filtered with polycarbonate membrane (pore size: 5 μm). The PTAPPNW was obtained and dispersed in water. Fig. 1 illustrates the scheme of the PTAPPNW fabrication by electrochemical polymerization.

Modification of the electrode. The SWNTs were treated by sonication in a mixture of HNO₃ and H₂SO₄ for 4 h at 60 °C, and then washed several times with distilled water carefully and filtered with a polycarbonate membrane (pore size: 5 μm). Meanwhile, the GCE surface was highly polished with alumina paste, sonicated with ultrasonic agitation for 5 min, washed with 1.0 M HCl solution, and then rinsed with distilled water several times and methanol finally. After being cleaned thoroughly, the GCE was coated with the 0.5% Nafion solution containing PTAPPNW (1 mg/mL) and SWNT (1 mg/mL), and then the solvents were evaporated in air at room temperature. The PTAPPNW + SWNT + Nafion/GCE have been used as the working electrode for the determination of H₂O₂. Meanwhile, 0.5% Nafion solutions of PTAPPNW (1 mg/mL) and of SWNT (1 mg/mL) were used for the modified electrodes of PTAPPNW + Nafion/GCE and SWNT + Nafion/GCE, respectively. Before and after each experiment, the PTAPPNW + SWNT + Nafion/GCE have been washed with distilled water. All experiments were carried out in a 15 mL electrolytic cell with 5 mL PBS solutions, while the dioxygen was continuously removed by purging with high-purity argon.

Results and Discussion

Electrochemical properties of PTAPPNW. The synthesizing process of PTAPPNWs is simple and easy to control. Before the electropolymerization starts, it is always essential that the AAO template be immersed into monomer solution for adequate time to ensure monomer diffusion in the narrow pores of the template. After 40 cycles of electropolymerization the deposition was completed. Fig. 2 represents the cyclic voltammograms (CVs) recorded during the electropolymerization of TAPP at AAO membrane by sweeping the potential from 2.0 to -1.5 V versus Ag/AgCl with a scan rate of 0.02 V/s for 40 cycles at room temperature in PBS solutions. The cathodic scan gave two peaks at about 0.0 V and -0.8 V. The two peaks were unclear at initial scan, but became clear after the 20th cycle. Until the 20th cycle, the reduction currents increased markedly, but after the 20th cycle they grew slightly. In this study, an applied potential of around 0.0 V was used for the determination of H₂O₂ by electrochemical reduction.

The AAO template was treated in a 1.0 M NaOH solution for 1 hour to obtain the PTAPPNW nanowire by removal of the AAO membrane. The PTAPPNWs were characterized using FE-SEM. Fig. 3 exhibits the FE-SEM image of the synthesized PTAPPNW. Figs. 3A and B show the very uniform shape of the PTAPPNW. The average length of the PTAPPNW was 40 μm and the diameter was about 200 nm, which corresponded to the diameter of the nanopores in the blank AAO membrane. Fig. 3C exhibits the binding shape of SWNT on the PTAPPNW. The prepared nanowires were straight, continuous, and smooth, indicating that the PTAPPNW can be prepared in a very uniform and controlled way.

Electrochemical response of H₂O₂ at the modified GCE. The CVs of 5.0 mM H₂O₂ at the bare GCE, PTAPPNW + Nafion/

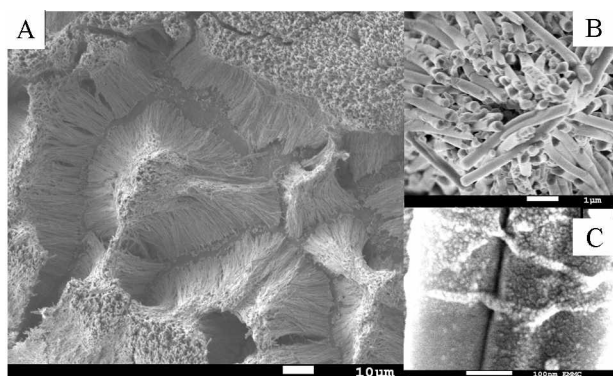


Figure 3. FE-SEM images of PTAPPNW (A; $\times 500$), (B; $\times 8,500$) and PTAPPNW + SWNT (C; $\times 150,000$).

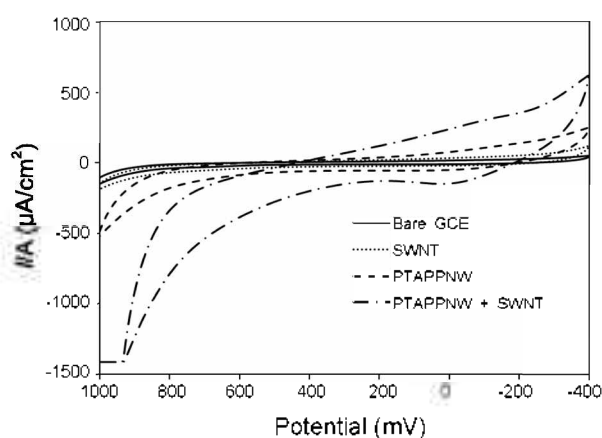


Figure 4. CVs obtained at the bare GCE, SWNT + Nafion/GCE, PTAPPNW + Nafion/GCE and PTAPPNW + SWNT + Nafion/GCE at pH 7.4 in a PBS solution containing 5.0 mM H_2O_2 . Scan rates: 0.1 V/s.

GCE, SWNT + Nafion/GCE, and PTAPPNW + SWNT + Nafion/GCE in the PBS solution at pH 7.4 are shown in Fig. 4. The reduction peak current of H_2O_2 at bare GCE was very small, but was slightly increased at SWNT and PTAPPNW. Based on the PPNW + SWNT curve of Fig. 4, the reduction peak current of H_2O_2 was increased markedly, with a broad shape around 0.0 V. The response of H_2O_2 at PTAPPNW + SWNT + Nafion/GCE gave a remarkable increase when compared with the other electrodes used in this work.

Fig. 5 illustrates the amperometric response of the SWNT + Nafion/GCE, PTAPPNW + Nafion/GCE, and PTAPPNW + SWNT + Nafion/GCE with 5 μL casting solution to subsequent additions of 1.0 mM H_2O_2 in PBS at an applied potential of 0.0 V. In Fig. 5, the amperometric response of H_2O_2 at the PTAPPNW + SWNT + Nafion/GCE was remarkably increased compared with the other electrodes studied. The PTAPPNW + SWNT + Nafion/GCE enhanced the amperometric response of H_2O_2 more than 5 or 10 times greater than those at the other modified electrodes. The PTAPPNW + SWNT + Nafion/GCE responded rapidly to the changing H_2O_2 concentration, with a response time of within 5 s. The inset of Fig. 5 shows the plot of the response currents vs. the H_2O_2 concentration.

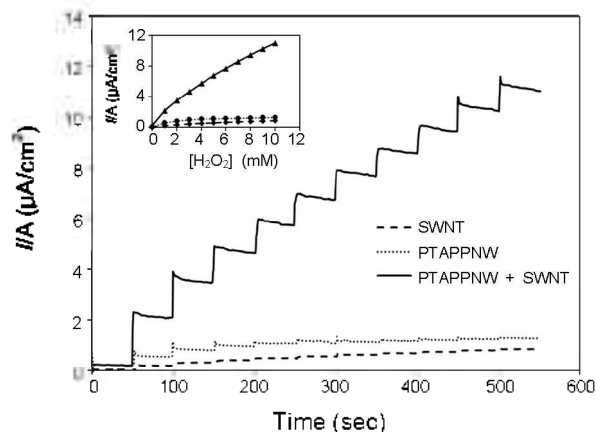


Figure 5. Amperometric response of 0.0 ~ 10.0 mM H_2O_2 (1.0 mM step) at SWNT + Nafion/GCE, PTAPPNW + Nafion/GCE and PTAPPNW + SWNT + Nafion/GCE with 5.0 μL casting solution at an applied potential of 0.0 V in 0.1 M PBS pH 7.4. Inset: plot of the response currents vs. the H_2O_2 concentration.

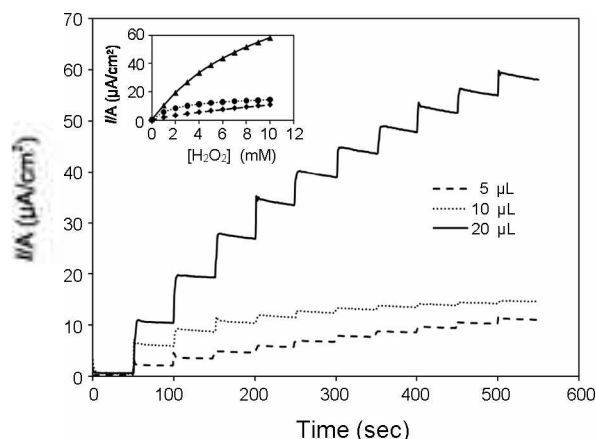


Figure 6. Amperometric response of 0.0 ~ 10.0 mM H_2O_2 (1.0 mM step) at 5, 10 and 20 μL casting mixture solutions and PTAPPNW + SWNT + Nafion/GCE at an applied potential of 0.0 V in 0.1 M PBS pH 7.4. Inset: plot of the response currents vs. the H_2O_2 concentration.

The composite of the SWNT and PTAPPNW in the electrode film (see Fig. 3C) should promote the sensitivity of H_2O_2 detection, because PTAPPNW as well as SWNT have high surface area and excellent electrical property, and Nafion is a negatively charged polymer and surfactant.

Fig. 6 illustrates the amperometric response of H_2O_2 at the PTAPPNW + SWNT + Nafion/GCE with 5, 10, and 20 μL casting solution to subsequent additions of 1.0 mM H_2O_2 in PBS at an applied potential of 0.0 V. In Fig. 6, the amperometric response of H_2O_2 at the PTAPPNW + SWNT + Nafion/GCE with 20 μL casting solution was remarkably increased, and was enhanced 4 ~ 6 times more than those at the other modified electrodes. This crucial enhancement may be attributed to the increase of the surface area of the modified electrodes due to the increase of the amount of catalysts as nanostructures. The inset of Fig. 6 provides a plot of the response currents vs. the H_2O_2 concentration. It was difficult to get a stable electrode in the modified GCE with a casting solution over 20 μL , and the

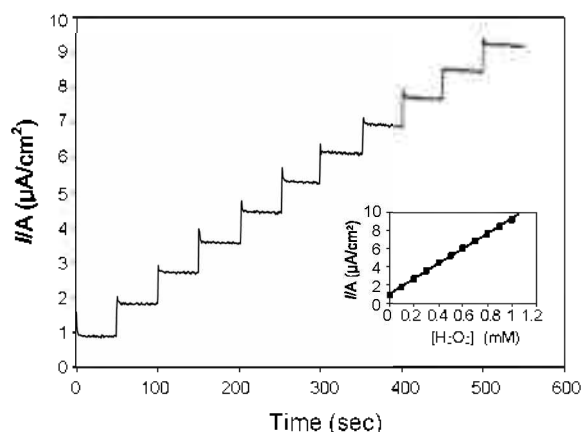


Figure 7. Amperometric response of 0.0 ~ 1.0 mM H_2O_2 (0.1 mM step) at 20 μL casting mixture solution and PTAPPNW + SWNT + Nafion/GCE at an applied potential of 0.0 V in 0.1 M PBS pH 7.4. Inset: plot of the response currents vs. the H_2O_2 concentration.

response current was saturated.

Fig. 7 illustrates the amperometric response of H_2O_2 at the PTAPPNW + SWNT + Nafion/GCE with 20 μL casting solution to subsequent additions of 0.1 mM H_2O_2 in PBS at an applied potential of 0.0 V. The inset of Fig. 7 gives the plot of the response currents vs. the H_2O_2 concentration. In the low concentration region between 100 μM and 1.0 mM, the linear regression equation was obtained as $I_p/\mu\text{A} = 0.589 [\text{C}]/\mu\text{M} + 0.072$, with a correlation coefficient of 0.999. The sensitivity is $8.41 \mu\text{A} \mu\text{M}^{-1} \text{cm}^{-2}$ which is higher value rather than $0.318 \mu\text{A} \mu\text{M}^{-1} \text{cm}^{-2}$ obtained from the literature.²¹ The detection limit (DL) was 1.0 μM at a signal-to-noise ratio (S/N) of 3 in the PBS solution at pH 7.4, representing a physiological pH. This DL is comparable to the previous results.^{19,21} The relative standard deviation of the same modified electrode in five successive scans was about 3% for 100 μM H_2O_2 , confirming the significant reproducibility of the PTAPPNW + SWNT + Nafion/GCE for the determination of H_2O_2 in biological pH.

The modified GCE prepared with SWNT, Nafion and poly-porphyrin nanowire can easily be determined H_2O_2 by amperometric method at low overpotential such as 0.0 V, and provides high sensitivity, good DL and reproducibility. Therefore, this modified electrode may be applied to the detection of H_2O_2 produced by enzyme catalytic reaction in physiological systems.

Conclusion

Nanowires of polytetrakis(*o*-aminophenyl)porphyrin (PTAPPNW) were fabricated by electrochemical polymerization with the cyclic voltammetric method in AAO membranes. PTAPPNW was easily prepared with a very uniform shape. The PTAPPNW + SWNT + Nafion/GCE exhibited strongly enhanced voltammetric and amperometric sensitivity towards H_2O_2 in a PBS solution at pH 7.4. Based on the results of the amperometric method, the response time was very short, and the linear regression equation was obtained in the very low concentration region of less than 1.0 mM at an applied potential of 0.0 V. Therefore, the PTAPPNW + SWNT + Nafion/GCE

can be used to monitor H_2O_2 at very low concentrations in biological pH as an efficient electrochemical H_2O_2 sensor. This result was attributed to the synergic effect between porphyrin-nanowire as an electroconducting polymer, SWNT as an effective electron transfer mediator, and Nafion as a negatively-charged binder.

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