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Water-soluble fluorescent conjugated polymer-enzyme hybrid system for the determination of both hydroquinone and hydrogen peroxide

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ABSTRACT

In this paper, a sensitive and simple detecting system was developed for quantitative analysis of both hydroquinone (H_2Q) and hydrogen peroxide (H_2O_2), based on the successful combination of horse radish peroxidase (HRP) and water-soluble conjugate fluorescence polymers $PPESO_3$. In the presence of HRP and H_2O_2, H_2Q could be oxidized to 1,4-benzoquinone (BQ), an intermediate, which plays the main role in the enhanced quenching of the photoluminescence (PL) intensity of $PPESO_3$. The quenching PL intensity of $PPESO_3$ (I_0/I) was proportional to the concentration of H_2Q and H_2O_2 in the range of 1.0×10^{-6} to 2.0×10^{-3} mol/L ($R^2 = 0.996$) and 6.0×10^{-6} to 2.0×10^{-3} mol/L ($R^2 = 0.999$), respectively. The detection limit for H_2Q and H_2O_2 was 5.0×10^{-7} mol/L and 1.0×10^{-6} mol/L, respectively. The present fluorescence quenching method was successfully applied for the determination of H_2Q in the lake water, rainwater, tap-water and chemical plant wastewater samples. Compared with previous reports, the fluorescence quenching approach described in this work is simple and rapid with high sensitivity, which has a potential application for detecting various analytes which can be translated into quinone.

1. Introduction

Phenol compounds such as hydroquinone (H_2Q) are among the major components of pollutants produced by industrial wastewater and agricultural activities. H_2Q is a potentially carcinogenic substance, which causes severe effects on the central nervous system [1]. H_2Q has extremely detrimental effects on humans through oral, dermal, or respiratory tracks and thus represent a serious environmental problems and health hazards [2,3]. Due to the high toxicity and persistence of H_2Q in the environment, the sensitive and fast determination of H_2Q becomes an important subject. Many methods have been reported for the determination of H_2Q, including gas chromatography [4], chemiluminescence [5], amperometric detection and flow injection analysis [6]. However, compared with these methods, optical biosensors based on absorbance and fluorescence detection are more advanced because of several advantages [7,8]. For example, the response mechanism of optical detection is very classic, simple and reliable, and the reactions taking place in sample solutions are not disturbed because optical transducers do not change the component in the solutions. Recently, optical sensors based on fluorescence quenching have been paid more attention. Wu et al. detected phenol optically using an oxygen-sensitive luminescent dye [7]. Wang et al. used a quantum dots-enzyme system to detect phenolic compounds and H_2O_2 [9]. Zhu et al. used molecularly imprinted polymer microspheres prepared through precipitation polymerization for H_2Q recognition [1].

In recent years, conjugated polymers (CPs) have been intensively studied for chemical and biological sensor applications owing to their superior signal amplification and superquenching compared to quantum dots and small molecule dyes [10]. These amplification and superquenching properties are due to the conjugated polymer backbone [11], on which one single quencher molecule can cause an effective and fast quenching. Thanks to these amplification and superquenching properties, CPs show some interesting and useful properties i.e. strong light absorption, strong fluorescence, electroactivity, and good transport properties for charge carriers and excitons [12]. So far, various analytes, such as metal ions [13], small molecules [14], enzymes [15], proteins [16], DNA [17] and RNA [18] have been detected in aqueous solution or on solid substrates. Since CPs quenched by phenol have not been reported previously and indeed might have wide potential application in the development of phenol sensor, we developed a H_2Q sensor based on CPs ($PPESO_3$) quenching.

Based on the enzymatic reaction product of H_2Q can efficiently quench the photoluminescence (PL) intensity of $PPESO_3$, we established a sensitive and simple H_2Q sensor. Meanwhile, the $PPESO_3$ PL intensity can be quenched with the addition of H_2O_2 in the presence of H_2Q, thus detection of H_2O_2 can also be achieved. The detection limits for H_2Q compounds (5.0×10^{-7} mol/L) are low enough to detect the common levels of phenolic pollutants in wastewater and lower than the U.S. Environmental Protection Agency

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estimated wastewater discharge limit of 0.5 mg/L (at 10^{-6} mol/L level) [19].

2. Experiment

2.1. Chemicals

1,4-Diethynylbenzene and Tetrakis(triPHenylPHosphine)palladium ($(PPh_3)_4$-Pd) were purchased from Aldrich Chemical Co. and Hangzhou Kaida Metal Catalyst & Compounds Co. Ltd. (Hangzhou, China), respectively. 2,5-Diiodohydroquinone, dioxane (Tianjin Guangfu Institute of elaborate chemical industry) and 1,3-propanesultone (J&K Chemica) were used as received. Horse radish peroxidase (HRP) was obtained from Sino-American Biotechnology Co. Ltd. H_2Q and benzoquinone (BQ) were purchased from Tianjin Guangfu Fine Chemical Research Institute. All the other chemicals, including CuI, Na_2HPO_4, NaH_2PO_4, H_2O_2 methanol, acetone, ether and DMF were obtained from Beijing Chemical Co. Ltd. Stock solutions of 0.1 mol/L H_2O_2, benzoquinone, hydroquinone, and HRP were freshly prepared daily. All chemicals used were of analytical reagent grade without further purification. The water used in all experiments had a resistivity of 18 MΩ/cm. All the water used in the experiments was deaerated by purging with N_2 for 30 min.

2.2. Instrumentation

All fluorescence measurements were carried out in a 1 cm pathlength quartz cuvette at ambient temperature with a Shimadzu RF-5301 spectrometer. In all optical experiments, the excitation wavelength was 400 nm and the fluorescence intensity referred to the maximum emission of $PPESO_3$ at 520 nm.

2.3. Experimental method

The synthesis of $PPESO_3$ was according to the previous paper [20]. For BQ quenching experiment, 1 mL of 2.0 μmol/L $PPESO_3$ in PBS (pH 7.0) solution and 1 mL of BQ solution with different concentrations from 2.0 μmol/L to 6.0 mmol/L were added to a quartz cuvette. The final volume of every sample is 2.0 mL and the final concentration of $PPESO_3$ is 1.0 μmol/L. Then the mixture was shaken evenly and the spectral information was recorded by spectrofluorophotometer. For the assay of H_2Q, the conjugated polyelectrolyte $PPESO_3$ was diluted to 1.0 μmol/L with PBS (pH 7.0), followed by the addition of 2.0 μg/mL HRP and 3.0 mmol/L H_2O_2. Then, various amounts of H_2Q were added, with the ultimate concentration of H_2Q covered a relatively wide range (1.0×10^{-6} to 2.0×10^{-3} mol/L). The resulting solution (2.0 mL) was shaken evenly kept at room temperature for 14 min before recording the spectral information by spectrofluorophotometer. The method of detecting H_2O_2 was same as that of H_2Q. As H_2Q is easily oxidized under environmental conditions, so deoxidation water were used in each H_2Q detecting experiment. For real samples detection, the system can be used to determine both H_2Q and BQ. Combination of the system and the hybrid without H_2O_2 and/or HRP would be useful to detect H_2Q and BQ individually. The molecular structures of BQ, H_2Q and $PPESO_3$ are shown in Scheme S1 (in supplementary material).

For real samples detection, we chose four kinds of water samples (lake water, rainwater, chemical plant wastewater and tap-water) and all the water samples were diluted 10 times with 0.02 mol/L PBS (pH 7.0). The diluted water samples were added with different concentrations of H_2Q (0.1 mmol/L, 1.0 mmol/L, 2.0 mmol/L) to prepare the spiked samples. Real samples detection was carried out using the procedure described above.

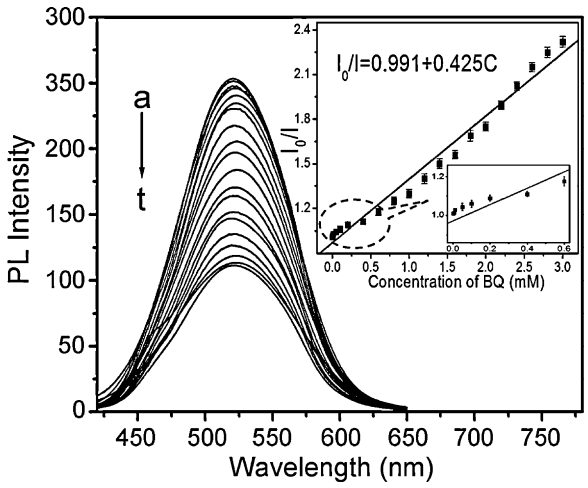

Fig. 1. The PL spectra of 1.0 μmol/L $PPESO_3$ in different concentrations of BQ. a–u represent the concentrations of BQ of 0.001, 0.006, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 mmol/L, respectively. The inset shows the linear relationship between I_0/I and concentration of BQ. The excitation wavelength was 400 nm.

3. Results and discussion

3.1. Superquenching of water-soluble $PPESO_3$ by 1,4-benzoquinone

1,4-benzoquinone (BQ) as a classic electron acceptor could shuttle the electron from the conduction band to the valence band of the excited luminescence material, resulting in the quenching of PL intensity [21]. In aqueous solution, $PPESO_3$ exhibited a strong green emission at around 520 nm. When BQ was added to $PPESO_3$ PBS solution, even at very low concentration, the fluorescence of $PPESO_3$ was quenched remarkably. The quenching was enhanced gradually with the increasing quantity of BQ. And the efficiency of fluorescence quenching can be evaluated quantitatively by the Stern–Volmer equation [22].

$$\frac{I_0}{I} = 1 + K_{SV}[Q]$$

where I and I_0 are the fluorescence intensities with and without the quencher, respectively, [Q] is the quencher concentration, and K_{SV} represents the Stern–Volmer constant. From the slope of the Stern–Volmer plot (Fig. 1 inset), K_{SV} is calculated to be 4.25×10^2 L/mol, which shows that BQ is an effective quencher by accepting electron from $PPESO_3$. A linear relationship between I_0/I and concentration of BQ was gained from 1.0×10^{-6} to 3.0×10^{-3} mol/L BQ. The PL intensity of $PPESO_3$ was quenched more than 95% by 8.0 mmol/L BQ (not shown in the figure).

The relationship between the reaction time and PL intensity of $PPESO_3$ was shown in Fig. 2. It can be seen that upon addition of BQ, the PL intensity of $PPESO_3$ quenched immediately due to electron transfer quickly from $PPESO_3$ to BQ, then the fluorescence intensity remained nearly unchanged with further increase in reaction time, which indicated that it was very rapid to reach equilibrium for the interaction between BQ and $PPESO_3$.

To distinguish between static and dynamic mechanisms, temperature dependence of Stern–Volmer equation should be addressed. Fig. S1 (in supplementary material) shows the Stern–Volmer plot for fluorescence quenching system of $PPESO_3$-BQ at two different temperatures. The K_{SV} is 1.10×10^3 L/mol at 313 K while the K_{SV} is 4.25×10^2 L/mol at 288 K. The results show that the Stern–Volmer quenching constant is in proportion to