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A colorimetric and ratiometric fluorescent probe for quantitative detection of GSH at physiologically relevant levels

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ABSTRACT

A naphthalene derivate containing piazselenole (NDP) has been developed as an example of colorimetric and ratiometric fluorescent probes for glutathione (GSH) at physiologically relevant concentration. Upon addition of GSH, the probe displayed a ratiometric fluorescent response with an enhancement of the ratios of emission intensities at 436 and 615 nm, accompanied with the solution color change from jacinth to colorlessness. The detection range is 0–80 mM with the detection limit of 0.178 mM. Moreover, the probe showed good selectivity and satisfying results in the determination of GSH. As the linear response range covers the concentration range of biological samples, the probe may have the potential to determine GSH directly in biological samples in the future.

1. Introduction

Glutathione (GSH) is a primary non-protein thiol compound in most mammalian cells [1]. It plays an important role in protecting cells from free radicals and reactive oxygen species and maintaining an adequate intracellular redox status. Also, GSH is involved in various physiological processes, detoxification of xenobiotics (for example oxoaldehydes, polyphenols), transportation of amino acids, stabilization of cell membranes, synthesis of proteins and DNA, and so on [2–6]. The absence of GSH in biological bodies may lead to many diseases, such as occlusive vascular, premature arteriosclerosis, leukemia, and diabetes [7]. Therefore, it is of extreme importance to develop appropriate methods to quantitatively detect GSH in biological samples.

Several methods are available for the determination of GSH, including electrochemistry [8,9], liquid chromatography [10–12] and mass spectrometry [13]. Though many methods have been reported to determine GSH, spectrofluorimetric method is commonly used high sensitivity [14–24]. Different spectrofluorimetric methods such as Zn(II)-8-hydroxyquinoline-5-sulphonic acid [25], Ce(III) with disulfide bonds [26], and naphthalene-2,3-dicarboxaldehyde [27], have been developed for the detection of GSH. However, most of them require complicated and cumbersome laboratory procedures. In particular, these technologies do not provide the proper concentration range of GSH under normal physiological conditions, for the concentration in biological samples is about 1–10 mM [7]. To the best of our knowledge, only two sensing systems for the detection of GSH at physiologically relevant levels have been reported. One based on quantum dot that used to detect GSH in accord with the requirement, but this probe only works on one wavelength band intensity with no shift of the absorbance and emission wavelength [28]. The other is our previous indicator that can detect the physiological level of GSH quantitatively, but the synthetic process of this indicator is relative complex [22].

Recently, colorimetric and ratiometric sensors have drawn more and more attention. Colorimetric sensors is a class of sensors that transform the molecular recognition events into color changes, so monitoring process can be judged by the naked eye instead of by complex expensive instruments [29–33]. Consequently, colorimetric method can be applied with very low experimental cost and simple experimental process. As to ratiometric method, it has a significant advantage in solving the problems produced by variabilities of sample environment and probe distribution in the quantitative measurement of analytes. Also, it can eliminate the effects of spurious fluctuations coming from the intensity of the excitation source [34–37].

To improve the synthetic process and obtain a proper detection range that can be used under normal physiological conditions, in

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this paper, we reported a simple naphthalene derivate containing piazselenole (NDP) for the detection of GSH, which includes many properties, such as high selectivity, naked-eye and ratiometric quantitative detection, and suitable working range. We hypothesized that the reaction of colored long-wavelength emission NDP with GSH would deliver colorless short-wavelength naphthalene-2,3-diamine (DAN), which might allowed the determination of GSH by naked-eye and ratiometric fluorescence methods.

2. Experimental

2.1. Apparatus and reagents

Fluorescence measurement was performed with a Cary Eclipse spectrofluorimeter (Varian Company, USA) equipped with 1.0 cm quartz cells. The UV–vis spectrum was recorded on a spectrophotometer (Shimadzu, Japan) equipped with a 1.0 cm quartz cell. The pH measurement was carried out with a PHSJ-3F pH acidmeter (ShangHai Precision & Scientific Instrument Co. Ltd.). ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker AMX400 spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard in DMSO-d_6. High-resolution mass data was measured with Fourier transform ion cyclotron resonance mass spectrometer (APEX IV).

All reagents were of at least analytical grade and the solutions were prepared with twice distilled water. Reduced glutathione (Amersco), L-cysteine (Sigma) and all amino acids were purchased from Beijing AoBoXing Biotech Company. A stock solution of 0.25 mM NDP was prepared by dissolving appropriate amount of NDP in ethanol. A stock solution of 0.1 M GSH was prepared by dissolving appropriate amount of GSH in twice distilled water and stored at 4 °C.

2.2. Synthesis of NDP

Naphthalene-2,3-diamine (316.4 mg, 2 mmol) and selenium dioxide (244.2 mg, 2 mmol) were ground respectively, and then mixed in a mortar at room temperature. After 30 min of grinding, the crude products were obtained and dissolved in *n*-hexane, and then filtered. The solvent was removed under reduced pressure to give the desired product (423 mg, 91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.355 (*d*, *J* = 9.6 Hz, 2H), 7.935 (*d*, *J* = 6.8 Hz, 2H), 8.538 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 119.795, 126.944, 128.088, 129.355, 134.353, 134.584, 135.374, 158.662, 160.266, 178.796. HRMS (ESI positive) calcd for $C_{10}H_7N_2Se$ $[M+H]^+$ 234.97690, found 234.97666.

2.3. Procedures

The spectral properties of NDP were measured in a mixture of ethanol and 20 mM phosphate buffered saline (PBS) (3:7, v/v) solution. All fluorescence spectra were recorded at room temperature in the range 400–750 nm with exciting wavelength at 380 nm. The width of the excitation and emission slit was set to 10 nm and 10 nm. The UV–vis spectrum was performed at room temperature in the range 190–600 nm.

3. Results and discussion

3.1. Absorption and fluorescence characteristic spectra

To investigate whether NDP can be a ratiometric and colorimetric GSH probe, a series of tests was carried out. Firstly, NDP solution was diluted in ethanol and 20 mM PBS solution (3:7, v/v, pH 7.4). With the addition of GSH, the solution showed a color change from

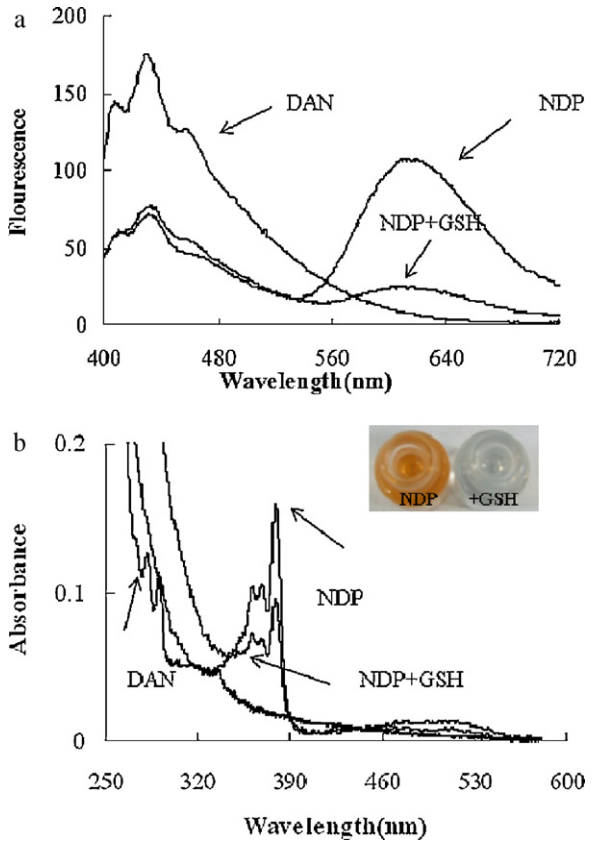

Fig. 1. Fluorescence emission (a) and absorption (b) spectra of the NDP (10 μM), NDP–GSH (80 mM) system in PBS (20 mM) solution (ethanol: PBS = 3:7, v/v, pH 7.4) and DAN (10 μM). Excitation wavelength was 380 nm. Insert photo is the photographs of the solution of NDP (75 μM, ethanol) in the absence (left) and presence (right) of GSH (80 mM, PBS, 20 mM, ethanol:PBS = 3:7, v/v, pH 7.4).

jacinth to colorlessness while the solution maintained its original color in the absence of GSH (Fig. 1). This phenomenon indicates that NDP can be used as a colorimetric GSH probe. Moreover, the color changed gradually with the GSH concentration increased (Fig. 5). This colorimetric assay for the determination of GSH is much simpler than the above-mentioned methods with no requirements of the instrument.

Secondly, to prove that NDP can be used as a ratiometric probe, the absorption and fluorescence emission spectra of NDP–GSH were recorded and the results were shown in Fig. 1. These results indicate that NDP shows notable changes in their absorption spectra and fluorescence spectra in the presence of GSH. The absorbance of 380 nm greatly decreased after reaction with GSH. In the presence of GSH, NDP shows an enhancement at 436 nm and a decrease at 615 nm in the fluorescence spectra. These results clearly indicate that along with the presence of GSH, NDP can be a good colorimetric and ratiometric probe for the determination of GSH.

3.2. Effect of pH

The pH value of the buffer solution is usually regarded as one of most important factors in evaluating the performance of the probe. To investigate the effect of the pH value on this reaction, Britton-Robinson (BR) buffers with different pH were employed to adjust the pH value. Emission spectra of GSH and NDP–GSH at varied pH were recorded. As shown in Fig. 2, the probe alone showed no distinct changes in the range of 3.0–10.0. With the addition of GSH, the fluorescence ratio was stable in the range of 3.0–10.0. Considering that pH 7.4 is normal physiological pH value condition, we