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RESEARCH ARTICLE

SYNTHESIS AND CHARACTERIZATION OF A Cu^{2+}-SELECTIVE FLUORESCENT PROBE BASED ON NAPHTHALIMIDE DERIVATIVE

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ABSTRACT

A Cu^{2+}-sensitive fluorescent probe **P** based on naphthalimide derivative was designed and characterized. Study showed that **P** had good selectivity to Cu^{2+} among the tested metal ions. The fluorescent intensity was proportional to the concentration of Cu^{2+} in the range of 1.5×10^{-6}-7.0×10^{-6} M with a detection limit of 5.0×10^{-7} M Cu^{2+}. The possible binding mode of **P** with Cu^{2+} was also proposed.

INTRODUCTION

Copper plays important roles in living bodies (1). However, high concentration of copper is toxic and dangerous to some organisms such as bacteria and viruses (2). So far as humans concerned, it can cause neurodegenerative diseases (e.g., Alzheimer's and Wilson's diseases) probably by its involvement in the production of reactive oxygen species (3). Thus, great efforts have been devoted to develop efficient methods to detect Cu^{2+} in the environment (4). Fluorescent probes allow nondestructive and prompt detection by a simple fluorescence enhancement (off-on) or quenching (on-off) response (5-7). Thus, it is of great interest to design and synthesize fluorescent probes which led to the change of fluorescence signals before and after the combination of analysts (7-9). In this work, an "on-off" type Cu^{2+}-fluorescent probe was synthesized and characterized (Scheme 1), studies showed that this probe has good fluorescent response to Cu^{2+} compared other tested metal ions.

Experimental Section

Reagents and Instruments: All reagents and solvents are of analytical grade and used without further purification. UV-Vis spectra were obtained on a Hitachi U-2910 spectrophotometer. Mass (MS) spectra were recorded on a Thermo TSQ Quantum Access Agillent 1100 system. Nuclear magnetic resonance (NMR) spectra were measured with a Bruker AV 400 instrument and chemical shifts are given in ppm from tetramethylsilane (TMS).

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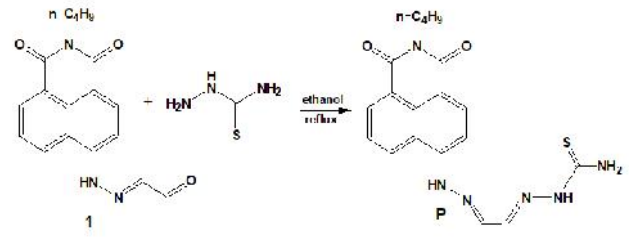
Synthesis of P: Compound **1** was synthesized according to the reported method [10]. Under N_2 gas, compound **1** (35.2 mg) and thiosemicarbazide (17.0 mg) were mixed in ethanol (30 mL) and stirred under reflux for 5 h, and then the solution was cooled to room temperature. The orange precipitate so obtained was filtered and washed with cold ethanol and used directly. Yields: 80.8%. MS m/z: 395.9 $(M\text{-}H^+)^-$. ^1H NMR (DMSO, d_6): 11.69 (s, 1H), 11.66 (s, 1H), 8.72 (s, 1H), 8.47 (t, 1H), 8.36 (d, 2H), 8.10 (t, 1H), 7.90 (d, 2H), 7.79 (d, 1H), 7.60 (t, 1H), 4.01 (t, 2H), 1.58 (m, 2H), 1.33 (m, 2H), 0.91 (t, 3H).

General Spectroscopic Methods: Metal ions and probe **P** were dissolved in deionized water and DMSO to obtain 1.0 mM stock solutions, respectively. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to the corresponding desired concentration. For all fluorescent measurements, the slit widths of emission and excitation were both 10 nm, excitation wavelength was set as 450 nm.

RESULTS AND DISCUSSION

Selectivity Measurement of P: Selectivity was an important property of probe. So, the selectivity measurement was firstly conducted in ethanol-water solution (1:1, v:v, pH7.0, 20 mM HEPES) (Figure 1), and the testing cations were Na^+, K^+, Ag^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cr^{3+} and Fe^{3+}. From the results we can include that the addition of Cu^{2+} to the solution of **P** caused obvious fluorescent quenchment at 550 nm, and only Hg^{2+} has little influence on the response of **P**. Thus, this compound was characterized as a Cu^{2+}-selective probe.



Scheme 1. Synthesis route of compound P

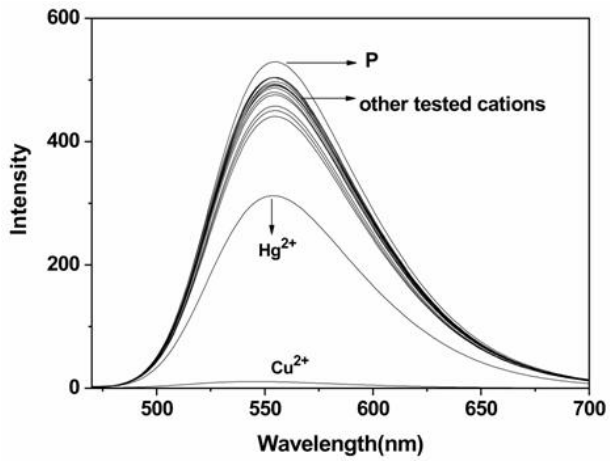


Figure 1. Fluorescent emission spectra of P (10 μM) to different metal ions (50 μM) in ethanol-water solution (1:1, v:v, pH7.0, 20 mM HEPES)

Water Effect on P and P-Cu^{2+} System: The content of water has big effect on the response of probe since it is organic molecule. According to the theory that similarities can be solvable easily in each other, the addition of water decreased the solubility of probe, which affected the performance of the probe. In this work, the content of water has bigger influence on **P** than that of on **P**-Cu^{2+} system (Figure 2). According to the results, water and ethanol in a ratio of 1:1 (v:v) was used throughout this work.

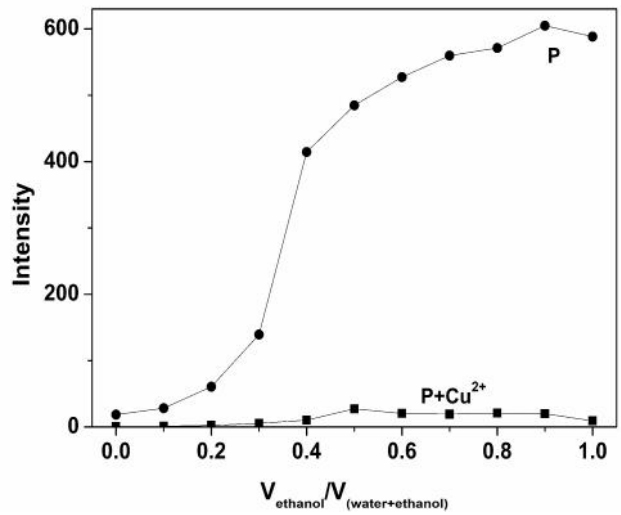


Figure 2. Effects of water content in the testing solution on the fluorescence intensity of P (10 μM) and P-Cu^{2+} (50 μM) system.

pH Effect on P and P-Cu^{2+} System: pH titration experiment was performed to investigate a suitable pH value for the sensing of **P** to Cu^{2+} (Figure 3).

The results showed that the fluorescent change of free probe **P** was not obvious in the range of pH 4.0–10.0. However, the addition of Cu^{2+} to the solution of **P** caused a fluorescent quenchment at 550 nm in the range of pH4.0–10.0, and the minimum was got at pH 6.0-10.0. For the real sample considered, pH7.0 was chosen for the fluorescent studies of **P**.

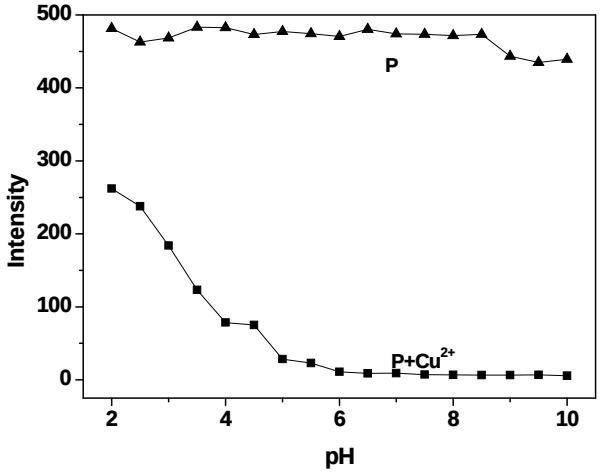


Figure 3. Influences of pH on the fluorescence spectra of P (10 μM) and P (10 μM) with Cu^{2+} (50 μM) in an ethanol-water solution (1:1, v:v). The pH was modulated by adding 1.0 M HCl or 1.0 M NaOH in HEPES buffers

Fluorescent Titration Experiment of P with Cu^{2+}: In order to study the reaction of **P** with Cu^{2+}, fluorescent titration experiment was carried out (Figure 4). With the addition of different concentration of Cu^{2+}, the fluorescent intensity at 550 nm decreased accordingly, and the intensity was proportional to the concentration of Cu^{2+} in the range of 1.5×10^{-6}-7.0×10^{-6} M with a detection limit of 5.0×10^{-7} M Cu^{2+}.

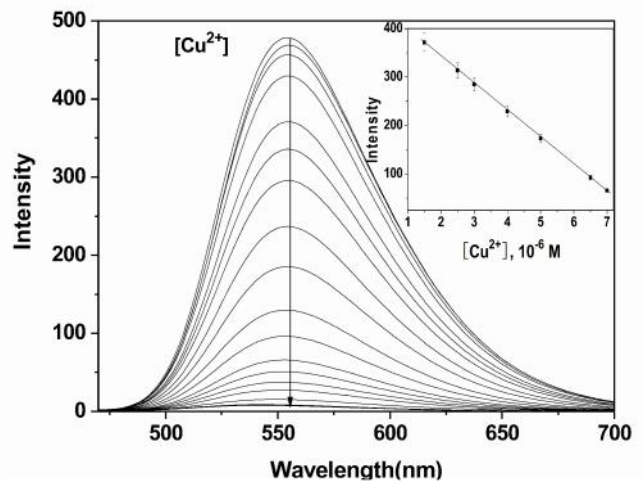


Figure 4. Fluorescence response of P (10 μM) to various concentrations of Cu^{2+} in ethanol-water solution (1:1, v:v, pH7.0, 20 mM HEPES)

Binding Mode of P with Cu^{2+}: According to the results obtained above, the binding mode of **P** with Cu^{2+} was studied with job's plot experiment (Figure 5). The total concentration of **P** and Cu^{2+} was kept as 50 μM, and with the ratio alter of **P** to Cu^{2+}, a regular fluorescent change took place, and the fluorescent intensity reached minimum at 0.5, which indicated a complex formed in 1:1 mole ratio, and the possible binding mode was shown in Scheme 2.

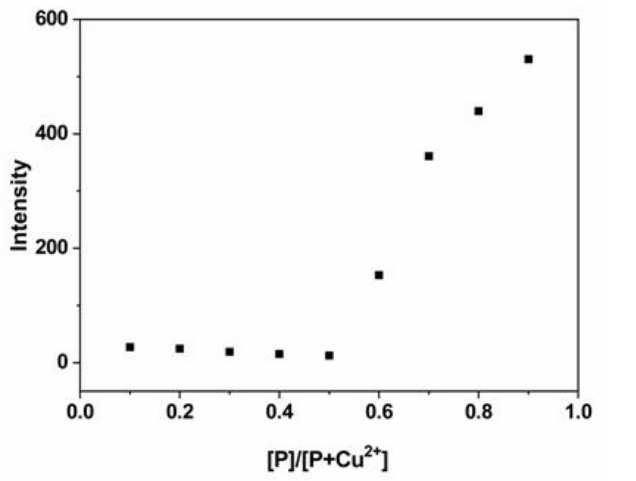
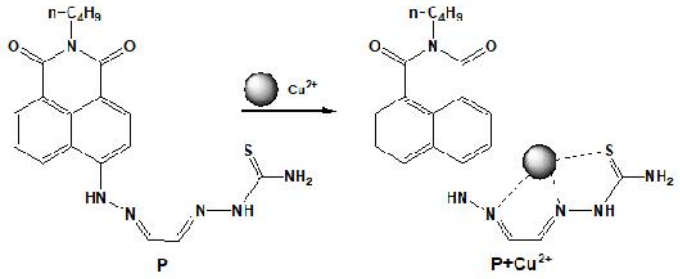


Figure 5. Job's plot of P with Cu^{2+}. Total concentration of P and Cu^{2+} was kept at fixed 50 μM



Scheme 2. Binding mode of P-Cu^{2+}

In order to study the binding mode further, reversibility of the coordination between **P** and Cu^{2+} was examined by the EDTA-adding experiments. Addition of EDTA to the solution containing **P** and Cu^{2+} led to immediate fluorescence enhancement, whereas readdition of excess Cu^{2+} could quenching the signal (Figure 6). This proved that the the reaction of **P** with Cu^{2+} was reversible.

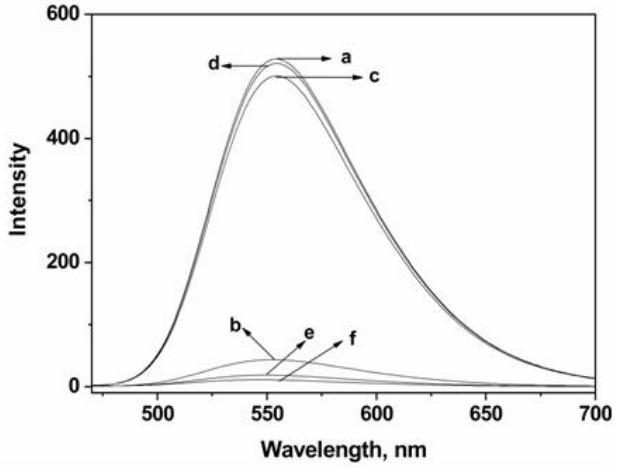


Figure 6 Reversible titration response of P to Cu^{2+} in ethanol-water solution (1:1, v:v, pH7.0, 20 mM HEPES). a: P (10 μM), b: P (10 μM) with Cu^{2+} (10 μM), c: P (10 μM) with Cu^{2+} (10 μM) and then adding EDTA (100 μM), d: P (10 μM) with Cu^{2+} (10 μM) and EDTA (100 μM) and then addition of Cu^{2+} (100 μM), f: P (10 μM) with Cu^{2+} (10 μM) and EDTA (100 μM) and then addition of Cu^{2+} (500 μM)

Acknowledgment

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