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Hemicyanine based fluorimetric and colorimetric pH probe and its application in bioimaging

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ABSTRACT

To obtain a better insight into the physiological and pathological functions of pH, probes with supra optical properties in water are still needed urgently. Focused on this situation, we herein reported a hemicyanine based probe for pH detection in aqueous solution. The probe has excellent water-solubility, high selectivity and sensitivity. Also, the structure of the probe from phenol to phenoxide induced a turn-on fluorescent response within a certain pH range of 4.5–10.7 and peaked with 30-fold enhancement, and the pK_a value was determined to be 8.5. Simultaneously with the pH increase, a prominent solution color of the system changed from yellow to amaranth. More importantly, the biological experiments demonstrated the applicable of the probe to monitor the pH changes in living cells. This work illustrates that the probe could be practical and ideal pH indicator with good biological significance.

1. Introduction

Acid-base homeostasis plays vital roles in normal physiological processes including cell metabolism, proliferation, apoptosis, ion transport and homeostasis, enzyme activity, drug resistance and endocytosis among all living species and life forms [1–5]. A series of diseases including disordered cell function, cancer, shock, rheumatoid arthritis and Alzheimer's disease [6–9] have already been confirmed to associate with abnormal intracellular pH values. However, the related functional mechanisms of pH values are still indefinable. To develop reliably techniques to monitor the pH values in both extracellular and intracellular would promote the further study of the pH modulated physiological and pathological processes.

Bedding for the requirements mentioned above, a variety of techniques such as microelectrodes [10], nuclear magnetic resonance [11], UV–Vis absorbance spectroscopy, fluorescence spectroscopy [12–23]have been developed to measure pH values. To date, optical probes are becoming one of the most powerful tools for monitoring intracellular pH among them, and possess numerous technical and practical superiorities over other techniques because of their non-invasive imaging in both cellular and subcellular regions [12–22]. The excellent properties of fluorescent probes including high sensitivity, fast responses, biological applicable, noninvasive test and low cost stimulated the development of pH probes [24–35]. Most of them were designed based on the protonation processes of aniline [36] and phenoxide moiety which displayed turn-off or turn-on fluorescent responses and had made great progresses in pH detection and bioimaging. For pH probes, however, properties including excellent optical performances, good water solubility and, most of all, approximate physiological pK_a values are needed. To develop fluorescent probes featured these properties are still badly needed for exploring the functional mechanisms of pH values in biological systems.

Holding this in mind, we designed and synthesized a hemicyanine based probe **1** and **2** ($\varepsilon = 60.64$ L/g·cm^{-1}) for pH detection in aqueous solution in this work. The synthesis route was summarized in Scheme 1. Probe **1** not only could be readily prepared, but also showed excellent sensing properties. Through the ^{1}H NMR titration experiments, we confirmed the pH sensing mechanism (Scheme 2). Owing to the indolium iodide moiety, probe **1** displayed an obvious advantage: it has excellent water-solubility compared with previously reported pH probes monitored in organic solvents [37–39]. Further, the structure changes of the probe from phenol to phenoxide induced a turn-on fluorescent response in the pH range of 4.5–10.7. And the pK_a value was

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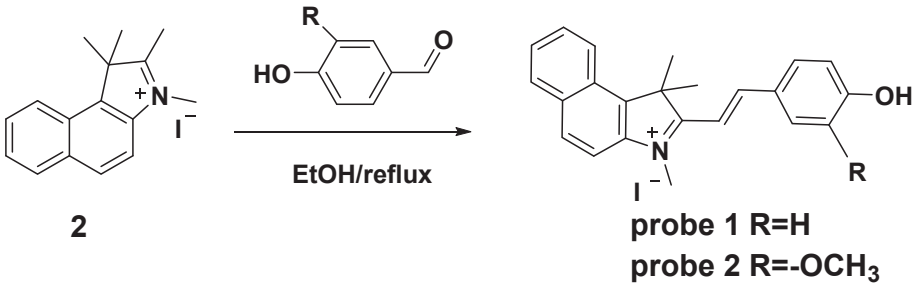
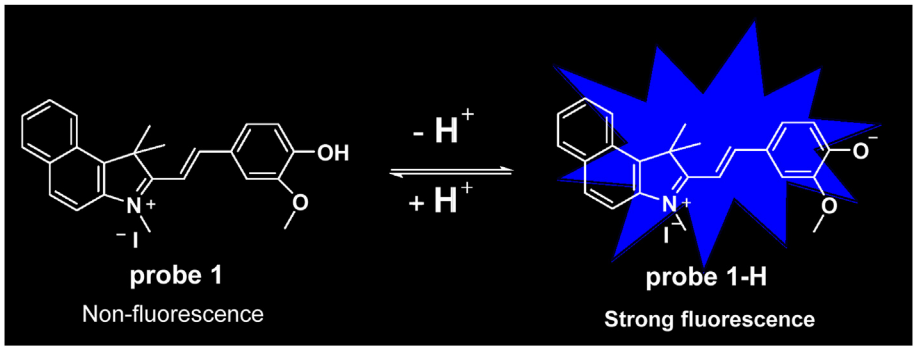


Scheme 1. The synthesis of probe **1** and probe **2**.



Scheme 2. Proposed response mechanism of probe **1** towards pH.

determined to be 8.5 that is similar to physiological pK_a value. Accompanied with the fluorimetric changes, the color of the system changed from yellow to amaranth in the naked eyes. Most important of all, the biological experiments demonstrated the applicable of probe **1** for monitor the pH changes in living cells. However, probe **2** lacking methoxy group (a well-known hyperchrome), has lower ε 50.85 L/g·cm^{-1} and high pK_a value of 8.9. So probe **1** has the higher sensitivity for UV detection.

2. Materials and methods

2.1. Materials

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were of analytical grade also without further purification. Distilled water was used after passing through a water ultrapurification system. All metal ions salts and amino acids were purchased from Shanghai Experiment Reagent Co., Ltd (Shanhai, China).

2.2. Instruments

TLC analysis was performed using precoated silica plates. A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–Visible (UV–Vis) spectra were recorded on an Agilent 8453 UV–Visible spectrophotometer. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanhai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm). ^{1}H NMR and ^{13}C NMR experiments were performed with a BRUKER AVANCE III HD 600 MHz and 151 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). Coupling constants (J values) are reported in hertz. ESI determinations were carried out on AB Triple TOF 5600 plus System (AB SCIEX, Framingham, USA). The ability of probe **1** reacting with different pH medium in the living cells was also evaluated by a Leica DMi8 fluorescence inversion microscope system.

2.3. Preparation and characterization of probe *1*

Compound **2** was conveniently synthesized according to our previous work [40]. Probe was synthesized as follows: vanilline (0.304 g, 2 mM) and compound **2** (1.2 equiv.) were dissolved in anhydrous ethanol (40 mL). The reaction mixture was then refluxed for 10 h. The solution was then removed under reduced pressure. The crude product was recrystallized from acetonitrile (10 mL) and

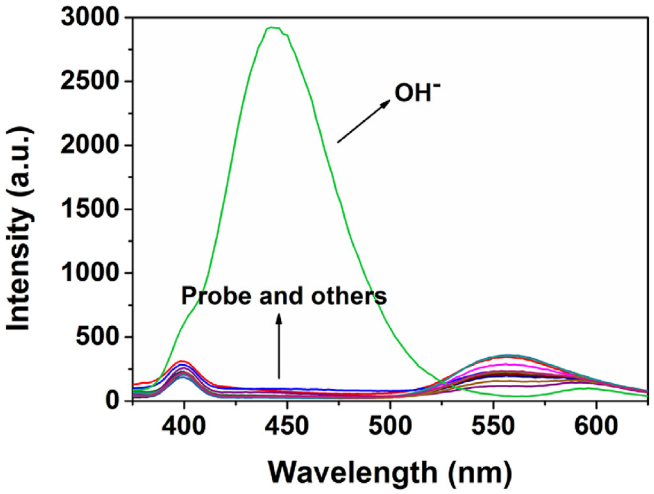


Fig. 1. Fluorescence spectra of the probe **1** (10 μM) in the presence of different metal ions and biologically relevant analytes. 1: blank; 2: GSH (10 mM); 3: Hcy; 4: Cys (3–4: 1 mM); 5: K^+; 6: Ca^{2+}; 7: Na^+; 8: Mg^{2+} (5–8: 10 mM); 9: Zn^{2+}; 10: Cu^{2+}; 11: Fe^{3+} (9–11: 10 μM); 12: H_2O_2; 13: ClO^-; 14: $ONOO^-$ (12–14: 100 μM); 15: OH^- (pH 10.5).