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A highly sensitive fluorescent acidic pH probe based on rhodamine B diethyl-2-aminobutenedioate conjugate and its application in living cells

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ABSTRACT

A highly sensitive fluorescent acidic pH probe based on rhodamine B-2-aminobutenedioate conjugate, was designed and synthesized. When the H^+ concentration falls within the pH range of 4.0–6.5, the spirolactam unit of rhodamine B-2-aminobutenedioate was opened, which resulted in the dramatic enhancement of both fluorescence and absorbance intensity as well as the color change of the solution. Background metal ions showed small or no interference with the detection of H^+. Confocal laser scanning microscopy experiments showed that rhodamine B-2-aminobutenedioate can be applied to detect acidic pH variations in living cells with a turn-on signal.

1. Introduction

Protons play an important role in many chemical and biological processes, and the development of methods that can monitor the change of pH are very important [1,2]. Many methods have been used for measurement of pH variations including microelectrodes, nuclear magnetic resonance, absorption and fluorescence spectroscopy [3–5]. Among these methods, fluorescence detection has become the promising strategy used for pH detection because of its operational simplicity, low cost, real time monitoring and high selectivity [6]. Moreover, the fluorescence microscopic imaging technique could map the distribution of H^+ within living cells [7–14]. Some fluorescent molecular pH sensors have been reported and widely used in analytical chemistry, physiology and bioscience. Most of them employ tunable acidity of phenol or amine derivatives as the pH-sensitive center [14–24]. Limitations of the currently available fluorescent pH probes include low sensitivity or excitation wavelength in the ultraviolet region. The developments of new fluorescent pH-probes with more desirable properties are still significant.

The rhodamine framework is an ideal model to construct fluorescent chemosensors due to its excellent photophysical properties such as long absorption and emission wavelength, large absorption coefficient and high fluorescence quantum yield [25]. Many rhodamine based probes for metal ions have been developed [26]. In contrast, only a few rhodamine based pH fluorescent sensors have been reported [27–31]. This may be due to the sensitivity of rhodamine derivatives to H^+. Herein, we reported a new rhodamine B-diethyl-2-aminobutenedioate conjugate derivative (**RBDAB**), which displays remarkable enhanced fluorescence intensities and clear color changes from colorless to pink over a pH range of 4.0–6.5.

2. Experimental

A Techcomp UV-8500 spectrophotometer (Shanghai, China) was used for absorption measurement. The fluorescence spectra were recorded with a Hitachi F-4500 spectrofluorimeter. NMR spectra were measured on a Bruker DMX-500 spectrometer at 500 MHz in CD_3CN. A Delta 320 pH-meter [Mettler-Toledo Instruments (Shanghai) Co., China] was used for pH measurements. Laser confocal scanning microscope [FluoView FV1000, Olympus] was used for detecting for H^+ in living cells. All other chemicals were local products of analytical grade. Distilled-deionized water was used throughout the experiment. All solvent used in spectroscopic analysis are spectrostropic grade.

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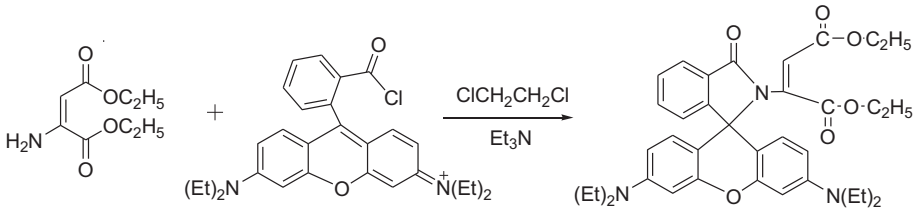

Scheme 1. Synthesis of **RBDAB**.

2.1. Synthesis

Rhodamine B chloride (2.5 g, 5.4 mmol) and diethyl-2-aminobutenedioate (2.02 g, 10.8 mmol) were dissolved in $ClCH_2CH_2Cl$ (25 ml) followed by addition of triethylamine (2 ml). After stirred in an ice bath for 3 h, the mixture was refluxed for 2.5 h. After the solvent was removed by evaporation, the residue was purified by silica gel column chromatography with EtOAc/Cyclohexane (1/6, v/v) as eluent, affording **RBDAB** as a white solid 1.32 g, yield 40%. M.p.: 219–222 °C. IR (cm^{-1}): 2972, 2931, 1729, 1714, 1697, 1635, 1617, 1549, 1515, 1467, 1375, 1356, 1222, 1118; ^1H NMR (500 MHz, CD_3CN): 7.92 (d, 1H, $J = 7.0$ Hz), 7.66–7.60 (m, 2H), 7.13 (d, 1H, $J = 7.5$ Hz), 6.43–6.39 (m, 3H), 6.33–6.29 (m, 4H), 3.88 (q, 2H, $J = 7.5$ Hz), 3.66 (q, 2H, $J = 7.5$ Hz), 3.34 (q, 8H, $J = 7.5$ Hz), 1.11 (t, 12H, $J = 7.5$ Hz), 1.07 (t, 3H, $J = 5$ Hz), 1.03 (t, 3H, $J = 7.5$ Hz); ^{13}C NMR (250 MHz, CD_3CN): 164.9, 163.3, 162.1, 154.0, 151.9, 148.9, 136.5, 132.8, 131.0, 130.5, 128.5, 127.1, 124.3, 122.9, 107.5, 105.8, 97.2, 97.1, 67.6, 61.4, 60.1, 44.0, 13.3, 13.2, 12.0; Anal. Calcd for $C_{36}H_{41}N_3O_6$: C, 70.68; H, 6.76; N, 6.87; Found: C, 70.51; H, 6.68; N, 6.72.

2.2. General procedure for H^+ detection

A 1.0×10^{-5} M stock solution of **RBDAB** and a 1.0×10^{-6} M stock solution of **RBDAB** were prepared in MeCN–water (10/90, v/v) buffer solution (0.1 M citrate buffer solutions). These solutions were modulated for different pH with sodium hydroxide and hydrochloric acid. The absorption and fluorescence sensing of H^+ ion was running immediately.

3. Results and analysis

3.1. Synthesis

As shown in Scheme 1, **RBDAB** can be easily obtained through the reaction of rhodamine B chloride with diethyl-2-aminobutenedioate in 40% yield. The structure of **RBDAB** was confirmed by its IR, ^1H NMR and ^{13}C NMR spectra (Fig S1 and 2) and by elemental analyses.

3.2. Uv–vis spectral responses of *RBDAB*

The absorption spectra of **RBDAB** (10 μM) in MeCN–water (10/90, v/v) buffer solution (0.1 M citrate buffer solutions) exhibited weak bands over 500 nm at pH = 7.0. Along with decreasing of the pH value from 6.5 to 4.0, the absorbance was significantly enhanced at 567 nm (Fig. 1 and Fig. S3). In the meanwhile, the color of the solution changed from colorless to red, which indicated that **RBDAB** could serve as a visual indicator for H^+ concentration (Fig. 2). However, when the pH value of system changed from 4.0 to 2.0, the absorbance was significantly decreased at 567 nm and the color changed from red to approximately colorless.

3.3. Fluorescence spectral responses of *RBDAB*

RBDAB (1 μM) showed a very weak fluorescence in MeCN–water (10/90, v/v) buffer solution (0.1 M citrate buffer solutions) when the pH value of system is above 6.5. Its fluorescence quantum yield is about 0.007 (pH = 7.0) with rhodamine B in methanol as standard. As shown in Fig. 3, with increasing of H^+ concentration to pH range of 6.5–4.0, both a red color and the strong fluorescence appeared, and the fluorescence quantum yield is 0.76 (pH = 4.5) with rhodamine B in methanol as standard. There is a more than 100-fold increase in the emission intensity (λ_{em} = 585 nm). When the pH value of system further decreases, the intense fluorescence of the system gradually diminished (Fig. S4). These indicated that **RBDAB** could serve as a sensitive acidic fluorescent probe that works on the pH range of 4.0–6.5.

3.4. Tolerance of *RBDAB* to H^+ over other metal ions

The effects of common metal ions on pH measurement were assessed through competitive experiments. There exists no obvious change of fluorescence intensity of **RBDAB** (1 μM) in MeCN–water (10/90, v/v) buffer (0.1 M citrate buffer solutions) at pH = 6.8 when 1000 equiv Hg^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Al^{3+} and Ca^{2+} ions were added, respectively. The relative fluorescence intensity of the probe in the absence or presence of the excess Ba^{2+}, Ce^{3+}, K^+, Hg^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Ag^+, Mg^{2+}, Ni^{2+}, Cd^{2+}, Fe^{2+} and Zn^{2+} ions at pH = 3.8 are shown in Fig. 4. The results indicate that the effect of such metal ions on pH measurement is negligible. The time course of fluorescence intensity of **RBDAB** (1 μM) in MeCN–water (10/90, v/v) buffer (0.1 M citrate buffer solutions) at pH = 3.8 was also be performed. The experiment result indicates that the probe can instantly respond to the change of H^+ concentration, and the probe solution is stable to the light and air (Fig. S5).

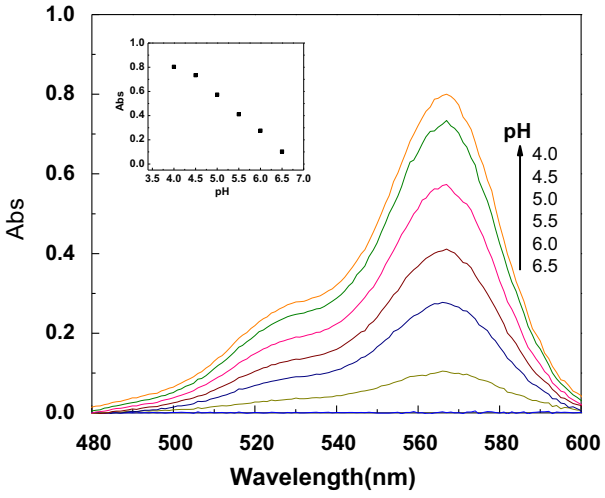

Fig. 1. UV–vis absorption spectra of 10 μM **RBDAB** at different pH. All samples measured in citrate buffer solution in the presence of 10% MeCN as a cosolvent.