Dyes and Pigments 126 (2016) 224–231

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

A novel pH fluorescent probe based on indocyanine for imaging of living cells

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ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form
27 October 2015
Accepted 25 November 2015
Available online 17 December 2015

Keywords:
pH fluorescent probe
Confocal laser scanning microscope
Cellular imaging
Indocyanine
Membrane permeability
Extreme acidity

ABSTRACT

In this paper, we report a novel pH fluorescent probe, 1,1-dimethyl-2-((E)-2-(pyridin-4-yl)vinyl)-1H-benzo[e]indole (PVBI), for imaging in living cells via ethylene bridging of indole derivatives and pyridine. The pH titration indicated that the probe exhibits sensitive pH-dependent behavior with pK_a 3.87 and responded linearly and rapidly to minor pH fluctuations within the range of 3.0–4.6 (linear coefficient of 0.9968). Thus PVBI could be potentially useful for quantitative determination of pH within the acidic window. Also, PVBI displays a notably large Stokes shift of 93 nm which could effectively reduce the excitation interference. Moreover, PVBI possesses highly selective response to H^+ over various metal ions, good photostability and excellent reversibility. The confocal laser scanning microscope images for PVBI diffusing into cells in real-time and detection of pH in living cells were achieved successfully, suggesting the probe has excellent cell membrane permeability and could be applied to monitor pH fluctuations in living cells.

1. Introduction

Intracellular pH (pH_i) plays a critical role in cellular events [1], including cell growth and apoptosis, calcium regulation, receptor-mediated signal transduction, ion transport, etc. Living cells are critically dependent upon pH_i homeostasis because most proteins have distinct pH ranges for function. Some organelles, e.g., endosomes and plant vacuoles, have intracompartmental pH of 4–6. In cell biology, low intracompartmental pH values can serve to denature proteins or to activate enzyme and protein functions that would be too slow around pH 7.0. For instance, the acidic environments in lysosomes (pH 4.5–5.5) can facilitate the degradation of proteins in cellular metabolism [2]. Moreover, numerous metabolic pathways require stringent regulation of the pH_i. The disruption of acid/base homeostasis is associated with a variety of diseases such as cancer, cystic fibrosis, and immune dysfunction [3–6]. The investigation of biological processes at a molecular level has received considerable attention for its potential application in early diagnosis and treatment of disease. Accordingly, the requirements for better understanding the effect of pH_i especially acidic condition on the physiological and pathological processes facilitate the development of methods for monitoring pH_i.

Optical probe, especially fluorescent probe, has been attractive and versatile tools for investigation of pH due to its high sensitive, rapid, nondestructive, easily miniaturized (down to sub-micrometer levels) and technical simple [7,8], etc. The combination of optical probe with fluorescent imaging based on confocal laser scanning microscope is one of the most effective assays for monitoring intracellular species using because of the high spatial and temporal resolution [2,9–12]. Currently, a variety of fluorescent pH probes have been reported in the literature (some are commercially available) for living cells [13–17], tissues [18] and organisms [19,20] imaging. However, some of pH probes still suffer from the severe excitation interference caused by a shorter Stokes shift [21,22]. The complicated synthesis [14,20,21], easy photobleaching and irreversible [23] also are expected to be improved. Furthermore, to the best of our knowledge, few research was involved on the fluorescent probes which are pH sensitive in the lower pH region (pH < 4) in some cases pH changes (e.g. within the stomach) can enter the ranges [24]. In spite of the extreme acidity is

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http://dx.doi.org/10.1016/j.dyepig.2015.11.027
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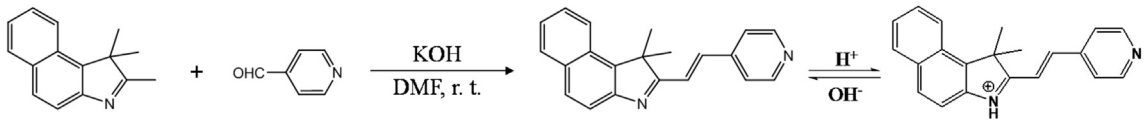

Scheme 1. Synthetic route and acid–base equilibrium of PVBI.

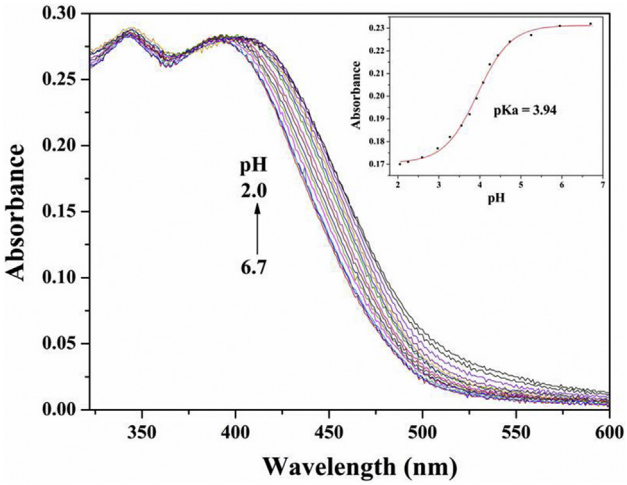

Fig. 1. Change of absorption spectra of PVBI with pH decreased from 6.7 to 2.0. Inset: Sigmoidal fitting of pH-dependent average absorbance at 410 nm, 430 nm and 450 nm.

fatal for the majority of living organisms, some microorganisms such as acidophiles and *helicobacter pylori* can live under such extreme conditions [25,26]. Therefore, we try to develop the extreme acidic pH probes toward large Stokes shift, high sensitivity and good photostability.

Indole derivatives with a D (donor)–π–A (accepter) structure are frequently adopted as the fluorophore to design intramolecular charge transfer (ICT) fluorescent probes displaying a large Stokes shift [15–17,27]. In this paper, we constructed a fluorescent pH probe, PVBI, based on ethylene bridging of benzoindole and pyridine. PVBI possesses high sensitivity and selective response to H^+ over metal ions, good photostability and excellent reversibility. More important that the probe has excellent cell membrane permeability and is applied successfully to monitor pH fluctuations in living cells.

2. Experimental

2.1. Materials and apparatus

All chemicals and solvents were of analytical grade and used without further purification. 1,1,2-trimethyl-1H-benzo[e]indole was purchased from J&K Chemical. 4-pyridinecarboxaldehyde was purchased from Aladdin Reagent Co., Ltd. Human renal carcinoma cells 7860 were kindly provided by Shanxi Medical University (China). All other chemicals were commercially available from Beijing Chemical Reagent Co.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer (Bruker biospin, Switzerland) using tetramethylsilane (TMS) as an internal standard in the solvent deuterated dimethyl sulfoxide (DMSO-d_6), respectively. Mass spectrometric data were obtained with an ESI mass spectrometer (PerkinElmer, America). Absorption spectra were taken on a TU-1901 double-beam UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., LTD, Beijing, China). Fluorescence spectra measurements were performed on a Cary Eclipse spectrofluorometer equipped with a 150 W xenon lamp source (Varian Australian PTY Ltd, Victoria, Australia). Fluorescent images were taken on an FV1000 confocal laser scanning microscope (Olympus Co., Ltd. Japan). Deionized water was obtained from a Milli-Q water purification system (Millipore). pH values were measured using a Beckman Φ 50 pH meter (Shanghai LeiCi Device Works, Shanghai, China). Gaussian 09 programs were used in theoretical calculations.

2.2. Synthesis and characterization of the fluorescent probe

The synthetic route of PVBI is depicted in Scheme 1. A mixture of 1,1,2-trimethyl-1H-benzo[e]indole (0.837 g, 4.0 mmol), 4-pyridinecarboxaldehyde (0.471 mL, 5.0 mmol) and powdered KOH (1.122 g, 20.0 mmol) in 18 mL DMF was stirred overnight at room temperature under nitrogen atmosphere. Then, the mixture

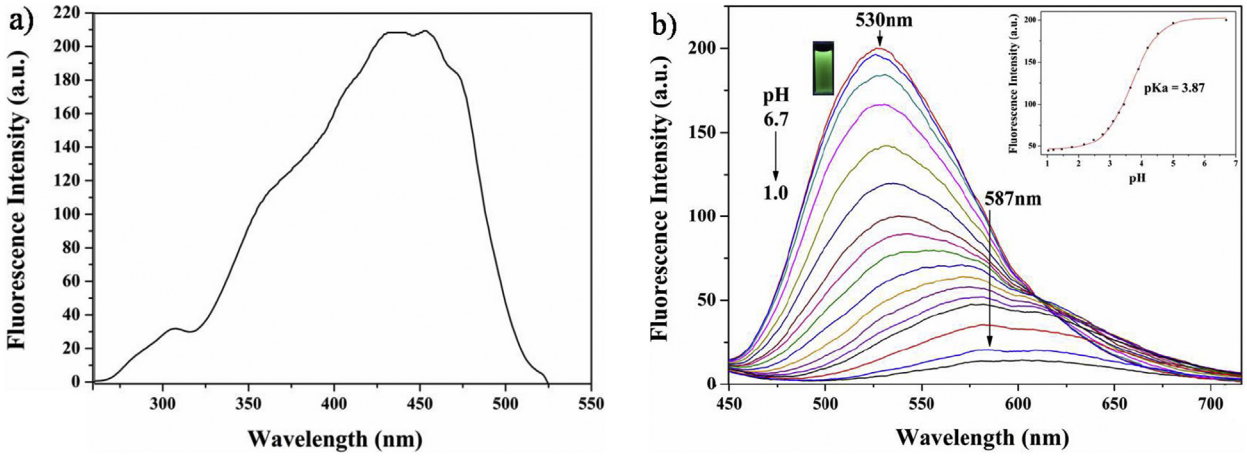

Fig. 2. a) The fluorescence excitation spectra of PVBI at pH 6.7; b) Change of fluorescence emission spectra of PVBI with pH decreased from 6.7 to 1.0 (λ_{ex} = 437 nm). Inset: sigmoidal fitting of pH-dependent fluorescence intensity at 530 nm.