A colorimetric proton receptor for low pH values based on a pyrene–spiropyran conjugate

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A novel colorimetric pH indicator based on a pyrene–spiropyran (SP) conjugate (1) was designed and synthesised. The acidic-sensing property of 1 was investigated by UV–vis absorption spectra, which showed a low pH-dependent (2.0–5.5) reversible equilibrium between colourless SP form (1) and brown-yellow merocynine form (2). This switch process can be observed by naked eye and was also determined by fluorescence spectra and 1H NMR spectra. A rational explanation for the spectroscopic changes of 1 was given by the computational studies.

Keywords: pH indicator; colorimetric; UV–vis absorption spectra; spiropyran; merocynine

Introduction

Protons play a central modulating role in maintaining pH homeostasis for all living organisms (1–3). Thus, protons have become one of the most important sensing targets (4) among the interesting species in vivo. Therefore, some sensors or probes are developed to measure pH changes using fluorescence (5–8) or absorption spectroscopy (10). Among these sensors, many of them work at a relatively narrow range and very few sensors can work within the acidic pH range (9). Although extreme acidity (pH < 4) is fatal for most of living species, many micro-organisms such as ‘acidophiles’ have lived under such harsh conditions (11). Thus sensing the pH, especially at a low pH value of <5, is still highly desirable and challenging.

Optical pH sensors based on the measurement of absorption wavelengths, intensity and, especially, the colour changes observed by naked eye in response to environmental acidity are very attractive since inherent superiority resulted from visual detection and relatively simple handling. In particular, spiropyrans (SPs) as one of the most fascinating families of photochromic compounds (12) have been widely used for the optical sensory applications (13–15) and would be a promising colorimetric candidate for pH sensing. They would undergo reversible structural transformation between a colourless SP form and a coloured merocyanine (MC) form upon light, heat or chemical stimulus (12, 16), which has been shown to exhibit extremely sensitive absorption and colour changes in the visible range (17–19).

In connection with our recent research on pH fluorescent probe based on pyrene–amino acid conjugate (20) and the above consideration in mind, herein we report a new pyrene–SP derivative 1 as a colorimetric pH indicator in aqueous media (Scheme 1).

Results and discussion

The designed indicator 1 could be easily synthesised by one-step condensation between 1-hydroxy-2-pyrenecarboxaldehyde and 1,3,3-trimethyl-2-methyleneindoline in boiling ethanol (Scheme 2). Its structure was identified by 1H, 1H–1H COSY, 13C NMR and ESI mass spectrometry (see ‘Experimental’ section and the Supporting Information, available online). Compound 1 is stable in the solid state and solution, even under UV light irradiation.

As shown in Figure 1, the UV–vis absorption spectra of 1 (5.0 × 10⁻⁵ M) were measured in EtOH–H₂O (50:50, v/v) buffer solution (Na₂HPO₄/NaH₂PO₄, 0.05 M) at various pH values. In general, the absorption range of SP form is from 200 to 370 nm and they are normally colourless (21). When pH value is 5.5, 1 shows a distinctive absorption band centred at 277 nm (ε = 2.94 × 10³ cm⁻¹ M⁻¹) and almost no absorption in the visible region, which suggested that the SP form was the main species. Upon increasing the acidity, the absorption of 1 decreased at 277 nm and a new absorption peak at 422 nm appeared. The absorption peak is assigned to the protonated MC form 2 (422 nm, ε = 2.30 × 10³ cm⁻¹ M⁻¹) (22). When pH value is 2.0, there is a distinctive absorption band centred at 422 nm, suggesting that the spirocycle-opened MC form 2 has become the main species. Moreover, a dramatical colour change from colourless to brown-yellow was observed by the naked-eye upon increasing acidity (Figure 2), and this colour ‘turn-on’ is in sharp contrast to previous colour ‘turn-off’ based on 9-(cycloheptatrienyl-
The solution of 1 is colourless at the pH range of 7.5–5.0. When the pH value is <4.5, the colour of the solution changed to yellow, then to brown-yellow. Moreover, the excellent reversibility of 1 was observed between pH 2.0 and 5.5 (Figure 3), accompanied by back and forth colour change. These results show a low pH-dependent (2.0–5.5) reversible equilibrium between colourless SP form (1) and brown-yellow merocynine form (2) and therefore 1 could be served as a colorimetric indicator for low pH value in aqueous solution.

To study the stability of 1 upon increasing acidity, the nonlinear fitting of absorption was carried out according to the Equation (1) (Figure 4) (22). The pKₐ of equilibrium from 1 to 2 was estimated to be 3.52.

\[
\text{pK}_{\text{a}} = \text{pH} - \frac{\log [A - A_1]}{[A_2 - A]}
\]

where \(A_1\) is the absorption of 1, \(A\) is the absorption of the solution at various pH values and \(A_2\) is the absorption of 2.

The fluorescence responses of 1 were also investigated (Figure 5). When excited at 226 nm, 1 only displays an emission band at 343 nm in different pH values. With the decrease of pH value, the fluorescence intensities at 343 nm were quenched, implying that 1 has transformed to...
by isomerisation and protonation process since MC form is very weak fluorescent (23).

$^1$H NMR experiment also supported this equilibrium shift triggered by acidity. As shown in Figure 6, the signals of aromatic protons of 1 shifted downfield to different extents upon the increase of acid concentration, clearly indicating that the protonated MC 2 with extension of conjugated system had been formed. Another obvious fact is the change of stereochemistry relationship (Z to E) between the double bond protons H13 and H14, which can be judged by the change of coupling constants (10.0–16.4 Hz).

To gain further insights into the optical response of 1 to proton and the related structure changes from 1 to 2, we carried out density function theory (DFT) and time-dependent DFT calculations with B3LYP/6–31G(d) basis set using the Gaussian 03 program (24) for the geometry optimisation and simulation of electronic spectrum (Figure 7). The main contribution transition of 1 for the $S_0 \rightarrow S_1$ energy state comes from HOMO $- 2 \rightarrow$ LUMO $+ 1$. As shown in Figure 7 (left), the electron density is mainly localised on the pyrene moiety; therefore, the fluorescence of 1 is contributed by this part. The energy gap value between the HOMO $- 2$ and LUMO $+ 1$ of 1 is 4.71 eV (278.41 nm), in good agreement with the observed maxima absorption band (277 nm). As for 2, the main transition for the $S_0 \rightarrow S_1$ energy state is HOMO $- 1 \rightarrow$ LUMO, and the electron density of both HOMO $- 1$ and LUMO is located in the whole molecule. Thus, the electron density on the pyrene group decreased, whereas the electron density on indolium moiety increased due to the intramolecular charge transfer (25), which could be the main reason for the fluorescence quench of 1 at low pH values. Moreover, the energy gap value between the HOMO $- 1$ and the LUMO of 2 is 2.94 eV (449.87 nm), which is also close to the measured maxima absorption band (422 nm) and is in good agreement with the bathochromic shift in the absorption spectra in comparison with 1.
Conclusion

In summary, a new colorimetric proton receptor 1 for low pH values has been demonstrated, which is resulted from special pH-dependent reversible equilibrium between colourless SP form (1) and brown-yellow merocynine form (2) under acidic condition. This low pH indicator is complementary to normal pH sensor and provides an alternative for extreme acidic regions. Utilising other SP conjugates, extension of the present work is ongoing and will be reported in due course.

Experimental

General methods

Column chromatography was carried out on silica gel (200–300 mesh). "H and 13C NMR spectra were measured on the Bruker 400 MHz instruments using TMS as an internal standard. ESI-MS were determined on a Bruker esquire 6000 spectrometer. Fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer equipped with quartz cuvettes of 1 cm path length. UV–vis absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. All pH measurements were made with a pH-10C digital pH meter.

HPLC grade ethanol and deionised water were used for each measurement. The pH titrations were investigated in EtOH–H2O (50:50, v/v) buffer solution (Na2HPO4/NaH2PO4, 0.05 M). For the pH titration, 1.0 M HCl or 1.0 M NaOH was added. During the titration, the solution was allowed to equilibrate for 3 min after each addition.

Synthesis of 1

A solution of 1-hydroxypyrene-2-carboxaldehyde (26) (0.25 g, 1 mmol) and 1,3,3-trimethyl-2-methyleneindoline (0.17 ml, 1 mmol) in EtOH (30 ml) was stirred at reflux for 6 h under N2. After cooling to room temperature, the solution was evaporated in vacuo, then the resulting solid was dissolved with CHCl3 (50 ml) and washed with H2O (3 × 50 ml) followed by drying over anhydrous Na2SO4. After filtration of sodium sulphate, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (petroleum ether–EtOAc = 10:1) to give 1 as a yellow solid (0.36 g, 90%). 1H NMR (d6-DMSO, 400 MHz): δ 8.14 (d, J = 7.6 Hz, 1H), 8.11 (s, 1H), 8.08 (d, J = 7.6 Hz, 1H), 8.02 (d, J = 8.8 Hz, 1H), 7.95 (t, J = 8.0 Hz, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.44 (d, J = 10.4 Hz, 1H), 7.19 (t, J = 6.8 Hz, 1H), 7.18 (d, J = 7.2 Hz, 1H), 6.86 (t, J = 7.2 Hz, 1H), 6.66 (d, J = 7.6 Hz, 1H), 6.06 (d, J = 10.0 Hz, 1H), 2.82 (s, 3H), 1.39 (s, 3H), 1.29 (s, 3H) ppm. 13C NMR (d6-DMSO, 100 MHz): δ 148.3 (2C), 136.8, 131.8, 131.7, 130.1, 127.6, 127.1, 126.5, 126.2, 126.1, 125.1, 124.9, 124.7, 124.3, 124.1, 122.8, 121.6, 120.8, 120.7, 119.2, 118.0, 116.3, 106.9, 105.3, 52.0, 29.1, 25.8, 20.4 ppm. ESI-MS: m/z = 402.3 [M + H]+, calcd for C29H24NO = 402.2.
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