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A dual-channel probe for selective fluoride determination and application in live cell imaging

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ABSTRACT

A dual-channel fluoride anions probe has been synthesized from 4-hydroxy-1,8-Naphthalimide and t-butyldiphenylsilyl derivative. The probe presents selective coloration for fluoride anion that can be recognized by naked-eye. And it can quantitatively determine fluoride anion in aqueous and organic media. In MeCN/H_2O (v/v, 50:50), the detection limit of the probe is found to be 0.35 mg L^{-1} which satisfied EPA's requirements. While density function theory and time-dependent density function theory calculations were conducted to rationalize the optical response of the probe. Furthermore, the biological application shows that it could be successfully employed as a selective fluoride probe in the fluorescence imaging of living cells.

1. Introduction

Fluoride sensing is one of the most attractive targets in anion recognition for its significance in health and environmental issues [1]. Excess intake of fluoride will lead to fluoride 'disease' [2–6]. And the fluoride level in drinking water standard regulated by EPA (United States Environmental Protection Agency) is 1.0–4.0 mg L^{-1}. Thus, it is necessary to develop fast, highly selective and sensitive method to determine fluoride level in drinking water. Compared to the costly and complicated ion-selective electrode and ion chromatography which were widely used in fluoride detection, fluorescence is a convenient and satisfactory method with simplicity and real-time detection for fluoride [7,8].

Over the past decades, a lot of neutral receptors and sensor molecules for fluorescent fluoride sensing have been reported [9–34]. They are mainly classified into three categories according to interaction mode: hydrogen bonding interaction, Lewis-acid and Lewis-base interaction and specific reactivity with silicon. Among them, the hydrogen bonding interaction is widely exploited in the development of artificial receptors. They were developed for fluoride sensing by using ureas/thioureas [9,10], amides [11,12], indoles [13], pyrroles [14,15] and hydroxys [16,17] as hydrogen bonding donors. In these sensors, deprotonation plays an important role in fluoride determination. Meanwhile, some chemosensors were developed with a Lewis-acid group in the recognition site for fluoride detection [18,19]. A remarkable contribution has been provided by Gabbarea's group [20–22], who adopted charged ammonium and phosphonium groups for fluoride detection in aqueous media. Recently, another strategy developed on the specific affinity between fluoride and silicon as a reaction-based sensor was introduced by Kim and Swager [24]. Subsequently, several chemodosimeters for F^- were reported [25–29]. Among them, it is impressive that Hong, Zhang, Du and their co-workers [26–28] introduced simple systems to selectively detect fluoride anion in living cell through fluorescent imaging. Though these reaction-based sensors are irreversible in nature, may need high concentration or long reaction time, their volatility can bring convenience in practical applications. Up to now, there are a lot of receptors reported to selectively detect fluoride anion, but for high hydration energy of fluoride anion in water, still very few receptors have been

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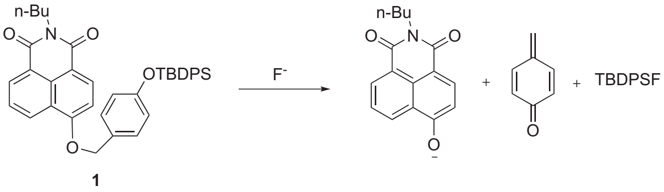
developed which can achieve highly selective fluoride binding in aqueous media. The reaction-based sensors with high selectivity and stability may solve the problem. Thus, it is of interest to develop reaction-based fluorescent sensors for fluoride anions.

With this in mind, we constructed probe **1** based on 4-hydroxyl-1,8-naphthalimide [35,36] for chromogenic and fluorescent fluoride determination. A t-butyldiphenylsilyl group was chosen as reactive site owing to its high affinity to fluoride [37]. We expected the silyl group is cleaved upon reaction with fluoride anion, and probe **1** could be converted into the de-protected product (compound 2 in the naphtholate form) (Scheme 1). The transformation will affect ICT (intramolecular charge transfer) process of probe **1** leading to changes in UV and fluorescence spectroscopy. Therefore, a dual-channel fluoride sensor was synthesized readily.



1

Scheme 1. The plausible mechanism for the spectroscopic changes of **1** in the presence of F⁻.

2. Experimental

2.1. Apparatus

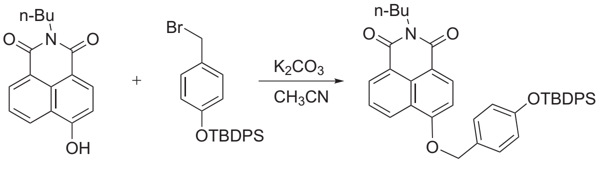
^{1}H NMR spectra were recorded on a Bruke AM-400 spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shift multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were obtained with Thermo LCQ DECA. XP^{Plus} ESI-MS. Elemental analysis was obtained with a Carlo-Erba 1106 elemental analyzer. The absorption spectra were measured with a Shimadzu UV-2550 spectrometer. The fluorescence spectra and relative fluorescence intensity were measured with a Hitachi FL-4500 fluorescence spectrophotometer with a 10 mm quartz cuvette. The pH value was detected using Sartorius Basic PH meter PB-10. The fluorescence imaging experiment was carried out using an inverted microscope with Olympus IX-71.

2.2. Chemicals

All reagents for synthesis were of analytical reagent grade obtained from commercial suppliers and used without further purification. Acetonitrile (MeCN) was dried over CaH_2 and over 4A molecular sieves. Silica gel (mesh 200–300) obtained from Qingdao Ocean Chemicals, China. Doubly distilled water was used throughout. In the titration experiments, all the anions were added in the form of tetrabutylammonium (TBA) or Na salt, which were purchased from Sigma–Aldrich Chemical, stored in a vacuum desiccator and dried fully before using. HEPES (4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid) is used as a buffer reagent was purchased from Sigma–Aldrich Chemical.

2.3. Synthesis of probe 1

Compound 2 and compound 3 were synthesized according to the reported method [38,39]. Probe **1** was synthesized readily (Scheme 2).



2 3 1

Scheme 2. Synthesis route of probe **1**.

To a solution of compound **2** (500 mg, 1.86 mM) in MeCN were added K_2CO_3 (385 mg, 2.8 mM) and **3** (765 mg, 1.86 mM). The reaction mixture was stirred at 20–30 °C overnight, some precipitates formed. The mixture was filtered and the cake was dissolved by EtOAc (30 mL), and then washed with brine (15 mL) and water (15 mL). The organic layer was dried by anhydrous Na_2SO_4, filtered and concentrated to dryness to afford desired product as yellow–green solid (0.7 g, 63.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.58 (d, $J = 8.0$ Hz, 1H), 8.54 (dd, $J = 8.0, 12.0$ Hz, 2H), 7.74 (d, $J = 8.0$ Hz, 4H), 7.68 (t, 1H), 7.37–7.47 (m, 6H), 7.25 (d, $J = 8.0$ Hz, 2H), 7.08 (d, $J = 8.0$ Hz, 1H), 6.83 (d, $J = 8.0$ Hz, 2H), 5.22 (s, 2H), 4.17 (t, $J = 8.0$ Hz, 2H), 1.72 (m, 2H), 1.45 (m, 2H), 1.12 (s, overlapped, 9H), 0.98 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.5, 163.9, 159.8, 155.9, 135.5, 133.3, 132.6, 131.5, 129.9, 129.4, 128.9, 128.7, 127.9, 127.8, 125.9, 123.6, 122.4, 120.0, 115.2, 106.3, 70.6, 40.1, 30.2, 26.4, 20.4, 19.4, 13.8. MS (ESI-MS): calculated for MH^-, 612.3; found 612.4. Analytical calculated for $C_{39}H_{39}NO_4Si$: C, 76.31; H, 6.40; N, 2.28; Found: C, 76.33; H, 6.42; N, 2.32.

2.4. Cell incubation and imaging

Human lung carcinoma (A549) cells were obtained from China center for type culture collection (CCTCC). A549 cells (1×10^4 cells mL^{-1}) were seeded on a Lab-Tek glass chamber slide in 35 mm cell culture dish. After 24 h, washed three times with PBS (phosphate buffered saline), then cells were treated with probe **1** (20 μM) in RPMI1640 (containing 0.5% acetone, *v*/*v*) for 24 h. After washed another three times with PBS, the cells were treated with RPMI1640 containing 50 mM of NaF for 4 h. Finally, the glass chamber slide was taken from culture dish and loaded on the fluorescent microscope. The fluorescent images were taken with green filter.

3. Results and discussion

3.1. Spectral properties

Probe **1** is easily dissolved in MeCN, and its free form has an absorption band maximum at 365 nm in MeCN. Fig. 1 presents the changes of adsorption spectra with the addition of fluoride anion. It was observed that the absorption peak at 365 nm gradually decreased and a new peak at 474 nm (inset of Fig. 1) emerged showing a large bathochromic shift. Two isosbestic points at 328 and 400 nm indicated that a new species with 1:1 stoichiometric relationship between probe **1** and fluoride anion formed (Fig. 1), which was further confirmed by Job plots of probe **1** and fluoride anion (Fig. S1).

To confirm whether the new species is naphtholate anion, a control experiment between probe **1** and sodium salt of compound **2** was executed. In MeCN, sodium salt of 4-hydroxy-1,8-naphthalimide shows UV absorbance maximum at about 475 nm, which is longer than its 4-alkoxy derivative at about 365 nm. After the addition of one equiv of fluoride anion to the solution of probe **1**, a characteristic absorption band centered at about 475 nm was also observed, which was in agreement with the absorbance excitation maximum of sodium salt of compound **2** (Fig. S2). It