Sensors and Actuators B 264 (2018) 304–311

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Substituent effect: A new strategy to construct a ratiometric fluorescent probe for detection of Al^{3+} and imaging in vivo

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ARTICLE INFO

Article history:
Received 20 October 2017
Received in revised form 2 March 2018
Accepted 5 March 2018
Available online 6 March 2018

Keywords:
Ratiometric fluorescent sensor
Normal-tautomer tautomerism
Excited-state charge transfer
Substituent effect
Flavone derivatives

ABSTRACT

Fluorescent sensors are important tools in environment and life science. Ratiometric fluorescent sensors are more advantageous than single intensity-based ones. Excited-state intramolecular proton transfer (ESIPT) molecules endow dual fluorescence from the excited enol and keto tautomers, providing excellent platforms for constructing ratiometric fluorescent sensors. However, the current ESIPT mechanism for ratiometric fluorescent sensors is relatively simple, resulting in that its application limits in given systems. Therefore, it is essential to construct a ratiometric fluorescent sensor that based on ESIPT molecule with reliable, controlled and general strategy. In this work, we employed a new strategy to construct a ratiometric fluorescent sensor allyl-(4′-methyl-3-hydroxyflavone) carbonate (FA) which is based on the finding that the electron-withdrawing substituted group could nearly block the normal-tautomer tautomerism taking place through excited-stated charge transfer (ESCT). FA exhibited highly selective and ratiometric fluorescent response to Al^{3+} and could detect Al^{3+} at a low concentration of 0.75 μM in aqueous solution. FA can detect Al^{3+} in tap and laker water samples, and in living cells. The proof-of-principle method provides a common strategy for design of ratiometric fluorescent sensors.

1. Introduction

Among various detection methods, fluorescence detection is more attractive as it offers several advantages including high sensitivity, simplicity of operation and non-invasiveness [1–4]. Usually the targets could be specifically recognized and quantified by mean of intensity change of fluorescent sensors. Although these sensors have been developed for detection and imaging with high spatial and temporal resolution, most of them mainly relied on the intensity change at single wavelength, which is sometimes problematic and disadvantageous to quantitative detection because this change is often influenced by the concentration of probe, autofluorescence and instrumental fluctuation [5]. In contrast, a ratiometric fluorescent sensor measuring the ratio of fluorescence intensities at two different wavelengths can effectively eliminate these effects, realizing reliable quantitative detection [6–8]. To this end, various molecule-design strategies such as intramolecular charge-transfer (ICT) and fluorescence resonance energy transfer (FRET) have been exploited to construct ratiometric fluorescent sensors. But some of them are easily affected by polarity of solution or strictly required spectra overlap and certain distance between the donor and the acceptor, which limited their wide application [9–12]. Excited-state intramolecular proton transfer (ESIPT) as a new sensing mechanism emerging in recent years has attracted more interests because molecules with ESIPT characteristic endow ratiometric fluorescence change derived from the excited enol and keto tautomers, and large Stokes shift from tautomerization upon excitation [13,14]. ESIPT molecules provide excellent platforms for the construction of ratiometric fluorescent sensors [15,16]. ESIPT sensors have been widely used to detect targets with ratiometric fluorescence change through protection and deprotection of hydroxyl group [17–24]. However, these mechanisms are relatively simple and often susceptible to microenvironment, especially in aqueous solution, due to the existence of intermolecular hydrogen bonding interactions [25,26]. In addition, the targets for sensing are totally confined to Zn^{2+} ions through the inherent recognition sites (N and O) [27–32]. Although the introduction of additional recognition groups could expand the species of targets, these molecules surely lose ESIPT property of ratiometric fluorescence

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https://doi.org/10.1016/j.snb.2018.03.017
0925-4005/

response. Therefore, an uncommon, reliable and controllable strategy is requisite to make the ESIPT molecules to retain ratiometric fluorescence response to targets as new recognition groups are added.

In this context, a new approach is explored, which is based on the finding that tautomerism of ESIPT-based molecule through excited-state charge transfer (ESCT) is affected by the substituent on hydroxyl group. In detailed, a group was introduced into the hydroxyl position of ESIPT molecule, ESIPT process is blocked and primary emission comes from enol (normal) species. If the substituted group is strongly electron-withdrawing, the tautomerism through ESCT would be seriously depressed and very weak tautomer emission would accordingly be observed. In the presence of target, the weak tautomer emission is shifted to that of newly formed complex while enol (normal) emission keeps unchanged. Due to the blockade of hydroxyl group, it is envisioned that this strategy could overcome the disadvantage of ESIPT sensors whose fluorescence is easily affected by aqueous environment. More importantly, it would provide a proof-of-principle strategy and common platform to produce ratiometric response through rational introduction of specific recognition group with electro-withdrawing property for detection of targets required.

In order to evaluate the feasibility of the new strategy, we designed a new ESIPT molecule-based ratiometric fluorescent sensor for Al^{3+} detection. Al^{3+}, an ion mainly coming from acid rain and human activities, poses serious threats to biospheres and human health [33–35]. Excessive intake of Al^{3+} in organs of human could cause various diseases including Alzheimer's disease and Parkinson's disease, and increase the risk of lung and bladder cancer [36–40]. The World Health Organization (WHO) has recommended that the average daily intake of Al^{3+} to be around 3–10 mg and the guidelines for drinking water no more than 7.41 μM [41,42]. Therefore, it is of importance to explore ratiometric sensors to selectively detect and accurately quantify Al^{3+} in environment and biological system [43–47].

2. Experimental section

2.1. Materials and instruments

All chemicals and reagents were used directly as obtained commercially unless otherwise noted. Water used was ultra filter deionized. Stock solution of probes (10 mM) were prepared in CH_3CN and diluted with aqueous solution (H_2O:CH_3CN = 3:1) to the concentration required for test experiments. The metal salts used in stock solutions were $Al_2(SO_4)_3$, $ZnCl_2$, $CaCl_2$, $MgSO_4$, KNO_3, $Ni(NO_3)_2{\cdot}6H_2O$, $Co(OAc)_2{\cdot}4H_2O$, $Mn(OAc)_2{\cdot}4H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Cd(OAc)_2{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, $CrCl_3{\cdot}6H_2O$, $AuCl_3$, $AgNO_3$, $Hg(OAc)_2$ and $Pb(OAc)_2{\cdot}3H_2O$, respectively. Stock solutions (10 mM) of various metal salts were prepared with deionized water. The fluorescence spectra of probes were performed in aqueous solution (H_2O:CH_3CN = 3:1) at room temperature. The excitation wavelength was at 348 nm and the emission was collected from 360 to 600 nm.

NMR spectra were collected on a Bruker 500 avance III spectrometer. Mass spectrum was obtained in ESI mode on a HP1100LC/MSD mass spectrometer. UV–vis spectra were acquired on a Shimadzu 1750 UV–vis spectrometer. Fluorescence spectra were obtained on a RF-5301 fluorescence spectrometer (Japan).

2.1.1. Calculation of binding constants and the limit of detection

The binding constants were calculated employing Benesi-Hildebrand method [28] using Eq. (1), where K_a is the binding constant, I_x is the fluorescence intensity of the free **FA**, I_0 is the observed fluorescence intensity of the **FA**-Al^{3+} complex, and I_{fc} is the fluorescence intensity at the saturation. The plot of $1/(I–I_0)$ vs $1/[Al^{3+}]^{0.5}$ gave a linear fitting, indicating a 2:1 stoichiometry between the **FA** and Al^{3+} ions. Limit of detection LOD = $K \times S_1/S$, where K = 3, S_1 is the standard derivation of the blank solution and S is the slope of the calibration curve.

$$1/(I_x - I_0) = 1/(I_x - I_{fc}) + 1/(I_\infty - I_0)K_a[Al^{3+}]^{0.5} \quad (1)$$

2.1.2. Fluorescence microscopic imaging in live cells

The cultured HeLa cells were incubated with probe **FA** (10 μM) for 1 h, subsequently, after addition of Al^{3+}, cells were incubated in DMEM at 37 °C for another 1 h. After incubation for the corresponding time, the cells were washed with PBS three times to remove free compound and ions before analysis. Cells were then analyzed by Laser Scanning Confocal Microscope (AIR).

2.2. Synthesis procedure

2.2.1. Synthesis of compound FA

3HF (0.252 g, 1 mmol), CH_2Cl_2 (10 mL), allyl chloroformate (0.24 g, 1 mmol), 15-crown-5 (5–10% mol) were added in a round-bottom flask (50 mL). After that, sodium hydroxide (25%, 1 mL) was gradually added into the mixture and the mixture was stirred at room temperature for 3 h until the color turned into colorless. After completion of reaction, the mixture was extracted with CH_2Cl_2 and combined. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: ethyl acetate = 20:1), the product **FA** was obtained as a white solid (302 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.11 (dd, J = 8.0, 1.4 Hz, 1H), 7.89 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 7.81 (d, J = 8.1 Hz, 3H), 7.59 – 7.54 (m, 1H), 7.44 (d, J = 8.1 Hz, 2H), 5.95 (ddd, J = 22.6, 10.6, 5.4 Hz, 1H), 5.38 (dd, J = 17.3, 1.5 Hz, 1H), 5.29 (dd, J = 10.6, 1.3 Hz, 1H), 4.74 (d, J = 5.4 Hz, 2H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.18, 156.04, 155.06, 151.63, 142.18, 134.88, 132.93, 131.45, 129.66, 128.15, 126.17, 125.86, 125.04, 122.80, 118.76, 118.71, 69.31, 21.10. m/z: Calcd. $[M+H]^+$ For $C_{20}H_{17}O_5$: 337.30, found: 337.03.

2.2.2. Synthesis of compound FM

3HF (126 mg, 0.5 mmol), K_2CO_3 (300 mg, 2.25 mmol), chloromethyl ether (0.2 mL, 2 mmol) and dry acetone (30 mL) were added in a round-bottom flask (50 mL) at room temperature, and then the mixture was refluxed for 6 h. After completion of reaction, the reaction mixture was cooled to the room temperature and filtered. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: ethyl acetate = 4:1), the product 4′-methyl-3-(methoxymethyl)hydroxyflavone (**FM**) was obtained (130 mg, 90%) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09 (dd, J = 8.0, 1.5 Hz, 1H), 7.98 (d, J = 8.2 Hz, 2H), 7.89–7.78 (m, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.55–7.44 (m, 1H), 7.40 (d, J = 8.1 Hz, 2H), 5.16 (s, 2H), 3.07 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.61, 155.91, 154.71, 140.88, 136.95, 134.06, 129.06, 128.62, 127.62, 125.07, 124.91, 123.31, 118.35, 96.74, 56.81, 21.04. m/z: Calcd. $[M+Na]^+$ For $C_{18}H_{16}O_4Na$: 319.09, found: 319.15.

3. Results and discussion

3.1. Design and synthesis of compounds

4′-methyl-3-hydroxyflavone (**3HF**) is the favorable building blocks for constructing fluorescent sensors due to its high fluorescence quantum yield, excellent photo-stability, and facile synthesis, as well as large Stokes shift from ESIPT [18]. An electron-withdrawing group allyl carbonate was introduced into its 3-hydroxy position to conduct fluorescent sensor **FA** (Scheme 1).