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Fluorescence *turn-on* detection of fluoride using HPQ-silyl ether reactive probes and its *in vivo* application

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ABSTRACT

Fluoride and its derivatives are implicated in a variety of pathological and physiological conditions and hence play an important role in our daily life. Thus a fluorescent probe that enables the detection and imaging of fluoride in living species is highly demanded. In this study, three *turn-on* fluorescent probes (HPQF1 ~ HPQF3) were designed for detecting fluoride with their sensibilities verified by different steric hindrances around silyl ethers. The three HPQ-based probes could quantitatively detect fluoride anion in acetonitrile as well as in water. The fluorescent response time was only a few seconds in acetonitrile, and two different fluorescent sensing channels in acetonitrile (blue) and water (green) were observed by naked eyes. This work also provides a useful method to fluorescently image the presence of fluoride anions in both living cells and specific zebrafish organelles.

1. Introduction

Fluoride has received considerable attention due to its great potential in biological and industrial applications [1]. At a moderate concentration, its derivatives can effectively prevent prophylactic tooth decay and treat osteoporosis [2]. However, excessive ingestion of fluoride was proved to cause dental or skeletal fluorosis, gastric and kidney disorders, urolithiasis in humans, as well as inhibit neurotransmitter biosynthesis in fetuses [3]. Furthermore, fluoride is everywhere in our daily life as it is added to tap water and toothpaste and can also be released upon hydrolysis of phosphorofluoridate nerve agents, both of which make it urgent to assess the possible toxicity [4]. Thus, rapid and specific probes for quantitative detection of fluoride in water and living systems are of increasing importance in chemosensor research.

Recently, great efforts have been directed to the development of fluorescent sensors or probes for detecting fluoride. Among the existing analytical methods, three main strategies have been utilized for optical detection of fluoride include: hydrogen bonding between fluoride and NH hydrogen (amide, pyrrole, indole, urea, tetrazole and thiourea) [5], boron-fluoride complexation [6], and fluoride mediated desilylation [7,9,10]. The ionic strength and hydration enthalpy of fluoride in water are increased significantly [8], which makes hydrogen bonding ineffective in *aqueous* media. Though Organoboron-derived chemosensors could monitor fluoride in drinking water and fluorinated chemical warfare agents (e.g. Sarin) [6b], they are unsuitable for biological applications due to their cytotoxicity and instability in cellular environments [7f]. Considering the great affinity of fluoride for silicon, Kim and Swager firstly exploited *aqueous* fluoride chemosensors by using fluoride-triggered Si-O cleavage [9], which can alleviate the problems faced by the first two strategies and thus has received growing interest. A probe that can work in *aqueous* conditions and permeate cell membranes has not been realized until Hong's group developed a silyl ether-coumarin probe for detecting fluoride with the aid of this strategy [10a,12,13]. Nevertheless, those probes still need to be modified from the following considerations: (1) Compared with excited state intramolecular proton transfer ESIPT-based probes [7b,11], silyl ether-coumarin based probes cannot minimize background fluorescence and thus their sensitivity is decreased; (2) The rapid single-wavelength fluorescence response and determination with a low detection limit are still rare in *aqueous* media; (3) The component of silyl ethers *via* steric hindrance factor that governs selectivity and sensitivity has never been discussed in designing fluoride probes; (4) Chemosensor-based methodology for monitoring the accumulated fluoride in living organisms has

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not been established, which makes it difficult to assess the fluoride level of individual organs. These issues should be addressed when designing fluoride probes for biological applications.

2. Experimental section

2.1. Reagents and apparatus

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried using standard procedures. Electrospray ionization mass spectra (*ESI-MS*) were measured on a Micromass LCTTM system. Fluorescence was measured at room temperature on a Perkin-Elmer LS 50B fluorescence spectrophotometer. ^{1}H NMR and ^{13}C NMR were measured on a BrukerAV-500 or BrukerAV-300 spectrometer with chemical shifts in ppm (in $CDCl_3$ or DMSO-d_6; TMS as internal standard). Data were presented as follows: Chemical shift (in ppm on the scale TMS as internal standard), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constant (J/Hz), and interpretation. pH measurements was made with a Sartorius basic pH meter PB-10. Φ_F was determined with rhodamine B as a standard (Φ_F = 0.69 in ethanol). TLC analysis was performed on silica gel plates. Column chromatography was conducted over silica gel (mesh 200–300), and both were obtained from the Qingdao Ocean Chemicals.

2.2. Synthesis and characterization of probes

2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone (**HPQ**): 2-Aminobenzamide (544 mg, 4.0 mmol) was dissolved in anhydrous EtOH (25 mL) and followed by the addition of salicylic aldehyde (488 mg, 4.0 mmol). The reaction mixture was refluxed in the presence of 2 drops of piperidine for 90 min. The resulting solution was cooled to 0 °C, and DDQ (2,3-dichloro-5,6-dicyano-1, 4-benzoquinone, 908 mg, 4.0 mmol) was added in 3 portions over 30 min. The mixture was slowly brought to room temperature for another 1 h and a greenish precipitate was formed. The solid was collected by filtration and washed with a small amount of cooled ethanol, which was purified by recrystallisation using ethanol to afford the desired pale yellow compound (819 mg, yield: 86%). M.p. 299.6–301.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.21 (m, 2H, Ar-H), 7.82 (m, 2H, Ar-H), 7.54 (m, 2H, Ar-H), 6.98 (m, 2H, Ar-H). *ESI*-MS: *m/z* 239.1 $[M+H]^+$, 261.1 $[M+Na]^+$.

2-(2′-tert-butyldimethylsilyloxy-phenyl)-4(3H)-quinazolinone (HPQF 1): To a solution of 2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone **HPQ** (476 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (40 mL), DMAP (4-dimethylamiopryidine, 30 mg) and triethylamine (0.8 mL) were added. The resulting mixture was cooled to 0 °C, and TBDMSCl (t-butyldimethylsilyl chloride, 450 mg, 3.0 mmol, in 10 ml CH_2Cl_2) was added in 3 portions over 30 min. The suspended mixture was slowly dissolved at room temperature with another 1 h. Then it was poured into the saturated $NaHCO_3$ (40 mL) solution, and the resulting mixture was extracted three times with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated under vacuum. Compound **HPQF 1** was isolated using a silica gel chromatographic column eluted with petroleum ether/Dichloromethane/ethyl acetate (v/v/v, 50:47:3), resulting a light yellow solid (R_f = 0.45, 148 mg, yield: 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.73 (s, 1H, N-H), 8.38 (d, J = 7.9 Hz, 1H, Ar-H), 8.31 (d, J = 7.9 Hz, 1H, Ar-H), 7.79 (m, 2H, Ar-H), 7.49 (t, J = 7.8 Hz, 1H, Ar-H), 7.41 (t, J = 7.8 Hz, 1H, Ar-H), 7.17 (t, J = 7.5 Hz, 1H, Ar-H), 6.98 (d, J = 8.2 Hz, 1H, Ar-H), 1.05 (s, 9H, -SiC$(CH_3)_3$), 0.338 (s, 6H, -Si$(CH_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$): 161.37, 154.01, 151.11, 149.43, 134.42, 132.62, 131.43, 129.98, 127.84, 126.48, 122.60, 122.45, 121.22, 120.37, 25.75, 18.37, −4.10. Anal. HPLC: 99.1% purity. Anal. Calcd for $C_{20}H_{24}N_2O_2Si$: C, 68.02; H, 6.51; N, 8.04. Found: C, 68.15; H, 6.86; N, 7.95. *ESI*-MS: *m/z* 353.1 $[M+H]^+$, 375.1 $[M+Na]^+$.

2-(2′-triisopropylsilyloxy-phenyl)-4(3H)-quinazolinone (**HPQF 2**): To a solution of 2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone **HPQ** (476 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (40 mL), DMAP (4-dimethylamiopryidine, 30 mg) and triethylamine (0.8 mL) were added. The resulting mixture was cooled to 0 °C, and TIPSCl (Triisopropylsilyl chloride, 576 mg, 3.0 mmol, in 10 ml CH_2Cl_2) was added in 3 portions over 30 min. The suspended mixture was slowly dissolved at room temperature with another 1 h. Then it was poured into the saturated $NaHCO_3$ (40 mL) solution, and the resulting mixture was extracted three times with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated under vacuum. Compound **HPQF 2** was isolated using a silica gel chromatographic column eluted with petroleum ether/Dichloromethane/ethyl acetate (v/v/v, 50:45:5), resulting a light yellow solid (R_f = 0.5, 292 mg, yield: 37%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 14.45 (s, 1H, N-H), 8.40 (d, J = 7.8 Hz, 1H, Ar-H), 8.19 (d, J = 8.1 Hz, 1H, Ar-H), 7.87 (d, J = 8.3 Hz, 1H, Ar-H), 7.82 (t, J = 7.2 Hz, 1H, Ar-H), 7.53 (t, J = 7.4 Hz, 1H, Ar-H), 7.38 (t, J = 7.5 Hz, 1H, Ar-H), 7.05 (d, J = 8.1 Hz, 1H, Ar-H), 6.96 (t, J = 7.4 Hz, 1H, Ar-H), 1.67 (q, J = 7.5 Hz, 3H, -SiCH$(CH_3)_2$), 1.20 (d, J = 7.5 Hz, 18H, -CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 166.36, 162.10, 161.38, 149.56, 134.28, 132.79, 129.35, 126.71, 126.15, 124.51, 119.25, 118.62, 117.80, 116.44, 18.12, 12.80. Anal. HPLC: 97.8% purity. Anal. Calcd for $C_{23}H_{30}N_2O_2Si$: C, 69.81; H, 7.56; N, 7.31. Found: C, 70.01; H, 7.66; N, 7.10. *ESI*-MS: *m/z* 395.4 $[M+H]^+$, 417.4 $[M+Na]^+$.

2-(2′-tert-butyldiphenylsilyloxy-phenyl)-4(3H)-quinazolinone (**HPQF 3**): To a solution of 2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone **HPQ** (476 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (40 mL), DMAP (4-dimethylamiopryidine, 30 mg) and triethylamine (1.5 mL) were added. The resulting mixture was cooled to 0 °C, and TBDPSCl (tert-butyldiphenylsilyl chloride, 822 mg, 3.0 mmol, in 10 ml CH_2Cl_2) was added in 3 portions over 30 min. The suspended mixture was slowly dissolved at room temperature with another 1 h. Then it was poured into the saturated $NaHCO_3$ (40 mL) solution, and the resulting mixture was extracted three times with CH_2Cl_2 (40 mL). The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated under vacuum. Compound **HPQF 3** was isolated using a silica gel chromatographic column eluted with petroleum ether/Dichloromethane/ethyl acetate (v/v/v, 50:45:5), resulting a light yellow solid (R_f = 0.42, 171 mg, yield: 18%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 14.03 (s, 1H, N-H), 8.39 (d, J = 8.1 Hz, 1H, Ar-H), 7.87 (t, J = 8.7 Hz, 2H, Ar-H), 7.82 (d, J = 7.2 Hz, 4H, Ar-H), 7.60 (t, J = 6.5 Hz, 1H, Ar-H), 7.43 (t, J = 7.4 Hz, 2H, Ar-H), 7.38 (t, J = 7.4 Hz, 4H, Ar-H), 7.32 (d, J = 7.9 Hz, 1H, Ar-H), 7.32 (d, J = 7.9 Hz, 1H, Ar-H), 7.22 (d, J = 7.1 Hz, 1H, Ar-H), 6.92 (d, J = 8.2 Hz, 1H, Ar-H), 6.57 (t, J = 7.6 Hz, 1H, Ar-H), 1.28 (s, 9H, -Si $(CH_3)_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 165.61, 161.72, 160.90, 149.90, 135.34, 134.38, 132.59, 132.06, 130.06, 129.88, 127.79, 126.84, 126.36, 124.30, 118.95, 118.23, 117.22, 116.34, 27.07, 19.74. Anal. HPLC: 99.4% purity. Anal. Calcd for $C_{30}H_{28}N_2O_2Si$: C, 75.51; H, 5.78; N, 5.91. Found: C, 75.60; H, 5.92; N, 5.88. *ESI*-MS: *m/z* 477.2 $[M+H]^+$, 499.1 $[M+Na]^+$.

Reaction probe **HPQF3** with NaF or TBAF (HPLC-MS positive ion mode): **HPQF3** (95.2 mg, 0.2 mmol) was dissolved in 50% CH_3CN/H_2O (100 mL) and followed by the addition of NaF (420 mg, 10.0 mmol) or TBAF (315 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 2 h and conversion was checked by analytical HPLC. (HPQ yield: > 80%) (4.6 mm × 150 mm 5 μm C18 column; 5 μL injection; 10% CH_3CN/H_2O, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); *ESI*-MS: *m/z* 239.1 $[M+H]^+$, 261.1 $[M+Na]^+$.

3. Results and discussion

3.1. Rational design of the HPQ-based fluorescent probes

2-(2′-hydroxy-phenyl)-4(3H)-quinazolinone (HPQ) is distinguished