Fluorescent probe for detection of fluoride in water and bioimaging in A549 human lung carcinoma cells†

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We have successfully developed a fluoride ion probe for fluorescence cell bioimaging—desirable properties include retention of the fluorophore inside cells, non-cytotoxicity to mammalian cells, appreciable solubility in water, and stoichiometric reaction with analytes.

Fluoride ions are widely used as an essential ingredient in toothpaste and pharmaceutical agents and are even added to drinking water owing to their tendency to prevent dental caries[1] and enamel demineralization resulting from wearing orthodontic appliances; they are also used for the treatment of osteoporosis.[2] However, a high intake of fluoride may cause fluorosis,[3] and also lead to nephrotoxic changes[4] and urolithiasis in humans.[5] In molecular and cell biology, NaF is known to influence various cell signaling processes[6] and to induce apoptosis at high concentrations for 24 h in mammalian cells.[7] For these reasons, considerable effort has been devoted to the development of novel methods for the detection of fluorides, particularly NaF.[8,9] In fact, the development of receptor-based sensors for the detection of fluoride ions is challenging owing to the small size, high electronegativity, and high hydration enthalpy of fluoride ion. Therefore, while most chemosensors recognize fluoride ions in a tetrabutylammonium fluoride (TBAF) salt in organic solvents,[8] only a few can detect fluoride ions, as in NaF, in aqueous solutions.[9] Recently, Gabbaï successfully demonstrated a borane-based fluoride receptor working in DMSO–H_2O (4 : 6, v/v).[9a] Swager, Yang and their co-workers also developed fluoride-detecting systems based on a coumarin moiety performing in organic solvents such as THF, CH_2Cl_2 or in acetone–water (7 : 3, v/v) through Si–O bond cleavage.[8e,9d] However, none of the fluoride chemosensors meet the requirements for biological applications, which are as follows; (1) to be able to selectively detect fluoride ions in 100% water, (2) to be capable of permeating the cell membrane, (3) to be non-toxic and display fluorescence upon the detection of fluoride ions in cellular systems. To the best of our knowledge, no fluoride chemosensors or probes satisfying the above-mentioned requirements have been reported.

In an effort to address these issues for biological applications, we have explored the possibility of developing a novel fluoride chemodosimeter in water. As part of previous contributions to the development of chemodosimeters,[8b,e,f,9] we recently demonstrated a resorufin-based chemodosimeter incorporating a *tert*-butyldiphenylsilyl (TBDPS) moiety for the detection of fluoride anions, with high selectivity in aqueous solution.[9b] However, our initial attempt to apply the resorufin-based system for the detection of fluoride ion in 100% water failed due to its poor solubility in water.

In our endeavour to develop a fluoride sensor in water, we moved forward to design a new fluoride detection system working under physiological conditions. In order to improve the water solubility, we planned to downsize our sensor system and introduce hydrophilic moieties. This analysis led us to design a 7-hydroxycoumarin-based system containing a *tert*-butyldimethylsilyl (TBDMS) moiety, **TBMCA** (**1**, *tert*-butyldimethylsilyl 7-hydroxycourmarin-4-acetic acid methyl ester), based on a previous approach.[9d] In addition, we introduced a methyl ester group to 4-acetic acid on the fluorescent coumarin moiety to increase water solubility and to enhance cell permeability as well as to detain the fluorophore inside the cell after hydrolysis by using a negatively charged carboxylate group.[10] **TBMCA** was prepared by silyl protection of 7-hydroxycoumarin-4-acetic acid methyl ester with TBDMSCl and imidazole in anhydrous DMF (see ESI†).[11] 7-Hydroxycoumarin-4-acetic acid methyl ester was synthesized in accordance with a known procedure.[12] Unfortunately, the fluorescence intensity of **TBMCA** immediately showed strong enhancement upon the exposure to phosphate buffered saline (PBS) (Fig. S4, ESI†). In addition, the fluorescence intensity of **TBMCA** was increased only 1.2-fold after treatment of 1 mM NaF for 3 h in PBS (Fig. S1 and S4, ESI†), which is consistent with a previous report.[9d] We initially conjectured that the strong enhancement of fluorescence intensity in PBS was due to the instability of the TBDMS moiety in **TBMCA**. However, the fluorescence intensity of **TBMCA** was barely changed in PBS over a 24 h period (Fig. S2, ESI†). Then, we examined fluorescence emission changes of **TBMCA** as a function of solvent polarity and observed increased fluorescence intensity of **TBMCA** in accordance with increased polarity of solvent (Fig. S3 and S4, ESI†). Based on this observation, we postulated that the covalent bond character of the Si–O bond of **TBMCA** is much weaker in water, due to its interaction with water molecules, which renders mimicking the

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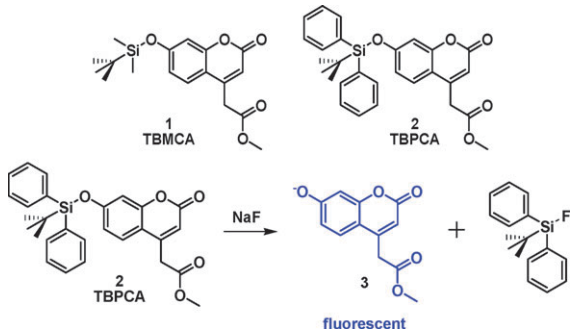

Scheme 1 Molecular structures of **TBMCA** and **TBPCA**, and the sensing mechanism of probe **TBPCA** for the detection of NaF.

intramolecular charge transfer (ICT) events *via* stronger Si–O bond polarization and leads to turn-on of fluorescence in water without actual desilylation. Consequently, there was only a marginal enhancement of fluorescence when **TBMCA** was treated with fluoride anion in water (Fig. S1, ESI†). Therefore, we decided to replace TBDMS (dimethyl) moiety with a bulkier TBDPS (diphenyl) moiety to reduce the accessibility of water molecules to the silicon atom.

TBPCA (**2**, *tert*-butyldiphenylsilyl 7-hydroxycourmarin-4-acetic acid methyl ester) was prepared using TBDPSCl by the same procedure as that for **TBMCA**. **TBPCA** was prepared for the selective turn-on of quenched fluorescence by an ICT mechanism when 7-hydroxycoumarin **3** was released upon the attack of fluoride ion on the silyl ether moiety (Scheme 1). The fluorescence intensity of **TBPCA** is also affected by the solvent polarity, but significantly less than that of **TBMCA** (Fig, S3 and S4, ESI†). In terms of sensitivity toward fluoride ion, we observed more than 4-fold enhancement of fluorescence intensity of **TBPCA** after treatment of 1 mM NaF for 3 h in PBS (Fig. S1, ESI†). Therefore, we selected **TBPCA** as a fluoride sensor with excellent properties for the application in physiological conditions.

The enhanced fluorescence intensity of **TBMCA** in water, but not in the case of **TBPCA**, is consistent with the computer-assisted rationalization with *ab initio* calculation and molecular modelling (see ESI†).[13] In fact, *ab initio* calculation intimates the importance of the electronic effect by the TBDPS and TBDMS group in polar solvents; the Si–O polarity difference ($|\Delta Q|$ = 0.021 C) and the Si–O bond length difference (0.007 Å) of **TBPCA** between hexane and water were significantly smaller than those ($|\Delta Q|$ = 0.103 C, 0.009 Å) of **TBMCA**. In addition, the water-accessible area on silicon atom of **TBPCA** was 0.23 Å^2, which is significantly smaller than that (1.67 Å^2) of **TBMCA**, as calculated by molecular modelling software, Naccess, which means that the Si–O bond of **TBMCA** is quite accessible to water. These results also support our initial rationale above.

We confirmed the linearity of the fluorescence emission intensity (λ_{em}: 461 nm) of **TBPCA** (2 μM) relative to fluoride concentrations in HEPES buffer at 25 °C (Fig. 1(a)). On the basis of this calibration curve at 461 nm, the concentration of NaF can be confidently predicted by measuring the fluorescence emission of **TBPCA**. Even though fluoride anions have high selectivity and affinity towards the silicon atoms of TBDPS, it is difficult to overcome the strong hydration effect

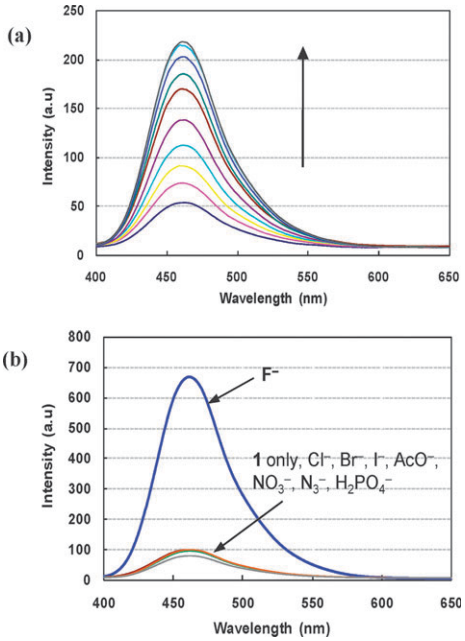

Fig. 1 (a) Fluorescence emission change of **TBPCA** (2 μM) recorded 4 h after reaction with various concentrations of NaF (0–1.3 mM) in 10 mM HEPES buffer (pH 7.4) at 25 °C. (b) Comparison of fluorescence emission intensity of **TBPCA** (2 μM) after 4 h for various sodium salts (1 mM) in 10 mM HEPES buffer (pH 7.4) at 25 °C (blue line represents NaF).

of fluoride in aqueous environments; therefore, an extended incubation time is required for the consistent determination of fluoride concentration through sufficient reaction of fluoride ions with **TBPCA**.[9b] For instance, the fluorescence emission intensity of **TBPCA** was maximized only 4 h after exposure to 1 mM NaF in the HEPES buffer at 25 °C (Fig. S6, ESI†). The selectivity of **TBPCA** for F^- was confirmed by treatment of various anions (1 mM) as sodium salts such as Cl^-, Br^-, I^-, AcO^-, NO_3^-, N_3^-, $H_2PO_4^-$. As shown in Fig. 1b, only NaF can efficiently exhibit fluorescence emission (λ_{ex}: 375 nm) and the fluorescence intensity in the case of NaF is more than seven times that produced in the case of the other anions. Fig. 2 clearly shows the difference between the optical image of **TBPCA** in the presence of NaF and UV light (λ_{ex}: 365 nm) and the optical image in the presence of other anions. Finally, we examined whether **TBPCA** can influence the cell viability in mammalian cells. After the treatment of the A549 human epithelial lung carcinoma cell line with **TBPCA** (20 μM) for 24 h, there was no reduction in the cell viability: the cell

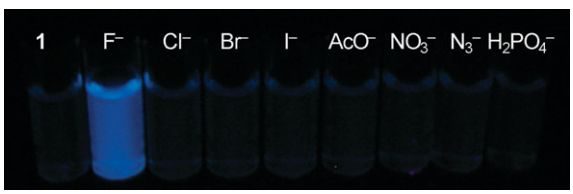

Fig. 2 Optical changes in fluorescence emission of **TBPCA** (2 μM) when **TBPCA** is excited by UV irradiation (λ_{ex}: 365 nm) after 4 h for various sodium salts (1 mM) in 10 mM HEPES buffer (pH 7.4) at 25 °C. Left to right: **TBPCA**, F^-, Cl^-, Br^-, I^-, AcO^-, NO_3^-, N_3^-, $H_2PO_4^-$ (sodium salts).

viability was measured by considering the mitochondrial function using Cell Counting Kit-8 (CCK8),[14] and this result indicates the possibility of using **TBPCA** for bioimaging live cells in aqueous media.[15]

TBPCA fulfills the requirements for displaying fluorescence in *in vitro* cell imaging: it can be retained in a cell, it is non-cytotoxic, and it can exhibit fluorescence upon sensing the appropriate physiological conditions. On the basis of these properties of **TBPCA**, which indicate that this probe is a selective chemodosimeter for fluoride anions, we explored the possibility of its use in biological systems by its application to A549 human lung carcinoma cell lines. The addition of 50 mM NaF to A549 cells loaded with **TBPCA** (20 μM) leads to a significant increase in the fluorescence intensity as compared to control experiments (Fig. 3(b) and (c)). Due to the slow rate of this reaction, the **TBPCA**-loaded A549 cells with NaF have to be incubated for 3 h at 37 °C to obtain the maximum fluorescence intensity; incubation for more than 3 h causes the deterioration of the fluorescence signal.

To take advantage of the chemodosimeter, **TBPCA** was also used for the quantification of fluoride ions in the cells. After 3 h incubation of the A549 cells with NaF (50 mM) under the physiological conditions, the cells were harvested and thoroughly washed. The harvested cells were sonicated and centrifuged for the preparation of cell lysate in PBS buffer. The resulting lysate was treated with **TBPCA** (2 μM) for 4 h at 25 °C to quantify the fluoride ions in the cell lysates; the quantification was performed on the basis of the fluorescence intensity and a standard curve prepared with NaF-doped cell lysates. This new quantification method reveals that 1.86×10^{-2} pmol fluoride anion is present in the cytoplasm per cell under physiological conditions (Fig. S12, ESI†).

In conclusion, we have successfully developed **TBPCA** as a fluoride ion probe for fluorescence cell bioimaging with desired properties, such as the detaining of the fluorophore inside a cell, non-cytotoxicity to mammalian cells, fluorescence upon sensing, appreciable solubility in water, and stoichiometric reaction with analytes. We also demonstrated fluorescence cell bioimaging using **TBPCA** for the detection of NaF in A549 human epithelial lung cancer cells under physiological conditions. Moreover, **TBPCA** can be utilized for the quantification of fluoride ions in living systems.

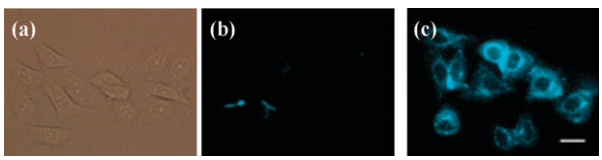

Fig. 3 Brightfield image and fluorescence cell images of A549, human epithelial lung carcinoma. (a) Bright-field image of A549 cells incubated with **TBPCA** (20 μM) for 30 min and subsequently incubated for 3 h at 37 °C. (b) Fluorescence image of A549 incubated with **TBPCA** (20 μM) for 30 min and subsequently incubated without NaF for 3 h at 37 °C. (c) Fluorescence image of A549 cells incubated with **TBPCA** (20 μM) for 30 min and subsequently treated with 50 mM NaF for 3 h at 37 °C. The scale bar represents 20 μm.

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