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Unmodified fluorescein as a fluorescent chemosensor for fluoride ion detection

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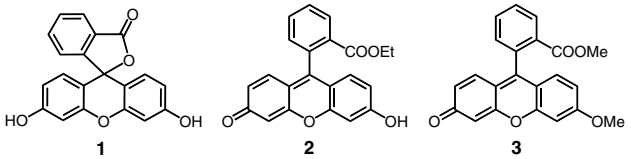
Abstract—Unmodified fluorescein (**1**) behaves as a fluorescent chemosensor for F^- detection, where the F^--induced fluorescence enhancement is driven by a transfer of the phenolic OH protons to F^-.

The recognition and sensing of anions have attracted a great deal of attention because of biological and environmental importance of anions.[1] Fluoride ion (F^-) is one of the most important anions due to its pivotal roles in dental care and the treatment of osteoporosis.[2] Design of fluorescent probe for F^- detection has therefore attracted much attention due to high sensitivity and simplicity of the fluorescence analysis.[3]

Fluorescein is a dye used extensively as bio-labeling reagents and fluorescent probes due to its excellent photophysical properties, such as long-wavelength absorption and emission, high fluorescence quantum yield, and high stability against light.[4] Considerable effort has been devoted to the development of fluorescent probes for reactive oxygen species (ROS)[5] and metal cations[6] based on the fluorescein platform. There are, however, only three reports of fluorescein-based fluorescent anion sensor.[7] Yoon et al.[7a] synthesized a fluorescein derivative conjugated with boronic acid and aminomethyl groups. This material shows a F^--induced emission enhancement due to a suppression of the photoinduced electron transfer from the amine nitrogen to the photoexcited fluorescein moiety by a cooperative coordination of F^- with the ligand groups. Yang et al.[7b] synthesized a fluorescein derivative whose two phenolic OH are protected by *tert*-butyldimethylsilyl (TBS) groups. This shows a F^--induced fluorescence enhancement due to the deprotection of TBS, leading to a formation of the spirocycle-opened 'emissive' fluorescein species. Very recently, a fluorescein bearing a thiourea group was synthesized by Kim et al.,[7c] which shows an anion-induced fluorescence enhancement. In that, hydrogen-bonding interaction between anion and thiourea group leads to a spirocycle opening of the fluorescein moiety, resulting in fluorescence enhancement. Needless to say, practical anion sensing requires inexpensive and easily preparable sensors;[8] however, all of these fluorescein-based anion sensors require a synthesis step for sensor preparation.[7]

Here we report that a commercially-available 'unmodified' fluorescein (**1**, Fig. 1) behaves as a fluorescent chemosensor for F^- detection, enabling selective F^- sensing among the halide anions. We describe here that the strong fluorescence enhancement of **1** by F^- is simply triggered by a transfer of the phenolic OH protons to F^-, leading to a formation of the spirocycle-opened emissive anionic species.

As shown in Figure 2, **1** (1 μM) dissolved in acetonitrile (MeCN) is nonfluorescent. Addition of 10 equiv of F^- (as a n-Bu_4N^+ salt) to the solution, however, leads to



1 2 3

Figure 1. Structures of fluorescein (**1**) and its derivatives (**2**, **3**).

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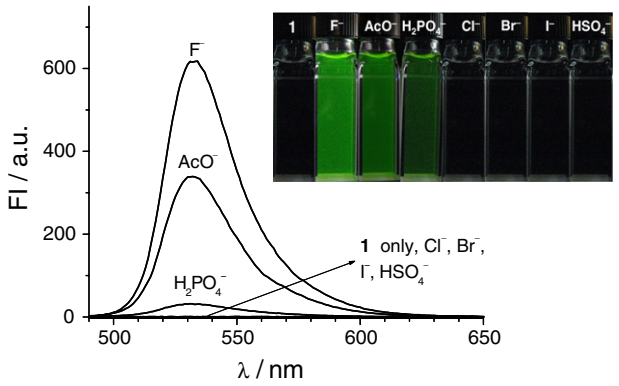


Figure 2. Fluorescence spectra of **1** (1 μM) in MeCN measured with 10 equiv of respective anions as *n*-Bu_4N^+ salt ($\lambda_{ex} = 480$ nm). (Inset) change in fluorescence color.

an appearance of a strong green fluorescence at 500–600 nm. Moderate and weak emission enhancement is observed for AcO^- and $H_2PO_4^-$, respectively, whereas no enhancement is observed for other anions (Cl^-, Br^-, I^-, and HSO_4^-). As shown in Figure 3, fluorescence titration with F^- reveals that the fluorescence enhancement is saturated upon the addition of 10 equiv of F^-, where the fluorescence quantum yield is 0.61.[9] The emission enhancement is determined to be 2100-fold, which is the highest value among the reported fluorescein-based fluorescent F^- sensors.[7] In contrast, addition of even 100 equiv of other halide anions (Cl^-, Br^-, and I^-) shows no emission enhancement (Fig. 3). Notably, as shown in Figure S1,[10] the fluorescence response of **1** toward F^- is unaffected by the presence of other halide anions (Cl^-, Br^-, and I^-), indicating that **1** is potentially available for selective F^- detection among the halide anions.

Figure 4 shows absorption spectra of **1** (10 μM) in MeCN. Without anions, **1** is colorless and exhibits almost no absorption at 400–550 nm, indicating that **1** exists as a spirocycle-closed form. This is confirmed by a distinctive spiro-carbon shift at 83.51 ppm in the ^{13}C NMR spectrum of **1**.[11] Addition of F^-, however, leads

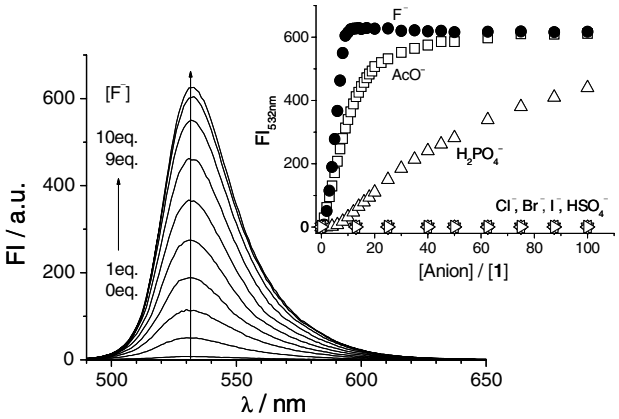


Figure 3. Fluorescence titration of **1** (1 μM) with F^- ($\lambda_{ex} = 480$ nm). (Inset) Change in fluorescence intensity ($\lambda_{em} = 532$ nm).

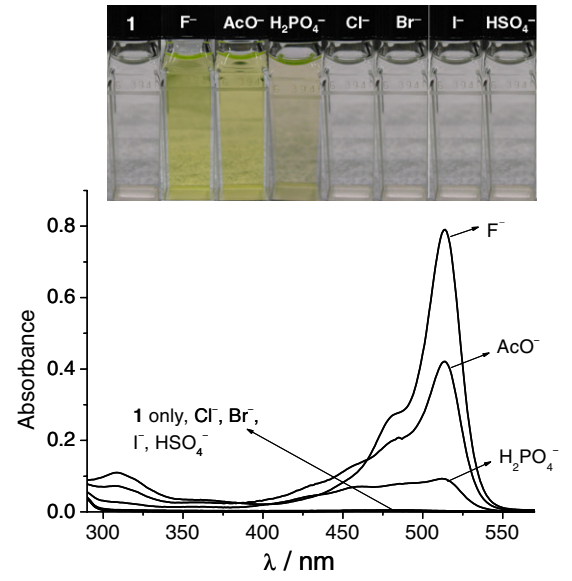


Figure 4. Absorption spectra of **1** (10 μM) measured in MeCN with 4 equiv of respective anions. (Inset) Color change.

to an appearance of a strong absorption ($\lambda_{max} = 514$ nm), along with a clear color change of the solution from colorless to yellow-green. Moderate and weak absorption increase is observed for AcO^- and $H_2PO_4^-$, respectively, but almost no change is observed for other anions (Cl^-, Br^-, I^-, and HSO_4^-).

As shown in Figure 5, absorption titration of **1** with F^- reveals that addition of first 2 equiv of F^- leads to an increase in the entire absorption at 400–550 nm (see blue line). Further addition of 2 equiv of F^- (see red line) leads to further increase in 465–550 nm absorbance, but also leads to a decrease in 400–465 nm absorbance with an isosbestic point at 465 nm. These findings imply that two kinds of fluorescein species form in response to the interaction between **1** and F^- with 1:2 and 1:4 stoichiometry. This is confirmed by the Job's plots of **1** with F^-: as shown in Figure 6, 460 nm absorbance shows a maximum at X ($=[F^-]/([F^-]+[\mathbf{1}])) = 0.66$, whereas

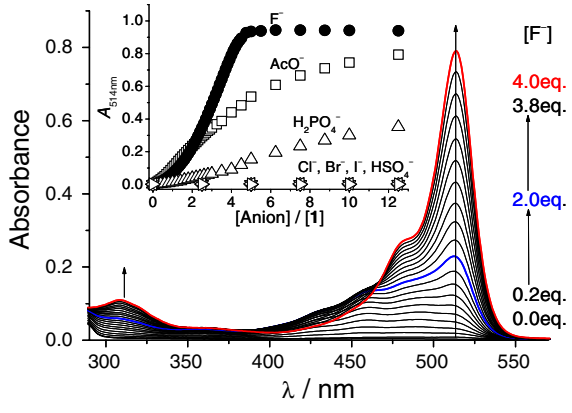


Figure 5. Absorption titration of **1** (10 μM) with F^-. (Inset) Change in absorbance monitored at 514 nm. The detailed absorption spectrum change: see Figure S2.[10]

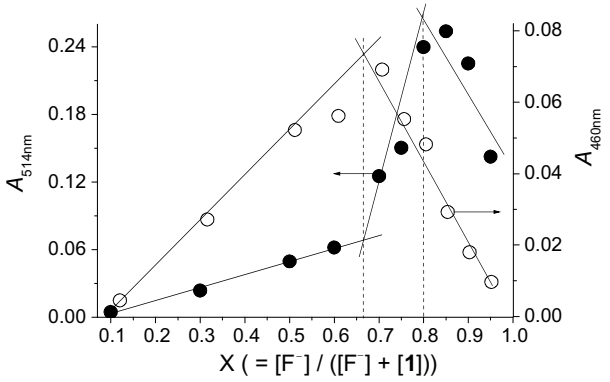
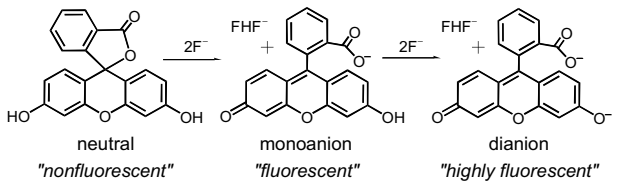


Figure 6. Job's plot for F^- versus **1** in MeCN measured (closed circle) at 514 nm and (open circle) at 460 nm. $[F^-] + [\mathbf{1}] = 20\ \mu M$.

514 nm absorbance shows a maximum at $X = 0.8$. The respective absorption spectra of **1** obtained with 2 and 4 equiv of F^- (Fig. 5) are similar to those of the monoanion and dianion species of **1** observed in water.[12] As reported,[12a,13] **1** is emissive in the anionic forms, especially in the dianion form. These findings suggest that, as proposed in Scheme 1, two phenolic OH protons of **1** are removed via the interaction with F^- in the ground state, resulting in the formation of 'emissive' two anionic species.

For further confirmation of the proposed F^- sensing mechanism of **1**, ^1H NMR titration of **1** was carried out with F^- in DMSO-d_6 (Figure S3[10]). Upon the addition of 0.2 equiv of F^-, phenolic OH proton of **1** (10.09 ppm) shifts downfield (11.02 ppm) with a significant intensity decrease. This indicates the occurrence of a hydrogen-bonding interaction between the phenolic OH protons of **1** and F^-.[14] Addition of 2 equiv of F^- leads to a complete disappearance of the OH proton. In addition, all of the aromatic protons of **1** shift upfield. This means that net electron density of the aromatic ring of **1** increases,[15] indicative of the removal of phenolic OH proton from **1**. The upfield shift of the aromatic protons almost stops upon the addition of 4 equiv of F^-, meaning that the two phenolic OH protons of **1** are completely removed at this stage. This result is consistent with the absorption titration and the Job's plot data (Figs. 5 and 6). In addition, upon the addition of >4 equiv of F^-, a new triplet signal appears at 16.1 ppm ($J = 121$ Hz), which is ascribed to a FHF^- dimer,[1h,3d,g,16] meaning that the phenolic OH protons of **1** are actually removed by F^-. The removal of the phenolic OH protons is also confirmed by ESI–MS analysis (Figure S4[10]): the MS chart obtained with **1** and 5 equiv of F^- in MeCN shows a strong peak at m/z 1056.8, which is ascribed to $([\mathbf{1}-2H] + 3[n\text{-}Bu_4N])^+$



Scheme 1. Proposed F^- sensing mechanism of **1**.

ion, in which both phenolic OH protons of **1** are removed. This means that anionic species of **1** are actually produced by the F^--induced proton removal. The F^- sensing mechanism of **1** can therefore be summarized as Scheme 1: first 2 equiv of F^- leads to removal of one phenolic OH proton of **1** via a hydrogen-bonding interaction, resulting in a formation of a FHF^- dimer and the emissive monoanion of **1**.[15] Another phenolic OH proton of the monoanion is removed continuously with further addition of 2 equiv of F^-, forming FHF^- dimer and the emissive dianion.

The proposed F^- sensing mechanism of **1** (Scheme 1) is further confirmed by absorption and fluorescence behaviors of the control compounds, **2** and **3** (Fig. 1; see Materials[10]). Compound **2** has a spirocycle-open form by the esterification of the carboxylic acid group of **1**; therefore, even without anions, **2** exhibits an absorption at 400–500 nm (Figure S5[10]). This absorption spectrum is similar to that of **1** obtained with <2 equiv of F^- (Fig. 5), meaning that the removal of the phenolic OH proton of **1** actually leads to the spirocycle opening (Scheme 1). Upon the addition of F^- to **2**, a new absorption appears at 524 nm, along with a decrease in 400–475 nm absorbance (isosbestic point: 475 nm), as is also the case for **1** with 2–4 equiv of F^-. This means that the phenolic OH proton of **2** is also removed by F^-. Job's plot of **2** with F^- (Figure S6[10]) shows a maximum absorption at $X = 0.66$, indicating that the proton removal of **2** by F^- occurs in a 1:2 stoichiometry.[17] These indicate that 2 equiv of F^- leads to removal of one phenolic OH proton; this fact supports the proposed deprotonation mechanism of **1** (Scheme 1). Compound **2** is weakly fluorescent, but addition of F^- leads to a fluorescence enhancement (Figure S7[10]).[18] This means that the removal of the phenolic OH proton produces stronger emitting species; this also supports the proposed mechanism (Scheme 1).

Compound **3** (Fig. 1) has a spirocycle-open form, where both carboxylic acid and phenolic OH groups of **1** are methylated. This compound does not show any absorption or fluorescence response to F^- (Figure S10[10]). This is because **3** does not produce anionic species due to the lack of a phenolic OH proton. This finding again support the proposed mechanism: the removal of the phenolic OH proton of **1** by F^- triggers the strong fluorescence enhancement (Scheme 1).

In conclusion, we found that the unmodified 'ready-made' fluorescein (**1**) behaves as a fluorescent F^- sensor, which shows potential for selective F^- detection among the halide anions. As is usually observed for the fluorescent anion sensors,[3,7] the present fluorescein system has difficulty in the application for aqueous samples.[19] However, the fluorescein is photoexcited by visible light and shows high fluorescence quantum yield and high sensitivity; therefore, the fluorescein may be applicable as a fluorescent F^- sensor. The sensing mechanism clarified here, which cleverly detects F^- by simple proton transfer processes, may contribute to the design of more effective and more sensitive fluorescent anion sensor based on the fluorescein platform.

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Supplementary data

Supplementary data (Materials and Figures S1–S16). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.10.086.

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17. The formation of the anion form of compound **2** is confirmed by ^{1}H NMR titration (Fig. S8) and ESI–MS (Fig. S9) analyses. Upon the addition of F^-, the phenolic OH proton of **2** at 11.06 ppm (in DMSO-d_6) disappears and all of the aromatic protons shift upfield, as is also the case for **1**. Triplet FHF^- dimer signals also appear at 16.1 ppm ($J = 121$ Hz). ESI–MS analysis of a MeCN solution containing **2** with 5 equiv of F^- shows a peak at m/z 843.5, assigned to $([\mathbf{2}-1H]+2[n\text{-}Bu_4N])^+$ ion. These data clearly support the formation of the anion form of **2** by the F^--induced removal of the phenolic OH proton.
18. The fluorescence quantum yield of **2** with 5 equiv of F^- is only 0.14.
19. Addition of 0.1% or 1% water to MeCN containing fluorescein and 20 equiv of F^- shows fluorescence intensity similar to that obtained without water, although the addition of 5% water to MeCN shows almost zero fluorescence intensity.