Highly Selective Fluoride Ion Detection Based on a Fluorescent Alizarin–*o*-Aminomethylphenylboronic Acid Ensemble in Aqueous MeOH Solution

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A self-organized fluorescent sensor consisting of alizarin and *o*-aminomethylphenylboronic acid **1** is presented, the ensemble shows highly selective fluoride ion-response in H_2O–MeOH (1:4 w/w) at pH 5.5. Compound **1** ($pK_a = 5.63$) at pH 5.5 in the solution follows the equilibrium: alizarin–**1** + $H^+ \rightleftharpoons$ **1**H^+ + alizarin. Thus, F^- added into the solution preferably binds to **1**H^+ to allow the ensemble to release alizarin, resulting in a fluorescence quenching.

Much attention has been devoted to the field of anion receptor chemistry,[1] where the design of chemosensors for anions is a significant focus due to the importance in the medical and environmental area for the detection and quantification of anions. In this context, monitoring anions with high Gibbs hydrogen enthalpy[2] in aqueous media is still a definite challenge; we have focused on fluoride ions (F^-) as a target analyte, due to it being the smallest negative species, and also being important in dental care[3] and treatment of osteoporosis.[4] The majority of the reported fluoride ion sensors work well in an organic solvent.[5] Many approaches to access anion-induced response involves hydrogen bonding and/or Lewis acid–base interactions with anions, and is often subject to interference from oxoanions such as AcO^-,[6] due to competitive Lewis basicity. The sensing of F^- in protic media, therefore, become an intriguing subject.[7]

One route to prepare simple chemosensors capable of avoiding extensive synthetic chemistry is through the development of self-organized receptor–reporter systems, attained by linking molecular units through reversible interactions.[8] For instance, the indicator displacement assay, pioneered by Anslyn, is a useful means of analyte-detection.[9] The use of well-tailored interactions between phenylboronic acid (PBA) and *cis*-diols (boronate esterification) leads to highly functional systems because this process is much faster than the human time-scale.[10] We have thus investigated the optical properties of *o*-aminomethylphenylboronic acid **1**[11] with alizarin in aqueous media, and found that fluorescence of the alizarin–**1** ensemble was selectively quenched upon addition of F^- in H_2O–MeOH (1:4 w/w) solution at pH 5.5. It is interesting to note that such a quenching was not observed upon addition of other anions tested. The intriguing results are described in this communication.

o-Aminomethylphenylboronic acid **1** has a neighboring nitrogen, providing an intramolecular Lewis acid–base interaction between the boron and the tertiary amine. The N–B coordinated and solvated species are in equilibrium,[12] and are both expected to facilitate boronate ester formation with alizarin and cause a change in the optical properties. The photophysical behavior of alizarin in the presence of **1** (9 equiv.) in H_2O–MeOH (1:4 w/w) was then investigated by carrying out spectrofluorometry at varying pH where a solution containing excess base was titrated with standard acid (Figure 1). Alizarin has no fluorescence over a large pH range from 2 to 12 (Figure 1, ●), whereas in the presence of **1**, the fluorescence intensity at 550 nm increases, and reaches a maximum at pH from 5 to 8 (Figure 1, □). We ascribe the observed fluorescence enhancement to the formation of alizarin–**1** ensemble caused by the alizarin binding to the boronic acid group. Evidence for the formation of the complex came from ^{1}H NMR data in D_2O–CD_3OD (1:4 w/w) under weakly acidic conditions (Figure S1).[13] Adding **1** (1 equiv.) into a solution of alizarin made the signals of alizarin somewhat broad and resulted in a significant up-field shift in one set of peaks ($\Delta\delta$[Ar–Ha] = 0.22 ppm. See Figure S1).[13] These observations confirmed the formation of a alizarin–**1** ensemble through the boronate esterification. These observations were supported by FAB MS spectroscopic data ($m/z = 460$ [**1** + alizarin − $2H_2O$ + H]$^+$). The presence of F^- in the solution involving alizarin and **1** produced a different pH-profile when compared to that observed for alizarin plus **1**; notably, the fluorescence intensity significantly decreased in the range of pH 5–7 (Figure 1, ■). The pronounced quenching profile under the weakly acidic conditions motivated us to set up the conditions for applying alizarin–**1** ensemble to F^- sensing. Firstly, under the conditions optimized at pH 5.5 using MES buffer the fluorescence titrations of alizarin (50 μM) at an excitation wavelength of 420 nm, upon incremental amounts of **1** up to 1.5 mM, caused a 95-fold enhancement of the spectra, the association constant being $(1.35 \pm 0.11) \times 10^4\ M^{-1}$ for the formation of alizarin–**1** complex (Figure S3(a)).[13] One can compute from the association constant that 92.3% of alizarin (50 μM) can be converted to alizarin–**1** ensemble in the presence of 9 equiv. of **1**. Subsequently, we carried out fluorescence titrations of alizarin with F^- in the presence of 9 equiv. of **1** in H_2O–MeOH (1:4 w/w) at pH 5.5.

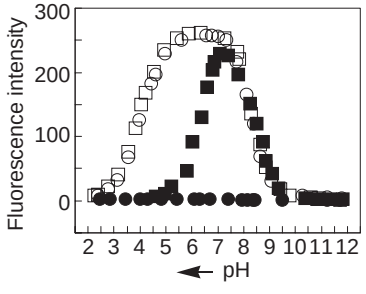

Figure 1. Spectrofluorimetric pH-titrations of alizarin (●); alizarin plus **1** (□); alizarin plus **1** with F^- (■); alizarin plus **1** with AcO^- (○) in H_2O–MeOH (1:4 w/w); 100 mM NaCl; [alizarin] = 50 μM; [**1**] = 459 μM; [KF] = [KOAc] = 30 mM; $\lambda_{ex} = 420$ nm; $\lambda_{em} = 550$ nm.

As expected, the fluorescence intensity at 550 nm decrease upon addition of F^-, as shown in Figure S3(b),[13] the behavior being detected by naked eye. The binding profile for the release of free alizarin as F^- competes for **1** in the solution, could be reproduced by a nonlinear curve fitting plot based on a plausible equation: alizarin–**1** + F^- ⇌ F^-–$\mathbf{1H^+}$ + alizarin (vide infra). Although multiple equilibriums will be involved in the equation, the presence of isosbestic point at 420 nm in the UV–vis spectra suggests that release of alizarin takes place by the interaction of F^-. The release of alizarin induces fluorescence quenching; fitting the decrease in fluorescence intensity at 550 nm as a function of F^- concentration gives apparent association constant of the boronic acid with F^- to be $360 \pm 72\ M^{-1}$. In the presence of AcO^- a profile perfectly superimposable on that of alizarin–**1** entity (Figure 1, ○) was obtained. This result is interesting because the basicity as well as hydration energy of AcO^- is comparable with F^-. Previously, James et al. reported that $\mathbf{1H^+}$ preferably binds to F^- through Lewis acid–base (B–F) interaction associated with NH···F hydrogen bonding.[11] Thus, we decided to check the pK_a value of **1** carefully under the employed conditions, the value being 5.63 ± 0.03 (Figure S5).[13] Judging from the pH dependency of **1**, a change from $\mathbf{1H^+}$ to **1** should occur in the pH region from 4 to 7. Taken together, a plausible mechanism is illustrated in Scheme 1 for F^--induced fluorescence quenching of alizarin in the presence of **1** at pH 5.5. Alizarin binds to **1** effectively to form a fluorescenct alizarin–**1** ensemble because **1** has a tetrahedral boronate segment. However, at pH 5.5 **1** equilibrates with $\mathbf{1H^+}$ as inferred from the pK_a value, where added F^- can bind to $\mathbf{1H^+}$ more favorably, accompanying with the equilibrium shift from alizarin–**1** ensemble to F^-–$\mathbf{1H^+}$. As a result, an incremental amount of F^- releases non-fluorescent alizarin from the equilibrium, leading to quenching of fluorescence. Indeed, the ^{1}H NMR signals in Figure S1[13] where an excess amount of KF was added to the solution involving alizarin and **1** indicate the presence of free alizarin. Further assessment was taken from a ^{19}F NMR study (Figure S6).[13] The signal at -137.7 ppm is assignable to F^-–$\mathbf{1H^+}$,[11] supporting plausible mechanism depicted in Scheme 1.

Anion selectivity of the present fluorescent system was investigated using F^-, Cl^-, Br^-, I^-, and AcO^- which represent families of biologically important anions. Figure 2 shows fluorescence quenching of alizarin when these anions (30 mM) were added to a H_2O–MeOH (1:4 w/w) solution at pH 5.5, in the presence of 9 equiv. of **1**. The measurement showed a strong quenching by F^-, which was estimated from the quenching ratio ($=(I_0 - I)/(I_0 - I_{alizarin})$), based on the fluorescence intensity of alizarin–**1** in the absence (I_0) and presence of 30 mM of F^- (I), to be 96%. It is noteworthy that the presence of anions other than F^- elicited no response of fluorescence: the slight change upon adding iodide ion can be ascribed to "heavy atom" effect. In this way, the experimental results suggest that alizarin–**1** ensemble shows highly selective detection of F^- in aqueous solution, being mainly attributed to the efficient affinity between $\mathbf{1H^+}$ and F^- in which F^- as "small base" can be accommodated through F–NH hydrogen bonding and F–B acid/base interactions.

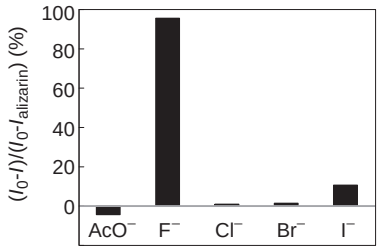

Figure 2. Fluorescence quenching for alizarin upon addition of various anions in the presence of **1** in H_2O–MeOH (1:4 w/w); 100 mM NaCl; MES buffer (pH 5.5); 25 °C, [alizarin] = 50 μM, [**1**] = 454 μM, λ_{ex} = 420 nm. I denotes the fluoresecnet intensity in the presnece of 30 mM of X^- (X^- = F^-, Cl^-, Br^-, I^-, and AcO^- as potassium salts).

In summary, alizarin–**1** ensemble shows a highly selective F^--induced fluorescence quenching at 550 nm in the visible region. We believe that our results are the first example of fluorescent sensing of F^- in aqueous media, based on a self-organized approach using boronic acid system.

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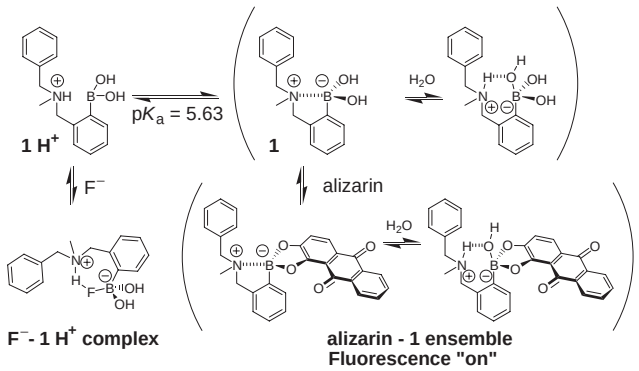

Scheme 1. Plausible mechanism for F^--induced fluorescence quenching.

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13 Supporting Information is available on the CSJ-Journal web site.

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