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Fluorescent Chemodosimeter for Selective Detection of Cyanide in Water

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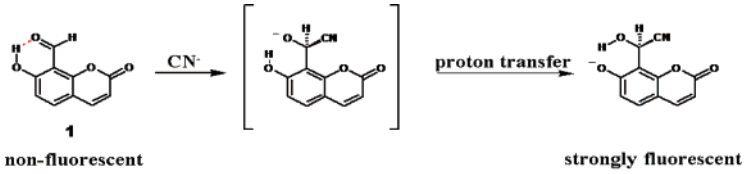
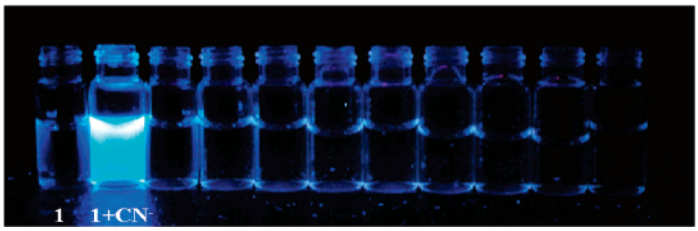
ABSTRACT





A coumarin-based fluorescent chemodosimeter with a salicylaldehyde functionality as a binding site has been developed for selective detection of cyanide anions over other anions in water at biological pH.

The cyanide ion is an extremely hazardous material that damages by absorption through the lungs, gastrointestinal track, and skin and can kill mammals upon binding to a heme unit.[1] The process of cellular respiration in mammals is inhibited by the cyanide anion, which interacts strongly with a heme unit in the active site of cytochrome a_3.[2] Although cyanides have been found in many foods and plants, most environmental cyanides are released by industries involved in gold mining, electroplating, and metallurgy.[3] Humans may be exposed to cyanides from dietary, industrial, environmental, and other sources. Consequently, there is a growing interest in sensing the presence of the toxic cyanide anion by coordination[4] or covalent bonds.[5]

Among the various chemosensors, fluorescent chemosensors present many advantages, including high sensitivity, low cost, easy detection, and suitability as a diagnostic tool for biological concern.[6,7] Unfortunately, few, if any, fluorescent cyanide anion sensors are capable of displaying high selectivity over other anions in pure water.[5e,8]

Salicylaldehyde is a popular reaction counterpart for nucleophilic addition reactions owing to its activated carbonyl

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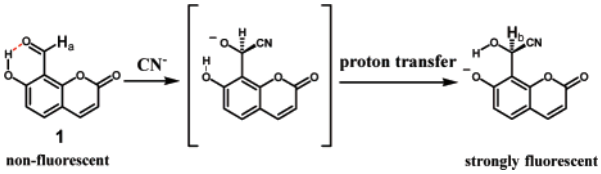
by a phenolic hydrogen through an intramolecular hydrogen bond.[9] Resonance-assisted hydrogen bonding (RAHB)[10] is one of the driving forces for the reactions, as observed in pyridoxal phosphate.[11]

Herein, we report a selective fluorescent chemodosimeter **1** for cyanide ion detection (Scheme 1). The dosimeter **1**

Scheme 1. Structure of the Dosimeter and Its Proposed Cyanide-Sensing Mechanism



has a coumarin group as a fluorescent signal unit and a salicylaldehyde functionality as a recognition or reaction unit. For this aim, the dosimeter **1** was synthesized according to the literature procedure.[12]

Cyanide is expected to be detectable by a nucleophilic attack toward a carbonyl functional group, which has been activated by the phenol proton of the dosimeter **1** through the intramolecular hydrogen bonding. Fast proton transfer of the phenol hydrogen to the developing alkoxide anion causes the strong fluorescence of the sensor (Scheme 1), as previously studied by a chromogenic cyanide dosimeter based on salicylaldehyde. It has been shown that the deprotonation of the phenol proton upon the addition of cyanides created a color change due to the bathochromic shift in the azo-based dosimeter.[13] However, with the present dosimeter, cyanide anions operate as a nucleophile toward the sensor, thereby inducing the hyperchromic effect and fluorescence enhancement.

We investigated the ^{1}H NMR spectra of the dosimeter **1** in the presence of cyanide anions and compared it with that of the sensor itself (Figure 1). The aldehyde proton (H_a) at

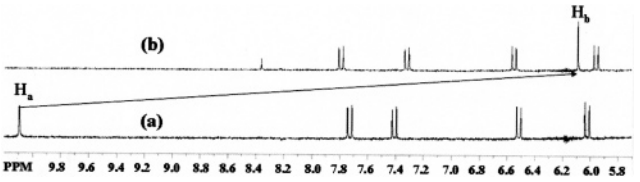


Figure 1. ^{1}H NMR spectral change of the dosimeter upon addition of cyanide anions: (a) sensor only and (b) sensor and 10 equiv of NaCN ([**1**] = 10 mM in D_2O at 25 °C).

around δ 10.1 ppm was dramatically shifted upfield toward δ 6.1 ppm (H_b) upon cyanide addition at room temperature. This chemical shift of H_b was consistent with a cyanohydrin form due to the nucleophilic attack of the cyanide anion toward the dosimeter's carbonyl group. ^{1}H NMR analysis indicated that the cyanide anion functions as a nucleophile in water.

These cyanide-sensing phenomena were monitored by fluorescence titration in an aqueous solvent (HEPES buffer at pH 7.4). Fluorescence monitoring of the cyanide addition reaction was performed by using a 10 μM solution of the dosimeter **1** in water under biological pH at room temperature. Upon addition of cyanide anions, the fluorescence emission intensity of the dosimeter at λ_{em} = 450 nm was increased 190-fold and was saturated at 1500 equiv of cyanide (Figure 2, Figure S1). Job analysis for the complex-

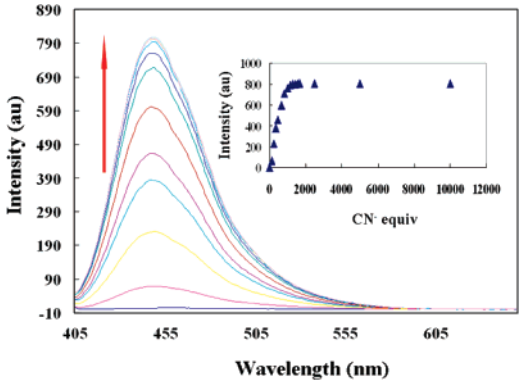


Figure 2. Fluorescence titration spectra of the dosimeter upon addition of cyanide anion. Inset: Fluorescence change of the sensor against [CN^-]/[**1**] ([**1**] = 10 μM, λ_{ex} = 360 nm, λ_{em} = 450 nm).

ation of the sensor and cyanide also corroborated the 1:1 binding stoichiometry (Figure S4).

To evaluate the selectivity of the dosimeter **1** for cyanide, we monitored the fluorescence intensities for various anions

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upon addition of excess guests in HEPES buffer solution at pH 7.4.

The dosimeter's fluorescence intensity was highly enhanced by cyanide. However, other anions such as F^-, $H_2PO_4^-$, AcO^-, ClO_4^-, Br^-, Cl^-, I^-, NO_3^-, and N_3^- did not cause any significant changes in the fluorescence emission intensity, even at a concentration of 1500 equiv of guests (Figure 3a). The fluorescence profiles at 450 nm of the

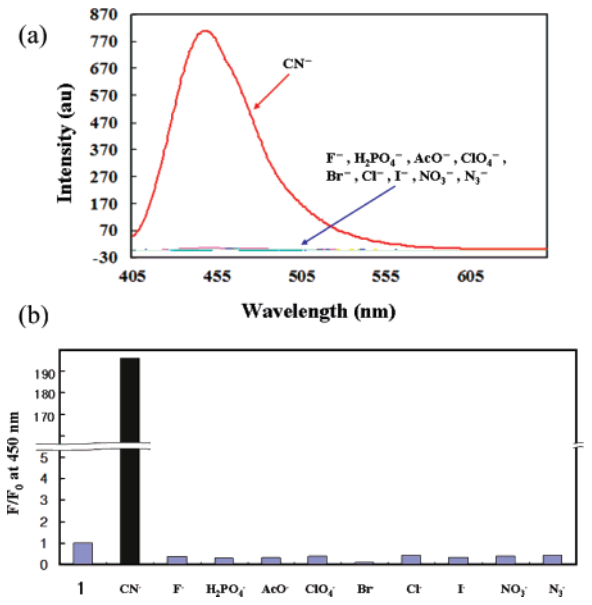

Figure 3. (a) Fluorescence spectra of the dosimeter (10 μM) in HEPES upon addition of 1500 equiv of various anions (from left to right: only the sensor, CN^-, F^-, $H_2PO_4^-$, AcO^-, ClO_4^-, Br^-, Cl^-, I^-, NO_3^-, N_3^-) (sodium salts). (b) Its relative fluorescence intensities.

chemodosimeter showed a remarkably higher selectivity for cyanide over the other anions in water (Figure 3b). We assume the cyanide selectivity of the dosimeter **1** is due to the nucleophilicity of cyanide in water. The nucleophilicity of cyanide in water seems to be the major contributor to the high selectivity of the dosimeter **1** for cyanide, while in DMSO, not only the nucleophilicity but also the basicity of anions (F^-, $H_2PO_4^-$, AcO^-) may cause large changes in the UV−vis spectra of the chromogenic chemodosimeter and therefore result in less selectivity for cyanide.[13] A large increase in the fluorescence intensity could be applied to the detection of cyanide anions by the naked eye. When the chemodosimeter was excited at 365 nm in the presence 1500 equiv of other anions in HEPES buffer at pH 7.4, a bright blue fluorescence response was selectively observed only in the presence of cyanide in the solution of 10 μM of the sensor (Figure 4).

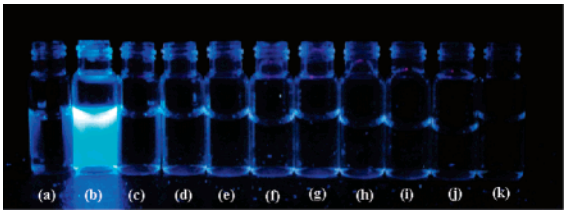

Figure 4. Fluorescence responses of the dosimeter (10 μM) in the presence of 1500 equiv of different anions: (a) only the sensor, (b) CN^-, (c) F^-, (d) $H_2PO_4^-$, (e) AcO^-, (f) ClO_4^-, (g) Br^-, (h) Cl^-, (i) I^-, (j) NO_3^-, and (k) N_3^- (sodium salts).

The in vivo selectivity of the dosimeter **1** for cyanide was also examined with living cells by using a fluorescence microplate reader. After P19 cells were incubated with 100 μM of the sensor for 1 h at 37 °C, the fluorescence images in the cells were monitored with increasing amounts of cyanide and compared with those in the cells without cyanide. Only the P19 cells treated with 1 mM cyanide showed a significant fluorescence intensity in the living cells (Figures S5 and S6).

In summary, a chemodosimeter having a salicylaldehyde moiety as a binding unit and a coumarin skeleton as a signaling unit was synthesized, and its fluorescence properties in the presence of anions were evaluated. The chemodosimeter displayed a dramatic change in fluorescence intensity selectively for cyanide anions over other anions in water at biological pH. This significantly enhanced fluorescence intensity was probably caused by the nucleophilicity of the cyanide anion and the carbonyl activation by the phenol proton of the salicyl functional group in the dosimeter through an intramolecular hydrogen bond.

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Supporting Information Available: Synthetic details, Job plot, cyanide selectivity over other anions, fluorescence response of the dosimeter to various anions in P19 cells, and fluorescence microplate reader images of living P19 cells in the presence of the chemodosimeter treated with CN^-. This material is available free of charge via the Internet at http://pubs.acs.org.

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