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Sensing cyanide ion via fluorescent change and its application to the microfluidic system

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ABSTRACT

We introduce a cyanide selective microfluidic platform, in which fluorescent chemodosimeter **1** displayed a selective green fluorescence upon the addition of cyanide. Our system was examined in aqueous solution, and the 'OFF–ON' type emission change can be monitored over 500 nm. In addition, colorimetric change was also observed upon the addition of cyanide. The practical use of the probe was demonstrated by its application to the detection of cyanide in the living cells.

The development of selective optical signaling systems for anions has received considerable attention in recent few decades due to their important roles in biological and environmental processes.[1] Especially, cyanide is a detrimental anion causing poisoning in biology and environment.[2] Cyanide ion is extremely toxic to mammals, leading to vomiting, loss of consciousness, and eventual death.[3] A significant proportion of fatalities among fire victims is due to cyanide poisonings, as blood cyanide concentrations reach a level of 23–26 μM.[3,4]

In this regard, various fluorescent and colorimetric sensors have been actively reported in recent 10 years. For example, one of the common approach is utilizing cyanide complexes with Zn(II)–porphyrin,[5] Ru(II)-pyridine,[6] boronic acid derivatives[7] and CdSe quantum dots.[8] Few hydrogen bonding approaches are also available.[9] On the other hand, nucleophilic addition of cyanide was recently utilized by various groups, in which the interference by other anions, such as fluoride and acetate, can be minimized. Nucleophilic addition of cyanide to oxazine,[10] pyrylium,[11] squarane,[12] trifluoroacetophenone,[13] acyltriazene,[14] acridinium[15] and salicylaldehyde[16] has been reported in recent few years. However, there have been still only few examples of 'OFF–ON' type sensors for cyanide, which work in aqueous solution.[7,16b]

On the other hand, there is growing interest in performing chemical reactions in microfluidic devices, which have one or more channels with at least one dimension <1 mm, to generate a laminar flow condition.[17] These microfluidic chemical analysis systems offer a variety of advantages over conventional analysis, bulk equipments: low fluid volume consumption, better process control, massive parallelization, compactness of the systems, etc. However, a rapid and efficient mixing is one of the most challenging tasks to overcome in the field of microfluidic analysis systems because chemical mixing in the laminar flow depends on molecular diffusion, which is a slow process. Thus, new types of active and passive micromixers have been invented for rapid and efficient mixing in the microfluidic systems.[18] Unlike the active micromixers which need external actuators, the passive mixers can be completed simply by modifying channel geometries into the following ones: zig-zag channels,[19] Tesla structures,[20] serpentine channels,[21] twisted channels,[22,23] and channels with patterned grooves.[24] These modifications can enhance stretching, folding and breaking of laminar flows, leading to efficient mixing of the flows.

Indeed, a combination of fluorescent chemosensor and microfluidic system can be a powerful tool for the development of lab-on-a-chip.[25] Even though there has been a microfluidic system for sensing cyanide,[26] there has not been an example of a combination of fluorescent chemosensor and microfluidic system for cyanide detection. In the present study, we introduce the cyanide selective microfluidic platform, in which fluorescent chemodosimeter **1** displayed a selective green fluorescence upon the addition of cyanide. The practical use of the probe was demonstrated by its application to the detection of cyanide in the living cells.

The cyanide probe **1** was synthesized following the reported procedure.[27] For the fluorescent study, various anions, such as CN^-, AcO^-, F^-, Cl^-, Br^-, I^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and ClO_4^- were evaluated in acetonitrile–HEPES (9:1, v/v, 0.01 M pH 7.4 HEPES). Using these anions (100 equiv), compound **1** (3 μM) showed a large fluorescent enhancement *only* with CN^-, even though there was a relatively small fluorescent enhancement with AcO^- (Fig. 1). The overall emission change upon the addition of cyanide ion

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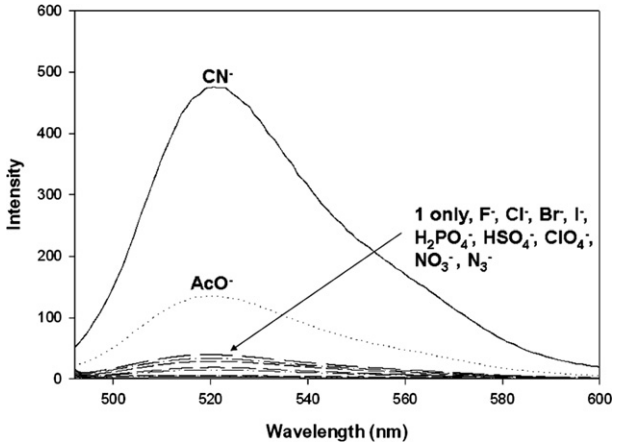


Figure 1. Fluorescent changes of **1** (3 μM) upon the addition of various anions (100 equiv) in CH_3CN-HEPES (0.01 M, pH 7.4) (9:1, v/v) at room temperature (excitation at 485 nm, excitation and emission slit: 5 nm).

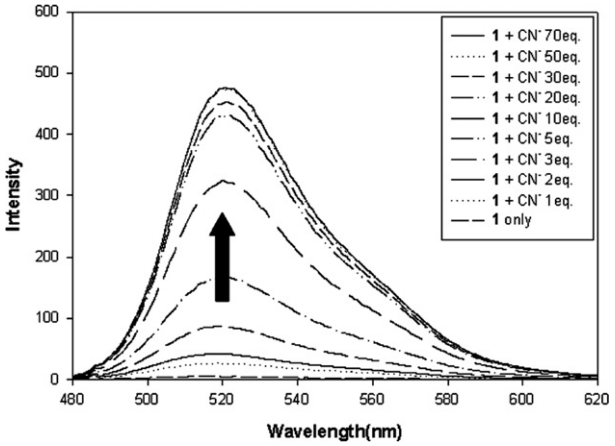


Figure 3. Fluorescence changes of **1** (3 μM) with different amounts of cyanide in CH_3CN–H_2O (9:1, v/v) at room temperature (excitation at 485 nm, excitation and emission slit: 1.5 nm).

was more than 200-fold. The fluorescent changes were observed within 1 min after the addition of anions.

The selectivity for cyanide can be attributed to the nucleophilicity of cyanide in aqueous solution. Figure 2 explains the partial ^1H NMR spectra of sensor **1** and **1** in the presence of cyanide ion (20 equiv) in CD_3CN–D_2O (9:1, v/v) at room temperature. The aldehyde proton (H_a) at δ 10.62 ppm disappeared and a new peak (H_b) at δ 6.16 ppm appeared upon the addition of cyanide, which can be attributed to a tetrahedral intermediate of addition adduct of cyanide anion to the carbonyl group as illustrated in Scheme 1. The aldehyde group in salicylaldehyde is known as an activated carbonyl group towards the nucleophilic attack since the carbonyl group can make an intramolecular hydrogen bonding.[28] Hong et al. recently utilized this event to the nice colorimetric sensor[16a] and fluorescent chemodosimeter for cyanide.[16b]

The fluorescent changes of probe **1** with various amounts of cyanide were examined in CH_3CN–H_2O (9:1, v/v) (Fig. 3 and S-Fig. 1). Our fluorescent chemodosimeter displayed a saturation point upon the addition of 50 equiv of cyanide (Fig. 3) and less than 10 μM of cyanide can be easily detected using our system. 'OFF–ON' type in aqueous solution, emission over 500 nm, working at room temperature and high sensitivity are certainly merits of our system compared to the previously reported examples. Furthermore, our system also displayed a colorimetric change upon the addition of cyanide. Figure 4 explains the colorimetric changes of **1** (30 μM) upon the addition of cyanide in CH_3CN–H_2O (9:1, v/v) at room temperature. The other anions examined did not induce any significant color change (Supplementary data).

The application of live cell-imaging to detect cyanide ion using the probe **1** was also studied. Human keratinocyte cell line, HaCaT cells were incubated with 20 μM of the sensor for 1 h at 37 °C. After washing with PBS, the cells were treated with 20 μM of sodium cyanide. Figure 5 shows the fluorescence images of HaCaT cells, differential interference contrast (DIC) images, and merged images, respectively.

The significant green fluorescence of probe **1** with cyanide was clearly observed and there was no fluorescence in cells treated with just probe **1** (not shown). This result demonstrated that probe **1** can be applied in live cell-imaging in aqueous condition.

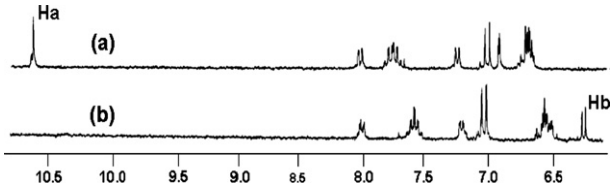


Figure 2. Partial ^1H NMR spectral change of compound **1** (3 mM) upon addition of cyanide in CD_3CN–D_2O (9:1, v/v) at 25 °C: (a) sensor only and (b) sensor and 20 - equiv of tetrabutylammonium cyanide.

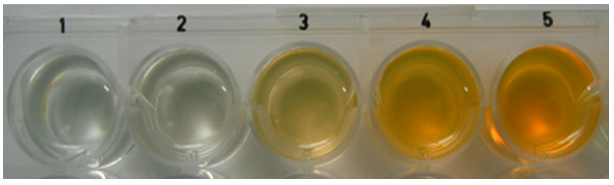
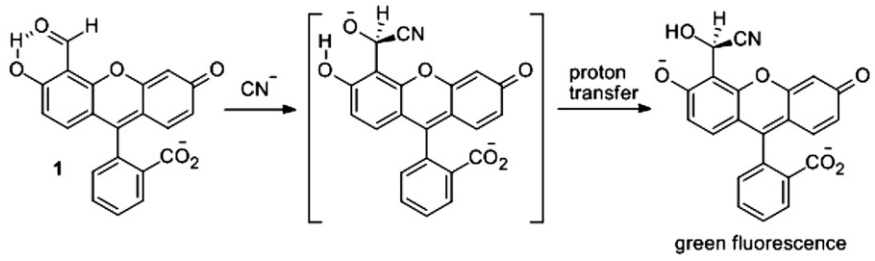


Figure 4. Colorimetric changes of probe **1** (30 μM) upon the addition of cyanide in CH_3CN–H_2O (9:1, v/v) (1: probe only, 2: 1 equiv of cyanide, 3: 5 equiv of cyanide, 4: 10 equiv of cyanide, 5: 50 equiv, of cyanide).



Scheme 1. Structure of the probe **1** and its proposed mechanism upon the addition of cyanide.

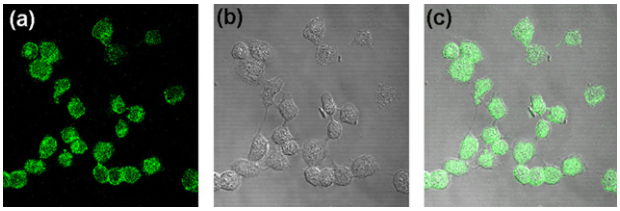


Figure 5. Images of HaCaT cells treated with 20 μM of NaCN after incubated with 20 μM of **1**. (a) Fluorescent images, (b) DIC images, (c) merged images. (excitation = 488 nm, emission = 505–530 nm).

Figure 6a shows the mixer comprising of three major functional parts marked in different colors: flow loading, chaotic mixing, and fluorescence measuring parts. In the flow loading part, the two inlets were connected to a syringe pump which controlled the flow rate of loaded sample. The microchannel in the chaotic mixing part had herring-bone shaped obstacles on its wall, which caused chaotic advection and enhanced mixing of laminar flows. The fluorescence measuring part consisted of a flat-wall microchannel for the easiness in the fluorescence measurement. After mixing with cyanide, the fluorescence images of the chemodosimeter were captured under a fluorescence microscope. Sensitivity of the chemodosimeter to cyanide was determined by analyzing these images using a Java-based image processing program, IMAGE-J.[25,29] Figure 6b shows a mixing result with food dyes in different colors run through the passive mixer. When they (yellow and blue dyes) met and ran through the chaotic mixing part, the thickness of green color gradually increased along the channel. Evenness in the green color purity indirectly indicates the level of mixing in the microchannel.

In order to compare and investigate the effect of fluorescent sensor sensing cyanide in this microfluidic device, a measure would be required to quantify the difference of sensor mixing with different concentrations of cyanide. Six images were captured because **1** mixing with five concentrations of cyanide (1 equiv, 5 equiv, 10 equiv, 20 equiv and 50 equiv) and a control mixing (**1** mixing with solvent only) have been checked. Their fluorescence intensities were measured by IMAGE-J and data were evaluated by Excel. Fluorescence intensity valve changing of **1** is correlated to the concentration of cyanide and has been shown in S-Figure 2.

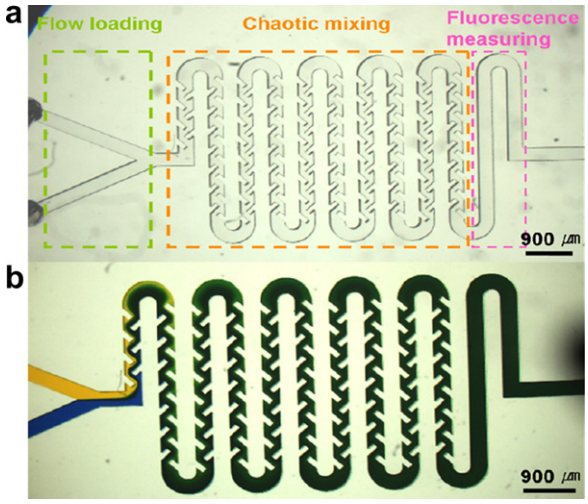


Figure 6. Optical images of the passive microfluidic mixer and mixing of food dyes in the mixer. (a) The mixer was composed of three parts: flow loading part (marked in green), chaotic mixing part (marked in orange) and fluorescence measuring part (marked in pink). (b) A mixing test was carried out by introducing yellow and blue food dyes into the micromixer.

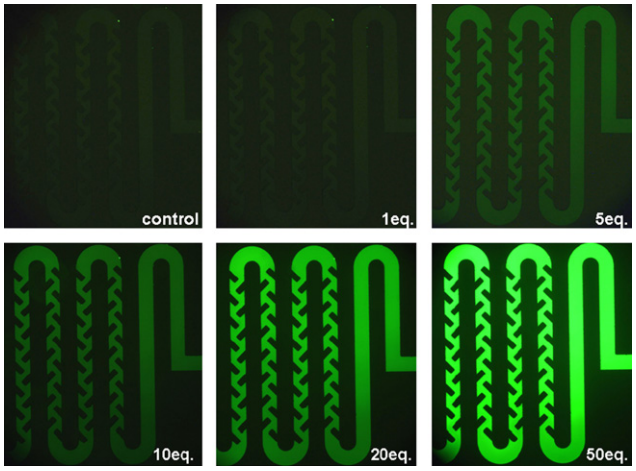


Figure 7. Fluorescence image of **1** (10 μM) mixing with cyanide at a flow rate of 10 μl/min.

In the present study, we introduced a cyanide selective microfluidic device, in which fluorescent chemodosimeter **1** displayed a selective green fluorescence upon the addition of cyanide. Our system was examined in aqueous solution, and the 'OFF–ON' type emission change can be monitored over 500 nm with a 200-fold enhancement of emission intensity. Furthermore, our probe **1** also displayed a colorimetric change with cyanide. The practical use of the probe was also demonstrated by its application to the detection of cyanide in the living cells (see Fig. 7).

Acknowledgments

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Supplementary data

Experimental details, the NMR spectra, fluorescence spectra. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.04.139.

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