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Coumarin-Cu(II) Ensemble-Based Cyanide Sensing Chemodosimeter

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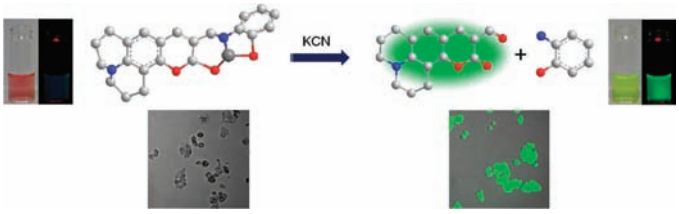
ABSTRACT



An "ensemble"-based chemodosimeter 1–Cu(II) for cyanide detection is reported. 1–Cu(II) can recognize a cyanide ion over other anionic species to show a marked fluorescence enhancement under aqueous conditions. "Off–on" fluorescence change of 1–Cu(II) is proceeded by addition of cyanide, which induces decomplexation of the Cu(II) ion from nonfluorescent 1 followed by hydrolytic cleavage of the resulted Schiff base to give a strongly fluorescent coumarinaldehyde (2). The selective detection of cyanide with 1–Cu(II) for biological application was also performed in HepG2 cells.

Cyanide is extremely toxic to mammals with even a small amount of this species, leading to vomiting, loss of consciousness, and eventually to death.[1] Nevertheless, it has been produced in large quantities and used in various industrial processes, which has led to environmental contamination as well.[2] Therefore, its concentration in drinking water is limited to less than 2 μM according to the World Health Organization.[3] In this regard, the sensitive detection method of cyanide such as fluorescence sensing probes has attracted considerable attention in recent years.

Fluorescence sensing of specific anions via suitable chemosensors is a valuable technique with high sensitivity, rapid response, and easy performance, offering application methods not only for in vitro assays but also for in vivo imaging studies.[4] Much effort has been drawn to develop the chemosensors for various biological substances such as cations, anions, sugars, and proteins. In particular, the fluorescent sensors have been mainly focused on cation targeting and on their corresponding bioimaging studies in living cells.[5] However, bioimaging studies of fluorescent chemosensors toward anions have been rarely reported, probably because of low solubility in aqueous media, low selectivity, and low sensitivity.[6]

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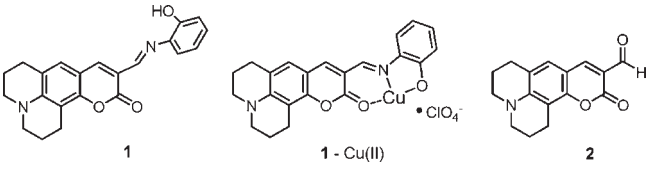
The detection principles of the most reported chemosensors for cyanide are based on nucleophilic addition,[7] hydrogen-bonding interactions,[8] cyanide complexes/addition,[9] or coordination of copper ion[10] with cyanide. However, there are still limitations regarding the sensitivity, selectivity, and compatibility within an aqueous or biological environment. Another consideration would be cation effect in sensing system for the CN^- detection, whereas tetrabutylammonium (TBA) has been usually used as a countercation in many reports.[7−10] Since potassium cyanide (KCN) is the toxic species defined by the U.S. Environmental Protection Agency (EPA) standard, detection of KCN rather than that of $(n\text{-Bu})_4N^+CN^-$ would be more meaningful in aspect of a practical application of the CN^- detection.

In light of these considerable drawbacks in the CN^- sensing reported so far, we have prepared an ensemble-based nonfluorescent **1**−Cu(II) able to selectively and sensitively detect the CN^- (particularly KCN) ion in the manner of "turn-on" fluorogenic sensing as well as naked-eye detection for the KCN. Taking advantage of this off−on fluorescence sensing system, it has been applied to intracellular CN^- detection in HepG2 cells in this study as well.

Probe **1**−Cu(II) was prepared by modification of our previously reported synthetic procedures (Scheme 1).[11] As in our earlier studies, we found that **1** is easily hydrolyzed to produce **2** strongly fluorescent in aqueous media. The crystal structure of **1**−Cu(II) (Figure S1, Supporting Information) confirms that the *o*-OH group in **1** serves as an additional binding site for the Cu(II) ion binding to provide a stable complex which gives a reduced fluorescence presumably due to MLCT-based heavy metal ion effect.

Scheme 1. Compounds **1**, **1**−Cu(II), and **2**



On the basis of our previous report,[11] it is clear that removal of Cu(II) from the **1**−Cu(II) complex will induce an emission via subsequent hydrolysis of its Schiff base by water. It is well-known that CN^- ion has strong binding ability to Cu(II) ion because of its strong back-bonding contribution to the metal ion to form a stable $[Cu(CN)_x]^{n-}$ complex.[12] On the basis of this ensemble system, it would be plausible to suggest that **1**−Cu(II) is hydrolyzed by CN^- ion with aid of water to give fluorescence enhancement as indicated in Scheme 2.

We found that **1**−Cu(II) is initially nonfluorescent (Φ_f = 0.02). Addition of CN^- ion to **1**−Cu(II) induces a 57 nm hypsochromic shift of the absorption maximum from 521 to 464 nm and a perceived color change from orange-red to green (Figure 1a). In addition, the fluorescence intensity at λ_{em} = 514 is concomitantly enhanced (Φ_f = 0.65) as a function of time as indicated in Figure 1b. A newly formed emission band by the addition of cyanide ion to a solution of **1**−Cu(II) is found to be the same as that of **2** (Table S1, Supporting Information). Therefore, it is reasonable that CN^- in a solution of **1**−Cu(II) causes a formation of stable $Cu(CN)_x$ to release free **1** followed by in turn easily hydrolyzed to provide **2** which is strongly fluorescent.

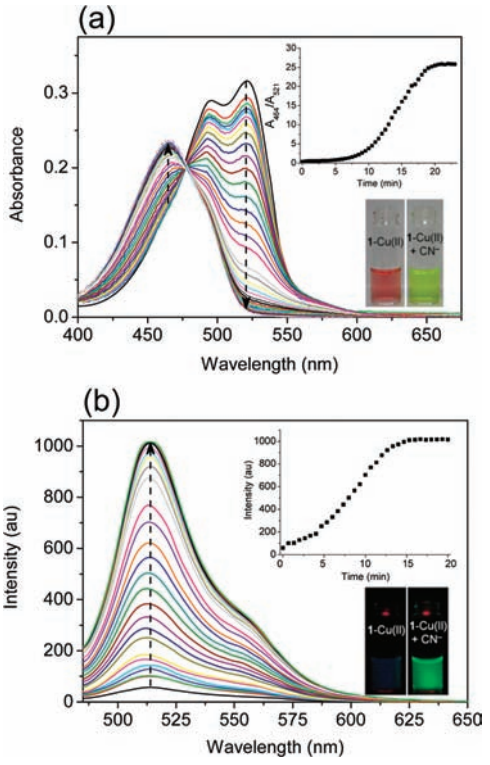


Figure 1. Time-dependent (a) UV−vis and (b) fluorescence spectra of **1**−Cu(II) (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) upon addition of cyanide (16 equiv). Excitation at 479 nm (slit = 1.5/1.5). Inset: plot of (a) A_{464}/A_{521} vs time and (b) emission intensity at 514 nm vs time.

To investigate compatibility of the ensemble-based anion detection ability of **1**−Cu(II) with the biological environment,

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the absorption and emission spectra of **1**−Cu(II) in an aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) were examined with respect to various anions commonly found in biological media. Indeed, as shown in Figure 2(a), **1**−Cu(II) revealed remarkable enhancement of emission intensity only in the presence of CN^-, whereas no noticeable changes were observed with other anions (F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, ClO_4^-, HCO_3^-, SCN^-, OH^-, CO_3^{2-}, HPO_4^{2-}, PO_4^{3-}, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP)). Competitive experiments shown in Figure 2 also confirm that **1**−Cu(II) shows a selective response to the CN^- even in the presence of other anions.

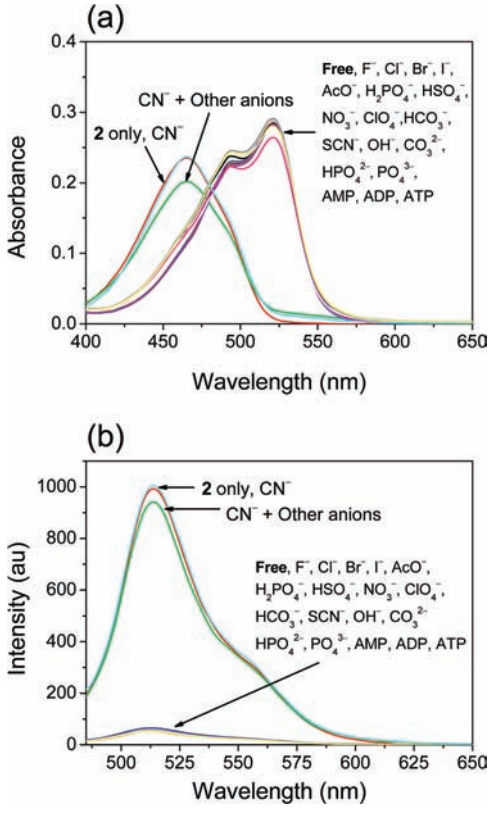


Figure 2. (a) UV−vis and (b) fluorescence spectra of **1**−Cu(II) (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) upon addition of various anions (16 equiv). Excitation at 479 nm after 20 min (slit = 1.5/1.5).

An ESI-MS spectrometric analysis of **1**−Cu(II) treated with CN^- in aqueous solution revealed signals for the formation of the expected **2** (m/z 270.2, $[2 + H]^+$) (Figure S2, Supporting Information). ^{1}H NMR spectroscopy of the reaction product also showed the same splitting patterns anticipated from the structure **2** (Figure S3, Supporting Information). In addition, the fluorescence quantum yield (Φ_f = 0.65) of a mixture of **1**−Cu(II) and CN^- is similar to that of **2** (Φ_f = 0.66) (Table S1, Supporting Information). These results again support that **1**−Cu(II) easily transforms into **2** by the addition of CN^- ion in aqueous media.

Figure S4 (Supporting Information) demonstrates that chemodosimetric reaction of **1**-Cu(II) with CN^- is linear and sensitive enough to detect the 10^{-8} M CN^- ion in aqueous solution. In pH variable test, this florescence "off−on" system is working properly in the pH range from 6 to 10 in aqueous solution (Figure S5, Supporting Information). Therefore, it should be noteworthy that probe **1**−Cu(II) in our study is a desirable fluorescence probe in that it is simple, reliable, selective, and biocompatible reaction-based CN^- sensor.

Figure S6 (Supporting Information) illustrates fluorescence spectral changes during the reaction of **1**−Cu(II) with varied [CN^-]. In their emission spectra, a significant enhancement of fluorescence intensity at 514 nm was observed upon excitation at 479 nm. Interestingly, the fluorescence intensity within the first 2 min shows a linear response to the [CN^-] whereas the fluorescence change within 15 min is sigmoidal. This apparent discrepancy between two different kinetic behaviors in 2 min and in 15 min may be related with the reaction mechanism as shown in Scheme 2. Time courses of the fluorescence intensity for the reaction of **1**-Cu(II) with various amount of CN^- are shown in Figure 3. The progress curve with 80 μM of CN^- clearly demonstrates biphasic responses composed of a fast phase within 3 min and a slow phase starting around 5 min. This biphasic time course is found in the samples at almost all range of CN^- concentrations. It would be interesting to remind that the fast phase linearly responds to the CN^- concentration, which may be important in quantification of CN^-.

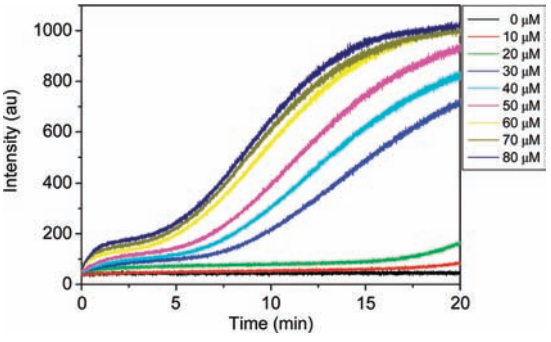
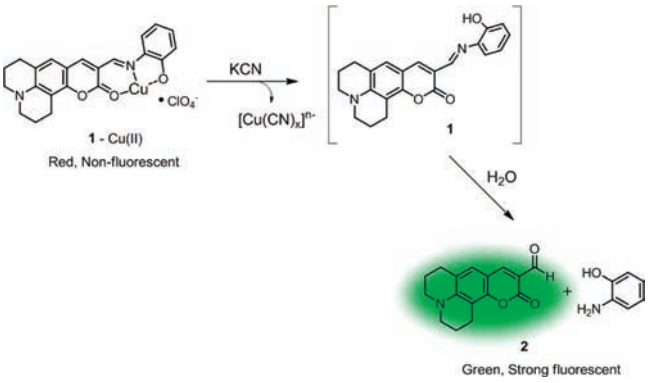


Figure 3. Time course of the fluorescence response of **1**−Cu(II) (5.0 μM) in aqueous solution (10 mM PBS buffer, pH 7.4, 1.0% DMSO) upon addition of various concentrations of cyanide (0−16 equiv). The excitation and emission wavelength were 479 and 514 nm, respectively.

The kinetic analysis implicates that both phases depend on CN^- concentration and the CN^- plays important roles in decomplexation of Cu(II) from **1**−Cu(II) as well as in hydrolysis of probe 1−Cu(II) to give the free **2**. (Figure S7, Supporting Information). This conclusion is further supported by the facts that fluorescence emission from

Scheme 2. Schematic Illustration of Turn-on Fluorescence Sensing Mechanism of **1**−Cu(II) for Cyanide



separately prepared **2** is unchanged in the presence of CN^- (Figure S8, Supporting Information) and that hydrolysis of **1** is accelerated with cyanide (Figure S9, Supporting Information). The latter reaction is presumed as an example of base-catalyzed Schiff base hydrolysis.[13] Also, an ESI-MS spectrometric analysis of **1**−Cu(II) treated with CN^- in aqueous solution from a fast phase within 3 min revealed signals corresponding to the formations of the expected **1** (m/z 361.2, $[\mathbf{1} + H]^+$), **2** (m/z 292.2, $[\mathbf{2} + Na]^+$), and **1** − Cu(II) (m/z 500.1, $[\mathbf{1} + Cu + DMSO - H]^+$) (Figure S10, Supporting Information). This results is also indicative of biphasic responses composed of **1**−Cu(II) for CN^- ion.

To demonstrate that **1**−Cu(II) is able to detect the CN^- ion in cells, the cells preloaded with **1**−Cu(II) complex were treated with various amounts of KCN (Figure 4). Time-dependent and $[CN^-]$-dependent increases of fluorescence intensity were observed inside of the cells, as shown in the inset, after the KCN treatment (Figure S11, Supporting Information, and Figure 4, respectively). Fluorescence was only slightly observable on the cells after 40 min of incubation prior to the KCN treatment (Figure S7, Supporting Information), and any significant change of the cell morphology or floating appearance of the cells was not observed. The fluorescence intensities in Figures 4 and S6 (Supporting Information) are apparently caused by chemodosimetric reaction of **1**−Cu(II) complex with CN^- ion in the cells to give a strongly fluorescent coumarinaldehyde **2**.

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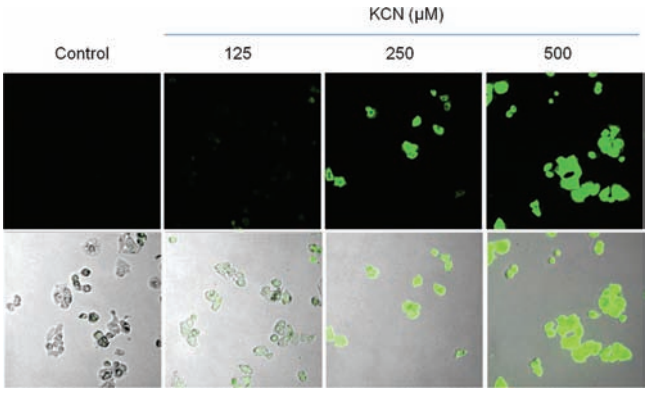

Figure 4. Confocal microscopic images of HepG2 cells in the presence of **1**−Cu(II) (1.0 μM). The fluorescence images were acquired after 1 min of treatment of KCN (125, 250, and 500 μM) on HepG2 cells. Bottom panels show an overlay of the image with a confocal phase contrast image.

In summary, we demonstrated that an "ensemble"-based chemodosimeter **1**−Cu(II) can selectively probe the cyanide ion in aqueous media with respect to a marked fluorescence enhancement over other anionic species under aqueous condition. The "off−on" fluorescence mechanism of **1**−Cu(II) with cyanide ion is suggested as a decomplexation of Cu(II) from nonfluorescent **1** followed by hydrolytic cleavage of the Schiff base to give a strongly fluorescent coumarinaldehyde **2**. Both reactions are contributed by the cyanide ion. The selective cyanide detection with **1**−Cu(II) for the biological application was also performed in HepG2 cells to show the "off−on" fluorescence cellular image as well.

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Supporting Information Available. Text and figures giving details of the X-ray crystal structure, additional spectral data, ^1H NMR spectra, and confocal microscopic images. This material is available free of charge via the Internet at http://pubs.acs.org.