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Short communication

A fluorescein derivative FLTC as a chemosensor for Hg^{2+} and Ag^{+} and its application in living-cell imaging

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ABSTRACT

FLTC was synthesized and used as a fluorescent chemosensor to detect Hg^{2+}. It showed high selectivity toward Hg^{2+} over many heavy metal ions in an ethanol–H_2O (3:2, v/v, HEPES buffer, 0.5 mM, pH 7.15) solution with a detection limit of 0.21 μM. After complexation with Hg^{2+}, FLTC showed extremely high selectivity toward Ag^{+} with a detection limit of 0.009 μM. Therefore, detection of Hg^{2+} and Ag^{+} could be realized using FLTC and the FLTC–Hg^{2+} complex, respectively. Cytotoxicity assays and fluorescence microscopy analysis showed that FLTC could be used as a fluorescent probe to detect Hg^{2+} and Ag^{+} in L-02 human liver cells.

Hg^{2+} and Ag^{+} are two of the heavy transitional metals that receive intensive attention because large quantities of them are released into environment each year. Ag^{+} is widely used in the electrical industry, photographic and imaging industry leading to environmental pollution [1]. Exposure to mercury, even in small dose, is a great danger to humans and wildlife. When mercury enters the body it acts as a neurotoxin, harming the brain and nervous system [2]. Mercury exposure is especially dangerous to pregnant women and young children [3]. Thus development of sensitive and selective methods to determine trace Hg^{2+} and Ag^{+} is of great interest. Conventional methods, such as atomic absorption spectroscopy [4] and inductively coupled plasma mass spectroscopy (ICPMS) [5], have been used in the past for this purpose. Although these methods are sensitive and accurate, advanced instrumentation and complicated time-consuming sample pre-treatments are needed. Fluorescence spectroscopy is a rapid nondestructive and relatively low cost method that can be used for real time tracking to detect ions in living systems [6,7].

Various fluorescent chemosensors for Hg^{2+} have been developed, such as those based on the receptor moiety of 3,9-dithia-6-azaundecane [8], 2,6-bis (amino- methyl)pyridine [9], oligonucleotide [10], thioether-rich crown [11], thiosemicarbazide [12], 1,4-disubsitued azine [13], thiol ligands on nanopartical surface [14], carbohydrazone [15],cyclen [16], thymine [17] and etc. Chemosensors designed for Hg^{2+} usually contain thio groups because their high affinity for Hg^{2+}. A number of fluorescence chemosensors for Ag^{+} containing receptor moiety of guanine [18], piperazine [19], benzoylthiourea [20], DNA [21], carbodithioate [22] and 1,2-diphenyldiselenide [23] have also been developed. Meanwhile there are several fluorescence chemosensors that can detect both Hg^{2+} and Ag^{+} simultaneously, but most of them cannot distinguish one from another [24–32].

In a previous study, FLTC was synthesized for the detection of HOCl [33]. In this study, we demonstrate that it can be used for the detection of Hg^{2+} and Ag^{+} in buffers and living cells, which may find applications in fast detection of Hg^{2+} and Ag^{+} or cell imaging (Fig. 1).

FLTC was synthesized according to a previously described procedure [33], and its structure was clearly confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (Figs. S1–S3). At certain pH values, fluorescein compounds may undergo a ring open reaction, and emit strong fluorescence, which causes background interference during detection [34,35]. As shown in Fig. S4, an acid-base titration study conducted in ethanol–H_2O (3:2, v/v) solution showed that FLTC did not emit any distinct and characteristic fluorescence in the pH range of 1.0–7.0, which indicates that FLTC mainly presents in its spirolactam form in acidic environments. When the pH was adjusted to values between 7.0 and 11.0, the fluorescence intensity at 524 nm was greatly enhanced, likely due to the ring-opening process of the spirocyclic moiety of fluorescein. At a pH was higher than 11.0, the

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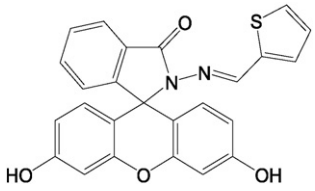

Fig. 1. Chemical structure of FLTC.

Table 1
Different detection limit of Hg^{2+}.

Compound	Detection limit of Hg^{2+}
FTLC	0.21 μM
1	0.010 μM [9]
MF1	0.060 μM [11]
2	4.60 μM [13]
Complex 1	0.118 μM [30]
SQ1	0.13 μM [31]

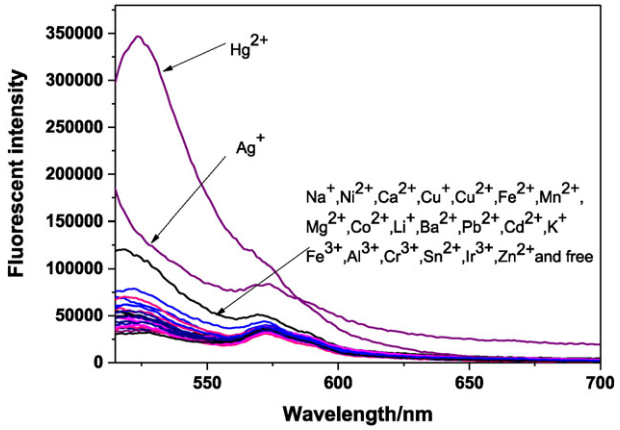

Fig. 2. The fluorescence spectra of FLTC (10 μM) in an ethanol–H_2O (3:2, v/v, pH 7.15, HEPES buffer, 0.5 mM) solution in the absence and presence of 10 equivalents of metal ions.

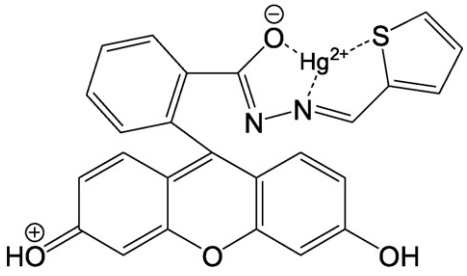

Fig. 3. Proposed FLTC–Hg^{2+} complex.

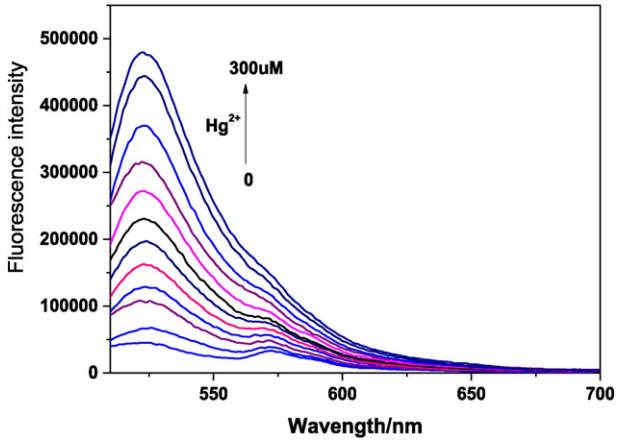

Fig. 4. Fluorescence spectra of FLTC (10 μM) in an ethanol–H_2O (3:2, v/v, pH 7.15, HEPES buffer, 0.5 mM) solution when different amounts of Hg^{2+} were added.

fluorescence intensity tended to stabilize, which indicates that the open-ring form of FLTC became a dominant species in solution. These results showed that FLTC is insensitive to pH changes from 1.0 to 7.0 and may work under physiological conditions with very low background fluorescence. Therefore, an ethanol–H_2O (3:2, v/v, pH 7.15, HEPES buffer, 0.5 mM) solution has been chosen for the following studies.

The fluorescence spectra of FLTC in the presence of metal ions (Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Ir^{3+}, Cu^+, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sn^{2+} and Zn^{2+}) are shown in Fig. 2. A remarkable enhancement of fluorescence was observed only in the presence of Hg^{2+} (50 μM, 5 eq.), suggesting that FLTC can be used to sense Hg^{2+} ions as a "turn on" chemosensor. The mechanism may be explained by the formation of a strongly fluorescent ring-opened FLTC–Hg^{2+} complex (Fig. 3) [36].

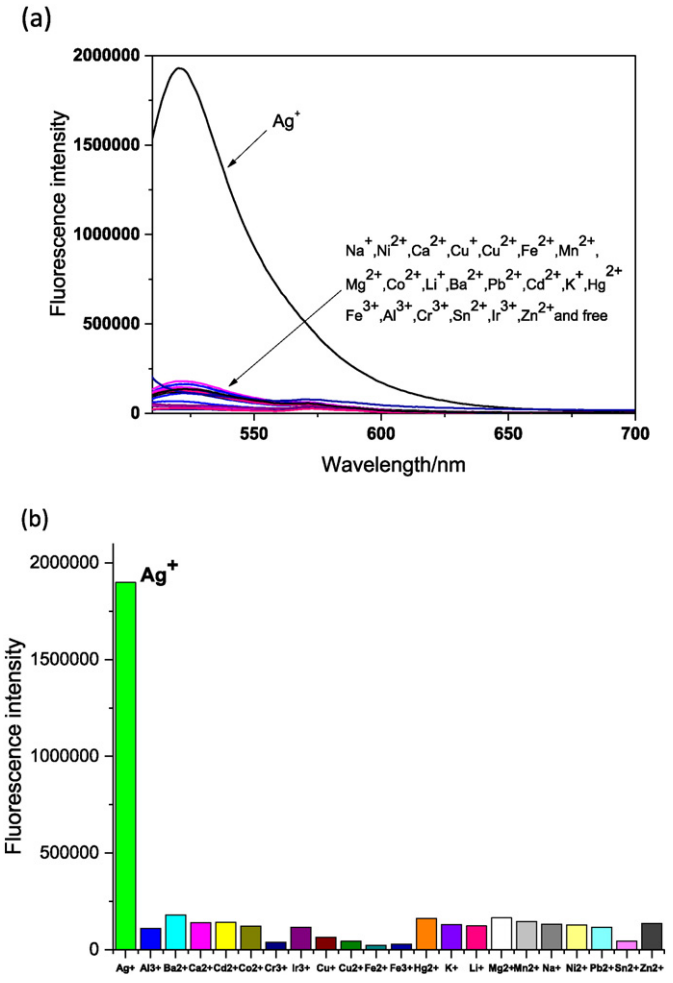

Fig. 5. Fluorescence spectra of FLTC (10 μM) with Hg^{2+} (50 μM,5 equiv) in ethanol–H_2O (3:2, v/v, pH 7.15, HEPES buffer, 0.5 mM) in the presence of 5 equiv of metal ions (a). Bar graph shows the relative emission intensity of FLTC·Hg^{2+} complex at 524 nm upon the addition of metal ions (b).