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Notes

A Ligand Exchange-based Fluorogenic Assay for Cartap Using Cu^{2+}-calcein Blue Complex

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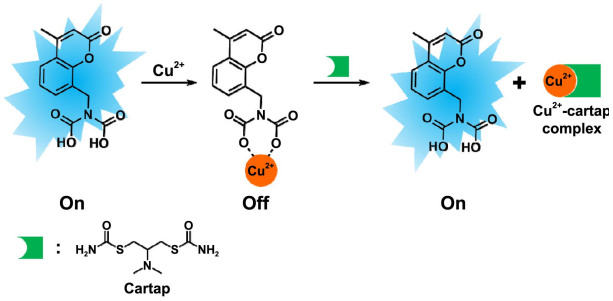
Cartap, an ion channel blocker of the insect nicotinic acetylcholine receptor, has been used as an insecticide since it was commercialized in 1967.[1] It is commonly used worldwide and is one of the most frequently used insecticides against pests in rice, tea trees, fruit trees, *etc.* because it has a high insecticidal activity and a low toxicity in humans.[2] The overuse of cartap can lead to dangerous levels of residues in various foods, which result in a hazard for human health because cartap causes significant neuromuscular toxicity, and eventually results in multisystem organ failure.[1,3] Owing to the toxicities of cartap, food administrations in various countries have defined the maximum residue limits for cartap. For example, the permissible limit for cartap in crops was set at 0.5 ppm by the WHO.[4] The toxicity of cartap and the restriction of residue limits have led to the development of various detection methods for cartap residues in food. A number of detection methods have been developed based on gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS).[5] These methods are very useful for the quantification of cartap in various samples with high sensitivity, but require expensive instruments and a laborious setup process including the determination of optimum eluent conditions, column selection, and flow rate optimization.

A chemosensor-based assay for cartap is one of the most desirable methods to address these problems because it has multiple advantages over other types of assays including low cost, ease of application, versatility, and high sensitivity.[6] Although various detection methods for cartap have been developed, only a limited number of chemosensor methods have been developed.[7] Recently, fluorescent chemosensors for cartap were developed based on nanocrystals and a combination of cucurbit[7]uril and palamatine, which can detect cartap in aqueous samples with high sensitivity and selectivity.[7a,b] However, these chemosensors are turn-off type chemosensors and produce a decreased fluorescence that is undesirable for analytical purposes. Therefore, it is highly desirable to develop a turn-on type chemosensor for cartap with high sensitivity and selectivity.



Scheme 1. Schematic illustration of the cartap assay.

Metal-ion fluorogenic dye complex-based chemosensors have been developed for the detection of analytes that have high binding affinities to metal ion in the complexes.[8] These detection methods are based on ligand exchanges in which the metal ions are removed from the complexes by analytes, thereby inducing a fluorescence change in the metal complexes. For example, a Cu^{2+}-gelatin complex and a Cu^{2+}-zincon complex were designed for the detection of phytate and cyanide ions, respectively.[8a,b] In this paper, we report a fluorogenic chemosensor for cartap with high selectivity and sensitivity, using a Cu^{2+}-calcein blue complex. Cartap has a high binding affinity for transition-metal ions because the molecule has two thiocarbamate moieties and one amine moiety. The fluorescence of calcein blue is quenched by Cu^{2+} due to mechanisms inherent for paramagnetic species.[9] Therefore, the Cu^{2+}-calcein blue complex would be converted to free calcein blue when exposed to cartap, which results in the enhancement of fluorescence in calcein blue as shown in Scheme 1.

A fluorescence titration of Cu^{2+} was carried out using a 100 nM solution of calcein blue in borate buffer at pH 8.0 to determine the optimal concentration of Cu^{2+} for the cartap assay. Fluorescence emission of calcein blue was measured after the addition of Cu^{2+} and the emission curves are shown in Figure 1. The addition of Cu^{2+} induced a reduction of fluorescence in calcein blue (Figure 1 inset), and the fluorescence was nearly proportional to the Cu^{2+} concentration until saturation occurred at 1.5 equiv of Cu^{2+}. The Cu^{2+}-cal-

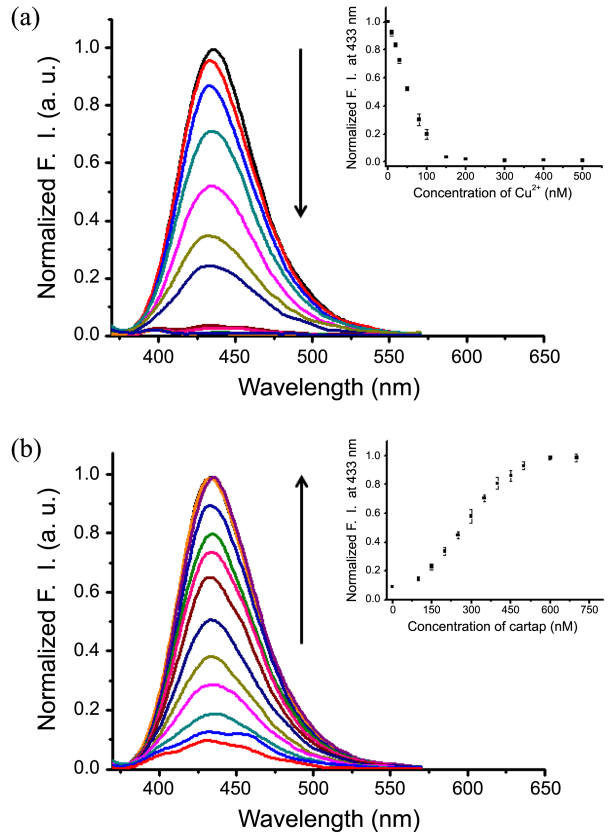


Figure 1. (a) Fluorescence emission spectra obtained by the addition of Cu^{2+} (0-500 nM) to borate buffer solution (pH 8.0, 10 mM) containing calcein blue (100 nM). Inset: Plot of fluorescence intensity of calcein blue at 433 nm *versus* Cu^{2+} concentration. (b) Fluorescence emission spectra obtained by the addition of cartap (0-700 nM) to borate buffer solution (pH 8.0, 10 mM) containing calcein blue (100 nM) and Cu^{2+} (150 nM). Inset: Plot of fluorescence intensity of calcein blue at 433 nm *versus* cartap concentration.

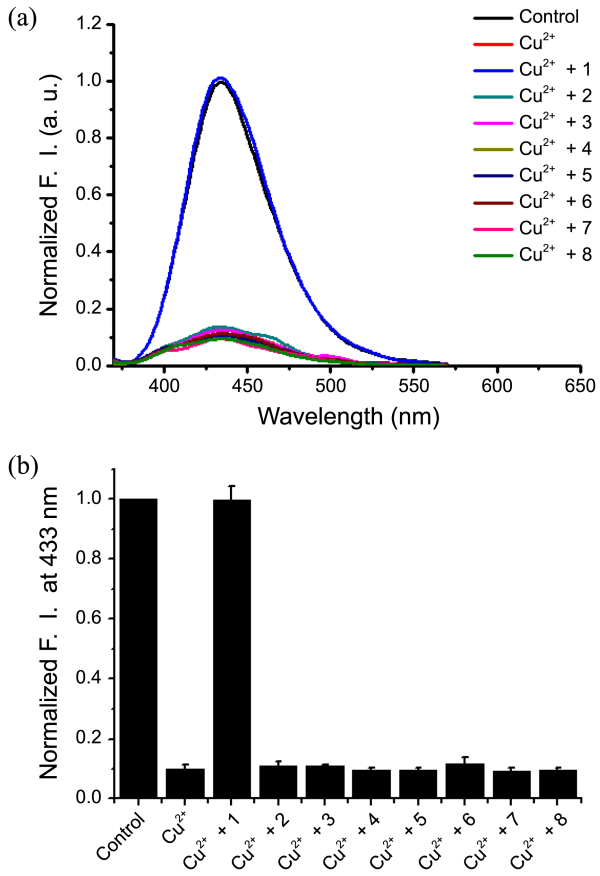


Figure 2. (a) Fluorescence emissions spectra obtained by the addition of various pesticides (500 nM) to a mixture of borate buffer solution (pH 8.0, 10 mM, 0.05 % acetonitrile, v/v) containing calcein blue (100 nM) and Cu^{2+} (150 nM). (b) Plot of fluorescence intensity of calcein blue at 433 nm *versus* various pesticides. 1: Cartap, 2: Acephate, 3: Thiocyclam, 4: Pendimethalin, 5: Bensultap, 6: Benfuracarb, 7: Iprobenfos, 8: Edifenphos.

cein blue complex, a chemosensor for cartap, was prepared by mixing calcein blue and Cu^{2+} (1.5 equiv.) in 10 mM borate buffer solution at pH 8.0; since the fluorescent quenching of calcein blue was saturated with 1.5 equiv. of Cu^{2+}. Fluorescence changes of the complex were measured after addition of various amounts of cartap. As expected, the addition of cartap to the copper-ion fluorogenic dye complex resulted in a large enhancement of fluorescence; the increase in fluorescence was nearly proportional to the cartap concentration. From the titration results, the detection limit of a Cu^{2+}-calcein blue complex for cartap was estimated at 0.036 ppm (see Supporting Information). This detection limit (0.036 ppm) is acceptable since it is an order of magnitude lower than the WHO's limit for cartap in crops of 0.5 ppm. In addition, to discover a more efficient chemosensor for cartap than the Cu^{2+}-calcein blue complex, other metal-ion fluorogenic dye complexes were prepared. Calcein blue and 1.5 equiv. of various metal ions were mixed, and fluorescence changes were measured upon addition of cartap. These experiments show that the Cu^{2+}-calcein blue complex is the most efficient chemosensor for cartap among the various metal-ion calcein blue complexes tested (see Supporting Information).

To evaluate the selectivity of this system, fluorescence changes were measured in the presence of various pesticides

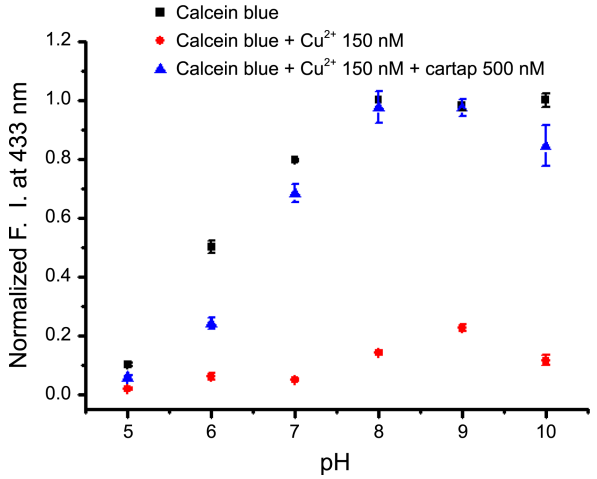


Figure 3. Plot of fluorescence intensity of calcein blue (100 nM) at 433 nm *versus* pH for various buffer solutions (10 mM); pH 5-6: acetate, pH 7: phosphate, pH 8-10: borate.

and the fluorescence changes are shown in Figure 2. This system has high selectivity for cartap over the other pesticides including bensultap, which has a similar structure to cartap. Also, no significant changes of fluorescence in the fluorescence assay were observed upon addition of the other pesticides.

A ligand exchange-based fluorogenic assay is sensitive to the pH of the assay medium, because the binding affinities of metal ions to ligands are sensitive to the pH.[8] Therefore, the pH effect of the medium in this assay was evaluated in the pH range of 5.0-10.0. As shown in Figure 3, this system did not work well in the pH range between 5 and 6; however, it showed a strong response to cartap in a broad pH range over pH 7.

In conclusion, we have developed a highly effective fluorogenic sensing system for cartap using a Cu^{2+}-calcein blue complex with high sensitivity and selectivity. The detection limit of this system is 0.036 ppm, which is acceptable and within the permissible limit of cartap for crops by the WHO (0.5 ppm). This sensing system has high selectivity for cartap over the other pesticides including bensultap, which has a similar structure, and works well in a broad pH range over pH 7. Unlike previously reported turn-off fluorogenic sensing systems, this system has a strong fluorescence enhancement in the presence of cartap.

Experimental

Chemicals and Instruments. All reagents used in this work were purchased from Sigma-Aldrich Chemical Co. and were used without further purification. All metal-ion solutions were prepared from the respective perchlorate salts. Fluorescence measurements were recorded in a 10 × 10-mm quartz cuvette (HELLMA) on a SCINCO FS-2 spectrophotometer at 25 °C.

Determination of Cu^{2+} Concentration. The stock solutions of calcein blue (1 μM) and borate buffer (0.1 M, pH 8.0) were prepared. Each stock solution was mixed with different concentrations of Cu^{2+} solutions. The final concentration of calcein blue was 100 nM. The fluorescence spectra of the solutions were recorded in 25 min after adding various concentrations of Cu^{2+} to the solutions of calcein blue respectively

Fluorogenic Assay for Cartap. The stock solutions of calcein blue (1 μM), Cu^{2+} (1.5 μM), and borate buffer (0.1 M, pH 8.0) were prepared. Each stock solution was mixed and incubated for 25 min in advance. Aqueous cartap solution was then added to the mixture. The final concentrations of calcein blue and Cu^{2+} were 100 nM and 150 nM, respectively. The fluorescence spectra of the solutions were recorded in 8 min after adding various concentrations of cartap to the solutions of mixture of calcein blue and Cu^{2+}.

Selectivity Test for Various Pesticides. The stock solutions of calcein blue (1 μM), Cu^{2+} (1.5 μM), and borate buffer (0.1 M, pH 8.0) were prepared. Each stock solution was mixed and incubated for 25 min in advance. A pesticide dissolved in aqueous acetonitrile solution was then added to the mixture. The final concentrations of calcein blue, Cu^{2+}, and pesticides were 100 nM, 150 nM, and 500 nM, respectively (0.05 % acetonitrile in H_2O, v/v). The fluorescence spectra of the solutions were recorded in 8 min after adding various pesticide to the solutions of mixture of calcein blue and Cu^{2+}.

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Supporting Information. This information is available free of charge *via* the Internet at http://kcsnet.or.kr.

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