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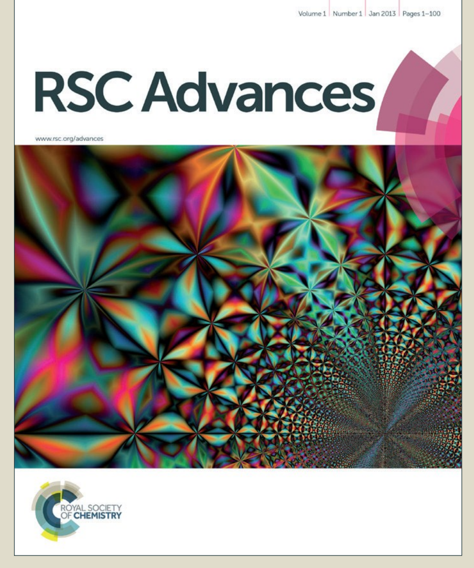

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A colorimetric chemosensor for the sequential detection of copper ion and amino acids (cysteine and histidine) in aqueous solution

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Abstract

A simple and selective colorimetric chemosensor **1** was reported for the sequential detection of Cu^{2+}, cysteine (Cys) and histidine (His) in aqueous solution. The presence of Cu^{2+} led to a distinct naked-eye color change from pale yellow to orange. Also, the receptor enabled analysis of Cu^{2+} ions with a sensitivity limit of 0.37 μM, which is far below the WHO acceptable limit (31.5 μM) in drinking water. **1** could be also used as a practical, visible colorimetric test strip for Cu^{2+} (down to 10 μM) in aqueous media. Moreover, the resulting **1**-Cu^{2+} complex sensed cysteine and histidine with an absorption change via UV-visible or a naked-eye color change, although His and Cys were indistinguishable with the method proposed herein.

Keywords: colorimetric chemosensor, sequential detection, copper, cysteine, histidine, julolidine

Introduction

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Copper is one of most vital metal ions in biological systems, because it functions as an important cofactor by taking an active part in a large variety of enzymes, including superoxide dismutase, cytochrome *c* oxidase and tyrosinase.[1-2] However, with excessive loading, copper ion can cause several diseases including Alzheimer's, Parkinson's and Wilson diseases.[3-4] Consequently, considerable efforts have been devoted to the development of colorimetric or fluorescent Cu^{2+}-selectivity chemosensors.[5-24]

Among many naturally existing amino acids, cysteine (Cys) and histidine (His) have attracted much attention because of their important biological functions. Cys has been proven to act as a potential neurotoxin, a biomarker for various medical conditions, and a disease-associated physiological regulator.[25-26] Cys deficiency would cause many troubles including retarded growth in children, hair depigmentation, hematopoiesis decrease, skin lesions and psoriasis.[27-28] Hence, it is of great importance to selectively detect and discriminate Cys as a disease-associated biomarker in biological systems. So far, various conventional detection processes such as high performance liquid chromatography (HPLC).[29-30] optical detections and capillary electrophoresis[31] have been exploited for the determination of Cys. Among these various methods, optical detection techniques have been proven to be the most convenient.[32]

His is essential for human growth and plays vital roles in the biological system such as control of transmission of metal and as repair of tissue.[33] Recent studies have shown that a deficiency of His in plasma may lead to an impaired nutritional state in patients with chronic kidney disease.[34] Therefore, the developments of sensory probes for an amino acid such as His are still highly demanded. Among them, the metal complex-based colorimetric compounds to selectively detect an amino acid have attracted much attention.[35-37]

In recent years, there has been a great emergence of interests in the development of sensors for the sequential detection of various cations and amino acids.[38-43] Among the different types of chemosensors, the probes based on colorimetric determination of the cations and amino acids have many advantages because of the simplicity, low cost, and rapid tracking of analytes.[44] Therefore, our group has been interested in the colorimetric sequential recognition of metal ions and amino acids.

Herein, we describe a new compound **1** based on a julolidine moiety, which was designed and synthesized as a colorimetric receptor of Cu^{2+} with the color change from pale yellow to

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orange in aqueous solution. Moreover, the resulting **1**-Cu^{2+} complex showed the colorimetric recognition of Cys and His.

Experimental Section

General information

All solvents and reagents (analytical grade and spectroscopic grade) were obtained from Sigma-Aldrich and used as received. Both ^{1}H NMR and ^{13}C NMR were performed on a Varian 400 MHz and 100 MHz spectrometer and chemical shifts are recorded in ppm. Electrospray ionization mass spectra (ESI-MS) were collected on a Thermo Finnigan (San Jose, CA, USA) LCQTM Advantage MAX quadruple ion trap instrument. Elemental analysis for carbon, nitrogen, and hydrogen was carried out by using a Flash EA 1112 elemental analyzer (thermo) in Organic Chemistry Research Center of Sogang University, Korea. Absorption spectra were recorded at room temperature using a Perkin Elmer model Lambda 2S UV/Vis spectrometer.

Synthesis of 1

8-Hydroxyjulolidine-9-carboxaldehyde (0.68 g, 3 mmol) and 4-nitro-1,2-phenylene diamine (0.50 g, 3.2 mmol) were dissolved in 20 mL of absolute ethanol. Then, three drops of phosphoric acid were added into the reaction mixture, which was stirred for 4 h at room temperature. The orange powder was produced, which was collected by filtration, washed with diethyl ether and air-dried. Yield : 0.814 g (77.0 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.76 (s, 1H), 8.59 (s, 1H), 7.87 (d, 1H), 7.82 (d, 1H), 6.99 (s, 1H), 6.76 (d, 1H), 6.49 (d, 2H), 3.24 (m, 4H), 2.62 (m, 4H), 1.86 (m, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.50, 157.93, 150.25, 147.53, 137.04, 135.07, 131.30, 123.69, 114.47, 113.46, 113.37, 108.85, 105.75, 50.08, 49.64, 27.32, 22.06, 21.13, 20.64 ppm. ESI-MS: *m/z* calcd for $C_{19}H_{20}N_4O_3$-H^+ ([M-H^+]), 351.146; found, 351.563. Anal. Calc. for $C_{19}H_{20}N_4O_3$: C 64.76; H, 5.72; N, 15.90; Found: C, 64.82; H, 5.96; N, 15.58.

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A colorimetric chemosensor

UV-vis titration measurements. Receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL) and 15 μL of **1** (3 mM) were diluted with 2.985 mL of DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. $Cu(NO_3)_2{\cdot}2.5H_2O$ (2.4 mg, 0.01 mmol) was dissolved in DMF (2 mL). 0.9-12.6 μL of the Cu^{2+} solution (5 mM) were transferred to each receptor solution (15 μM) prepared above. After mixing them for a few seconds, UV-vis absorption spectra were taken at room temperature.

For Cys, receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL) and 15 μL of **1** (3 mM) were diluted with 2.985 mL of DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. $Cu(NO_3)_2{\cdot}2.5H_2O$ (9.6 mg, 0.04 mmol) was dissolved in DMF (2 mL) and 3.2 μL of this Cu^{2+} solution (20 mM) were transferred to each receptor solution (15 μM) to give 1.4 equiv. Then, Cys (4.8 mg, 0.04 mmol) was dissolved in bis-tris buffer (2 mL) and 0-13.5 μL of this Cys solution (20 mM) were transferred to a mixture of **1** and Cu^{2+} (15 μM) to give 6 equiv. After mixing them for a few seconds, UV-vis spectra were taken at room temperature.

For His, receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL) and 15 μL of **1** (3 mM) were diluted with 2.985 mL of DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. $Cu(NO_3)_2{\cdot}2.5H_2O$ (9.6 mg, 0.04 mmol) was dissolved in DMF and 3.2 μL of this Cu^{2+} solution (20 mM) were transferred to each receptor solution (15 μM) to give 1.4 equiv. Then, His (6.2 mg, 0.04 mmol) was dissolved in bis-tris buffer (2 mL) and 0-16.9 μL of this His solution (20 mM) were transferred to a mixture of **1** and Cu^{2+} (15 μM) to give 7.5 equiv. After mixing them for a few seconds, UV-vis spectra were taken at room temperature.

Job plot measurements. Receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL). 15, 13.5, 12, 10.5, 9, 7.5, 6, 4.5, 3, 1.5, and 0 μL of the receptor solution (3 mM) were taken and transferred to vials. Each vial was diluted with DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make a total volume of 2.985 mL. $Cu(NO_3)_2{\cdot}2.5H_2O$ (1.4 mg, 0.006 mmol) was dissolved in DMF (2 mL). 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, and 15 μL of

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$Cu(NO_3)_2 \cdot 2.5H_2O$ solution (3 mM) were added to each diluted receptor solution. Each vial had a total volume of 3 mL. After shaking the vials for a few minutes, UV-vis absorption spectra were taken at room temperature.

For Cys, **1** (2.1 mg, 0.003 mmol) and $Cu(NO_3)_2 \cdot 2.5H_2O$ (1.4 mg, 0.006 mmol) were dissolved in DMF (2 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex. 15, 13.5, 12, 10.5, 9, 7.5, 6, 4.5, 3, 1.5, and 0 μL of the **1**-Cu^{2+} complex solution (3 mM) were taken and transferred to vials. Each vial was diluted with DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make a total volume of 2.985 mL. Cys (0.7 mg, 0.006 mmol) was dissolved in bis-tris buffer (2 mL). 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, and 15 μL of the Cys solution were added to each diluted **1**-Cu^{2+} solution. Each vial had a total volume of 3 mL. After reacting them for a few seconds, UV-vis spectra were taken at room temperature.

For His, **1** (2.1 mg, 0.003 mmol) and $Cu(NO_3)_2 \cdot 2.5H_2O$ (1.4 mg, 0.006 mmol) were dissolved in DMF (2 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex. 15, 13.5, 12, 10.5, 9, 7.5, 6, 4.5, 3, 1.5, and 0 μL of the **1**-Cu^{2+} complex solution (3 mM) were taken and transferred to vials. Each vial was diluted with DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make a total volume of 2.985 mL. His (0.9 mg, 0.006 mmol) was dissolved in bis-tris buffer (2 mL). 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, and 15 μL of the His solution were added to each diluted **1**-Cu^{2+} solution. Each vial had a total volume of 3 mL. After reacting them for a few seconds, UV-vis spectra were taken at room temperature.

Competition of 1 towards various metal ions. Receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL) and 15 μL of **1** (3 mM) were diluted to 2.985 mL DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. MNO_3 (M = Na, K; 0.02 mmol), $M(NO_3)_2$ (M = Mn, Co, Ni, Cu, Zn, Cd, Mg, Ca, Pb; 0.02 mmol), $M(ClO_3)_2$ (M = Fe; 0.02 mmol) or $M(NO_3)_3$ (M = Al, Fe, Cr, Ga, In; 0.02 mmol) were dissolved in DMF (1 mL), respectively. 3.2 μL of each metal solution (20 mM) were taken and added into 3 mL of each **1** solution (15 μM) prepared above to make 1.4 equiv. Then, 3.2 μL of the $Cu(NO_3)_2 \cdot 2.5H_2O$ solution (20 mM) were added into the mixed solution of each metal ion and **1** to make 1.4 equiv. After mixing them for a minute, UV-vis absorption spectra

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were taken at room temperature.

Competition of 1-Cu^{2+} complex towards various amino acids. For Cys, **1** (2.1 mg, 0.003 mmol) and $Cu(NO_3)_2 \cdot 2.5H_2O$ (9.6 mg, 0.04 mmol) were dissolved in DMF (2 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex (3 mM). Various amino acids and peptide such as Gly, Ala, Ser, Thr, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg or GSH (0.02 mmol) were dissolved in 10 mM bis-tris (1 mL). 13.5 μL of each amino acid or GSH solution (20 mM) were taken and added into 3 mL of the solution of Cys (90 μM) to give 6 equiv of amino acids or GSH. Then, 15 μL of **1**-Cu^{2+} solution (3 mM) were added into the mixed solution of each amino acid or GSH and Cys to make 1 equiv. After the procedure was carried out, the mixed solutions were diluted with 2.985 mL of DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. After mixing them for a few seconds, UV-vis spectra were taken at room temperature.

For His, **1** (2.1 mg, 0.003 mmol) and $Cu(NO_3)_2 \cdot 2.5H_2O$ (9.6 mg, 0.04 mmol) were dissolved in DMF (2 mL), respectively. The two solutions were mixed to make **1**-Cu^{2+} complex (3 mM). Various amino acids and peptide such as Gly, Ala, Ser, Thr, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg or GSH (0.02 mmol) were dissolved in 10 mM bis-tris (1 mL). 16.9 μL of each amino acid and GSH solution (20 mM) were taken and added into 3 mL of the solution of His (112.5 μM) to give 7.5 equiv of amino acids or GSH. Then, 15 μL of **1**-Cu^{2+} solution (3 mM) were added into the mixed solution of each amino acid or GSH and His to make 1 equiv. After the procedure was carried out, the mixed solutions were diluted with 2.985 mL of DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 μM. After mixing them for a few seconds, UV-vis spectra were taken at room temperature.

Reversible test of 1 toward Cu^{2+} by using EDTA. Receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL) and 15 μL (3 mM) of it were diluted with 2.985 mL DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make a final concentration of 15 μM. $Cu(NO_3)_2 \cdot 2.5H_2O$ (9.6 mg, 0.04 mmol) was dissolved in DMF (2 mL) and 3.2 μL of the Cu^{2+} ion solution (20 mM) were added to the solution of **1** (15 μM) prepared above. After mixing it for a few seconds, UV-vis spectrum was taken at room temperature.

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Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, 0.04 mmol) was dissolved in buffer solution (2 mL) and 3.2 μL of the EDTA solution (20 mM) were added to the solution of **1**-Cu^{2+} complex (15 μM) prepared above. After mixing it for a minute, UV-vis spectrum was taken. For the reversibility study, another 3.2 μL of the Cu^{2+} ion solution (20 mM) was added to the above solution. After mixing it for a minute, UV-vis spectrum was taken at room temperature. The same experimental procedure was repeated one more time.

pH effect toward Cu^{2+} ion. A series of solutions with pH values ranging from 2 to 12 was prepared by mixing sodium hydroxide solution and hydrochloric acid. After the solution with a desired pH was achieved, receptor **1** (2.1 mg, 0.003 mmol) was dissolved in DMF (2 mL), and then 15 μL of the receptor (3 mM) were diluted with 2.985 mL DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0) to make the final concentration of 15 □M. $Cu(NO_3)_2 \cdot 2.5H_2O$ (9.6 mg, 0.04 mmol) was dissolved in DMF (2 mL). 3.2 μL of the Cu^{2+} solution (20 mM) were transferred to each receptor solution (15 μM) prepared above. After mixing them for a few seconds, UV-vis spectra were taken at room temperature.

pH effect toward Cys and His. A series of solutions with pH values ranging from 2 to 12 was prepared by mixing sodium hydroxide solution and hydrochloric acid. After the solution with a desired pH was achieved, receptor **1** solution (2.1 mg, 0.003 mmol) and $Cu(NO_3)_2 \cdot 2.5H_2O$ (9.6 mg, 0.04 mmol) was dissolved in DMF (2 mL), respectively. Then both 15 μL of the receptor (3 mM) and 3.2 μL of the Cu^{2+} solution (20 mM) were transferred to each complex solution (15 μM) prepared. 16.9 μL of the Cys solution (20 mM) was transferred to **1**-Cu^{2+} complex solution prepared above. After mixing them for a few seconds, UV-vis spectra were taken at room temperature. The same experimental procedure was also carried out for His.

Results and discussion

Synthesis of 1

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The receptor **1** was obtained by the condensation reaction of 4-nitro-1,2-phenylenediamine and 8-hydroxyjulolidine-9-carboxaldehyde with a 77 % yield in ethanol (Scheme 1), and characterized by ^{1}H NMR, ^{13}C NMR, ESI-mass spectrometry and elemental analysis.

EtOH

RT

1

Scheme 1. Synthetic procedure of **1**.

Absorption spectroscopic studies of 1 toward Cu^{2+} ion

The chromogenic sensing ability of receptor **1** was studied in the presence of nineteen different cations such as Cu^{2+}, Al^{3+}, Ga^{3+}, In^{3+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Co^{2+}, Ni^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mn^{2+} and Pb^{2+} in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0). Upon the addition of 1.4 equiv of each metal ion, only Cu^{2+} induced a distinct spectral change while other metal ions showed either no or small changes in the absorption spectra relative to the free **1** (Fig. 1a). Consistent with the change of the UV-vis spectrum, the solution color of **1** changed from pale yellow to orange with copper ion (Fig. 1b), indicating that the **1** could serve as a potential candidate of colorimetric chemosensor for Cu^{2+}.

(a)

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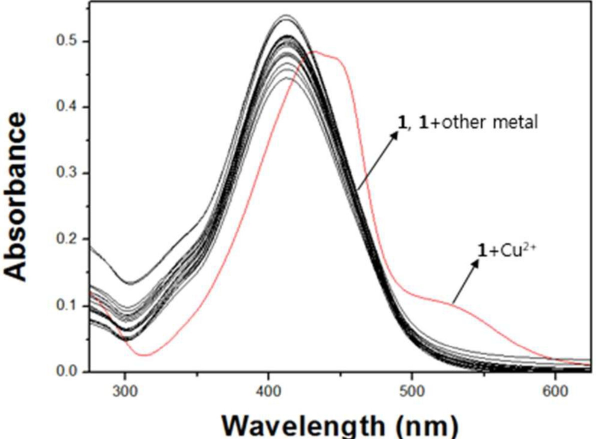

(b)

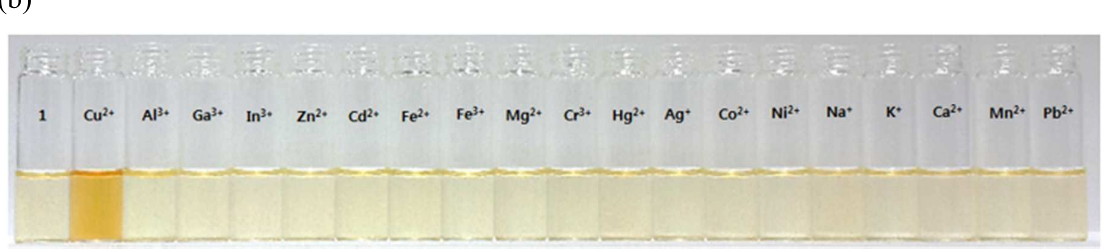

Fig. 1 (a) UV-vis spectra of **1** (15 μM) upon addition of 1.4 equiv of various metal ions in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0). (b) Color changes observed for **1** (30 μM) upon the addition of 1.4 equiv of various metal ions in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0).

The sensing mechanism of **1** to Cu^{2+} might be explained by intramolecular charge transfer (ICT) and ligand-to-metal charge-transfer (LMCT) mechanisms. Kaur *et al.* suggested that ICT mechanism referred to the push-pull effect of the electron-donating and electron-withdrawing groups.[45] That is, the red shift indicates that the energy gap of ICT band decreases, upon binding metal ions to the electron withdrawing moieties.[46] Likewise, we assume that the red shift of **1**-Cu^{2+} complex at 430 nm was induced by binding of Cu^{2+} to the electron withdrawing groups such as the C=N group. In addition, the color change could be explained by LMCT mechanism. The band with the molar extinction coefficient in the thousands, 6.3 x 10^3 $M^{-1}cm^{-1}$ at 525 nm, is too large to be Cu-based d-d transitions and thus

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must be ligand-based transitions.[22, 47] Therefore, the change of ICT and LMCT bands might be responsible for the dramatic color change from pale yellow to orange.

The binding properties of **1** with Cu^{2+} were further studied by UV-vis titration experiments. On the treatment with Cu^{2+} to the solution of **1**, the absorption band at 410 nm significantly decreased, and a new band at 525 nm gradually reached maximum at 1.4 equiv of Cu^{2+} (Fig. 2).

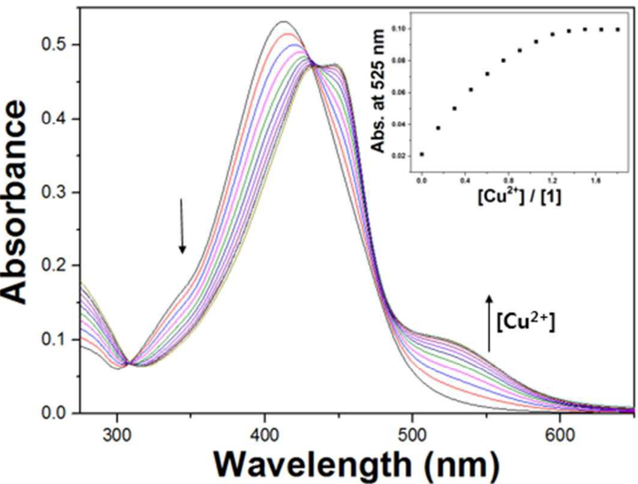

Fig. 2 UV-vis titration of **1** (15 μM) with Cu^{2+} (0-1.4 equiv). Inset: Absorption titration profile of **1** with Cu^{2+} at 525 nm.

The Job plot revealed a 1:1 stoichiometric ratio between the **1** and Cu^{2+} (Fig. S1), which was confirmed by ESI-mass spectrometry analysis.

The positive ion mass spectrum indicated the 1:1 binding mode between **1** and Cu^{2+} [m/z 486.933; calcd, 487.128] (Fig. 3).

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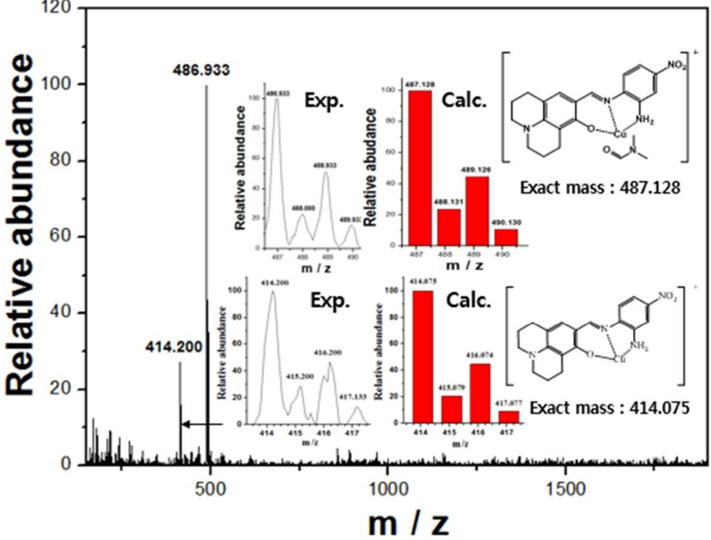

Fig. 3 Positive-ion ESI-mass spectrum of **1** (100 μM) upon addition of 1 equiv of Cu^{2+}.

Based on Job plot and ESI-mass spectrometry analysis, we propose that the oxygen and the two nitrogen atoms of **1** might bind to Cu^{2+} as shown in Scheme 2.

Donor
Acceptor
1
$Cu(NO_3)_2$
LMCT
(X = solvent or NO_3^-)

Scheme 2. Proposed binding mode of **1**-Cu^{2+} complex.

Based on the UV-vis titration, the binding ability (K = 6.9 x 10^4 M^{-1}) of **1** for Cu^{2+} was determined from Benesi-Hildebrand equation (Fig. S2).[48] This value is within those (10^4 ~10^5 M^{-1}) previously reported for Cu^{2+}-binding cheomsensors.[49-50] The detection limit (DL) was

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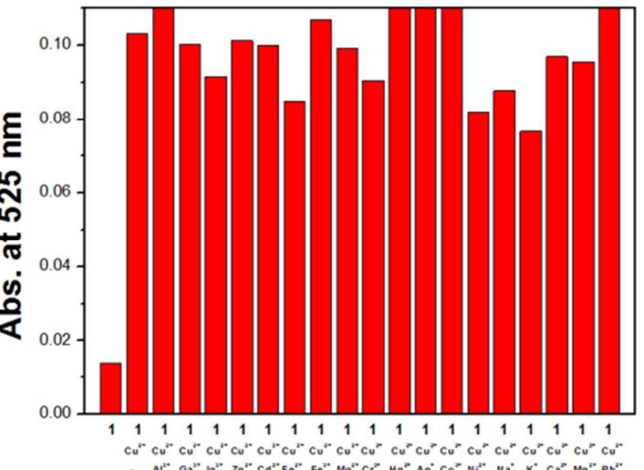
calculated to be 0.37 μM, using the equation DL = 3σ/m, where σ is the relative standard deviation and m is the slope of the calibration graph.[51] This value is far below the WHO acceptable limit (31.5 μM) in drinking water (Fig. S3).[52-53]

To study further the ability of **1** for Cu^{2+} detection, competitive measurements were carried out in the presence of various metal ions. As shown in Figure 4, the presence of other background metal ions showed no or a little change of absorbance.

(a)



(b)

Fig. 4 (a) Competitive selectivity of **1** (15 μM) toward Cu^{2+} (1.4 equiv) in the presence of other metal ions (1.4 equiv). (b) Color changes of **1** (30 μM) in the presence of Cu^{2+} (1.4 equiv) and other metal ions (1.4 equiv).

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To examine the reversibility of **1** toward Cu^{2+} in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0), ethylenediaminetetraacetic acid (EDTA, 1.4 equiv) was added to the mixed solution of **1** and Cu^{2+} (Fig. S4). The solution color changed from pale yellow to orange (the original color of **1**). Upon addition of Cu^{2+} into the mixture solution again, the absorbance at 525 nm appeared accompanied by a color change. These results indicate that **1** could be recyclable easily through treatment with a proper reagent such as EDTA. Such reversibility and regeneration might be important for the fabrication of devices to sense the Cu^{2+} in aqueous environmental solution.

We studied the effect of pH on the absorption response of **1** to Cu^{2+} ion in a series of solutions with pH values ranging from 2 to 12 (Fig. S5). The color of the **1**-Cu^{2+} complex remained in the orange region between pH 7 and 12, while its color changed to the pale yellow between pH 2 and 6. The absorbance increase between pH 6 and 7 indicated that the hydroxyl proton of phenol group in **1**-Cu^{2+} complex began to deprotonate. These results showed that Cu^{2+} could be clearly detected by naked eye using **1** over the environmentally relevant pH range of 7.0-12.0.

For the practical application of **1**, test kits were prepared by immersing filter papers in a DMF solution of **1** and then dried in oven. These test strips were used to detect Cu^{2+} among various cations. As shown Fig. 5, when the test kits coated with **1** were added to other cation solutions (10 μM), an obvious color change was observed only with Cu^{2+} in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0). Hence, the test kits coated with **1** could be conveniently used to detect Cu^{2+} down to 10 μM much below the WHO guideline (31.5 μM). Importantly, this is the first example that **1** could detect the lowest concentration of copper by using the test strip in aqueous solution, to the best of our knowledge.

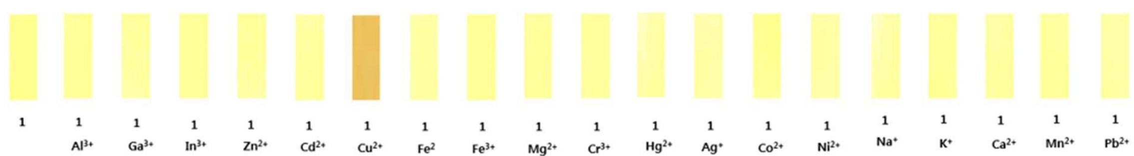

Fig. 5 Photographs of the test kits coated with **1** (1 mM) in the presence of various metal

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ions (10 μM).

We also constructed a calibration curve for the determination of Cu^{2+} by **1** (Fig. S6). Receptor **1** exhibited a good linear relationship between the UV-vis absorbance of **1** and Cu^{2+} concentration (7.5-21.0 μM) with a correlation coefficient of $R^2 = 0.9895$ (n = 3), which means that **1** is suitable for quantitative detection of Cu^{2+}. In order to examine the applicability of the chemosensor **1** in environmental samples, the chemosensor was applied to the determination of Cu^{2+} in water samples. We prepared artificial polluted water samples by adding various metal ions known as being involved in industrial processes into deionized water. The results were summarized in Table S1, which exhibited satisfactory recovery and R.S.D. values for the water samples.

Absorption spectroscopic studies of 1-Cu^{2+} complex toward Cys and His

Because it has been reported that the thiol-containing amino acid and peptide strongly bind to Cu^{2+} ions,[55-58] we examined the selectivity of **1**-Cu^{2+} complex to Cys using copper-sulfur affinity. The absorbance spectral study of **1**-Cu^{2+} complex with 19 different amino acids and peptide such as Gly, Ala, Ser, Thr, Cys, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg and glutathione (GSH) was carried out in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0). Upon the addition of 7.5 equiv of each amino acid or GSH, **1**-Cu^{2+} complex showed little or no spectra changes in absorption peaks in the presence of Gly, Ala, Ser, Thr, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg and GSH as shown in Fig. S7a. In contrast, the addition of Cys and His to **1**-Cu^{2+} complex showed significant spectral changes. Consistent with the changes of UV-vis spectra, the addition of Cys and His to **1**-Cu^{2+} complex instantly color changes from orange to pale yellow, respectively (Fig. S7b). This result indicates that **1**-Cu^{2+} could be used as a "naked-eye" sensor for Cys and His in aqueous media, respectively, although His and Cys were indistinguishable with the method proposed herein.

Importantly, this is the first example of the sequential colorimetric detection of Cys and His by using copper complex as a receptor, to the best of our knowledge (Table 1).[27, 40-43, 55-59]

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Table 1. Examples for the sequential detection of Cu^{2+}, Cys and His by organic chemosensors.

Sensors	Detection Limit (μM)	Interference	Percent of water in solution	Method of detection	Analyte	Reference
	No data	No data	50%	Fluorescence	Cys, Hcy	[27]
	3.1	None [a]	99.5 %	Fluorescence	His	[40]
	26 (His)	None [a]	80 %	Fluorescence	His	[41]
	None	None [a]	10 %	Fluorescence	His	[42]
	1.6	No data	100 %	Fluorescence	His	[43]
	No data	Arg, Lys	50%	Fluorescence	Cys, Hcy, GSH	[55]
	9	No data	99.9%	Fluorescence	Cys	[56]
	No data	No data	70%	Fluorescence	Cys	[57]

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	0.065	None[a]	100%	Fluorescence	Cys	[58]
	0.04	GSH	100%	Fluorescence	Cys	[59]
	Cys : 3.6 His : 9	None[a]	83 %	Naked eye	Cys, His	[This work]

a. None means that there is no interference among the common amino acids and GSH.

The binding properties of **1**-Cu^{2+} complex with both Cys and His were further studied by UV-vis titration experiments. On the treatment with Cys to the solution of **1**-Cu^{2+}, the absorption band at 525 nm significantly decreased, and the band gradually reached minimum at 6 equiv of Cys (Fig. S8a). Only one clear isosbestic point at 313 nm indicates the evident reaction of **1**-Cu^{2+} complex with Cys. The titration of **1**-Cu^{2+} with His also blue-shifted with an isosbestic point at 309 nm, when the concentration of His reached to 7.5 equiv (Fig. S8b).

The binding modes between **1**-Cu^{2+} complex and the two amino acids, Cys and His, were revealed by using Job plot analysis. The Job plots for the **1**-Cu^{2+} complex with Cys (Fig. S9a) and His (Fig. S9b) exhibited a 1:1 stoichiometry, respectively.

The interactions of **1**-Cu^{2+} complex with Cys or His were further analyzed by ESI-mass spectrometry analysis. The negative ion mass spectrum of **1**-Cu^{2+} complex upon addition of 1 equiv of Cys indicated the 1:1 binding between Cu^{2+} and Cys $[(Cys\text{-}H^{+})^{-} + Cu^{2+} + 2NO_3^{-}]^{-}$ [m/z 306.933; calcd, 306.917] (Fig. S10a). For His, the negative ion mass spectrum showed that a peak at m/z = 450.207 was assignable to $[(His\text{-}H^{+})^{-} + Cu^{2+} + 2NO_3^{-} + H_2O + DMF$ [calcd, 450.041] (Fig. S10b). Based on the Job plot and ESI-mass spectrometry analysis, we propose that the **1**-Cu^{2+} complex might undergo the demetallation by Cys and His, respectively (Scheme 3).

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(X = solvent or NO_3^-)

Cys / His

+ CuCys / CuHis

Scheme 3. Approach for the detection of Cys and His by **1**-Cu^{2+} complex.

From the results of UV-vis titration, the dissociation constants of the **1**-Cu^{2+} for Cys and His were determined as 4.7 x 10^3 M^{-1} and 2.4 x 10^4 M^{-1}, respectively, on the basis of Benesi–Hildebrand equation (Fig. S11). For practical application, the detection limit was also an important parameter. Thus, the detection limits of **1**-Cu^{2+} for the analysis of Cys and His were determined to be 3.6 μM and 9.0 μM using of the basis 3σ/m, respectively (Fig. S12).

To further study the ability of **1**-Cu^{2+} for Cys and His detection, inhibition tests were performed with 19 various amino acids and peptide such as Gly, Ala, Ser, Thr, Val, Leu, Ile, Met, Pro, Phe, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg and GSH. When **1**-Cu^{2+} was treated with 6 equiv of Cys in the presence of the same concentration of other amino acids (Fig. 6), there was no inhibition for the detection of Cys by **1**-Cu^{2+} in DMF-buffer solution (1:5, v/v, 10 mM, bis-tris, pH 7.0).[54] These results demonstrate that **1**-Cu^{2+} could be an excellent chromogenic sensor with high selectivity for Cys over competing amino acids. Similarly, we conducted the preferential selectivity of **1**-Cu^{2+} as a colorimetric sensor for the detection of His in the presence of various amino acids. His was also not inhibited by the competing other amino acids as shown in Fig. S13.

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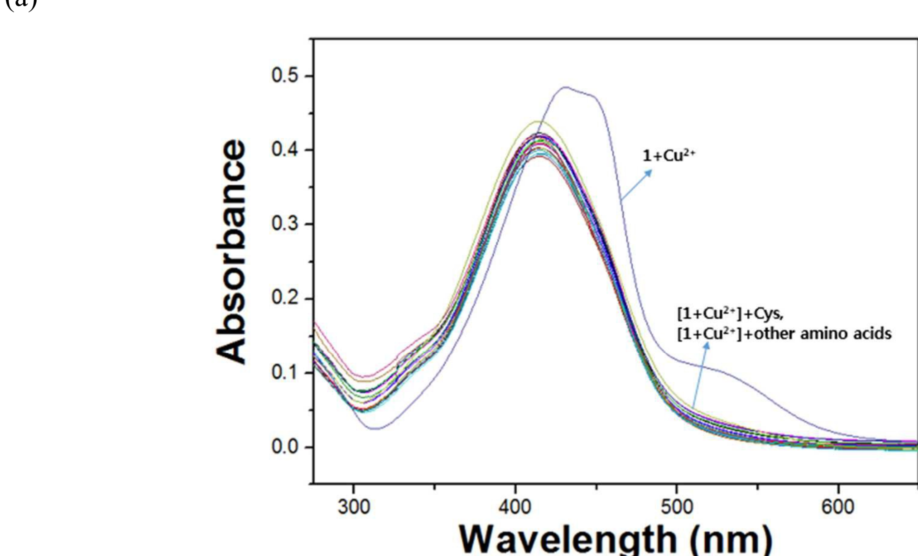
(a)



(b)

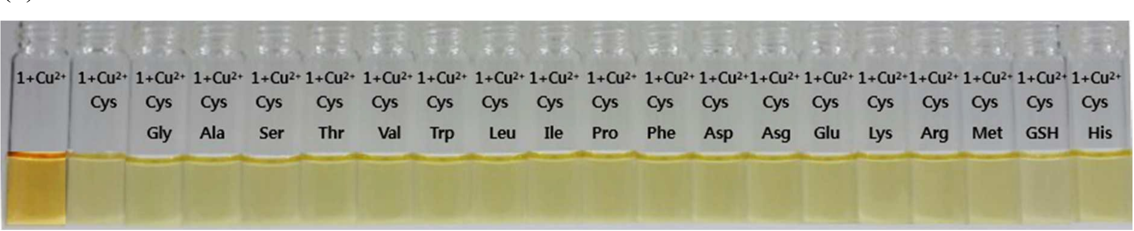


Fig. 6 (a) Competitive selectivity of **1**-Cu^{2+} complex (15 μM) toward Cys (6 equiv) in the presence of other amino acids (6 equiv). (b) Color changes of **1**-Cu^{2+} complex (30 μM) in the presence of Cys (6 equiv) and other amino acids (6 equiv).

To verify its potential usage in environment, we investigated the effect of pH on the absorption responses of **1**-Cu^{2+} complex to Cys and His in a series of solutions with pH values ranging from 2 to 12, respectively (Fig. S14). The colors of the **1**-Cu^{2+} complex with both Cys and His certainly remained in the orange region between pH 7 and 12, whereas its

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color changed to the pale yellow between pH 2 and 6. Therefore, these results indicate that both Cys and His could be definitely detected by naked eye using **1**-Cu^{2+} complex over the environmentally relevant pH range of 7-12.

Conclusions

We have developed a new colorimetric sensor **1** for the sequential detection of copper ion and amino acids (cysteine and histidine). **1** induced an obvious color change from pale yellow to orange in the presence of Cu^{2+} ion, and the bathochromic shift was explained by the change of ICT band. The receptor enabled analysis of Cu^{2+} ions with a sensitivity limit of 0.37 μM, which is far below the WHO acceptable limit (31.5 μM) in drinking water. Besides, **1** could be used as a practical, visible colorimetric test strip for quantifying Cu^{2+} (10 μM) in aqueous environment. Moreover, chemosensing ensemble **1**-Cu^{2+} complex was used as a colorimetric sensor for cysteine and histidine, although His and Cys were indistinguishable with the method proposed herein. Importantly, this is the first example of the sequential colorimetric chemosensor for Cys and His by using copper complex as a receptor. This sequential sensing system was simple in design, fast in operation, and more promising and convenient than other methods. Thus, these results may contribute to the development of a novel type of the colorimetric sequential recognition of copper ion and amino acids.

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Supplementary Material

Supplementary data related to this article can be found at http: //

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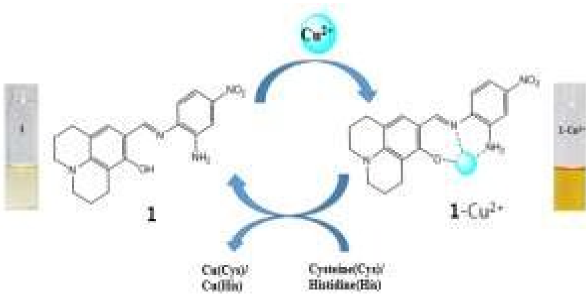
Graphical Abstract



A simple and selective colorimetric chemosensor was reported for the sequential detection of Cu^{2+}, cysteine and histidine.

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