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Fluorescent Sensor Based on BINOL for Recognition of Cysteine, Homocysteine, and Glutathione

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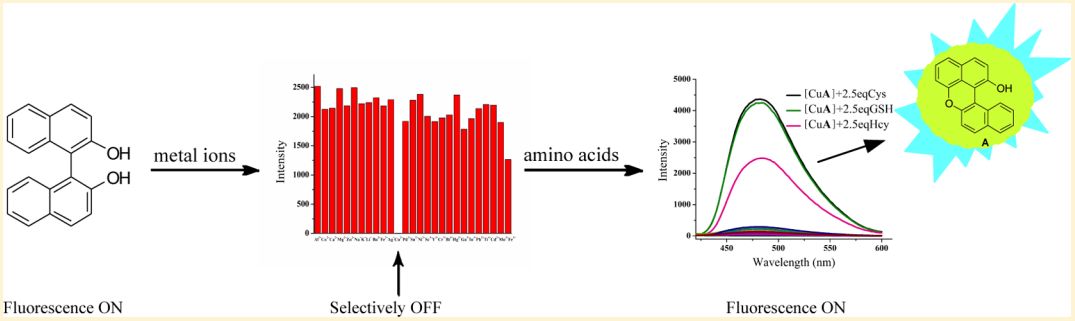
Supporting Information



ABSTRACT: A new and simple fluorescent sensor based on unmodified BINOL was developed. The fluorescence of BINOL could be turned off with high selectivity toward Cu(II) among 27 metal ions. Meanwhile, it was found that BINOL was oxidized to dibenzo[*a*,*kl*]xanthen-1-ol (**A**) by $Cu(NO_3)_2$. A new peak appeared at ~482 nm upon addition of thiol-containing cysteine (Cys), homocysteine (Hcy), and glutathione (GSH). Thus, a cascade recognition of Cu(II) and Cys, Hcy, and GSH are well presented.

Cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) as intracellular thiol-containing amino acids play critical roles in biological systems.[1] For instance, Cys has been proven to act as a potential neurotoxin,[2] a biomarker for various medical conditions,[3] and a disease-associated physiological regulator.[4] The concentration of Hcy in vivo has been recognized as an independent risk factor for cardiovascular disease.[5] GSH has the potential to prevent cancer and other mutation-related diseases.[6] Therefore, the detection of thiol-containing molecules is of great importance. In the past decades, various strategies have been developed for the recognition of these thiol-containing amino acids, including electrochemical voltammetry,[7] colorimetric,[8] phosphorescent,[9] and high-performance liquid chromatography (HPLC).[10] As an alternative technique, fluorescent probes have attracted great attention in recent years because of their efficient and real-time features.[11] Recently, the 1,3-alternate thiacalix[4]arene-Hg(II),[12a] triazole-based calix[4]arene-Cd(II),[12b] 2-(benzo[*d*]-thiazol-2-yl)-6-methoxyphenyl acrylate,[12c] and iminocoumarin-Cu(II)[12d] probes were found efficient to recognize thiols. However, developing simple, efficient, and inexpensive probes for the fluorescent recognition of these critical thiol-containing amino acids are still highly desirable.

BINOL and its derivatives represent a class of important molecules that have been widely applied in fluorescence-based molecule recognition.[13] For example, Pu's group reported that a chiral molecular gel prepared by sonication of a BINOL-based tripyridine-Cu(II) complex was useful for visual discrimination of amino alcohols.[14] Zhu and Cheng et al. synthesized a chiral perazamacrocycle featuring BINOL and *trans*-DACH units which could serve as a fluorescent sensor for cascade recognition of Cu(II) and unmodified α-amino acids.[15] To the best of our knowledge, BINOL and its derivatives have not been utilized to date in the recognition of thiols. Herein, we report our new discovery that BINOL displayed on−off-mode fluorescence change with $Cu(NO_3)_2$; in the process, BINOL was oxidized to dibenzo[*a*,*kl*]xanthen-1-ol (**A**). Thus, a new probe was formed and this new probe showed high sensitivity and high selectivity for the fluorescent recognition of Cys, Hcy, and GSH against other amino acids through off−on-mode.

In the fluorescence spectrum of BINOL, excitation and strong emission wavelengths were observed at 305 and 360 nm in CH_3CN/H_2O (v/v = 1:1), respectively. Initially, we investigated the selective fluorescent properties of BINOL toward Li^+, Na^+, K^+, Pd^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+}, Cr^{3+}, Co^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Ga^{3+}, In^{3+}, Pb^{2+}, Ti^{4+}, Sn^{2+}, Sc^{3+}, Y^{3+}, Bi^{3+}, Ag^+, and Zn^{2+}. The fluorescence of BINOL could be completely quenched only by $Cu(NO_3)_2$ (Figure 1). The fluorescent emission of BINOL toward Cu(II) was also investigated in different media, such as THF, DMF, DMSO, CH_3OH, and CH_3COCH_3 with H_2O (v/v = 1:1) under the same conditions. The maximum fluorescence quenching was observed in CH_3CN/H_2O (v/v = 1:1) medium (Supporting

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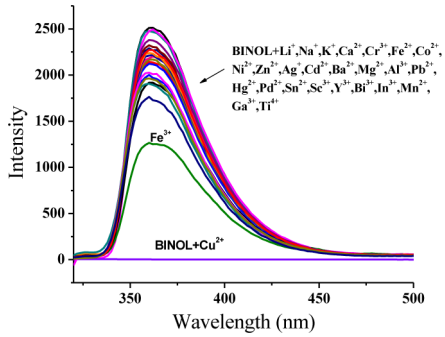
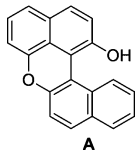


Figure 1. Fluorescence responses of BINOL (2.0×10^{-4} M, λ_{ex} = 305 nm) to various metal ions. (2.0×10^{-4} M) in CH_3CN/H_2O (v/v = 1:1).

Information Figure S1). Titration of BINOL with $Cu(NO_3)_2$ showed that complete quenching of the fluorescent occurred when 1 equiv $Cu(NO_3)_2$ was added (quenching efficiency = 99%). Besides, we have conducted a CD titration for (*R*)-BINOL with $Cu(NO_3)_2$ (Supporting Information Figure S4). It was interesting to find that the CD signals of chiral BINOL disappeared after the addition of 1 equiv $Cu(NO_3)_2$, which suggested that a new achiral compound was formed. In order to confirm the structure of the new compound, detethylene diamine tetraacetic acid (EDTA) was used as a copper chelating agent to release the new compound, and then it was isolated by column chromatography. ^{1}H NMR, ^{13}C NMR, HRMS, and IR spectrum studies showed that the new compound was dibenzo[*a*,*kl*]xanthen-1-ol **A**[16] (Scheme 1), which was the oxide of BINOL. So, we suspected that after mixing the BINOL with $Cu(NO_3)_2$, a new **A**-copper complex ([Cu**A**]) might be formed and quenched the fluorescence due to metal-to-ligand charge transfer that was discovered by UV spectra experiments (Supporting Information Figure S10).

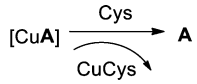
Scheme 1. Structure for BINOL Oxidation Product



A

We further tested the fluorescence response of [Cu**A**] complex toward amino acids and found that [Cu**A**] complex was inactive toward other amino acids except for Cys, Hcy, and GSH (Figure 2a). Amino acids such as Ser and Met did not exhibit any detectable fluorescence changes although they have similar structures as Cys. When the [Cu**A**] complex was treated with Cys, Hcy, and GSH, a large fluorescence enhancement was observed at ~482 nm, which was identical with the fluorescence of **A**. So, the fluorescence enhancement should be caused by release of **A** from the [Cu**A**] complex through the chelation of thiol-containing amino acids with the copper (Scheme 2). This was also further confirmed by the separation of **A** from the system in the presence of Cys. The prominent fluorescence changes of [Cu**A**] complex were observable by the naked eye. Upon addition of Cys, Hcy, and GSH, the solution displayed a strong fluorescence whereas other natural amino acids did not induce any fluorescence changes under UV light (Figure 2b). Quantitative analysis of the in situ generated [Cu**A**] complex ensemble toward Cys was investigated by fluorescent titration (Figure 2c). The binding constant of [Cu**A**] complex with Cys was calculated using the Benesi-Hilderand equation, and the association constant (K) was up to 1.521×10^3 M^{-1}. Moreover, the addition of Cys to the system still resulted in similar fluorescence changes in the presence of competitive amino acids, indicating that the probe displayed a high selectivity for Cys even involving other amino acids (Figure 3).

Scheme 2. Proposed Mechanism of Ligand Displacement



Afterwards, Hcy and GSH were engaged in the titration experiments (Supporting Information Figure S13–S16). The

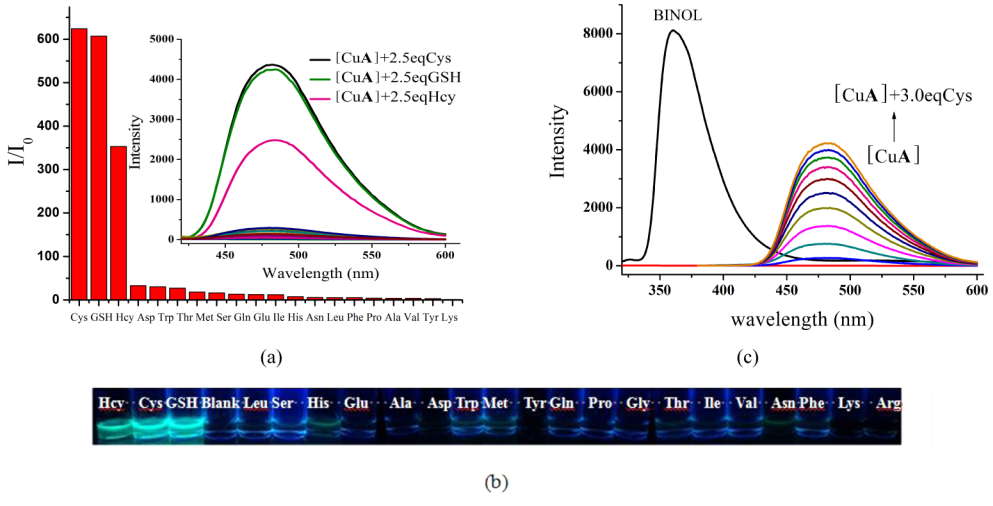


Figure 2. (a) Fluorescence responses of [Cu**A**] complex (2.0×10^{-4} M, λ_{ex} = 305 nm) toward various amino acids (5.0×10^{-4} M) in CH_3CN/H_2O (v/v = 1:1). The inset histogram shows the fluorescence changes of [Cu**A**] complex with Cys, Hcy, GSH, and other amino acids. (b) Visual fluorescent color change of [Cu**A**] complex with different amino acids under UV light (λ_{ex} = 365 nm). (c) Fluorescence responses of BINOL (2.0×10^{-4} M) and [Cu**A**] complex (2.0×10^{-4} M) toward Cys in CH_3CN/H_2O (v/v = 1:1) (0, 0.05, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, and 3.00 equiv) (λ_{ex} = 305 nm, slits: 5/5 nm).

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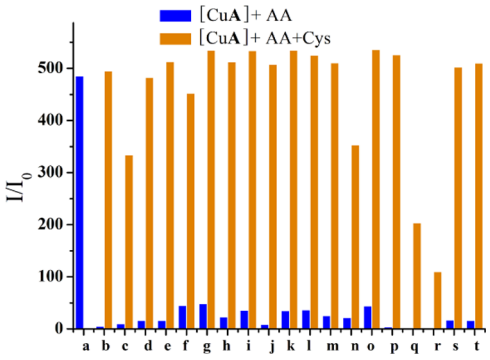

Figure 3. Fluorescence ratio of [Cu**A**] complex (2.0×10^{-4} M, λ_{ex} = 305 nm) to various species of amino acids (AA), and its competition graph with Cys in CH_3CN/H_2O (v/v = 1:1). Blue bar: [Cu**A**] + AA. Orange bar: [Cu**A**] + AA + Cys. (λ_{em} = 482 nm. Slits, 5 nm/5 nm). Key: (a) Cys; (b) Cys + Ala; (c) Cys + His; (d) Cys + Leu; (e) Cys + lle; (f) Cys + Trp; (g) Cys + Asn; (h) Cys + Val; (i) Cys + Phe; (j) Cys + Pro; (k) Cys + Glu; (l) Cys + Thr; (m) Cys + Met; (n) Cys + Ser; (o) Cys + Asp; (p) Cys + Tyr; (q) Cys + Arg; (r) Cys + Lys; (s) Cys + Gln; (t) Cys + Gly.

analytes Hcy and GSH enhanced the fluorescence intensity of [Cu**A**] complex (I_0) as much as I/I_0 316.4- and 531.3-fold, respectively. A linear relationship was observed between the fluorescence intensity and the concentration of GSH/Hcy with R correlation coefficient of 0.999/0.998, respectively. Furthermore, analysis of fluorescence spectra of [Cu**A**] complex with other amino acids and peptides suggested recognition ability in the following order: Cys/GSH > Hcy ≫ other amino acids. Thus, [Cu**A**] complex could be used as a good turn-on fluorescent sensor for recognition of Cys, Hcy, and GSH.

In summary, we found that commercially available BINOL combined with $Cu(NO_3)_2$ could serve as a fluorescent off–on sensor for Cys, Hcy, and GSH. First, the BINOL showed a turn-off fluorescent change in the presence of $Cu(NO_3)_2$. During the processes, a new BINOL oxide-copper complex [Cu**A**] was formed. Consequently, the [Cu**A**] complex acted a turn-on-mode fluorescence recognition for Cys, Hcy, and GSH over other naturally occurring amino acids via a ligand displacement mechanism. Thus, an off–on-mode fluorescent sensor based on BINOL for recognition of Cys, Hcy, and GSH has been well presented.

EXPERIMENTAL SECTION

Materials and Methods. Absorption spectra were recorded at 298 K between 220 and 400 nm. Fluorescence emission spectra were measured in the front face mode at 298 K. The ^{1}H NMR and ^{13}C NMR spectra were measured on a 400 MHz spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). All the fluorescence titrations were contained in 1 cm quartz cell. All solvents and reagents were commercially available and analytical-reagent-grade.

Benesi-Hilderand Method. The association constant K of the complex was then calculated with a linear relationship by Benesi-Hilderand method (eq 1).

$$\frac{I_0}{I - I_0} = \frac{b}{a - b}\left\{\frac{1}{K[M]} + 1\right\} \quad (1)$$

Here I_0 is the fluorescence intensity of the receptor in the absence of guest; I is the fluorescence intensity in the presence of guest; [M] is the concentration of guest; K is the association constant between receptor and guest. In the equation, both a and b are constants.

Preparation of [CuA] Complex for Fluorescence Measurement. A mixture of BINOL (5.7 mg) and $Cu(NO_3)_2 \cdot 3H_2O$ (4.8 mg) was stirred in CH_3CN (5 mL) at room temperature for 30 min until the color of solution became terra-cotta. The solutions of the sensor were freshly prepared for each measurement. The [Cu**A**] complex was used for the titration with different samples for fluorescence.

Isolation of Compound A from the Copper Complex Using EDTA. Three equiv EDTA was added to the solution of copper complex (4 mM) in 100 mL of 1:1 mixture of CH_3CN and water. The resulting solution was stirred for 10 min at room temperature. Upon completion of the reaction, the organic layer was separated and the aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic layer was washed with water and then with brine (2 × 50 mL) and dried over anhydrous Na_2SO_4. The product **A** was isolated by column chromatography.

Dibenzo[*a,kl*]xanthen-1-ol A. ^{1}H NMR (400 MHz, DMSO) δ 10.22 (s, 1H), 7.99–7.88 (m, 3H), 7.70 (d, J = 8.8 Hz, 1H), 7.54–7.45 (m, 2H), 7.41 (m, 2H), 7.34 (d, J = 8.8 Hz, 1H), 7.26 (t, J = 8.0 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ = 152.5, 149.6, 148.6, 130.9, 130.0, 129.1, 128.8, 127.6, 127.4, 127.0, 126.1, 124.7, 124.2, 121.7, 120.0, 116.8, 114.3, 108.3, 107.6 ppm; EI-HRMS: $C_{20}H_{12}O_2$ $[M+H]^+$ 285.0911 (calcd 285.0916); FT-IR (KBr, cm^{-1}) 3390, 3035, 1600, 1505, 1370, 1260, 1100, 810.

ASSOCIATED CONTENT

Supporting Information

Additional fluorescence, UV, CD, IR, HRMS, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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