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A high-efficiency salamo-based copper(II) complex double-channel fluorescent probet

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In this paper, a salamo-based copper(11) complex probe L-Cu²⁺ was synthesized, which combined with copper(II) ions to form L-Cu²⁺ for the detection of S²⁻ and had a good fluorescence chemical response. Through spectral analysis, we found that S^{2-} could be identified with high sensitivity and selectivity in the presence of various anions and could be used for the detection of S^{2-} by the naked eye. With the addition of S²⁻, the solution color changed from colorless to bright yellow. UV absorption, fluorescence and other characterization methods were carried out, and the mechanism of action was determined. In addition, we performed a visual inspection of H_2S gas, and the probe $L-Cu^{2+}$ could detect S^{2-} in the gas molecules, revealing its potential application value in biology and medicine.

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Introduction

As is well known, Cu^{2+} and S^{2-} play vital roles in the entire life system, and sulfides exist widely in nature and have certain toxicity.1 The discharge of sulfur-containing pollutants has seriously threatened the environment and human health.² This not only pollutes soil and water resources, but also releases hydrogen sulfide gas in acidic environments, resulting in air pollution. Due to the nature of the sulfur ion itself, its detection of environmental and physiology has become a very meaningful task.3 Among the many detection methods, fluorescence detection⁴ has the advantages of simple operation, high sensitivity, and good selectivity, and it is widely used in the field of ion detection optical imaging.5 The sensitive detection of the hydrogen sulfide content in living cells will help understand the pathogenesis of hydrogen sulfide.6 Based on the chemical and biological properties of salen7 and salamo-based complexes,8-10 we synthesized a highly efficient selective and sensitive complex to detect sulfur ions.¹¹ Fluorescence properties change significantly when responding to a specific analyte; the fluorescence signal is used as a detection means to convert the identification information into an optical signal,12 and the analyte is quantitatively and qualitatively detected.13 The probe molecule L-Cu2+ had an obvious color effect on S^{2-} , which could bind S^{2-} well in the presence of various anions, and the UV absorbance showed obvious changes;¹⁴ this indicated that S²⁻ can be recognized by L-Cu²⁺ double channels. In addition, after entering H_2S , it was found that $L-Cu^{2+}$ could detect S^{2-} in H_2S with naked eye recognition.

Experiment

Materials and measurements

The drugs and instruments used in this paper are shown in the ESI.†

The synthetic method of the ligands H₂L

H₂L was synthesized by the same method published by our lab and therefore, it is not repeated here¹⁵ (Scheme 1).

The synthetic method of the probe L-Cu²⁺

 H_2L and copper(II) nitrate were weighed in an equimolar ratio, dissolved in an ethanol solution, stirred for 4 hours and then made up to 100 mL and formulated into 1.0×10^{-4} mol L⁻¹ ethanol solution. The ligand H_2L was used as a 1.0 \times 10^{-4} mol L⁻¹ ethanol solution and anions as a 1.0 imes 10^{-3} mol L⁻¹ EtOH/H₂O (10 : 1) solution.

Results and discussion

UV-vis absorption spectroscopy

UV absorption spectrum anion full-scanning experiment: 1 mL of $L-Cu^{2+}$ stock solution was added to the sample cell and then, 0.9 mL of ethanol and 0.1 mL of deionized water were added to test its UV absorption spectrum as a control experiment. The absorption band of **L-Cu²⁺** at around 357 nm was attributed to H₂L and the d-d electron transition of the ligand to Cu²⁺ after Cu²⁺ coordination. Various anion storage solutions of 1 mL were added to the solution of $L-Cu^{2+}$ (1 mL), and the changes in the UV absorption spectra were measured. It was found that only when S²⁻ or CN⁻ was added, there was a significant change in the UV-vis absorption spectrum, and there were two new peaks at 343 and 413 nm. In addition, the UV absorption peaks of the H_2L EtOH/ H_2O (10 : 1) solution were used as the

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Scheme 1 Synthetic route to the probe H_2L .

control experiment. It was found that the peak shape and height of H₂L in the EtOH/H₂O solution were consistent with those for the $L-Cu^{2+}$ solution after adding S^{2-} ions. The experimental results showed that the probe molecules could detect S^{2-} or CN^{-} efficiently in an EtOH/H₂O (10 : 1) solution. As shown in Fig. 1, the solution color varied significantly from colorless to bright yellow. The color of the solution and the UV absorption intensity after the addition of other anions did not change significantly but they changed only upon increasing the single addition of S^{2-} ; this indicated that the probe molecules have good sensitivity and selectivity for the recognition of S²⁻ and other common anions do not affect the recognition results (Fig. 2). The intensity of the absorption peak at 413 nm was taken from S²⁻ and the other anions were added to make a column chart (Fig. 3). It can be clearly seen from this diagram that the increase in other anions causes little interference with the recognition of S^{2-} . The probe molecule can conveniently and effectively detect S^{2-} in the EtOH/H₂O (10:1) solution, which has a good application prospect.

In the ultraviolet spectrum titration experiment, the sample was accumulated to 2 mL of the sensor EtOH solution by the same method. As shown in Fig. 4, it could be seen that as the S^{2-} concentration increased, new absorption peaks appeared at 343 and 413 nm; the intensity gradually changed, and the titration reached the end point until the ion concentration reached 1 equivalent.



Fig. 2 A variety of anions (Cl⁻, ClO₄⁻, CN⁻, CO₃²⁻, H₂PO₄⁻, HCO₃⁻, HPO₄⁻, HS⁻, I⁻, NO₂⁻, NO₃⁻, CH₃COO⁻, P₂O₇⁴⁻, SiO₃²⁻, Br⁻ and S²⁻) was added in the presence of sulfur ions, and the peak at 413 nm did not change at all.

Fluorescence spectroscopy

When 1 equivalent of a 10-fold concentration of sixteen anions of the EtOH/H₂O (10:1) solution was added to the ethanol



Fig. 1 Absorption spectra of $L-Cu^{2+}$ solution (5 × 10⁻⁵ M) in the absence and presence of various anions (Cl⁻, ClO₄⁻, CN⁻, CO₃²⁻, H₂PO₄⁻, HCO₃⁻, HPO₄⁻, HS⁻, I⁻, NO₂⁻, NO₃⁻, CH₃COO⁻, P₂O₇⁴⁻, SiO₃²⁻, Br⁻ and S²⁻).



Fig. 3 The black strip represents the absorbance intensity of the probe $L-Cu^{2+}$ solution at 413 nm when S^{2-} was added, and the red strip represents the absorbance intensity of the probe $L-Cu^{2+}$ solution at 413 nm when various anions and S^{2-} were added.



Fig. 4 Changes in the absorption spectra of the probe $L-Cu^{2+}$ solution (5 \times 10⁻⁵ M) upon the increase of S²⁻ (0–1.0 equiv.). Inset: before and after the addition of S²⁻ (1 equivalent), the bright yellow change of the probe $L-Cu^{2+}$ solution can be observed by the naked eye.

solution of the probe molecules, a bright yellow color change could be observed by the naked eve due to the action of the probe molecule and S^{2-} (Fig. 5). Furthermore, when the excitation wavelength was 375 nm, the fluorescence emission intensity was weak, but the addition of S^{2-} caused a strong fluorescence emission peak at 455 nm and a redshift of 25 nm (Fig. 6). It was proven that the sensor molecule can detect S^{2-} through two channels. In addition, the fluorescence emission peak of H_2L in the EtOH/H₂O (10:1) solution was tested as a control experiment, and it was found that the addition of S^{2-} in the L-Cu²⁺ ethanol solution could restore the fluorescence emission peak of H₂L. It can be seen from Fig. 7 that the addition of other anions to the solution has no significant effect on the ability of the probe molecule to recognize S^{2-} . It was concluded that the probe molecule has a good anti-interference performance against other anions in the fluorescence emission spectrum of S^{2-} . In order to verify the binding ratio between the probe molecule and S²⁻, a fluorescence titration experiment was performed.

In the titration experiment, the excitation wavelength was 375 nm. As shown in Fig. 8, as different amounts of S^{2-} were



Fig. 6 1 mL of L-Cu²⁺ solution (5 × 10⁻⁵ M) was taken in the sample cell, and 1 mL solutions of different anions (Cl⁻, ClO₄⁻, CN⁻, CO₃²⁻, H₂PO₄⁻, HCO₃⁻, HPO₄⁻, HS⁻, I⁻, NO₂⁻, NO₃⁻, CH₃COO⁻, P₂O₇⁴⁻, SiO₃²⁻, Br⁻ and S²⁻) were added with a pipette to test the change in the fluorescence spectrum.

added dropwise to the ethanol solution of the probe molecule, the fluorescence intensity of the main peak gradually increased with a gradual redshift. When 1.0 equivalent of S^{2-} was added, the fluorescence intensity did not increase, indicating the end



Fig. 7 500 μ L of different anions (Cl⁻, ClO₄⁻, CN⁻, CO₃²⁻, H₂PO₄⁻, HCO₃⁻, HPO₄⁻, HS⁻, I⁻, NO₂⁻, NO₃⁻, CH₃COO⁻, P₂O₇⁴⁻, SiO₃²⁻, Br⁻ and S²⁻) were added to 1 mL of L-Cu²⁺ solution, and 500 μ L of S²⁻ was added to test the effect on the fluorescence intensity of the system.



Fig. 5 (a) The photograph shows the status of $L-Cu^{2+}$ when 1 mL of anions was added. (b) The picture shows the color changes of the probe solutions with the sequential addition of 1 mL anion solutions and 1 mL S^{2-} .

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Fig. 8 Fluorescence spectra of probe L-Cu²⁺ solution (5 \times 10⁻⁵) upon the increase in S²⁻ (0–1.0 equivalent).

of the fluorescence titration. As depicted in Fig. S1,[†] the binding constant of the receptor molecule **L-Cu**²⁺ and S²⁻ was $K = 0.5 \times 10^4$, and the lowest detection limit was LOD = 3.27×10^{-8} .¹⁵ In addition, pH control experiments showed that the sensor **L-Cu**²⁺ can effectively identify S²⁻ in the range of pH = 3-8 (Fig. 9).



Fig. 9 The black dotted line diagram shows the $L-Cu^{2+}$ fluorescence spectrum at different pH conditions; the red dotted line shows the fluorescence spectrum test results with the addition of S^{2-} .

Mechanism research

The probe molecule has an absorption peak at 357 nm. With the addition of S²⁻, Cu²⁺ was bound by S²⁻, and absorption peaks appeared at 343 and 413 nm. On increasing the S²⁻ concentration, the peak at 343 nm decreased, the absorption peak at 413 nm was enhanced, and a ratio-type response to S^{2-} was achieved. In the linear range, the ratio signal changed by a factor of 20. This probe has no response to various anions outside S²⁻ and CN⁻ and has good selectivity. The solution changed from colorless to bright yellow before and after the reaction; thus, S²⁻ can be determined by colorimetry. In the fluorescence experiment, the ligand acted as a donor, and Cu²⁺ acted as a receptor to form the L-Cu²⁺ complex. The fluorescence disappeared due to the quenching effect of Cu²⁺. Excess S^{2-} was added, and the fluorescence was recovered, exhibiting the stronger binding ability of S²⁻ and Cu²⁺, which caused the ligand fluorescence to be restored.

By mass spectrometry, it can be seen that a significant peak at m/z 398.2 was in agreement with the molecular ion peak of the ligand (Fig. S2(a)†), and a peak appeared at m/z 437.1 (Fig. S2(b)†), which should be attributed to **L**-Cu²⁺, indicating the line of the complex **L**-Cu²⁺. Then, NaS was added and further mass spectrometry was performed. As can be seen from Fig. S2(c),† a molecular ion peak appeared at m/z 398.2 that corresponded to the ligand. It is fully demonstrated that S²⁻ combines with Cu²⁺ in the complex to form CuS. At the same time, **L**-Cu²⁺ released Cu²⁺ therein and returned to the ligand state (Fig. 10).

H₂S gas detection

 H_2SO_4 (60 mL, 3 mol L⁻¹) was added to Na₂S to prepare H_2S gas, which was then introduced into pure ethanol and the L-Cu²⁺ ethanol solution sequentially. In the presence of H_2S , the probe solution deepened gradually from pale yellow, and it was easily recognized by the naked eye. However, the pure ethanol solution did not change significantly. Fig. 11 shows the color change trends one minute and three minutes after H_2S gas was introduced into the ethanol solution and L-Cu²⁺ solution. The color remained almost unchanged for 3 h, which indicated that the probe had good stability in identifying H_2S and could meet the needs for analysis and detection. The formation of an acidic environment by the introduction of H_2S gas and the lack of water in the solution that hindered the formation of intramolecular hydrogen bonds broke the rigid environment of the molecules and turned the solution brown. As shown in Fig. 12,



Fig. 10 Schematic diagram of the structure of the probe molecule and its two-channel visualization detection of S^{2-}



Fig. 11 H_2S gas was introduced into ethanol solution and $L-Cu^{2+}$ ethanol solution, respectively. The color changes with the increase in the H_2S concentration and contact time under solar conditions are shown.



Fig. 12 120 μ L to 3 mL of water was added into L-Cu²⁺ solution containing H₂S gas, and the color of the solution gradually returned to bright yellow.

No.	Probe	Solvent	Dual channel	Naked eye	Reversible	LOD	Reference
1		DMSO/H ₂ O (7 : 3 v/v)	No	Yes	No	$5.2 imes 10^{-9}$	16 <i>a</i>
2	G-Quadruplex DNAzyme	Tris buffer	No	Yes	No	4.1×10^{-10}	16 <i>b</i>
3	TA-CuNCs	H_2O	Yes	Yes	No	0.1×10^{-6}	13 <i>c</i>
4		$EtOH/H_2O(1:1 \text{ v/v})$	Yes	Yes	Yes	3.27×10^{-9}	This work

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when water was added to the probe $L-Cu^{2+}$ solution containing H_2S gas, the color of the solution changed from brown to yellow.

Conclusions

In summary, we synthesized a salamo-based Cu^{2+} complex probe $L-Cu^{2+}$ according to the literature, which could be identified with S^{2-} in the UV absorption spectrum and fluorescence spectrum. Mass spectrometry showed that S^{2-} and S^{2-} -bound Cu^{2+} when added to $L-Cu^{2+}$ formed stable CuS. As shown in Fig. 1, after the addition of S^{2-} , the absorption peak at 357 nm disappeared, and new absorption peaks appeared at 343 and 413 nm, thereby establishing a ratio type probe for measuring S^{2-} . Similarly, in the fluorescence experiment, as S^{2-} was added to **L-Cu²⁺**, the reaction system produced a new fluorescence peak at 455 nm, and the intensity was continuously enhanced. The fluorescence intensity at 455 nm was plotted as a bar graph, as shown in Fig. 7. After adding various anions to **L-Cu²⁺**, S^{2-} was added, and the fluorescence intensity did not significantly interfere. This indicated that **L-Cu²⁺** has good selectivity for S^{2-}

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in fluorescence experiments. Our experimental results showed that the probe molecule has the property of detecting S^{2-} in the gas. Also, when H_2S was introduced into the ethanol solution of the probe molecule, the color of the solution changed with time to form a brown solution. Our experiments showed that it can achieve fast, accurate and real-time monitoring of H_2S gas and it has potential application values. In addition, we compared other S^{2-} recognizing probes with our work, as shown in Table 1.

Conflicts of interest

There are no conflicts to declare.

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