A Tetrathenylethylene-based Fluorescent Chemosensor for Cu²⁺ in Aqueous Solution and Its Potential Application to Detect Histidine

Yan ZENG,*† Guanxin ZHANG,** and Deqing ZHANG**

*National Engineering Laboratory for Industrial Enzymes, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 West 7th Avenue, Tianjin Airport Economic Area, Tianjin 300308, P. R. China
**Beijing National Laboratory for Molecular Sciences, Organic Solids Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Zhongguancun North First Street 2, Beijing 100190, P. R. China

A tetrathenylethylene-based compound with the property of aggregation-induced emission was designed and synthesized. It exhibited good sensitivity and selectivity to Cu²⁺ over other metal ions (Zn²⁺, Cd²⁺, Ba²⁺, Ni²⁺, Mg²⁺, Ca²⁺, Co²⁺, Fe³⁺, Pb²⁺, Mn²⁺, Fe²⁺, Ag⁺, K⁺, and Hg²⁺) in an aqueous solution by the “turn-off” UV-vis absorbance and fluorescence signals. More interesting, with the subsequent addition of histidine rather than other amino acids such as proline, alanine, aspartic acid, lysine, arginine, leucine, glutamine or cysteine, the complex between the compound and Cu²⁺ was disrupted, and the UV-vis absorbance and fluorescence signals were recovered, indicating that the complex from the compound and Cu²⁺ could be used as a “turn-on” fluorescent probe for histidine.

Keywords: Tetrathenylethylene, aggregation-induced emission, Cu²⁺, histidine

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Introduction

Copper is the third most abundant trace metal (behind iron and zinc) found in the human body. It is a cofactor for a variety of oxidative enzymes and plays an important role in various biological processes.¹ On the other hand, copper ion is a significant metal pollutant due to its widespread use and the toxic impact of its excess on microorganisms even at submicromolar concentrations.² It has been found that the alteration in the cellular homeostasis of copper is connected to serious neurodegenerative diseases, including Menkes disorder, Wilson’s disease, familial amyotrophic lateral sclerosis, and Alzheimer’s disease.³,⁴ Thus, chemosensors for copper ions,⁵–⁸ especially those used in environment friendly aqueous solvents with sensitive and fast fluorescent response signals,⁹–¹¹ have received much attention in recent years.

Histidine (His) not only plays a key role in the formation of the myelin sheath, the protective barrier that surrounds neural cells and supports the transmission of brain signals to different parts of the body, but also participates in the detoxification of heavy metals and other cellular debris for elimination through the liver and kidneys.¹² Thus, the detection of His is important in biochemistry and molecular biology. Numerous methods have been reported for the determination of His,¹³–¹⁶ therein, the indicator-displacement assay with Cu²⁺ is a representative design principle.¹⁷–¹⁹ In the indicator-displacement assay, the absorbance or fluorescence of the indicator, which is bound to the receptor through non-covalent interactions, is either quenched or enhanced by the receptor, but then recovers when the analyte displaces the indicator. For example, Zhou et al.²⁰ has employed a DNA-scaffolded silver nanocluster/Cu²⁺ ensemble as a turn-on fluorescent probe for His based on the fact that His has better coordination ability than the nucleic acids of the DNA-AgNCs to Cu²⁺.

Since the phenomenon named aggregation-induced emission (AIE) was reported,²¹ in which non-emissive dyes can be induced to emit efficiently by the aggregate formation, a large number of AIE-active dyes, such as tetraphenylethylene-based compounds, have been developed in the fields of organic light-emitting diodes and chemo/bioprobes.²²–²⁶ Though some tetraphenylethylene-based sensors for metal ions have been reported,²⁷–³⁰ and a tetraphenylethylene-based zinc complex was reported as a sensitive DNA probe by coordination interaction,³¹ there is still few related studies on the detection of Cu²⁺ or amino acids by the indicator-displacement assay with Cu²⁺.³² In this report, a tetraphenylethene-based fluorescent compound I was synthesized and used to detect Cu²⁺ in an aqueous solution. The compound I showed a strong AIE effect in the aqueous solution but after the addition of Cu²⁺ its fluorescence was quenched. Moreover, the fluorescence quenching was reversible with the subsequent addition of His, indicating that in the presence of Cu²⁺ the compound I can be also used as a potential “turn-on” fluorescent probe for His.

¹ To whom correspondence should be addressed.
E-mail: zeng_y@tib.cas.cn
Experimental

Reagents and chemicals
All reagents were of the highest grade available and used without further purification, except toluene and tetrahydrofuran (THF) which were pre-dried with Na then purified by distillation. The solutions of Zn$^{2+}$, Cd$^{2+}$, Ba$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Fe$^{3+}$, Pb$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Ag$^+$, K$^+$, and Hg$^{2+}$ were prepared from their hydrated perchlorate salts.

Synthesis of compound 1
Compound 1 was synthesized by Suzuki coupling reaction (Scheme 1). Under the nitrogen atmosphere, 4-bromo-$N,N$-bis(pyridylmethyl)aniline (0.28 g, 0.94 mmol), 1,2,2-triphenylvinylboronic acid (0.22 g, 0.61 mmol), Aliquat 336 (42 mg), K$_2$CO$_3$ (0.60 g, 4.4 mmol), 25 mL distilled toluene, 4 mL ultrapure water and 20 mg Pd(PPh$_3$)$_4$ were sequentially added into the three-necked flask and heated at 120$^\circ$C for 18 h. Then after cooling, the resulting mixture was extracted with CH$_2$Cl$_2$. The organic layer was dried over anhydrous Na$_2$SO$_4$, and the solvents were evaporated in vacuum. The residue was chromatographed on silica gel with petroleum ether (60 – 90$^\circ$C)/ethyl acetate (1:1, v/v) as eluent to give 0.29 g (yield: 91%) of the product as a pale yellow solid.

Characterization of compound 1
$^1$H NMR (400 MHz, CDCl$_3$, ppm) $\delta$: 8.56 (2 H, d, $J$ = 8.4 Hz), 7.60 (2 H, d, $J$ = 8.4 Hz), 7.22 (2 H, d, $J$ = 7.6 Hz), 7.15 – 6.99 (17 H, m), 6.82 (2 H, d, $J$ = 8.4 Hz), 6.47 (2 H, d, $J$ = 8.8 Hz), 4.78 (4 H, s); $^{13}$C NMR(100 MHz, CDCl$_3$, ppm) $\delta$: 158.8, 150.0, 147.0, 144.5, 144.3, 144.2, 140.9, 139.2, 136.7, 132.4, 131.54, 131.43, 131.38, 127.6, 127.5, 126.3, 126.0, 122.0, 121.0, 111.6, 57.2. MS-ESI ($m/z$): 530.4 M$^+$, Elemental analysis (Calcd %) for C$_{32}$H$_{31}$N$_3$: C, 86.17, H, 5.90, N, 7.93. Found: C, 85.68, H, 5.95, N, 7.72.

Measurements
UV-visible spectra were measured on a JASCO V-570 spectrometer in a 1-cm quartz cell. Fluorescence measurements were carried out with a JASCO-FP6600 spectrometer in a 1-cm quartz cell.

Results and Discussion

The AIE phenomenon of compound 1
At the diluted concentration (20 $\mu$M), compound 1 in THF was non-luminescent, but by the addition of water its emission was turned on. As shown in Fig. 1, after addition of water, its maximum absorption shifted from 334 to 344 nm and its fluorescence intensity at 499 nm (excited at 344 nm) was increased by 310 fold at 99% volume fraction of water. The fluorescence enhancement could also be detected with the naked eyes (inset of Fig. 1).

The UV-visible absorbance and fluorescence change by the addition of Cu$^{2+}$
As illustrated in Fig. 2a, compound 1 (20 $\mu$M) in the THF/water mixture (1:99, v/v) showed its highest absorbance at about 344 nm. However, the addition of Cu(ClO$_4$)$_2$ led to a decrease of its absorbance intensity and a slightly blueshift of its maxima absorbance. Moreover, the intensity of the absorbance was decreased linearly with the concentration of Cu$^{2+}$ up to a ligand/Cu$^{2+}$ ratio of 1. And when even more Cu$^{2+}$ was added the absorbance remained almost unchanged, implying a 1:1 complex was formed between compound 1 and Cu$^{2+}$ (inset of Fig. 2a).

The fluorescence emission spectra of compound 1 in the THF/water mixture (1:99 v/v) in the presence of Cu$^{2+}$ was also examined. Similarly to the UV-visible absorbance change but more obviously, the fluorescence emission was quenched gradually upon the addition of Cu(ClO$_4$)$_2$, as shown in Fig. 2b. A linear decrease of the fluorescence intensity at 499 nm was

![Scheme 1](image1)

**Scheme 1**: The synthesis route of compound 1.

![Fig. 1](image2)

**Fig. 1**: The absorption and emission spectra of compound 1 (20 $\mu$M) in THF (dotted line) and THF-water mixture (1:99 v/v, solid line). Inset: photographs of compound 1 in (a) THF-water mixture and (b) THF taken under illumination of UV lamp.
also observed until one equiv. of Cu²⁺ was added. Further addition of Cu²⁺ led to only a nominal decrease in fluorescence intensity (inset of Fig. 2b). The TOF-MS result of the solution containing compound 1 and Cu²⁺ with a mole ratio of more than 1 also supported that their complex was formed as a 1:1 ratio, because only the peak at a mass/charge (m/z) unit of 592.2 assigned to the formed complex between compound 1 and Cu²⁺, and the peak at m/z of 528.1 likely due to the free compound 1 by decomplexation were observed by TOF-MS. Moreover, based on the Stern-Volmer equation:33,34

\[
\frac{I_0}{I} = 1 + K_{sv}[Q] 
\]

where \(I_0\) and \(I\) were the fluorescence intensity at 499 nm in the absence and presence of the quencher Cu²⁺, respectively, and [Q] was the concentration of the quencher Cu²⁺, the Stern-Volmer constant \((K_{sv})\) was determined at about 2.12 \(\times\) 10⁵ M⁻¹ and was comparable to some earlier reports.33,34 The detection limit, taken as the concentration of Cu²⁺ which produced a signal equivalent to three times the standard deviation of the blank, was calculated to be 0.17 μM and was also satisfactory for Cu²⁺ detection in drinking water within U.S. EPA limit (~20 μM).35

Selectivity over metal ions

Compound 1 exhibited excellent selectivity for Cu²⁺ over many other metal ions (Zn²⁺, Cd²⁺, Ba²⁺, Ni²⁺, Mg²⁺, Ca²⁺, Co²⁺, Fe³⁺, Pb²⁺, Mn²⁺, Fe²⁺, Ag⁺, K⁺, and Hg²⁺). As shown in Fig. 3, an excess of these hydrated perchlorate salts (100 μM) had little effect on the fluorescence intensity of compound 1 (20 μM in THF/water, 1:99, v/v), while 1.0 equiv. Cu²⁺ was added subsequently, its fluorescence intensity was almost quenched completely. The similar phenomenon was reported by Guo et al., using bis(2-pyridylmethyl)amine as the Cu²⁺ ligand of their fluorescent sensor for Cu²⁺.34 Additionally, to explore the effects of anionic counterions, fluorescence response of compound 1 to bivalent copper salts with different cations (Cu(ClO₄)₂, CuCl₂, Cu(OAc)₂, and Cu(NO₃)₂) was examined in an aqueous solution. The results showed that there were no obvious differences in the fluorescence sensing behavior of compound 1 to these perchlorate, chloride, acetate, and nitrate salts. The metal ion Cu²⁺ was bound with the bis(2-pyridylmethyl)amine group in compound 1 and the fluorescence was efficiently quenched due to the paramagnetic properties of Cu²⁺ via electron or energy transfer.20

The UV-visible absorbance and fluorescence change by the subsequent addition of His

The above results indicated that compound 1 was a “turn-off” fluorescent sensor for Cu²⁺. Considering that some complexes of Cu²⁺ were used to detect His based on the indicator-displacement assay principle, the UV-visible absorbance and fluorescence change of the complex between the compound 1 and Cu²⁺ upon the addition of His was also tested. As shown in Fig. 4a, when His was gradually added, the absorbance of compound 1 in the presence of Cu²⁺ increase accordingly. Almost no change of absorbance was observed after the addition of 2.0 equiv. of His. A similar change was also observed in the fluorescence. The fluorescence signal of compound 1 caused by AIE effect gradually increased with the amount of His, and changed faintly when more than 2.0 equiv. of His was added (shown in Fig. 4b). It is well-known that the
imidazole moieties can help His snatch the copper ions from other complexes. Thus, the increase in the absorbance and fluorescence by the addition of His both indicated that His pulled Cu^{2+} from the coordination sphere of the initial complex, and coordinated with the metal ion in a 2:1 ratio by its imidazole group, so that the electron or energy transfer between the compound and Cu^{2+} was inhibited and the AIE phenomenon attributed to the tetraphenylethylene structure in compound was recovered.

The selectivity of the complex from the chemosensor and Cu^{2+} over different amino acids.

To examine the selectivity of the complex from compound 1 and Cu^{2+} to His, the responses of the complex to other different amine acids containing proline (Pro), alanine (Ala), aspartic acid (Asp), lysine (Lys), arginine (Arg), leucine (Leu), glutamine (Gln), cysteine (Cys), and tyrosine (Tyr) were also recorded in the aqueous solution. The results (Fig. 5) showed the addition of other amino acids had a slight effect on the fluorescence of compound 1. The reason was that His had a better coordination ability than the bis(2-pyridylmethyl)amine unit of compound 1 and could bind Cu^{2+} in a 2:1 association process. Whereas, for the case of the other amino acids, Cu^{2+} was still coordinated to compound 1 and the electron or energy transfer still existed so that the enhancement in fluorescence could not be observed.

Conclusions

A compound with the tetraphenylethene and bis(2-pyridylmethyl) amine units was synthesized to detect Cu^{2+} in an aqueous solution. Therein, its tetraphenylethene structure was the indicator and its bis(2-pyridylmethyl)amine unit was the receptor. The result showed that the compound could detect Cu^{2+} in an aqueous solution with high selectivity and sensitivity. Moreover, the addition of histidine could disrupt the complex between the compound and Cu^{2+}, and induced a recovery of the absorbance and fluorescence of the aggregated compound, indicating that the complex from the compound and Cu^{2+} could be used as a “turn-on” fluorescent probe for histidine.

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References

1. J. Y. Uriu-Adams and C. L. Keen, Mol. Aspects Med., 2005, 26, 268.
2. Y. Zhou, F. Wang, Y. Kim, S. J. Kim, and J. Yoon, Org. Lett., 2009, 11, 4442.
3. L. M. Gaetke and C. K. Chow, Toxicology, 2003, 189, 147.
4. H. Kozlowski, A. Janicka-Klos, and J. Brasun, Coord. Chem. Rev., 2009, 253, 2665.
5. V. Chandrasekhar, S. Das, C. R. Yadav, S. Hossain, R. Parihar, G. Subramaniam, and P. Sen, Inorg. Chem., 2012, 51, 8664.
6. D. Maity, A. K. Manna, D. Karthigeyan, T. K. Kundu, S. K. Pati, and T. Govindaraju, Chem. Eur. J., 2011, 17, 11152.
7. D. W. Domaille, L. Zeng, and C. J. Chang, *J. Am. Chem. Soc.*, 2010, 132, 1194.
8. R. F. H. Vigier and A. N. Hulme, *J. Am. Chem. Soc.*, 2006, 128, 11370.
9. Y. K. Yue, C. X. Yin, and F. J. Huo, *J. Coord. Chem.*, 2014, 67, 2039.
10. F. J. Huo, C. X. Yin, Y. T. Yang, J. Su, J. B. Chao, and D. S. Liu, *Anal. Chem.*, 2012, 84, 2219.
11. M. Zhao, X. F. Yang, S. He, and L. Wang, *Sens. Actuators*, B, 2009, 135, 625.
12. C. R. Harding and I. R. Scott, *J. Mol. Biol.*, 1983, 170, 651.
13. S. K. Sun, K. X. Tu, and X. P. Yan, *Analyst*, 2012, 137, 2124.
14. F. Pu, Z. Huang, J. Ren, and X. Qu, *Anal. Chem.*, 2010, 82, 8211.
15. D. L. Ma, W. L. Wong, W. H. Chung, F. Y. Chan, P. K. So, T. S. Lai, Z. Y. Zhou, Y. C. Leaung, and K. Y. Wong, *Angew. Chem., Int. Ed.*, 2008, 120, 3795.
16. D. Xiong, M. Chen, and H. Li, *Chem. Commun.*, 2008, 880.
17. Q. H. You, A. W. M. Lee, W. H. Chan, X. M. Zhu, and K. C. F. Leung, *Chem. Commun.*, 2014, 50, 6207.
18. J. T. Hou, K. Li, K. K. Yu, M. Y. Wu, and X. Q. Yu, *Org. Biomol. Chem.*, 2013, 11, 717.
19. H. Li, J. Liu, Y. Fang, Y. Qin, S. Xu, Y. Liu, and E. Wang, *Biosens. Bioelectron.*, 2013, 41, 563.
20. Y. Zhou, T. Zhou, M. Zhang, and G. Shi, *Analyst*, 2014, 139, 3122.
21. J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. W. Zhan, Y. Liu, D. Zhu, and B. Z. Tang, *Chem. Commun.*, 2001, 1740.
22. Y. Hong, J. W. Y. Lam, and B. Z. Tang, *Chem. Soc. Rev.*, 2011, 40, 5361.
23. M. Wang, G. Zhang, D. Zhang, D. Zhu, and B. Z. Tang, *Mater. Chem.*, 2010, 20, 1858.
24. A. Qin, J. W. Y. Lam, and B. Z. Tang, *Prog. Polym. Sci.*, 2012, 37, 182.
25. S. Chen, Y. Hong, Y. Liu, J. Liu, C. W. T. Leung, M. Li, R. T. K. Kwok, E. Zhao, J. W. Y. Lam, Y. Yu, and B. Z. Tang, *J. Am. Chem. Soc.*, 2013, 135, 4926.
26. J. Wang, J. Mei, R. Hu, J. Z. Sun, A. Qin, and B. Z. Tang, *J. Am. Chem. Soc.*, 2012, 134, 9956.
27. Y. Hong, S. Chen, C. W. T. Leung, J. W. Y. Lam, J. Liu, N. W. Tseng, R. T. K. Kwok, Y. Yu, Z. Wang, and B. Z. Tang, *ACS Appl. Mater. Interfaces*, 2011, 3, 3411.
28. S. Fei, G. Zhang, D. Zhang, and H. Jiang, *Org. Lett.*, 2011, 13, 6738.
29. N. Bian, Q. Chen, X. L. Qiu, A. D. Qi, and B. H. Han, *New J. Chem.*, 2011, 35, 1667.
30. J. H. Ye, J. Liu, Z. Wang, Y. Bai, W. Zhang, and W. He, *Tetrahedron Lett.*, 2014, 55, 3688.
31. Z. Zhu, L. Xu, H. Li, X. Zhou, J. Qin, and C. Yang, *Chem. Commun.*, 2014, 50, 7060.
32. H. T. Feng, S. Song, Y. C. Chen, C. H. Shen, and Y. S. Zheng, *J. Mater. Chem. C*, 2014, 2, 2353.
33. L. Shang and S. Dong, *J. Mater. Chem.*, 2008, 18, 4636.
34. Z. Guo, W. Zhu, and H. Tiang, *Macromolecules*, 2010, 43, 739.
35. H. S. Jung, P. S. Kwon, J. W. Lee, J. Kim, C. S. Hong, J. W. Kim, S. Yan, J. Y. Lee, J. H. Lee, T. Joo, and J. S. Kim, *J. Am. Chem. Soc.*, 2009, 131, 2008.
36. S. Parthasarathy, F. Long, Y. Miller, Y. Xiao, D. McElheny, K. Thurber, B. Ma, R. Nussinov, and Y. Ishii, *J. Am. Chem. Soc.*, 2011, 133, 3390.
37. X. Lou, L. Zhang, J. Qin, and Z. Li, *Langmuir*, 2010, 26, 1566.