Short communication

A new copper(II) selective fluorescence probe based on naphthalimide: Synthesis, mechanism and application in living cells

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A new fluorescence probe L based on naphthalimide has been synthesized for selective and quantitative detection of Cu2+ in CH3CN:H2O (4:1, v/v) solution. L exhibited a strong green fluorescence. Upon addition of 2 equiv. of Cu2+, the fluorescence emission shows a steady and smooth decrease until a plateau is reached with a 30-fold quenching of fluorescence intensity. In the presence of Cu2+, the absorbance peak of L maximum at 466 nm decreased, and a new absorption band at 600 nm appeared. Under the identical conditions, other physiological and environmental important metal ions induced negligible spectroscopic changes. The 1:2 stoichiometry binding mode of L with Cu2+ was supported by the Benesi–Hildebrand analysis and ESI-MS spectra studies. The detection limit for Cu2+ was estimated to be 64 ppb. Fluorescence microscopy experiments showed that L has practical application in living cells.

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1. Introduction

The development of molecular fluorescence probes for the detection of environmentally and biologically important species has always been of particular importance, and usually involves the design and synthesis of molecules that contain binding sites and signaling subunits able to display selective changes in fluorescence emission upon guest binding [1–4]. Metal-selective fluorescence probes as useful tools have been widely exploited to detect biologically relevant metal ions [5–14]. Especially, the detection of Cu2+ is attracting continuous attention, as copper is a significant metal pollutant due to its widespread use, but it is also an essential trace element that plays a pivotal role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals [15]. For example, the copper ion plays a critical role as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome oxidase and tyrosinase [16], Its concentration in the neuronal cytoplasm may contribute to the etiology of Alzheimer’s [17] or Parkinson’s disease [18]. However, exposure to a high level of copper can cause gastrointestinal disturbance even liver or kidney damage [19]. The average concentration of blood copper in the normal group is 100–150 μg/L (15.7–23.6 μM) [20]. The U.S. Environmental Protection Agency (EPA) has set the limit of copper in drinking water to be 1.3 ppm (20 μM) [21]. By virtue of its highly sensitive and high-speed spatial analysis of cells, fluorescence bioimaging has provided a facile and less cell-damaging means of visualizing analytes of biological interest in living cells [22]. To image intracellular metal ions, highly sensitive and selective probes that exhibit visible fluorescent emission changes in aqueous media need to be developed. Thus, the development of highly selective and sensitive fluorescent probes for monitoring Cu2+ in living cells remains highly required [23]. Herein, we would like to report a new naphthalimide-based Cu2+-selective fluorescence probe 4-tert-Butyl-2,6-diformylphenol-6-hydrazino-benzo[de]isoquinoline-1,3-diones (L), which could be used for rapid, highly selective and sensitive detection of Cu2+, and the biological application of L to monitor Cu2+ in cultured cells was also presented.

2. Experimental

The synthetic routes were shown in Scheme 1. Compounds 1, 2 and 3 were synthesized according to the literatures [24–26]. Compound L was synthesized conveniently from the reaction of 6-hydrazino-benzo[de]isoquinoline-1,3-diones with 4-tert-Butyl-2,6-diformylphenol [27,Fig. S1]. Cu–L was synthesized from L and Cu(ClO4)2·6H2O under reflux condition for 3 h.

3. Results and discussions

Free L displayed an absorption band with a maximum absorbance peak at about 466 nm (log e = 5.34) (Fig. 1). Upon addition of Cu(ClO4)2·6H2O in the CH3CN:H2O (4:1, v/v) solution of L (20 μM), the absorption band centered around 466 nm decreased, finally remained constant...
after approximate 2 equivalents of Cu²⁺ were added (Fig. S2). Meanwhile, a new absorption band centered around 600 nm appeared (Fig. 1). The former band should be assigned to the π–π* transition of naphthalimide chromophore, while the latter should be attributed to a metal-to-ligand charge transfer (MLCT) band caused by Cu²⁺-binding. A Benesi–Hildebrand analysis established a 1:2 stoichiometry for the L–Cu²⁺ complexation species, with an association constant (K) being calculated as $K = 1.58 \pm 0.45 \times 10^9 \text{M}^{-2}$ ($R = 0.999$) (Fig. S3)[28].

However, for L (20 μM) solutions, no significant absorbance changes around 466 nm were observed in the presence of 10 equivalents of alkali, alkaline earth and the other transition metal ions (Figs. S4 and S5), and even rare earth metal ions (except radioactive element promethium, Figs. S6 and S7), implying that L could have special binding ability toward Cu²⁺. The high selectivity of the L for Cu²⁺ over other metal ions should be in part contributing to the strong coordination ability of Cu²⁺ and its larger association constant.

When excited at 466 nm, free L exhibited an emission band centered about 560 nm ($\Phi_f = 0.38$) in CH₃CN:H₂O (4:1, v/v) solution (Fig. 2) [29]. Upon the addition of Cu²⁺ ions, the fluorescence was quenched significantly. The titration measurements showed a steady and smooth decrease until a plateau was reached ($\Phi_f = 0.021$). The overall effect upon addition of 2 equiv. of Cu²⁺ was a 30-fold quenching of fluorescence at 560 nm (Fig. S8).

To further explore the availability of L as a highly selective probe for Cu²⁺, fluorescent responses of L to the other metal ions that probably affect the fluorescence intensity were examined. No significant spectra

Fig. 1. UV−vis spectra of L (20 μM) in CH₃CN:H₂O (4:1, v/v) solution upon addition of increasing concentrations of Cu(ClO₄)₂.

Fig. 2. Fluorescence emission spectra of L (20 μM) in CH₃CN:H₂O (4:1, v/v) solution upon addition of increasing concentrations of Cu(ClO₄)₂ with an excitation wavelength at 466 nm.
changes of L (20 μM) were observed in the presence of 10 equivalents alkali and alkaline earth metals, such as Na⁺, K⁺, Mg²⁺, and Ca²⁺, and the first row transition metals Cr³⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺ and even group 12 metals Zn²⁺, Cd²⁺ and Pb²⁺ as well as Hg²⁺, Ag⁺ and Cu⁺, respectively (Fig. S9). Spectra changes of L (20 μM) increased little in the presence of Co²⁺, Ni²⁺, Cd²⁺ and Hg²⁺. In addition, the fluorescence responses of L (20 μM) to the rare earth metal ions were also examined, and the result indicated that the fluorescence intensity of L was hardly disturbed even in the presence of 10 equivalents rare earth metal ions (Except radioactive element promethium, Fig. S10). Furthermore, the competition experiments revealed that the Cu²⁺-induced fluorescence response was unaffected in the presence of the other metal ions mentioned above (Figs. 3 and S11). Thus the Cu²⁺-selective binding and On-Off response could take place in the coexistence of the competitive metal ions. It should be noted that L was only quenched by Cu²⁺, which was quite different from the reported Cu²⁺ probe [30].

The further fluorescent titration profile of L with Cu²⁺ demonstrated that the detection limit of Cu²⁺ was at 1 μM (64 ppb, decreasing 11.72%) level when L was employed at 1 μM (Fig. S12). Meanwhile, under the optimized condition, the fluorescence intensities of the solution of L were nearly proportional to the amount of Cu²⁺ with EC₅₀ = 3 μM (192 ppb), indicating that the limit of detection of L to Cu²⁺ met the limit of copper in drinking water according to the U.S. EPA standard. [21] Furthermore, the response of L to Cu²⁺ was irreversible rather than a ion-catalyzed reaction [31]. The fluorescence signals of naphthalimide group in the L-Cu²⁺ complexation species would be recovered upon the addition of EDTA. When excess Cu²⁺ was added, the signals would be quenched again.

From a mechanistic viewpoint, it was likely that there were a number of combined influences achieving the unique selectivity for the Cu²⁺ ion, including the suitable coordination conformation of the Schiff-based receptor, the nitrogen(oxygen)-affinity character of the Cu²⁺ ion, and the amide deprotonation ability of the Cu²⁺ ion. The fluorescence quenching of naphthalimide moiety by Cu²⁺ ion could be ascribed to a PET (photo-induced energy transfer) mechanism and/or a d-d electron paramagnetic quenching mechanism [32].

The evidence of complexation behavior of L (C₄₄H₄₂N₆O₅) with Cu²⁺ came from ESI-MS spectra. As shown in Fig. S13, upon the addition of 2 equivalents of Cu²⁺ in L in CH₃CN:HO (4:1, v/v) solution, on the one hand, the peak at m/z = 798.5848 corresponding to [Cu(C₄₄H₄₂N₆O₅)]⁺ was observed. The isotopic patterns fit very well with the isotope distribution patterns calculated by using the IsoPro 3.0 program for [Cu(C₄₄H₄₂N₆O₅)]⁺. On the other hand, a peak at m/z = 961.5223 corresponding to [Cu₂(C₄₄H₄₀N₆O₅)(ClO₄)]⁺ was observed and the isotopic patterns fit very well with the isotope distribution patterns calculated (Fig. S14).

To investigate the effect of anions on the probe, the UV–vis and fluorescence titration experiments of L (20 μM) in CH₃CN:H₂O (4:1, v/v) solution upon addition of increasing concentrations of Cu(NO₃)₂, CuCl₂ and Cu(OAc)₂ were also performed, respectively. Compared with Cu(ClO₄)₂, the addition of Cu(NO₃)₂, Cu(OAc)₂ or CuCl₂ gave rise to similar responses in UV–vis and fluorescence titration spectra (Figs. S15–S20). However, it was required approximately 3 times Cu(OAc)₂ to reach the balance in fluorescence titration (Fig. S18). The possible reason might be ascribed that Cu(OAc)₂ was a weak electrolyte. In general, the vast majority of perchlorates were soluble in water, organic solvents and even their mixed solutions. In addition, the coordination ability of perchlorates was very weak due to the steric hindrance and charge dispersion, thus the complexation behavior between the ligand (probe) and metal ions would hardly be disturbed by perchlorate anions. Therefore, perchlorates were preferred in our research.

For the biological application of the fluorescence probe, the sensing should be operated in a wide range of pH. Fig. S21 showed that the suitable pH range for Cu²⁺ determination is pH 3–9 where the fluorescence “on-off” could be operated by the copper ion binding. Consequently, our present Cu²⁺-selective receptor would be an ideal probe for monitoring physiological Cu²⁺.

The ability of biosensing molecules to selectively monitor guest species in living cells is of great importance for biological application [33]. Fluorescence images of MCF-7 cells were observed under a Nikon eclipse TE2000-5 inverted fluorescence microscope with a 20 × objective lens (Fig. 4). MCF-7 cells incubated with L (10 μm) for 30 min in 0.01 M PBS buffer showed a clear green intracellular fluorescence (Fig. 4, a), which suggested that L was cell permeable. After stained with L for 30 min and rinsed with PBS three times, MCF-7 cells were supplemented with 10 and 20 equivalents Cu²⁺ for another 30 min, respectively. A partial quenching of the green fluorescence intensity (Fig. 4, b, 10 equiv. Cu²⁺) and remarkable decrease fluorescence intensity (Fig. 4, c, 20 equiv. Cu²⁺) were observed. The cells remained viable and showed no apparent toxicity and side effects throughout the imaging experiments (about 3 – 4 h). These experiments indicated that L can detect intracellular Cu²⁺ ions. Therefore, it could be a useful molecular probe for investigate biological processes involving Cu²⁺ ions within living cells.

4. Conclusions

In conclusion, a new naphthalimide-based fluorescent probe L by the combination of 6-hydrazino-benzojdeisosquinoline-1,3-diones with 4-tetetyl-2,6-diformylphenol was developed. L exhibited significant changes of optical property and excellent selectivity toward Cu²⁺ over other metal ions under physiological conditions. The Benesi–Hildebrand analysis and ESI-MS spectra results indicated a 1:2 stoichiometry of L/Cu²⁺ complex. The fluorescence probe L had a detection limit of ppb scale. Preliminary fluorescence microscopy experiments showed that L has practical application in living cells and further investigations on its applications in life science are still underway.

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Fig. 4. Fluorescence images of MCF-7 cells incubated with L (20 μM) and further incubated with addition of various concentrations of Cu(ClO$_4$)$_2$ [(a) 0, (b) 10, and (c) 20 equiv., respectively] and their corresponding bright field transmission image [(d) 0, (e) 10, and (f) 20 equiv., respectively].

References

[31] L. H NMR (DMSO-d6, ppm): 8.73 (s, 1 H), 8.71 (d, 2 H), 8.30 (d, 2 H), 7.76 (q, 2 H), 7.72 (s, 2 H), 7.40 (d, 2 H), 3.58 (t, 4 H), 1.59 (m, 4 H), 1.42 (s, 9 H), 1.36 (m, 4 H), 0.95 (t, 6 H).
[32] C NMR (DMSO-d6, ppm): 163.45, 162.76, 162.74, 153.15, 143.29, 133.26, 130.63, 128.98, 128.03, 125.58, 124.92, 120.14, 113.76, 111.34, 106.20, 100.40, 33.79, 31.03, 29.60, 19.59, 13.44. API-MS: m/z: 737.3 for [L + H$^+$], m/z: 759.3 for [L + Na$^+$]. Anal. Calcd. for Cu$_2$Na$_2$O$_7$: C, 71.72; H, 6.02; N, 11.40%. Found: C, 71.63; H, 6.09; N, 11.31%.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2015.10.022.

Appendix A. Supplementary data

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