A ratiometric and exclusively selective Cu\textsuperscript{II} fluorescent probe based on internal charge transfer (ICT)

Xiufu Chen, Jingyun Wang, Jingnan Cui*, Zhaochao Xu, Xiaojun Peng*

State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China

1. Introduction

The development of highly selective and ratiometric fluorescent probes capable of reporting transition—metal ions has attracted considerable attention.\textsuperscript{1} In particular, the design of ratiometric fluorescent sensors for copper ions in the presence of a variety of other metal ions is actively investigated.\textsuperscript{2–5} As is well-known, copper is a vital trace element, the third most abundant in humans, and is present at low levels in a variety of cells and tissues with the highest concentrations in the liver.\textsuperscript{6} Cu\textsuperscript{II} plays an important role in living systems, such as those occurring in the human nervous system, gene expression, and the functional and structural enhancement of proteins.\textsuperscript{7} Therefore fast detection of Cu\textsuperscript{II} in aqueous solution or biosystems is significant for life sciences and environmental sciences. However, design and synthesis of excellent fluorescent probes for selective detection of transition metal ions in biosystems remain a great challenge, because they often coexist and most of them have a similar reactivity with common fluorescent probes. To achieve Cu\textsuperscript{II}—only ratiometric sensing in aqueous solution or biosystems, fluorescent probes require deliberate design.

1,8-Naphthalimide with an electron donor and an acceptor (EDA) group is characteristic of an internal charge transfer (ICT) chromophore.\textsuperscript{4} In our previous study, two 2-(aminomethyl)pyridine ligands had been introduced to 4 and 5 positions of 1,8-naphthalimide (Fig. 1, A),\textsuperscript{4} forming a tetradeutate receptor, which displayed a special cavity and had a strong binding with Cu\textsuperscript{II}. As a result ratiometric fluorescence responses based on ICT mechanism were obtained. Soon afterward, it was observed that the amines conjugated to 1,8-naphthalimide can be deprotonated in the presence of Cu\textsuperscript{II}, which resulted in the color change from primrose yellow to pink (probe B).\textsuperscript{4b} But some disadvantages exist in these two probes, probe A is not a highly selective fluorescent sensor, it is interfered by other metal ions, such as Co\textsuperscript{II}, Fe\textsuperscript{II}, Ni\textsuperscript{II}, Ag\textsuperscript{I}; probe B has a lower sensitivity for Cu\textsuperscript{II} and poor fluorescent quantum yield because of the PET from aniline to fluorophore. Recently, Xu et al. have shown that the addition of a carbonyl group between 1,8-naphthalimide and di-2-picolylamine (probe C)\textsuperscript{8} demonstrated the ability to not only block heavy and transition metal (HTM) ions from interacting with the naphthalimide but also increase the oxidation potential of naphthalimide and to act as a sacrificial donor in order to maintain fluorescence.\textsuperscript{9} For probe 1 in this work, two 2-amino-N-phenylacetamide ligands were introduced to 4 and 5 positions of 1,8-naphthalimide bases on probe B for the high selectivity with the similar tetradeutate receptor, the addition of a carbonyl group at the side of aniline not only provides more Lewis basic binding site but also reduces the steric hindrance by avoiding the interaction of two aniline groups. Besides, it repressed PET from aniline to fluorophore, thus increasing the fluorescent quantum yield.

2. Results and discussion

2.1. Synthesis

Probe 1 synthesized by conjugating compound 2 and 3 was shown in Scheme 1. The intermediate compound 2 was synthesized...
from acenaphthene following a literature procedure.  

2-Azido-N-phenylacetamide was prepared in 90% yield by the condensation of 2-bromine-N-phenylacetamide with sodium azide and was subsequently reduced into compound 3 in 100% yield, probe 1 was easily synthesized by conjugating compound 2 and compound 3 in 83.5% yield.


2.2. The effect of pH

Fluoroionophores are usually disturbed by a proton in the detection of metal ions. Thus, the influence of pH on the fluorescence of 1 was first determined by fluorescence titration (Fig. 2). The fluorescence of 1 at 521 nm remains unaffected between pH 12.56–1.99 and rapidly decreases from pH 1.99 to 0.96 and pH 12.56 to 13.04, leading to a vaulted curve. The fluorescence quenching was most likely caused by the photoinduced electron transfer (PET) from the fluorophore to protonated aniline in strong acids condition, which was similar to the findings of de Silva in the design of an ‘off-on-off’ fluorescent PET sensor. And PET from deprotonated aniline to the fluorophore in strong alkali condition. Therefore, further fluorescence studies were carried out at pH 7.4 maintained with HEPES buffer (30 mM).

2.3. Optical behavior of 1 with Cu²⁺

The emission spectra of 1 and its fluorescence titration with Cu²⁺ were recorded in an ethanol–water solution (90:10, v/v) (Fig. 3), and the emission spectrum of free 1 displays a broad band with a maximum at 521 nm. When Cu²⁺ was added to the solution of 1, the fluorescence emission intensity at 521 nm decreased significantly and a blue-shifted emission band centered at 471 nm showed up, which was attributed to the formation of a 1/Cu²⁺ complex. The inset in Fig. 3 exhibits the dependence of the intensity ratios of emission at 471 nm to that at 521 nm (I₄₇₁/I₅₂₁) on the concentrations of Cu²⁺, which indicates the formation of a 1/Cu²⁺ adduct of 1:1 stoichiometry. The Φₑ values of free 1 and 1/Cu²⁺ adduct (1:1) are 0.5378 and 0.1138, respectively. In the UV–vis absorption spectra of 1, no significant changes was found with the addition of Cu²⁺. This would indicate that the blue shift of fluorescence spectra was caused by a change of the charge-transfer character of the emissive species.

Fig. 2. Influence of pH on the fluorescence of 1 in the ethanol–water solution (90:10, v/v). Excitation wavelength is 447 nm, [1] = 10 μM. The pH was modified by adding 75% HClO₄ or 25% N(CH₃)₄OH.

Fig. 3. Fluorescent emission spectra of 1 in the presence of different concentrations of Cu²⁺(0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 50 μM) in ethanol–water solution (90:10, v/v, 30 mM HEPES buffer, pH 7.4). Excitation wavelength was 447 nm, and emission was at 471 and 521 nm. The concentration of 1 was 10 μM. Inset: ratiometric calibration curve I₄₇₁/I₅₂₁ as a function of Cu²⁺ concentration.
The fluorescence titration of 1 with various metal ions in ethanol–water solution was conducted to examine the selectivity. As shown in Fig. 4b, the addition of Cu^{2+} induced a selective increase in emission band at 471 nm. The addition of other metal ions, such as Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Ag^+, Hg^{2+}, Pb^{2+}, Ce^{3+}, and Ba^{2+}, produced a negligible change in the fluorescence spectra of 1. Fig. 4a shows the dependence of the intensity ratios \( \frac{I(475)}{I(525)} \) on the metal ions. The competition experiments were conducted in the presence of Cu^{2+} at 10 \( \mu \)M mixed with other metal ions at 30 \( \mu \)M, as well as in a mixture of the metal ions, respectively, no significant variation in the intensity ratios \( \frac{I(475)}{I(525)} \) was found by comparison with that without the other metal ions besides Cu^{2+} (Fig. 4c). Therefore, in aqueous solutions, probe 1 is an outstandingly high selectivity fluorescent sensor for Cu^{II}.

Proposed mechanism of binding mode of probe 1 with Cu^{2+} is shown in Fig. 5, which is similar to probe A’s binding way. Probe 1 bases on probe B for the high selectivity with the similar tetradentate receptor, but the addition of a carbonyl group at the side of aniline lead to forming a tetradentate receptor site with the carbonyl oxygen donors, which provides the more Lewis basic competition between M and F (fluorescence). Also, the amide linkage can increase the in flexibility of the receptor to reduce the steric hindrance by avoiding the interaction of two aniline groups. The capture of Cu^{2+} by the tetradentate receptor resulted in the reduction of the electron-donating ability of the two amino groups conjugated to the naphthalene ring, thus, the receptor showed a 50 nm blue shift of fluorescence emission base on the internal charge transfer (ICT).

2.4. Cell imaging of 1 with Cu^{II}

The utility of probe 1 for fluorescence imaging of Cu^{2+} in living cells was investigated. To determine the cell permeability of 1, MCF7 cells were incubated with 10 \( \mu \)M 1 for 30 min at 37 °C and washed with PBS to remove the remaining 1. A clear yellow fluorescent image could be observed obviously from fluorescence microscopy as shown in Fig. 6c. In Fig. 6d, the cells were pretreated with Cu^{2+} in the growth medium for 30 min. The cells were then washed with PBS to remove the remaining Cu^{2+} and further incubated with probe 1 for 30 min. The resulting bright green fluorescence image demonstrates that probe 1 is cell membrane permeable and able to display a fluorescence blue-shift response to Cu^{2+} in the living cells. It should be potentially useful for the study of the toxicity or bioactivity of Cu^{2+} in living cells.
3. Conclusions

Probe 1 was designed with the extra carbonyl group based on strategies that not only provides more Lewis basic binding site but also reduces the steric hindrance by avoiding the interaction of two aniline groups, and we have demonstrated that probe 1 displayed colorimetric response with fluorescence spectra from yellow to green, which was useful for easy detection of CuII with a ratio-metrically and exclusively selectivity in the presence of a variety of other metal ions in aqueous solutions. And especially, probe 1 can be used to successfully detect CuII in cultured cells with the same fluorescence change. Blue shift emission (50 nm) was attributed to the reduction of the electron-donating ability of the two amino groups conjugated to the naphthalene ring. The design strategy of the sensor with the addition of a carbonyl group will help to improve the development of fluorescent sensors for detect other metal ions.

4. Experimental

4.1. Materials and methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. 1H NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as parts per million (in CDCl3/DMSO-D6). Mass spectra were measured on an HP 1100 LC−MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Nexus 770 spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-20. Fluorescence spectra were determined on a Hitachi F-4500. Absorption spectra were determined on a PGENERAL TU-1901 UV−vis Spectrophotometer.

4.2. Preparation of fluorometric metal ion titration solutions

All the solvents were of analytic grade and used as received. The solutions of metal ions were prepared from LiClO4·3H2O, NaClO4, KClO4, BaCl2·2H2O, CaCl2, FeCl3·4H2O, MnCl2·4H2O, CoCl3·6H2O, NiCl2·6H2O, ZnCl2, CdCl2·1/2H2O, HgCl2, AlCl3, FeCl3·6H2O, AgNO3, Pb(NO3)2, Mg(NO3)2·2H2O, Cu(NO3)2·3H2O, Ce(NO3)3·6H2O, Cr(NO3)3·9H2O, respectively, and were dissolved in distilled water. Stock solutions of host (0.01 M) in DMSO were also prepared. Test solutions were prepared by placing 4−40 mL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 4 mL with 0.3 M HEPES (pH 7.4). For all measurements, excitation was at 447 nm. Both excitation and emission slit widths were 3 nm or 5 nm.

4.3. Synthesis of 2-amino-N-phenylacetamide 3

2-Bromine-N-phenylacetamide (1.07 g, 1.0 equiv) was added to a reaction vessel containing a solution of NaN3 in DMSO (1.1 equiv, 0.5 M). The reaction was monitored by NMR analysis. Upon completion of the reaction, water (50 mL) was added and the product was extracted with ether (3×50 mL). The combined organic layers were washed with water (2×50 mL) and brine (50 mL) and dried with natrium sulfate. The organic solvent was removed to provide 2-azido-N-phenylacetamide as a colorless liquid in 90% yield. 1H NMR (400 MHz, CDCl3) δ 8.00 (bd s, 1H), 7.54 (d, J=8.6 Hz, 2H), 7.35 (dd, J1=8.4 Hz, J2=7.5 Hz, 2H), 7.16 (d, J=8.6 Hz, 1H), 4.15 (s, 2H).15

A solution of 2-azido-N-phenylacetamide (5 mmol) in 40 mL of methanol was hydrogenated over 3.5% Pd/C (300 mg) in autoclave (1 Mp) for overnight, and starting material disappeared, then catalyst was removed by filtration through Celite and washed with 5 mL of methanol. The filtered solution was concentrated by rotary evaporation to give 2-amino-N-phenylacetamide 3 as colorless liquid (100% yield). 1H NMR (CDCl3, 400 MHz) δ 1.69 (s, N−H), 3.32 (s, 2H), 7.03 (t, J=7.2 Hz, 1H), 7.27 (t, J=7.6 Hz, 2H), 7.55 (d, J=8.0 Hz, 2H), 9.45 (s, N−H); 13C NMR (CDCl3, 100 MHz) δ 45.12, 119.58, 124.05, 128.90, 137.80, 171.46; IR (KBr, cm−1): 3320, 3275, 3058, 2915, 1655, 1600, 1498, 1447, 1313, 1076, 755, 692; HRMS (ES) calcd for C16H13N3O3 [M+H]+ 278.0957, found 278.0956.

4.4. Synthesis of probe 1

2-Amino-N-phenylacetamide 3 (7.72 mmol) was added dropwise to a solution of 145 mg (0.38 mmol) N-butyl-4-bromo-5-nitro-1,8-naphthalimide 2 and 128 μL (2.0 equiv) N,N-disopropylethylamine (DIPEA) in 3 mL 2-methoxyethanol, and then the mixture was heated to reflux for 3 h and monitored by TLC. After the reaction was completed, the solution was cooled at room temperature to give yellow needle crystals. The product was filtered off, washed with 2-methoxyethanol, and then dried in the air in 83.5% yield (177 mg). Mp: 276.2−278.4 °C; 1H NMR (DMSO-d6, 400 MHz) δ 0.90 (t, J=7.2 Hz, 3H), 1.28−1.34 (m, J=7.6 Hz, 2H), 1.54−1.57 (m, J=7.6 Hz, 2H), 3.98 (t, J=7.2 Hz, 2H), 4.21 (s, 4H), 6.75 (d, J=8.8 Hz, 2H), 7.07 (t, J=7.2 Hz, 2H), 7.32 (t, J=8.0 Hz, 4H), 7.59 (d, J=7.6 Hz, 4H), 7.71 (s, N−H), 8.25 (d, J=8.4 Hz 2H), 10.22 (s, N−H); 13C NMR (DMSO-d6, 100 MHz) δ 14.23, 20.29, 30.35, 40.03, 48.20, 107.20, 110.73, 110.86, 119.99, 124.06, 129.26, 132.19, 133.66, 139.03, 152.67, 163.75, 168.15; IR (KBr, cm−1): 3342, 2956, 2872, 1666, 1626, 1600, 1557, 1499, 1446, 1314, 1107, 748, 691; HRMS (EI) calcd for C12H13N3O4 [M]+ 549.2376, found 549.2368.

Acknowledgements

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References and notes


13. Fluorescence quantum yields (FF) were estimated with N-butyl-4-butylamino-1,8-naphthalimide in absolute ethanol as a standard (FF = 0.81) Guo, X.; Qian, X.; Jia, L. J. Am. Chem. Soc. 2004, 126, 2272.
