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A lipid droplet-targeted fluorescence probe for visualizing exogenous copper (II) based on LLCT and LMCT

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ABSTRACT

A simple and sensitive probe, pyridine-based Schiff base (PBSB), is prepared based on the previous studies, which shows a rapid response to copper (II) through a fluorescence enhancement process. On association with copper (II), PBSB immediately presents a large red-shift (130 nm) in emission spectrum simultaneously accompanying with increasing emission intensity. The mechanism and binding sites of PBSB detecting copper (II) have been established by theoretical calculations and ^{1}HNMR study, respectively. More importantly, in the complex biological system, PBSB having an excellent membrane permeability and a great photostability can visualize copper (II) in lipid droplet by bio-imaging, which display so great promising advantages in the issues causing by disorder of copper ions that could be convenient for prevention and control problems with human health and complex bio-system.

1. Introduction

With the progress of materials science and life science, more and more scientists are attracted to take part in explorations of subcellular organelle. Particularly, for lipid droplet – a dynamic organelle, which are formed and degraded, move inside cells and also may undergo fusion [1]. More importantly, lipid droplets are most abundant in adipose tissue, where stored triacylglycerol provides the primary energy reserve for the organism [2]. In addition, there are evidences indicating that copper deficiency are linked with various cellular processes including apoptosis, aging, and differentiation, which would trigger lipid droplet dysfunctions causing extensive diseases such as fatty liver diseases, hyperlipidaemia and type II diabetes [3–5]. On the other aspect, intracellular copper, mostly as monovalent Cu (I) than divalent Cu (II), is not free but bound to various transporters, chaperones and small molecules, which have the difficulty in detecting intracellular Cu^{2+}. That is what urgent to contribute our attention to develop a novel way for rapidly detecting divalent Cu (II) in lipid droplet.

Fluorescent methods have been used for detecting Cu^{2+} in recent years [6,7]. Specially, fluorescence probes taking advantage of specificity, high sensitivity and simple instrumentation for general imaging of Cu^{2+} in living systems have been proposed [11], to our surprise, it is extremely rare that the above-mentioned copper (II) probes which can be used for specific imaging in lipid droplet. And that, most of these reported conventional copper ions probes are based on quenching of fluorescence signals due to its paramagnetic nature [8–10], meanwhile they process some weakness, which couldn't obtain accurate concentration, eliminate light collection efficiency and evaluate systematic errors created by the sample environment. As a consequence, that is an opportunity to devise a turn-on probe targeting lipid droplet for visualizing copper (II) in view of these requirements [12–17].

Fortunately, a new compound pyridine-based Schiff base PBSB (Scheme 1 for the chemical structure) was synthesized, in PBSB, triphenylamine as a basic skeleton provided rich charge and exhibited great optical property, also the pyridine moiety was introduced for coordinating with metal ions, and ultimately through a flexible "C = N" moiety linking them together. In the spectrogram of PBSB, the fluorescence intensities presented rapidly tremendous change on 449 nm and 579 nm before and after copper ion's appearance. Namely, PBSB showed a fast response to copper (II) through a fluorescence enhancement process at 579 nm. Meanwhile, the biological assays displayed that PBSB had a well photostability, excellent membrane permeability

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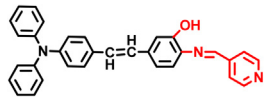

Scheme 1. The structure of PBSB.

and could detect exogenous Cu^{2+} in lipid droplet by bio-imaging.

2. Experimental section

We had provided the routes of intermediates with consulting previous methods [18]; **PBSB**: dissolved 4-pyridine aldehyde (0.074 g, 1.586 mmol) in 35 mL ethanol solution and dropwise added into 30 mL ethanol solution of **M** (0.40 g, 1.184 mmol) (Figure S). At room temperature, stirred the mixed solution and then red solid precipitated gradually after 0.500 h. Next, filtrated solid after reaction finished and recrystallized with ethanol to produce 0.240 g solid. The yield was 86.2%. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.30 (s, 1 H),8.86 (s, 1 H), 8.72 (J = 4.00, d, 2 H), 7.98 (J = 4.00, d, 2 H), 7.54 (J = 8.00, d, 2 H), 7.16–7.36 (m, 5 H), 7.04–7.15 (m, 9 H), 6.95–6.97 (J = 8.00, d, 2 H) 5.77 (s, 1 H),.^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 156.17, 152.26, 150.25, 146.90, 146.77, 142.99, 138.07, 135.55, 131.22, 129.56, 127.68, 126.39, 124.14, 123.28, 122.89, 122.29, 119.48, 117.99, 113.70. FT-IR (KBr, cm^{-1}): 3380.32 (m), 3018.50 (w), 1609.83 (s), 1521.73 (w), 1489.94 (w), 1433.48 (m), 1327.18 (m), 1273.02 (m), 1178.32 (s), 956.81 (s), 828.63 (m), 752.39 (m), 695.09 (m), 617.97 (s), 539.97 (s), 494.46 (s). MS (ESI): calcd for [M]$^+$: 468.2013, found, 468.2078.

3. Results and discussion

3.1. Recognition ability toward copper ions in vitro

Firstly, selectivity behavior were evaluated by adding equal quantity's different metal ions into common solutions of PBSB, at room temperature after heavily screening, in acetonitrile PBSB presented a weak fluorescence under excited at 396 nm in Fig. 1; then adding 1.5 equiv. physiologically and environmentally vital anions, biomolecules and metal ions such as F^-, Cl^-, S^{2-}, CO_3^{2-}, HCO_3^-, HPO_4^{2-}, ClO_4^-, Br^-, SO_4^{2-},HSO_4^-, Ni^{2+}, Hg^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Pb^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Ba^{2+}, Ag^+, Li^+, K^+, Na^+ and Arg, Ser, Phe, Val, Tyr, Gly, ILe, Asp, Hcy, Ala, Thr, Lys, Leu, Cys, Gsh, there were no apparent change. And when trivalent cations (Fe^{3+}, Al^{3+}, Cr^{3+}) were added into it, there was also no distinct fluorescence intensity change at 579 nm (Fig. S5), so do Cu^+ (Fig. S15). However, under the same condition, when added

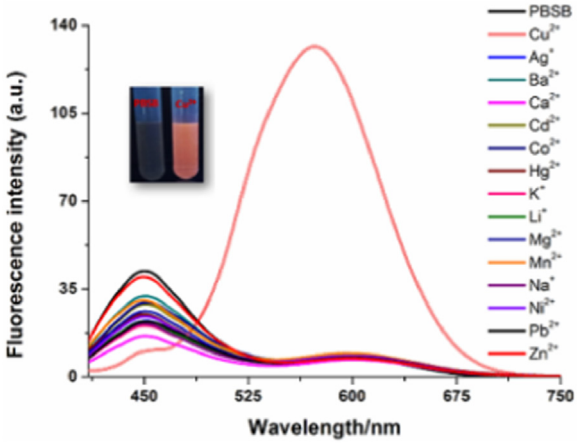

Fig. 1. Fluorescence spectra of PBSB (10 μM) in acetonitrile upon addition of 1.5 equiv. various nitrate salts. Inset: color of PBSB and PBSB + Cu^{2+} under UV lamp at 365 nm.

metal ion was copper (II), it would have a dramatic difference that **PBSB** for Cu^{2+} at about 579 nm displayed a noteworthy increase of fluorescence intensity. At the same time, the fluorescence lifetime was increased from $2.35 * 10^{-10}$ to $9.40 * 10^{-10}$ s, the fluorescence quantum yields was from almost nothing to 0.49% ($C_{(Cu)^{2+}}$ = 15 μM). Moreover, we also carried out the interference assay which could make further exploration on selectivity of PBSB. The results revealed that PBSB could detect copper (II) without other metal ions' interference (Fig. S6). Meanwhile, the reversibility experiments indicated a recyclable coordination between **PBSB** and Cu^{2+} (Fig. S14).

The second step is to evaluate the sensitivity of probe **PBSB**. Firstly, kinetic curves of **PBSB** reacting with Cu^{2+} at varying concentrations were provided in Fig. S9, the result demonstrated that fluorescence increase of **PBSB** fast arrived in plateau about 1.5 min, namely **PBSB** could realize a quick response for Cu^{2+}. And then the **PBSB** were titrated by various concentrations of Cu^{2+}, as shown in Fig. 2, upon excitation at 396 nm, the free **PBSB** mainly at 449 nm showed a emission band, according to the density functional theory (DFT) calculation in Fig. 3, it presented an intramolecular charge transfer (ICT) from triphenylamine group to pyridine group in **PBSB** (the calculated λ_{em} = 447 nm), which is in good agreement with experimental emission spectra. In contrast, the appearance of Cu^{2+} could heavily decrease the fluorescence intensity at 449 nm and concurrently a new red-shifted emission band at 579 nm was also arising, ascribed to the ligand-to-ligand charge transfer (LLCT) and ligand-to-metal charge transfer (LMCT) from **PBSB** to **PBSB** and **PBSB** to Cu^{2+} based on the DFT calculation result (the calculated λ_{em} = 578 nm), which also confirmed with the experimental emission spectra. Notably, when excited at 396 nm, there were titration variations of two emission band at low and relatively high concentration of Cu^{2+}, which would be more advantageous than single emission band, especially, the large red-shifted emission band to 579 nm in the visible region is easy to detect Cu^{2+} simply by naked eye (Fig. S14). Additionally, the association constant between **PBSB** and Cu^{2+} was calculated in Fig. S8, and the detection limit (DL) of PBSB to Cu^{2+} was $3.4 * 10^{-6}$ M (R^2 = 0.9785) (Fig. S7).

Next, in Fig. 4, we utilized **PBSB** (0.01 M , DMSO-d_6) with Cu^{2+} (0.1 M, DMSO-d_6) as analytes to complete ^{1}H NMR titration assays for further exploring the binding mechanism between probe **PBSB** and Cu^{2+}. Interestingly, with addition of Cu^{2+}, the -OH signal (H_a) of probe **PBSB** was enhanced at first and then gradually weaken, which could attribute to the hydrogen-atom of hydroxy (-OH) group and the nitrogen-atom of -C=N- together formed an intramolecular hydrogen bond firstly, then appearance of copper ion with electron-withdrawing interaction could decrease the electron density of N (-C=N-), which would cut off the formed hydrogen bond leading to the increase of Ha, at the same time, there was a sharply decrease of Hb, Hc, Hd, He, it might be ascribed to that Cu^{2+} gradually coordinated with the N atom of 4-pyridine moiety and its intense chemical shielding interaction could directly shield the H signal near the N atom of 4-pyridine moiety, meanwhile, the signal of Hg was gradually weakened. Here, we also observed that all the signals broaden with addition of Cu (II), and the benzene ring area had a slightly shift towards up-field. For the phenomena might be caused by the diamagnetic Cu (II) and its low-spin complex Cu-**PBSB**. Based on all of above results, Job's plot (Fig. S10) and association constant between, a plausible binding mode could be proposed in Fig. S11.

3.2. Recognition ability toward copper ions in vivo

Considering **PBSB's** promising properties in vitro, we also attempt to explore its ability of fluorescence imaging application in vivo by using HeLa cell model.

3.3. Imaging of PBSB in biological

In vivo assays, cytotoxity test firstly required to be preformed by

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