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Preparation, regulation and biological application of a Schiff base fluorescence probe

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ABSTRACT

A facile fluorescence switch with Schiff base units was designed and achieved by nucleophilic addition and dehydration reaction. The fluorescence of the probe can be regulated by metal ions (Al^{3+} and Cu^{2+}). The whole process shows that the weak fluorescence of the probe enhances with the addition of Al^{3+}, and then the strong fluorescence of the probe/Al^{3+} ensemble reduces by introducing Cu^{2+}. Meanwhile, the solution color changes of the probe with metal ions can be observed under 365 nm UV–vis light from weak light, pale green, green, pale green to weak light. Noticeably, the photo regulation processes of the probe by metal ions can be realized in the biological system and applied in cells imaging. The work provides a new strategy for designing facile regulation probe and develops a new application for Schiff base derivatives.

1. Introduction

Fluorescence probes based on Schiff base units have been developed and applied in molecular recognition field in recent years [1–5]. Many researches display that an ideal fluorescence probe depends on the facile design and matched bonding space for various analytes [6–10]. Compared with transition metal ions, to realize excellent detection for Al^{3+} ion is hard because of its poor coordination ability and spectroscopic characteristics [11–14]. Fortunately, the development and application of Schiff base compounds with N and O as hard-base donor sites provides a facile pathway for determining Al^{3+} with high sensitivity and selectivity. To date, many well-property probes with Schiff bases units for Al^{3+} have been obtained and applied in analysis and testing fields [15–19]. These studies mainly focused on improving the parameter values of sensitivity and selectivity of the probes for Al^{3+}. Meanwhile, a more meaningful aspect for these probes was ignored. We believe that the development of a probe with regulation and control property through different metal ions will be more important and meaningful because of its wide applied range and facile switch function in biological field.

In order to achieve regulation probes by metal ions, it is indispensable for designing and preparing a probe with high recognition property for one metal ion. In the aspect, Liu and coworkers reported a simple Schiff base probe for Al^{3+} in 2011 [19]. Though the probe (HBSB, Scheme 1) with a very simple structure, it displays higher selectivity and sensitivity for Al^{3+}. For the speculated binding action, they think that the hydroxyl groups in 4-position of benzene ring do not take part in combining with Al^{3+}. However, we design and synthesize a similar probe MBSB (Scheme 1), which is only a difference in hydroxyl group changing to methoxy group for 4-position of benzene ring. Unexpectedly, the MBSB probe has no response for Al^{3+} detection. Based on the fact, we think that all of the hydroxyl groups of HBSB participate in the binding action with Al^{3+}. And, multiple heteroatoms (N or O) in the probe are favorable for occurring effective bonding with Al^{3+}. Herein, we design and synthesize a new Schiff base probe (HMBSB, Scheme 1) with two = N–NH bonds, in which –N–H groups are used to bind Al^{3+}. As expected, due to forming six-membered ring and then leading to excited-state intramolecular proton transfer, the probe has very weak fluorescence [20–22]. With introduction of Al^{3+} to the probe, it shows an obvious fluorescence "turn-on" response. It's worth noting that the bright fluorescence of HMBSB/Al^{3+} ensemble can be quenched by Cu^{2+}. In this case, a facile regulation probe by Al^{3+} and Cu^{2+} ions is realized. The probe not only acts as a chemosensor for Al^{3+}, but also is a photo regulation switch, which is desired for applying in cells imaging.

2. Experimental

2.1. Materials and instruments

Unless otherwise stated, all chemical reagents were obtained from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods prior to use. Terephthaloyl dichloride, hydrazine (85%), 2-hydroxy-4-

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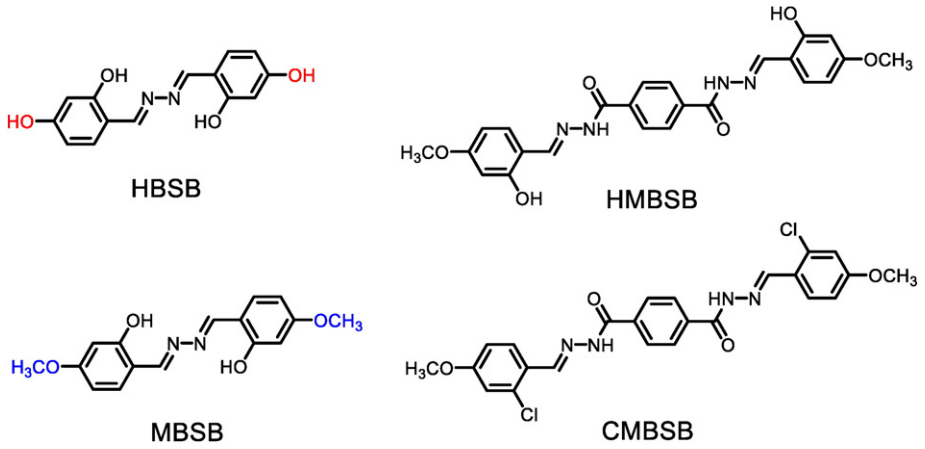

Scheme 1. The chemical structures of four Schiff base compounds.

methoxybenzaldehyde and 2-chloro-4-methoxybenzaldehyde were purchased from Aldrich (Steinheim, Germany). Metal ions both were nitrates and were provided from Alfa Aesar (Tianjin, China). ^{1}H NMR and ^{13}C NMR were measured on a Bruker ARX400 spectrometer with chemical shifts reported as ppm (TMS as an internal standard). High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. Fluorescence spectra were acquired with a Hitachi F-4600 fluorescence spectrophotometer, the excitation and emission slit widths both were 5.0 nm. Confocal laser scanning microscopy (CLSM) imaging was taken on a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan).

2.2. Fluorescence measurements

All experiments were in mixture solvents (DMSO/H_2O = 6/4, v/v). The stock solutions (1.0×10^{-3} M) of these compounds were diluted in 1.0 L measuring flask with DMSO/H_2O to afford the working solution (1.0×10^{-5} M). The stock solutions of metal ions were both 1.0×10^{-3} M. The standard stock solutions of lower concentrations were prepared by suitable dilution of the stock solution. All spectra analysis studies were carried out in a quartz cuvette with 1 cm path. The total volume of working solutions is 2.0 mL. The excitation wavelength was set in 375 nm according to experimental requirements. All of the experiments were performed at room temperature.

2.3. Synthesis of the compounds

A solution of 2.03 g terephthaloyl dichloride (0.01 mol) in 20 mL THF was slowly added to 20 mL THF with 10 mL hydrazine (85%) at room temperature. The mixture was stirred for 5 h at the room temperature. Then, the mixture was poured into 200 mL iced water and extracted three times with dichloromethane (3 × 100 mL). The obtained organic phase was washed by redistilled water (2 × 100 mL) and dried by anhydrous sodium sulfate. The organic solvent was removed and obtained terephthalohydrazide. Then, 0.76 g 2-hydroxy-4-methoxybenzaldehyde was added to a solution of 0.97 g the above terephthalohydrazide (0.05 mol) in 20 mL ethanol and the mixture was stirred for 12 h at 60 °C. The separated solid was filtered, washed and dried and then gave the product N^1,N^4-bis[(*E*)-2-hydroxy-4-methoxybenzylidene]-terephthalohydrazide (HMBSB) in 58.6% yield. ^{1}H NMR (*d*-DMSO, 400 MHz) δ 12.31 (b, 1H), 11.97 (b, 1H), 10.82 (s, 2H), 8.87 (s, 2H), 8.11 (m, 4H), 7.57 (d, 2H), 6.58 (m, 4H), 3.80 (s, 6H); ^{13}C NMR (*d*-DMSO, 100 MHz) δ 168.65, 165.92, 164.87, 137.51, 134.82, 132.97, 116.59, 112.34, 106.18, 60.69; EI-MS ($C_{24}H_{22}N_4O_6$, 462, m/z) 463 [M + 1].

The detail synthesis routes of these compounds were showed in Scheme S1. The MBSB compound was prepared according to the reported literature [23]. The CMBSB was synthesized according to the HMBSB method and these characterization data were displayed in supplementary data.

2.4. Cells culture and imaging

Under a humidified atmosphere containing 5% CO_2, SiHa cells were grown in DMEM medium containing 10% FBS routinely, then harvested for subculture using trypsin (0.05%, Gibco/Invitrogen) at 37 °C. SiHa cells were subculture onto a 35 mm × 35 mm Petri dish with a glass bottom, then allowed to grow for 24 h for attachment, after which 1 mL of DMEM medium containing 10% 20 μM HMBSB was used to incubate the SiHa cells at 37 °C for 5 h. The medium was replaced and phosphate-buffered saline (PBS, pH = 7.4) was used to wash the cells thrice. And different equivalent metal ions in PBS buffer solution were added into the dish and the cells were cultured at 37 °C for 1 h. The medium was replaced and phosphate-buffered saline (PBS, pH 7.4) was used to wash the cells thrice. Then fresh medium with cytoplasm located dye (Lyso tracker red) was added and incubated. After washing thrice with PBS, the images of the cells were recorded on confocal laser scanning microscopy.

3. Results and discussion

3.1. The fluorescence properties and bonding processes of the probes with Al^{3+} ion

Based on the previously reported result [19], the type probes usually have low fluorescence emission because of the six-ring formation and excited-state intramolecular proton transfer. Hence, the excitation and emission spectra of HMBSB are 310 nm and 420 nm with low fluorescence intensity, respectively (Fig. 1). When introducing Al^{3+} to HMSBS in DMSO/H_2O, the fluorescence excitation and emission spectra take obvious changes. An obvious red-shift from 420 nm to 495 nm of emission peak was observed with the increase of Al^{3+} (Fig. S1). Corresponding, a new maximum excitation peak at 375 nm also was investigated (Fig. 1). From these results, we believe that the complex of HMBSB and Al^{3+} has been formed and the maximum excitation and emission peaks are 375 nm and 495 nm, respectively. In order to verify the combination way of HMBSB with Al^{3+}, other two compounds (MBSB and CMBSB, Scheme 1) were synthesized and studied in the interaction with Al^{3+} in the same condition. The results show that the fluorescence has no change with the adding of Al^{3+} to MBSB or CMBSB in DMSO/H_2O. Compared to the structure with previously reported HBSB (Scheme 1), the only difference of MBSM lies in hydroxyl group in 4-position