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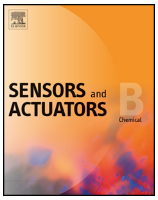

A new pyrene-based aggregation induced ratiometric emission probe for selective detections of trivalent metal ions and its living cell application

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ABSTRACT

We synthesize a newly designed pyrene-based fluorescent probe **FBP** containing a Schiff base and amide linkage as a receptor, which demonstrates selective UV–vis and fluorometric detections of trivalent metal ions in various semi-aqueous solution conditions. Interestingly, by increasing water contents the aggregation induced ratiometric emissions (AIREs) of photoluminescence in **FBP** solutions (in DMSO/H_2O) are enhanced from blue to green due to the excimer formation of the aggregates in water. In addition, the enhanced aggregated ratiometric green fluorescence behaviors of **FBP** in the presence of trivalent metal ions (Al^{3+}, Fe^{3+}, and Cr^{3+}) are observed in semi-aqueous solutions as well as the solid state, which is well utilized to detect trivalent metal ions in living cells. Further verification of the probe detections, their interactions toward trivalent metal ions are well characterized by the time-resolved photoluminescence (TRPL) of spectroscopic responses as well as theoretical calculations. Therefore, we believe the application of such a new AIRE-based approach is first reported among Schiff base and pyrene-based fluorescent chemo- and bio-sensors.

1. Introduction

Owing to the vital roles of trivalent metal ions in living organisms, selective sensing of Al^{3+}, Fe^{3+}, and Cr^{3+} have received great interests, but only few trivalent metal ions sensors were reported in the literature [1–3]. Myriads of synthetic probes for mono and divalent metal ion detections have been presented previously. However, synthetic probes for selective detection of trivalent metal ions without interfering from other counter ions are rare. According to the widespread applications of these trivalent metal ions in biological and environmental importance [4,5], it deserves to proceed more researches.

The trivalent form of chromium is an indispensable nutrient for human beings, and its deficiency causes disorders in the glucose levels and lipid metabolism which is based on the cadence of the action for insulin through glucose tolerance factors (GTF). By that process it stimulates certain enzymes, stabilizes proteins and nucleic acids, but on the contrary, a variety of diseases, including diabetes and cardiovascular diseases, will be induced by its oversupplies [6–9]. Moreover, chromium is an ecological pollutant, and its grown-up due to various industrial and agricultural endeavors is also a matter of concern. Iron is needed in the molecular level for a number of highly complex processes. Its trivalent form provides the oxygen-carrying capacity of heme, e.g., the transportation of oxygen around the body, acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain. Iron is also concerned in the conversion of blood sugar to energy at the cellular level extending from oxygen metabolism to DNA and RNA synthesis. Its deficiency and excess result in various pathological disorders [10–13]. Al^{3+} grounds an oxidative stress within brain tissues caused by drinking water contagion, which is abundantly found in nature. Moreover, gastrointestinal problems, interferences with Ca^{2+} metabolism, shrunk liver and malfunctioned kidney can be instigated by aluminum toxicity. It plays a role toward leading to the formation of Alzheimer-like neurofibrillary tangles [14–16].

Recently organic molecules having aggregation induced emission (AIE) behavior have been gained more interests across materials community as this area further illuminates molecular

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sensor functions. Tang et al. have extensively studied the AIE phenomena over the last decade and reported the first AIE-based sensors, in which tetraphenylethene moiety is the most frequently used example of AIE [2,17–29]. Some moieties with AIE appearances in stimuli-responsive nano-materials and active layers of efficient organic light emitting diodes have also been found to serve as sensors [30,31]. Specific colorimetric and ratiometric fluorescence responses toward trivalent metals have been exhibited by pyridinyl-functionalized tetraphenylethene [2]. Moreover, only a few reports of AIE behavior were demonstrated by excimer formations of fluorophores containing pyrene rings [32–41]. Surprisingly, present results of our pyrene-based fluorophore with Schiff-base probes showed another special aggregation induced ratiometric emission (AIRE) processes upon increasing water content. Moreover, similar effect was highly pronounced in the presence of trivalent metal ions such as (Al^{3+}, Fe^{3+}, and Cr^{3+}) in semi aqueous conditions at physiological pH.

Herein, we successfully synthesized three novel sensor molecules in good yields, i.e., **FBP** and **FBA**, which were verified by ^1H NMR, mass and elementary analysis. Anthracene moiety is also well known for the excimer formation and shows the AIE as well [36,39]. Thus, the anthracene fluorogen was introduced to analogous **FBA** to compare the sensing capability with that of **FBP**. Interestingly, only pyrene-based **FBP** was found to show high selectivity toward trivalent metal ions (Al^{3+}, Fe^{3+}, and Cr^{3+}) with excimer formation. Moreover, **FBP** exhibited special aggregation induced ratiometric emission (AIRE) via stoke shift from blue to green emissions upon adding waters, which has never been reported previously.

2. Experimental

2.1. Materials

All the reagents were purchased from commercial sources and used without further purification, from ACROS, Aldrich, TCI, Fluka, TEDIA, and Lancaster Chemical Co. DMSO was used highly purified company grade. The solvents were degassed by nitrogen 1 h prior to use before reaction. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DX-300 (300 MHz for 1H and 75 MHz for 13C) spectrometer using D6-DMSO solvent (1H: 300, MHz; 13C: 75, 100, 125, and 150 MHz) at a constant temperature of 298 K. Chemical shifts were reported in parts per million from low to high field and referenced to residual solvent DMSO-*d*6 $\delta = 2.49$ ppm and $\delta = 39.56$ ppm. Coupling constant (J) were reported in hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: s = singlet, d = doublet, t = triplet, m = multiples, br = broad. Fluorescence measurements were conducted with HITACHI 4000 Series Spectrophotometer. All emission and excitation spectra were corrected for the detector response and the lamp output. Melting points were determined using a Fargo MP-2D apparatus and are uncorrected. Elemental analyses were conducted on HERAEUS CHN-OS RAPID elemental analyser. Thin layer chromatographies (TLC) were performed on Glass plate coated with silica 60 F24 (Merck). The plates were visualized using ultra-violet light (256 nm) and developed using I2 chamber. Flash chromatographies were performed on Merck silica gel 60 (230–400 mesh) under pressure using desired solvents. Time-resolved photoluminescence (TRPL) spectra were measured using a home-built single photon counting system. Excitation was performed using a 443 nm diode laser (Picoquant PDL-200, 50 ps fwhm, 2 MHz). The signals collected at the excitonic emissions of solutions were connected to a time-correlated single photon counting card (TCSPC, Picoquant Timeharp 200). The emission decay data were analyzed with the biexponential kinetics in which two decay components were derived. The lifetime values (τ_1 and τ_2) and pre-exponential factors A_1 and A_2) were determined and summarized.

2.2. Stock solution preparation

The receptor (**FBP**) solutions were prepared using dried and degassed solvents with a concentration of 50 μM and 10 μM in DMSO/HEPES buffer (8/2) and all cations solutions (Ag^+, Ag^{2+}, Cd^{2+}, Cu^+, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, and Fe^{3+}) were prepared from their respective chlorides with the concentration of 1 mM. Among the previous metal ions, due to the solubility problem of AgCl in the aqueous solution, the PL titration results were confirmed to have little difference as replaced with $AgNO_3$. Before titration studies receptors and cations were diluted to water [51]. The solutions of **FBP** (1.0 ml) were placed in a quartz cell (10.0 mm width) and the fluorescence as well as absorption spectra were recorded.

2.3. Cell imaging

Human cervical cancer cell lines (HeLa cells) were utilized (2×10^5) for seeding **FBP** with a concentration of 500 μM in DMSO:HEPES (80:20, v/v). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and 10% fetal bovine serum in an incubator (37 °C, 5% CO_2). Cell imaging procedure was performed as follows: initially HeLa cells were incubated with 500 μM (30 μl) of probe **FBP** alone in DMEM (1 ml) for 30 min at 37 °C and were observed under microscope to get a blue fluorescence image. Subsequently samples containing **FBP** with fixed ratio of metal ions were also incubated for 30 min at 37 °C and images under confocal microscope were recorded. Furthermore, the confocal images of all samples were again recorded after 3 h.

2.4. Synthesis procedures

2.4.1. Synthesis of (N'^1E,N'^3E)-N'^1,N'^3-bis(pyren-1-ylmethylene)isophthalohydrazide (**FBP**)

To a solution of 1-pyrenecarboxaldehyde (0.6 g, 25 mmol) in toluene (20 ml), isophthalohydrazide (0.25, 12 mmol) was added followed by addition of ethanol (3 ml), and which was stirred to react for 5 min at room temperature. Then, trifluoroacetic acid (TFA) was added as a catalytic amount into reaction mass. After that, the reaction was refluxed for 2 h at 110 °C resulting in a yellow solid. The reaction was monitored by TLC. After completion, the reaction mixture was cooled and filtered on Buckner funnel. Furthermore, the yellow solid was stirred and washed thoroughly with ethanol (50 ml) at 80 °C for 30 min and filtered again on Buckner funnel. After purification with ethanol, pure compound **FBP** as a bright yellow solid was afforded with a yield of 90%. ^1H NMR (300 MHz, DMSO-D6) δ 12.27 (2H, s), 9.59 (2H, s), 8.82 (2H, d, $J = 9.3$ Hz), 8.61 (3H, m), 8.36 (8H, m), 8.25 (6H, m), 8.1 (2H, t, $J = 7.5$ Hz), 7.78 (1H, t, $J = 7.8$ Hz). EI-MS (m/z): calculated for $C_{42}H_{26}N_4O_2$, 618; found, 619 (M + 1). Elementary analysis: calculated: C = 81.54%, N = 9.06%, H = 4.24%, found: C = 81.57%, N = 9.03%, H = 4.21%. M.P.: 326–328 °C.

2.4.2. Synthesis of (N'^1E,N'^3E)-N'^1,N'^3-bis(anthracen-1-ylmethylene)isophthalohydrazide (**FBA**)

To a solution of 9-anthraldehyde (0.53 g, 25 mmol) in toluene (20 ml), isophthalohydrazide (0.25 g, 12 mmol) was added followed by addition of ethanol (3 ml), which was stirred to react for 5 min at room temperature. Then, trifluoro-acetic acid (TFA) was added as a catalytic amount into reaction mass. After that, the reaction was refluxed for 2 h at 110 °C to obtain a yellow solid. The reaction was monitored by TLC. After completion, the reaction mixture