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Rational design of an "on-off-on" fluorescent switch for Cu^{2+} and histidine based on chiral macrocyclic dioxopolyamine

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ABSTRACT

A novel chemosensor **1** having a chiral macrocyclic dioxopolyamine of C_2 symmetry as a receptor and anthracene as a signal unit has been designed and synthesized for cations and α-amino acids recognition in DMSO-HEPES buffer (1:9, v/v, pH 7.2). The ligand exhibited selective response to Cu^{2+} even in the presence of other metal ions with a fluorescence "*switch-off*" behavior. Additionally, the *in situ* generated **1-Cu^{2+}** ensemble displayed specific recognition to histidine by a "*switch-on*" fluorescence response. For this dual functional switch, its sensing behavior *via* a displacement mode was confirmed by ^{1}H NMR titration and ESI mass spectroscopy. Sequential "on-off-on" fluorescence responses of **1** to Cu^{2+} and histidine are successfully applied in HeLa cells.

1. Introduction

In recent decades, great attention has been drawn to explore the systems for specific detection of biologically relevant cations [1], anions [2] and neutral α-amino acids [3]. As the third most abundant transition metal ions after iron and zinc, the deficiency of Cu^{II} can affect enzyme activity, while its excess may cause the damage of the kidneys and liver [4]. Thus, the distribution of Cu^{2+} ion in life system has to be highly controlled, and different categories of sensors have been explored for Cu^{II} recognition with exceptional selectivity and sensitivity [1,4–7]. To further exploit the paramagnetic ability of Cu^{2+} induced fluorescence quenching, sensing ensemble systems have been developed for selective detection of cyanide [8], glutathione [9], histidine [10], ATP/ADP [11,12], cysteine [13] and sulfide [4] detection with a sequential "On-Off-On" response.

Being an essential α-amino acid in a biological system, histidine serves as a neurotransmitter in the central nervous system and regulates metal transmission process. The deficiency of histidine could impair the nutritional state with chronic kidney disease, whereas excessive histidine could affect a variety of functions in humans [14,15]. Thus, detection of histidine especially in biological samples has attracted considerable attention. Among the techniques generally employed for histidine detection, including colorimetric detection [16], electrochemical methods [17], and circular dichroism (CD) spectra [14], fluorescence-based sensing is of great advantage due to its high sensitivity, easy portability and *in situ* monitoring with rapid response [10,15,18–22]. In fact, a variety of fluorophore-crafted systems have been utilized for histidine detection, such as metal complexes [15,23], polymers [14], MOFs [20], carbon nanoparticle [24], *etc.* However, it is worth noting that the histidine detection is always interfered by cysteine [15,25,26] and tryptophan [18]. Therefore, the development of fluorescent probes for specific detection of histidine in aqueous solution is always in demand.

Macrocyclic compounds are an important category in the field of supramolecular chemistry [27]. By delicate design of the structures, the rigid macrocycles always have superspecific recognition abilities. At the same time, metal-macrocycle ensemble system is preferred by a significant improvement in the water solubility, which favored their biological application. Utilization of metal-chiral macrocycle complexes has been rarely explored for α-amino acids recognition. Herein, the rigid C_2 symmetric macrocycle was combined with anthracene moiety to develop a fluorescent ligand **1**, which shows strong affinity for Cu^{2+} in aqueous solution. The *in situ* generated **1-Cu^{2+}** ensemble exhibited specific selectivity towards histidine, which may be ascribed to the unique property of the chiral macrocycle structure.

2. Experimental Sections

2.1. Materials and Instruments

The perchlorate salts of metal ions, such as Cu^{2+}, Fe^{3+}, Cd^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Al^{3+} and Co^{2+} were purchased from Aldrich chemicals. Eight α-amino acids and their enantiomer, such as L/D-Alanine (Ala), L/D-Phenylalanine (Phe), L/D-Histidine (His), L/D-Arginine (Arg), L/D-Tryptophan (Trp), L/D-Aspartic acid (Asp), L/D-cysteine and L/D-cystine were detected directly without modification. Acetonitrile was purified

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prior to use and other reagents were purchased and used without further purifications. The macrocyclic dioxopolyamine was obtained following published procedures [28]. NMR spectra were recorded on a Varian Mercury 300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C relative to tetramethylsilane as internal standard. Absorption spectra were identified on UV-2501 PC spectrophotometer.

2.2. Synthesis of Compound **1**

A mixture of 9-(chloromethyl) anthracene (0.25 g, 0.85 mmol), the macrocyclic dioxopolyamine (0.25 g, 0.77 mmol), potassium iodide (0.3 g, 1.81 mmol) and dipotassium hydrogen phosphate (0.3 g, 1.40 mmol) in 25 mL anhydrous acetonitrile was stirred and heated under reflux for 2 days. The solvent was removed under reduced pressure to obtain a solid residue, which was taken up in dichloromethane, washed with water and brine. The organic layer was dried and evaporated to furnish the crude product, purified using silica gel column chromatography with ethyl acetate/methanol (70:1) as eluting solvent (74.7% yield) to get a pure product **1**. ^{1}H NMR (DMSO d_6, 300 MHz): δ 8.57 (s, 1H), 8.41–8.44 (m, 2H), 8.06–8.10 (m, 2H), 7.51–7.52 (m, 4H), 7.41 (s, 2H) 4.69–4.73 (d, J = 12.8 Hz, 1H), 4.24–4.28 (d, J = 13.1 Hz, 1H), 2.99–3.02 (m, 2H), 2.78–2.91 (m, 3H), 2.71–2.78 (m, 6H), 2.32–2.38 (m, 2H), 2.02–2.05 (m, 2H), 1.68–1.91 (m, 2H), 1.53–1.56 (m, 2H), 1.19–1.23 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.35, 134.08, 131.38, 129.15, 128.70, 127.94, 127.19, 126.15, 124.80, 124.53, 68.42, 55.85, 53.91, 53.38, 50.85, 36.37, 23.78 ESI-MS m/z (%): 514.17 ($(M + H)^+$, 100).

2.3. Cell Cultures and Imaging

HeLa cells were cultured in a medium consisted of 10% fetal bovine serum (FBS, Sigma) and 80 μg/mL penicillin-streptomycin, which were grown at 37 °C in a humidified 5% CO_2 atmosphere. Confocal fluorescence images were obtained by a TCS SP5 laser scanning microscope equipped with a 63× oil immersion objective (Leica, Germany).

3. Results and Discussion

3.1. Synthesis and Structural Characteristics of 1

The compound **1** was obtained from the reaction of 9-(chloromethyl) anthracene with the C_2 symmetric macrocyclic dioxopolyamine [28] in anhydrous acetonitrile with $KHPO_4$ and KI (Scheme 1). Its structure was identified by a combination of ^{1}H NMR, ^{13}C NMR and ESI-MS (Fig. S1-S3).

3.2. Binding Studies of 1 to Metal Ions

Firstly, the recognition ability of receptor **1** towards various metal ions was investigated by the fluorescence titration in DMSO-HEPES buffer (1:9, v/v, pH 7.2). The results indicated that compared to other

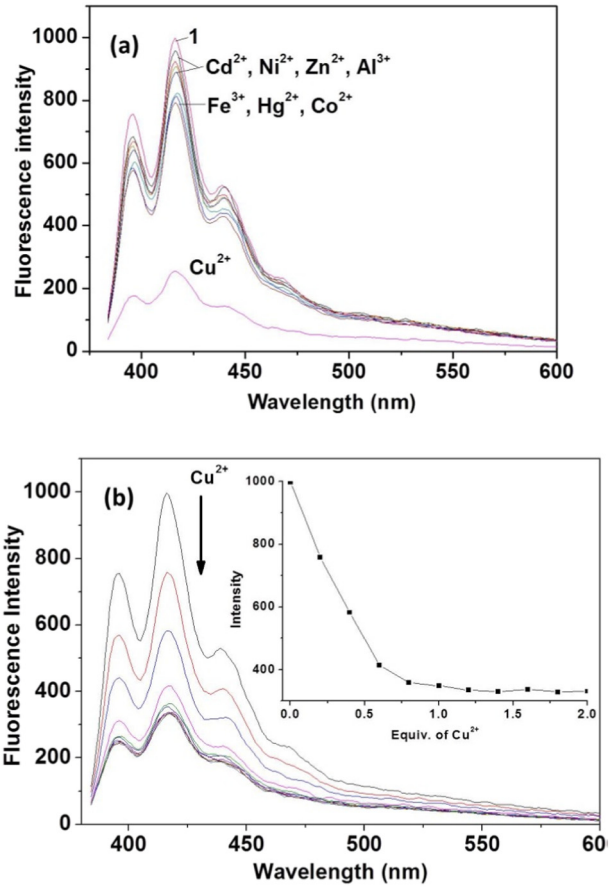
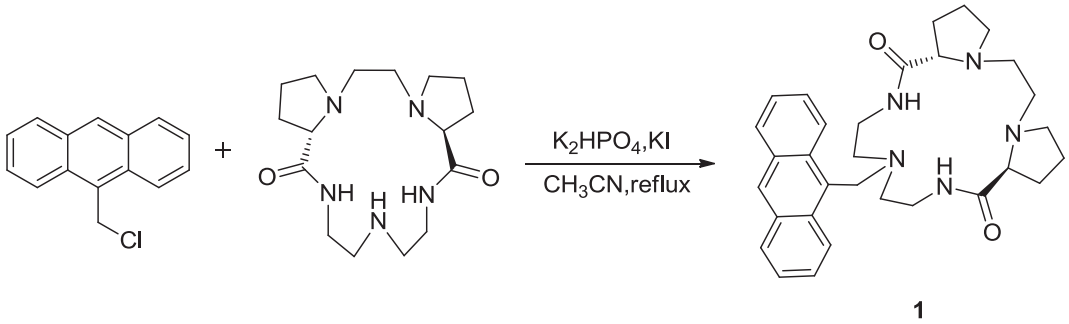


Fig. 1. (a) Fluorescence spectra of **1** (50 μM) before and after addition of an equivalent amount of various metal ions (DMSO-HEPES buffer, 1:9, v/v, pH 7.2). (b) Fluorescence titration profile of **1** (50 μM) upon addition of copper ions from 0 to 2.0 equiv. Inset: plot of fluorescence intensity at 416 nm *versus* the equivalents of copper ions added.

metal ions, Cu^{2+} induced the most significant fluorescence reduction as displayed in Fig. 1a. With an increase of Cu^{2+} concentration, the fluorescence of **1** was quenched gradually (Fig. 1b). Its intensity is reduced to around 30% after the equivalent amount of Cu^{2+} addition as listed in the insert of Fig. 1b, which is due to the paramagnetic fluorescence quenching ability of Cu^{2+} [19,29]. UV–Vis titration experiments of **1** with Cu^{2+} were conducted under the same conditions (Fig. S4). Even after 20 equiv. of Cu^{2+} addition, no significant change of the UV–vis absorption bands of the anthracene moiety was observed, indicating a classic PET process occurred [30].

In order to further confirm the selectivity of **1** towards Cu^{2+}, competition experiments were carried out by addition of 1 equiv. of Cu^{2+} to the probe solutions in the presence of 10 equiv. of other common metal ions. It seemed that the coexisting ions did not show obvious



Scheme 1. Synthesis of ligand 1.