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A novel peptide-based fluorescence chemosensor for selective imaging of hydrogen sulfide both in living cells and zebrafish

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ABSTRACT

Hydrogen sulfide (H_2S) plays an important role as a signaling compound (gasotransmitter) in living systems. However, the development of an efficient imaging chemosensor of H_2S in live animals is a challenging field for chemists. Herein, a novel peptide-based fluorescence chemosensor **L**-Cu was designed and synthesized on the basis of the copper chelating with the peptide ligand (FITC–Ahx–Ser–Pro–Gly–His–NH_2, **L**), and its H_2S sensing ability has been evaluated both in living cells and zebrafish. The peptide backbone and Cu^{2+}-removal sensing mechanism are used to deliver rapid response time, high sensitivity, and good biocompatibility. After a fast fluorescence quench by Cu^{2+} coordinated with **L**, the fluorescence of **L** is recovered by adding S^{2-} to form insoluble copper sulfide in aqueous solution with a detection limit for hydrogen sulfide measured to be 31 nM. Furthermore, the fluorescence chemosensor **L**-Cu showed excellent cell permeation and low biotoxicity to realize the intracellular biosensing, **L**-Cu has also been applied to image hydrogen sulfide in live zebrafish larvae. We expect that this peptide-based fluorescence chemosensor **L**-Cu can be used to study H_2S-related chemical biology in physiological and pathological events.

1. Introduction

Molecular tools for spying signaling molecules, and biologically important ions inside the cells play a vital role in cell biology (Liu et al., 2016; Sengupta et al., 2014; Tracey et al., 2015). Much attention has been given by chemists and biologists to develop various fluorescence chemosensors that would allow real-time tracking of a small molecule of interest in living cells and animals (Adhikari et al., 2016; Bajaj et al., 2009; Brunoud et al., 2012; Cotruvo et al., 2015; Ding and Fang, 2010; Nakano et al., 2013; Strutt et al., 2011; Zhang et al., 2011).

Recently, hydrogen sulfide (H_2S) has gained tremendous attention as an important endogenously produced gasotransmitter after nitric oxide (NO) and carbon monoxide (CO), and also it has been implicated in a variety of physiological functions, such as anti-apoptosis, vasodilation, antioxidation, and anti-inflammation (Basabe-Desmonts et al., 2007; Cortese-Krott et al., 2015; Mishanina et al., 2015; Szabo, 2007; Wallace and Wang, 2015). When the regulation of hydrogen sulfide level is abnormal, it may cause serious diseases, like Alzheimer's disease (Papapetropoulos et al., 2009). Taken together, understanding the biological functions of hydrogen sulfide (H_2S) in the cell has potential valuable application in disease treatment, and the ability to image hydrogen sulfide in the cell is a powerful way to dig out its genesis, trans-location, and action.

Recognition of intra-cellular H_2S is an active and fruitful research area in chemical biology and has attracted more and more talents. Several approaches have been utilized to achieve this goal, such as chemical reaction based probes (Chen et al., 2013; Dai et al., 2014; Feng et al., 2016; Lin et al., 2015; Lippert et al., 2011; Peng et al., 2014; Wang et al., 2013, 2015a; Zhang et al., 2013; Zhu et al., 2016), quencher (such as Cu^{2+}), removal (Hou et al., 2012a, 2012b), florescence protein (Chen et al., 2012), and nanoparticles (Zhang et al., 2014; Zhu et al., 2014). Yang et al. have already reported a novel fluorescent probe displays highly sensitive and selective fluorescence response to H_2S under lysosomal pH environment but is out of operation in neutral cytosol and other organelles, what more, the probe is membrane-permeable and suitable for visualization of both the exogenous and endogenous H_2S in lysosomes of living cells (Yang et al., 2014). However, most of these techniques display a long response time

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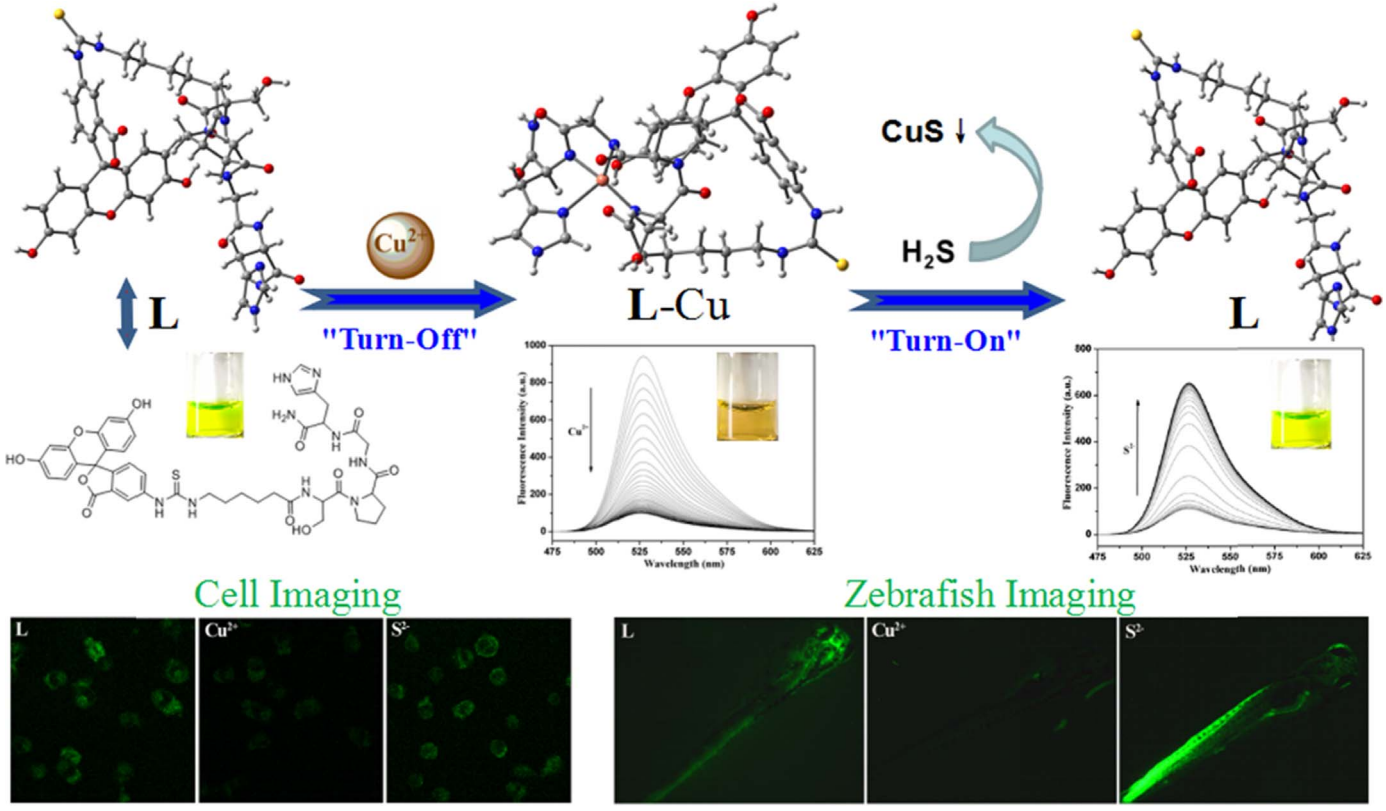

Scheme 1. Schematic illustration of peptide ligand **L** (FITC–Ahx–Ser–Pro–Gly–His–NH_2) structure and the peptide-based fluorescence chemosensor **L**-Cu (**L** chelate with copper ions) for H_2S detection in aqueous solution, which featured a rapid response time with high sensitivity and good biocompatibility, and displayed high specificity for hydrogen sulfide in live cells and zebrafish larvae. The insert photos are the solutions of **L**, **L**-Cu, and **L**-Cu + H_2S under ambient light.

and use exogenous molecule which may cause cell toxicity or has low biocompatibility except florescence protein. To address this issue, efforts have concentrated on peptide-based fluorescence chemosensors, the endogenous molecule, because of the mature synthetic method, their modular nature, and biomolecular properties (Heller et al., 2011; Neupane et al., 2016; Pazos et al., 2009; Wu et al., 2010; Zhao et al., 2011; Zondlo et al., 2010).

Herein, we reported a peptide ligand **L** (FITC–Ahx–Ser–Pro–Gly–His–NH_2) using FITC as fluorophore, Pro–Gly as spacer, Histidine (His) and Serine (Ser) as ionophore, which has been designed and synthesized by solid phase peptide synthesis (SPPS), to chelate with Cu^{2+} to obtain the fluorescence chemosensor **L**-Cu for H_2S detection in live cells and zebrafish larvae, which featured a rapid response time with high sensitivity and good biocompatibility. The Cu^{2+}-removal sensing mechanism was used to regenerate fluorescence response of the chemosensor as shown in Scheme 1. Compared with our previous work (Wang et al., 2015b, 2015c, 2015d, 2016), after the dansyl group was replaced by fluorescein isothiocyanate (FITC), the signal over background noise was increased to 10-fold and the excitation wavelength moved to 495 nm which was well-suited for cell imaging studies. Preliminary attempts to apply our chemosensor to model organism (live zebrafish larvae) have been carried out. Because of the rapid and specific response to H_2S, this peptide-based fluorescence chemosensor can be employed for preferential imaging of H_2S both in living cells and model organism.

2. Experimental section

2.1. Materials and instruments

The main materials for solid phase peptide synthesis, including Rink Amide resin (0.45 mmol/g), Fmoc-His(Trt)-OH, Fmoc-Gly-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, 6-Fmoc-amino hexanoic acid (Fmoc-6-Ahx-OH), and fluorescein isothiocyanate (FITC) were purchased from Top-peptide Co., Ltd. (Shanghai, China). The other synthesis reagents, including 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophospha (HBTU) , N,N-diisopropylcarbodiimide, N,N-dimethylformamide (DMF), dichloromethane (DCM), trifluoroacetic acid (TFA), ethyl alcohol, diethyl ether, triethylamine, triisopropylsilane (TIS), piperidine and acetonitrile were purchased from commercial suppliers with analytical grade and used without further purification. Stock solutions of the perchlorate or nitrate salts of the respective ions (Ag^+, K^+, Na^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, as perchlorates, Hg^{2+} as a nitrate) were prepared with double-distilled water (dd H_2O), and stock solutions of the sodium or potassium salts of the respective anions were also prepared with dd H_2O, which was also used throughout the study.

The crude product was purified by HPLC with a Vydac C18 column. The freeze drying of the sample was performed using an FD-1 Ultra-low freeze dryer. Electrospray ionization MS (ESI-MS) spectra were determined on a Bruker Maxis 4G spectrometer. Absorption spectra were measured using a Varian UV-Cary 5000 spectrophotometer. Fluorescence spectra and quantum yields measurements were performed on a Hitachi F-4500 spectrofluorometer. The lifetime measurements of the samples were determined on an Edinburgh Instrument FSL920. All measurements were carried out at room temperature. All pH measurements were made with a pH-10C digital pH meter. Cell experiment was operated on Zeiss LSM 710 confocal microscope. All the photographs were taken using a Canon digital camera under the illumination of a 365 nm UV lamp.

2.2. Solid phase peptide synthesis of *L*

L was synthesized using a solid phase peptide synthesis according to the reported literatures (Fields and Noble, 1990). Based on the Scheme S1, first of all, Fmoc-His(Trt)-OH (0.2479g, 0.1 mmol×4