Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 207 (2019) 276–283

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

A fluorescent dansyl-based peptide probe for highly selective and sensitive detect Cd^{2+} ions and its application in living cell imaging

Peng Wang *, Dagang Zhou, Bo Chen

Chemical Synthesis and Pollution Control Key Laboratory of Sichuan Province, College of Chemistry and Chemical Engineering, China West Normal University, Shida Road 1#, Nanchong 637002, PR China

ARTICLE INFO

Article history:
Received 25 May 2018
Received in revised form 14 September 2018
Accepted 15 September 2018
Available online 22 September 2018

Keywords:
Peptide
Dansyl group
Fluorescent probe
Cd^{2+} ions
Cell imaging

ABSTRACT

We reported a novel and readily synthesized fluorescent "turn-on" probe **L** (Dansyl-Glu-Pro-Gly-Cys) based on dansyl group combine tetrapeptide. The probe **L** exhibited highly sensitive fluorescent recognition to Cd^{2+} ions in HEPES buffer solutions (10.0 mM, pH 7.4). The 2:1 binding stoichiometry of **L** with Cd^{2+} were determined based on fluorescence titration and the Job's plot investigation. The competitive experiments were found to be highly selective for the Cd^{2+} ions even in the existence of excess competing metal ions including Zn^{2+}, Pb^{2+}, Hg^{2+} and Cu^{2+} ions. The binding constant of the complex **L**-Cd was calculated to be $5.18 \times 10^{10}\ M^{-2}$, and showed great affinity compared to other probes. In addition, the detection limit of the probe **L** for Cd^{2+} ions was calculated to be 45 nM, which presented a pronounced sensitivity toward Cd^{2+} ions. Most importantly, the probe **L** had wide range of pH and good biocompatibility, and were successfully applied to selectively detected Cd^{2+} ions within pH range of 7–12 and bioimaging studies in live cells.

1. Introduction

Heavy and transition metals (HTM) can cause serious problems in the environment and human health, because they are not biodegradable and remain indefinitely in ecological systems [1–5]. Cadmium, one of the very important metallic elements, is widely used in many fields such as metallurgy, pigments, electroplating, nickel cadmium batteries and the war industry. Excess or absence Cd^{2+} causes many lesions in organs and tissues, such as the brain, kidney, liver and bone. Cadmium is also a very toxic element and easily absorbed and accumulated by plants and other organisms [6–12]. In addition, Cd^{2+} and some metal ions, especially Zn^{2+} ions, have many similar properties, and these ions frequently produce similar changes of fluorescence intensity and shifts in wavelengths when coordinated with probes [10–12]. Therefore, it plays an extremely important role of the timely and efficient detection of cadmium ions in human health and social development.

Among all analytical methods, fluorescent sensing detection technology displays paramount sensitivity and affords quick responses in a high spatial and temporal resolution, which makes it a most popular method [13–16,18,19]. What's more, fluorescence probes have also outstanding advantages in situ measurement (such as fluorescence imaging technology) and biological applications. Recently, many fluorescent probes for Cd^{2+} ions and other metal ions have been reported in the literature [13–16,18–26]. However, most fluorescent probes for Cd^{2+} ions have been studied in organic solvents or organic/aqueous mixtures solutions, limiting their practical application, and some probes cannot rule out the interference of Zn^{2+} ions in the detection of Cd^{2+} ions. Peptide fluorescent probes are composed by short peptides and fluorescent groups, and it consists of three parts: the identifying group, the connecting body and the fluorescence group. Compared with other fluorescent probes, peptide fluorescent probes have many unique advantages, such as good water solubility, good biocompatibility, low toxicity and cell permeability. In addition, The peptide fluorescent probes can be easily designed by different sensing mechanisms, such as photoinduced electron transfer (PET), fluorescence resonance energy transfer (FRET) and chelation enhanced fluorescence (CHEF) [27–40]. What's more, the PET mechanism is a practical control over the signaling of a fluorophore and has yielded numerous sensitive "turn-on" model probes. Therefore, it is still very important to develop novel and practical peptide fluorescent probe based on PET mechanism for rapid, sensitive and selective detection of Cd^{2+} ions in 100% aqueous solution and living cells.

Herein, we reported a simple peptide fluorescent probe **L** (Dansyl-Glu-Pro-Gly-Cys) based on dansyl group combine tetrapeptide by solid phase peptide synthesis (SPPS). for probe **L**, dansyl groups are fluorophore (electron acceptors), Pro-Gly (β-turn) is spacer and Cysteine (Cys) and Glutamic acid (Glu) is ionophore (electron donors). As

* Corresponding author.
E-mail address: wangpchem17@163.com (P. Wang).

https://doi.org/10.1016/j.saa.2018.09.029
1386-1425/

designed, the Cd^{2+} ions can binds to **L** via two coordination probes (sulfydryl of Cys and oxygen atoms of Glu respectively) to build the **L**-Cd complex based on Hard-Soft-Acid-Base (HSAB) theory. The probe **L** displayed high selectivity, a rapid response time and excellent water solubility for Cd^{2+} ions due to introduction of the extremely dansyl group, and an outstanding anti-interference ability for its large stokes shift of 225 nm (λ_{ex} = 330 nm, λ_{em} = 545 nm). Based on photo-induced electron transfer (PET) fluorescence quenching mechanism [38–41], the inherent fluorescence of free **L** was quenched in HEPES buffer (10.0 mM, pH 7.4) solutions, and fluorescence was then recovered when **L** combine to Cd^{2+} ions. The results demonstrated that **L** is very sensitivity for Cd^{2+} ions with a detection limit of 45 nM. Furthermore, cellular imaging experiment demonstrated that **L** could be used as a fluorescent probe for reliably detecting Cd^{2+} ions in living cells.

2. Experimental

2.1. Materials

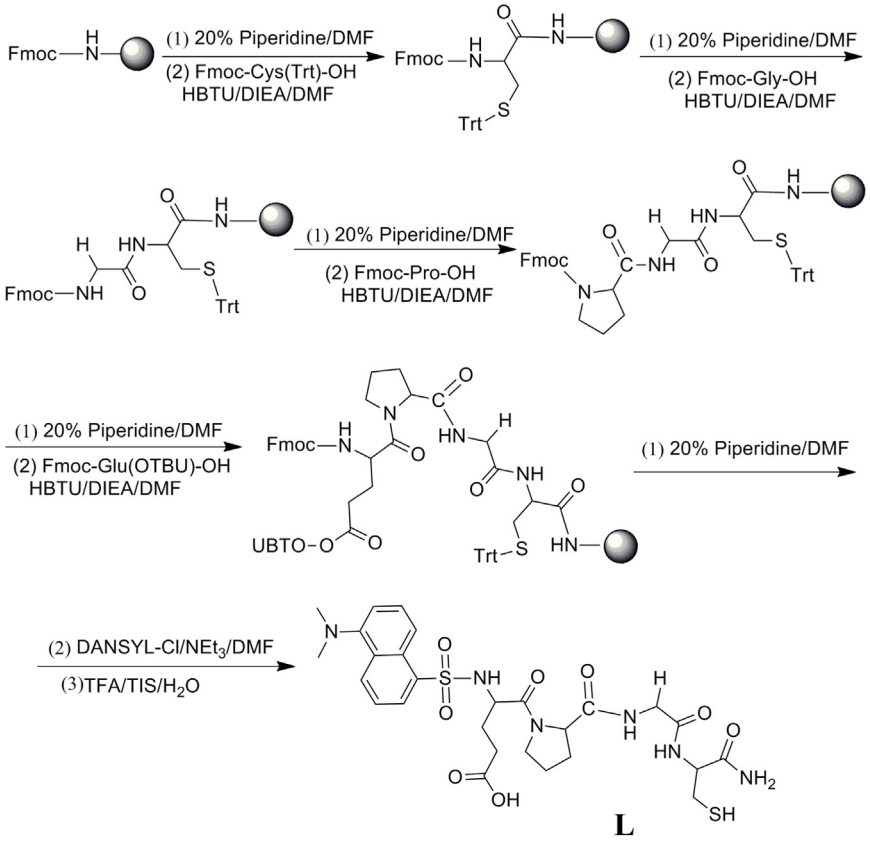
All analytic grade solvents were procured and used without extra purification. All Fmoc-L-amino acids, Fmoc-Rink Amide resin and dansyl chloride were purchased from Top-peptide Co., Ltd. (Nanjing, China). The solutions of various testing metal ions with a concentration of 10^{-3} M were prepared from $CaCl_2$, $CdCl_2$, $CoCl_2$, $ZnCl_2$, $HgCl_2$, KCl, NaCl, $MnCl_2$, $NiCl_2$, $FeCl_3$, $Cu(NO_3)_2$, $(NH)_4Fe(SO_4)_2$, $AgNO_3$, $Al(NO_3)_2$, $Pb(NO_3)_2$, $Cr(NO_3)_3$ and $Mg(NO_3)_2$ in distilled water, respectively.

2.2. Instruments

The sample was dried using an FD-1A-50 Ultra-low freeze dryer, and was purified using HPLC with a Vydac C18 column. All pH HEPES buffer solution were prepared using NaOH and HCl by pHS-3E digital pH meter. Mass spectra were measured on Bruker Daltonics Esquire 6000 spectrometer. Absorption spectra were determined on a Shimadzu UV-2550 spectrophotometer. Fluorescence spectra were recorded using a Cary eclipse spectrofluorometer, including quantum yield. HeLa cells were procured from the School of Life Sciences of University of Sciences and Technology of China (Hefei, China). Fluorescence images of HeLa cells were obtained using a Zeiss LSM 710 confocal microscope. The fluorescence photos of the samples were taken under the 365 nm ultraviolet lamp.

2.3. Synthesis and Characterization

As shown in Scheme 1, The probe **L** (0.1 mmol) was synthesized from Fmoc-Rink Amide resin (0.2222 g, 0.1 mmol) using solid phase peptide synthesis (SPPS) referenced to our previous literature [36–40]. Fmoc-L-Cys (Trt)-OH (0.2343 g, 0.4 mmol), Fmoc-L-Gly-OH (0.1189 g, 0.4 mmol), Fmoc-L-Pro-OH (0.1345 g, 0.4 mmol) and Fmoc-L-Glu (OtBu)-OH (0.1702 g, 0.4 mmol) were assembled successively in Rink Amide resin, the Fmoc group of Glu was then deprotected When tetrapeptides were synthesized. Dansyl chloride (0.2158 g, 0.4 mmol), DMF (5.0 mL) and triethylamine (40.0 μL) were added at room temperature 4 h after removal of the terminal Fmoc group. The Rink Amide resin were dried using circulating water pump after reaction completion. The coarse product **L** was cleaved from Rink Amide resin using the mixture cleavage solution (3.8 mL TFA, 0.1 mL TIS and 0.1 mL H_2O) for 4 h, and then extracted with diethyl ether (−20 °C). The coarse product **L** was purified using HPLC with a Vydac C18 column, and the probe **L** purity was 95.65% (Fig. S1 and Table S1). Probe **L**: Yellowish solid; Synthetic yield: 81%; ESI-MS (m/z) of **L** calculated value: 637.1365; Observed value: 638.1930 ($[\mathbf{L} + H^+]^+$, Fig. S2). ^1H NMR (400 MHz, DMSO, ppm): δ = 12.00 (br, 1H), 8.46 (d, J = 8.0 Hz, 1H), 8.31 (d, J = 4.0 Hz, 2H), 8.14–8.10 (m, 2H), 7.86 (d, J = 8.0 Hz,1H), 7.61–7.55 (m, 2H), 7.25 (d, J



Scheme 1. Synthesis of **L** by solid phase peptide synthesis (SPPS).