Sensors and Actuators B 259 (2018) 484–491

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A water-soluble fluorescent chemosensor having a high affinity and sensitivity for Zn^{2+} and its biological application

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ARTICLE INFO

Article history:
Received 13 October 2017
Received in revised form
13 December 2017
Accepted 15 December 2017
Available online 16 December 2017

Keywords:
Zinc ions
Fluorescence imaging
Water soluble
8-aminoquinoline
Carboxyl groups

ABSTRACT

A water soluble 8-aminoquinoline based fluorescent Zn^{2+} chemosensor (AQZ-2COOH) containing two carboxylic groups has been synthesized. The designed two carboxyl groups not only greatly improved the water solubility of this chemosensor but also increased its affinity for Zn^{2+}. It has been proved that AQZ-2COOH, a pentadentate N, O-chelating ligand was able to coordinate with Zn^{2+} through three nitrogen atoms and two oxygen atoms, resulting in the formation of a stable Zn^{2+} complex, and the binding constant between AQZ-2COOH and Zn^{2+} was determined to be 3.6×10^9 M^{-1}. Furthermore, AQZ-2COOH shows 39-fold fluorescence enhancement upon binding of Zn^{2+} in aqueous solution. However, this remarkable emission enhancement did not occur when other metal ions were present. Further study revealed that AQZ-2COOH had low biotoxicity, and live cell imaging showed that AQZ-2COOH can be efficiently phagocytized by HeLa cells, a visible green fluorescence from the intracellular area was observed after incubation with AQZ-2COOH and Zn^{2+} for 3 h, suggesting that AQZ-2COOH can be a promising candidate for detection of mobile Zn^{2+} in biological systems.

1. Introduction

Zinc, one of the essential trace elements in the human body, plays an extremely crucial role in some important physiological processes such as human growth and development, immunity, endocrine, reproductive inheritance and so on [1–3]. It is known that zinc deficiency can cause an increased risk for growth retardation, neurological disorders and infectious diseases [4–6]. Moreover, zinc is also involved in the processes of the auxin (indole acetic acid) production and CO_2 fixation in plant, thus, zinc deficiency in plants can cause a significant effect on the nutritional quality and productivity of crops [7]. In addition, excessive intake of zinc can cause an imbalance in zinc dependent cellular process, resulting in some neurodegenerative diseases and acute renal failure. Therefore, the detection of zinc in living organisms is of great significance for several disease diagnosis.

Recently, many researchers have been increasingly concerned about fluorescence detection of Zn^{2+} because of its high-sensitivity, instantaneous response and easy operation [8–17]. In fact, numerous fluorescent sensors based on 2,2′-dipicolylamine, [18–25] 8-aminoquinoline [26–30], schiff base [31–34], etc have been designed to detect Zn^{2+}. For example, Qian group synthesized a series of 8-aminoquinoline-based Zn^{2+} fluorescent sensors and found that the sensitivity of these sensors to Zn^{2+} largely depends on the substituents and their positions on the quinoline ring [35]. Guo and co-workers reviewed the fluorescence imaging of Zn^{2+} in living systems [3]. Liu et al. reported a highly water soluble and selective Zn^{2+} fluorescent chemosensor based on β-cyclodextrin and its application in bio-imaging [36]. While taking account of the biological applicatioin, several aspects must be considered in the design of a suitable Zn^{2+} chemosensor: (1) water-solubility, as organic solvents can cause a variety of toxic effects on the organism; (2) the affinity for Zn^{2+}, as most Zn^{2+} ions in vivo are tightly bound to proteins and enzymes through coordination; (3) biocompatibility, as toxic substances will affect cell growth and viability; (4) size of the chemosensors, as larger particles are hardly phago-

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https://doi.org/10.1016/j.snb.2017.12.099
0925-4005/

cytized by living cells. Inorganic-organic hybrid fluorescent sensors show some advantages in Zn^{2+} detection, while they are rarely used for cell imaging due to their larger size [37–40].

However, as a spectroscopically silent metal ion ($3d^{10}$), it is still a challenge to develop a satisfactory fluorescence sensing system for Zn^{2+}. In this regard, chemosensors based on chelation induced fluorescence changes provide an optimal choice to detect Zn^{2+}, and this method is mainly on the basis of two mechanisms: (1) internal charge-transfer (ICT); (2) photoinduced electron-transfer (PET) [41,42]. If using 8-aminoquinoline as an example, upon complexation with Zn^{2+}, the intramolecular hydrogen bond in 8-aminoquinoline is broken, thus a large chelation induced fluorescence enhancement effect is observed because the chelation abrogates the intramolecular electron-transfer process [35]. Unfortunately, many of these chemosensors have suffered from relatively poor water solubility or low affinity for Zn^{2+}. Herein, we report a new polyamino polycarboxylic 8-aminoquinoline-based chemosensor for Zn^{2+} detection. Especially, the designed two carboxyl groups allow the sensor to be completely dissolved in pure water and the carboxyl groups also involved in the coordination of Zn^{2+}. Meanwhile, this chemosensor can selective detect of Zn^{2+} via a chelation induced enhanced fluorescence effect. *In vitro* assessments using human mesenchymal stem cells (hMSCs) revealed that this chemosensor had low biological toxicity and live cell imaging demonstrated that it has great potential for imaging Zn^{2+} in living cells.

2. Experimental section

2.1. Synthesis of the chemosensor

2.1.1. Synthesis of compound 1

Compound 1 was synthesized according to Ref. [35]. Firstly, a mixture of 8-aminoquinoline (0.288 g), anhydrous triethylamine (0.416 mL) and anhydrous $CHCl_3$ (20 mL) was stirred for about 5 min under cooling with ice-water, and then chloroacetyl chloride (0.246 mL) was added drop by drop to the above solution. After stirring for another 5 min, the reaction solution was warmed to room temperature and stirred for 24 h. Finally, the solvent was removed under vacuum using a rotary evaporator, and the resulting solid was purified by column chromatography (silica gel) using ethyl acetate/petroleum ether (1:2 by volume) as eluent to yield a white solid (0.36 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 10.90 (s, 1H), 8.86 (dd, 1H), 8.75 (dd, 1H), 8.18 (dd, 1H), 7.58-7.52 (m, 2H), 7.48 (dd, 1H), 4.31 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 164.5, 148.7, 138.8, 136.4, 133.6, 128.1, 127.2, 122.6, 121.9, 116.7, 43.4.

2.1.2. Synthesis of compound 2

Compound 1 (0.5 g), diethyl iminodiacetate (0.51 mL), N,N-diisopropylethylamine (0.47 mL), KI (10 mg) were firstly dissolved in anhydrous acetonitrile (15 mL), and then the reaction solution was refluxed under an atmosphere of N_2 for about 36 h. After cooling down, the mixture was evaporated under reduced pressure. The obtained yellowish oil was purified by column chromatography (silica gel, ethyl acetate: petroleum ether: triethylamine = 1:5:0.02 by volume) to give compound 2 as a pale yellow oil (0.56 g, 66%). ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm: 11.14 (s, 1H), 8.88 (dd, 1H), 8.70 (dd, 1H), 8.39 (dd, 1H), 7.68-7.55 (m, 3H), 4.07 (q, 4H), 3.73 (s, 4H), 3.59 (s, 2H), 1.12 (t, 6H). ^{13}C NMR (300/4 MHz, DMSO-*d*6) δ ppm: 170.8, 169.6, 149.5, 138.6, 136.9, 134.6, 128.3, 127.4, 122.6, 122.3, 116.2, 60.6, 59.3, 55.8, 14.4.

AQZ-C_2H_5 was synthesized using ethylamine hydrochloride as raw material by the similar synthetic route (Fig. S1). ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm: 11.45 (s, 1H), 8.91 (dd, 1H), 8.71 (dd, 1H), 8.39 (dd, 1H), 7.67–7.54 (m, 3H), 3.36 (s, 2H), 2.64 (q, 2H), 1.12 (t, 3H). ^{13}C NMR (300/4 MHz, DMSO-*d*6) δ ppm: 171.3, 149.5, 138.5, 137.0, 134.6, 128.3, 127.5, 122.6, 122.1, 115.8, 53.6, 44.4, 15.6. ESI–MS: M/Z calcd for $C_{13}H_{15}N_3O$: 229.1215, $[M+1]^+$, found: 230.1294. (Fig. S2 and 3).

2.1.3. Synthesis of AQZ-2COOH

Compound 2 (0.4 g) was dissolved in 6 mL of a mixture of alcohol and water (1:1 by volume) to form a clear solution, and then NaOH solution (2 mol L^{-1}, 1.4 mL) was added slowly. After stirring for about 5 h, the pH value of the above solution was adjusted to 3 by H^+ ion-exchange resin. The solution was removed under reduced pressure and the obtained solid was suspended in anhydrous acetone (15 mL) to give the product as a white solid (0.24 g, 71%). ^{1}H NMR (400 MHz, D_2O, pH >12) δ ppm: 8.62 (d, 1H), 8.09 (d, 1H), 7.93 (d, 1H), 7.53 (d, 1H), 7.41-7.34 (m, 2H), 4.05 (s, 2H), 3.70 (s, 4H). ^{13}C NMR (100 MHz, D_2O, pH >12) δ ppm: 174.1, 168.4, 149.8, 139.4, 137.3, 131.6, 128.3, 126.4, 125.3, 121.9, 121.6, 58.0, 57.5. ESI–MS: M/Z calcd for $C_{15}H_{15}N_3O_5$: 317.1012, $[M+1]^+$, found: 318.1092. (Fig. S4 and 5).

2.2. General procedure for analysis

Parent stock solution of AQZ-2COOH (1.0 mM) was prepared in Tris-HCl buffer (20 mM, pH 7.2), and diluted to an appropriate concentration with Tris-HCl buffer before use. Zn^{2+} was obtained by zinc sulphate heptahydrate, the stock solution of Na^+, K^+, Mg^{2+}, Ba^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+} and Co^{2+}, Pb^{2+} and Cr^{3+} Mn^{2+} and Cu^{2+} were prepared from their nitrate salts, the stock solutions of F^-, Br^-, Cl^-, I^-, NO_3^-, NO_2^-, SO_4^{2-}, SO_3^{2-}, $H_2PO_4^-$, HCO_3^-, and CH_3COO^- were prepared by using their sodium salt, respectively. All optical tests were performed in Tris-HCl buffer.

2.3. Cellular uptake [43,44]

Cellular uptake of AQZ-2COOH by HeLa cells was studied by using a confocal laser scanning microscope (Leica SP8). Firstly, HeLa cells (5×10^4 per well) were plated into a 6-well culture plates and grown for 24 h at 37 °C before cellular uptake study. And then HeLa cells were incubated with AQZ-2COOH and Zn^{2+} for different times (0.5, 1, 3 h) at 37 °C, following by rinsing with phosphate-buffered saline (PBS) three times. Finally, the harvested HeLa cells were fixed with formaldehyde solution (2.5%, 1 mL/well) for 10 min at 37 °C and rinsed with PBS solution three times again.

3. Results and discussion

The synthesis procedure of AQZ-2COOH is illustrated in Scheme 1, and the designed compounds have been characterized by NMR spectroscopy and mass spectrometry. Firstly, compound 1 was synthesized as previously reported [35], and then compound 2 was obtained by a simple nucleophilic reaction using diethyl iminodiacetate as the nucleophilic reagent. Finally, compound 2 was hydrolyzed with NaOH and acidified with H^+ exchange resin to give the title compound as a white power (AQZ-2COOH). It should be pointed out that AQZ-2COOH, a pentadentate N, O-chelating ligand, can form a stable complex with Zn^{2+} through two oxygen atoms from carboxyl groups and three nitrogen atoms from pyridine, amide and amine units.

The bonding condition of AQZ-2COOH with Zn^{2+} was firstly investigated by UV–vis absorbance spectroscopy. As shown in Fig. 1, the absorption curves showed a significant red-shift upon titration of AQZ-2COOH with Zn^{2+}. As the Zn^{2+} concentration was increased, the intensity of the absorption peak at 300 nm gradually decreased. Meanwhile, a new absorbance peak emerged at 350 nm, whose intensity continuously increased until a Zn^{2+} to ligand molar ratio of