Sensors and Actuators B 213 (2015) 268–275

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A fluorescence sensor for Zn^{2+} that also acts as a visible sensor for Co^{2+} and Cu^{2+}

Eun Joo Song[a,b], Gyeong Jin Park[a,b], Jae Jun Lee[a,b], Suyeon Lee[c], Insup Noh[c], Youngmee Kim[d], Sung-Jin Kim[d], Cheal Kim[a,b,**], Roger G. Harrison[e,*]

[a] Department of Fine Chemistry, Seoul National University of Science & Technology, Seoul 139-743, Republic of Korea
[b] Department of Interdisciplinary Bio IT Materials, Seoul National University of Science & Technology, Seoul 139-743, Republic of Korea
[c] Department of Chemical Engineering, Seoul National University of Science & Technology, Seoul 139-743, Republic of Korea
[d] Department of Chemistry and Nano Science, Ewha Womans University, Seoul 120-750, Republic of Korea
[e] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA

ARTICLE INFO

Article history:
Received 12 November 2014
Received in revised form 16 February 2015
Accepted 17 February 2015
Available online 28 February 2015

Keywords:
Zinc fluorescent sensor
Visible cobalt
Copper sensor

ABSTRACT

Monitoring Zn^{2+} levels in biological environments with fluorescent sensors is important. This paper gives the synthesis and properties of a new Zn^{2+} sensor based on quinoline and pyridylaminophenol. The sensor is selective for Zn^{2+} and remains fluorescent when bound to Zn^{2} even in the presence of other metal ions. Along with fluorescing when bound to Zn^{2+}, the sensor becomes colored when Cu^{2+} or Co^{2+} is added to it. These two metal ions result in the sensor becoming yellow. The crystal structure of the Cu–sensor complex shows that all of the sensor's nitrogens are bound to the metal ion. In studies with living cells, the fluorescence intensity of the sensor correlates to the concentration of Zn^{2+}.

1. Introduction

Zinc ions in biological environments do not have a color, are redox inactive, and are magnetically silent. Small molecules that change fluorescence upon binding to Zn^{2+} have proven successful in their ability to monitor Zn^{2+} concentrations and locations in cells and organisms [1]. To perform their function as a Zn^{2+} sensor in biological environments, a sensor must bind Zn^{2+} in preference to other metal ions, be water soluble, and respond to Zn^{2+} with intense fluorescence [2].

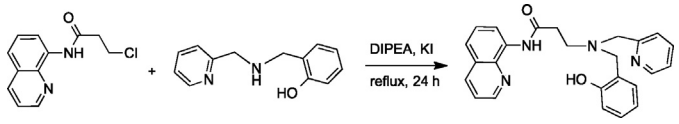
Visible sensors are also significant for the detection and quantification of metal ions. Copper is another essential metal in biology. Cobalt, although to a lesser extent, also occurs in organisms. Although Co^{2+} and Cu^{2+} have *d–d* transitions in the visible region, the transitions are weak and render dilute solutions of the metal ions colorless. Along with the few fluorescent sensors for Co [3], there are a few visible sensors as well. The visible sensors for Co are based on compounds with conjugated rings and nanoparticles [4]. Cu has also been detected with visible sensors. These sensors are based on a variety of molecules including dyes [5], guanidine [6], Schiff bases [7], and other molecules [8].

Multifunctional sensors are becoming more important due to one molecule being able to be used for the detection of more than one metal ion [9]. They include transition metal sensors that give a different response for the following pairs: Cr/Al [10] Cr/Fe [11], Mn/Ag [12], Fe^{2+}/Fe^{3+} [13], Fe/Cu [14], Cu/Zn [15], Cu/Hg [16], Zn/Al [17], and Co/Zn [18]. Often the response is either a visible or fluorescence change for both metals, but in a few cases the change is visible form one metal and fluorescence for the other.

In this paper we present the properties of a dual sensor that fluoresces in the presence of Zn^{2+} and changes color in the presence of Co^{2+} and Cu^{2+}. The sensor relies on quinoline as the origin of fluorescence and pyridylaminophenol [19] as the metal ion-binding group. Quinoline-based receptors have proven to be successful Zn^{2+} sensors [20]. When Zn^{2+} binds the sensor, it fluoresces, making for a way to detect Zn^{2+} in an aqueous environment. The fluorescence due to Zn^{2+} is selective for Zn^{2+} and remains even when other metal ions are present. When Co^{2+} binds to the sensor a new visible absorption band grows in, which also remains in the presence of other metal ions. A crystal structure of the Cu–receptor complex shows the Cu^{2+} binding to the nitrogen atoms of the receptor. The results of using the receptor in living cells are presented.

* Corresponding author. Tel.: +1 801 422 8096.
** Corresponding author. Tel.: +82 2 970 6693.
E-mail addresses: chealkim@seoultech.ac.kr (C. Kim), roger_harrison@byu.edu (R.G. Harrison).

http://dx.doi.org/10.1016/j.snb.2015.02.094
0925-4005/



Scheme 1. Receptor synthesis.

2. Results and discussion

The new receptor was synthesized by adding 3-chloro-N-(quinoline-8-yl)propanamide to functionalized amine (Scheme 1). This receptor is similar in structure to receptors with dipicolylamine (dpa) as the binding group, but it differs by having a phenol group in place of a pyridine [21]. The binding to zinc by this receptor should still be strong, even though one oxygen is replacing a nitrogen and a six membered metal–ligand ring will form instead of the five membered metal–ligand ring. This receptor, which has two carbons separating the amine from the phenol, is similar to the sensor that has the same binding domain, but only one carbon between the phenol and the amine [22]. It is expected that this change will result in a weaker binding strength to Zn^{2+} and different binding properties with other metal ions.

The sensor does not fluoresce on its own, however when Zn^{2+} is added to it a fluorescence band at 520 nm grows until one equivalent of Zn^{2+} has been added (Fig. 1). The fluorescence enhancement only occurs with Zn^{2+} ions and does not happen with other metal ions such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Ga^{3+}, In^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, and Hg^{2+} (Fig. 2). This fluorescence response to only Zn^{2+} in aqueous solution will serve to allow Zn^{2+} sensing under biological conditions. The binding of Zn^{2+} stops the amide lone pair of electrons from quenching the fluorescence of the quinoline, but unlike with the other transition metals, which also bind to the amide electrons, Zn^{2+} does not quench the fluorescence. This unique property of Zn^{2+} results in the receptor fluorescing with Zn^{2+} and not with other metal ions. The fluorescence due to Zn^{2+} is maintained for hours and is above fifty percent of its original value after 24 h (ESM). The selectivity for Zn^{2+} matches what has been observed for several other receptors [20].

Not only is fluorescence only seen with Zn^{2+}, it is resistant to change by other metal ions. The fluorescence due to the Zn–receptor complex persisted when many other metal ions were added to the complex (Fig. 3). Cu^{2+} and to a smaller extent for Co^{2+} resulted in a decrease in fluorescence intensity. This implies the binding constant of Zn^{2+} to the receptor is stronger than that of many metal ions and comparable to Cu^{2+}. The binding constant for Zn^{2+} was found to be $1.0 \times 10^4\ M^{-1}$ in aqueous-acetonitrile

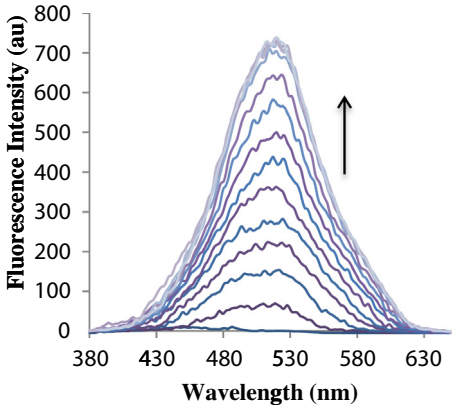


Fig. 1. Fluorescence increase due to Zn^{2+}. (A) Acetonitrile–buffer solution of Zn^{2+} was added in 0.1 equiv. portions to 10 μM receptor in acetonitrile–buffer solution (10 mM HEPES, pH 7.4, 1:1 acetonitrile/buffer, 356 nm excitation).

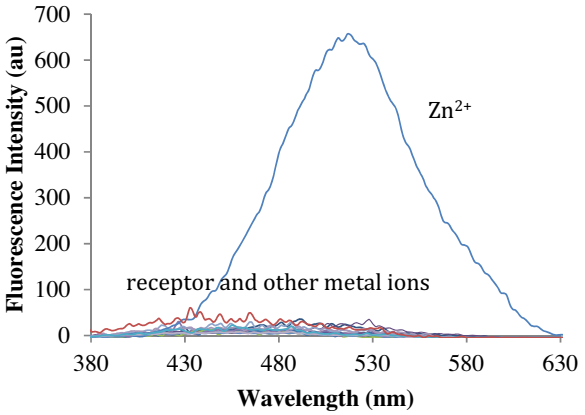


Fig. 2. Receptor fluorescence with metal ions. Zn^{2+} (top spectra) causes fluorescence enhancement, while the receptor shows no fluorescence with other metal ions (spectra near the base line) (10 μM receptor acetonitrile–HEPES buffer solutions, excited at 356 nm).

solutions. This binding constant is similar to what was found for Cu^{2+} ($7.9 \times 10^3\ M^{-1}$) and Co^{2+} ($2.3 \times 10^3\ M^{-1}$) in the same solution. As expected, the binding constant to Zn^{2+} for the receptor was lower than sensors with dipicolylamine, which have binding constants of $4.1 \times 10^{10}\ M^{-1}$ [21]. It is also lower than the binding constant of the sensor with a methylene in place of the ethylene between the phenol and amine, which has a binding constant of $4.0 \times 10^6\ M^{-1}$ [22]. Even with this smaller binding strength, due to the intense fluorescence by the receptor, the Zn^{2+} detection limit of 8.14 nM was found, which concentration is within the range of biological Zn^{2+} concentrations.

The fluorescence due to Zn^{2+} can be turned off and on. When EDTA is added to the Zn^{2+}–receptor complex, the fluorescence is distinguished (Fig. 4). When more Zn^{2+} is added to the solution, fluorescence reappears. Upon the addition of more EDTA, the fluorescence is again stopped. Low pH also causes the fluorescence to be erased. When the pH is below 6, there is not fluorescence, most likely due to the receptor being protonated and not binding to Zn^{2+}. However, when the pH is above 6 and less than 12, fluorescence is strong. The receptor without Zn^{2+} does not fluoresce at any pH. Thus, unlike Zn^{2+} binding, proton binding does not cause fluorescence.

Along with the fluorescent data, absorption changes show a 1:1 binding of Zn^{2+} to receptor. The absorption change of the receptor due to Zn^{2+} binding levels off after one equivalent of Zn^{2+} (Fig. 5). Two absorption bands (245 and 325 nm) decrease and two bands (270 and 375 nm) increase upon Zn^{2+} binding. The 375 nm band tails off into the visible region of light, implying the complex could be exited with visible light. A Job plot also shows 1:1 receptor to Zn^{2+} binding. These absorption changes might be due to

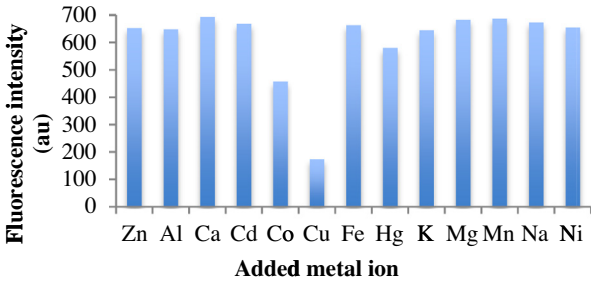


Fig. 3. Fluorescence inhibition by metal ions. The fluorescence (356 nm excitation) of the Zn^{2+}–receptor complex (10 μM acetonitrile–HEPES buffer solution, pH 7.4) remains when most other metal ions (1 equiv.) are added.