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Selective zinc sensor based on pyrazoles and quinoline used to image cells

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ABSTRACT

The synthesis, Zn^{2+} binding, crystal structure, and cell imaging studies of a new pyrazole amine quinoline receptor with a flexible binding pocket are described. Upon coordination to Zn^{2+}, the absorption of the receptor increases at 364 nm and it fluoresces at 500 nm. The fluorescence response to Zn^{2+} is selective for Zn^{2+} and does not occur with other metal ions, not even Cd^{2+}. In solution, the receptor forms 1:1 complexes with Zn^{2+}, but in the solid-state two Zn^{2+} ions coordinate to the receptor. The aqueous solubility of the receptor allows for imaging of Zn^{2+} in living cells. Cells exposed to receptor and Zn^{2+} fluoresce when excited with visible light.

1. Introduction

Zinc has a multitude of uses in organisms, including acting as a Lewis acid in hydrolytic enzymes, as a structure component of proteins, and as a signal in brain function. Due to the prevalence of Zn^{2+} in organisms, its detection and monitoring are essential to understand its role in biological organisms [1]. Without being colored or redox active it has been difficult to detect Zn^{2+} ions in biological environments. With its *d* orbitals full of electrons and those electrons stable, Zn^{2+} resembles Ca^{2+} more than other transition metal ions. Cellular levels of Zn^{2+} are not the same and thus different concentrations of cellular zinc need to be monitored. Therefore, receptors with high and low affinity for zinc are important [2]. However, no matter what the binding strength of the receptor is, selectivity for Zn^{2+} over other metal ions is critical.

The development of zinc sensors is an active research field. Most sensors have two components, a fluorophore and a Zn^{2+} binding site. Various molecules have been used as fluorophores, one of which has been quinoline [3]. We have developed receptors with the amidoquinoline fluorophore due to the large enhanced fluorescence of quinoline after the amide binds to Zn^{2+} [4]. Several of the sensors are biocompatible and have been used to image cells [5].

The Zn^{2+} binding domain in sensors must chelate Zn^{2+} and thus often has several nitrogen atoms. Such ligands as dipicolylamine (DPA) have been employed as the chelates [6] and some cases they have been included with quinoline to make receptors [7]. A ligand that hasn't been used is the dipyrazolylamine. The dipyrazolylamine, with its pyrazole nitrogens separated from its amine nitrogen by three atoms, is able to form six-membered metal containing rings [8]. Metal ions such as Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} have been coordinated to dipyrazolylamine ligands [9]. And in some cases the dipyrazolylamine ligand coordinates strongly to Zn^{2+} due to the flexible coordination of Zn^{2+}, but less strongly to other transition metal ions, due to their preference to one definite geometry, such as octahedral geometry. The unique ability of Zn^{2+} to be a strong Lewis acid and yet to be stable in various geometric conformations renders it able to coordinate strongly to ligands to which other metal ions bind more weakly.

In this paper we present the synthesis and properties of a new zinc receptor that has a flexible binding site composed of pyrazoles. The receptor has an amidoquinoline unit as its fluorophore. The

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new receptor fluoresces in the presence of Zn^{2+}, but not with other metal ions. A crystal structure of the receptor bonded to Zn^{2+} shows coordination to the Zn^{2+} through pyrazole nitrogens and the amide oxygen and nitrogen. The new receptor-Zn^{2+} complex has the important properties of being soluble in water and fluorescing when excited with visible light. The receptor is able to induce fluorescence in living cells that have been exposed to Zn^{2+}.

2. Results and discussion

2.1. Synthesis

The new receptor **1** was synthesized by adding 2-chloro-*N*-(quinolin-8-yl)acetamide to bis[2-(3,5-dimethylpyrazol-1-yl) ethyl]-amine in the presence of base (Scheme 1). Column chromatography was used to isolate pure product, which showed methylene proton NMR signals next to the carbonyl group to be at 3.4 ppm, signifying receptor assembly. The molecule is colorless and does not fluoresce.

2.2. Fluorescence due to Zn^{2+}

Upon addition of Zn^{2+} to an aqueous solution of receptor, the receptor fluoresces. The fluorescence at 500 nm increases upon excitation at 356 nm until one equivalent of Zn^{2+} has been added (Fig. 1). Importantly, the fluorescence response is selective for Zn^{2+} and the sensor doesn't fluoresce when other metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Ga^{3+}, and In^{3+} are present (Fig. 2). Remarkably, unlike many other Zn^{2+} sensors, the receptor does not fluoresce in the presence of Cd^{2+}. Not only is the receptor selective for Zn^{2+}, but other metal ions do not quench the fluorescence caused by Zn^{2+}. The fluorescence of the Zn-receptor complex is not affected when one equivalent of metal ion, such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Ga^{3+}, and In^{3+} is present (Fig. 3). Larger equivalents (2, 5, and 10 equivalents) of Cr^{3+}, Fe^{3+}, Co^{2+}, and Cu^{2+} do reduce the fluorescence intensity of the Zn-receptor complex, however, it still remains over fifty percent of its original value.

2.3. pH range of fluorescence

Fluorescing at biologically relevant pH is important for the usefulness of the receptor. The fluorescence enhancement of the receptor caused by Zn^{2+} is maintained over a pH range from 6 to 11 (Fig. 4). The continuous fluorescence over five pH units implies that the Zn-receptor complex is stable over this pH range.

2.4. Fluorescence cycling

The receptor also shows chelation ability over several binding episodes. The fluorescence of the Zn-receptor complex is quenched when EDTA is added to it, but when more Zn^{2+} is added to the solution, the fluorescence returns (Fig. 5). This fluorescence quenching and emission can be cycled several times without loss of fluorescence intensity. The binding constant of EDTA to Zn^{2+} is of the order of 10^{16} M^{-1} and is much larger than the 1.1×10^7 M^{-1} binding constant for receptor to Zn^{2+}. Thus EDTA removes Zn^{2+} from the Zn-receptor complex. With this binding constant for the receptor-Zn^{2+} complex and the strong fluorescence intensity of the complex, the detection limit of Zn^{2+} by the receptor is 30 nM.

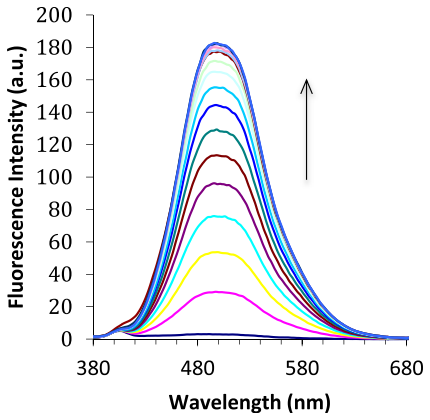


Fig. 1. Fluorescence intensity increase due to Zn^{2+} addition to receptor **1**. Conditions: 10 μM receptor in bis-tris aqueous solution, 356 nm excitation, 0 to 2 equivalents of Zn^{2+} added in 0.1 equiv. portions.

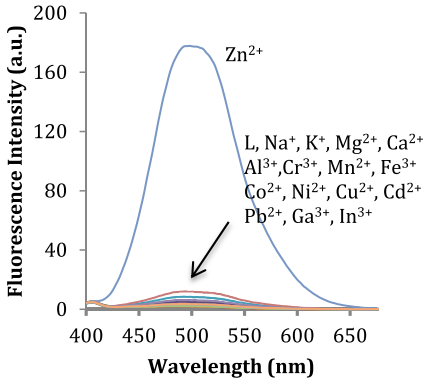
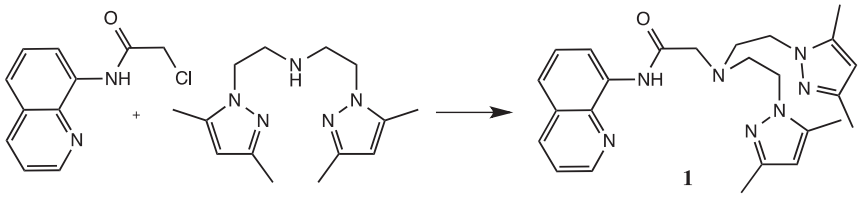


Fig. 2. Receptor fluorescence due to Zn^{2+}. Other metal ions do not cause fluorescence. Conditions: 10 μM receptor in bis-tris aqueous solution, 356 nm excitation, 1 equiv. metal nitrate.

2.5. Absorption change upon Zn^{2+} binding

The receptor has absorption bands at 220 and 325 nm. Both of these bands decrease in intensity when Zn^{2+} is added and new bands at 274 nm ($\varepsilon = 2.4 \times 10^4$ $M^{-1}cm^{-1}$) and 364 nm ($\varepsilon = 3.7 \times 10^3$ $M^{-1}cm^{-1}$) develop (Fig. 6). This red shift in the amide and aromatic π to π* transitions upon Zn^{2+} binding has been noted before with quinoline receptors. We attribute it to a greater lowing



Scheme 1. Synthesis of receptor **1**. Conditions: reflux in acetonitrile with triethylamine.