Rhodamine–Pyridyl Probe: A Selective Optical Reporter for Biologically Important Zn^{2+}

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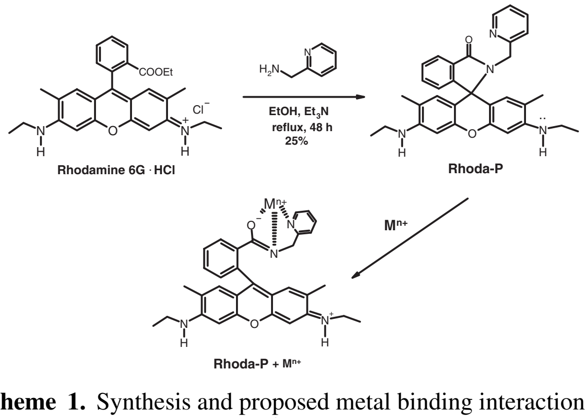
A new Rhodamine-based probe, Rhoda-P affords remarkably selective chromo- and fluorogenic responses in the presence of Zn^{2+} with the color turning from colorless ($\lambda_{max} = 302$ nm) to orange ($\lambda_{max} = 528$ nm) and the fluorescence output increased by 160-fold at 1.5×10^{-3} M of Zn^{2+}. In contrast, Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} did not significantly alter the photophysical properties of the probe even at relatively higher concentrations than Zn^{2+}.

Zinc is one of the essential elements for humans[1] with approximately 300 enzymes containing Zn^{2+} known either for cellular or catalytic functions.[2] The presence of zinc in seawater[3] is crucial for the survival of many sea organisms including plankton.[4] However, zinc is also a pollutant of the environment and toxic to soil microbes.[5] Consequently, over the past decades, numerous fluorescent "*on–off*" or "*off–on*" type sensors and a few examples of colorimetric Zn^{2+} sensors have been reported.[6–9]

Recently, an array of chemosensors based on Rhodamine dyes for targeting Cu, Fe, Pb, and Hg ions have been described.[10] Based on the Rhodamine motif, presently, we report a new pyridyl-incorporated Rhodamine probe, Rhoda-P that delivers highly selective dual colorimetric and fluorogenic responses for targeting the physiologically important Zn^{2+}.

Synthesis of Rhoda-P was readily achieved as shown in Scheme 1.[11] A proposed sensing mechanism involves chelation of metal ions with the "spiroamide" and "pyridyl nitrogen" functions that could provoke electron participation from the amino group(s) of the xanthene moiety, thereby triggering the spiroring opening (Scheme 1). The altered photophysical properties of the resultant ring-opened structure, in comparison to that of the Rhoda-P would provide a basis for the optical detection of the interacting metal ion(s).

The UV–vis spectrum of Rhoda-P displayed an absorption maximum at 302 nm ($\varepsilon_m = 1.36 \times 10^4\,M^{-1}\,cm^{-1}$) with a very weak absorption also discernible in the 500–600 nm region due to the presence of a small fraction of the open form.[10f] Figure 1 shows the UV–vis spectral responses of the probe in the presence of Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} as their perchlorate salt. Addition of Zn^{2+} at 100 equivalent with respect to the concentration of Rhoda-P gave rise to a new intense absorption band at 528 nm with isosbestic points at 296 and 322 nm. However, Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} even up to 3000 equivalents failed to induce changes in the original UV–vis spectral profile of the probe.



Scheme 1. Synthesis and proposed metal binding interaction of Rhoda-P with metal ions, M^{n+}.

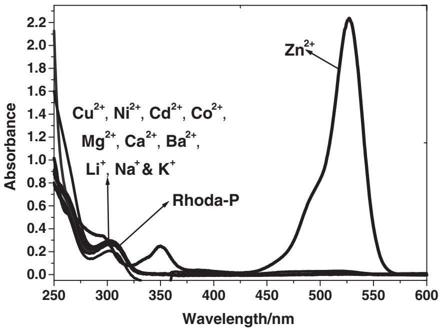


Figure 1. Absorption spectra of Rhoda-P (2.0×10^{-5} M) in the presence of selected metal ions (2.0×10^{-3} M) in CH_3CN.

Spectrophotometric titration of Rhoda-P (2×10^{-5} M) with incremental additions of Zn^{2+} ($0–2.0 \times 10^{-3}$ M) is shown in Figure 2, and the inset shows a graphical representation of the steady changes in the UV–vis profile of Rhoda-P upon adding Zn^{2+}. At a limiting concentration of 2.0×10^{-3} M of Zn^{2+}, the absorbance at 528 nm reached a maximum ε_m of $11.28 \times 10^4\,M^{-1}\,cm^{-1}$ corresponding to a ca. 313-fold absorption enhancement over that of the residual absorption associated with the probe.

The binding constant, $\log K_s$ of 2.41 calculated using nonlinear curve fitting[12] indicates the potential of the probe for sensing micromolar concentrations Zn^{2+}. A shift of the –NHCO– band in the IR spectrum from 1685 cm^{-1} in Rhoda-P to 1649 cm^{-1} in the Rhoda-P–Zn^{2+} complex is consistent with the anticipated spirolactam ring-opening, as envisaged in Scheme 1. The $\log K_s$ for other metal ions could not be determined due to insignificant interaction with the probe. The color of the probe changes from colorless to orange in the presence of Zn^{2+}, whereas other metal ions examined did not induce perceptible color change.[11] Clearly, the probe, Rhoda-P offers a highly selective discrimination of Zn^{2+} allowing a naked eye detection of this ion.

Excitation of Rhoda-P at 302 nm gave rise to a weak emission band at 560 nm with a quantum yield of 0.063 determined with reference to coumarin **1** in MeCN.[13] Introduction of Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} up

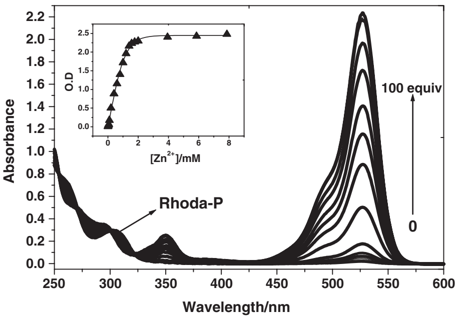


Figure 2. Spectrophotometric titration of Rhoda-P (2.0×10^{-5} M) with Zn^{2+} ($0–2.0 \times 10^{-3}$ M) in CH_3CN. Inset: Absorption plot of Rhoda-P against increasing concentration of Zn^{2+} at 528 nm.

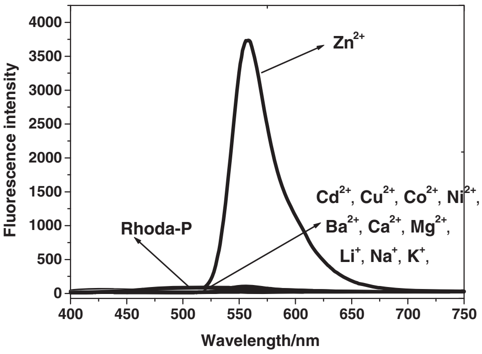


Figure 3. Fluorescence spectra of Rhoda-P (1.0×10^{-5} M) in the absence and presence of selected metal perchlorates (1.5×10^{-3} M) in CH_3CN ($\lambda_{ex} = 320$ nm).

to 3×10^{-3} M induced relatively insignificant (≤ 2 fold) emission enhancements (Figure 3). On the other hand, the fluorescence intensity increased linearly with increasing Zn^{2+} concentration. At a limiting concentration of 1.5×10^{-3} M of Zn^{2+}, a dramatic emission enhancement of ca. 160-fold was observed.[11] This is one of the highest fluorescence modulations among the known Zn^{2+} probes. The Job plot indicated 1:1 binding stoichiometry,[11] and from the fluorimetric titration, the detection limit of Zn^{2+} was calculated to be 4.78×10^{-7} M.[11]

In order to validate the binding selectivity of Rhoda-P toward Zn^{2+}, we performed a competitive fluorescence spectral measurement in the presence of different metal ions. No detectable perturbations in the emission profile of Rhoda-P containing Zn^{2+} (1.5×10^{-3} M) were observed upon adding 1.5×10^{-2} M each of Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+}. This experiment clearly implies strong complexing ability of Rhoda-P only toward Zn^{2+} even in the presence of relatively higher concentrations of other added metal ions. Presumably, factors such as moderately hard nature and smaller ionic radii of Zn^{2+} could in part account for its relatively stronger affinity toward the moderately hard "amide and pyridyl" chelating site of the probe.

In conclusion, easily accessible Rhoda-P offers naked eye detection as well as remarkably high "turn-on" signaling capability for the detection of Zn^{2+}. Importantly, coordinatively competing Ba^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+} do not cause significant optical perturbations, thus excluding any potential interferences from these ions. These attributes augur well for future work aimed at modifying the probe to make it compatible for physiological detection of Zn^{2+}.

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11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html. Supporting Information includes synthesis of Rhoda-P, binding stoichiometry determination by Job plot, spectrofluorimetric titration graph, detection limit determination, and competitive experimental graph.

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