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Colorimetric and orange light-emitting fluorescent probe for pyrophosphate in water

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ABSTRACT

A dual-mode probe based on a benzothiazolium hemicyanine chromophore was designed and synthesized for the detection of pyrophosphate (PPi) in water. The use of a fluorescent probe for colorimetric and long-wavelength fluorescence detection of PPi could be suitable for both rapid in-field and bioimaging experiments.

Pyrophosphate (PPi) is involved in a number of important cellular metabolic processes such as DNA and RNA polymerization reactions and ATP hydrolysis.[1] In addition, researchers have recently reported a relationship between PPi concentration and cancer.[2] In this regard, there is a growing interest in using different detection methods to monitor PPi in biological processes, with fluorescence techniques being the most popular.[3–5] Although many fluorescent probes for PPi have already been reported, both by our group and others,[6–11] there is a continuous need for the development of novel probes for this species that can facilitate the study of biological processes involving its release.

It is well known that a fluorophore with near infrared (NIR) or long-wavelength emission is desirable for the detection of cellular PPi because of its favorable cell penetrating ability and the minimal overlap with the wavelengths at which cells exhibit autofluorescence.[12,13] In addition, a probe that can detect PPi using a colorimetric method could facilitate rapid in-field analysis without sophisticated instrumentation.[14] Currently, there is considerable interest in developing chemosensors that emit at longer wavelengths and can be visualized with the naked eye.[15–17] However, there are only a few successful PPi sensors reported to date.[18,19] Herein, we report a colorimetric and orange light-emitting fluorescent probe for the detection of PPi in cells.

It was expected that benzothiazolium hemicyanine dye would be a suitable signaling unit for use in biological applications because of its cell permeability, non-toxicity, and long-wavelength emission ($\lambda_{em} \sim 560$ nm).[20] Thus, by combining this with a Zn(II)·2,2′-dipycolylamine (DPA) complex as a binding site, an effective fluorescent probe for PPi could be achieved (Fig. 1).[4,7] Treatment of 4-hydroxybenzaldehyde with DPA in the presence of formaldehyde gave compound **2**. The benzothiazolium hemicyanine fluorophore was then introduced by Knoevenagel condensation with 3-ethyl-2-methylbenzothiazolium iodide. Sensor **1**·2Zn(II) was obtained by the addition of $Zn(NO_3)_2$ to a solution of **1** in DMSO (See Supplementary Material).

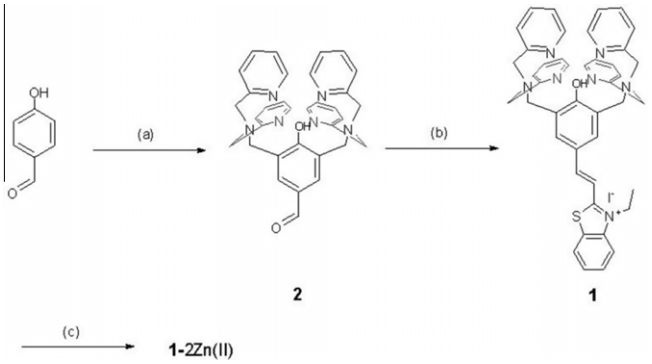

Figure 1. Synthesis of **1**·2Zn(II): (a) 2,2′−Dipicolylamine, aq formaldehyde, 1,4-dioxane, 110 °C, 2 days, 48% yield (b) 3-Ethyl-2-methylbenzothiazolium iodide, pyridine, EtOH, 80 °C, 1 day, 12% yield (c) $Zn(NO_3)_2$, DMSO, RT, 30 min, quantitative yield.

Photophysical properties of **1**·2Zn(II) in the presence of PPi were monitored by UV−vis and fluorescence spectrometry. UV−vis

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titration of **1**·2Zn(II) revealed that the maximum intensity wavelength (λ = 454 nm) underwent a 27 nm bathochromic shift with an increased intensity upon the addition of PPi in 10 mM HEPES buffer (pH 7.4). The intensity of the fluorescence emission of **1**·2Zn(II) at 548 nm was relatively weak (Φ_f = 0.08), whereas the addition of 2 equiv. of PPi caused a prominent increase (ca. 5 times) in fluorescence centered at 558 nm (Φ_f = 0.10)[21] (Fig. 2A). The fluorescence intensity exhibited a sharp increase upon the addition of an increasing amount of PPi to the **1**·2Zn(II) solution, with the maximum intensity being exhibited when 1 equiv of PPi was added (Fig. 2B). The binding constant was estimated to be 4.4×10^7 M^{-1} (See Supplementary Material).

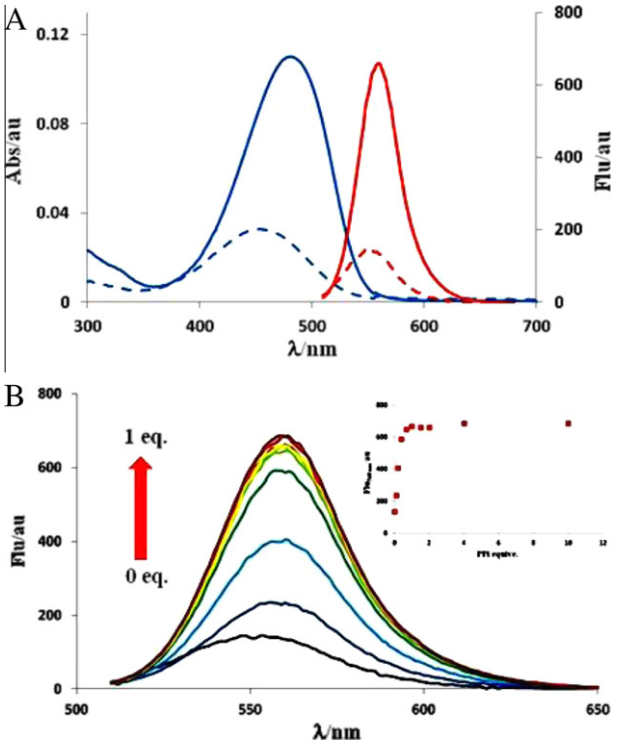

Figure 2. (A) UV–vis absorption (blue dashed line) and fluorescence emission (red dashed line) spectra of **1**·2Zn(II) (10 μM) in the presence of PPi (2 equiv., solid line) in 10 mM HEPES buffer (pH 7.4). (B) Fluorescence emission intensity of **1**·2Zn(II) (10 μM) in various concentrations of PPi (1 ~ 100 μM, pH 7.4, 10 mM HEPES buffer). Inset shows increasing fluorescence intensities of **1**·2Zn(II), which are measured at 560 nm with excitation at 500 nm.

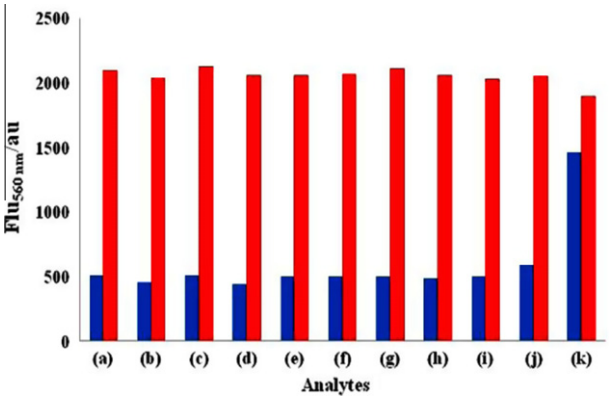

Figure 3. Fluorescence emission intensity of **1**·2Zn(II) (5 μM, pH 7.4, 10 mM HEPES buffer) in the presence of (a) probe only, (b) Pi, (c) CN^-, (d) citrate, (e) Cl^-, (f) F^-, (g) HCO_3^-, (h) OAc^-, (i) N_3^-, (j) AMP, (k) ATP. Blue bars represent the addition of analytes (20 equiv.). Red bars represent the subsequent addition of PPi (2 equiv.) to the mixture. All data were measured using a fluorescence spectrometer at 560 nm with excitation at 500 nm.

To evaluate the selectivity of **1**·2Zn(II) toward PPi, various biological competitive analytes were prepared. As expected, there were no significant changes in the fluorescence intensity upon the addition of an excess amount of phosphate (Pi), CN^-, citrate, Cl^-, F^-, HCO_3^-, OAc^-, N_3^-, and AMP, whereas a detectable response was observed due to ATP. This competitive experiment clearly demonstrated that **1**·2Zn(II) has a high selectivity for PPi over many other biological competitive analytes, apart from nucleoside triphosphates (NTPs) that are present in cells (Fig. 3).

Furthermore, the selective recognition of PPi by **1**·2Zn(II) can be visualized by the naked eye owing to the colorimetric change. Solutions of **1**·2Zn(II) alone and those of it mixed with other analytes, except PPi and ATP, in 10 mM HEPES buffer, appeared yellow,

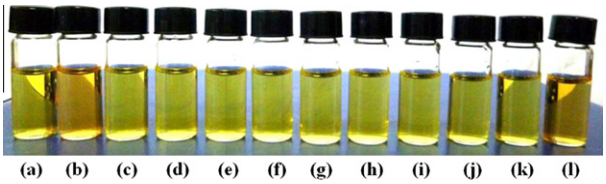

Figure 4. Colorimetric detection of **1**·2Zn(II) (5 μM, pH 7.4, 10 mM HEPES buffer) in the presence of (a) probe only, (b) PPi, (c) PO_4^{3-}, (d) CN^-, (e) citrate, (f) Cl^-, (g) F^-, (h) HCO_3^-, (i) OAc^-, (j) N_3^-, (k) AMP, (l) ATP. 20 equiv. of analyte were added except for PPi (2 equiv.).

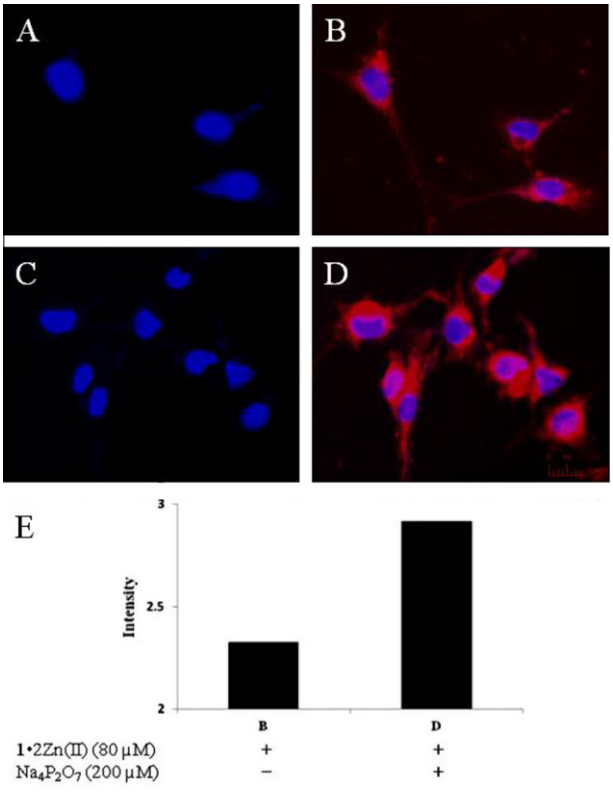

Figure 5. Fluorescence live-cell pseudo-color images of C2C12 myoblast cells. (A and C) Cells stained by Hoechst nuclear dye. (B) Cells incubated with Hoechst nuclear dye and then with **1**·2Zn(II) (80 μM) for 30 min. (D) Cells incubated with Hoechst nuclear dye followed by **1**·2Zn(II) (80 μM) for 30 min, and subsequently, with PPi ($Na_4P_2O_7$, 200 μM) for 30 min. Emission was collected at (A, C) blue channel and (B, D) Cy3 channel upon excitation at (A, C) 350 ± 25 nm (200 W metal halide arc lamp) and (B, D) 543 ± 11 nm (200 W metal halide arc lamp). All images were acquired using a 20× objective. (E) Quantification data from (B) and (D).

whereas mixtures of **1**·2Zn(II) and either PPi or ATP were pale brown. Therefore, it is clear that **1**·2Zn(II) can distinguish PPi or ATP from other anions (Fig. 4). The color change may result from the observable bathochromic shift[22,23] of **1**·2Zn(II) triggered by the addition of either PPi or ATP (Fig. 2A).

To determine whether **1**·2Zn(II) was suitable for use in biological applications, in vitro testing using the C2C12 myoblast cell line was carried out (Fig. 5). The cells were incubated with **1**·2Zn(II) (80 μM) for 30 min, and subsequently with PPi ($Na_4P_2O_7$, 200 μM) for 30 min. Prior to the addition of PPi, the cells showed only a weak level of fluorescence (Fig. 5B),[24] however, a clear intracellular fluorescence increase was observed after the addition (Fig. 5D). The viability of the cells after treatment with the probe was verified using the Hoechst nuclear stain (Fig. 5A, C).[25] These results show the potential for using **1**·2Zn(II) for the detection of PPi within cells.

In conclusion, we have developed a highly selective colorimetric and fluorescent probe for PPi. This probe can be applied in biological fluorescence imaging, and its orange emitting light and good cell permeability make it highly desirable for other bio-studies. This would be attractive to biological and medical researchers who are studying the biological roles and diagnostic relevance of PPi.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.08.018.

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24. We believe that the observed weak fluorescence prior to PPi addition may be due to the presence of cellular PPi and NTPs.
25. The cell imaging experiments with lower concentrations of **1**·2Zn(II) (⩽40 μM) were not successful as the fluorescence was not high enough for monitoring the fluorescence differences. After treatment with higher concentrations of **1**·2Zn(II) (⩾160 μM) and PPi (⩾400 μM), denaturation of cells was occasionally observed.