Tetrahedron Letters 53 (2012) 6598–6601

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

A selective fluorescent probe for the detection of mercury (II) in aqueous media and its applications in living cells

Olimpo García-Beltrán [a,b,e,*], Natalia Mena [c,e], Tania A. Berríos [d], Enrique A. Castro [d], Bruce K. Cassels [a,e], Marco T. Núñez [c,e], Margarita E. Aliaga [d,*]

[a] Department of Chemistry, Faculty of Sciences, University of Chile, Santiago 7800024, Chile
[b] Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andrés Bello, Avenida República 275, Piso 3, Santiago, Chile
[c] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[d] Facultad de Química, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago 6094411, Chile
[e] Institute for Cell Dynamics and Biotechnology, University of Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 9 September 2012
Revised 21 September 2012
Accepted 24 September 2012
Available online 29 September 2012

Keywords:
Hg^{2+}-selectivity
Fluorescent probe
Coumarin derivatives
Biothiols

ABSTRACT

In this Letter we present a new probe, 2-amino-3-hydroxy-2-(hydroxymethyl)propyl 2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetate (**PMR**), which can reversibly detect mercuric ions (Hg^{2+}) in HEPES buffer under physiological conditions. Possible interference with other analytes was examined. **PMR** displays a highly selective decrease of its fluorescence at 460 nm when it reacts with Hg^{2+}. Interestingly, the probe can also be used as a fluorescent turn-on sensor for biologically relevant thiols such as glutathione and cysteine. **PMR** can be used to determine mercury in living cells.

The detection of heavy and transition metal ions is a task that is attracting a considerable amount of attention,[1] principally for biological or environmental applications. Among the heavy metals, mercury is considered one of the most toxic and dangerous elements; specifically, in its +2 state it has a very high affinity for sulfhydryl groups such as those present in enzymes, other proteins and endogenous thiols.[2] Mercuric ions can easily pass through biological membranes and cause serious damage to the central nervous and endocrine systems.[3] Thus, considerable efforts have been made to synthesize probes that can detect these ions in a selective and sensitive manner. Probes based on changes in fluorescence induced by such ions are particularly attractive and are one of the first choices because of the simplicity of their use and the low detection limit of fluorescence.[4]

Recently, many sensitive fluorescent probes based on rhodamine,[5] coumarin[6] or squaraine derivatives,[7] as well as other fluorophores,[8] have been developed to detect mercury ions. It is noteworthy that most of these only work in organic solvents, which severely limits their potential for biological and environmental applications. A few of them have been reported as water-soluble,[5a,8b,9] but the development of highly selective probes for Hg(II) detection in aqueous solution with little or no interference from other metal ions is still a highly desirable goal.

Herein we report a new probe, 2-amino-3-hydroxy-2-(hydroxymethyl)propyl 2-(7-hydroxy-2-oxo-2*H*-chromen-4-yl)acetate (**PMR**), which is water soluble and has the desired photophysical features. **PMR** was prepared through a three-step (Scheme S1; Supplementary data) process and was characterized by ^{1}H NMR and ^{13}C NMR spectroscopy (Figs. S3 (A) and (B)).

The absorption spectrum of **PMR** shows a maximum at 330 nm and a shoulder near 375 nm (Fig. S4; Supplementary data), a molar extinction coefficient (ε) of 14460 M^{-1} cm^{-1} and an emission band at 460 nm (Fig. S5; Supplementary data). The quantum yield of this probe is 0.32, with a lifetime (τ) of 5.86 ns and a Stokes shift of 8022 cm^{-1}. Figure 1 shows the Hg^{2+} concentration-dependent emission fluorescence spectra of **PMR** (20 μM). When excited at 330 nm, the emission fluorescence intensity at 460 nm decreases about eightfold upon increasing the concentration of Hg^{2+} from 0 to 100 μM (Fig. 1). A good linear relationship between fluorescence intensity and Hg^{2+} concentration was observed, with a correlation coefficient as high as 0.9916 (Inset to Fig. 1). According to these results, the detection limit of **PMR** for Hg^{2+} is 1.1×10^{-9} M. This result is comparable to those reported for other probes[7b,10] and suggests the use of **PMR** in biological and environmental applications.

The changes observed in the fluorescence intensity of **PMR** after the addition of Hg^{2+} ions is presumably due to the chelation of

* Corresponding authors. Tel.: +56 2 978 7253; fax: +56 2 271 3888.
E-mail addresses: ojgarciab@ug.uchile.cl (O. García-Beltrán), mealiaga@uc.cl (M.E. Aliaga).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.09.111

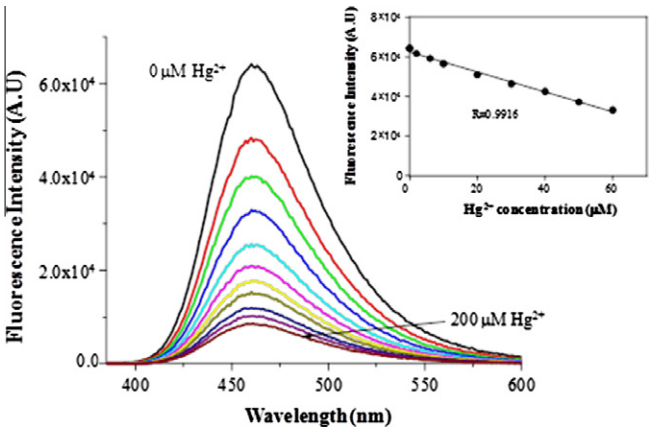


Figure 1. Fluorescence emission spectra (λ_{ex} = 330 nm) of **PMR** (20 μM) upon addition of $HgCl_2$ (0–200 μM) in 20 mM HEPES buffer, pH 7.4. Inset shows the relationship between fluorescence intensity and Hg^{2+} concentration (2–60 μM).

these ions by the oxygen (hydroxyl and coumarin carbonyl) and nitrogen atoms of the probe. Typically, when heavy metals such as Hg^{2+} are chelated by heteroatoms in close proximity to a fluorophore, they quench the fluorescence by enhanced spin-orbit coupling.[11]

The stability constant K_a of the **PMR**-Hg^{2+} interaction was determined using the Benesi-Hildebrand equation, which gives a value of 1.29×10^4 M^{-1} (Fig. S6; Supplementary data). The binding stoichiometry of the **PMR**-Hg^{2+} complex was determined from the Job plot (Fig. S7; Supplementary data). Maximum emission intensity was observed when the mole fraction of Hg^{2+} was close to 0.5, which indicates the formation of a 1:1 complex between **PMR** and Hg^{2+} with a total concentration of 100 μM. This was further confirmed by the appearance of a peak at *m/z* 524.0482 assignable to $[PMR+Hg(II)]^+$ in the ESI spectrum (Fig. S8; Supplementary data). A possible binding mode between **PMR** and Hg^{2+} is proposed in Scheme 1. This was tested by ^1H NMR spectroscopy of the probe in the absence and presence of Hg^{2+}. Upon addition of Hg^{2+}, the recognizable CH_2 resonances of the Tris moiety (δ 4.10 and 3.78) undergo slight downfield shifts (between δ 4.20 and 3.92, respectively, with considerable broadening). The OH proton resonance (δ 4.82) is shifted upfield (δ 4.40) and a signal appears at δ 3.10, assigned to the NH_2 protons which in the **PMR** spectrum are obscured by the H_2O signal in standard conditions (Fig. S9; Supplementary data). Surprisingly, however, the coumarin signals are practically unchanged.

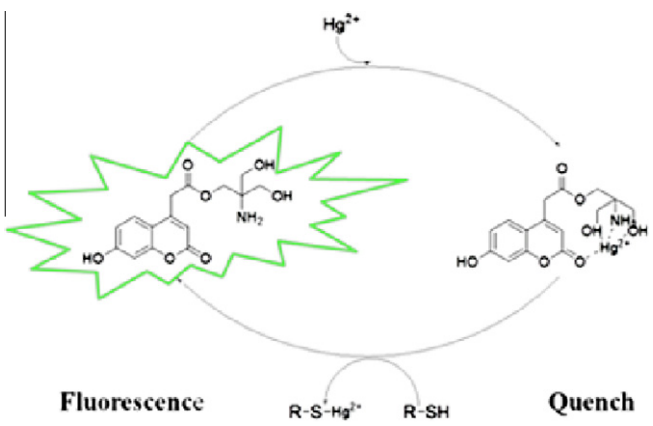
In addition, kinetic studies of the response of **PMR** to Hg^{2+} were performed using a spectrofluorimeter under pseudo-first order reaction conditions (10 μM **PMR** and 100–800 μM $HgCl_2$ in HEPES buffer) and an ionic strength of 0.2 M (maintained with $NaNO_3$) at 30 °C. The time-dependent response of **PMR** to mercury ions was monitored using fluorescence spectroscopy (Fig. 2A). The observed rate constants (k_{obs}) were found to vary from 1.4×10^{-3} s^{-1} to 3.0×10^{-3} s^{-1} for 100 μM and 800 μM Hg^{2+}, respectively, as shown in Figure 2B.

In order to evaluate the selectivity of the PMR probe, different metal ions (Hg^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+} or Cd^{2+}) were tested. Among these ions, only Hg^{2+} significantly affected the fluorescence intensity of PMR (Fig. 3).

To further check the practical applicability of the **PMR** probe as a Hg^{2+}-selective fluorescent sensor, we carried out competitive experiments in the presence of 20 μM Hg^{2+}, mixed with other metal ions at 200 μM. As shown in Figure 4, these experiments demonstrate that none of the selected ions interfere to any obvious extent with the detection of Hg^{2+}.

As is well known, reversibility is an important feature of excellent chemical probes. Thus, experiments were conducted by adding glutathione (GSH) to examine the reversibility of the **PMR**-Hg^{2+} interaction (Fig. 5A). This figure clearly shows that the fluorescence intensity of a solution containing **PMR** and Hg^{2+} increases with the addition of GSH. The latter can be explained considering the high affinity of the sulfhydryl group of GSH towards Hg^{2+};[12] thus, this biothiol can remove the Hg^{2+} ions bound to the probe and release the latter, leading to the recovery of the fluorescence quenched by Hg^{2+} (Fig. 5A). Similar results were obtained when cysteine (Cys), another biothiol, was used instead of GSH (not shown). We also observed this behaviour when the test was performed in SH-SY5Y human neuroblastoma cells. **PMR** permeates into the living cells and is thus suited for fluorescence imaging of Hg^{2+} in such systems. Thus, cultured SH-SY5Y cells were incubated with 5 μM **PMR** for 20 min at room temperature, and a significant **PMR** fluorescence was detected in the interior of the cells (Fig. 5B). However, when 10 μM $HgCl_2$ was included in the incubation medium for another 3 h, a loss of fluorescence from the intracellular area was evident (Fig. 5C). Interestingly, an increment of fluorescence was observed when the cells previously exposed to $HgCl_2$ were treated with 20 mM *N*-acetylcysteine (NAC) (Fig. 5D). This result is consistent with that presented in Figure 5(A) and is based on the ability of NAC to increase GSH levels.

In conclusion, we have developed a new, highly sensitive and selective 'turn-off' fluorescent probe for mercuric ion detection in an aqueous environment. The method proved to be simple, selective and sensitive. Most importantly, this probe was successfully applied to the imaging of Hg^{2+} ions in cells.



Scheme 1. Possible binding mode of PMR with Hg^{2+}.

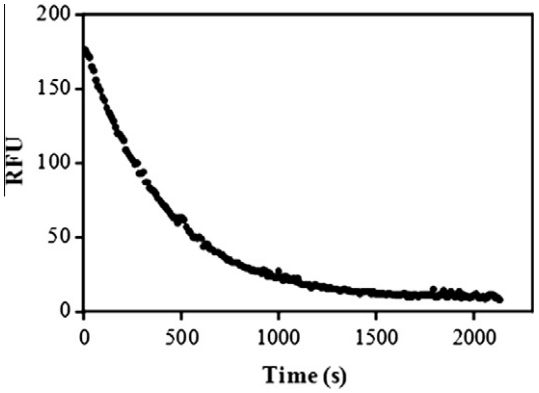


Figure 2A. Kinetic profile for the interaction between **PMR** (10 μM) and Hg^{2+} (600 μM). Results are expressed as Relative Fluorescence Units (RFU) at λ_{em} = 460 nm.