A chemodosimeter approach to fluorescent sensing and imaging of inorganic and methylmercury species†

Mithun Santra,[a] Dowook Ryu,[a] Amrita Chatterjee,[a] Sung-Kyun Ko,[b] Injae Shin*[b] and Kyo Han Ahn*[a]

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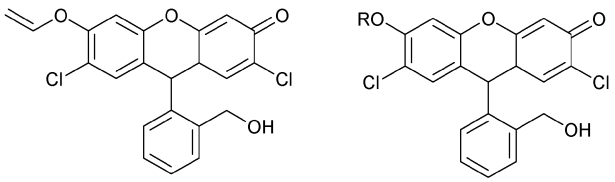
A highly sensitive fluorescent turn-on probe specific for methylmercury species as well as inorganic mercury ions has been developed on the basis of mercury ion-promoted hydrolysis of a fluorescein-derived aryl vinyl ether.

Mercury is a highly poisonous element and widespread pollutant, occurring from natural and anthropogenic sources. Organic forms of mercury, typically, methylmercury species (CH_3HgX; X = halides, *etc.*) are much more toxic than inorganic mercury species (HgX_2).[1] Because of their lipid solubility, methylmercury species readily cross the blood–brain barrier, accumulate in the brain, and cause damage to the central nervous system,[1,2] as well as other organs. The epidemics of Minamata Bay in Japan demonstrated the fatal threat of methylmercury to human health.[3]

A variety of fluorescent molecular probes for the detection of mercury ions with ease and high sensitivity have flourished in recent years.[4] Surprisingly, most of these probes only sense inorganic mercury ions and those that sense methylmercury are rare.[5] Furthermore, none of the fluorescent probes can be applied to evaluate the accumulation of methylmercury in living species. For example, the rhodamine-based fluorescent chemodosimeter reported by Tae and co-workers shows high sensitivity and selectivity towards inorganic mercury ions, and has been used to monitor them both in cells and in a living vertebrate organism. However, the chemodosimeter showed a weak response to CH_3HgCl in contrast to $HgCl_2$.[4g,k]

One plausible reason for the low sensitivity of the heteroatom-based molecular probes towards methylmercury may arise from the weak molecular interactions between them. To develop a fluorescent probe for the notoriously poisonous methylmercury ions, we have designed a chemodosimeter based on a chemical reaction that avoids the popular mercury ion-coordination to heteroatoms.[6] The chemodosimeter thus designed is fluorescein-based vinyl ether, **1**, which reacts only with organomercury and inorganic mercury ions in the presence of other metal ions, and shows a "turn-on"-type fluorescent response with high sensitivity. The fluorescent imaging of mammalian cells and vertebrate organisms treated with methylmercury is also demonstrated for the first time.

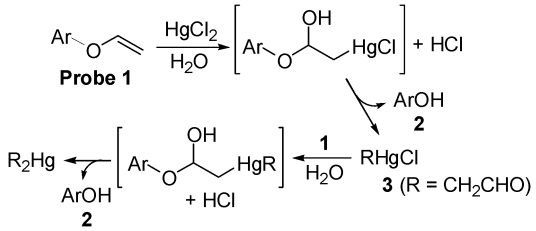
The design of probe **1** is based on the mercury ion-promoted hydrolysis reaction of vinyl ethers: the oxymercuration of vinyl ether **1** generates the corresponding hemiacetal intermediate, which undergoes fragmentation into alcohol **2** and aldehyde **3** (Scheme 1).



Such a mercury ion-promoted hydrolysis of vinyl ether **1** is also expected to occur with methylmercury species (CH_3HgX), because a similar oxymercuration intermediate is envisioned on the basis of the reaction mechanism. Because vinyl ether **1** is very weakly fluorescent, but alcohol **2** is highly fluorescent, we may realize fluorescent turn-on sensing of methylmercury as well as inorganic mercury ions in aqueous media.

The required vinyl ether **1** was directly synthesized from known compound **4** through deallylation and vinylation steps.[7]

A solution of probe **1** (5.0 μM) in PBS buffer (pH 7.4) containing 5% DMSO became strongly fluorescent upon addition of $HgCl_2$ (Fig. 1(a)), as it is converted to fluorescein **2** (Φ_F = 0.89)[7a] through hydrolysis. The generation of **2** was confirmed by isolation and characterization of the final product after titration. Importantly, the titration of probe **1** with $HgCl_2$ (from 0 to 1.0 equiv.) showed saturation behavior at 0.5 equiv. of $HgCl_2$ (Fig. 1(b)), which can be explained by the mechanism in Scheme 1. The mechanism suggests that an organomercury species such as **3** also promotes the hydrolysis.



Scheme 1 A plausible hydrolysis mechanism of probe **1** by $HgCl_2$ and RHgCl in water.

[a] *Department of Chemistry and Center for Electro-Photo Behaviors in Advance Molecular Systems, POSTECH, San 31 Hyoja-dong, Pohang, 790-784, Republic of Korea. E-mail: ahn@postech.ac.kr*
[b] *Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea. E-mail: injae@yonsei.ac.kr*

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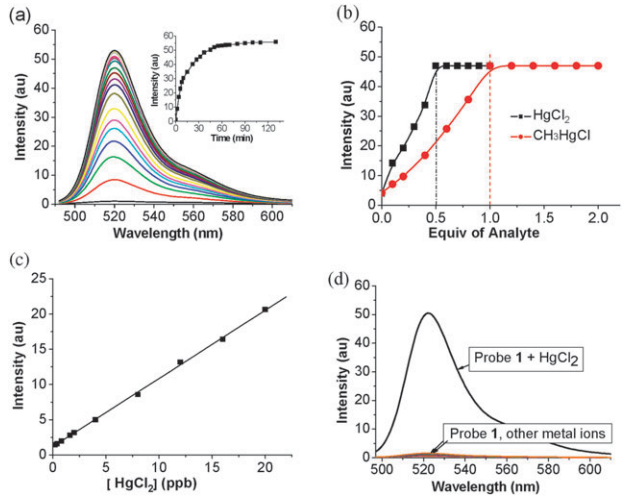

Fig. 1 (a) The time-dependent fluorescence change acquired for a 2 : 1 mixture of probe **1** and $HgCl_2$; inset: a plot of the fluorescence intensity change as a function of the reaction time. (b) The fluorescence intensity change of probe **1** as a function of equiv. of $HgCl_2$ (■) and CH_3HgCl (●), taken after 10 min for each addition. (c) A plot of fluorescence intensity *vs.* [$HgCl_2$] obtained for a 2 : 1 mixture of **1** and $HgCl_2$ for the range of 0.25–20 ppb [$HgCl_2$], taken after 1 h of each mixing. (d) The fluorescence change after 1 h acquired for a 2 : 1 mixture of probe **1** and various metal ions (Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ag^{+} and Hg^{2+}). All measurements were taken with 5.0 μM of probe **1** in PBS buffer (pH 7.4) containing 5% DMSO (excitation at 480 nm; the intensity was estimated by the peak height at λ = 520 nm).

Certainly, probe **1** shows fluorescence recovery upon treatment with CH_3HgCl in the PBS buffer.† In this case, the fluorescence saturation is reached with 1.0 equiv. of CH_3HgCl (Fig. 1(b)), which can be also explained by the mechanism.

Similar fluorescence titrations at different pH conditions (pH 4.0–9.0) were also carried out.† In the absence of $HgCl_2$, probe **1** itself did not show any fluorescence change at pH 4.0, even after 24 h, which indicates that the observed fluorescence change is mainly due to catalysis by mercury species. Comparing the fluorescence recovery time for the cases of $HgCl_2$ and CH_3HgCl, the former reacts with probe **1** a little faster than the latter.

When 1 equiv. of $HgCl_2$ was added to probe **1** in the PBS buffer, the fluorescence saturation took about 1 h (about 70% recovery after 10 min), while it took about 1.5 h in the case of CH_3HgCl under the same conditions.† However, it is not necessary to wait for the full recovery of fluorescence for quantification purposes, as we can obtain a linear relationship between the concentration of mercury ions and the fluorescence intensity over an arbitrary time span. A linear relationship between the fluorescence intensity and the concentration of $HgCl_2$ is obtained for a wide concentration range (1.0×10^{-9}–1.0×10^{-5} M; 0.2–2000 ppb, a lower concentration part of which is shown in Fig. 1(c)), and a detection limit of below 1 ppb level is obtained.†‡ If an assay of fast and full response is needed, the molar ratio of the probe to the mercury ions can be increased, because an excess amount of the probe gives negligible fluorescence and hence does not affect quantification of the data. This is an advantageous property of probe **1**.

Probe **1** is specific towards mercury ions, while other metal ions (Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Ag^{+}) show essentially no fluorescence change, owing to little hydrolysis of the vinyl ether with these metals (Fig. 1(d)). Also, probe **1** shows full a fluorescence response to the mercury ions, even in the presence of all other metal ions.† Other mercury compounds, such as $Hg(OAc)_2$, gave the same results.

Next, we evaluated the effectiveness of probe **1** for fluorescence monitoring of both CH_3HgCl-contaminated cells and a living vertebrate organism, zebrafish. Human lung cancer cells (A549 cells) incubated with probe **1** and CH_3HgCl clearly show green fluorescence, indicating that probe **1** can enter cell membranes and react with the organomercury species to form fluorescent **2** (Fig. 2(a)).

However, the cells do not exhibit fluorescence in the absence of the external organomercury species (Fig. 2(b)). We explored further whether **1** could be used to monitor organomercury species in living organisms. For this study, a three-day-old zebrafish was exposed to **1** in the presence and absence of CH_3HgCl. The results of the fluorescence microscopy analysis show that the organomercury species in zebrafish is fluorescently detected by **1** (Fig. 2(c) and (d)). Encouraged by this result, we analyzed methylmercury accumulation in grown zebrafish with probe **1**. Interestingly, the methylmercury accumulated in the eye, heart, fin, gall bladder, and eggs, but not in the brain and liver under the experimental conditions (Fig. 3). These preliminary *in vivo* studies clearly demonstrate that the probe has potential for studying the accumulation of methylmercury species in other cells and organisms.

In summary, we have devised a structurally simple yet efficient fluorescent probe for organomercury as well as inorganic mercury ions. The vinyl ether probe **1** thus shows specific response to the mercury species and turn-on fluorescence with high sensitivity, as the vinyl ether undergoes mercury ion-promoted hydrolysis to give the strongly fluorescent **2**. With this probe, the fluorescent imaging of mammalian cells and organisms incubated with organomercury species has been demonstrated for the first time.

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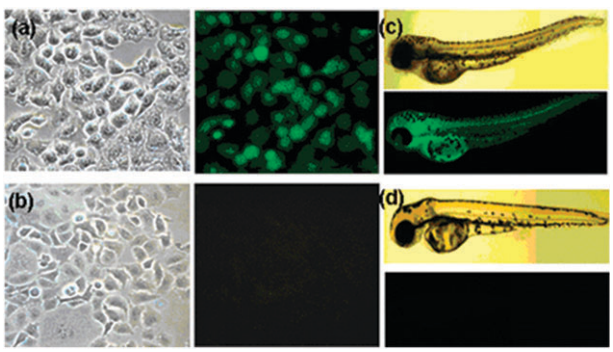

Fig. 2 Cells and organisms incubated with 50 mM probe **1** and 100 mM CH_3HgCl in 5% CH_3CN–water. Images of A549 cells (a) in the presence and (b) absence of CH_3HgCl. Images of three-day-old zebrafish (c) in the presence and (d) absence of CH_3HgCl.

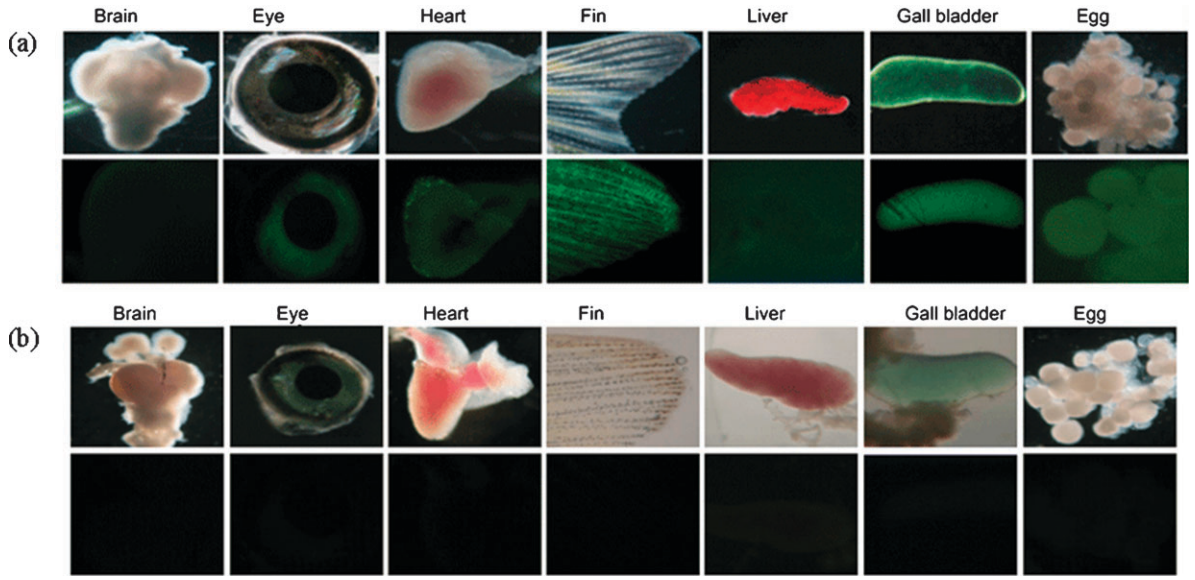

Fig. 3 (a) Images of zebrafish organs treated with 200 nM of CH_3HgCl and 50 μM of probe **1** (top: microscope images, bottom: fluorescence images). (b) Images of zebrafish organs treated with 50 μM of probe **1** in the absence of external CH_3HgCl (top: microscope images, bottom: fluorescence images).

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‡ The maximum concentration level of mercury ions in drinking water set by the US EPA is 2 ppb.

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