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Fluorescence turn-on chemodosimeter for rapid detection of mercury (II) ions in aqueous solution and blood from mice with toxicosis

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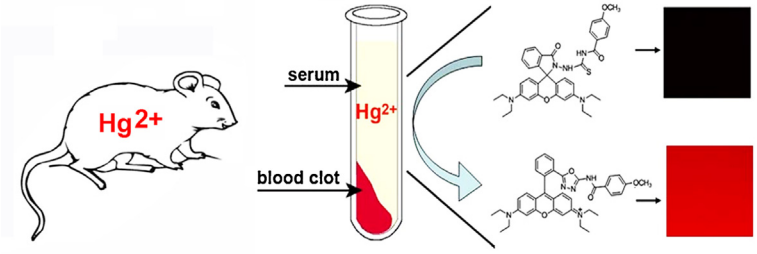
HIGHLIGHTS

- A new fluorescence probe for detection of Hg^{2+} ions was developed.
- The probe can monitor Hg^{2+} ions in aqueous media and in blood in ppb level.
- The probe facilitates naked-eye detection of Hg^{2+} ions.

GRAPHICAL ABSTRACT

A rhodamine-based turn-on fluorescence probe can monitor the blood Hg^{2+} ions in toxicosis mice with high sensitivity and selectivity, and facilitates naked-eye detection of Hg^{2+} ions.



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ABSTRACT

The heavy metal mercury (Hg) is a threat to the health of people and wildlife in many environments. Among various chemical forms, Hg^{2+} salts are usually more toxic than their counterparts because of their greater solubility in water; thus, they are more readily absorbed from the gastrointestinal tract into circulation. Therefore, new chemical receptors for detecting Hg^{2+} ions in circulation are needed. In this study, we developed a rhodamine-based turn-on fluorescence probe to monitor Hg^{2+} in aqueous solution and in blood of mice with toxicosis. The chemodosimeter responds to Hg^{2+} ions stoichiometrically, rapidly, and irreversibly at room temperature as a result of a chemical reaction that produces strongly fluorescent oxadiazole. The new fluorescent probe shows good fluorescence response, with high sensitivity and selectivity, toward Hg^{2+} ions in aqueous solution and in blood from mice with toxicosis and facilitates the naked-eye detection of Hg^{2+} ions.

1. Introduction

Mercury (Hg), one of the most toxic heavy metals, is released into the environment from volcanic emission, gold mining, solid waste incineration, and combustion of fossil fuels. It poses a huge threat to human beings and the environment. It exists in various forms: metallic, ionic, and as a part of organic salts and complexes. Mercuric ion (Hg^{2+}), one of the most stable inorganic forms, can be transformed by microbial biomethylation in the environment into methylmercury, which then bioaccumulates through the food chain. Even exposure to low doses of Hg may lead to digestive, kidney and especially neurological diseases [1–3]. Therefore, a convenient and rapid method to detect Hg is desirable; especially, highly sensitive and selective detection and imaging of Hg^{2+} ions in tissues and organisms is crucial.

Although sophisticated analytical techniques, including atomic absorption, atomic emission and inductively coupled plasma spectroscopy, are currently used for Hg detection, inexpensive and

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real-time monitoring methods are needed for detecting Hg^{2+}. Various fluorescent and colorimetric Hg^{2+} chemosensors based on small molecules [4–8], conjugated polymers [9–11], nanoparticles [12–21], and biomolecules [22–25] have been reported. However, these probes have low solubility in aqueous media, unsatisfactory sensitivity and selectivity for detection at the parts-per-billion level, narrow pH span, slow response time, poor cell permeability or difficulty in transforming to the corresponding cell-permeable forms, poor accessibility due to expensive and complex preparation.

Given the US Environmental Protection Agency (EPA)-mandated limit of 2 ppb (10 nM) for inorganic Hg^{2+} in drinking water, new probes with detection limits in the parts-per-billion to low parts-per-billion range are required to analyze natural water samples. In addition, estimating the normal level of toxic metals in the human body is important. Individuals vary substantially in their blood concentrations of Hg depending on age, sex and residence; for instance, coastal residents show significantly higher mean total Hg levels in blood (80.5 nmol L^{-1}, range 4.5–924.5 nmol L^{-1}) than inland residents (59 nmol L^{-1}, range 4–733 nmol L^{-1}) [26]. The Hg concentration in blood in Cambodians ranges from 26 to 290 nmol L^{-1}, lower than that in Hg-contaminated or high fish-intake regions [27]. The mean Hg level in blood in normal volunteers in Tehran was 42.4 ± 22.1 nmol L^{-1} [28]. In Sweden, 10–50 nmol L^{-1} Hg in whole blood is used as normal range in risk assessments with Hg exposure [29]. Therefore, developing simple Hg^{2+} ion sensing schemes to improve assessment is important.

The rhodamine framework is an ideal mode for constructing off–on fluorescent and chromophoric chemosensors because of the large molar extinction coefficient (ε), high fluorescence quantum yield (Φ) and long absorption and emission wavelength elongated to the visible region [5,30–45,]. As well, because Hg^{2+} promotes an irreversible reaction of thiosemicarbazides to form 1,3,4-oxadiazoles, some chemodosimeters have been based on combining the spirolactam ring opening of rhodamine derivatives and the mild Hg^{2+}-promoted reaction of thiosemicarbazides [46–48]. Recently, excellent spirolactam-based chemodosimeters were developed with an Hg^{2+}-induced irreversible chemical reaction, with the detection of Hg^{2+} at parts-per-billion levels at high selectivity [49–54].

Although a variety of publications have described fluorescence imaging of Hg^{2+} ions in cells and tissues [30,50,51,53,55–59], few probes have been developed for rapid detection of Hg^{2+} in blood serum [60]. Detecting Hg^{2+} in blood from animals with toxicosis remains challenging. We developed a colorimetric and fluorescent chemodosimeter system that is highly sensitive and selective for detecting Hg^{2+} in aqueous solution and in blood from mice with toxicosis.

2. Experimental

2.1. Instrumentation

^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. Melting points were determined on an XD-4 digital micro-melting-point apparatus. Infrared (IR) spectra were recorded with use of an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). UV–vis spectra were recorded on a U-4100 UV-Vis-NIR Spectrometer (Hitachi). Fluorescent measurements were recorded on a Hitachi F-4500 fluorescence spectrophotometer.

2.2. Chemicals and reagents

Deionized water was used to determine absorption and fluorescence. All reagents were purchased from commercial suppliers and used without further purification. The solutions of metal ions were prepared from $NaNO_3$, $Mg(NO_3)_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Cr(NO_3)_3{\cdot}9H_2O$, 50% (wt.) $Mn(NO_3)_2$ (aq.), $Fe(NO_3)_3{\cdot}9H_2O$, $FeSO_4{\cdot}7H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $AgNO_3$, $Cd(NO_3)_2{\cdot}4H_2O$, $Ba(NO_3)_2$, $HgCl_2$, $Pb(NO_3)_2$, $CuCl_2{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$, $Cu(Ac)_2{\cdot}H_2O$, NaCl, NaAc and Na_2SO_4 with deionized water. All samples were prepared at room temperature, shaken for 10 s and stored for 18 h before UV–vis and fluorescence determination. Thin-layer chromatography (TLC) involved use of silica gel $60F_{254}$ plates (Merck KGaA). HEPES buffer solutions (pH 7.2) were prepared with 20 mM HEPES and the appropriate amount of aqueous sodium hydroxide with use of a pH meter.

2.3. Synthesis of N-((3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl) carbamothioyl)-4-methoxybenzamide (probe **1**)

Probe **1** was synthesized as we previously described [61]. 2-Amino-3′,6′-bis(diethylamino)spiro[isoindoline-1,9′-xanthen]-3-one (4.50 g, 9.85 mmol) was added to the solution of 4-methoxybenzoyl isothiocyanate (2.09 g, 10.81 mmol) in acetonitrile (130 mL). The mixture was refluxed for 20 min, then cooled to room temperature. After being filtered under reduced pressure, the solid was washed with cold acetonitrile, dried to give the probe **1** as a white or light pink solid: 6.26 g (97.7%); mp 237–239 °C; IR (KBr), υ: 3282, 3081, 2970, 2931, 2893, 2870, 1711, 1662, 1633, 1612, 1510, 1425, 1375, 1356, 1330, 1261, 1221, 1186, 1118, 1079, 1023, 857, 816, 785, 763, 698, 635, 606, 578 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.16 (t, 12 H, NCH_2CH_3, J = 7.0 Hz), 3.34 (q, 8H, NCH_2CH_3, J = 7.1 Hz), 3.84 (s, 3H, OCH_3), 6.33 (dd, 2H, Xanthene–H, J = 8.9, 2.1 Hz), 6.36 (d, 2H, Xanthene–H, J = 2.0 Hz), 6.78 (d, 2H, Xanthene–H, J = 7.0 Hz), 6.90 (d, 2H, Ar–H, J = 8.9 Hz), 7.14 (d, 1H, Ar–H, J = 7.1 Hz), 7.48–7.52 (m, 2H, Ar–H), 7.59 (t, 1H, Ar–H, J = 7.5 Hz), 7.67 (d, 2H, Ar–H, J = 8.8 Hz), 8.01 (d, 1H, Ar–H, J = 7.0 Hz), 8.84 (s, 1H, NH), 11.65 (s, 1H, NH) (Fig. S12, ESI); ^{13}C NMR($CDCl_3$, 100 MHz), δ (ppm): 181.4, 165.2, 164.0, 163.7, 153.8, 151.8, 149.2, 133.3, 129.7, 128.7, 128.3, 124.1, 123.7, 123.6, 114.2, 108.1, 103.8, 97.8, 66.7, 55.5, 44.4, 12.6; HRESIMS calculated for $[M+H]^+$ $C_{37}H_{40}N_5O_4S^+$: 650.2801 found: 650.2793.

2.4. Synthesis of N-(6-(diethylamino)-9-(2-(5-(4-methoxybenzamido)-1,3,4-oxadiazol-2-yl) phenyl)-3H-xanthen-3-ylidene)-N-ethylethanaminium (compound **2**)

The solution of *N*-((3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl) carbamothioyl)-4-methoxybenzamide (**1**) (650 mg, 1.0 mmol) in dichloromethane (50 mL) was added to the solution of $HgCl_2$ (543 mg, 2.0 mmol) in 30 mL water, then tetrabutylammonium bromide (67 mg, 0.2 mmol) was added. The mixture was stirred at reflux for 1.5 h, then the cooled mixture was diluted with dichloromethane (50 mL). The organic layer was washed with water and dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated to give the product (445 mg, 68.3%); mp 180–182 °C; IR (KBr), υ: 2973, 2929, 2870, 1701, 1649, 1589, 1528, 1465, 1413, 1392, 1336, 1273, 1246, 1179, 1131, 1072, 1010, 976, 921, 820, 759, 722, 682 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ (ppm): 1.30 (t, 12H, NCH_2CH_3, J = 7.2 Hz), 3.58 (q, 8H, NCH_2CH_3, J = 7.2 Hz), 3.81 (s, 3H, OCH_3), 6.76–6.78 (m, 4H, Xanthene–H), 6.90 (d, 2H, Ar–H, J = 8.8 Hz), 7.09–7.11 (m, 2H, Xanthene–H), 7.39 (d,