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Simple immobilization of mercury ion chemosensors to solid substrate

Q1 Chang Hoon Jeon[a,b], Chul Soon Park[a], Chang Soo Lee[a,b,*], Tai Hwan Ha[a,b,*]

[a] Hazards Monitoring BNT Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, South Korea
[b] Nanobiotechnology and Bioinformatics (Major), University of Science & Technology (UST), Daejeon 34141, South Korea

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ABSTRACT

To develop selective and disposable mercury chemosensors, four different mercury chelators were synthesized and grafted onto cellulose filter paper. Three colorimetric sensors were azobenzene derivatives bearing a dithiaazadioxo crown ether moiety as a core chelator for Hg^{2+} ions. The fourth chelator was a rhodamine-based *turn-on* mercury binder that responded to both methylmercury and Hg^{2+} ions. This sensor develops the typical pink color of rhodamine (colorimetric) and exhibits strong fluorescence (fluorometric). For paper immobilization, the cellulose filter paper was first primed with 3-(aminopropyl)trimethoxysilane (APTMS) so that all the four ligands were covalently immobilized, simply by soaking them in each solution.

Introduction

Because of the high toxicity of mercury ions (Hg^{2+}), sensitive detection methods have been sought to prevent human exposure, which causes health problems affecting the cardio-vascular and central nervous systems [1]. Considering the low limit for mercury in drinking water specified by Environmental Protection Agency (EPA) regulations (i.e., 2 ppb), very sensitive detection schemes, such as electrochemical voltammetry (or amperometry) [2,3], atomic absorption spectroscopy (AAS) [4], and inductively coupled plasma mass spectroscopy (ICP-MS) [5], have been developed in recent decades. These methods usually require sophisticated and expensive instrumentation and specially trained operators. The use of mercury has been extensively banned from many industrial processes, and related regulations have been tightened in most countries. However, the accumulated concentrations in marine food chains and the relatively loose regulations in developing countries still necessitate simple and cost-effective monitoring systems for mercury ions. Therefore, metal-chelating chemosensors [6,7] that change their fluorescent characteristics or exhibit unique colors upon binding with metal ions are promising alternatives to expensive instrumentation. In a previous study, we synthesized an azobenzene-based colorimetric sensor with a dithiaazadioxo crown ether as a specific binder of Hg^{2+} ions that showed selective chelating capability and typical solvato-chromic behavior in aqueous and organic solvents [8]. Here, as a practical extension of this previous investigation, mercury-binding ligands were tethered to cellulose paper to create a disposable mercury sensor based on a concept similar to that of ordinary pH paper. In addition to the dithiaazadioxo crown ether, a rhodamine-based dye with a thiosemicarbazide group [9] was newly synthesized and tethered, and its performance was compared to that of the azobenzene-based chelators.

Experimental

Instruments

^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were collected for the structural analysis of the ligands using Varian Inova 600 NB spectrometers (USA). Mass spectra were obtained using a mass spectrometer coupling electrospray ionization and a time-of-flight analyzer (ESI-MS) from Agilent Technologies Inc. 1100 + G1958 (USA). Ultraviolet/visible (UV/Vis) and fluorescence spectra were collected using a PerkinElmer VT 3000 spectrophotometer (USA) and a Scinco Fluoromate FS-2 spectrometer (South Korea), respectively.

Q2 * Corresponding authors at: Hazards Monitoring BNT Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, South Korea.
E-mail addresses: cslee@kribb.re.kr (C.S. Lee), taihwan@kribb.re.kr (T.H. Ha).

Materials

2,4-Dinitro-5-fluoroaniline (DNFA) (Japan), 4,5-difluoro-2-nitroaniline (DFNA) (Japan), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl) (China) and triethylamine (TEA) (Japen) were purchased from TCI. Sodium nitrite (Japan), rhodamine B (India), hydrazine hydrate (South Korea), 1,4-phenylene diisothiocyanate (Poland), N-hydroxysuccinimide (NHS) (USA), (3-aminopropyl)trimethoxysilane (APTMS) (USA), N,N′-dimethylformamide (DMF) (Germany), and all metal perchlorates (USA) were purchased from Sigma–Aldrich and used without further purification. Qualitative cellulose filter paper was purchased from Whatman (No. 2) (China), and solvents for NMR experiments, including chloroform-d (USA) and dimethylsulfoxide-d_6 (USA), were purchased from Cambridge Isotope Laboratories.

10-(4-((4,5-Difluoro-2-nitrophenyl)diazenyl)phenyl)-1,4-dioxa-7,13-dithia-10-azacyclo-pentadecane (L2) and 10-(4-((5-fluoro-2,4-dinitrophenyl)diazenyl)phenyl)-1,4-dioxa-7,13-dithia-10-azacyclopentadecane (L3)

Sodium nitrite (69.0 mg, 1.0 mmol) was dissolved in ice-cold water at 0 °C (10 mL) and added dropwise into another ice-cooled 40-mL solution of DFNA (174.1 mg, 1.0 mmol) or DNFA (201.0 mg, 1.0 mmol) in a mixture of H_2SO_4/acetic acid (1/3, v/v). The mixture was stirred for 20 min. The diazonium salt solution was added dropwise to a DMF solution (50 mL) of 1,4-dioxa-7,13-dithia-10-phenyl-10-azacyclopentadecane (327 mg, 1.0 mmol) [8]. After additional stirring at 0 °C for 6 h, 20 mL of $CHCl_3$ and 10 mL of water were sequentially added to the reaction vessel, and the solution was transferred to a separatory funnel. The organic layer was extracted from the aqueous layer three times, and the organic extracts were combined, dried over anhydrous $MgSO_4$, and filtered. After evaporating the solvent in vacuo, the crude product was chromatographed on silica gel using a $CHCl_3$/MeOH mixture (20/1, v/v) to yield L2 as an orange solid (353.8 mg, 69.1% yield) and L3 as a purple solid (311.5 mg, 57.8% yield). L2: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.85 (d, J = 9.2 Hz, 1H), 7.75 (dd, J = 9.0, 7.1 Hz, 1H), 7.63 (dd, J = 10.7, 7.6 Hz, 1H), 6.70 (d, J = 9.3 Hz, 1H), 3.83–3.80 (m, 1H), 3.78–3.75 (m, 1H), 3.66 (s, J = 4.2 Hz, 1H), 2.96–2.92 (m, 1H), 2.79–2.77 (m, 1H); ^{13}C NMR ($CDCl_3$, 600 MHz): δ 150.98, 143.58, 143.39, 142.70, 129.46, 126.87, 113.52, 111.61, 111.57, 106.92, 106.72, 74.35, 74.29, 70.70, 70.66, 70.37, 52.21, 52.17, 51.75, 31.50, 31.45, 29.54, 29.49; MS (ESI) m/z calcd for $C_{22}H_{26}F_2N_4O_4S_2$ [M + H]$^+$ 513.14, found 513.0. L3: ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.65 (d, J = 6.8 Hz, 1H), 7.89 (d, J = 9.3 Hz, 3H), 7.66 (d, J = 11.5 Hz, 2H), 6.73 (d, J = 9.4 Hz, 4H), 3.84–3.79 (m, 16H), 3.66 (s, J = 5.6 Hz, 8H), 2.97–2.91 (m, 8H), 2.81–2.76 (m, 8H); ^{13}C NMR ($CDCl_3$, 600 MHz): δ 160.03, 152.46, 144.23, 127.38, 126.31, 123.07, 111.98, 110.39, 107.67, 107.51, 105.74, 105.57, 74.21, 70.60, 52.33, 31.60, 29.55; MS (ESI) m/z calcd for $C_{22}H_{26}FN_5O_6S_2$ [M + H]$^+$ 540.13, found 540.1.

1-(3′,6′-Bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl)-3-(4-isothiocyanatophenyl)thiourea (L4)

Rhodamine B hydrazide 1 (457.2 mg, 1.0 mmol) [11,12] was dissolved in 50 mL of DMF, and then 1,4-phenylene diisothiocyanate (959.9 g, 5.0 mmol) was added. After stirring overnight at room temperature (rt), the solvent was evaporated in vacuo. The crude residue was chromatographed on silica gel using a $CHCl_3$/MeOH mixture (50/1, v/v) to yield L4 as a white solid (588.3 mg, 91% yield). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.98 (d, J = 7.6 Hz, 1H), 7.65 (td, J = 7.6, 1.2 Hz, 1H), 6.60–7.56 (m, 1H), 7.56 (s, 1H), 7.27 (d, J = 7.7 Hz, 1H), 7.12–7.06 (m, 2H), 7.04–6.99 (m, 2H), 6.97 (s, J = 6.0 Hz, 1H), 6.47 (d, J = 8.8 Hz, 2H), 6.42 (d, J = 2.6 Hz, 2H), 6.27 (dd, J = 8.9, 2.6 Hz, 2H), 3.44–3.21 (m, 10H), 1.15 (t, J = 7.1 Hz, 16H); ^{13}C NMR ($CDCl_3$, 600 MHz): δ 182.46, 167.27, 154.30, 149.98, 149.34, 136.79, 134.38, 129.12, 128.99, 128.28, 127.57, 125.72, 125.52, 124.77, 123.89, 108.30, 104.07, 98.29, 67.36, 44.40, 12.56; MS (ESI) m/z calcd for $C_{36}H_{36}N_6O_2S_2$ [M + H]$^+$ 649.23, found 649.2.

Immobilization of mercury binder molecules (L1–L4) onto amine-modified cellulose paper

Cellulose filter paper with a diameter of 90 mm was soaked in an ethanolic APTMS solution (1 wt%) for 1 h. The amine-primed cellulose paper was washed with copious ethanol three times and dried under vacuum at 40 °C for 6 h. To tether L1 onto the amine-modified cellulose paper, L1 was activated with the NHS ester in advance by dissolving L1 (47.5 mg, 0.1 mmol), EDC·HCl (22.9 mg, 0.12 mmol) and NHS (13.8 mg, 0.12 mmol) in DMF (20 mL) [8]. After stirring at rt for 3 h, TEA (30.3 mg, 0.3 mmol) was added to the reaction mixture, and the amine-modified cellulose paper was soaked in it overnight. After washing with copious ethanol three times and drying under vacuum at 40 °C for 6 h, L1 was immobilized on amine-modified cellulose paper (L1-AP). For the immobilization of L2 and L3, a piece of amine-modified cellulose paper was simply dipped into the above-described DMF solution (0.1 mmol in 20 mL) containing TEA (0.3 mmol) overnight. After incubation at rt, the cellulose paper was washed with copious ethanol three times and dried for 6 h in vacuo at 40 °C to yield the corresponding L2-AP and L3-AP. We were unable to tether L2 under these conditions based on the color of the resultant L2-AP compared to that of L3-AP, which exhibited a color characteristic of L3. For L4 immobilization, a piece of amine-modified cellulose paper was simply dipped into a DMF solution of L4 (0.1 mmol in 20 mL) overnight (without TEA). The resultant L4-AP was washed with copious ethanol three times and dried in vacuo at 40 °C for 6 h.

Results and discussion

Synthesis of L1, L2, and L3 and the estimation of the selective Hg^{2+}-binding capability

In addition to the previous azobenzene-based chelator (compound L1 in Fig. 1(a)) that bears a carboxylate group in the para-position [8], we also synthesized mercury chelators (L2 and L3) with backbones similar to that of L1 but different terminal functionalities, as shown in Fig. 1(a). The introduction of electron-withdrawing nitro- or fluoro-groups was intended to tune the spectral characteristics by modulating the internal charge-transfer (ICT) dipoles [11]. Specifically, two fluoronitroaniline compounds—DFNA and DNFA—were diazotized in acidic conditions and coupled to a dithiaazadioxo crown ether moiety. Both fluoronitroanilines increase the electron withdrawal capability, yielding a fortified push–pull dipole for the azobenzene [12]. The diazotization reactions were moderately successful, yielding L2 (~69% yield) and L3 (~58% yield) after silica gel column chromatography. Because of the nitro- (or fluoro-) group in the azobenzene moiety, which increases the ICT dipole moment, the maximum absorption wavelengths of L2 (468 nm) and L3 (533 nm) were red-shifted compared to that of L1 (Δl_{max}: L2 ~30 nm, L3 ~95 nm), yielding deep yellow and magenta colors for L2 and L3, respectively. The binding behaviors of L2 and L3 with Hg^{2+} were considered to be the same as that of L1 in a mixed solvent of acetonitrile and water, and the coordination of a single Hg^{2+} ion in the dithiaazadioxo ring of L1 was established by NMR titration [8]. Notably, the newly synthesized L2 and L3 have additional advantages for solid immobilization because the highly negative aromatic fluoride may be prone to aromatic nucleophilic substitution by suitable nucleophiles, such as amines or alkoxides (see below) [14].

As shown in Fig. 2(b), the colorimetric changes of L2 in response to various metal ions were first examined by dissolving it in solutions containing excess metal (5 eq.) in a mixed solvent of