ORGANIC LETTERS

2009
Vol. 11, No. 13
2740−2743

Resonance Energy Transfer Approach and a New Ratiometric Probe for Hg^{2+} in Aqueous Media and Living Organism

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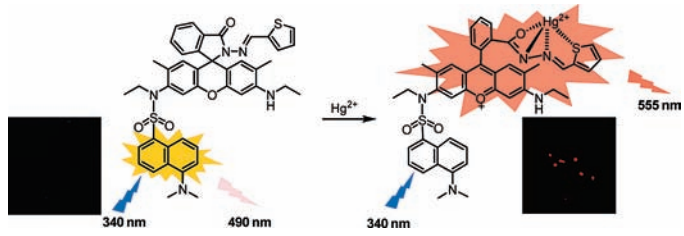
Received April 14, 2009

ABSTRACT



Resonance energy transfer from dansyl to the rhodamine moiety in a newly synthesized chemosensor L_2 has been utilized successfully for detection of Hg^{2+} in aqueous solution and living cells such as *Pseudomonas putida*.

A fluorescent chemosensor capable of sensing a specific analyte has potential application in chemistry and biology,[1] as this generally allows detection of an analyte present in ultratrace quantity. Such detection of heavy transition metal ions is of paramount interest due to the high toxicity of these metal ions toward human health and the environment.[2] Usually, there are three different photoinduced processes that are involved in the signaling or response phenomena of luminescence based chemosensors, namely, PET (photoinduced electron transfer),[3] PCT (photoinduced charge transfer),[4] and RET (resonance energy transfer).[5] RET is a

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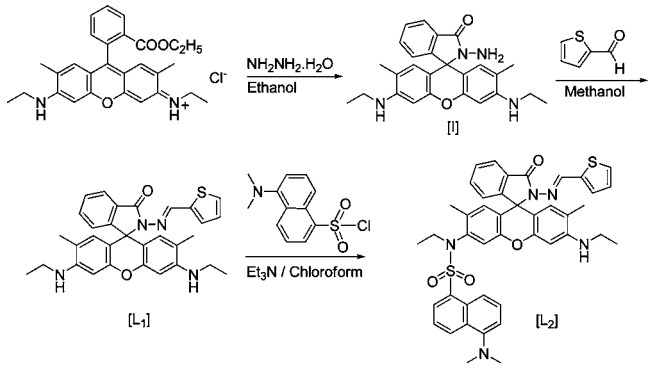
10.1021/ol900810q CCC: $40.75
Published on Web 06/09/2009

nonradiative energy transfer process in which the excitation energy of the donor is transferred to the nearby acceptor via long-range dipole−dipole interaction and/or short-range multipolar interaction. Donor and acceptor units with appreciable spectral overlap between the donor emission and acceptor absorption spectra constitute an appropriate RET pair. For commonly used luminescence-based probe molecules, quantitative measurements are possible if a linear relationship exists between luminescence intensity and the fluorophore concentration. However, this may not be known with sufficient accuracy in the case of aggregation or photobleaching. For biological applications RET-based probe molecules are generally more useful than single dye-based probes, as the RET-based process is independent of the concentration of a single fluorescent dye and one can quantify the analyte concentration by using the ratio of intensities of the well resolved fluorescence peaks with reasonable intensities at two different wavelengths for analyte-free and analyte-bound probe.[6] However, despite many advantages, examples of RET-based *off−on* fluorogenic sensors for Hg^{2+} in aqueous solution is not common in the literature.[7]

Herein, we report a new chemosensor (**L$_2$**), which was obtained following the synthetic methodology shown in Scheme 1. A newly synthesized thiophene derivative of

Scheme 1. Synthetic Route of **L$_1$** and **L$_2$**



rhodamine (**L$_1$**) was used as an intermediate. Both **L$_1$** and **L$_2$** were characterized using various analytical, spectroscopic techniques (see Supporting Information), which agreed well with the proposed structures for **L$_1$** and **L$_2$**. Molecular structure for **L$_1$** was also confirmed by single crystal X-ray analysis.[8] Ability of the receptor **L$_2$** to bind specifically to Hg^{2+} in mixed aqueous media with associated fluorescence *on* response has given this molecule an edge over some of the earlier reported RET-based receptor molecules.[5k,l,7a,c]

UV−vis spectra recorded for **L$_1$** (CH_3CN-H_2O, 1:1, v/v) shows an absorption maxima at 309 and 232 nm with two distinct shoulders at 340 and 260 nm. Absorption bands/shoulders were predominantly due to intraligand $\pi-\pi^*$ charge transfer transitions. Binding ability of **L$_1$** toward various metal ions (Li^+, Na^+, K^+, Cs^+, Sr^{2+}, Mg^{2+}, Ca^{2+}, Cr^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}) was checked. Spectral studies, using a solution of **L$_1$** (CH_3CN-H_2O, 1:1, v/v), revealed a significant change in electronic spectral pattern only when Cu^{2+} or Hg^{2+} was added: a new absorption band around 530 nm was developed with detectable change in solution color (Figure 1a and Supporting Information). While an enhancement

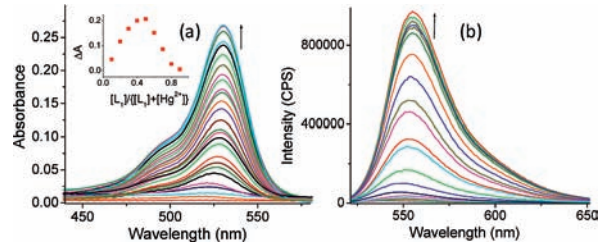


Figure 1. (a) Absorption spectra of **L$_1$** (20 μM) in H_2O-CH_3CN (1:1, v/v) with varying [Hg^{2+}] (0−600 μM). Inset: Job's plot that indicates the 1:1 stoichiometry. (b) Fluorescence spectra of **L$_1$** (10 μM) in the presence of varying [Hg^{2+}] (λ_{ext} = 500 nm).

in fluorescence intensity of **L$_1$** (Φ = 0.006; λ_{ext} = 500 nm) at ~557 nm was observed on addition of either of these two metal ions, this enhancement was more significant (105-fold) for Hg^{2+} (Φ = 0.62) as compared to that for Cu^{2+} (12-fold, Φ = 0.07) (Figure 1b and Supporting Information). Binding constants for two respective metal ions with **L$_1$** were evaluated and found to be (K_{Hg2+} = (1.45 ± 0.1) × 10^4 and K_{Cu2+} = (3.1 ± 0.2) × 10^3 M^{-1} at 25 °C), and in both cases 1:1 complex formation was evident. A similar formation constant value for these two respective metal ions was reported earlier for related rhodamine derivatives.[9] Appearance of a new absorption spectral band at around 530 nm and an enhancement in fluorescence intensity at around 555 nm on binding to Hg^{2+}/Cu^{2+} suggest opening of the spirolactam ring in **L$_1$** on metal ion coordination.[10] Spectral studies using a freshly prepared solution of dansyl phenyl amide (DNPA),

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(8) Crystal data for the compound: CCDC No. 695747; molecular formula $C_{31}H_{30}N_4O_2S$, M = 522.65, crystal size 0.34 × 0.25 × 0.20 mm^3, triclinic, space group P-1 with a = 9.657(5) Å, b = 11.328(5) Å, c =12.770(6) Å, α = 93.435(8)°, β = 109.501(8)°, γ = 92.979(9)°, V = 1310.7(11) $Å^3$, Z = 2, D_{calcd} = 1.324 g/cm^3, T = 293(2) K, F(000) = 590, absorption coefficient = 0.160 mm^{-1}, λ = 0.71073 Å, 10230 reflections were collected, 5091 observed reflections, R(int) = 0.0349, no. of parameters = 347, $R1$ = 0.0930, w$R2$ = 0.2000 with ($I \geq 2\sigma(I)$), goodness of fit on $F2$ = 1.174. Largest difference peak and hole: 0.337 and −0.473 e$Å^{-3}$, respectively.

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synthesized following a known procedure,[11] revealed that its emission spectra had a significant overlap (inset, Figure 2a) with the absorption spectra of the Hg^{2+}-bound

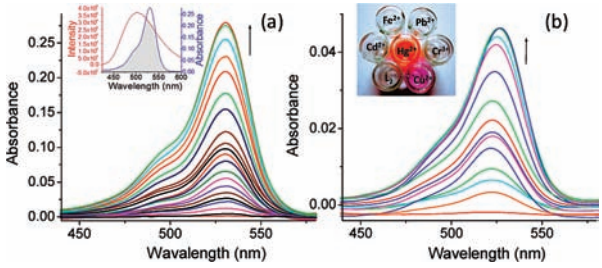

Figure 2. Absorption spectra of $\mathbf{L_2}$ (1.0×10^{-5} M) in H_2O-CH_3CN (1:1, v/v) in the presence of varying (a) $[Hg^{2+}]$ ($0-7 \times 10^{-4}$ M) (inset: spectral overlap between emission spectrum of the donor and absorption spectrum of the acceptor) and (b) $[Cu^{2+}]$ ($0-6.14 \times 10^{-4}$ M). Inset: visible color changes of $\mathbf{L_2}$.

xanthene moiety of $\mathbf{L_1}$ ($Hg^{2+}\cdot\mathbf{L_1}$), and this led us to explore the possibility of using dansyl functionality in combination with $\mathbf{L_1}$ for ratiometric sensing of Hg^{2+}.

Electronic spectra recorded for $\mathbf{L_2}$ (CH_3CN-H_2O, 1:1, v/v) was basically dominated by the absorption bands that belong to the $\mathbf{L_1}$ moiety, while a shoulder at 340 nm became more prominent presumably due to contribution from the dansyl moiety in $\mathbf{L_2}$. On excitation of the CH_3CN-H_2O solution of $\mathbf{L_2}$ at 340 nm, a relatively weak and very broad emission band ($\lambda_{ems} = 500$ nm) was observed (Figure 3), which could

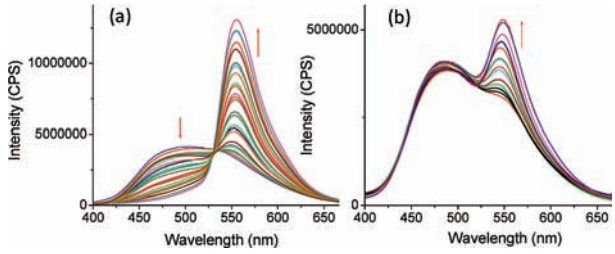

Figure 3. Fluorescence spectra of $\mathbf{L_2}$ (1.0×10^{-5} M) in H_2O-CH_3CN (1:1, v/v) with varying (a) $[Hg^{2+}]$ ($0-7.0 \times 10^{-4}$ M) and (b) $[Cu^{2+}]$ (0–6.14×10^{-4} M) using λ_{ext} of 340 nm.

be assigned to the dansyl unit based emission. This was further confirmed by time correlated single photon counting (TCSPC) studies using a 340 nm nano LED as an excitation source. Emission decay traces ($\lambda_{mon} = 539$ nm) for $\mathbf{L_2}$ could be best fitted with a biexponential function with time constants ($\tau_1 = 3.9 \pm 0.2$ ns (14.3%) and ($\tau_2 = 17.2 \pm 0.2$ ns) (85.7%), ($\chi^2 = 1.16$), whereas for the DNPA this was found to be a single exponential with τ being 13.6 ns ($\chi^2 = 1.71$). The shorter and minor component was assigned to the xanthene fragment (minor fraction that may exist in equilibrium along with the spirolactam form),[10a] and a longer and major component was assigned to the dansyl unit present in $\mathbf{L_2}$. Thus, comparison of the decay time constants for the DNPA and $\mathbf{L_2}$ revealed the predominant decay of the excited singlet state based on the dansyl moiety. Further, absence of any xanthene-center based emission at ~560 nm and absorption band at ~530 nm signified its spirolactam structure. Preliminary studies reveal that when a solution of Hg^{2+} or Cu^{2+} (CH_3CN-H_2O, 1:1, v/v) was added to a solution of $\mathbf{L_2}$ (CH_3CN-H_2O, 1:1, v/v), a distinct color change could be noticed by the naked eye (inset, Figure 2b). Electronic spectral studies reveal that a new spectral band appeared at around 530 nm on addition of the respective metal ion (10 molar equiv) solution to that of $\mathbf{L_2}$ (see Supporting Information), an observation similar to that for $Hg^{2+}\cdot\mathbf{L_1}$. The absorption band for $\mathbf{L_2}$ in the presence of either Hg^{2+} or Cu^{2+} was dominated by the new charge transfer band. Intensity of the new absorption at 530 nm was more intense for Hg^{2+} as compared to that for Cu^{2+}. The association constant for respective complexes $Hg^{2+}\cdot\mathbf{L_2}$ and $Cu^{2+}\cdot\mathbf{L_2}$ was evaluated from systematic spectrophotometric titrations (Figure 2) using the Benasi–Hildebrand equation and was found to be (3.9 ± 0.1) $\times 10^4$ M^{-1} ($K_{Hg^{2+}\cdot\mathbf{L_2}}$) and ($6.8 \pm 0.2$) $\times 10^3$ M^{-1} ($K_{Cu^{2+}\cdot\mathbf{L_2}}$) at 25 °C. Stoichiometry for the complexes formed for both metal ions were evaluated on the basis of the Job's plot and was found to be 1:1. Reversible binding of $\mathbf{L_2}$ with Hg^{2+} and Cu^{2+} was also examined. Addition of 10 equiv of $EDTA^{2-}$ to a mixture of $M^{2+}\cdot\mathbf{L_2}$ (5.0×10^{-5} M, M^{2+} is Hg^{2+}/Cu^{2+}) results in bleaching of the absorption band at 530 nm, which signifies the regeneration of the spirolactam structure (see Supporting Information).

Emission spectra recorded for $\mathbf{L_2}$ ($\lambda_{ext} = 340$ nm, dansyl moiety absorbs predominantly at this wavelength) in the presence of Hg^{2+} and Cu^{2+} showed respective emission bands at 555 and 545 nm. Further, $\mathbf{L_2}$ was found to be almost nonluminescent when excited at 500 nm (where the xanthene moiety absorbs predominantly). However, a significant increase in emission intensity at 555 nm was observed when a similar experiment was repeated in the presence of Hg^{2+}/Cu^{2+} (5 molar equiv). Quantum yield for $\mathbf{L_2}$, $Hg^{2+}\cdot\mathbf{L_2}$, and $Cu^{2+}\cdot\mathbf{L_2}$ was found to be 0.009, 0.35, and 0.16, respectively, for $\lambda_{ext} = 500$ nm. More interestingly, appreciable enhancement in emission intensity at 555 nm was also registered following excitation at 340 nm, a wavelength where dansyl units absorb predominantly. This tends to demonstrate the RET process in the presence of only the Hg^{2+} ion (Figure 3). Appearance of the absorption band at 530 nm for $Hg^{2+}\cdot\mathbf{L_2}$ /$Cu^{2+}\cdot\mathbf{L_2}$ and the emission band at ~555 nm suggested the opening of the spirolactam ring and generation of the delocalized xanthene moiety.[10] Figure 2a (inset) clearly shows that absorption spectra of the related xanthene moiety ($Hg^{2+}\cdot\mathbf{L_1}$) and emission spectra of the dansyl unit has an appreciable overlap, which makes nonradiative transfer of excitation energy between donor dansyl to acceptor xanthene moiety feasible. Systematic fluorescence spectral titration of $\mathbf{L_2}$ (CH_3CN-H_2O, 1:1, v/v) on excitation at 340 nm in the presence of varying $[Hg^{2+}]$ revealed a gradual decrease in the dansyl unit based emission at 483 nm along with a concomitant increase in the new emission band at 555 nm

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with a well-defined isoemissive point at 539 nm (Figure 3). These binding constant values were also evaluated from the luminescence titration studies using λ_{ext} of 340 and 555 nm as the monitoring wavelengths (λ_{mon}). Respective binding constants for the two metal ions thus obtained agreed well ($K_{Hg^{2+}\cdot L_2} = (5.0 \pm 0.2) \times 10^4\ M^{-1}$ and $K_{Cu^{2+}\cdot L_2} = (7.9 \pm 0.1) \times 10^3\ M^{-1}$ at 25 °C) with those obtained from absorption spectral studies. Further, spectral studies revealed that a lower detection limit for Hg^{2+} was 0.1 ppm (for signal-to-noise ratio of 3:1), and thus $\mathbf{L_2}$ could be used as a sensitive and selective chemosensor for Hg^{2+} using the RET process.

The ratio of acceptor-to-donor emission ($\lambda_{ext} = 340$ nm) intensity ($\lambda_{555}/\lambda_{483}$), in the absence and presence of varying [Hg^{2+}], varied from 0.83 to 22.46, and this 27-fold enhancement was attributed to the RET process. Optical spectral studies revealed that $\mathbf{L_2}$ could also bind Cu^{2+}; however, no significant enhancement in emission intensity at 545 nm was observed. The quenching of the fluorescence of the xanthene moiety in $\mathbf{L_2}$ by Cu^{2+} could be explained on the basis of the well-known paramagnetic effect of the d^9 Cu(II) system.[12]

To probe the singlet−singlet resonance energy transfer from the dansyl unit to the xanthene moiety in $Hg^{2+}\cdot\mathbf{L_2}$, time-resolved fluorescence decay studies were also undertaken using the TCSPC technique. A solution of $\mathbf{L_2}$ (CH_3CN-H_2O, 1:1, v/v) in the absence and presence of 1 molar equiv of Hg^{2+} was used for following excitation with a 340 nm nano LED source. Fluorescence decay was monitored at 539 nm, the iso-emissive point for two fluorophores (dansyl unit and $Hg^{2+}\cdot\mathbf{L_1}$). The emission decay curve for $Hg^{2+}\cdot\mathbf{L_2}$ could be best fitted to a biexponential decay function with $\tau_1 = 4.0 \pm 0.4$ ns (89%) and $\tau_2 = 15.0 \pm 0.5$ ns (11%) ($\chi^2 = 1.14$). A larger component with a lifetime of 4 ns was attributed to the xanthene unit in $Hg^{2+}\cdot\mathbf{L_2}$, while the smaller and slower component was assigned for the dansyl unit. Thus, TCSPC studies reveal that in the presence of Hg^{2+}, the situation is completely different and deactivation of the excited state occurs predominantly through the excited state that belongs to the xanthene moiety in $Hg^{2+}\cdot\mathbf{L_2}$ rather than the dansyl unit in $\mathbf{L_2}$, although the excitation wavelength of 340 nm, specific for the dansyl unit, was used as the excitation source. Thus, results of the time-resolved emission studies also corroborate the RET process.

The singlet−singlet excitation energy-transfer efficiency (Φ_{ET}) and rate constant for the energy-transfer process (k_{ET}) between donor and acceptor were evaluated from steady-state and time-resolved fluorescence data (see Supporting Information, eqs 1 and 2).[5a,13] The Φ_{ET} for the present study was found to be 83%, and while k_{ET} was found to be $2.84 \times 10^8\ s^{-1}$. The Förster critical distance (R_0) was calculated (see Supporting Information) using eq 1 and was found to be 74.3 Å. R_0 is the distance at which 50% energy transfer takes place between donor and acceptor.

$$R_0 = 9.79 \times 10^3[(J)Q(n^{-4})(\kappa^2)]^{1/6} \quad (1)$$

To explore the possibility of using $\mathbf{L_2}$ as a probable reagent for bioimaging application, Gram-negative bacterial cells (*Pseudomonas putida*) were studied by confocal laser microscopy (Olympus 1 × 81 with FV1000 confocal laser microscope) before (control) and after exposing these microbes to an aqueous solution of Hg^{2+}. Then, subsequently the control and cells exposed to Hg^{2+} solution were further exposed to the aqueous solution of the reagent $\mathbf{L_2}$. *Pseudomonas putida* is known to adsorb Hg^{2+} ion[14,15] and appeared either colorless or nonfluorescent (Figure 4). However, cells

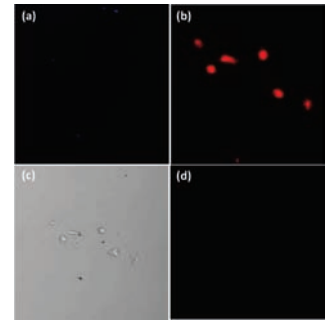

Figure 4. Confocal laser microscope images of (a) blank cells of *Pseudomonas putida*; (b) and (c) confocal image ($\lambda_{moni} = 560$ nm) and bright field image for the cells, exposed to Hg^{2+} and (10 μM) and then to $\mathbf{L_2}$ (20 μM), respectively; (d) confocal image of the cells exposed to Hg^{2+} and $\mathbf{L_2}$ and the fluorescence intensity monitored at 490 nm with $\lambda_{exc} = 405$ nm.

exposed to Hg^{2+} (10 μM) and then stained with $\mathbf{L_2}$ (20 μM, in 1:1 v/v water−ethanol) at 25 °C appeared as a pink-red color when observed under the optical microscope and red fluorescent when viewed through a confocal microscope using 405 nm excitation source (Figure 4).

Appearance of the red fluorescence confirmed the formation of $Hg^{2+}\cdot\mathbf{L_2}$ with Hg^{2+} that was adsorbed within the bacterial cells. Thus, the reagent $\mathbf{L_2}$ could be used as an optical and fluorescent staining agent for detection of the Hg^{2+} uptake in bacteria.

Thus we could demonstrate that $\mathbf{L_2}$ could be used as a staining agent for detection of Hg^{2+} uptake in bacteria and can be used in combination with dansyl moiety as ratiometric sensitizer for the Hg^{2+} ion in aqueous solution. As desired for the development of chemosensors that show ratiometric RET response to a specific and biologically important metal ion, $\mathbf{L_2}$ has a large crosssection for energy absorption, solubility in water, and high quantum yield.

Acknowledgment. DST and CSIR have supported this work. M.S., A.K.M., S.K.M., and A.S. acknowledge CSIR for research fellowship.

Supporting Information Available: Synthetic details, characterization of $\mathbf{L_1}$ and $\mathbf{L_2}$, and selected spectroscopic data for $\mathbf{L_1}$ and $\mathbf{L_2}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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