Luminescent sensor for Cd2+, Hg2+ and Ag+ in water based on a sulphur-containing receptor: Quantitative binding–softness relationship

Luminescent sensor for Cd²⁺, Hg²⁺ and Ag⁺ in water based on a sulphur-containing receptor: quantitative binding–softness relationship

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ABSTRACT: A water-soluble, high-output fluorescent sensor, based on a lumazine ligand with a thiophene substituent for Cd^{2+}, Hg^{2+} and Ag^+ metal ions, is reported. The sensor displays fluorescence enhancement upon Cd^{2+} binding (log $\beta = 2.79 \pm 0.08$) and fluorescence quenching by chelating with Ag^+ and Hg^{2+} (log $\beta = 4.31 \pm 0.15$ and 5.42 ± 0.1, respectively). The mechanism of quenching is static and occurs by formation of a ground-state non-fluorescent complex followed by rapid intersystem crossing. The value of the Stern–Volmer quenching rate constant (k_q) by Ag^+ ions is close to 6.71×10^{12} mol/L/s at 298 K. The thermodynamic parameters (ΔG, ΔH and ΔS) were also evaluated and indicated that the complexation process is spontaneous, exothermic and entropically favourable. The quantitative linear relationship between the softness values of Klopman (σ_K) or Ahrland (σ_A) and the experimental binding constants (β) being in the order of $Hg^{2+} > Ag^+ > Cd^{2+}$ suggests that soft–soft interactions are the key for the observed sensitivity and selectivity in the presence of other metal ions, such as: Pb^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} and Mg^{2+} ions. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: lumazine; fluorescent sensor; static quenching; Stern–Volmer plot; softness; HSAB theory

Introduction

Because of the high demand in analytical chemistry, clinical biochemistry, medicine, the environment, etc., numerous studies have reported fluorescent chemosensors for cations (1, 2). The potential for progress in the development of fluorescent sensors can be explained by the distinct advantages offered by fluorescence detection in terms of sensitivity, selectivity, response time, local observation, etc. It turns out that most of the recent research progress (3, 4) in this area has focused on sensing and selectively removing heavy transition metal (HTM) ions that have high toxicity to living plants and animals, even at very low concentration, such as Hg^{2+} (5) and Cd^{2+} (6, 7).

Numerous examples of fluorescent chemosensors for Hg^{2+}, Ag^+ and Cd^{2+} have been reported (1–4). Some of these sensors were reported to undergo fluorescence enhancement upon metal binding [chelation enhancement of fluorescence(CEF) type] and some have their fluorescence quenched upon binding [chelation enhancement of quenching (CEQ) type]. Such quenching is disadvantageous because it hampers a temporal separation of spectrally similar complexes with time-resolved fluorometry and because it produces lower output signal upon complexation. Moreover, among these literature findings, many researchers report water-soluble sensors for Hg^{2+} (8–19), but few report sensors in pure water for Ag^+ (19–21) and Cd^{2+} (19, 20, 22, 23). Although HTM ions are relatively easy to chelate and detect in organic solvents, they are rather difficult to recognize directly in aqueous environments, due to their strong hydration. These limitations, together with the fluorescence output signal of sensors, need to be addressed when designing a metal sensor for biological and environmental practical applications. It is also noted that most of the reported fluoroionophores for the indicated HTM ions consist of a receptor that contains a sulphur atom.

According to the hard and soft acids and bases (HSAB) theory (24), the softness of the sulphur atom is expected to be the key for the observed selectivity towards the soft acids: Hg^{2+} (25), Ag^+ (26) and Cd^{2+}.

The lumazine moiety has recently been employed as a platform for the construction of functional fluoroionophores for the recognition of Ca^{2+} (27) and Zn^{2+} (28) metal ions. Inspired by these findings, a thiophene receptor was attached to the lumazine platform and successfully obtained 6-thienyllumazine (TLM; see Fig. 1), a highly selective and sensitive fluorescent sensor for Cd^{2+} ion in a totally water environment, via enhanced fluorescence signal (i.e. a *fluorogenic* or CEF-type sensor); and for Hg^{2+} and Ag^+ ions via fluorescence quenching (i.e. CEQ-type sensor). Such a design strategy might be of great interest in the practical application of chemosensors. The structural, theoretical and photophysical characterizations of the TLM compound were reported previously (29). Here the focus is on the TLM cation-sensing characterizations. Although Czarnik *et al.* (19) reported a water-soluble fluorescent sensor based on the anthrylaza macrocycle that displays both CEF and CEQ sensing for Cd^{2+} and Hg^{2+}, the fluorescent sensing that is reported here is based on a sulphur-containing receptor, rather than a macrocycle unit, which is interpreted in the present work by the HSAB theory. Thus, the present study offers a good example of soft–soft interactions in chemical reactions. In addition, the pure water solubility and high fluorescence output signal should give the presented sensor an advantage for practical applications in toxic metal recognitions,

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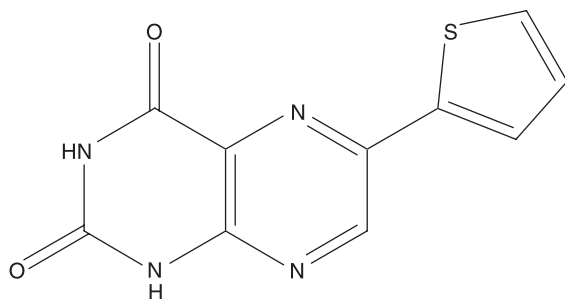Department of Chemistry, Yarmouk University, 21163 Irbid, Jordan

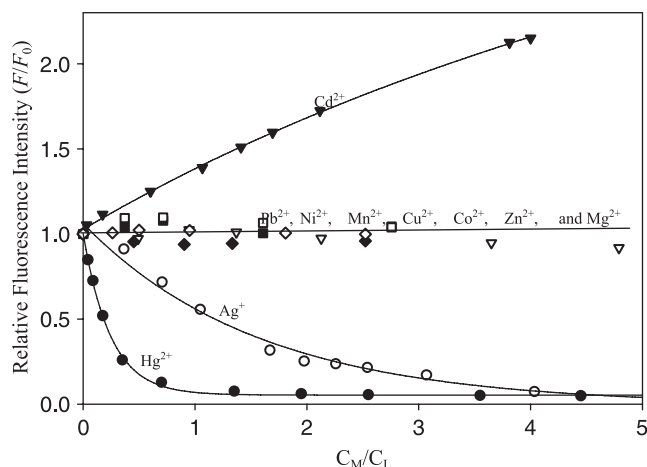**Figure 1.** Molecular structure of 6-thienyl lumazine (TLM).

Figure 2. Fluorescence titration plots of TLM as a function of molar ratio of metal to ligand in pure water (pH 5) at 298 K. Excitation was made at 360 nm and emission was monitored at 436 nm.

as few examples of water-soluble sensors with high output have been reported for Cd^{2+} and Ag$^+$.

Materials and methods

The TLM compound was synthesized in previous work (29). The absorption spectra were collected using a Labomed Double PC UV-Vis spectrophotometer, while the fluorescence spectra were measured using an Edinburgh FS-900CDT fluorometer with excitation and emission bandwidth of 4 ± 0.5 nm; the instrument's full description is available elsewhere (29). The absorption of each chromophore was checked to be less than 0.05 absorbance unit at the excitation wavelength. Double-distilled and deionized water was used.

The sensing properties of TLM molecules to metal ions were measured using a fluorescence technique in aqueous solutions, as follows. A 2500 μL water solution containing a certain concentration of TLM at pH 5.0 ± 1 was prepared, and an initial fluorescence measurement was made. Aliquots (0–1500 μL) of M^{2+} ion from a stock solution of M(NO$_3$)$_2$ or MCl$_2$ were added into the solution to give a final concentrations of 0–300 μmol/L, and the fluorescence spectra were recorded again.

Results and discussion

Figure 2 illustrates the fluorescence responses of TLM in pure water after the addition of several nitrate or chloride salts of metal ions. Upon the addition of an equivalent amount of Hg^{2+}, Cd^{2+}, Ag$^+$, Pb^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+} and Mg^{2+} to aqueous solutions of TLM compound, only Cd^{2+} enhances the fluorescence signal, while the addition of Hg^{2+} and Ag$^+$ ions causes marked fluorescence quenching. None of the other cations yielded significant changes on the emission intensity under identical conditions. The data in Fig. 2 were then analysed to obtain the binding stoichiometry and overall affinity constants (β) for each of the three HTM–TLM complexes. It should be emphasized that, although the conceptual approach of applying fluorescent molecules to sense soft metal ions with high selectivity and sensitivity is not new and has been used in various occasions (as outlined in the introduction), the presented sensor gains its value by its ability to sense, simultaneously, three toxic soft metal ions, viz. Hg^{2+}, Ag$^+$ and Cd^{2+}, by applying opposite approaches (CEQ and CEF) with reasonable selectivity and high sensitivity (detection limit of about 10 nmol/L) (20).

Measurements were also performed at different temperatures to obtain the thermodynamic parameters for Hg^{2+} and Ag$^+$ complexes, as summarized in Table 1.
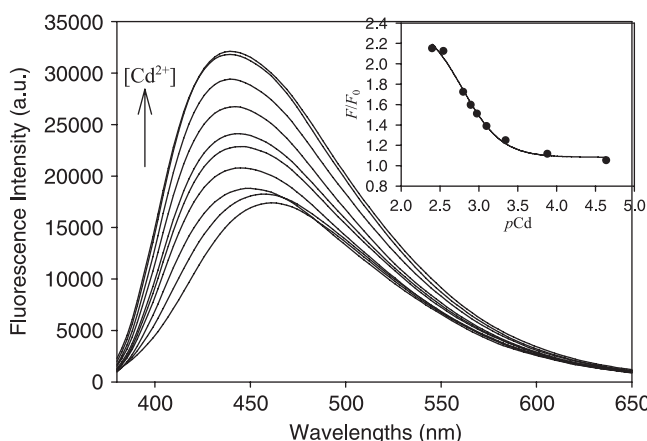
Figure 3. Fluorescence spectra of TLM (750 μmol/L) as a function of Cd^{2+} concentrations at room temperature and pH 5. (Inset) Relative fluorescence changes at 436 nm as a function of pDd [pCd = $-$ log(Cd^{2+})].

It might be suggested from the profiles of the binding titration curves in Fig. 2 that Hg^{2+} and Cd^{2+} ions form 1:2 complexes with TLM ligand, while Ag$^+$ forms a 1:1 complex. More evidence for the structures of the proposed complexes might be obtained from other techniques, such as X-ray (if a single crystal can be obtained), NMR, IR, etc. Such an issue is under investigation in our laboratory. However, a number of studies report similar stoichiometry for the complexation of lumazine-like ligands in their ground state with Ag$^+$ (30, 31), Hg^{2+} (32) and Cd^{2+} (33). In addition, it is well known that Cd^{2+}, Hg^{2+} and Ag$^+$ (soft acids) can preferentially interact with the sulphur atom (soft base) according to the Pearson's HSAB theory (24). So we can deduce that the variations in the fluorescence spectrum of TLM are induced through the interaction of Cd^{2+}, Hg^{2+} and Ag$^+$ with the lone-pair electrons on the sulphur atom.

Fluorescence enhancement of TLM by Cd^{2+}

Figure 3 shows a two-fold enhancement of TLM fluorescence intensity with a blue shift from 472 nm to 436 nm in the emission

Table 1. Thermodynamics data for the complex equilibrium of TLM with soft HTM ions in water at 298 K together with three metal softness parameters

HTM ions	Hg^{2+}	Ag^+	Cd^{2+}
[1]Log β	5.42 ± 0.1	4.31 ± 0.15	2.79 ± 0.08
[2]$\Delta H°$ (kJ/mol) ± 0.002	-40.1	-16.2	
[2]$\Delta S°$ (J/mol K) ± 0.002	-31.1	-28.1	
[3]$\Delta G°$ (kJ/mol) ± 0.002	-30.8	-7.8	
[4]Parameter softness, σ_P	0.064	0.073	0.081
[5]Parameter softness, σ_K	4.64	2.82	2.04
[6]Parameter softness, σ_A	4.60	4.20	3.50

[1]Determined by fitting the binding titration data, see text.

[2]Calculated using the classical van't Hoff equation, $\text{Ln}\,\beta = \dfrac{-\Delta H°}{RT} + \dfrac{\Delta S°}{R}$

[3]Calculated using the fundamental thermodynamic equation, $\Delta G° = \Delta H° - T\,\Delta S°$.

[4]Obtained from reference 36.

[5]Obtained from reference 37.

[6]Obtained from reference 38.

maximum with the addition of four equivalents of Cd^{2+} ions. The data were fitted by a sigmoidal function ($R = 0.997$) and a binding constant log β of 2.79 ± 0.08 was obtained.

The Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) distributions of TLM should help in better understanding the influence of Cd^{2+} ions on the fluorescence spectrum of TLM, which were calculated previously (29) by Semiempirical Austin Model 1 (AM1) and *ab initio* Hartree-Fock (HF) methods. It can be seen from the contours of the HOMO and LUMO orbitals that HOMO resides on the sulphur (thiophene ring) and the lumazine ring, while LUMO only resides on the lumazine part. Hence, the interaction of Cd^{2+} with the sulphur atom can significantly influence the HOMO energy level whereas the influence on LUMO energy level is negligible. Following metal coordination or protonation, the oxidation potential of the sulphur atom is shifted to more positive potential because the lone pair of sulphur is involved in coordination or protonation. Therefore, interaction of Cd^{2+} with the sulphur atom reduces electron density on the thiophene subunit and weakens its electron–electron donating ability. As a result, the HOMO energy level is lowered and the energy gap between HOMO and LUMO is increased, leading to the blue-shift fluorescence. Our previous findings (29) indicated that the emission of TLM is a characteristic of local excited state ($S_1 \rightarrow S_0$) emission of the lumazine ring and that the possibility of any other intermolecular or intramolecular photophysical de-excitation was eliminated. The destabilization of the HOMO energy state might increase the energy gap between the initial and final electronic states, which should decrease the efficiency of internal conversions and hence the fluorescence signal should be enhanced. It is assumed that the Cd^{2+}–TLM complex is not capable of absorbing the emitted light.

Fluorescence quenching of TLM by Ag^+ and Hg^{2+}

As shown in Fig. 4, it was possible to analyse the fluorescence quenching by Ag^+ using Stern–Volmer (SV) kinetics by assuming the formation of a 1:1 non-fluorescent Ag^+–TLM complex in the ground state. Linear fitting of the titration plots gives formation constants, log β, of 4.31 ± 0.15 ($R = 0.998$) and 4.22 ± 0.12
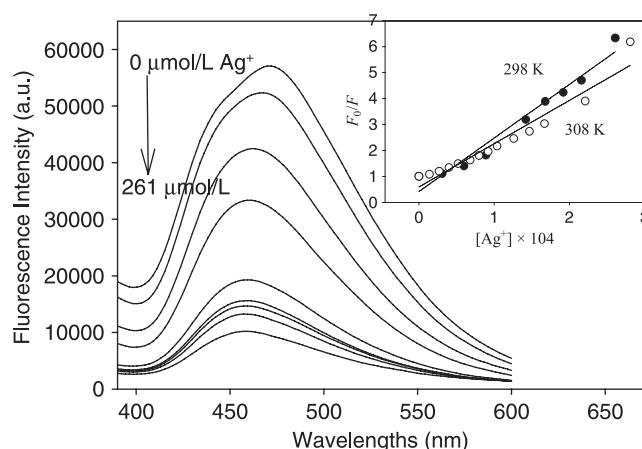

Figure 4. Fluorescence spectra of TLM (75 μmol/L) as a function of Ag^+ concentrations at room temperature at pH 5. (Inset) Stern–Volmer titration plot at 472 nm for 1:1 stoichiometry of TLM complex with Ag^+ in pure water.

($R = 0.997$) at 298 K and 308 K, respectively. The linear SV titration plot implies static quenching, but dynamic quenching (diffusion controlled) also gives a linear SV plot and so static and dynamic mechanisms are distinguished from each other by their differing dependences on temperature. An increase in temperature leads to an increase in the diffusion constant of quencher and will generally lead to an increase in dynamic (collisional) quenching. In contrast, an increase in temperature will generally lead to a decrease in the binding constant of quencher for fluorophore and will result in a decrease in quenching for a static quencher. The binding constant for Ag^+ decreases upon temperature increases inconsistent with static mechanism by formation of a ground-state 1:1 non-fluorescent complex. Thus, the possibility of dynamic quenching (collision with a paramagnetic species or heavy atoms) was excluded. Experimentally, it was possible to observe the formation of a black precipitate upon metal ion mixing with the ligand at low temperature. Pure static quenching might also be suggested, due to the absence of upward curvature. In addition, the plot of $(F_0/F - 1)/(Ag^+)$ against (Ag^+) was found to
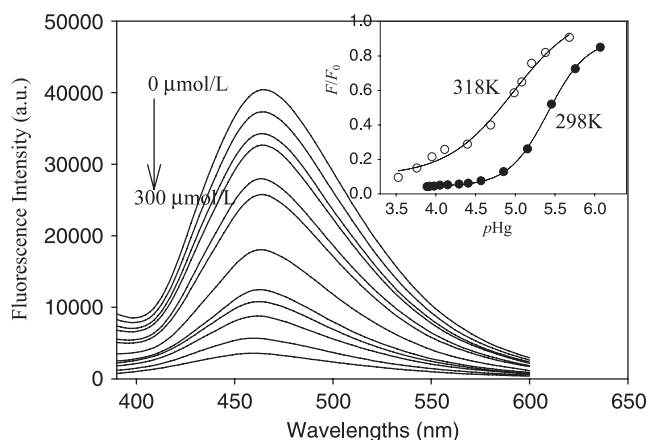
Figure 5. Fluorescence spectra of TLM (25 µmol/L) as a function of Hg²⁺ concentration at 318 K and pH 5. (Inset) Relative fluorescence changes at 436 nm as a function of pHg [pHg = − log(Hg²⁺)].

be non-linear, eliminating the possibility of simultaneous occurrence of static and dynamic quenching. It is reasonable to reject the dynamic mechanism, given the relatively short lifetime of TLM in water (3.1 ± 0.5 ns at 298 K) (29) as well as the relatively large value of the bimolecular quenching rate constant (k_q), which was calculated to be 6.71×10^{12} mol/L/s at 298 K, using the SV slope and the lifetime of TLM.

Regarding Hg²⁺ titration plots, the estimated 1:2 Hg²⁺–TLM association constants (log β) were found to be 5.42 ± 0.1 ($R = 0.998$), 5.18 ± 0.1 ($R = 0.997$) and 4.97 ± 0.1 ($R = 0.996$) at 298 K, 308 K and 318 K, respectively, as shown by Fig. 5. Thus, the quenching of TLM emission by the Hg²⁺ metal ion was higher than that of Ag⁺ addition. Again, the possibility of dynamic quenching was excluded, given the inverse temperature dependence of the binding constants.

Applying the classical van't Hoff equation results in a Gibbs energy value at 298 K of $\Delta G° = -7.8$ kJ ± 0.002 and $\Delta G° = -30.8$ kJ ± 0.003 for the Ag⁺–TLM and Hg²⁺–TLM complexes, respectively (Table 1). Thus, Hg²⁺–TLM complex formation is more energetically favoured. The entropy values were negative in sign, but it is important to remember that, in accordance with the second law of thermodynamics, ΔS_{univ}, not ΔS_{sys}, must be positive for a process to be spontaneous. Since the enthalpy is exothermic, ΔS_{surr} has larger magnitudes than the ΔS_{sys} and the process is spontaneous at temperatures below 1289 K and 1106 K for Hg²⁺ and Ag⁺ complexes, respectively.

It is interesting to compare our results with previous reports concerning the quenching mechanisms of excited states by metal ions. Masuhara et al. (34) reported a static mechanism with the involvement of intersystem crossing for the fluorescence quenching of some aromatic hydrocarbons by Hg²⁺ and Ag⁺ ions. The authors rejected other possible quenching mechanisms, such as dynamic (collisional) quenching, electron transfer or energy transfer. They argued that the spin multiplicity of these metal ions is singlet and no paramagnetic interactions are involved. The heavy atom effect was also excluded, since the quenching rates were not correlated with atomic number of ions. Their absorption band is in a wavelength region shorter than that of aromatic hydrocarbons, which excludes an efficient energy transfer to metal ions. In the present study, it was not possible to reject other quenching pathways, such as charge transfer or the involvement of intersystem crossing, without

knowing the exact electronic and geometrical structures of the complexes, which is under investigation. Nevertheless, the contribution from the intersystem crossing (35) in the quenching mechanism might be suggested, since the degree of quenching of Ag⁺ ($Z = 47$) and Hg²⁺ ($Z = 80$) depends on the metal atomic number (Z). The spin–orbit coupling (35) varies with the fourth power of Z and thus intersystem crossing becomes efficient (internal heavy atom effects). Given this fact, it might be suggested that rapid intersystem crossing followed the formation of the non-fluorescent complex.

Stability constant and softness relationship

It is important to correlate the trend in metal binding by TLM ligand with the metal softness. As shown in Table 1, the order of binding of the examined HTM ions (a soft acceptor) to the sulphur atom in the TLM molecule (a soft donor) is Hg²⁺ > Ag⁺ > Cd²⁺. Soft applies to species that are big, have low charge states and are strongly polarizable (24). Based on work by Pearson and Mawby (36), it was possible to estimate the softness, σ_P, for a large number of metal ions. Pearson and Mawby defined σ_P for a metal ion in terms of the coordinate bond energies of its fluoride and iodide. For ions of a given charge, softness increases as σ_P decreases. In the present study, Pearson and Mawby parameters were not suitable to reflect the degree of softness for the studied metal ions, since they did not provide a comparative scale of softness for ions with different charges. Klopman (37), however, introduced his softness parameter, σ_K, from a theoretical treatment based on polyelectronic perturbation theory, in which σ_K values increase with increasing softness. Third softness parameters, σ_A, were presented by Ahrland (38), based on the difference between the total ionization energy of a given acceptor and its dehydration energy. In contrast to Pearson and Mawby parameters, both Klopman and Ahrland parameters were defined so that their values were independent of the charges on metal ions.

A linear-regression correlation between the stability constants for the studied metal complexes at 298 K and σ_K and σ_A softness parameters in Table 1 were found with a correlation coefficient, R, of 0.974 and 0.999, respectively, as shown by the following linear equations:

$$\text{Log } \beta = 0.9963 \, \sigma_K + 1.0016 \qquad (1)$$

$$\text{Log } \beta = 2.3629 \, \sigma_A - 5.5746 \qquad (2)$$

The linear relationships indicate that the order of metal complexation is parallel to their softness. The σ_P-softness parameters (36) for all the non-interacting metal ions with TLM are as follows: Mg²⁺ (0.167), Pb²⁺ (0.131), Co²⁺ (0.130), Ni²⁺ (0.126), Mn²⁺ (0.124), Zn²⁺ (0.115) and Cu²⁺ (0.104). By comparing these values with the data in Table 1, it can be deduced that such metal ions are not soft enough to bind the soft TLM ligand.

Conclusion

TLM is clearly demonstrated as a water-soluble compound whose fluorescence is enhanced by the addition of Cd²⁺ and quenched by the addition of Ag⁺ and Hg²⁺ in the presence of many other metal ions. Such an approach might be of great interest in the practical application of chemosensors for molecular recognitions of HTM ion in many fields: biology, medicine, the environment, etc.

In addition, the article presents a clear example of soft–soft interactions and demonstrates softness in the order of Hg^{2+} > Ag^+ > Cd^{2+}. In contrast to numerous experiments that have been done using the HSAB theory to predict what adducts will form in a complex mixture of potential Lewis acids and bases, the present study makes the theory quantitative rather than qualitative. This is very useful to get a quantitative description in understanding the predominant factors which drive chemical properties and reactions.

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