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Fluorescence Turn On of Coumarin Derivatives by Metal Cations: A New Signaling Mechanism Based on C═N Isomerization

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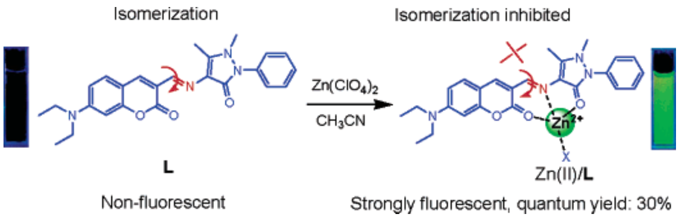
ABSTRACT



A new sensing mechanism based on C═N isomerization, which shows a very significant fluorescence enhancement to the metal cations in a simple and efficient way, is demonstrated. A coumarin derivative (L) containing a C═N group was designed as an example for illustration. The free ligand L is almost nonfluorescent due to the isomerization of C═N double bond in the excited state. However, the solution of ligand shows about a 200-fold increase of fluorescence quantum yield (about 30%) upon addition of $Zn(ClO_4)_2$.

Fluorescent probes/chemosensors capable of selectively recognizing guest species are of particular interest in supramolecular chemistry because of their high selectivity, sensitivity, and simplicity.[1,2] A fluorescent probe/chemosensor is a molecular system for which the physicochemical properties change upon interaction with a chemical species in such a way as to produce a detectable fluorescent signal.[3] Exploration of new interaction mechanisms between recognition and signal reporting units is of continuing interest for design of new fluorescent probes/chemosensors. Indeed, until now, a number of signaling mechanisms have been developed and widely applied for the optical detection of different

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species. These include photoinduced electron/energy transfer (PET),[4] metal−ligand charge transfer (MLCT),[5] intramolecular charge transfer (ICT),[6] excimer/exciplex formation,[7] and excited-state intra-/intermolecular proton transfer (ESPT),[8] etc. However, it should be noted that C═N isomerization has not been used as a signaling mechanism for fluorescent probes/chemosensors, although it has been documented previously.[9]

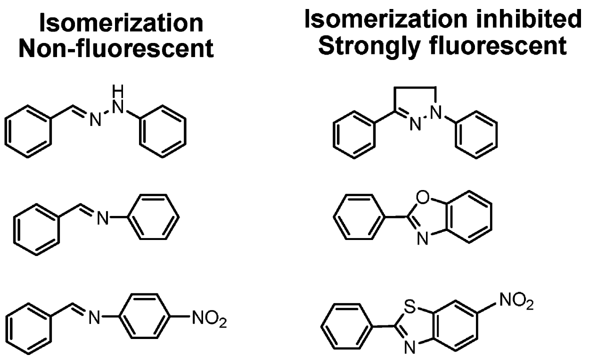

Figure 1. Molecular structures of unbridged and bridged C═N compounds.

In our previous work, as shown in Figure 1, we found that C═N isomerization is the predominant decay process of the excited states for compounds with an unbridged C═N structure so that those compounds are often nonfluorescent. In contrast, the fluorescence of its analogues with a covalently bridged C═N structure increases dramatically due to the suppression of C═N isomerization in the excited states.[10] With this in mind, we can reasonably expect that the C═N isomerization may also be inhibited by guest species through complexation in a sophisticatedly designed fluorescent-sensing molecule rather than covalent bridging of C═N bond. Herein, we report a novel signaling system via C═N isomerization as part of our continuing efforts for exploration of new sensing mechanisms for fluorescent probes/chemosensors.[7e] Fluorescence quantum yield of the coumarin derivative—compound **L** increases about 200-fold upon complexation with Zn^{2+}.

In the present work, a typically fluorescent dye, coumarin, was chosen as a fluorophore, and an antipyrine derivative acts as an additional chelating moiety. Both parts are linked by a C═N bond to form a potential fluorescent-sensing molecule (**L**) for metal cations. Compound **L** was synthesized according to the methodology as shown in Figure 2 and characterized by ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis.

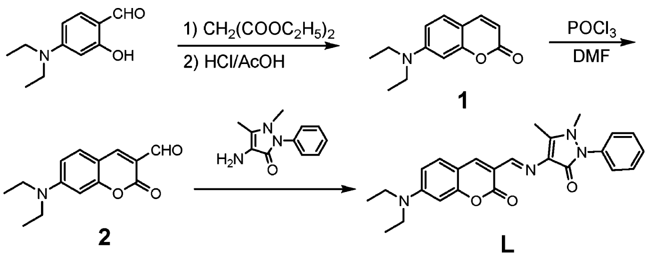

Figure 2. Synthetic method of compound **L**.

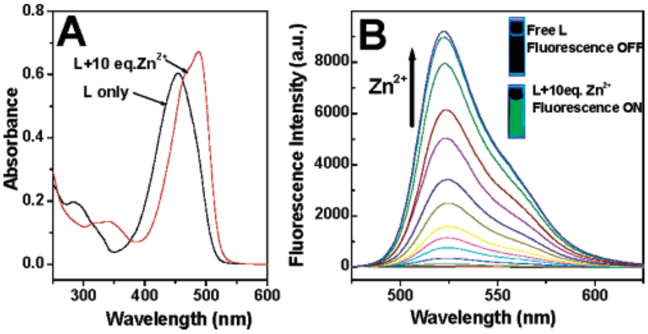

Figure 3. (A) UV−vis spectra of **L** (10 μM) and **L** (10 μM) upon addition of 10 equiv of $Zn(ClO_4)_2$ in CH_3CN. (B) Fluorescence spectra of **L** (10 μM) in CH_3CN upon addition of increasing concentrations of $Zn(ClO_4)_2$ (0, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μM) with an excitation wavelength of 450 nm. Inset: Photos of **L** in CH_3CN with and without addition of $Zn(ClO_4)_2$.

Figure 3 shows variations of UV−vis and fluorescence spectra of **L** upon addition of zinc cation in CH_3CN. It was found that the maximum absorption wavelength of **L** in CH_3CN shifts from 453 to 490 nm upon addition of 10 equiv of $Zn(ClO_4)_2$ (Figure 3A). On the other hand, the fluorescence of the solution increases dramatically with increasing concentrations of Zn^{2+}; concomitantly, the maximum emissive wavelength shifts from 500 to 522 nm (Figure 3B). Actually, an obviously green emission of the solution can be easily observed by the naked eye, as shown in Figure 3B. Fluorescence quantum yield (Φ_f) of free **L** in CH_3CN is 0.15%, whereas it reaches 30% when **L** binds with Zn^{2+}

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(fluorescein in 0.1 N NaOH as a reference, $\Phi_f = 0.85$).[11] In other words, the free ligand **L** is non-emissive, when it encounters zinc cation in CH_3CN, its fluorescence can be turned from "off" to "on", resulting in a 200-fold enhancement of the fluorescence quantum yield.[12]

It is generally understood that chelating groups C═N and C═O exhibit a high affinity to transition and post-transition metal cations, but less binding affinity toward alkali metal and alkaline earth metal cations due to the difference of electronic structures. Variation of fluorescence spectra of ligand **L** upon addition of different metal cations including Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, alkali metal and alkaline earth metal cations, is shown in Supporting Information (Figure S1). Addition of 10 equiv Fe^{2+}, Co^{2+} and Ni^{2+} can only induce very little changes of fluorescence spectra, whereas 10 equiv Zn^{2+}, Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+} can induce an obvious fluorescence enhancement. Photophysical data of **L** upon addition of different metal cations are also shown in Table 1. It can be found that Zn^{2+} gives rise to the largest fluorescence enhancement among these metal cations, although Cd^{2+}, Cu^{2+}, Pb^{2+}, and Hg^{2+} can also induce about 27-, 14-, 8-, and 13-fold increases of fluorescence quantum yields, respectively. Such a variation in fluorescence quantum yields is relatively small compared with Zn^{2+} (200-fold), indicating that **L** shows the strongest response in fluorescence spectrum to Zn^{2+} among these metal cations. In general, transition and post-transition cations with open shell d-orbitals often quench the fluorescence of fluorophores due to the electron or energy transfer between the metal cations and fluorophores, providing a very fast and efficient nonradiative decay of the excited states. In contrast, the transition cations with close shell d-orbitals, such as Zn^{2+}, do not introduce low-energy metal-centered or charge-separated excited states so energy and electron-transfer processes cannot take place.[13] In the present work, we observed an obvious enhancement of fluorescence quantum yields even in the presence of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Hg^{2+}. The reason can be explained by the competition between the fluorescence enhancement due to the inhibition of C═N isomerization and quenching of fluorescence from the metal cation-induced electron or energy transfer processes. This result indicates that the C═N isomerization plays a predominant role, although the restriction of s-cis versus s-trans conformation in the fluorophore may also be a little partial cause of the observed fluorescence turn-on (vide infra) and gives rise to more significantly spectral and photophysical response to transition metal cations.

Table 1. Photophysical Data of **L** upon Addition of Different Metal Cations[a]

$L + M^{2+}$	none	Fe^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Pb^{2+}	Cd^{2+}	Hg^{2+}
λ_{max} (nm)	500	513	516	512	500	522	530	524	521
I_m/I_0	1.0	1.7	1.2	1.0	26	339	9.4	43	21
Φ_f (%)	0.15	0.24	0.16	0.18	2.2	30	1.2	4.1	2.0

[a] λ_{max}: maximum emissive wavelength. I_m/I_0: ratio of maximum fluorescence intensity with and without metal cation. Φ_f: fluorescence quantum yield (fluorescein in 0.1 N NaOH as a reference, $\Phi_f = 0.85$).[11]

In order to get insight into the decay process, the lifetime of **L** in CH_3CN was measured by single photon counting, and it shows a good single-exponential decay. The lifetime of free **L** is quite short (0.15 ns). The radiative decay rate constant (k_r) and nonradiative decay rate constant (k_{nr}) are calculated to be 1.0×10^7 and $6.6 \times 10^9\ s^{-1}$, respectively, indicating that the nonradiative decay is the predominant process in the excited states.[14] When 10 equiv of Zn^{2+} was added, the lifetime of compound **L** increased to 0.72 ns, which is longer than that of free **L**, and the radiative and nonradiative decay rate constants changed to 4.2×10^8 and $9.7 \times 10^8\ s^{-1}$, respectively; both the radiative and nonradiative decay processes became comparative so that a strong fluorescence was observed.

The stability constant between **L** and Zn^{2+} in CH_3CN was calculated to be $(1.09 \pm 0.08) \times 10^6\ M^{-1}$ by the nonlinear least-squares fit according to the fluorescent titration data in Figure 3B with a good relationship ($R = 0.991$), implying that the formation of the complex with a 1:1 stoichiometry.[3,15] Since the free ligand **L** is relatively flexible, Zn^{2+} should be easy to fit into the pseudocavity formed between the C═N structure and two carbonyl groups of coumarin and antipyrine moieties. The fourth coordination site of Zn^{2+} may be occupied by the counteranion ClO_4^- or acetonitrile molecule. The proposed interaction mode between **L** and Zn^{2+} was shown in Figure 4 (X = ClO_4^- or CH_3CN).

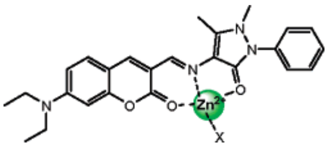

Figure 4. Proposed interaction mode between **L** and Zn^{2+}.

To further support the proposed complexation model, and also evaluate the role of oxygen atom of carbonyl group in coumarin it plays in the interaction process with metal cations, compound **M** (inset of Figure 5) was also synthesized[16] and characterized by ^1H NMR, ^{13}C NMR, and TOF-

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(12) It is generally understood that the variation of fluorescence quantum yield should be in the same order as that in fluorescence intensity if the fluorescence spectra do not shift or change the shape too much. However, in this case, the emission spectra shift from 500 to 522 nm in the presence of Zn^{2+}; more importantly, the shape of spectrum is also changed, and a new shoulder appears at about 560 nm, which should be the main reason for the difference of variations between the fluorescence quantum yield and fluorescence intensity.

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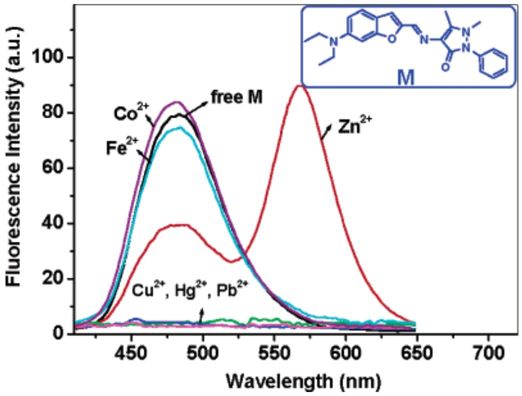

Figure 5. Fluorescence spectra of **M** (10 μM) in CH_3CN upon addition of different metal cations including Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, and Co^{2+} (each concentration was 100 μM) with an excitation wavelength of 400 nm. Inset: molecular structure of **M**.

HRMS (Supporting Information). It is closely similar to **L** in structure except for lack of carbonyl group of coumarin group. Figure 5 shows the fluorescence spectra of compound **M** in CH_3CN in the presence of different metal cations such as Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Co^{2+}, etc. The emission of **M** peaked at 480 nm was very weak (the fluorescence quantum yield is 0.69%)[11] and quenched dramatically in the presence of Cu^{2+}, Hg^{2+}, Pb^{2+}, whereas only a small change in fluorescence was observed upon addition of 10 equiv of Fe^{2+}, Co^{2+}. Interestingly, upon addition of 10 equiv of Zn^{2+}, a new peak at about 565 nm appeared while the peak at 480 nm decreased, and concomitantly, a slight increase of fluorescence was found probably due to the restriction of s-cis versus s-trans conformation in this fluorophore. However, no significant enhancement of fluorescence, as found for compound **L**, was observed because C═N bond is not included in the formed chelating complex as in compound **L**, and the isomerization of C═N is not suppressed dramatically. This is completely different from the behaviors for **L**, implying that the carbonyl group of coumarin moiety of **L** plays a very important role in the interaction process with metal cations.

In summary, we have demonstrated a new sensing mechanism based on C═N isomerization, which shows a very significant fluorescence enhancement of the compound—a coumarin derivative to the metal cations in a simple and efficient way. We believe that it can be extended to other sensing systems for recognition of different species. The present work also provides a novel concept for design of fluorescent probes/chemosensors with remarkable changes in fluorescence. Investigations along these lines for the development of more sophisticated systems based on the same mechanism for recognition of cations or anions, especially in aqueous solution, are in progress in our laboratory.

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Supporting Information Available: Synthesis of compound **L** and **M**, experimental details, and additional spectrum. This material is available free of charge via the Internet at http://pubs.acs.org.

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