Benzimidazole–Chalcone: A Selective Intramolecular Charge-transfer Probe for Biologically Important Zinc Ions

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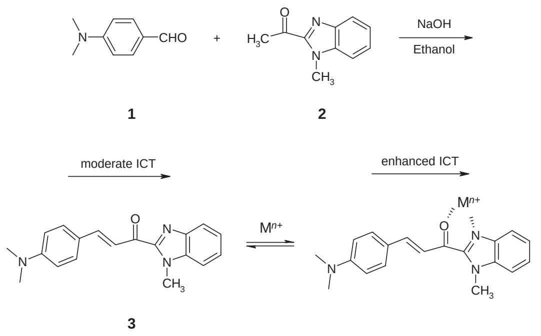
Benzimidazole–chalcone **3** functions as an optical probe on the basis of intramolecular charge-transfer (ICT) interaction involving bi-dentate chelation. Dramatic changes were observed both in the UV–vis and fluorescence behaviors of **3** in the presence of Zn^{2+}, while other metal ions e.g. Li^+, Na^+, K^+, Ca^{2+}, and Mg^{2+} produced relatively insignificant optical changes.

Study of perturbation of photophysical properties of optical probes linking a selective binding domain constitutes one of the fundamental approaches for the analyte determination. Zn^{2+} ions are well known to play important roles in biological process[1] such as a structural cofactor in metalloproteins, regulation of gene expression, and cellular apoptosis.[2] In addition, Zn^{2+} are also present in most DNA and RNA polymerases.[3] A disorder of zinc metabolism is associated with Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, hypoxia-ischemia, and epilepsy.[4–7] However, a majority of fluorosensors for Zn^{2+} are based on photoinduced electron-transfer mechanism.[8–13] To our knowledge, optical probes operating on the basis of ICT are quite uncommon.[14,15] ICT probes are interesting since molecules could be designed to possess excitation in the visible region to avert UV light impairment of living tissues.[4]

We report synthesis of benzimidazole–chalcone **3** that is designed to serve as an ICT probe as illustrated in Scheme 1.[16] The design principle is based on the premise that metal ion chelation via N,O coordination with **3** would induce enhanced CT thereby producing detectable changes in the optical properties of **3**.[17] To evaluate metal-binding potential, optical properties of **3** were investigated both by UV–vis and fluorescence spectroscopies in the presence of selected alkali and alkaline-earth metal ions as well as zinc ions. Compound **3** exhibited a broad charge-transfer band at λ_{max} 436 nm with an extiction coefficient ε_m of $2.69 \times 10^4\,M^{-1}\,cm^{-1}$ in CH_3CN. Addition of Li^+, Na^+, K^+, and Ca^{2+} perchlorates up to 300 molar equivalents did not affect the energy of the CT band, while the band intensity dropped by less than 5% (Figure 1).



Scheme 1. Synthesis and proposed ICT interaction of **3** with metal ions.

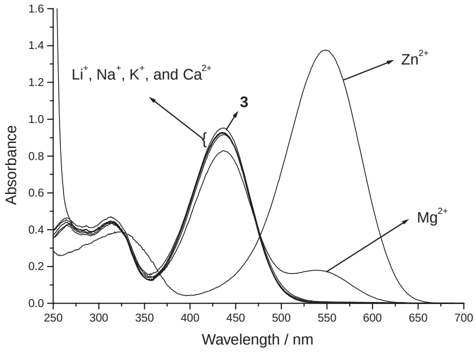


Figure 1. Absorption spectra of **3** (3.44×10^{-5} M) in the absence and presence of metal perchlorates (1.03×10^{-2} M) in acetonitrile.

However, addition of Zn^{2+} and Mg^{2+} ions resulted in marked changes both in the energy and shape of the absorption spectra of **3**. Most dramatic changes occurred upon addition of 300 molar equivalent Zn^{2+} which produced an intense new purple ICT band at 550 nm at the total expense of original yellow band at 436 nm. In contrast to Zn^{2+}, a much weaker ICT band at 540 nm is detected in the presence of comparable concentration of Mg^{2+} with the original band at 436 nm diminishing in intensity only by 15%. It is worth noting that complete complexation of **3** with Mg^{2+} required as high as 40,000 equivalents of $Mg(ClO_4)_2$ which is ca. 130 times higher than $Zn(ClO_4)_2$ concentration. This result demonstrates much stronger binding affinity of Zn^{2+} towards **3** even at much lower concentration relative to that of Mg^{2+}.

Spectrophotometric titration of **3** (10^{-5} M, CH_3CN) with respect to added $Zn(ClO_4)_2$ is depicted in Figure 2. A well-defined isosbestic point (λ_{isos} 476 nm) indicates 1:1 binding stoichiometry which was also confirmed by the Job's plot method.[16]

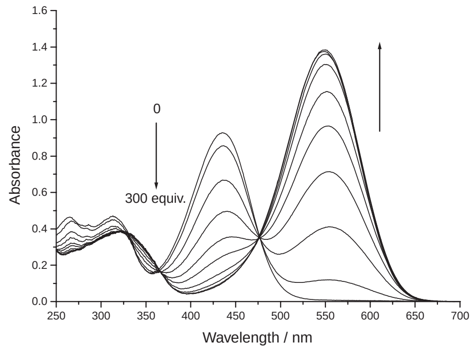


Figure 2. Spectrophotometric titration of **3** (3.44×10^{-5} M) with Zn^{2+} (0 to 1.03×10^{-2} M) in acetonitrile.

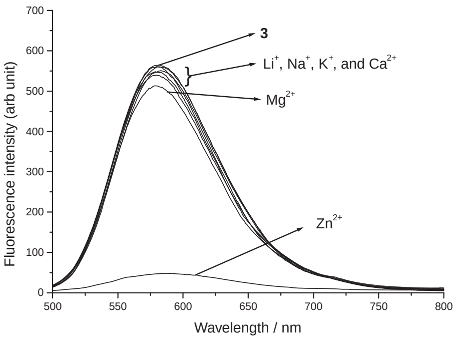


Figure 3. Fluorescence spectra of **3** (5.41×10^{-6} M) in the absence and presence of metal perchlorates (1.62×10^{-3} M) in acetonitrile.

Bi-dentate chelation involving benzimidazole nitrogen and carbonyl oxygen of **3** is supported by the shift of 1652 cm^{-1} band to 1597 cm^{-1} and downfield shifts of –NMe$_2$ and methyl-benzimidazole by δ 0.25 and 0.13, respectively.

When excited at its $\lambda_{\max}$ 436 nm, compound **3** emitted at 580 nm with a quantum yield Φ_f of 0.025. The λ_{em} of **3** was not altered upon addition of Li$^+$, Na$^+$, K$^+$, Ca^{2+}, and Mg^{2+} ions (up to 1.62×10^{-3} M), while the emission suffered <10% quenching. Thus, both UV–vis and fluorescence data indicate insignificant binding of these ions with **3** (Figure 3). However, Zn^{2+} (λ_{ex} at 476 nm) significantly affected the emission behavior of **3**. Not only λ_{em} was red shifted from 580 to 591 nm, but the chelation enhancement of emission quenching (CHEQ) led to ca. 92% reduction in the emission intensity at 1.62×10^{-3} M of Zn(ClO$_4$)$_2$. To give an indication of selectivity, 92% quenching by Mg^{2+} required ca. 150 fold higher concentration than zinc ions. Good agreement is found in the stability constants $\log K_s$ (Table 1) measured by both spectrophotometrically and fluorimetrically. Owing to poor binding, the $\log K_s$ for alkali metal ions could not be reliably determined. The $\log K_s$ follow the order Zn^{2+} $\gg$ Mg^{2+} > Ca^{2+} > Na$^+$ $\approx$ Li$^+$ $\approx$ K$^+$. From the UV–vis and emission data (band shifts and quenching) and high stability constant, we can conclude that chemosensor **3** manifests highly selective binding with Zn^{2+} compared to alkali and alkaline-earth metal ions investigated.

With few exceptions, most known zinc chemo- and fluoroionophores display emission enhancement.[8–13] The present case constitutes one of the rare cases where the binding event leads to quenching. Though excited-states dynamics are currently being investigated, a tentative explanation is proposed to account for the mechanism of CHEQ. It is known that in many heterocyclic chromophores, the n–π^* and π–π^* states are quite close in energy. It is conceivable that metal ion complexation via cooperative "N" and "C=O" chelation could enhanced the energy of the n–π^*, thereby rendering the π–π^* as the prominent excited state.[19,20] This shift in the nature of the excited states could lead to mixing of their vibronic states giving rise radiationless decay upon metal ion complexation.

Table 1. Stability constants and quantum yields of **3** and the complexes in acetonitrile

Compound	Φ_f[a]	$\log K_s$[b] (absorption)	$\log K_s$[b] (fluorescence)
3	0.025	—	—
3 ⊂ Li$^+$	0.0155	>0.1	>0.1
3 ⊂ Na$^+$	0.0153	>0.1	>0.1
3 ⊂ K$^+$	0.0158	>0.1	>0.1
3 ⊂ Ca^{2+}	0.0113	1.09	0.89
3 ⊂ Mg^{2+}	0.0072	1.56	1.28
3 ⊂ Zn^{2+}	0.0021	3.79	3.78

[a] Coumarin 153 in acetonitrile ($\Phi_f = 0.56$)[18] was used as a referecence standard for calculating $\log K_s$. [b] $\log K_s$ were determined by the nonlinear-square regression method.

In conclusion, we have reported a simple design of an ICT probe for selective recognition of zinc ions among common biological interfering ions. Unlike the widely reported PET sensors for Zn^{2+} which show detectable changes only in the emission, the ICT probe **3** offers detection both by UV–vis and fluorescence spectral monitoring. From sensor design point of view, the visible absorption of **3** ($\lambda_{\max}$ 436 nm) is of immence importance. Work is underway to design ICT probes with hydrophilic substituent(s) to increase their water solubility for biological applications.

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html. Supporting information includes synthesis of compound **3**, selective complexation of **3** in the presence of Mg(ClO$_4$)$_2$ and Zn(ClO$_4$)$_2$, spectrofluorimetric titration curve of **3** with Zn(ClO$_4$)$_2$ and Mg(ClO$_4$)$_2$ and binding stoichiometry by the Job's plot method.

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