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Novel Ca^{2+}-specific receptors derived from 3-formyl-2-hydroxybenzoic acid

Chien-Chung Cheng* and Ming-Chang Liu

Institute of Chemistry, Academia Sinica, Taipei 11529, Taiwan, ROC

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Abstract

Modified salen-type compounds consisting of 3-formyl-2-hydroxybenzoic acid were found to be novel receptors for the Ca^{2+}-specific recognition in water via the induction of a large red shift of the absorption band and an intensity quenching of the fluorescence emission. © 2000 Elsevier Science Ltd. All rights reserved.

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Chemical receptors with highly specific recognition of Group I and II metal ions are of interest in the development of molecular sensors and in biological applications.[1] The use of the photoinduced electron transfer (PET) method[2] provides the high sensitivity and distinguishable fluorescence observed upon the binding of alkaline and alkali–earth metal ions. Up until now, most of the Ca^{2+}-specific receptors with high selectivity and sensitivity are based on Tsien's important and sensual works[3]. These novel Ca^{2+}-fluorescent indicators were generally achieved by the use of the different fluorophores to attach the nonfluorescent Ca^{2+} chelators BAPTA[3]. Beside Tsien's compounds, new receptors specific for the detection of Ca^{2+} ion are still rare. Here, we report a new type of receptors derived from 3-formyl-2-hydroxybenzoic acid (FHBA) with Ca^{2+}-specific recognition in water. The specific recognition was examined by observing a decrease in fluorescent emission as well as a bathochromic shift in absorption spectra.

FHBA was prepared by modified procedures in a 30% yield.[4] When FHBA was dissolved in water, a green–blue fluorescence was observed at 475 nm with the excitation on 340 nm. The physical properties of FHBA and its derivatives are summarized in Table 1. Variation in the pH titration did not cause a significant change in fluorescence intensity of FHBA until a pH greater 10 was reached. The p*K*a of FHBA is 5.5 and 11.2 as determined by the change of UV absorption at various pH values and followed by the Henderson–Hasselbalch equation.[5] The

* Corresponding author. Tel: +886-2-27898578; fax: +886-2-27831237; e-mail: cccheng@chem.sinica.edu.tw

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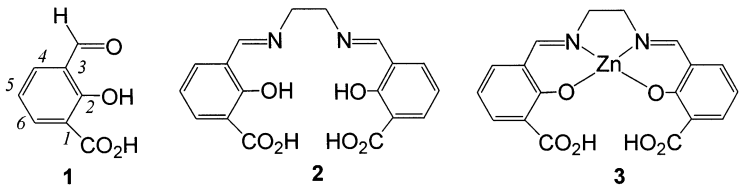
functional group and its position on the aromatic ring are found to be crucial factors in the generation of its fluorescence and intensity. For instance, 2-hydroxybenzoic acid, where the molecule lacks of the formyl group, shows no fluorescence emission at all. However, in salicylaldehyde, where the carboxylic group has been eliminated from FHBA, the fluorescence emission is preserved but weaker compared to that of FHBA. Moreover, when carboxylate group was introduced *para* to the phenolic group to form 3-formyl-4-hydroxybenzoic acid, the fluorescent intensity was found to be at least 60 times weaker than that of FHBA in the absence of Ca^{2+}.

Table 1



Compound	λ_{abs} (nm) ($\varepsilon \times 10^4$)[a]	λ'_{abs} (nm) (with Ca^{2+})[a,b]	λ_{em} (nm) (intensity)[a,b]	p*K*a[c]
1	337 (1.5)	383	470 (59.6)	5.5, 11.2
2	338 (1.8)	385	474 (61.1)	–
3	341 (1.2)	386	474 (99.5)	–

[a] Measured in water.

[b] Uncorrected spectra and [compound] ~ 0.6 μM in aerated water at 25°C. The excitation wavelength is fixed at 340 nm.

[c] Calculated using Henderson–Hasselbalch type mass action equations: $pKa = \log[(A_{max} - A)/(A - A_{min})] + pH$. Note: quantum yield for these three compounds were estimated around 0.2 in comparison with an aerated solution of coumarin I dye in water ($\Phi = 0.055$).[8] Since the maximum absorption wavelength is at 385 nm for the standard compound, it gives a large deviation error in comparison with their relative quantum yield.

The interaction between Ca^{2+} and these receptors was monitored by fluorescence emission spectrophotometer. A decrease in the fluorescence emission intensity was observed in the aqueous FHBA solution (0.6 μM) with an increasing $CaCl_2$ concentration (0–300 mM) at ambient temperature when the excitation wavelength is at 340 nm in water as well as in 10 mM sodium phosphate buffer (pH 7). The ratio of fluorescence quenching with the increasing concentration of Ca^{2+} ion is shown in Fig. 1A. Similar results were also observed when the excitation wavelength of 350 nm (the isosbestic point) was used. Under similar conditions, a variety of chloride salts of Group I and II metal ions, including Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Sr^{2+} and Ba^{2+}, revealed no significant intensity diminution. As a result, the selectivity for Ca^{2+} is at least 40 times greater than that of the rest of metal ions in water suggesting that the fluorescence intensity of FHBA is specifically quenched by Ca^{2+} ion.

In order to enhance the fluorescence intensity, salen-type ligands were also prepared by Schiff-base condensation using two equivalents of FHBA and ethylenediamine.[4] Compared to **1**, the fluorescence intensity of **2** in DMF was significantly enhanced by one order of magnitude. Nevertheless, in Ca^{2+} binding experiments, salen derivatives were found to be unstable in water (pH around 5) and were readily hydrolyzed to the starting material as disclosed by NMR

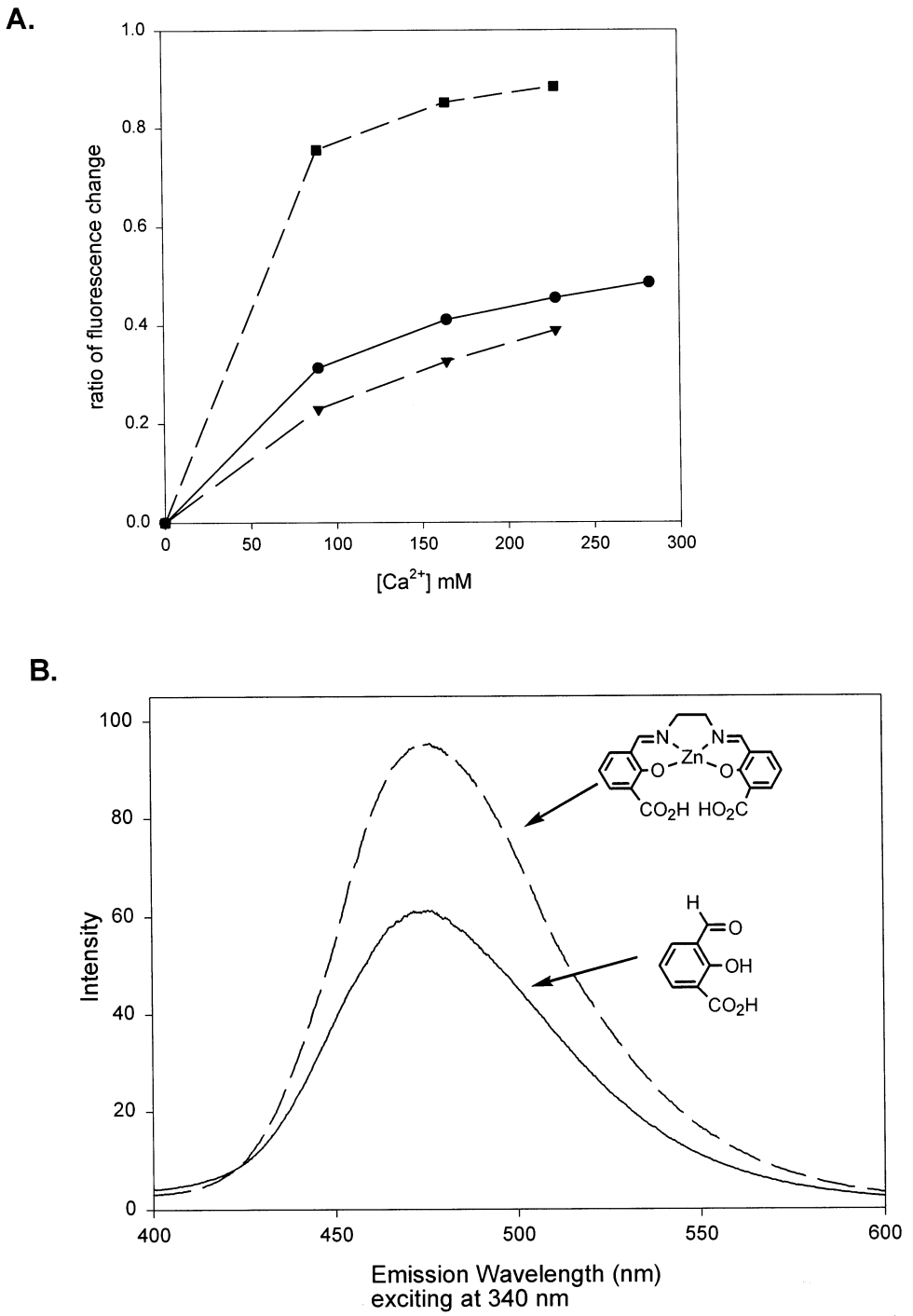


Figure 1. (**A**) Comparison of the quenching fluorescence emission induced by Ca^{2+} ion using receptors 1, 2, and 3 in sodium phosphate buffer (10 mM, pH 7). Symbols -▼-, -●-, and -■- represent compounds **1**, **2** and **3** (0.6 μM) in water. Ratio of fluorescence quenching = [the change of intensity upon adding Ca^{2+}/the total intensity of receptor (without Ca^{2+})]. The excitation wavelength is fixed at 350 nm. (**B**) Comparison of the fluorescence intensity between compounds **1** and **3** in water. The emission spectra are obtained under the same concentration of compound **1** and **3** (0.6 μM) and the excitation wavelength at 340 nm in water at 25°C

spectra. To prevent the imine bond from hydrolysis under acid condition, a Zn–salen type complex was also prepared. This Zn–salen type complex was obtained by refluxing with FHBA, ethylenediamine, and $Zn(OAc)_2$ in EtOH with a 65–70% isolated yield. The coordination of Zn^{2+} ion to a salen ligand has been known to stabilize the Schiff-base structures and to enhance the fluorescence intensity in the preparation of industrial pigments and studies of electroluminescence.[6] As expected, this Zn–salen complex was found to be more stable than salen ligand in water. Furthermore, the fluorescence intensity of **3** was also enhanced by 1.5 orders of magnitude compared to that of FHBA in water under the same conditions such as the concentration of 0.6 μM, excitation wavelength at 340 nm, and the same integration area from 400 to 600 nm (Fig. 1B). In addition, receptor **3** was demonstrated to be as much as two times more sensitive than **2** in association with Ca^{2+} ion. This modified Zn complex also exhibited Ca^{2+}-specific fluorescence quenching with Group I and II metal ions in Fig. 2. It should be noted that Ca^{2+} ion is known to be capable of binding to the phenolic group in Ni^{II}– and Cu^{II}–salen complexes resulting in the formation of bimetallic complexes.[7] Nevertheless, Zn–salen type complex was demonstrated here with improving fluorescence intensity as well as their sensitivity toward Ca^{2+} ion in water.

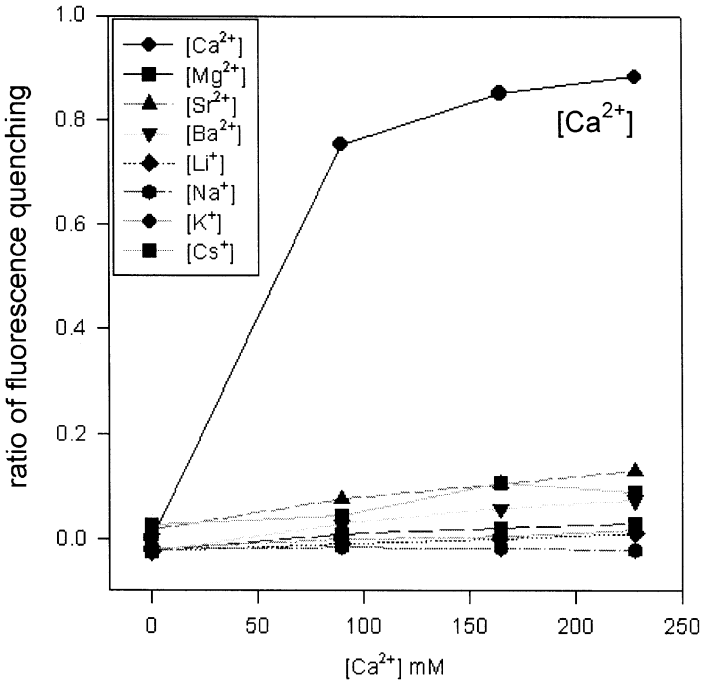


Figure 2. Demonstration of the Ca^{2+}-specific quenching of the fluorescent intensity of compound **3** (0.6 μM) using various alkali and alkaline–earth metal ions in water at 25°C

We also found that the color of the FHBA solutions was changed to a deeper yellow in the presence of Ca^{2+} ion. Thus, UV–vis spectroscopy may provide an alternative and simple tool in the visualization of Ca^{2+}-specific recognition for these receptors. Upon increasing the concentration of Ca^{2+} ion in each of the solutions of **1**, **2**, or **3**, a new absorption band appeared at 385 nm, which is approximately 50 nm red-shifted relative to the absorption wavelength of the starting materials indicated in Fig. 3. Under similar conditions, other alkaline or alkaline–earth

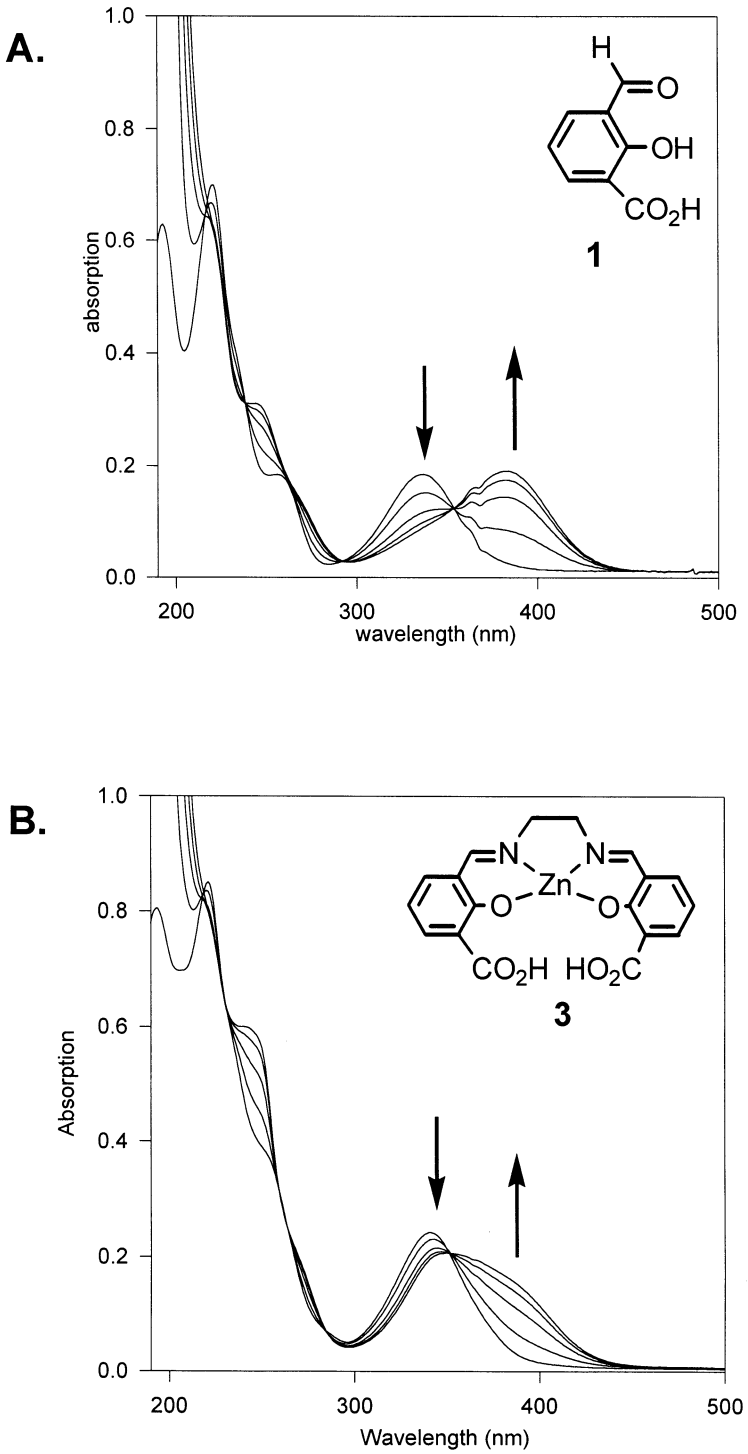


Figure 3. UV–vis titration of compounds **1** and **3** with the increasing Ca^{2+} ion concentration in water as well as in 10 mM sodium phosphate buffer at pH 7 at 25°C. [**1**] and [**3**] = 28 μM in water, and [Ca^{2+} = 29, 58, 81 and 110 μM. The isosbestic point is located at 350 nm in these absorption spectra

metal ions failed to induce the red-shift in the UV–vis spectra of these receptors, suggesting that the red-shift in the absorption is specifically due to the binding of the receptor with Ca^{2+} ion.

Considering that Ca^{2+} ion acts as a cation–proton exchange species (–OH···M→–OM···H) of the phenolic proton, an aqueous NaOH solution (10 mM) was used to neutralize the protons in **1**. After the deprotonation process has reached at pH>10, a red-shift was also observed in the absorption band as mentioned above. Furthermore, we noted that a correlation between the UV and fluorescence of **1** under various pH values, in support of the importance of phenolic proton in **1**, as illustrated in Fig. 4. When pH is adjusted to greater than 10, which is close to the p*K*a value of the phenolic proton, the intensity of the UV–vis absorption at 380 nm increases while the emission intensity at 485 nm decreases in the absence of Ca^{2+}. A similar correlation was also observed in the presence of Ca^{2+} ion. On the other hand, 2-hydroxybenzaldehyde reacted with Ca^{2+} ion shows a red-shift in the UV–vis spectra, but no fluorescence quenching was observed under the same conditions. This suggests that the deprotonation of the phenolic proton is corresponding to the large UV red-shift; meanwhile, the presence of carboxylate group of **1** is important to the fluorescence quenching process inducing by the specific Ca^{2+}-binding.

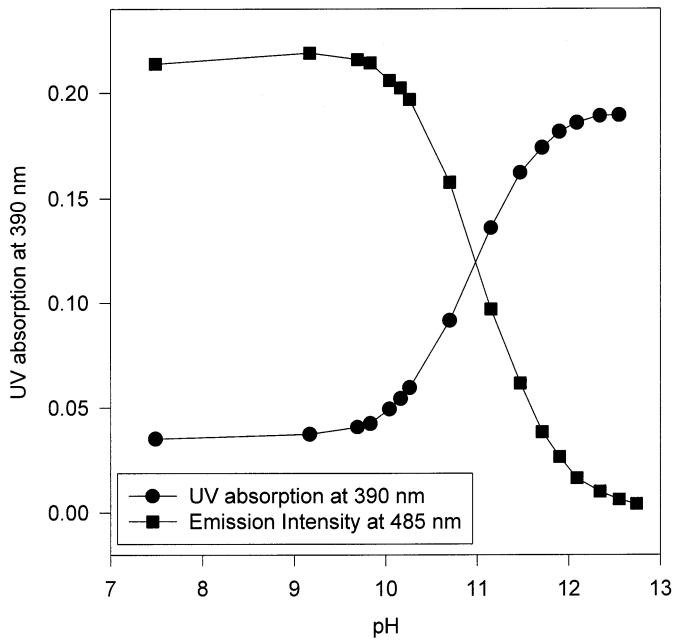


Figure 4. Correlation between the increase of the UV absorption at 390 nm and the quenching intensity of the fluorescence at 485 nm (excitation wavelength at 340 nm) of **1** (28 μM) along with pH variation (7–12) adjusted by NaOH in water at 25°C

Overall, FHBA and its derivatives were demonstrated to serve as novel Ca^{2+}-specific binding indicators via the induction of a red-shift of the absorption band and an intensity quenching of the fluorescence emission. Even though these receptors are specific binding to Ca^{2+} ion, the sensitivity of the receptors to Ca^{2+} ion is in the millimole range concentration. Hopefully, one may improve the sensitivity toward Ca^{2+} ion binding by increasing the number of the binding moiety and the flexibility of the bridging linker of the salen ligand. This approach is under investigation.

Acknowledgements

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