An OFF−OFF−ON Fluorescence Sensor for Metal Ions in Stepwise Complex Formation of 2,3,5,6-Tetrakis(2-pyridyl)pyrazine with Metal Ions

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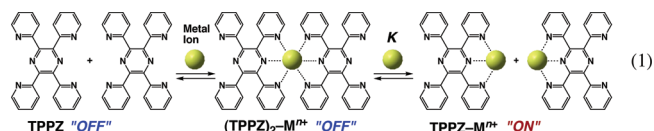
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Fluorescence sensors for metal ions are one of the common analytic tools for obtaining quantitative information about the amount of those metal ions.[1−5] Simple 1:1 stoichiometric host (probe molecule)−guest (metal ion) recognition is the most popular use for this technique, which affords "OFF−ON" switchability, where a probe molecule recognizes a metal ion emitting specific fluorescence upon binding the metal ion.[1−5] On one hand, the fluorescence intensity due to the complex formation with the metal ion (M^{n+}) increases linearly with increasing metal ion concentration from 0 M ([M^{n+}] > 0 M). On the other hand, development of detection tools for metal ions in a broad concentration range (such as Zn^{2+} in cellular systems where its concentration varies from 10^{-10} to 10^{-4} M in some vesicles) is highly desired.[6−8] Judicious choice of fluorescence sensors with appropriate affinities for metal ions has made it possible to obtain the useful fluorescence assay; however, those fluorescence sensors bind with metal ions within a limited concentration range of metal ions.[8] Development of new fluorescence assay systems, where a probe molecule begins to fluoresce at certain concentrations of metal ions, would expand the scope of the availability of fluorescence techniques for routine analysis of metal ions. In such a case, nonlinear OFF−OFF−ON switchability would be required in fluorescence sensors.

We report herein an OFF−OFF−ON fluorescence sensor for metal ions by using stepwise complex formation equilibria of 2,3,5,6-tetrakis(2-pyridyl)pyrazine (TPPZ) with metal ions. TPPZ used as a probe molecule in this work has recently been utilized in a variety of molecular wire complexes as a bridging ligand.[9]

Upon addition of scandium triflate [Sc(OTf)$_3$][10] (OTf = OSO$_2$CF$_3$) [0−5.9 × 10^{-5} M] to an acetonitrile (MeCN) solution of TPPZ (5.7 × 10^{-5} M), TPPZ shows stepwise UV−vis spectral changes in response to the ratio of Sc^{3+} concentration to the initial concentration of TPPZ ([Sc^{3+}]/[TPPZ]$_0$) at 0−0.5 (red line−blue line) and 0.5−1.0 (blue line−green line), with isosbestic points at 262, 276, 302, 311 nm and at 237 and 278 nm, respectively (Figure 1a). Such stepwise spectral changes of TPPZ with respect to [Sc^{3+}]/[TPPZ]$_0$ at 0−0.5 and 0.5−1.0 are ascribed to a 2:1 complex formation of TPPZ with Sc^{3+} [(TPPZ)$_2$−Sc^{3+}] and a 1:1 complex formation of TPPZ with Sc^{3+} (TPPZ−Sc^{3+}), respectively (eq 1).[11]

$$\text{TPPZ "OFF"} + \text{Metal Ion} \rightleftharpoons \text{(TPPZ)}_2\text{-M}^{n+}\text{ "OFF"} \overset{K}{\rightleftharpoons} \text{TPPZ-M}^{n+}\text{ "ON"} \quad (1)$$

Plots of absorbance changes (ΔAbs) at λ = 358 and 303 nm versus [Sc^{3+}]/[TPPZ]$_0$ [blue and red circles, respectively, Figure 1a (inset)] confirm the stoichiometry of (TPPZ)$_2$−Sc^{3+} and TPPZ−Sc^{3+} in eq 1. TPPZ forms the (TPPZ)$_2$−Sc^{3+} complex at Sc^{3+} concentrations below the 1:2 ratio of [Sc^{3+}] to [TPPZ]$_0$ ([Sc^{3+}]/[TPPZ]$_0$ < 0.5), which is converted to the TPPZ−Sc^{3+} complex at Sc^{3+} concentrations above the 1:2 ratio ([Sc^{3+}]/[TPPZ]$_0$ > 0.5).
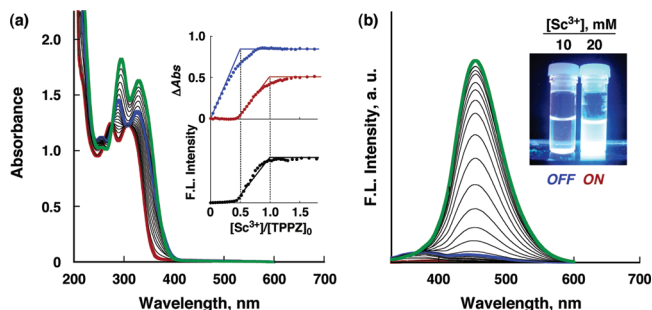
Figure 1. (a) UV−vis absorption and (b) fluorescence spectra of TPPZ (5.7 × 10^{-5} M) in the presence of Sc^{3+} [0 M (red line) to 2.8 × 10^{-5} M (blue line) to 5.9 × 10^{-5} M (green line)] in MeCN at 298 K. Excitation wavelength λ = 315 nm. Insets show (a) plots of ΔAbs at λ = 358 (●, blue) and 303 nm (●, red) vs [Sc^{3+}]/[TPPZ]$_0$ and a plot of fluorescence intensity at λ = 453 nm (●) vs [Sc^{3+}]/[TPPZ]$_0$, and (b) photograph of MeCN solutions of TPPZ (2.0 × 10^{-2} M) in the presence of Sc^{3+} (1.0 × 10^{-2} M and 2.0 × 10^{-2} M) under UV−vis irradiation.

The visible fluorescence photograph of MeCN solutions of TPPZ in the presence of 0.5 and 1 equiv of Sc^{3+} is shown in Figure 1b (inset, left and right, respectively). The 1:1 complex (TPPZ−Sc^{3+}) fluoresces strongly (right in photograph).[12] Since TPPZ itself hardly fluoresces, the fluorescence of TPPZ−Sc^{3+} may result from the change in the lowest excited-state from the n,π^* triplet to the π,π^* singlet which becomes lower in energy than the n,π^* triplet because of the complexation with Sc^{3+} that acts as a strong Lewis acid.[13,14] In contrast, the (TPPZ)$_2$−Sc^{3+} complex hardly fluoresces (left in photograph) as in the case of free TPPZ. The strong binding of Sc^{3+} ions in the TPPZ−Sc^{3+} complex may be significantly weakened in the 2:1 complex [(TPPZ)$_2$−Sc^{3+}], in which the lowest excited-state is changed from the π,π^* singlet to the n,π^* triplet again. This may be the reason why the (TPPZ)$_2$−Sc^{3+} complex hardly exhibits fluorescence, whereas the TPPZ−Sc^{3+} complex fluoresces strongly.

The fluorescence spectral titration of TPPZ by Sc^{3+} is shown in Figure 1b under same conditions as for the UV−vis spectral titration (Figure 1a). Stepwise fluorescence spectral changes in response to [Sc^{3+}]/[TPPZ]$_0$ at 0−0.5 (red line−blue line) and 0.5−1.0 (blue line−green line) are also observed as in the case of the UV−vis spectral titration of TPPZ by Sc^{3+}. There is very weak fluorescence at λ = 380 nm owing to the (TPPZ)$_2$−Sc^{3+} complex at Sc^{3+} concentrations below the 1:2 ratio ([Sc^{3+}]/[TPPZ]$_0$ < 0.5: Figure 1b red line−blue line). At Sc^{3+} concentrations above the 1:2 ratio ([Sc^{3+}]/[TPPZ]$_0$ > 0.5), however, strong fluorescence starts to appear at λ = 453 nm (Figure 1b blue line−green line), which is ascribed to formation of the TPPZ−Sc^{3+} complex. The fluorescence intensity (F.L. intensity) at λ = 453 nm (black circles [Figure 1a (inset)]) due to the TPPZ−Sc^{3+} complex is plotted against [Sc^{3+}]/[TPPZ]$_0$, where fluorescence intensity starts to increase at Sc^{3+} concentrations above the 1:2 ratio ([Sc^{3+}]/[TPPZ]$_0$ > 0.5).

When Zn^{2+} is employed instead of Sc^{3+}, TPPZ also shows stepwise UV−vis and fluorescence spectral changes in response
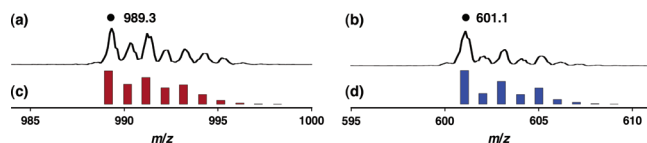
Figure 2. Positive-ion ESI MS of a MeCN solution of TPPZ (3.9×10^{-5} M) in the presence of Zn^{2+} [(a) 3.0×10^{-5} M and (b) 6.8×10^{-5} M]. The signals at m/z (a) 989.3 and (b) 601.1 correspond, respectively, to {[Zn-(TPPZ)$_2$](OSO$_2$CF$_3$)}$^+$ and {[Zn(TPPZ)](OSO$_2$CF$_3$)}$^+$. Calculated isotopic distributions for (c) {[Zn(TPPZ)$_2$](OSO$_2$CF$_3$)}$^+$ and (d) {[Zn(TPPZ)](OSO$_2$-CF$_3$)}$^+$ are shown.
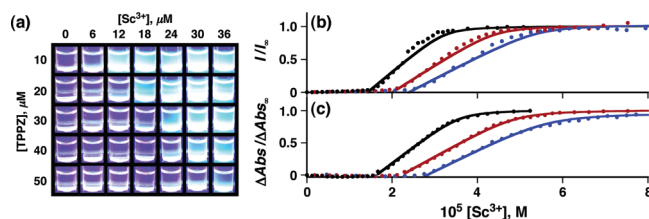
Figure 3. (a) Change in visible fluorescence of MeCN solutions of TPPZ (1.0×10^{-5}–5.0×10^{-5} M) in the presence of Sc^{3+} (0–3.6×10^{-5} M). Plots of (b) I/I_∞ at 453 nm and (c) $\Delta Abs/\Delta Abs_\infty$ [$= (A - A_0)/(A_\infty - A_0)$] at 303 nm vs [$Sc^{3+}$] for the titration of TPPZ [3.4×10^{-5} M (●), 4.7×10^{-5} M (●, red), and 5.7×10^{-5} M (●, blue)] by Sc^{3+} in MeCN at 298 K.

to the ratio of [Zn^{2+}]/[TPPZ]$_0$ at 0–0.5 and 0.5–50 (see Supporting Information S2).[10,15] Such stepwise UV–vis and fluorescence spectral changes with respect to [Zn^{2+}]/[TPPZ]$_0$ are also ascribed to formation of a 2:1 complex [(TPPZ)$_2$–Zn^{2+}] and a 1:1 complex (TPPZ–Zn^{2+}), respectively. These complexes are detected by positive-ion ESI mass spectrum of TPPZ (3.9×10^{-5} M) in MeCN in the presence of low (3.0×10^{-5} M: Figure 2a) and high (6.8×10^{-5} M: Figure 2b) concentrations of Zn^{2+}, respectively.[16–19]

To confirm the availability of such an OFF–OFF–ON fluorescence sensor for detection of metal ions in a wide concentration range, the fluorescence in the presence of various concentrations of TPPZ (1.0–5.0×10^{-5} M) and Sc^{3+} (0–3.6×10^{-5} M) were visualized as shown in Figure 3a. The TPPZ fluoresces strongly in the presence of >0.5 equiv of Sc^{3+} at each concentration of TPPZ. Dependence of the ratio of the fluorescence intensity to the final fluorescence intensity (I/I_∞) on [Sc^{3+}] is shown in Figure 3b, where I/I_∞ starts to increase at Sc^{3+} concentrations above the 1:2 ratio ([Sc^{3+}]/[TPPZ]$_0$ > 0.5), which is shifted to the larger value with increase of initial concentration of TPPZ [3.4×10^{-5} M (black circles), 4.7×10^{-5} M (red circles), and 5.7×10^{-5} M (blue circles)]. The dependence of I/I_∞ (Figure 3b) on [Sc^{3+}] agrees with that of $\Delta Abs/\Delta Abs_\infty$ on [Sc^{3+}] (Figure 3c). Such absorbance and fluorescence intensity changes due to the TPPZ–M^{n+} complex formation is expressed by eq 2, where A_0 and A_∞ are absorbances ascribed to (TPPZ)$_2$–M^{n+} and TPPZ–M^{n+}, respectively (for the derivation of eq 2 see Supporting Information S4). The formation constant (K) of TPPZ–Sc^{3+} is determined as $(1.4 \pm 0.1) \times 10^2$ from the best-fit lines in Figure 3, parts b and c.

$$(A - A_0) = (A_\infty - A_0) \times$$
$$\text{(or } I) \qquad \text{(or } I_\infty)$$
$$\frac{-K[M^{n+}] + \sqrt{K^2[M^{n+}]^2 + (4 - K)K[TPPZ]_0(2[M^{n+}] - [TPPZ]_0)}}{4 - K} \quad (2)$$

In conclusion, we have developed an OFF–OFF–ON fluorescence sensor for metal ions. The OFF–OFF–ON fluorescence sensor has potential uses for biological applications because our concept should be general enough to be applied to other probe molecules that exhibit stepwise complex formation with metal ions.

Supporting Information Available: UV–vis spectra of TPPZ in the presence of Sc^{3+} (8.0×10^{-4}–1.6×10^{-2} M) [S1], UV–vis and fluorescence titration of TPPZ by Zn^{2+} (S2), visible fluorescence of TPPZ–Zn^{2+} in water (S3), and the derivation of eq 2 (S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) TPPZ–Sc^{3+} is converted to TPPZ–$(Sc^{3+})_2$ at a high concentrations of Sc^{3+} (8.0×10^{-4} to 1.6×10^{-2} M) [see Supporting Information S1], and the TPPZ–$(Sc^{3+})_2$ complex fluoresces as strong as TPPZ–Sc^{3+}.

(12) The fluorescence lifetime of the TPPZ–Sc^{3+} complex was too short to be determined accurately (τ_s < 2 ns).

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(17) TPPZ should form a complex with Sc^{3+}, because even Zn^{2+} (divalent cation) that is a much weaker Lewis acid than Sc^{3+} (trivalent cation) can form the 2:1 and 1:1 complexes with TPPZ, (TPPZ)$_2$–Zn^{2+} and TPPZ–Zn^{2+}, respectively. Formation of (TPPZ)$_2$–Sc^{3+} and TPPZ–Sc^{3+} is confirmed by ^{1}H NMR, the signals of which exhibit a downfield shift in the presence of 0.5 equiv of Sc^{3+}, demonstrating development of positive charge by formation of (TPPZ)$_2$–Sc^{3+}. The ^{1}H NMR signals of (TPPZ)$_2$–Sc^{3+} exhibit a further downfield shift in the presence of 1 equiv of Sc^{3+} because of the additional binding of Sc^{3+} to yield the 1:1 complex, TPPZ–Sc^{3+}. However, (TPPZ)$_2$–Sc^{3+} and TPPZ–Sc^{3+} cannot be detected by positive-ion ESI MS, due possibly to multivalency of the Sc^{3+} complex of TPPZ.

(18) TPPZ also forms fluorescent TPPZ–Zn^{2+} in water (see Supporting Information S3).

(19) Complex formation of TPPZ with potentially interfering metal ions such as Fe(II) has been examined. TPPZ also forms a complex with Fe(II) in MeCN; however, this complex exhibits no fluorescence. Fluorescence sensors for other metal ions will be reported elsewhere.

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