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The Fluorescence Behavior of the Responsive Macrocycle by Aromatic Imine Molecules

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The macrocycle L exhibited a switch on-off behavior through the fluorescent responses by aromatic imine molecule **1** (X=H) / trifluoroacetic acid (TFA). In the "switch on" state, it was supposed that the aromatic imine molecule **1** is in the cavity of macrocycle L and a photoinduced electron transfer (PET) from the nitrogen of azacrown part to the anthryl group is inhibited by the interaction between the aromatic imine molecule **1** and the azacrown part of macrocycle L. In the "switch off" state, it was supposed that the protonated imine molecule **1** is induced by the continuous addition of TFA and a repulsion between the protonated azacrown part and the protonated imine molecule **1** is occurred. It was considered that this process induces the intermolecular PET from the protonated imine molecule **1** to the anthryl group of macrocycle L because of a proximity effect between the anthryl group and the protonated imine molecule **1**. From the investigation of the transient emission decay curve, the macrocycle L showed three components (3.45 ns (79.72%), 0.61 ns (14.53%), and 0.10 ns (5.75%)). When the imine molecule **1** was added in the macrocycle L as molar ratio=1:1, the first main component showed a little longer lifetime as 3.68 ns (82.75%) although the other two components were similar as 0.64 ns (14.28%) and 0.08 ns (2.96%). On the contrary, when the imine molecule **3** (X=Cl) was added in the macrocycle L as molar ratio=1:1, all the three components were decreased such as 3.27 ns (69.83%), 0.44 ns (13.24%), and 0.06 ns (16.93%). The fluorescent pH titration of macrocycle L was carried out from pH=3 to pH=9. The macrocycle L and Cu^{2+}-macrocycle L complex were intersected at about pH=5, while the Eu^{3+}-macrocycle L complex was intersected at about pH=5.5. In addtion, we investigated the fluorescence change of macrocycle L as a function of the substituent constant (σ_p^o) showing in the para-substituent with electron withdrawing groups (X=F, Cl) and electron donating groups (X= CH_3, OCH_3, $N(CH_3)_2$), respectively, as well as non-substituent (X=H).

Key words: macrocycle L, aromatic imine molecule, fluorescence behavior, transient emission decay curve, fluorescent pH titration, substituent constant

INTRODUCTION

In recent years, fluorescent receptors have gained much attention because of their usefulness in analysis and clarification of the roles of biomolecules in living systems [1]. They have served as chemosensors for stoichiometric hostguest molecular recognition [2]. Accordingly, various fluorescent supramolecules including crown ethers have been designed and synthesized [3]. Although numerous fluorescent receptors have been prepared, there have been limited report on the recognition of neutral molecules by fluorescent receptors. This study is crucial in developing biomimic systems for elucidating the roles of biomolecules in living systems. We have been recently studying the development of a functionalized dianthryl tetraaza macrocycle with the recognizing and switching ability on the neutral molecules as well as various metal ions [4]. Furthermore, we obtained some interesting results on the fluorescence behavior of macrocycle L. Thus, in this work we investigated the switch on-off capability of the macrocycle L by imine molecule **1**/ trifluoroacetic acid (TFA), by using fluorescence spectroscopic methods.

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MATERIALS AND METHODS

The macrocycle L and aromatic imine molecules were synthesized by the same procecure as reported previously [4].

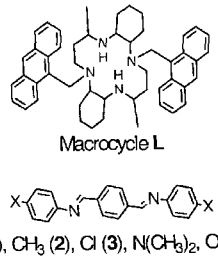

Figure 1. The structure of macrocycle L and aromatic imine molecules.

Acetonitrile was used as the spectrophotometric grade of Aldrich for the spectroscopic measurement. The salts $CuSO_4 \cdot 6H_2O$ and $EuCl_3$ were used for the spectroscopic measurement. The fluorescence ratios (F/Fo) were decided as the ratios between the fluorescence area (F) after adding the imine molecule and one (Fo) before adding it.

The absorption spectra were measured using a Hitachi U-3300 spectrophotometer. Fluorescent pH titration was measured by using pH Scan BNC (Singapore). The fluorescence spectra were measured using SLM 8100 spectrofluorometer (Aminco, USA) with Xe-arc lamp light source using 4 or 8 nm band pass excitation and emission monochromators, in which the rhodamine B solution was used as a reference to correct the variation of the Xe light source with time and wavelength. The time-resolved emission measurements were performed with an Edingburgh FL-900 and a femtosecond mode-locked Ti-sapphire laser pumped by a Nd : YVO_4 laser. Laser output has a 200fs pulse width, and it can span the excitation wavelength in the range of 235 – 300 and 350 – 490 nm by using nonlinear optical crystals. Temporal profiles of the fluorescence decays were measured by using time-correlated single photon counting method (TCSPC). The instrumental response function was measured by detecting the scattered laser pulse of ca. 1 ps with quartz crystal. The resultant FWHM is 40 ps. This method allows a time resolution of about 20 ps after deconvolution.

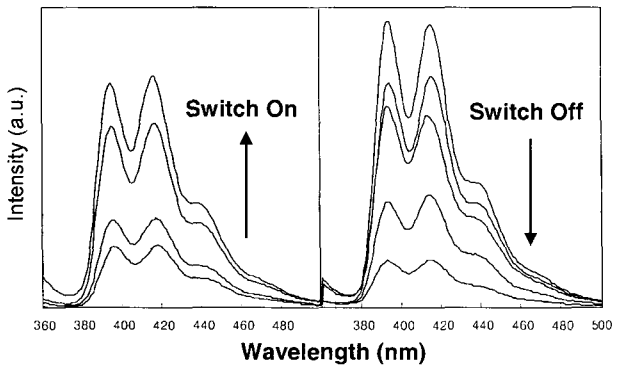

Figure 2. Fluorescence switching of macrocycle L by aromatic imine molecule 1/ TFA.

RESULTS AND DISCUSSION

The aromatic imine molecules were simply synthesized through the imine condensation reaction of 1,4-phthalaldehyde with aniline derivatives in distilled toluene or methylene chloride at the refluxing condition, as reported previously [4]. In addition, macrocycle L was prepared in the moderate yield by one-pot reaction of 9-chloromethylanthracence with 3,14-dimethyl-2,6,13,17-tetraazatricyclo[14,4,$0^{1,18}$,$0^{7,12}$] docosane, as reported previously [5]. The aromatic imine molecule **1** (X =H) was added in 1×10^{-6} mol dm^{-3} acetonitrile solution of macrocycle L as a cumulative concentration and the fluorescence measurement was carried out at λ_{ex}=260 nm. As shown in Fig. 2 and Fig. 3, the fluorescence was enhanced remarkably by the addition of the aromatic imine molecule **1** (*switch on*). In the "switch on" state, it was supposed that the aromatic imine molecule **1** is in the cavity of macrocycle L and a photo-induced electron transfer (PET) [6] from the nitrogen of the azacrown part to the anthryl group is inhibited by interaction between the aromatic imine molecule **1** and azacrown part of macrocycle L. After the saturation of the enhanced fluorescence, trifluoroacetic acid (TFA) was added. The initial addition of TFA enhanced the fluorescence a little more but decreased when TFA was continuously added (*switch off*), as shown in Fig. 2. This may be caused by the protonation of the nitrogen of the azacrown part in the intial addition of TFA, by which the PET from the nitrogen of the azacrown part to the anthryl group is inhibited completely. In the "switch off" state, it was supposed that the protonated imine molecule **1** is induced by the continuous addition of TFA and a repulsion between the protonated azacrown part and the protonated imine molecule **1** is occurred. Based on the above result, this process is induced to intermolecular PET from the protonated imine molecule **1** to the anthryl group of macrocycle L because of a

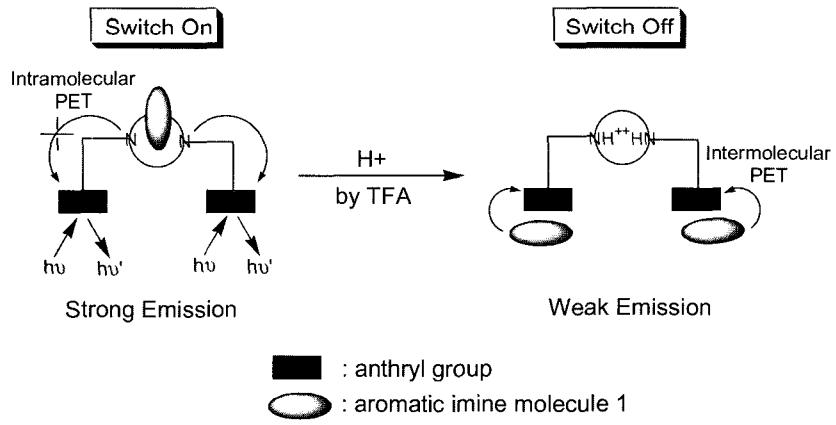

Figure 3. A proposed scheme of the switch on-off.

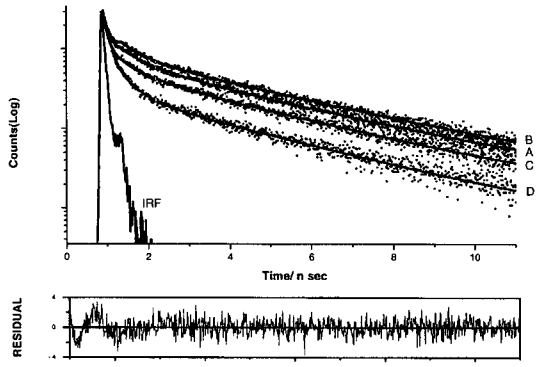

Figure 4. Fluorescence emission decay profiles of macrocycle L monitored at λ_{em}=420 nm; (A) only macrocycle L, (B) the molar ratio of macrocycle L and aromatic imine molecule **1**=1:1, (C) the molar ratio of those=1:10, and (D) the molar ratio of those=1:20.

proximity effect between the anthryl group and the protonated imine molecule **1**.

As shown in Fig. 4 and Table 1, from the investigation of the transient emission decay curve, the macrocycle L showed three components (3.45 ns (79.72%), 0.61 ns (14.53%), and 0.10 ns (5.75%)). When the imine molecule **1** was added in the macrocycle L as molar ratio=1:1, the first main component showed a little longer lifetime, increasing to 3.68 ns (82.75%) although the other two components were similar as 0.64 ns (14.28%) and 0.08 ns (2.96%). When the molar ratios of macrocycle L and the imine molecule **1** were 1:10 and 1:20, the lifetimes of the first main component were decreased to 3.38 ns (76.40%) and 3.25 ns (60.16%), respectively. Also, in the case of the transient emission decay curve of the imine molecule **2** (X=CH_3), the lifetimes of the first main component were decreased in the same way mentioned. It is considered that these results are ascribed to the PET inhibition from the nitrogen of the azacrown part to the anthryl group by interaction between the aromatic imine molecule and the azacrown part of macrocycle L in the molar ratio=1:1 condition and intermolecular PET appearance from the imine molecule to the anthryl group of macrocycle L by a proximity effect between the anthryl group and the imine mocule in the molar ratio=1:10 or 1:20 condition [7]. On the contrary, as shown in Fig. 5 and Table 2, when the imine molecule **3** (X=Cl) was added in the macrocycle L as molar ratio=1:1, all the three components were decreased such as 3.27 ns (69.83%), 0.44 ns (13.24%), and 0.06 ns (16.93%). It was considered that this result is ascribed to the PET appearance from the nitrogen of the azacrown part to the anthryl group because of a non-interaction between the aromatic imine molecule and the azacrown part of macrocycle L by the functional Cl group.

Table 1. Life times obtained from the fluorescence emission decay profiles of macrocycle L

macrocycle L: imine molecule 1 (molar ratio)	life time/ns τ_1	τ_2	τ_3
only macrocycle L	3.45(79.72%)	0.61(14.53%)	0.10(5.75%)
1:1	3.68(82.75%)	0.64(14.28)	0.08(2.96%)
1:10	3.38(76.40%)	0.619(13.39%)	0.11(10.21%)
1:20	3.25(60.16%)	0.50(17.10%)	0.13(22.70%)

cf)The parentheses indicate the component percent of each life time.

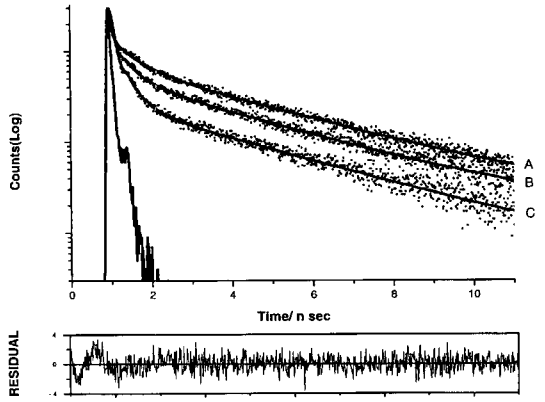

Figure 5. Fluorescence emission decay profiles of macrocycle L monitored at λ_{em}=420 nm; (A) only macrocycle L, (B) the molar ratio of macrocycle L and aromatic imine molecule **3**=1:1, and (C) the molar ratio of those=1:10.

In addition, when the molar ratio of macrocycle L and the imine molecule **3** was 1:10, the first and second component showed a shorter lifetime relative to the decay curve in the molar ratio=1:1 such as 3.12 ns (55.86%), 0.36 ns (17.37%), and 0.06 ns (26.77%).

As shown in Fig. 6, the fluorescent pH titration was carried out from pH=3 to pH=9 by the addition of 0.1 mol dm^{-3} aqueous NaOH solution in the 1×10^{-5} mol dm^{-3} acetonitrile : H_2O (4:1) solution of sample kept at pH=3 by the addition of 0.1 mol dm^{-3} aqueous HCl solution. The macrocycle L and Cu^{2+}-macrocycle L complex intersected at about pH=5 with beginning of fluorescence change at about pH=3.5 while the deprotonation of the macrocycle L and demetallation of Cu^{2+}-macrocycle L complex occurred completely at about pH=7, respectively. However, the Eu^{3+}-macrocycle L complex was intersected at about pH=5.5 with fluorescence change beginning at about pH=4 and then demetallation of Eu^{3+}-macrocycle L complex occurred completely at pH>8.

To investigate the fluorescence change as a function of the substituent constant, we selected the values of substituent constants (σ_p^o) showing in the para-substituent with electron

Table 2. Life times obtained from the fluorescence emission decay profiles of macrocycle L

macrocycle L: imine molecule 3 (molar ratio)	life time/ns τ_1	τ_2	τ_3
only macrocycle L	3.45(79.72%)	0.61(14.53%)	0.10(5.75%)
1:1	3.27(69.83%)	0.44(13.24)	0.06(16.93%)
1:10	3.12(55.86%)	0.36(17.37%)	0.06(26.77%)

cf)The parentheses indicate the component percent of each life time.

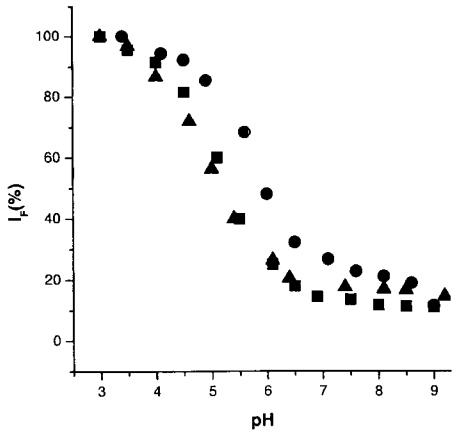

Figure 6. Fluorescent pH titration of macrocycle L and metal complexes; ■ (only macrocycle L), ●(Eu^{3+} complex), and ▲ (Cu^{2+} complex).

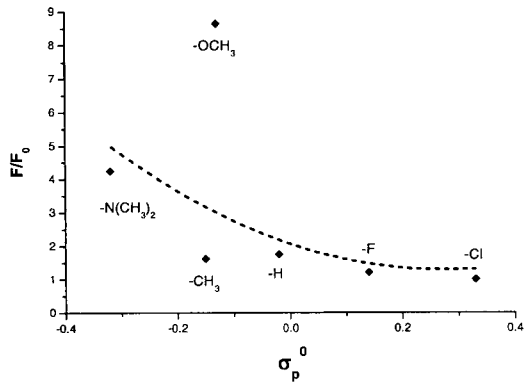

Figure 7. Fluorescence ratios (F/Fo) as a function of the substituent constants of aromatic imine molecules (σ_p^o).

withdrawing groups (X=F, Cl) and electron donating groups (X=CH_3, OCH_3, $N(CH_3)_2$), respectively, as well as non-substituent (X=H) [8]. As shown in Fig. 7, when it was plotted as the relation between fluorescence ratio (F/Fo) and substituent constant, a reciprocal proportional curve appeared. This result showed that the methoxy (X=OCH_3) substituent produced exceptional fluorescence ratios. This may be due to the strong hydrogen bonding between the oxygen of the methoxy group and the amine group of the azacrown part. In other words, the HOMO level of the azacrown part may be stabilized by strong hydrogen bonding, while the intramolecular PET was efficiently prohibited, efficiently inducing the strong emission.

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7. We obtained an experimental tendency that the emission enhancement induced to a little longer lifetime and the emission decrease induced to a little shorter lifetime in the first main component. It could not be excluded completely that the tendency of lifetime change is related to one of emission change.
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