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Fluorescence PET (photo-induced electron transfer) sensor for water based on anthracene-amino acid

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ABSTRACT

An anthracene-amino acid system with two carboxyl groups has been designed and synthesized as a new class of fluorescence PET (photo-induced electron transfer) sensor for detection of water in organic solvents. An enhancement in fluorescence is observed with increasing water content in 1,4-dioxane, THF, acetonitrile and ethanol, which is attributable to the suppression of PET by the intramolecular proton transfer of the carboxyl proton to the amino group. The detection limit and quantitation limit are, respectively, 0.1 and 0.3 wt% for 1,4-dioxane, 0.4 and 1.2 wt% for THF, 0.1 and 0.3 wt% for acetonitrile and 0.1 and 0.3 wt% for ethanol.

1. Introduction

Fluorescence sensors have received a considerable attention for their potential applications to biochemical and medical analyses and optoelectronic devises [1–8]. Among them, fluorescence sensors for detection and quantification of water in organic solvents and in the atmosphere are of a major importance in the construction of environmental monitoring system in industry as well as in the fundamental analytical chemistry [9–20]. In most of these fluorescence water sensors developed so far, however, the fluorescence intensity decreases with an increase of water in organic solvents and a significant fluorescence quenching occurs in polar solvents, in particular [15–20]. Thus, it is difficult to detect a trace amount of water in polar solvents by using these fluorescence sensors. In order to improve detection limits of fluorescence water sensors, a new detection principle is required.

Recently, we have designed and synthesized an anthracene-amino acid **1** to create a new class of fluorescence PET (photo-induced electron transfer) sensor for detection of water in organic solvents (Scheme 1a) [21]. Fluorescence PET sensors, in general, have been developed as pH sensor or cation sensors for detecting cations such as H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} as well as neutral organic species such as saccharides in biochemical analyses [5–8,22–29]. They are composed of a fluorophore skeleton linked to a cation binding site such as an amino moiety via a methylene spacer (fluorophore–spacer–receptor structure). The PET takes place from the nitrogen atom of amino moiety to fluorophore skeleton, leading to fluorescence quenching of the fluorophore. When the nitrogen atom is protonated or strongly interacts with a cation, the PET is inhibited and a drastic enhancement of fluorescence is observed. In our fluorescence PET sensor **1**, on the other hand, addition of water to organic solvents containing **1** promotes dissociation of the carboxyl proton of **1**, followed by the formation of fluorescent zwitterionic structure **1a** by the protonation of the amino group. As a result, the intramolecular proton transfer of the carboxyl proton to the amino group suppresses PET and thus an enhancement in fluorescence is observed with increasing water content in polar and less polar organic solvents. This provides a new concept of the molecular design for a fluorescence water sensor based on PET. The detection limit (DL) and quantitation limit (QL) are, respectively, 0.1 and 0.4 wt% for acetonitrile and 0.1 and 0.2 wt% for ethanol. However, the DL and QL in less polar organic solvents are lower than those in polar organic solvents.

In order to further improve sensing abilities, we have designed and synthesized a new fluorescence PET sensor for water based on anthracene-amino acid **4** having two carboxyl groups (Scheme 1b). We expected that the addition of water to organic solvents containing **4** causes an efficient formation of fluorescent zwitterionic compounds **4a**, because of a more acidic nature of **4** having two carboxyl groups than **1** which has one carboxyl group. Herein we report the high-sensitive detection of a trace amount of water in a various organic solvents based on the PET characteristics of anthracene-amino acid **4**.

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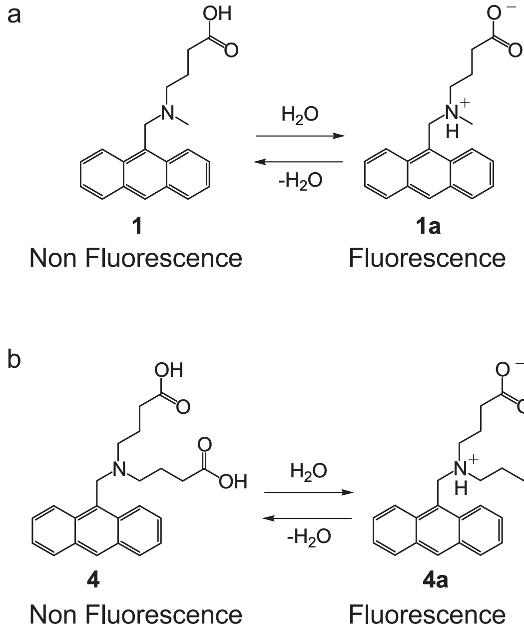


Scheme 1. Mechanism of fluorescence PET sensor (a) **1** and (b) **4** for detection of water in organic solvents.

2. Experimental

Melting points were measured with a Yanaco micro melting point apparatus MP model. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer by ATR method. Absorption spectra were observed with a Shimadzu UV-3150 spectrophotometer and fluorescence spectra were measured with a Hitachi F-4500 spectrophotometer. High resolution mass spectral data by ESI were acquired on a Thermo Fisher Scientific LTQ Orbitrap XL. ^1H NMR spectra were recorded on a JNM-LA-400 (400 MHz) FT NMR spectrometer with tetramethylsilane as an internal standard. Column chromatography was performed on silica gel (KANTO CHEMICAL, 60 N, spherical, neutral). The determination of water in 1,4-dioxane, THF, acetonitrile and ethanol solution was done with a MKC-610 and MKA-610 Karl Fischer moisture titrator (Kyoto Electronics manufucturing Co., Ltd.) based on Karl Fischer coulometric titration for below 1.5 wt% and volumetric titration for above 1.5 wt%, respectively.

*2.1. Synthesis of 4-(anthracen-9-ylmethyl)-amino-butyric acid ethyl ester (**2**)*

To a solution of 9-anthracenecarboxyaldehyde (8.0 g, 38.8 mmol) in 25% MeOH/CH_2Cl_2 (100 ml) was added a CH_2Cl_2 solution (100 ml) of ethyl 4-aminobutanoate hydrochloride (7.8 g, 46.5 mmol) pretreated with Na_2CO_3 solution (30% aq, 100 ml). The mixture was stirred at room temperature for 43 h under an argon atmosphere. The solution cooled to 0 °C, and then $NaBH_4$ (4.4 g, 0.12 mol) was added in small portions to the solution. The mixture was stirred at room temperature for 5 h. The solvent was removed in vacuo and the solid residue dissolved in CH_2Cl_2 (300 ml) and washed with Na_2CO_3 solution (10% aq, 3 × 100 ml). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed in vacuo to give an oily residue. The residue was chromatographed on silica gel (CH_2Cl_2/MeOH = 9:1 as eluent) to give **2** (3.7 g, yield 29%) as a light yellow oil; ^1H NMR (400 MHz, acetone-d_6, TMS) $\delta = 1.17$ (t, $J = 7.2$ Hz, 3H), 1.83–1.90 (m, 2H), 2.41 (t, $J = 7.4$ Hz, 2H), 2.93 (t, $J = 6.8$ Hz, 2H), 4.05 (q, 2H), 4.71 (s, 2H), 7.47–7.57 (m, 4H), 8.06 (d, $J = 8.2$ Hz, 2H), 8.45 (d, $J = 9.0$ Hz, 2H), 8.54 (s, 1H); IR (ATR): $\nu = 1726$ cm^{-1}; HRMS (ESI, m/z) 322.1797 [M+H]$^+$.

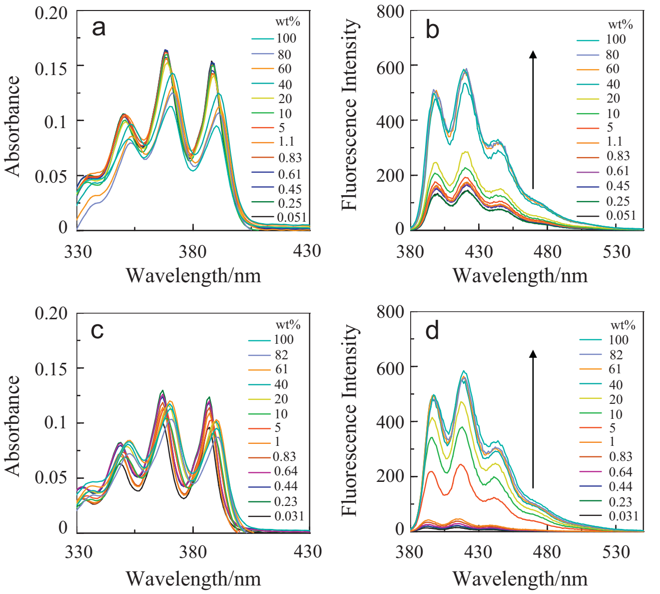


Fig. 1. (a) Absorption and (b) fluorescence spectra ($\lambda_{ex} = 366$ nm) of **4** ($c = 2.0 \times 10^{-5}$ M) in 1,4-dioxane containing water (0.051–100 wt%). (c) Absorption and (d) fluorescence spectra ($\lambda_{ex} = 366$ nm) of **4** ($c = 2.0 \times 10^{-5}$ M) in acetonitrile containing water (0.031–100 wt%).

*2.2. Synthesis of 4-anthracen-9-ylmethyl-(3-ethoxycarbonyl-propyl)-amino-butyric acid ethyl ester (**3**)*

A solution of **2** (3.5 g, 10.9 mmol) in acetonitrile (50 ml) was treated with sodium hydride (60%, 1.7 g, 43.6 mmol) and stirred for 1 h at room temperature. Ethyl 4-bromobutyrate (10.6 g, 54.4 mmol) was added dropwise over 20 min and the solution was stirred at room temperature for 1 h. After concentrating under reduced pressure, the resulting residue was dissolved in dichloromethane, and washed with water. The residue was chromatographed on silica gel (CH_2Cl_2/ethyl acetate = 3:1 as eluent) to give **3** (4.0 g, yield 84%) as a yellow oil; ^1H NMR (400 MHz, DMSO-d_6, TMS) $\delta = 1.18$ (t, $J = 6.3$ Hz, 6H), 1.80–1.86 (m, 4H), 2.10 (t, $J = 5.8$ Hz, 4H), 2.36 (t, $J = 5.8$ Hz, 4H), 4.03 (q, 4H), 4.48 (s, 2H), 7.49–7.55 (m, 4H), 8.07 (d, $J = 6.1$ Hz, 2H), 8.47 (d, $J = 7.1$ Hz, 2H), 8.55 (s, 1H); IR (ATR): $\nu = 1726$ cm^{-1}; HRMS (ESI, m/z) 436.2473 [M+H]$^+$.

*2.3. Synthesis of 4-anthracen-9-ylmethyl-(3-carboxy-propyl)-amino-butyric acid (**4**)*

A solution of **3** (3.0 g, 6.9 mmol) in ethanol (100 ml) was added dropwise aqueous NaOH (3 g, 75 mmol, 50 mL) with stirring at 60 °C. After further stirring for 8 h under reflux, the solution was acidified to pH 4 with 2 N HCl, and concentrated under reduced pressure. The residue was dissolved in dichloromethane, and washed with water. The residue was chromatographed on silica gel (CH_2Cl_2/MeOH = 5:2 as eluent) to give **4** (1.15 g, yield 44%) as a white solid; mp 169–171 °C; ^1H NMR (400 MHz, D_2O) $\delta = 1.85–2.08$ (br, 4H), 2.20–2.33 (br, 4H), 3.15–3.38 (br, 4H), 5.13 (s, 2H), 7.62–7.65 (m, 2H), 7.72–7.76 (m, 2H), 8.10–8.15 (m, 4H), 8.65 (s, 1H); IR (ATR): $\nu = 1721$ cm^{-1}; HRMS (ESI, m/z) 380.1859 [M+H]$^+$.

3. Results and discussion

The synthetic pathway of a fluorescence PET sensor **4** is shown in Scheme 2. The reductive amination of 9-

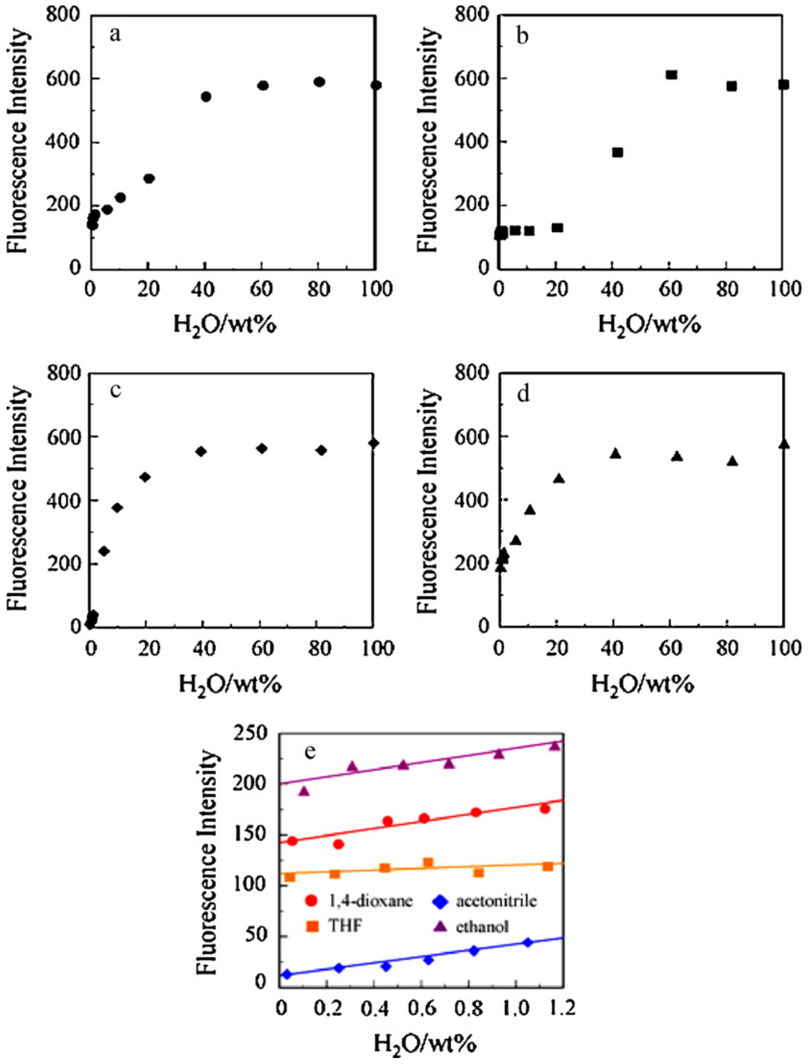
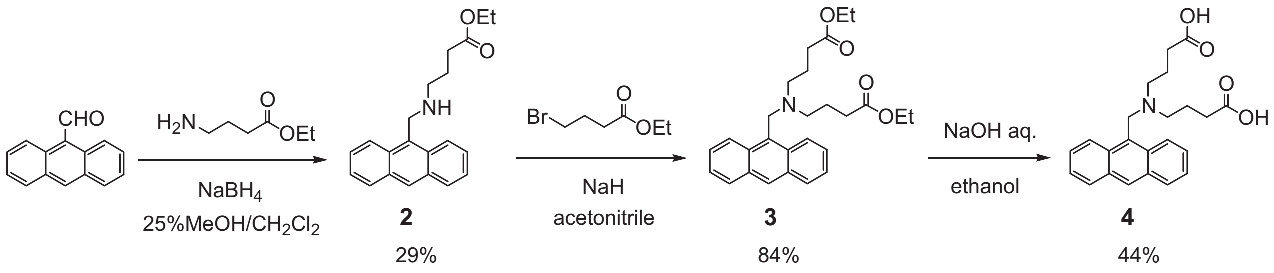


Fig. 2. Fluorescence peak intensity at around 418 nm (λ_{ex} = 366 nm) of **4** as a function of water content in (a) 1,4-dioxane, (b) THF, (c) acetonitrile, and (d) ethanol. (e) Fluorescence peak intensity of **4** in 1,4-dioxane, THF, acetonitrile, and ethanol solutions in the low water content region below 1.2 wt%.

anthracenecarboxyaldehyde with ethyl 4-aminobutanoate gave a monoester derivative **2** [30,31]. The reaction of monoester derivative **2** with ethyl 4-bromobutyrate in the presence of sodium hydride yielded a diester derivative **3**. Anthracene-amino acid **4** was obtained by hydrolysis of **3**.

The detection of water in 1,4-dioxane, THF, acetonitrile and ethanol was attempted by measuring absorption and fluorescence spectra of **4**. As are shown typically in Fig. 1a and c for 1,4-dioxane and acetonitrile, in all the four solvents the absorption spectra of **4** undergo only small changes in intensity and shape by increasing water content. On the other hand, the corresponding fluorescence spectra exhibit significant changes in intensity with a negligible change in their spectral shapes (Fig. 1b and d). The changes in the fluorescence peak intensity are plotted in Fig. 2a–d against water fraction in these four organic solvents. It is seen in the low water content limit below 1.2 wt% that the fluorescence intensities are increased linearly in 1,4-dioxane, acetonitrile and ethanol, while that in THF exhibits only a slight increase (Fig. 2e). When the water content ranges between 5 and 20 wt%, the fluorescence intensity is increased dramatically in acetonitrile and ethanol, but gradually in 1,4-dioxane and THF. The fluorescence intensity in the range between 20 and 60 wt% is increased dramatically in 1,4-dioxane



Scheme 2. Synthesis of fluorescence PET sensor **4**.

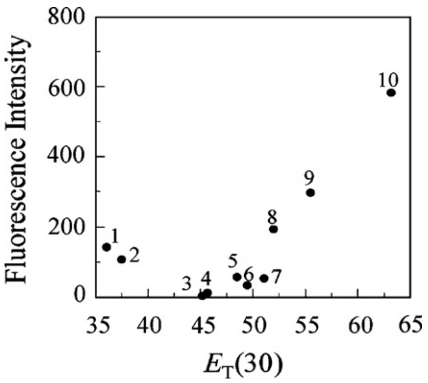


Fig. 3. Fluorescence peak intensity at around 418 nm (λ_{ex} = 366 nm) of **4** plotted against $E_T(30)$ of solvents. The numbers 1–10 correspond to 1,4-dioxane, THF, DMSO, acetonitrile, 2-propanol, ethyl acetoacetate, 2-ethoxyethanol, ethanol, methanol, and water, respectively.

and THF. The fluorescence intensity shows a saturation at 60 wt% for 1,4-dioxane and THF, and at 40 wt% for acetonitrile and ethanol. It is reasonable to presume here that a fluorescence enhancement of **4** with the increase of water content between 0 and 20 wt% is attributable to suppression of PET by the intramolecular proton transfer of the carboxyl proton to amino group (Scheme 1b).

The fluorescence peak intensities for almost dry organic solvents are plotted in Fig. 3 against the solvent polarity parameter, $E_T(30)$ [32]. It is interesting to note that the florescence intensity decreases with increasing $E_T(30)$ values going from 1,4-dioxane to DMSO and is almost zero for the solvents having $E_T(30)$ values at around 45, whereas it starts to increase when the $E_T(30)$ increases beyond 45. Thus, the plot is a parabola with a bottom at $E_T(30) = 45$. In fluorescence PET sensors reported earlier, the fluorescence intensities in neat organic solvents are found to decrease monotonously as the solvent polarity increases [33,34]. The parabolic dependence of the fluorescence intensity on the solvent polarity in the present PET sensor **4** is in marked contrast to that observed with the previous fluorescence sensors, suggesting the involvement of complicated processes in the PET activity of **4**. More detailed studies are in progress to account for the effects of solvent polarity on the PET processes.

We estimated the detection limit (DL) and quantitation limit (QL) by the following equations: $\mathrm{DL} = 3.3\sigma/m_{\mathrm{sl}}$ and $\mathrm{QL} = 10\sigma/m_{\mathrm{sl}}$, where σ is the standard deviation of blank sample and m_{sl} is the slope of calibration curve in the region of the low water content (below 1.0 wt%), respectively. The DL and QL of **4** are, respectively, 0.1 and 0.3 wt% for 1,4-dioxane, 0.4 and 1.2 wt% for THF, 0.1 and 0.3 wt% for acetonitrile and 0.1 and 0.3 wt% for ethanol, which are higher than those of **1**. This result indicates that the higher DL and QL values of **4** are attributable to a more acidic nature of **4** than **1**. In fact, the pH value of a 1.0×10^4 M solution of **4** is 4.2, which is smaller that that of **1** (pH = 4.9).

4. Conclusions

As a new class of fluorescence PET sensor for detection of water in organic solvents, we have designed and synthesized an anthracene-amino acid **4** having two carboxyl groups. An enhancement in fluorescence is observed with increasing water content in organic solvents, which is attributable to the suppression of PET by the intramolecular proton transfer of the carboxyl proton to the amino group. The DL and QL of **4** are higher than those of an anthracene-amino acid **1** having one carboxyl group. We demonstrate that the anthracene-amino acid system is one of the most promising classes of fluorescence PET sensor for detection of a trace amount of water.

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