PERKIN 2 COMMUNICATION

Fluorescence ratio sensing of alkali metal ions based on control of the intramolecular exciplex formation

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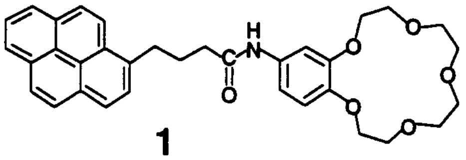
A new class of fluorescent PET (photoinduced electron transfer) sensors for alkali metal cations, 1 shows a significant change in the ratio of the monomer to the exciplex emissions upon complexation, allowing emission ratio sensing of alkali metal cations.

The development of fluorescent indicators sensitive to biologically important substrates such as alkali metal cations is of great interest in biomedical research. Among recent approaches to the design and fabrication of such fluorescent sensors and switches, photoinduced electron transfer (PET) has attracted much attention as a promising operating principle.[1,2] A number of excellent fluorescent signaling systems based on the PET strategy have been proposed. However, only a few of these PET systems are capable of ratiometric analysis.[3,4] We have recently reported a new class of fluorescent PET sensors for anions, where formation of an intramolecular exciplex between the excited fluorophore and the receptor was found to be controlled by anion binding.[5] This leads to anion-induced change in the ratio of the exciplex to the monomer emissions. Therefore, calibration *via* ratiometry becomes possible by exploiting the emission of the intramolecular exciplex formed in the PET path.[6] Although exciplex-mediated photoinduced charge transfer phenomena have been extensively studied due to their important role in photochemistry and photobiology,[6,7] there have been astonishingly few reports to exploit the exciplexes for the design of fluorescent signaling systems.[5,8–10]

We report herein the first ratiometric fluorescent sensing of alkali metal ions by control of the intramolecular exciplex formation in the novel PET system **1** in which pyrene (Py) is linked to amidobenzo-15-crown-5 (CE) *via* a propylene spacer. In this donor–spacer–acceptor system,† the intramolecular exciplex forms between the excited Py and CE in the cation-free state, while the exciplex formation is suppressed by metal ions when bound to the crown moiety. The present PET system based on intramolecular exciplex formation[13] is clearly different in principle from some fluorescent sensors with internal charge transfer (ICT) excited states, which have been applied to ratiometric analysis of alkali cations.[1c,14] Another class of ratiometric fluorescent signaling systems for these cations is based on the biasing of monomer–excimer equilibria.[1c,15]



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Compound **1** was synthesized by reacting 1-pyrenebutyric acid with 4′-aminobenzo-15-crown-5 in CH_2Cl_2 in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and purified by column chromatography with CH_2Cl_2–MeOH (9 : 1) as eluent on a first column and with CH_2Cl_2–MeOH–EtOAc (10 : 1 : 1) on a second one and by recrystallization from CH_2Cl_2–MeOH.‡

The absorption spectrum of **1** in MeCN agreed well with a simple sum of the spectra of 1-methylpyrene and 4′-acetylaminobenzo-15-crown-5, showing no significant interaction between Py and CE moieties in the ground state, *i.e.*, no charge-transfer interaction between the two chromophores. The fluorescence spectrum of **1** in non-degassed MeCN, however, differed from that of 1-methylpyrene (Fig. 1). While the monomer emission of **1** almost coincided in shape with that of 1-methylpyrene, the fluorescence intensity of the monomer emission was reduced to *ca.* one fourth relative to that of 1-methylpyrene. In addition, a structureless red-shifted band was observed at around 430–600 nm. Since the excitation spectrum monitored at 475 nm (structureless emission) was completely identical to that monitored at 397 nm (monomer emission), the structureless band did not originate from a ground state association. Thus, the effect of solvent polarity on the absorption and fluorescence spectra of **1** was examined in order to assign the origin of the long-wavelength emission.

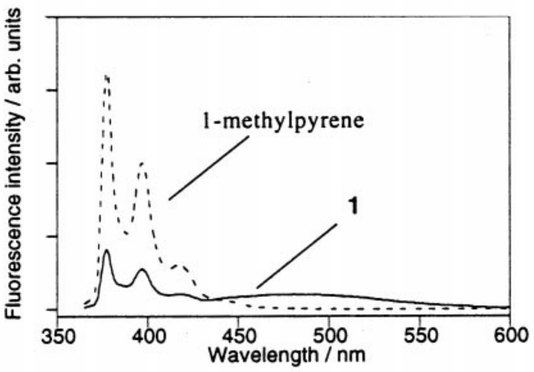

Fig. 1 Fluorescence spectra of **1** and 1-methylpyrene in non-degassed MeCN. [**1**] = [1-methylpyrene] = 5.0 × 10^{-7} M. Excitation wavelength: 326 nm.

The absorption spectrum of **1** was found to show little dependence on the solvent polarity, but the fluorescence spectrum strongly depended on the solvent polarity (Fig. 2). Two effects of increasing the relative permittivity (dielectric constant) of a solvent on the long-wavelength emission could be noticed: a decrease in intensity and a considerable red-shift. The maximum of the long-wavelength emission shifted from 453 nm in EtOAc to 478 nm in MeCN (Fig. 2A). These results clearly demonstrated the charge-transfer nature of the long-wavelength emission. The dipole moment μ_{ct} of the excited complex could be evaluated by eqn. (1)[12] which is derived from

$$\bar{\nu}_{ct} = \bar{\nu}_{ct}(0) - \frac{2\mu_{ct}^2}{hca^3}\Delta f + \text{constant} \qquad (1)$$

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{4n^2 + 2}$$

the Lippert–Mataga equation.[16] In this equation, *h* is Planck's constant, *c* is the speed of light, and *a* is the radius of the cavity in which the fluorophore resides. Δf is a solvent polarity parameter defined by the relative permittivity (dielectric constant) ε and the optical refractive index *n*. By plotting the band maxi-

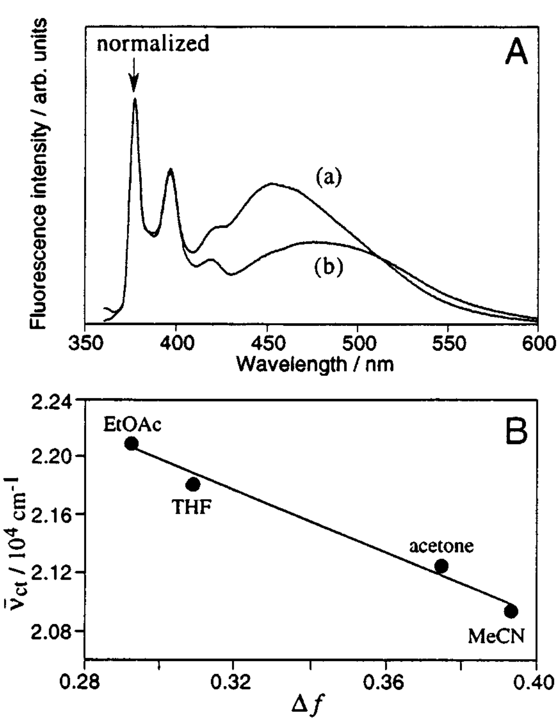

Fig. 2 (A) Fluorescence spectra of **1** (a) in EtOAc and (b) in MeCN. Sample solutions were degassed by the freeze–pump–thaw technique. [**1**] = 5.0 × 10^{-7} M. Excitation wavelength: 326 nm. (B) Wavenumber of the red-shifted emission of **1** as a function of solvent polarity parameter Δ*f*. Solvent (Δ*f*): EtOAc (0.293); THF (0.309); acetone (0.375); MeCN (0.393).

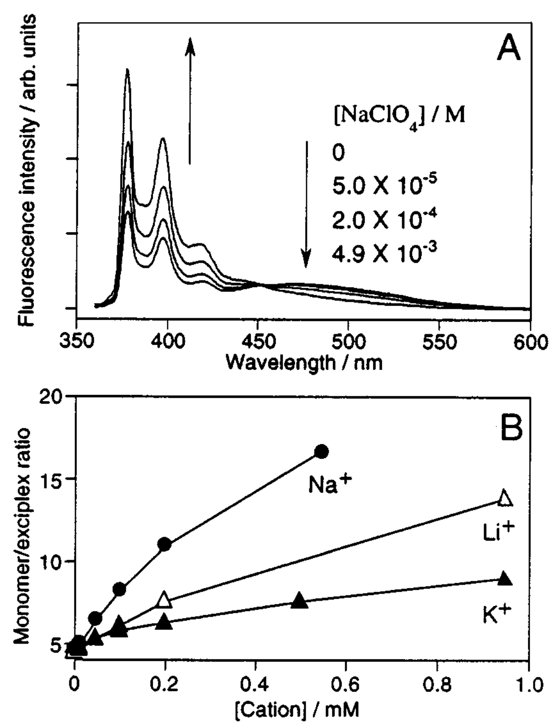

Fig. 3 (A) Effect of Na$^+$ (as ClO$_4^-$ salt) on emission spectra of **1** in non-degassed acetone. (B) Change in the monomer : exciplex ratio for **1** in non-degassed acetone upon addition of alkali metal ions (as ClO$_4^-$ salt). [**1**] = 5.0 × 10^{-7} M. Excitation wavelength: 350 nm; monomer emission: 378 nm; exciplex emission: 500 nm.

mum wavenumber $\tilde{\nu}_{ct}$ of the long-wavelength emission against Δ*f* (Fig. 2B), the magnitude of μ_{ct} could be calculated as 12 Debye (D), where *a* was estimated as 5.1 Å in the present case. The calculated μ_{ct} of 12 D was comparable with dipole moments of typical exciplex systems, which ranged from 10 D for the anthracene–diethylaniline complex to 13.5 D for the biphenyl–diethylaniline complex.[12] On the basis of these results, the structureless band was assigned to the emission of the intramolecular exciplex formed between the excited Py and CE moieties. The formation of the intermolecular exciplex can be disregarded under the present conditions ([**1**] = 5.0 × 10^{-7} M).

The effect of alkali metal ions (as ClO$_4^-$ salts) on the absorption and fluorescence spectra of **1** was examined in acetone. Upon addition of alkali metal ions, significant changes were observed in the fluorescence spectra of **1**, while the absorption spectra did not change at all irrespective of the presence or absence of these cations. Fig. 3A shows fluorescence spectra of **1** in acetone as a function of Na$^+$ concentration, when excited at 350 nm. Increasing the Na$^+$ concentration resulted in a decrease in the intensity of the exciplex emission, while the monomer fluorescence intensity increased. A clear iso-emissive point was observed at 454 nm. The Benesi–Hildebrand plot[17] obtained by using the fluorescence intensity change at 398 nm was linear, indicating 1 : 1 complex formation. The association constant K_{11} calculated from the slope was 4.0 × 10^3 dm^3 mol^{-1}. Similar responses were observed upon 1 : 1 complexation with Li$^+$ and K$^+$ [K_{11} (dm^3 mol^{-1}): Li$^+$: 1.7 × 10^3; K$^+$: 1.6 × 10^3]. It is worth noting that the ratio of monomer emission to exciplex emission followed in the order of Na$^+$ > Li$^+$ > K$^+$ (Fig. 3B), reflecting the stabilities of the 1 : 1 complexes.

The observed response could be qualitatively rationalized in terms of the change in the redox properties of the CE moiety. The PET from the CE to the excited Py was suppressed by the entry of a cation into the CE moiety because of the cation-induced increase in the ionization/oxidation potential of the CE moiety.[1] Such a suppression of the PET process also means that formation of the intramolecular exciplex becomes impossible. The monomer emission was therefore intensified upon complexation of alkali metal cations accompanied by a decrease in intensity of the exciplex fluorescence.

In summary, we have shown that **1** acts as a ratiometric fluorescent cation sensor based on the regulation of the intramolecular exciplex formation. Further work on the kinetics in the present system is in progress in our laboratory.

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Notes and references

† The feasibility of PET within this system was roughly assessed according to the Rehm–Weller equation: Δ*G*/kcal mol^{-1} = 23.06 [E_{ox}(D) − E_{red}(A)] − w_p − ΔG_{00}(A) where E_{ox}(D), E_{red}(A), w_p, and G_{00}(A) are the oxidation potential of a donor, the reduction potential of an acceptor, the ion-pairing energy, and the excitation energy of the acceptor, respectively.[6,11,12] E_{red} (Py)[11] = −2.09 V in DMF *vs.* SCE, and ΔG_{00}(Py)[6] = 77 kcal mol^{-1}. E_{ox}(CE) = *ca.* 0.8 V *vs.* Ag/Ag$^+$ electrode, which was measured in 0.1 M N(C$_4$H$_9$)$_4$BF$_4$ acetonitrile solution by cyclic voltammetry. A glassy carbon electrode was used as a working electrode. The free energy change, Δ*G*, of electron transfer within the present system calculated from these values is negative. However, accurate determination of the Δ*G* was impossible because the oxidation of CE was found to be an irreversible process.[11]

‡ Identifying data for **1**: ^{1}H NMR (DMSO-*d*$_6$) δ 2.10 (quint., *J* = 7.5 Hz, 2H, py–CH$_2$C*H*$_2$), 2.42 (t, *J* = 7.3 Hz, 2H, py–C*H*$_2$), 3.37 (t, *J* = 7.8 Hz, 2H, NHCOC*H*$_2$), 3.54–3.64 (m, 8H, O–C*H*$_2$C*H*$_2$–O), 3.70–3.78 (m, 4H, phenyl–O–CH$_2$C*H*$_2$), 3.94–4.02 (m, 4H, phenyl–O–C*H*$_2$CH$_2$), 6.86 (d, *J* = 8.8 Hz, 1H, NHCCHC*H*), 7.07 (dd, *J* = 8.5 Hz, 2.5 Hz, 1H, NHCC*H*CH), 7.27 (d, *J* = 2.2 Hz, 1H, NHCC*H*C), 7.96 (d, *J* = 7.8 Hz, 1H, py), 8.05 (t, *J* = 7.6 Hz, 1H, py), 8.12 (d, *J* = 9.0 Hz, 1H, py), 8.14 (d, *J* = 9.0 Hz, 1H, py), 8.21 (d, *J* = 9.5 Hz, 1H, py), 8.23 (d, *J* = 7.8 Hz, 1H, py), 8.26 (d, *J* = 6.8 Hz, 1H, py), 8.27 (d, *J* = 6.8 Hz, 1H, py), 8.40 (d, *J* = 9.3 Hz, 1H, py), 9.75 (s, 1H, NH). Anal. Calcd. for C$_{34}$H$_{35}$NO$_6$ (553.66): C, 73.76; H, 6.37; N, 2.53. Found: C, 73.47; H, 6.49; N, 2.62%.

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