Phosphorescent PET (Photoinduced Electron Transfer) Sensors: Prototypical Examples for Proton Monitoring and a 'Message in a Bottle' Enhancement Strategy with Cyclodextrins

Richard A. Bissell and A. Prasanna de Silva

School of Chemistry, Queen's University, Belfast BT9 5AG, Northern Ireland, UK

The bromonaphthalene derivatives **1–4** with proximal but non-adjacent tertiary amine groups show various degrees of pH-controlled phosphorescence in the millisecond range in aqueous solution: β- and γ-cyclodextrins cause notable enhancements in the emissions of some cases.

We demonstrate that room temperature phosphorescence in fluid solution[1] can be harnessed as a sensory parameter for pH (in the first instance) by generalizing the fluorescent PET (photoinduced electron transfer) sensor logic[2] [Fig. 1(*a*)]. This allows the exploitation of organic phosphors with long-lived (millisecond) emission [Fig. 1(*b*)] for interference-free ion-sensing in host systems with intrinsic–native fluorescence.† Such systems occur commonly in biology.[6] Cyclodextrins have been used for enhancing the phosphorescence of guests by encapsulation.[7] So we employ cyclodextrins as transparent shields to protect the phosphor module sterically from material contact with its environment while allowing access to communication photons. Sensing remains viable because the proton receptor module is not enveloped [Fig. 1(*c*)]. This is reminiscent of a 'message in a bottle',[8] where the cork is responsive to its environment and induces a change in the message. Sensors **1–4** are prototypes of this approach.

The base form of **1** is insoluble in water and in aqueous α-cyclodextrin solution, but is easily solubilized in β- and

† The long-lived emissions of organic phosphors and lanthanide ion complexes have been exploited for the study of slow micellar processes[3] and labelling in biophysics[4]/immunoassay[5] respectively, but not for ion-sensing.

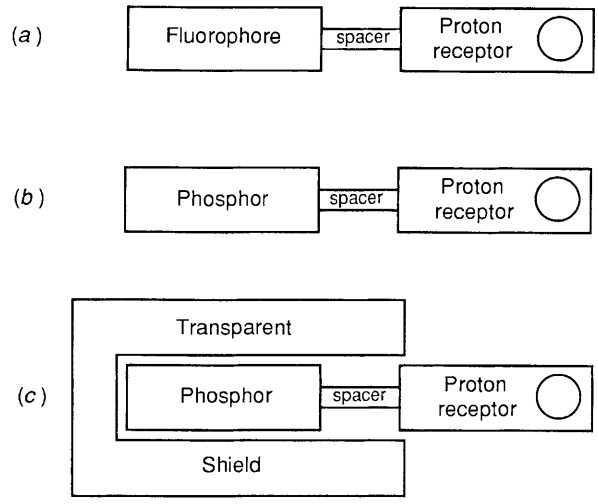

Fig. 1 Schematic representations of a (*a*) fluorescent PET pH sensor, (*b*) phosphorescent PET pH sensor and (*c*) sterically protected phosphorescent PET pH sensor

γ-cyclodextrin solutions. Sensor **1** also causes paramagnetic shifts by up to 0.2 ppm in the PMR (PMR = proton magnetic resonance) signals of β-cyclodextrin[7b] (both at 2.8×10^{-2} mol dm^{-3}) at acidic and basic pH. Such evidence for the inclusion of **1** by β-cyclodextrin can be supported by inspection of space filling Corey–Pauling–Koltun (CPK) molecular models. β-Cyclodextrin is known to enhance the phosphorescence of 1-bromonaphthalene[7a] and the triplet excited state of 1-chloronaphthalene is quenched by tertiary aliphatic amines (presumably by electron transfer) in acetonitrile.[9] So the phosphorescence of the bromonaphthyl module of **1** can be expected to be quenched by the diethylamino moiety.[10] Retrieval of phosphorescence is expected upon protonation of the amine electron pair.[2a,b,11] The results in Fig. 2 nicely bear out these expectations with a proton-induced room temperature phosphorescence enhancement (PE_H^+) of 81 for **1** in β-cyclodextrin solution. The phosphorescence life-time (τ_P), measured *via* eqn. (1), is 0.40 ms.

$$\log(I_P/I_{P0}) = -t/2.3\tau_P \quad (1)$$

where I_P and I_{P0} are the phosphorescence intensities at time t and zero time following the excitation flash.

$$\log[I_{Pmax} - I_P)/(I_P - I_{Pmin})] = pH - pK_a \quad (2)$$

The phosphorescence of **1** senses pH over the range 5.5–8.0. Analysis according to eqn. (2)[2a,b] yields a pK_a (T_1)[12] value of 6.9. On the other hand, a similar analysis of the significant UV absorption spectral changes occurring in **1** in β-cyclodextrin solution as a function of pH gives pK_a (S_0) = 7.9. This difference in pK_a values[12] is due to the significant internal charge transfer character of the T_1 state of **1** which is a substituent-perturbed aromatic hydrocarbon.[13] β-Cyclodextrin encapsulation has a remarkable effect on the phosphorescence of **1**. When compared to an aqueous solution of pH 3.0, the β-cyclodextrin induced phosphorescence enhancement (PE_{CD/H_2O}) is 4.0. Examination of the phosphorescence-pH profile of **1** in water with 10% methanol (added for adequate solubility at basic pH) gives $PE_H^+ > 100$, pK_a (T_1) = 7.2 and τ_P = 0.26 ms. The similarity of pK_a (T_1) values in β-cyclodextrin and water with 10% methanol implies that the proton receptor diethylamino unit is not enveloped by the host macrocycle and accesses essentially bulk water, supporting the representation in Fig. 1(*c*).[7b,14] It is notable tha β-cyclodextrin is beneficial but not essential for the employment of **1** as a phosphorescent pH sensor in aqueous solution.[3,7b,15] The reason for this observation is that triplet–triplet annihilation, the major phosphorescence quenching pathway in deaerated fluid solution, can also be inhibited by the electrostatic charge

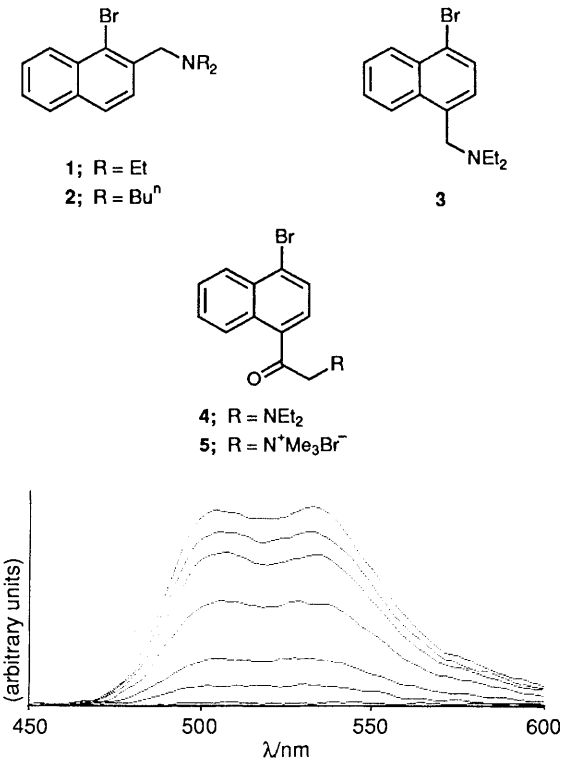

Fig. 2 Phosphorescence emission spectra of 5×10^{-5} mol dm^{-3} **1** in nitrogen-purged water with 5×10^{-3} mol dm^{-3} β-cyclodextrin, gathered with a delay time of 0.1 ms, gate time of 4.0 ms and excitation wavelength of 283 nm with pH (phosphate buffers) 3.1, 5.6, 6.6, 6.9, 7.5, 8.0 and 9.0 (in order of decreasing intensity). It is notable that fluorescence and second-order scattering signals are completely rejected. Sensor **1** in other media and also **2** and **3** have essentially identical spectral positions and shapes. Sensor **4** has a single phosphorescence maximum at 553 nm (excitation wavelength = 311 nm). The phosphorescence in all these cases is quenched essentially completely by air.

on the emissive protonated **1**. Examination of **1** in γ-cyclodextrin solution gave pK_a (T_1) = 6.9, τ_P = 0.15 ms, PE_H^+ = 23 and PE_{CD/H_2O} = 0.84. This last datum shows that there is no significant complexation under the concentration conditions employed for phosphorescence spectroscopy (5×10^{-5} **1** and 5×10^{-3} mol dm^{-3} γ-cyclodextrin).[16]

The results obtained with **2** illustrate the influence of hydrophobicity[17] on sensor action. In β-cyclodextrin solution, the phosphorescence of **2** displayed no significant pH dependence in the range pH 2.5–11, though different life-times (0.34 ms at pH 3.0 and 0.89 ms at pH 11.0) with concomitant small changes of spectral shape indicate the presence of protonated and unprotonated forms, respectively. This inference is also corroborated by the similarity of the pH dependence of UV absorption spectra of **2** in β-cyclodextrin with that of **1** and gives pK_a(S_0) = 5.3. Also, β-cyclodextrin does enhance the phosphorescence of **2** relative to pH 3.0 water, with PE_{CD/H_2O} = 3.1. The lack of phosphorescence quenching at high pH is rationalised as the retardation of photoinduced electron transfer in the relatively apolar location[9d,18] occupied by the rather hydrophobic **2**. Such a 'deeper' inclusion in the β-cyclodextrin cavity is also supported by the pK_a(S_0) value of 5.3. This is considerably lower than that for **1** (7.9) with an electronically similar substituent and would result from steric inhibition to aqueous solvation of the protonated amine moiety because of the cyclodextrin.[19]

Compound **3**, a structural isomer of **1**, shows no binding with β-cyclodextrin in dilute solution as evidenced by the nearly identical phosphorescence life-times in water and in β-cyclodextrin solution at pH 3.0 (τ_P = 0.45 and 0.48 ms, respectively), and also by the fact that PE_{CD/H_2O} = 1.0. Also

pK_a (S_o) = 8.0. The strong pH-dependent phosphorescence that is observed here originates mainly in the phosphate pH buffers employed, with the protonation equilibrium of the amine relegated to a minor role. Significant quenching of **3** is seen over the pH range 3–11 due to $H_2PO_4^-$ and HPO_4^{2-} ions (but not by H_3PO_4) *via* ion-pairing to the protonated amine followed by hydrogen-bonding[20] with the aromatic π electron system of the triplet excited state. On the other hand, **3** in γ-cyclodextrin solution shows PE_{CD/H_2O} = 1.3 suggesting significant complexation. Under these conditions, the phosphorescence is smoothly pH-dependent with pK_a (T_1) = 7.6, τ_P = 0.42 and 1.32 ms at pH 3.0 and 11.0, respectively. No complications are seen from the phosphate buffers. Thus, **3** in γ-cyclodextrin is a useful phosphorescence sensor system, even though the emission is not fully 'switched off' at basic pH (PE_H^+ = 2.1) owing to retardation of quenching as in **2**–β-cyclodextrin. Such drastically different pH-dependent phosphorescence behaviour of isomeric **1** and **3** points to the importance of geometric complementarity of the prospective host–guest pair.

Compound **4**, which has been adapted from Turro's microenvironmental probe **5**,[3] is less hydrophobic[17] than **1** or **3**. Compound **4** shows no inclusion in β- or γ-cyclodextrin under phosphorimetric conditions (PE_{CD/H_2O} = 1.0), but nevertheless exhibits useful phosphorescent pH sensing behaviour [λ_P = 553 nm, $PE_H^+ > 100$, τ_P = 0.45 ms, pK_a (T_1) = 6.0]. It is also excitable at longer wavelengths (311 nm).

In summary, **1**–**4**, with or without cyclodextrin enhancement, serve as phosphorescent pH sensors in anaerobic situations[21] with varying efficiencies. Their mode of action is most simply rationalized by a competition between slow endergonic electron transfer‡ from the lowest excited triplet state and phosphorescence. However, a fast exergonic electron transfer‡ from the lowest excited singlet state in competition with fluorescence and fast, heavy-atom enhanced intersystem crossing[27] can also reduce the formation of the triplet state precursor of phosphorescence. The photostability of the sensors appear adequate, presumably owing to rapid intramolecular back-electron transfer[2c–e], though debromination has been previously noted in intermolecular situations.[9] Covalently bound sensor–cyclodextrin systems would be a natural extension of the present work.[28]

We thank the SERC, Dr R. Pilgrim (CIRD, Valbonne, France) and Irene Campbell for support and help.

Received, 16th May 1991; Com. 1/02330F

References

1 K. Kalyanasundaram, *Photochemistry in Microheterogeneous Systems*, Academic, Orlando, 1987, chs. 3 and 9; T. Vo-Dinh, *Room Temperature Phosphorimetry for Chemical Analysis*, Wiley, New York, 1984; R. J. Hurtubise, in *Molecular Luminescence Spectroscopy: Methods and Applications* Part 2, ed. S. G. Schulman, Wiley, New York, 1988, p. 1.

2 (*a*) A. J. Bryan, A. P. de Silva, S. A. de Silva, R. A. D. D. Rupasinghe and K. R. A. S. Sandanayake, *Biosensors*, 1989, **4**, 169; (b) A. P. de Silva and K. R. A. S. Sandanayake, *Tetrahedron Lett.*, 1991, **32**, 421 and references cited therein. Photoinduced electron transfer is treated extensively in, (*c*) R. S. Davidson, *Adv. Phys. Org. Chem.*, 1983, **19**, 1; (*d*) G. J. Kavarnos and N. J. Turro, *Chem. Rev.*, 1986, **86**, 401; (*e*) *Photoinduced Electron Transfer* eds. M. A. Fox and M. Chanon, Elsevier, Amsterdam, 1988, parts A–D.

3 J. D. Bolt and N. J. Turro, *Photochem. Photobiol.*, 1982, **35**, 305.

4 V. de W. Horrocks and D. R. Sudnick, *Acc. Chem. Res.*, 1981, **14**, 384; F. S. Richardson, *Chem. Rev.*, 1982, **82**, 541; C. F. Meares and T. G. Wensel, *Acc. Chem. Res.*, 1984, **17**, 202; A. Abusaleh and C. F. Meares, *Photochem. Photobiol.*, 1986, **39**, 362; V. Balzani, N. Sabbatini and F. Scandola, *Chem. Rev.*, 1986, **86**, 319.

5 M. P. Bailey, B. F. Rocks and C. Riley, *Analyst*, 1985, **110**, 603; B. Alpha, V. Balzani, J.-M. Lehn, S. Perathoner and N. Sabbatini, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 1266; R. Ekins, F. Chu and J. Micallef, *J. Chemilumin. Biolumin.*, 1989, **4**, 59; G. Barnard, F. Kohen, H. Mikkola and T. Lovgren, *J. Chemilumin. Biolumin.*, 1989, **4**, 177.

6 *E.g., Biochemical Fluorescence: Concepts*, eds. R. F. Chen and H. Edelhoch, Dekker, New York, 1976, vol. 2.

7 (*a*) N. J. Turro, J. D. Bolt, Y. Kuroda and I. Tabushi, *Photochem. Photobiol.*, 1982, **35**, 69; (*b*) N. J. Turro, T. Okubo and C. Chung, *J. Am. Chem. Soc.*, 1982, **104**, 1789; (*c*) N. J. Turro, G. S. Cox and X. Li, *Photochem. Photobiol.*, 1983, **37**, 149; (*d*), (*e*) S. Scypinski and L. J. Cline Love, *Anal. Chem.*, 1984, **56**, 322; 1984, **56**, 331; (*f*) R. A. Femia and L. J. Cline Love, *J. Colloid Interfac. Sci.*, 1985, **108**, 271; (**g**) S. Hamai, *J. Am. Chem. Soc.*, 1989, **111**, 3954.

8 G. M. Sumner, *Message in a Bottle*, Virgin Music (Publishers), 1979. For other molecular analogues of bottles and corks, see Y. Okahata, *Acc. Chem. Res.*, 1986, **19**, 57; X. G. Lei, C. E. Doubleday, M. B. Zimmt and N. J. Turro, *J. Am. Chem. Soc.*, 1986, **108**, 2444; J.-H. Fuhrhop and D. Fritsch, *Acc. Chem. Res.*, 1986, **19**, 130; H. Ringsdorf, B. Schlarb and J. Venzmer, *Angew. Chem., Int., Ed. Engl.*, 1988, **27**, 113.

9 (*a*) R. A. Beecroft, R. S. Davidson and D. Goodwin, *Tetrahedron Lett.*, 1983, **24**, 5673; (*b*) R. A. Beecroft, R. S. Davidson, D. Goodwin, J. E. Pratt and X. J. Luo, *J. Chem. Soc., Faraday 2*, 1986, **82**, 2393. See also; (*c*) F. J. DeLuccia and L. J. Cline Love, *Anal. Chem.*, 1984, **56**, 2811; (*d*) M. Julliard in ref. 2(*e*), ch. 2.5 and references cited therein.

10 K. Kobayashi, M. Takahashi, Y. Muramatsu and T. Morita, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 2815; K. Kano, I. Takenoshita and T. Ogawa, *J. Phys. Chem.*, 1982, **86**, 1833; S. Hamai, *J. Phys. Chem.*, 1988, **92**, 6140 are cases of fluorescence quenching of aromatic hydrocarbons and amines co-included in cyclodextrins.

11 G. S. Cox, N. J. Turro, N. C. Yang and M. J. Chen, *J. Am. Chem. Soc.*, 1984, **106**, 422 and ref. 7(*e*) are two relevant, but different, examples of pH dependent luminescence in cyclodextrin solutions.

12 See J. F. Ireland and P. A. H. Wyatt, *Adv. Phys. Org. Chem.*, 1976, **12**, 131 for determination of pK_a (T_1) values *via* (*i*) flash photolysis and (*ii*) Forster cycles with phosphorescence spectra in acidic and basic glasses at low temperature.

13 H. Shizuka, *Acc. Chem. Res.*, 1985, **18**, 141.

14 M. Dhaenens, L. Lacombe, J.-M. Lehn and J.-P. Vigneron, *J. Chem. Soc., Chem. Commun.*, 1984, 1097.

15 T. Okubo and N. J Turro, *J. Phys. Chem.*, 1981, **85**, 4034.

16 *E.g.*, A. P. de Silva and K. R. A. S. Sandanayake, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1173 notes differences in complexation behaviour under the conditions of PMR and fluorescence spectroscopy.

17 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.

18 R. A. Bissell, A. P. de Silva, W. T. M. L. Fernando, S. T. Patuwathavithana and T. K. S. D. Samarasinghe, *Tetrahedron Lett.*, 1991, **32**, 425 is an analogous case for fluorescence.

19 R. W. Alder, *Chem. Rev.*, 1989, **89**, 1215; D. J. Cram, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 1039.

20 M. E. Huston, E. V. Akkaya and A. W. Czarnik, *J. Am. Chem. Soc.*, 1989, **111**, 8735.

21 See L. J. Cline Love and R. Weinberger, *Spectrochim. Acta*, 1983, **38B**, 1421; references 7(*c*) and 7(*d*) for examples of phosphors with notable insensitivity to oxygen.

22 H. Siegerman, in *Technique of Electroorganic Synthesis*, ed., N. L. Weinberg, Wiley, New York, 1975, part II, p. 667.

23 C. K. Mann and K. K. Barnes, *Electrochemical Reactions in Nonaqueous Systems*, Dekker, New York, 1970.

24 S. L. Murov, '*Handbook of Photochemistry*', Dekker, New York, 1973.

25 D. Rehm and A. Weller, *Isr. J. Chem.*, 1970, **8**, 259.

26 Z. R. Grabowski and J. Dobkowski, *Pure Appl. Chem.*, 1983, **55**, 245.

27 M. Kasha, *Radiat. Res.*, 1960, Suppl. **2**, 243.

28 See, A. Ueno, I. Suzuki and T. Osa, *J. Am. Chem. Soc.*, 1989, **111**, 6391 and references cited therein for fluorescent cases.

‡ The thermodynamic driving force for electron transfer from triethylamine (oxidation potential, E_{ox} = 1.19 V[22]) to 1-bromonaphthalene (reduction potential, E_{red} = −1.96 V,[23] triplet energy, E_T = 2.60 eV,[24] singlet energy, E_S = 3.87 eV[24]) in the triplet and singlet exicited states would be 0.45 eV (endergonic) and −0.82 eV (exergonic), respectively, upon application of the Weller equation,[25] $\Delta G_{ET} = -E_{S\text{ or }T} - E_{red} + E_{ox} - E_{ion\ pair}$. $E_{ion\ pair}$ was taken as 0.1 eV.[26]