Fluorescent PET (Photoinduced Electron Transfer) Sensors Selective for Submicromolar Calcium with Quantitatively Predictable Spectral and Ion-binding Properties

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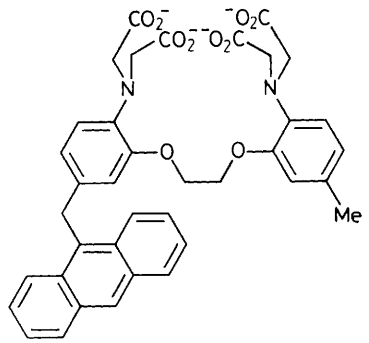
The anthracene (QCa1) and pyrazoline (QCa2) derivatives display Ca^{2+}- induced enhancements of fluorescence quantum yields (ϕ_F), by factors of 16 and 92, respectively; the binding selectivities (*vs.* Mg^{2+} and H^+) and characteristics of the electronic (absorption and emission) spectra (except $\phi_{Fmax.}$) are reasonably predictable from simpler model compounds.

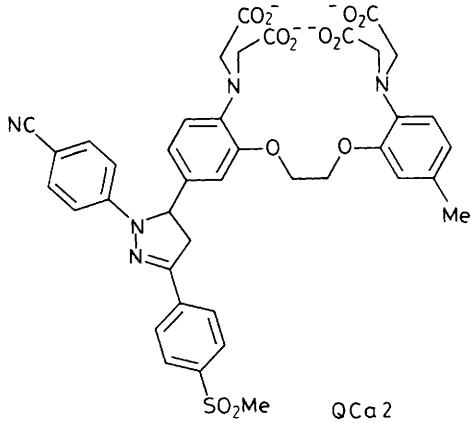
We present the sensors QCa1 and QCa2, of which the latter shows the largest fluorescence enhancement, in an organic molecule, caused by physiological levels of Ca^{2+} in neutral water.[1—4] The biological significance of these sensors lies in the fact that Ca^{2+} is an intracellular messenger of wide scope,[5] and the principle of photoinduced electron transfer (PET)[6] is first exploited here for the design of Ca^{2+} sensors. Several elegant systems are currently used in biological contexts.[1—3,7] However, QCa1 and QCa2 possess some remarkable and complementary features (Figure 1 and Table 1) due to their novel design.[8]

These features are as follows. (a) The successful use of both hydrocarbon and heterocyclic fluor units illustrates the flexibility of the present design logic.[8] These fluors have different polarities, hydrophobicities, and spectral properties. It is also notable that the fluorescence of these units is intrinsically independent of Ca^{2+}, Mg^{2+}, and H^+ over a wide concentration range. (b) The 'switched on' state induced by Ca^{2+} binding gives moderate fluorescence quantum yields ($\phi_{Fmax.} \leq 0.1$). The large fluorescence enhancement of QCa2 by nearly two orders of magnitude may allow direct qualitative visualization of $[Ca^{2+}]$ fields in real time, despite local variations in the optical path, the sensor concentration, and quenching effects.[7] (c) Reasonable extinction coefficients ($\varepsilon_{max.} \sim 10^4$ mol^{-1} dm^3 cm^{-1}) are observed in the near-VIS region, allowing excitation with minimal perturbation of the host/matrix.[2] (d) All electronic absorption ($S_0 \rightarrow S_1$) and emission parameters (except ϕ_F) are virtually invariant with the concentrations of Ca^{2+}, Mg^{2+}, and H^+. However, some hypochromicity and (for QCa1 only) loss of resolution in the absorption ($S_0 \rightarrow S_1$) spectrum is seen at pH <4. (e) All these optical properties (except ϕ_F) are predictable with a high degree of accuracy from simpler model fluors. The availability of literature data for (**2a**)[9] and (**3a**)[10] suggested their use as model fluors. Closer models (**2b**) and (**3b**) were subsequently prepared for a more exact comparison with the sensors QCa1 and QCa2, respectively. (f) Fluorescence intensity (I_F)–pM (M = Ca, Mg, or H) profiles agree with equation (1),[11] except

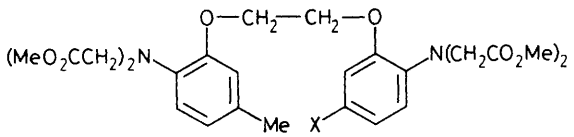
$$\log \left(\frac{I_{Fmax} - I_F}{I_F - I_{Fmin}} \right) = pM - \log \beta \qquad (1)$$



QCa1

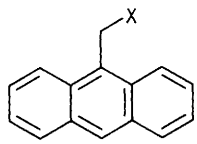


QCa2



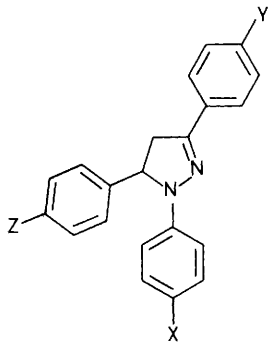
(1)

a; X = H
b; X = CHO
c; X = 9-anthryl methyl
d; X = 1-(4′-cyanophenyl), 3-(4″-methyl sulphonyl phenyl), -Δ^2-pyrazolin-5-yl



(2)

a; X = H
b; X = 4′-[*N*,*N*-di(carboxymethyl)amino]phenyl



(3)

a; X = SO_2Me, Y = CN, Z = H
b; X = CN, Y = SO_2Me, Z = CO_2^-

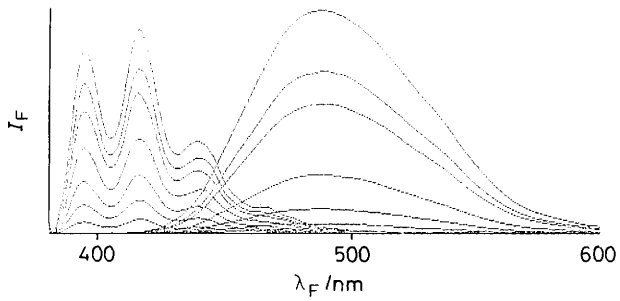


Figure 1. Fluorescence emission spectra of QCa1 (left) and QCa2 (right) with pCa 3.0, 6.0, 6.5, 7.0, 7.5, 8.0, and ∞ (in order of decreasing intensity). The detector gain has been scaled so that both sensors give similar maximum intensities at pCa 3.0.

that I_F–pMg profiles at pMg<1.2 are complicated by Mg : sensor binding (2 : 1) which is incomplete at pMg 1.[3] (g) Ion-binding constants for QCa1 and QCa2 are comparable with those predicted from our Ca^{2+} receptor module (which was Tsien's) bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA).[3] Together with (e), this demonstrates that QCa1 and QCa2 inherit their optical and ion-binding properties from their parent modules with high fidelity.[12] (h) In both cases, the sensor response is highly selective for physiological levels of intracellular Ca^{2+} (pCa ~7) against those of Mg^{2+} (pMg ~3) and H^+ (pH ~7).[1] The $\phi_{Fmax.}$ for Ca^{2+} (pCa 3) is five- and twenty-six-fold greater than for Mg^{2+} (pMg 1.2) for QCa1 and QCa2, respectively. Both anisidine units of the sensors are involved in 1 : 1 Ca^{2+} binding, whereas 1 : 1 binding of the smaller Mg^{2+} ion would occur preferentially at the distal anisidine unit, leading to smaller effects on the fluor. Protons usually produce the largest ϕ_F enhancements in PET sensors since they alone transform nitrogen (lone) electron pairs into covalent bonds with a much higher oxidation potential (E_{ox}).[11] Nevertheless QCa2 shows a four-fold greater $\phi_{Fmax.}$ for Ca^{2+} (pCa 3) *vs.* 2 : 1 H^+ at pH 4.5; further protonation (pH 1) causes the largest enhancements (120—230). The absence of any detectable effect on ϕ_F upon 1 : 1 protonation (around the expected[3] log β = 6.36) is due to monocation binding at the distal anisidine unit.

Compound (**1c**)† was prepared by mild Friedel–Crafts alkylation[13] of (**1a**).[14] Compound (**1d**)† was prepared by acid-catalysed cyclisation of the corresponding chalcone of (**1b**)[14] with the appropriate phenyl hydrazine.[10] The sensors QCa1 and QCa2 are then accessible by alkaline hydrolysis of (**1c**) and (**1d**), respectively.

† *Spectroscopic data* for (**1c**): ^1H NMR (300 MHz; $CDCl_3$) δ 7.39—8.36 (m, 9H, anthryl ArH), 6.57—6.68 (m, 6H, ArH), 4.88 (s, 2H, $ArCH_2$), 4.06 (br. s, 12H, OCH_2 and NCH_2), 3.47 (s, 6H, CO_2Me), 3.38 (s, 6H, CO_2Me), 2.23 (s, 3H, ArMe). For (**1d**): ^1H NMR (300 MHz; $CDCl_3$) δ 7.87—7.98 (2d, 4H, MeO_2S–Ar*H*), 7.08—7.45 (2d, 4H, NC–ArH), 6.62—6.74 (m, 6H, ArH), 5.34 (dd, 1H, N–CH, *J* 6.05, 12.3 Hz), 4.19 (br. s, 4H, OCH_2), 4.10 (2s, 8H, NCH_2), 3.87 (dd, 1H, CH–C=N, *J* 17.4, 12.3 Hz), 3.55 (s, 6H, CO_2Me), 3.50 (s, 6H, CO_2Me), 3.18 (dd, 1H, CH–C=N, *J* 6.05, 17.4 Hz), 3.08 (s, 3H, SO_2Me), 2.25 (s, 3H, ArMe).

Table 1. Electronic (absorption and emission) spectra and ion-binding properties of QCa1 and QCa2.[a]

Property	QCa1	QCa2
$\lambda_{abs.}$/nm, $S_0 \rightarrow S_1$ ($\varepsilon_{max.}$) /mol^{-1} dm^3 cm^{-1}	390 (8100)[b] 368 (8300) 352 (5400)	388 (27 000)[c]
$\lambda_{abs.}$ onset[d]/nm	400	436
λ_{Flu}/nm($\phi_{Fmin.}$)	440 (0.0014)[b] 416 394	489 (0.0011)[c]
$\phi_{Fmax.}$ Ca^{2+} [e] $(FE)_{Ca^{2+}}$ [f]	0.023 (16)	0.100 (92)
$\phi_{Fmax.}$ Mg^{2+} $(FE)_{Mg^{2+}}$	0.0043 (3)	0.0039 (3.5)
$\phi_{Fmax.}$ H^+ $(FE)_{H^+}$	0.034 (24) 0.32 (230)	0.026 (24) 0.13 (120)
log β Ca^{2+}/mol^{-1} dm^3	6.6[g]	6.5[g]
log β Mg^{2+}	2.0[g]	1.9[g]
log β H^+	—[h], 5.5, 3.1[g]	—[h], 5.7, 3.0[g]

[a] Fluors (5×10^{-6}—10^{-5} M) in aerated water at pH 7.3 with pCa and pMg buffers [ethylene glycol bis(2-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA), 4-morpholine propanesulphonic acid (MOPS)].[3] pH variations conducted with various pH buffers[18] and EGTA (10^{-2} M). Spectrofluorimetry performed with 2.5 nm slits and low detector gain. Thus applications with $<10^{-6}$ M fluors are feasible. λ_{exc} = 366 (QCa1) and 390 nm (QCa2), but other wavelengths within the absorption bands may be used. The ϕ_F values are obtained from corrected spectra after comparison with 9,10-diphenyl anthracene in methanol.[19] [b] A model fluor (literature), 9-methyl anthracene (**2a**), in cyclohexane:[9] λ_{abs} ($\varepsilon_{max.}$) and λ_{Flu} (ϕ_F) 385 (10 300), 366 (10 300), 347 (6000) and 436, 413, 390 (0.35, deaerated solution). A closer model is N-4′-(anthracene-9-yl methyl)phenyl iminodiacetic acid (**2b**) in water (pH 1.0), for which the corresponding data are: 388 (8900), 368 (9500), 350 (6100) and 439, 415, 393 (0.50). [c] A model fluor available in the literature is 1-(4′-methylsulphonyl phenyl), 3-(4″-cyanophenyl), 5-phenyl-Δ^2-pyrazoline (**3a**) in methanol.[10] $\lambda_{abs.}$ ($\varepsilon_{max.}$) and λ_{Flu} (ϕ_F) 385 (33 000) and 470 (0.78, deaerated solution). A closer model is 1-(4′-cyanophenyl), 3-(4″-methylsulphonyl phenyl), 5-(4‴-carboxyphenyl)-Δ^2-pyrazoline (**3b**) in water (pH 7.3) for which the corresponding data are: 388 (29 700) and 490 (0.54). [d] Wavelength (at lowest energy) at which $\varepsilon = 0.1\ \varepsilon_{max.}$.[20] [e] *N,N,N′,N*-tetramethylethylene diamine (10^{-2} M) caused no noticeable quenching. [f] The fluorescence enhancement factor (FE) = $\phi_{Fmax.}/\phi_{Fmin.}$ for a given ion. [g] Calculated according to equation (1) from I_F–pM profiles. Average gradient, 1.06. Average correlation coefficient, 0.988 (average *n*, 7). The model cation receptor is BAPTA, which has log $\beta_{Ca^{2+}}$ = 6.97, log $\beta_{Mg^{2+}}$ = 1.77 and ~0.5, log β_{H^+} (pK_a) = 6.36, 5.47, and <4.0.[3] [h] No detectable inflection in the I_F–pH profile in the pH range 6—7.

The selection of fluor and Ca^{2+} receptor modules can be straightforward when appropriate electrochemical data are available. For instance, E_{ox} of the receptor BAPTA in the cation-free state can be estimated as 0.82 V [*vs.* standard calomel electrode (SCE)], the corresponding E_{ox} value for *N,N*-dimethyl 2-anisidine.[15] Since E_{ox} is 0.96 V for 9-methyl anthracene,[15] a rapid electron transfer is expected[8] from the cation-receptor module across the single carbon spacer to the photoexcited anthracene unit of QCa1, rendering it virtually non-fluorescent (ϕ_{Fmin} ~10^{-3}). A rapid back electron transfer[16] would return the sensors to their initial state. Ca^{2+} binding to QCa1 and QCa2 'switches on' the fluorescence by significantly retarding the PET process. This takes place *via* the Ca^{2+}-induced conformational change[3] which deconjugates and effectively isolates the anisole moiety (E_{ox} 1.76 V)[15] from the iminodiacetate nitrogen electron pair, whose E_{ox} (estimated as 1.19 V, the corresponding value for triethylamine)[15] is further increased by the proximity of the dication.[8] Another contributory factor is the increased distance between the effective electrophores,[17] anthracene/pyrazoline and Ca^{2+} bound iminodiacetate. Further rational variations of fluor- and Ca^{2+}-receptor modules are in progress.

We thank the S.E.R.C., Drs. W. D. J. A. Norbert and K. R. A. S. Sandanayake, Mr. W. Harra, and Miss H. Johnston for support and help with aspects of this work.

Received, 6th October 1989; Com. 9/04295D

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