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Thermally-activated chemiluminescent squaraine rotaxane endoperoxide with green emission†

Carleton G. Collins, Jeffrey M. Baumes and Bradley D. Smith*

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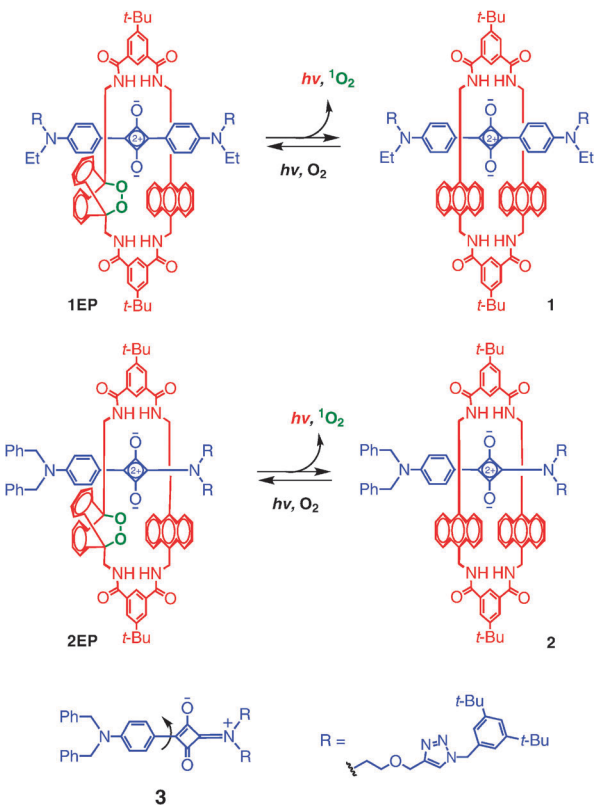
A squaraine rotaxane endoperoxide with a truncated squaraine chromophore undergoes a cycloreversion reaction and emits green light that can be transferred to red acceptor dyes. The results demonstrate that chemiluminescence emission for squaraine rotaxane endoperoxides comes from the excited squaraine inside the rotaxane.

Recently, we reported the synthesis and photochemical properties of squaraine rotaxane endoperoxides, such as **1EP** (Scheme 1).[1,2] We prepared **1EP** by direct photooxidation of the parent squaraine rotaxane, **1**, and showed that it can be stored indefinitely at low temperatures.[1] Upon warming to body temperature, **1EP** undergoes a quantitative cycloreversion reaction that regenerates **1**, releases molecular oxygen (primarily in its excited singlet state), and emits near-infrared light (~733 nm).

This discovery of a thermally activated chemiluminescent reaction that does not require external stimulation is of interest for both practical and theoretical reasons.[3] Our preliminary studies indicated that the chemiluminescence was mediated by the high energy singlet oxygen that was released during the reaction, however, it appeared that the emission was not coming from the singlet oxygen itself but from the excited squaraine fluorophore inside **1EP**.[1] To confirm this important mechanistic question, we decided to prepare an analogue of **1EP** that encapsulates a structurally different squaraine dye with altered excitation energy and look for a concomitant change in chemiluminescence emission wavelength. Here we report the synthesis and properties of **2EP**, a squaraine rotaxane endoperoxide with the same surrounding macrocycle as **1EP** but differs by having the truncated squaraine chromophore **3**. We find that **2EP** undergoes the expected thermally-activated cycloreversion reaction and releases singlet oxygen, but in this case the sample emits green light that matches the emission wavelength of the encapsulated squaraine. The paper concludes by briefly discussing the conundrum of an apparent uphill energy transfer from singlet oxygen to a significantly higher energy squaraine chromophore.

The precursor to **2EP** is squaraine rotaxane **2**, which was prepared by a clicked capping reaction that encapsulated squaraine **3** inside the anthracene-containing tetralactam macrocycle.[4] A comparison of the absorption/emission properties in chloroform for unencapsulated squaraine dye **3** (λ_{abs} = 451 nm, log ε = 4.46, λ_{em} = 481 nm, Φ_f = 0.09) and squaraine rotaxane **2** (λ_{abs} = 479 nm, log ε = 4.67, λ_{em} = 522 nm, Φ_f = 0.74) shows a 40 nm red-shift in fluorescence maxima and an eight-fold enhancement in fluorescence quantum yield. The large increase in quantum yield has been observed before with a related green, fluorescent squaraine rotaxane system and attributed to a decrease in internal mobility of the encapsulated squaraine.[5] A likely source of non-radiative decay for excited squaraine **3** is rotation of the C–C single bond that is



Scheme 1

Department of Chemistry and Biochemistry, University of Notre Dame, 236 Nieuwland Science Hall, Notre Dame, IN, USA. E-mail: smith.115@nd.edu; Tel: +1 574-631-8632

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indicated by the arrow in Scheme 1, and this process is inhibited when **3** is encapsulated inside rotaxane **2**.

The previously studied endoperoxide **1EP** was prepared in straightforward fashion by simply irradiating an aerated sample of the precursor squaraine rotaxane **1** with red light.[1] Apparently irradiation of **1** produces a moderate amount of singlet oxygen which reacts cleanly with the anthracene-containing macrocyle to generate **1EP** in quantitative yield.[2b,6] We initially tried the same approach to make **2EP** but found that irradiation of an aerated sample of pure **2** in $CDCl_3$ at 0 °C only resulted in slow decomposition. We reasoned that **2** was a poor oxygen photosensitizer and thus added a catalytic amount of the efficient photosensitizer, Rose Bengal, to the sample and repeated the irradiation. This led to smooth and nearly quantitative conversion of **2** into **2EP** as verified by standard spectrometric methods including multidimensional ^{1}H NMR spectroscopy. A rapid, low temperature purification step removed the Rose Bengal from the sample of **2EP** prior to kinetic and photophysical studies.

The cycloreversion of **2EP** in $CDCl_3$ was monitored by ^{1}H NMR spectroscopy and found to cleanly regenerate **2** with first order kinetics.[7] The first-order rate constant at 40 °C was 0.64 h^{-1} (half-life of 1.1 h) which is about three times faster than the rate constant for **1EP**.‡ The fraction of oxygen that was released as singlet oxygen was determined by the same chemical trapping experiment that we employed previously.[1] The cycloreversion was allowed to proceed in the presence of excess 2,3-dimethyl-2-butene, which reacted with the released singlet oxygen to form a hydroperoxide product that was quantified by integration of its olefin signals at 4.9–5.1 ppm.[8] The ratio of hydroperoxide to regenerated rotaxane **2** indicated that at least 61 ± 10% of the released oxygen was singlet oxygen, a fraction that is very similar to that released during the cycloreversion of **1EP**.

The chemiluminescent properties of the cycloreversion reaction of **2EP** were characterized using an optical imaging station that was equipped with a sensitive CCD camera. A cooled vial containing **2EP** in $C_2D_2Cl_4$ (1.5 mM) was warmed to 38 °C on a heated stage inside the imaging station and the chemiluminescence intensity was imaged as pixel intensity maps using four different filters. As shown in Fig. 1, the chemiluminescence was observed in the green filter window of 515–575 nm, which contrasts to the near-infrared emission of **1EP** that appears in 695–770 nm window.[1]

A more vivid demonstration of the difference in emission wavelengths was gained by preparing and visualizing multicolor chemiluminescent patterns on a solid surface. The patterns were generated by spotting separate $CDCl_3$ solutions of **1EP** and **2EP** (each 1.5 mM) onto a reverse phase TLC plate and allowing the plate to dry for 5 min. The representative pattern in Fig. 2 is an overlapping "ND" with the "N" composed of near-infrared emitting **1EP** and the "D" composed of green emitting **2EP**. Fig. 2a is a brightfield image of the pattern and Fig. 2b shows an unfiltered chemiluminescence pixel intensity map of all photons emitted from the plate over a 60 s period. The higher pixel intensity values for the "N" relative to the "D" reflect a significantly higher chemiluminescence quantum yield for **1EP**. Fig. 2c and d show the filtered chemiluminescence images detected in the near-infrared 695–770 nm and green 515–575 nm channels, respectively, and Fig. 2e is an overlaid image of panels *c* and *d*. These filtered pixel intensity maps show clearly that the two chemiluminescence emission wavelengths can be detected separately with no bleedthrough of the green emission into the near-infrared channel. At the locations where the near-infrared "N" overlaps with the green "D" there is enhancement of the near-infrared pixel intensity and loss of the green signal. This is indicative of energy transfer from **2EP** (donor) to **1EP** (acceptor). In other words, the green emission from **2EP** is diminished because it is absorbed by nearby molecules of **1EP** and re-emitted as near-infrared light.

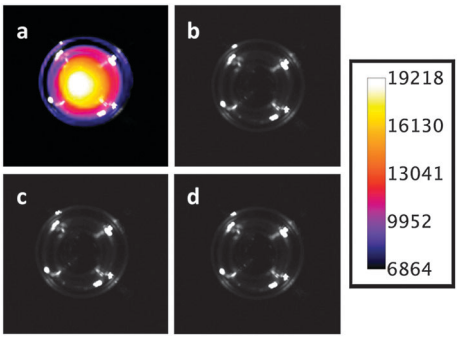

Fig. 1 False coloured pixel intensity maps of a vial containing a solution of chemiluminescent **2EP** (1.5 mM, $C_2D_2Cl_4$) and observed using four different filters: (a) 515–575 nm, (b) 575–650 nm, (c) 695–770, (d) 810–875 nm. Chemiluminescence acquisition parameters: exposure time = 60 s, large binning, fstop = 1, T = 38 °C, intensity scale in arbitrary units.

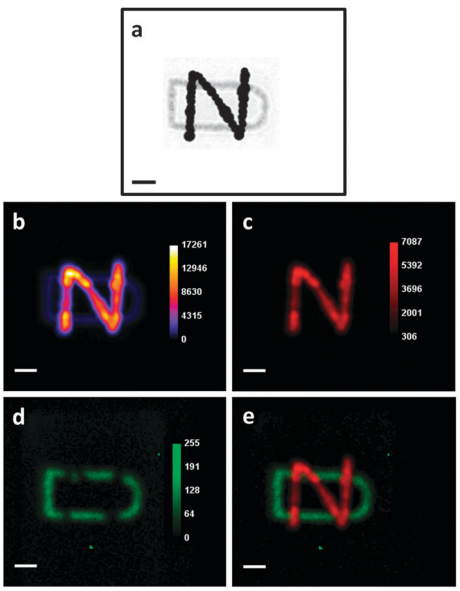

Fig. 2 Images of a multicolor "ND" pattern spotted on a reverse phase TLC plate (coated with C18 hydrocarbon) with the "N" composed of near-infrared emitting **1EP** and the "D" composed of green emitting **2EP**. (a) Bright field image, (b) unfiltered chemiluminescence pixel intensity map of all photons emitted from the plate, (c) chemiluminescence intensity map acquired using near-infrared 695–770 nm filter, (d) chemiluminescence intensity map acquired using green 515–575 nm filter, (e) overlay of panels *c* and *d*. Length scale bar corresponds to 5.0 mm; chemiluminescence intensity scales in arbitrary units. Chemiluminescence acquisition parameters: exposure time = 60 s, large binning, fstop = 1, T = 38 °C.

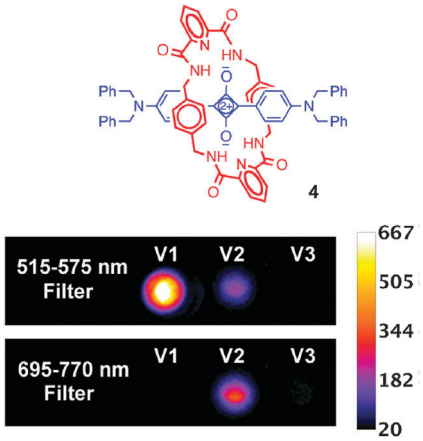

Fig. 3 (*top*) Chemical structure of fluorescent squaraine rotaxane **4** as energy acceptor for **2EP** chemiluminescence. (*bottom*) Chemiluminescence intensity maps of three vials (V1, V2, V3) containing separate $C_2D_2Cl_4$ solutions of: V1, **2EP** (1.5 mM); V2, binary admixture of **2EP** (1.5 mM) and **4** (3.0 mM); and V3, **4** (1.5 mM). The images were acquired using a green filter (515–575 nm) or near-infrared filter (695–770 nm). Chemiluminescence acquisition parameters: exposure time = 60 s, large binning, fstop = 1, T = 38 °C, intensity scale in arbitrary units.

An additional demonstration of this donor/acceptor energy transfer effect was gained by showing that the green emission of **2EP** can be transferred to a fluorescent but non-chemiluminescent acceptor dye. The inert fluorescent squaraine rotaxane **4** (with a non-reactive phenylene-containing macrocycle) was employed as the acceptor dye and in Fig. 3 are two sets of pixel intensity maps of three vials containing the following $C_2D_2Cl_4$ solutions: V1, **2EP** (1.5 mM); V2, binary admixture of **2EP** (1.5 mM) and **4** (3.0 mM); V3, **4** (1.5 mM). Green and near-infrared emission intensity maps were acquired using the 515–575 nm and 695–770 nm filters, respectively. As expected there is no chemiluminescence emission from vial V3 containing only the squaraine rotaxane **4**. A comparison of vials V1 and V2 shows that the green chemiluminescence intensity of **2EP** is diminished by the presence of energy acceptor **4** and that the excited **4** re-emits the transferred energy as near-infrared fluorescence. While the chemluminescence energy transfer effect is clear, additional studies are needed to elucidate the transfer mechanism (*e.g.*, trivial, Dexter, or Förster processes[9]). This information will facilitate our efforts to manipulate the energy transfer process and create new diagnostic chemiluminescent methods for biotechnology and imaging agents for biomedical science.[3]

We have previously reported evidence that the singlet oxygen released during squaraine rotaxane endoperoxide cycloreversion is a mediator of the chemiluminescence.[1] For example, the chemiluminescence intensity for **1EP** is greatly reduced by the presence of chemical additives that do not quench squaraine rotaxane fluorescence but do reduce singlet oxygen lifetime. The decay of singlet oxygen ($^1\Delta$) to triplet ground state ($^3\Sigma$) produces a weak dimol ($2{\cdot}^1O_2$) emission at 633 and 703 nm.[10] While these wavelengths are similar to the near-infrared emission of **1EP** they are much longer than the green emission reported here for **2EP** (~530 nm). Based on this evidence, we conclude that the chemiluminescence emission for squaraine rotaxane endoperoxides comes from the excited state squaraine chromophore that is encapsulated inside the rotaxane. This raises the interesting mechanistic question of how the decay energy of the lowest excited state of singlet oxygen (94 kJ mol^{-1}) can produce a squaraine excited singlet state, which in the case of **2EP** is about 230 kJ mol^{-1} above ground state. Previous literature studies of intermolecular sensitization of dye fluorescence by singlet oxygen have proposed several related mechanisms that involve dye excitation (including excitation of green emitting dyes[11]) by multiple molecules of singlet oxygen,[12] or singlet oxygen molecules in the second excited state.§ We have recently discussed if and how these proposed mechanisms relate to the chemiluminescent cycloreversion process described here.[13] A goal of our ongoing work is to test hypotheses based on these theories and elucidate the mechanism of squaraine rotaxane endoperoxide chemiluminescence. This work was supported by the National Science Foundation (USA) and the University of Notre Dame Integrated Imaging Facility.

Notes and references

‡ The cycloreversion rate constant for **2EP** at the lower temperature of 10 °C was 0.012 h^{-1} (half-life of 56 h) which indictes an activation energy of about 98 kJ mol^{-1}. This is 15 kJ mol^{-1} lower than the activation barrier for cycloreversion of **1EP**.

§ The second excited state of singlet oxygen is known to have an extremely short lifetime, and thus is typically discounted as a viable energy donor (see ref. 10*a*). However, it may be possible that the cycloreversion reaction releases singlet oxygen in close enough proximity to the encapsulated squaraine chromophore to allow a measurable amount of energy transfer from the second excited state. It is also worth emphasizing that the chemiluminescence quantum yield for green emitting **1EP** is significantly lower than near-infrared **2EP** (see Fig. 2) and it is possible that the chemiluminescence mechanism changes with different chromophore emission energies.

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