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A selective and sensitive chemiluminescence reaction of 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio]tetrathiafulvalene with singlet oxygen†

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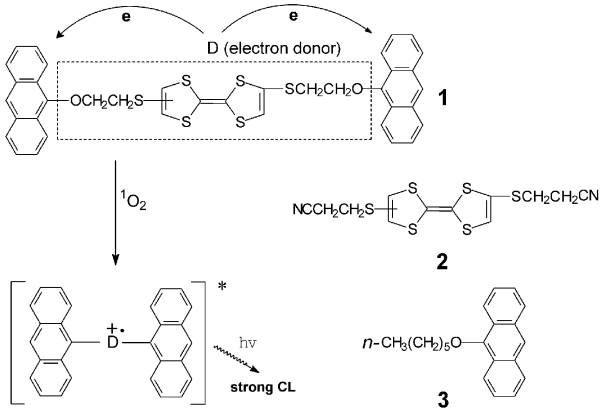
4,4′(5′)-Bis[2-(9-anthryloxy)ethylthio]tetrathiafulvalene bearing an electron-rich tetrathiafulvalene unit and a luminophore of anthracene shows a highly selective and sensitive chemiluminescence response to singlet oxygen.

Singlet oxygen ($^1O_2$), an excited state of molecular oxygen, has drawn much attention as a chemical and biological oxidant. However, it is usually difficult to corroborate the involvement of $^1O_2$ in many chemical and biological reactions because of the lack of a reliable detection method.[1] On the one hand, to distinguish $^1O_2$ from a variety of other ROS (reactive oxygen species) such as superoxide ion, hydroxyl radical and hydrogen peroxide, a technique with high selectivity is required; on the other hand, the technique should also possess high sensitivity due to low production and the short lifetime (~3 μs)[1a] of $^1O_2$ in aqueous environments.

Monitoring of the direct emission of $^1O_2$ at 1270 nm is very specific, but its use is sometimes problematic because of the low efficiency of $^1O_2$ emission.[1a] Chemical trapping by spectroscopic probes is also specific and much more sensitive than the detection of the 1270 nm emission.[2] The commonly used $^1O_2$ trap is 9,10-diphenylanthracene, which reacts specifically with $^1O_2$ to form an endoperoxide accompanying the decrease in absorbance at 355 nm as a sign of $^1O_2$ production.[3] Nevertheless, this method is still not very sensitive because it is based on the measurement of absorbance. To improve the sensitivity, Nagano *et al.* developed fluorescein-based probes, which react with $^1O_2$ to yield a sensitive fluorescence response.[1b,c] Alternatively, chemiluminescent traps for $^1O_2$, not requiring excitation light sources, can be applied in certain cases (*e.g.*, eliminating background fluorescence and various light scattering to improve signal-to-noise ratio). Furthermore, due to the high sensitivity of chemiluminescence (CL) detection, relatively low probe concentrations are necessary, thereby decreasing the likelihood of artifactual interference of secondary reactions.[2] However, only limited CL probes are available so far,[4] particularly for $^1O_2$ selective detection.[5] Herein we report 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio]tetrathiafulvalene **1** (Scheme 1), initially prepared to test its redox fluorescence property,[6] as a highly selective and sensitive CL trap for this purpose.

The probe **1** consists of a strong electron donor of a tetrathiafulvalene (TTF) unit[7] and a luminophore of anthracene. Because $^1O_2$ manifests substantial reactivity towards electron-rich organic molecules,[8] and the anthracene skeleton reacts specifically with $^1O_2$ producing CL,[1,3] we expected that **1** might have some special properties when reacted with $^1O_2$. As shown in Table 1, the reaction of **1** with either H_2O_2, OCl^-, $^{\bullet}OH$ or O_2^- does not generate noticeable CL, whereas an extremely strong CL is produced in the case of $^1O_2$,[1b,c,9] indicating that **1** exhibits indeed a highly selective CL response to $^1O_2$ only, but not to the other ROS. Moreover, from this CL reaction with 20 μM of **1**, $^1O_2$ at a concentration even down to 81 nM can still give a detectable signal (S/N = 3), also showing a highly sensitive character. The



Scheme 1 Possible CL reaction mechanism of **1** with $^1O_2$ and structures of reference compounds.

$^1O_2$-initiated CL spectrum of **1** matches well not only with the fluorescence spectrum of its reaction product (Fig. 1) but also with that of **1** itself,[6] suggesting that the emitting species should hold an anthracene core.

The probe **1** has a rather low fluorescence quantum yield of $\Phi = 3.3 \times 10^{-4}$ (quinine sulfate as a reference, $\Phi = 0.55$ in 0.05 M H_2SO_4[10]), which arises from the effective photoinduced electron transfer (PET) between TTF and anthracene units.[6] This is conducive to affording a low background signal. After reaction with $^1O_2$, however, more than 500-fold increase in fluorescence ($\Phi = 0.17$) was observed. As discussed previously,[6] this is owing to the oxidation of the TTF unit into the corresponding radical cation, which prohibits the PET process. In general, the strong CL of a species is associated with its strong fluorescence. Consequently, these results suggest that the oxidation of the TTF unit in **1** by $^1O_2$ not only is responsible for the fluorescence enhancement, but also contributes to the observed strong CL.

To have an insight into the role of the TTF moiety in activating the anthracene reactivity towards $^1O_2$, the CL behavior of reference

Table 1 Comparison of relative CL intensities from the reaction of **1** with different ROS[a]

Control	$^1O_2$[b]	H_2O_2[c]	OCl^-[d]	$^{\bullet}OH$[e]	O_2^-[f]	$^1O_2$[g]
2.5×10^{-5}	1.0	6.3×10^{-5}	5.0×10^{-4}	2.9×10^{-4}	1.8×10^{-4}	1.5

[a] The CL intensity [1.12×10^6 RLU (relative light units)] from the reaction of **1** with $^1O_2$ generated by the NaOCl/H_2O_2 system, giving a linear equation of log CL = $1.00 \times \log [^1O_2] + 6.01$ ($n = 9$, $\gamma = 0.999$) at least in the range of 3.2×10^{-4} – 1 mM of $^1O_2$, was defined as 1.0. The CL reaction was initiated by injecting ROS into 50 mM sodium phosphate buffer (pH 7) containing 20 μM of **1** and 50% (v/v) tetrahydrofuran as a cosolvent at 25 °C. Each of the data was the mean of three determinations with a relative error of less than ±5%. [b] 1 mM H_2O_2 + 10 mM NaOCl. [c] 1 mM H_2O_2. [d] 10 mM NaOCl. [e] 1 mM H_2O_2 + 0.1 mM ferrous ammonium sulfate. [f] 0.1 ml of superoxide solution (1 mg KO_2/ml dimethyl sulfoxide). [g] 1 mM H_2O_2 + 1 mM MoO_4^{2-} (working only in basic solutions) at pH 10.

† Electronic supplementary information (ESI) available: absorption spectra in the absence and presence of $^1O_2$, HPLC analysis, MS spectra, and evidence for $^1O_2$ involvement in the NaOCl/H_2O_2/**1** system. See http://www.rsc.org/suppdata/cc/b4/b405272b/

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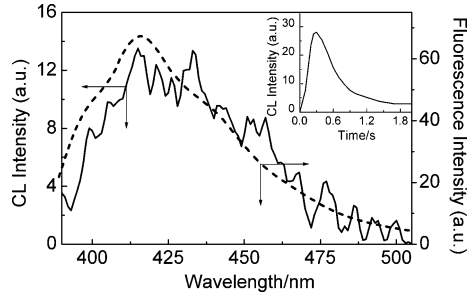

Fig. 1 The CL spectrum (—) from **1** and its fluorescence spectrum (···) excited at 370 nm after reaction with $^1O_2$. The concentrations of reactants were 200 μM of **1**, 10 mM of H_2O_2 and 30 mM of NaOCl. After oxidation and an appropriate dilution the reaction solution was then used to measure the fluorescence spectrum. The inset also shows the CL kinetic curve of the NaOCl/H_2O_2/**1** system with 1 mM of H_2O_2, 10 mM of NaOCl and 20 μM of **1**, illustrating a fast CL reaction.

molecules anthracene and **3** was also tested under the same conditions. Anthracene and **3** had a relative CL intensity of 0.012 and 0.032, respectively, which are far lower than that (1.0 in Table 1) of **1**, clearly showing that the presence of the TTF unit in **1** is crucial for the production of strong CL. In other words, the TTF unit enhances the anthracene reactivity towards $^1O_2$.

In order to explore the CL mechanism of the present system, the absorption spectra of **1–3** before and after oxidation were first examined.† A noticeable decrease in the absorption representing the anthracene moiety occurred for compound **3** which has an electron-donating alkoxyl in the 9-position. It is apparent that **3** lacks any reactive unit except the anthracene core. Based on previous studies,[11] in this molecule the most active sites for $^1O_2$ addition are the electron-rich carbons in the 9,10-positions, therefore the decrease of the absorption bands indicative of anthracene can be explained by the possible formation of endoperoxides. Moreover, the decrease in absorbance was also observed for the solution of **2**, indicating its reaction with $^1O_2$. For probe **1**, however, the absorption in the same wavelength range showed a larger decrease,† which may result from the sum of the decreased absorbances caused by the reactions of both the TTF and anthracene units.

To further confirm the above postulations, the reaction products of probe **1** and compound **3** with $^1O_2$ were then subjected to HPLC analysis.† The area of the peak with a retention time of 6.40 min corresponding to **3** was diminished to about 45% of the original value after reaction, concomitant with the emergence of a new peak at 3.17 min, which was characterized to be the predicted endoperoxide (*m*/*z* 311 $[M + H]^+$) by MS analysis.† This is consistent with the above spectral studies. But, the peak at 17.89 min indicative of probe **1** almost vanished after reacting with $^1O_2$ and a new one at 2.10 min appeared, also showing that **1** is more reactive towards $^1O_2$ than **3**. The separated product with the retention time of 2.10 min, when subjected to MS analysis, surprisingly gave the same molecular ion peak (*m*/*z* 708)† as the probe **1** rather than the predicted endoperoxide, implying that the endoperoxide of **1**, different from that of **3**, may be rather unstable. This may be associated with the presence of the TTF unit and responsible for the strong CL production. Moreover, the reaction product retained the characteristic absorption bands of anthracene,† and had stronger polarity because its retention time became much shorter than that of **1**, supporting the anticipation that it existed as a cation species, which can be generated from the decomposition of the formed unstable endoperoxide of **1** with $^1O_2$.

On the other hand, when the TTF unit in **1** was first oxidized into radical cation by $Fe(ClO_4)_3$, followed by reaction with $^1O_2$, a much weaker CL (about $\frac{1}{3}$ of the original value) was observed.

Taken together, we propose that the CL reaction in the present system might proceed through the following route (Scheme 1): the anthracene unit first traps $^1O_2$ to yield an unstable endoperoxide, whose decomposition causes not only excitation of the anthracene core which in turn emits light through radiative decay, but also oxidation of the electron-rich TTF moiety into a cation species. In such a reaction the formation of the final cation species of **1** promotes the proceeding of the CL reaction forward, and meanwhile prohibits the PET process between the TTF and excited anthracene units, thus enhancing CL. It should be noted that the reaction stoichiometry of **1** with $^1O_2$ is unclear. Besides, it cannot be ruled out that the oxidation of the TTF unit by $^1O_2$ provides chemical energy to excite directly the anthracene core which then emits light, but this pathway, if existing, would be a minor one since the probe displayed a specific CL response only to $^1O_2$ rather than to the other ROS. Otherwise, unselective reactions would occur and stronger CL would be observed with stronger oxidants such as $^{\bullet}OH$.

In conclusion, the results described here clearly demonstrate that **1** exhibits both high selectivity for and high sensitivity to $^1O_2$. The unique feature of the electron-rich TTF unit not only facilitates the PET process giving a desired low background signal, but also increases the anthracene reactivity towards $^1O_2$, thereby benefiting the $^1O_2$ trapping. This makes it possible for the probe to be used widely for $^1O_2$ detection in many chemical and biological systems.

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