J|A|C|S
COMMUNICATIONS

In Search of a Chemiluminescence 1,4-Dioxy Biradical

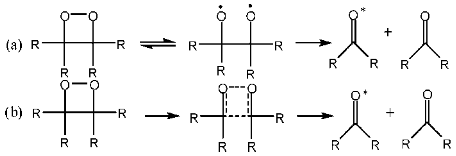
Richard Bos,[†] Sarah A. Tonkin,[†] Graeme R. Hanson,[‡] Christopher M. Hindson,[†] Kieran F. Lim,[†] and Neil W. Barnett*[,†]

Deakin University, School of Life and Environmental Sciences, Pigdons Road, Waurn Ponds 3217, Victoria, Australia, and University of Queensland, Centre for Magnetic Resonance, St. Lucia 4072, Brisbane, Australia

Received October 27, 2008; E-mail: barnie@deakin.edu.au

The thermal decomposition mechanism of 1,2-dioxetanes has been the subject of extensive experimental[1−6] and theoretical[7,8] investigations owing partially to the role of this class of compounds in bioluminescent and chemiluminescent light producing pathways.[9] Perhaps the most challenging mechanistic aspect has been proving the existence of a 1,4-dioxy biradical species, first postulated by Richardson and O'Neal over 30 years ago[1] (Scheme 1a). Around the same time, McCapra,[2] and subsequently Kearns,[3] developed a concerted mechanism that did not call for the involvement of a biradical (Scheme 1b). In the years following, these contrasting ideas were combined by Turro and Lechtken[4] and expanded by Adam and Baader[5] to rationalize certain kinetic observations and explain the yields of excited-state products. This work[4,5] became known as the asynchronous concerted or merged mechanism, involving simultaneous O−O′ and C−C′ bond extensions followed by rupture of the former to generate a biradical intermediate and subsequent decomposition to excited-state products.

Scheme 1. Some Proposed Mechanisms for the Thermal Decomposition of 1,2-Dioxetanes[a]

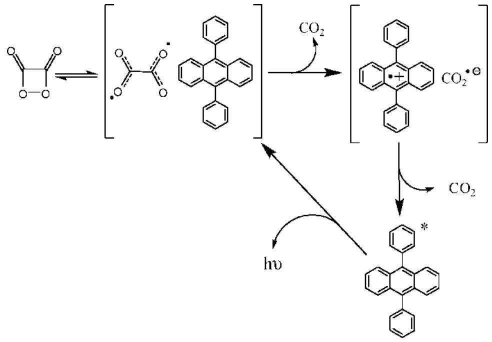


[a] (a) Stepwise biradical process;[1] (b) concerted process.[2]

To offer some specific evidence for 1,4-dioxy biradical formation, we have employed EPR spectroscopy to monitor the low temperature decomposition of a strained peroxyoxalate chemiluminescence intermediate (1,2-dioxetanedione) previously identified by ^{13}C NMR spectroscopy with ^{13}C-labeled oxalyl chloride as the reagent and *ab initio* calculated chemical shifts.[10] This type of chemiluminescence is thought to be governed by an intermolecular chemically initiated electron exchange luminescence (CIEEL) mechanism, first proposed by Schuster 30 years ago[11] (Scheme 2). Although some of the compounds invoked by the CIEEL postulate have since been spectroscopically identified (1,2-dioxetanedione, CO_2),[10,12,13] the paramagnetic species long thought to be directly responsible for fluorophore excitation have hitherto evaded definitive characterization. To address this current lack of spectroscopic evidence, further attention has been given to radical species other than the 1,4-dioxy biradical appearing in this reaction (Scheme 2).

Here we investigate the proposal of earlier groups[1,14] who assumed analogous decomposition pathways for 1,2-dioxetanes and 1,2-dioxetanedione and relate it to the postulated CIEEL mechanism (Scheme 2). To do so, two quartz EPR sample tubes (3 mm i.d.) containing 0.4 mL of THF and solvent and either 0.03 mL of oxalyl chloride or 0.1 mL of hydrogen peroxide with 1 mM 9,10-diphenylanthracene (DPA) were cooled at 180 K in an acetone/liquid nitrogen slush bath. The tube bearing oxalyl chloride was fitted with a Young valve, and after cooling both reactants, the hydrogen peroxide was transferred quantitatively (0.5 mL) using a syringe with gentle mixing. The sample tube was then inserted into the EPR spectrometer at 180 K where the extent of the reaction could be controlled by temperature adjustment and the amount of mixing time allowed, prior to cooling to 130 K for collection of X-band EPR spectra (compare Figures 1a, 1c).[15]

Scheme 2. Simplified Intermolecular CIEEL Sequence for Peroxyoxalate Chemiluminescence[11a]



[a] Thermolysis of 1,2-dioxetanedione[10,12] generates the $S = 1$ oxalate biradical[1,14] (Figure 1a: $\Delta M_S = \pm1$: 300 mT, $\Delta M_S = \pm2$: 160 mT) which oxidizes DPA to DPA$^{•+}$ and decomposes to give CO_2[10,13] and $S = {}^1/_2$ $CO_2^{•-}$ (Figure 1a, "|"). Back electron transfer (BET) ensues to yield CO_2 and excited DPA* which emits upon relaxation.

It is important to note that mixing the reagents at low temperature (180 K) ensures the decomposition of 1,2-dioxetanedione to the corresponding oxalate biradical is slow enough to be monitored by EPR. This stems from the relative temporal stability exhibited by the chemi-excitation source (1,2-dioxetanedione) at reduced temperature. As we have demonstrated previously[10] when oxalyl chloride and anhydrous hydrogen peroxide are reacted in the presence of DPA, light emission persists for more than 30 min at 200 K. Having also shown that 1,2-dioxetanedione decomposition is directly related to fluorophore excitation,[10,11] we propose that the oxalate biradical is continuously produced while chemiluminescence occurs. Furthermore, the generation of intense chemiluminescence under these conditions for more than 30 min[10] confirms the lack of reactivity between tetrahydrofuran and either the anhydrous hydrogen peroxide or the oxalyl chloride.

Typical experimental spectra (Figure 1a) of the chemiluminescent reaction at 130 K, acquired at a short mixing time, showed resonances around 300 and 160 mT arising from allowed ($\Delta M_S = \pm 1$) and formally forbidden ($\Delta M_S = \pm 2$) transitions from an $S = 1$ spin system arising from a biradical. In addition to the resonances

---

[†] Deakin University.
[‡] University of Queensland.

10.1021/ja808401p CCC: $40.75 © 2009 American Chemical Society

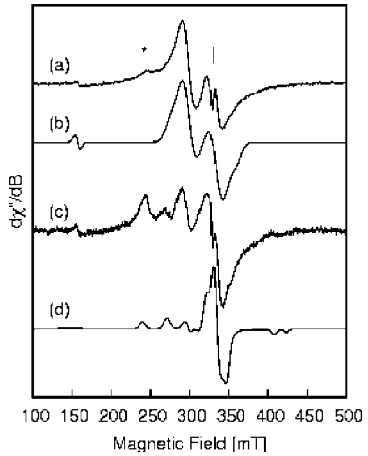


Figure 1. EPR spectra of the reaction of oxalyl chloride and anhydrous hydrogen peroxide in THF recorded at (a) short mixing times, $T = 130$ K, $\upsilon = 9.430\,752$ GHz and (c) after longer mixing times, $T = 130$ K, $\upsilon = 9.431\,13$ GHz.[16] The resonances labeled "|" and "*" arise from small proportions of $S = {}^1/_2$ and $S = {}^3/_2$ species, respectively, which are present in greater amounts at longer times. Computer simulations (XSophe-Sophe-XeprView)[17] of the (b) $S = 1$ and (d) $S = {}^3/_2$ species in (a, c).

belonging to the $S = 1$ spin system, resonances were also observed from $S = {}^1/_2$ ("|", Figure 1a) and $S = {}^3/_2$ ("*", Figure 1a) species, the latter occurring in larger proportion at longer mixing times (Figure 1c). The formation of an $S = {}^3/_2$ radical species is consistent with the reaction of a CO_2 radical anion ($S = {}^1/_2$) with the oxalate biradical ($S = 1$), where the appearance of the former is thought to arise from reduction and rapid decomposition of the biradical (Scheme 2).[1,14] The high energy $CO_2^{\bullet-}$ species has long been thought to be the source of fluorophore excitation *via* an intermolecular back electron transfer (BET) process.[11] We suggest that some $CO_2^{\bullet-}$ may also react with the continuously produced[10] oxalate biradical to produce a yet uncharacterized $S = {}^3/_2$ species ("*", Figure 1a,c) in a competitive dark reaction. Such alternate pathways undoubtedly contribute to the relatively low quantum yields of chemiluminescence systems compared to the enzyme mediated bioluminescence.[13]

Computer simulation of the EPR spectra (Figure 1a,c) with a second-order fine structure spin Hamiltonian[17]

$$H = \beta B\cdot g\cdot S + D\left(S_{z^2} - \frac{1}{3}S(S+1)\right) + E(S_{x^2} - S_{y^2}) \qquad (1)$$

and the spin Hamiltonian parameters ($S = 1$: $g = 2.1200$, $D = 0.041$ cm^{-1}, and $E/D = 0.1$; $S = {}^3/_2$: $g_x = 2.06$, $g_y = 1.98$, $g_z = 2.032$, $D = 0.0433$ cm^{-1}, and $E/D = 0.1502$)[18] yields the spectra shown in Figure 1b,d which are in excellent agreement with the experimental spectra (Figure 1a,c). Δg ($\Delta g = g - g_e$) = 0.1176, 0.02168 for the $S = 1$ and ${}^3/_2$ radicals is typical of oxygen centered radicals and is significantly larger than that found for carbon centered radicals.[19] The axial zero field splitting parameter, D, for the biradical corresponds to a maximum internuclear distance of 4.14 Å which is compatible with a trans separation of the O atoms within an $-O-C-C-O-$ fragment (Scheme 1). The structurally related tetramethylene$-$ethane biradical has $D = 0.025$ cm^{-1} corresponding to a slightly longer distance.[20] The large line widths of the resonances (ΔB_{pp}: $S = 1$: 17.3 mT; $S = {}^3/_2$: 11.6 mT) arise from shorter spin lattice relaxation times associated with the presence of closely lying excited states and are significantly greater than that expected for anisotropic $S = {}^1/_2$ radical species.

The validity of the assumption mentioned earlier[1,14] requires consideration of the similarities of 1,2-dioxetanes and 1,2-dioxetanedione. Both species are four-membered rings and would be expected to generate the biradical *via* analogous homolytic bond fission of the isoelectronic $C-O-O'-C'$ fragment. This being said, the doubly sp^2 hybridized 1,2-dioxetanedione is not entirely isoelectronic with commonly studied 1,2-dioxetanes or cyclobutane where the latter has been shown to proceed through a 1,4-tetramethylene biradical during ring opening.[8] As noted by De Vico et al.,[9] the situation for the 1,4-tetramethylene biradical is somewhat simpler than that proposed for either the 1,2-dioxetanes or 1,2-dioxetanedione. The 1,4-tetramethylene has two potential states compared with four singlet and four triplets possible for species containing a dioxetane moiety.[9] Finally, the 1,2-dioxetanedione and the corresponding oxalate biradical have planar geometries and contain an extended π-system.

Because of the above-mentioned differences, this study confirms the postulation of previous groups[1,14] that the decomposition of 1,2-dioxetanedione during peroxyoxalate chemiluminescence produces an oxalate biradical intermediate within what has now been confirmed as the light producing pathway.[10,12] The identification of such paramagnetic species during chemi-excitation offers much needed spectroscopic evidence supporting an intermolecular CIEEL mechanism.

References

(1) (a) O'Neal, H. E.; Richardson, W. H. *J. Am. Chem. Soc.* **1970**, *92*, 6553–6557. (b) O'Neal, H. E.; Richardson, W. H. *J. Am. Chem. Soc.* **1971**, *93*, 1828. (c) Richardson, W. H.; O'Neal, H. E. *J. Am. Chem. Soc.* **1972**, *94*, 8665–8668.
(2) (a) McCapra, F. *Chem. Commun.* **1968**, *3*, 155. (b) McCapra, F. *Pure Appl. Chem.* **1970**, *24*, 611–630.
(3) (a) Kearns, D. R. *J. Am. Chem. Soc.* **1969**, *91*, 6554–6563. (b) Kearns, D. R. *Chem. Rev.* **1971**, *71*, 395–427.
(4) (a) Turro, N. J.; Lechtken, P. *J. Am. Chem. Soc.* **1973**, *95*, 264–266. (b) Turro, N. J.; Lechtken, P. *Pure Appl. Chem.* **1973**, *33*, 363–389.
(5) Adam, W.; Baader, W. J. *J. Am. Chem. Soc.* **1985**, *107*, 410–416.
(6) Murphy, S.; Adam, W. *J. Am. Chem. Soc.* **1996**, *118*, 12916–12921.
(7) Wisley, S.; Bernardi, F.; Olivucci, M.; Robb, M. A.; Murphy, S.; Adam, W. *J. Phys. Chem. A* **1999**, *103*, 1669–1677.
(8) De Feyter, S.; Diau, E. W. G.; Scala, A. A.; Zewail, A. H. *Chem. Phys. Lett.* **1999**, *303*, 249–260.
(9) De Vico, L.; Liu, Y. J.; Wisborg Krogh, J.; Lindh, R. *J. Phys. Chem. A* **2007**, *111*, 8013–8019.
(10) Bos, R.; Barnett, N. W.; Dyson, G. A.; Lim, K. F.; Russell, R. A.; Watson, S. P. *Anal. Chim. Acta* **2004**, *502*, 141–147.
(11) Schuster, G. B. *Acc. Chem. Res.* **1979**, *12*, 366–373.
(12) Tonkin, S. A.; Bos, R.; Dyson, G. A.; Lim, K. F.; Russell, R. A.; Watson, S. P.; Hindson, C. M.; Barnett, N. W. *Anal. Chim. Acta* **2008**, *614*, 173–181.
(13) (a) Stigbrand, M.; Jonsson, T.; Ponten, E.; Irgum, K.; Bos, R. *Chemiluminescence in Analytical Chemistry*; Garcia-Campana, A. M., Baeyens, W. R. G., Eds.; Marcel-Dekker: New York, 2001; pp 181. (b) Rauhut, M. M. *Acc. Chem. Res.* **1969**, *2*, 80–87.
(14) Mann, B.; Grayeski, M. L. *Anal. Chem.* **1990**, *62*, 1532–1536.
(15) (a) Stevani, C. V.; Silva, S. M.; Baader, W. J. *Eur. J. Org. Chem.* **2000**, *24*, 4037–4036. (b) Silva, S. M.; Wagner, K.; Weiss, D.; Beckert, R.; Stevani, C. V.; Baader, W. J. *Luminescence* **2002**, *17*, 362–369. (c) Bastos, E. L.; Ciscato, L. F. M. L.; Bartoloni, F. H.; Baader, W. J. *Luminescence* **2006**, *21*, 270.
(16) X-Band EPR spectra were recorded on a Bruker Elexsys E500 spectrometer fitted with a Super High Q cavity (spectrometer settings for Figure 1: receiver gain = 96 dB; modulation amplitude = 6 G; microwave power 6 mW; 74 and 37 scans averaged for (a, c respectively)). Calibrations of the magnetic field and microwave frequency were achieved with a Bruker ER 035M Gaussmeter and an EIP 548B microwave frequency counter, respectively. Low temperature spectra were recorded using a liquid nitrogen flow-through cryostat in conjunction with a Eurotherm B-VT2000 variable temperature controller.
(17) Hanson, G. R.; Gates, K. E.; Noble, C. J.; Griffin, M.; Mitchell, A.; Benson, S. J. *Inorg. Biochem.* **2004**, *98*, 903–916. Strain distributions of D and E were not included in the simulations.
(18) The *E*/*D* ratio 0.1 for the biradical represents an upper limit and is more likely to be closer to zero, but without resolution of the axial resonances, a precise value cannot be determined.
(19) Gerson, F.; Huber W. In *Electron Spin Resonance of Organic Radicals*; VCH: Weinheim, 2003; pp 169−210.
(20) Dowd, P.; Chang, W.; Paik, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7416–7417.

JA808401P