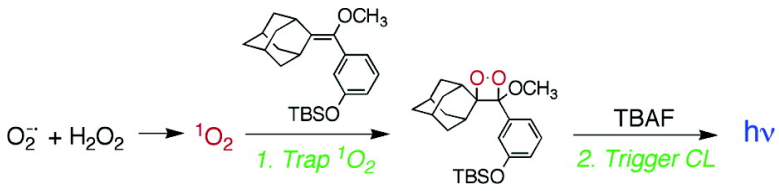
Communication

Quantification of Singlet Oxygen Production in the Reaction of Superoxide with Hydrogen Peroxide Using a Selective Chemiluminescent Probe

Laura A. MacManus-Spencer, and Kristopher McNeill

J. Am. Chem. Soc., **2005**, 127 (25), 8954-8955• DOI: 10.1021/ja052045b • Publication Date (Web): 04 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/04/2005

Quantification of Singlet Oxygen Production in the Reaction of Superoxide with Hydrogen Peroxide Using a Selective Chemiluminescent Probe

Laura A. MacManus-Spencer and Kristopher McNeill*

Department of Chemistry, University of Minnesota, 207 Pleasant Street SE, Minneapolis, Minnesota 55455

Received March 31, 2005; E-mail: mcneill@chem.umn.edu

Superoxide radical anion ($O_2^{-\bullet}$)[1] and hydrogen peroxide (H_2O_2)[2] are formed intracellularly, and the reaction of these two reactive oxygen species (ROS) has been studied for decades in an effort to explain their observed "toxic synergism".[3,4] Both singlet oxygen ($^1O_2$) and hydroxyl radical ($^\bullet$OH) have been proposed as highly cytotoxic products of this reaction. Singlet oxygen is known to oxidize a variety of biological substrates, such as proteins,[5] certain amino acids,[6] and nucleic acids.[7] Hydroxyl radical is a nonspecific oxidant, reacting with proteins and free amino acids with rate constants ranging from 10^7 to 10^{10} M^{-1} s^{-1}.[8]

The formation of both $^1O_2$ and $^\bullet$OH in the reaction of $O_2^{-\bullet}$ with H_2O_2 has been proposed to occur via the so-called Haber−Weiss or Haber−Willstätter reaction[9] (eq 1), in which a fraction of the O_2 is produced as $^1O_2$. The biological relevance of this reaction has been debated for at least four decades.[4,10−12]

$$H_2O_2 + O_2^{-\bullet} \rightarrow O_2 + {}^\bullet OH + {}^-OH \qquad (1)$$

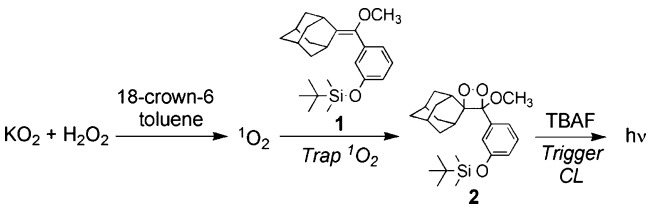
In 1975, Kellogg and Fridovich suggested that $^1O_2$ could be formed in the Haber−Weiss reaction and provided experimental evidence of its production in the reaction of xanthine oxidase with acetaldehyde in an aqueous system, which simultaneously generates $O_2^{-\bullet}$ and H_2O_2.[13] Experimental evidence of $^1O_2$ production in the Haber−Weiss reaction has also been found in aprotic solvents,[14] which are useful models of the nonaqueous hydrophobic environment of lipid bilayers.[15]

In this study, we used a sensitive chemiluminescent probe that selectively reacts with $^1O_2$ in the presence of $O_2^{-\bullet}$ and H_2O_2 to quantify the production of $^1O_2$ in the reaction of these two ROS. The trap-and-trigger probe, which we have described previously,[16] is based on a stable dioxetane precursor (**1**, Scheme 1). This detection method builds upon the work of Schaap,[17] Adam,[18] and others,[19] who have reported a series of spiroadamantylidene-substituted dioxetanes that are unusually stable and require a chemical trigger to initiate their chemiluminescent decomposition. In this detection scheme, $^1O_2$ is trapped in the form of a stable dioxetane (**2**, Scheme 1), which is quantified by its chemiluminescence (CL) signal, triggered by the addition of tetra-*n*-butylammonium fluoride (TBAF).

The reaction of $O_2^{-\bullet}$ with H_2O_2 was carried out in aprotic solvent (toluene) to take advantage of the greater stability and higher reactivity of $O_2^{-\bullet}$ under such conditions.[20] To circumvent the complications associated with heterogeneous reaction conditions or the presence of water, a homogeneous solution of H_2O_2 (0.032 M) was prepared in toluene by the oxidation of 2-ethylanthrahydroquinone (2-EAHQ) and purified by distillation (see Supporting Information).

The formation of dioxetane **2** in the reaction of KO_2 (2 mM, solubilized with 18-crown-6 ether; see Supporting Information for the determination of the dissolved $O_2^{-\bullet}$ concentration) with H_2O_2 (10 mM) in toluene at 25 °C in the presence of 100 μM probe **1** was followed by analyzing aliquots of the reaction mixture over a period of 90 min (Figure 1, circles). The formation of dioxetane **2** followed apparent first-order kinetics with an observed rate constant, k_{obs}, of $(2.1 \pm 0.3) \times 10^{-3}$ s^{-1}. The yield of $^1O_2$ under these conditions was calculated to be $(4.0 \pm 0.4) \times 10^{-6}$ M, which corresponds to a yield of (0.20 ± 0.03)% relative to the initial $O_2^{-\bullet}$ concentration.

Scheme 1



The formation of $^1O_2$ in this system was supported by experiments in which its lifetime was increased through the use of deuterated solvent and decreased by the addition of a $^1O_2$ quencher. The possible reaction and deactivation pathways of $^1O_2$ in this system are illustrated in Scheme 2. When the reaction of $O_2^{-\bullet}$ with H_2O_2 was carried out in 57% toluene-d_8 (Figure 1, squares), the observed enhancement in the $^1O_2$ yield (1.7) closely matched the enhancement predicted from the$^1O_2$ lifetimes in the two solvent mixtures (2.0). The formation of $^1O_2$ was also predictably inhibited by the addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), a known $^1O_2$ quencher[21] (Figure 1, triangles).

It has been proposed that the Haber−Weiss reaction (eq 1) does not occur in the absence of a metal catalyst.[4,11] We found no evidence for the participation of trace metal impurities in the formation of $^1O_2$ from KO_2 and H_2O_2, as there were no differences in the apparent kinetics or $^1O_2$ yield in the presence and in the absence of diethylenetriaminepentaacetic acid (DTPA), a metal chelator. However, in the presence of a relatively high amount of *added* Fe(II) acetate (100 μM), a 6-fold increase in the $^1O_2$ yield and an almost 2-fold increase in the apparent first-order rate constant were observed (see Supporting Information), suggesting that the reaction of $O_2^{-\bullet}$ with H_2O_2 to produce $^1O_2$ can be catalyzed by Fe(II) and possibly other redox-active metals. It is unclear from these experiments how the Fe(II) facilitates the production of $^1O_2$, although it has been suggested that it can catalyze the reaction by acting as a redox mediator.[4,9]

The potential formation of $^\bullet$OH in the reaction of $O_2^{-\bullet}$ with H_2O_2 under the conditions used in this study was also investigated. Hydroxyl radical is known to react with toluene with a rate constant of 8.1×10^9 M^{-1} s^{-1} to preferentially form *o*-, *m*-, and *p*-cresols (as opposed to benzyl radical);[23] the relative yields of these products are reported to be 0.84, 0.41, and 1.0, respectively.[24] A spectrophotometric detection method using Gibbs's reagent[25] (2,6-dichloroquinone-4-chloroimide) was used to quantify the total cresol concentration as an indicator of $^\bullet$OH production. By this method,

10.1021/ja052045b CCC: $30.25

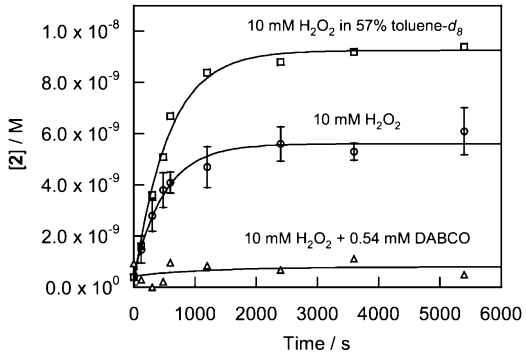
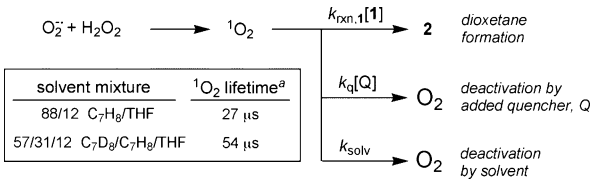


Figure 1. Production of dioxetane **2** during exposure of **1** (100 μM) to $O_2^{-\bullet}$ (2 mM) and H_2O_2 (10 mM) at 25 °C in toluene (○), in 57% toluene-d_8 (□), or in toluene with 0.54 mM DABCO (△). Data are fit to a monoexponential growth function. Error bars represent one standard deviation from four replicate measurements.

Scheme 2



solvent mixture	$^1O_2$ lifetime[a]
88/12 C_7H_8/THF	27 μs
57/31/12 C_7D_8/C_7H_8/THF	54 μs

[a] Lifetimes for $^1O_2$ were calculated from the fractional solvent composition and published lifetimes in pure solvents.[22]

the total cresol concentration after the reaction of $O_2^{-\bullet}$ with H_2O_2 in toluene was found to be less than the detection limit, 200 nM (see Supporting Information). With an upper limit of 200 nM, the yield of $^\bullet$OH can be no more than 0.01% relative to the initial $O_2^{-\bullet}$ concentration. This result suggests that the Haber−Weiss mechanism is not important in the reaction of $O_2^{-\bullet}$ with H_2O_2 under these conditions.

Another mechanism has been proposed for the reaction of $O_2^{-\bullet}$ with H_2O_2 in aprotic solvent in which H_2O_2 acts as a proton donor for $O_2^{-\bullet}$ (eqs 2 and 3).[26]

$$H_2O_2 + O_2^{-\bullet} \rightarrow HO_2^- + HO_2^\bullet \qquad (2)$$

$$HO_2^\bullet + O_2^{-\bullet} \rightarrow HO_2^- + O_2 \qquad (\text{or } {}^1O_2) \qquad (3)$$

If this mechanism is the source of $^1O_2$ in this study, one would expect any acid with a pK_a similar to that of H_2O_2 (10.7 in *N*,*N*-dimethylformamide (DMF)[27]) to also react with $O_2^{-\bullet}$ to produce $^1O_2$. However, when H_2O_2 was replaced with 2-nitrobenzoic acid, which has a pK_a of 9.9 in DMF,[28] no $^1O_2$ production was observed. Additionally, no $^1O_2$ was observed in the reaction of $O_2^{-\bullet}$ with another soluble proton donor, *tert*-butyl alcohol (see Supporting Information). Thus, a simple acid−base reaction between $O_2^{-\bullet}$ and H_2O_2 does not appear to be sufficient to describe the mechanism of $^1O_2$ production in this system.

Estimated biological concentrations of H_2O_2 and $O_2^{-\bullet}$ have been reported as $\leq 10^{-5}$ M and ca. 10^{-9} M (pH 7, aqueous), respectively.[29] As H_2O_2 is produced in the disproportionation of $O_2^{-\bullet}$, these species can also be expected to be co-localized in biological systems. Our results indicate that these species react to produce $^1O_2$ but with very low efficiency, and we found no evidence for the production of $^\bullet$OH. The low yields of $^1O_2$ and $^\bullet$OH suggest that their formation in the uncatalyzed reaction of $O_2^{-\bullet}$ with H_2O_2 should be relatively unimportant in biological systems, even in water-free hydrophobic environments where the stabilities and reactivities of these ROS may be greater than in aqueous environments.

Acknowledgment. Support has been provided by a Grant-in-Aid from the University of Minnesota.

Supporting Information Available: Experimental details for the preparation of 2-EAHQ and the generation of H_2O_2 in toluene; the reactions of $O_2^{-\bullet}$ with H_2O_2, 2-nitrobenzoic acid, and *tert*-butyl alcohol; and the detection of cresols using Gibbs's method. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1968**, *243*, 5753−5760. (b) Massey, V.; Strickland, S.; Mayhew, S. G.; Howell, L. G.; Engel, P. C.; Matthews, R. G.; Schuman, M.; Sullivan, P. A. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 891−897. (c) Knowles, P. F.; Gibson, J. F.; Pick, F. M.; Bray, R. C. *Biochem. J* **1969**, *111*, 53−58. (d) Bernacchia, A.; Biondi, A.; Genova, M. L.; Lenaz, G.; Falasca, A. *Toxicol. Mech. Methods* **2004**, *14*, 25−30.

(2) Halliwell, B.; Clement, M. V.; Long, L. H. *FEBS Lett.* **2000**, *486*, 10−13.

(3) Peters, J. W.; Foote, C. S. *J. Am. Chem. Soc.* **1976**, *98*, 873−875.

(4) Liochev, S. I.; Fridovich, I. *Redox Rep.* **2002**, *7*, 55−57.

(5) Davies, M. J. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 761−770.

(6) (a) Nilsson, R.; Merkel, P. B.; Kearns, D. R. *Photochem. Photobiol.* **1972**, *16*, 117−124. (b) Matheson, I. B. C.; Lee, J. *Photochem. Photobiol.* **1979**, *29*, 879−881. (c) Michaeli, A.; Feitelson, J. *Photochem. Photobiol.* **1994**, *59*, 284−289.

(7) (a) Hallett, F. R.; Hallett, B. P.; Snipes, W. *Biophys. J.* **1970**, *10*, 305−315. (b) Rosenthal, I.; Pitts, J. N., Jr. *Biophys. J.* **1971**, *11*, 963−966. (c) Clagett, D. C.; Galen, T. J. *Arch. Biochem. Biophys.* **1971**, *146*, 196−201. (d) Canva, J. J.; Balny, C. *Int. J. Radiat. Phys. Chem.* **1971**, *3*, 451−455. (e) Sysak, P. K.; Foote, C. S.; Ching, T.-Y. *Photochem. Photobiol.* **1977**, *26*, 19−27.

(8) Davies, M. J. *Biochim. Biophys. Acta* **2005**, *1703*, 93−109.

(9) Haber, F.; Weiss, J. *Proc. R. Soc.* **1934**, *A147*, 332−351.

(10) (a) Koppenol, W. H. *Nature* **1976**, *262*, 420−421. (b) Koppenol, W. H.; Butler, J.; Van Leeuwen, J. W. *Photochem. Photobiol.* **1978**, *28*, 655−660. (c) Afanas'ev, I. B.; Kupriyanova, N. S.; Letuchaya, A. V. *Oxygen Radicals Chem. Biol., Proc., Int. Conf., 3rd* **1984**, 17−23. (d) Kehrer, J. P. *Toxicology* **2000**, *149*, 43−50.

(11) Koppenol, W. H. *Redox Rep.* **2001**, *6*, 229−234.

(12) Koppenol, W. H. *Redox Rep.* **2002**, *7*, 59−60.

(13) Kellogg, E. W., III; Fridovich, I. *J. Biol. Chem.* **1975**, *250*, 8812−8817.

(14) (a) Kobayashi, S.; Ando, W. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 676−681. (b) Khan, A. U.; Kasha, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12365−12367.

(15) (a) White, S. H. *Nature* **1976**, *262*, 421−422. (b) Sawyer, D. T.; Roberts, J. L., Jr.; Calderwood, T. S.; Sugimoto, H.; McDowell, M. S. *Philos. Trans. R. Soc. London, Ser. B* **1985**, *311*, 483−503.

(16) MacManus-Spencer, L. A.; Latch, D. E.; Kroncke, K. M.; McNeill, K. *Anal. Chem.* **2005**, *77*, 1200−1205.

(17) (a) Schaap, A. P.; Chen, T. S.; Handley, R. S.; DeSilva, R.; Giri, P. P. *Tetrahedron Lett.* **1987**, *28*, 1155−1158. (b) Schaap, A. P.; Handley, R. S.; Giri, B. P. *Tetrahedron Lett.* **1987**, *28*, 935−938.

(18) (a) Adam, W.; Fell, R.; Schulz, M. H. *Tetrahedron* **1993**, *49*, 2227−2238. (b) Adam, W.; Bronstein, I.; Edwards, B.; Engel, T.; Reinhardt, D.; Schneider, F. W.; Trofimov, A. V.; Vasil'ev, R. F. *J. Am. Chem. Soc.* **1996**, *118*, 10400−10407.

(19) (a) Matsumoto, M.; Watanabe, N.; Shiono, T.; Suganuma, H.; Matsubara, J. *Tetrahedron Lett.* **1997**, *38*, 5825−5828. (b) Watanabe, N.; Suganuma, H.; Kobayashi, H.; Mutoh, H.; Katao, Y.; Matsumoto, M. *Tetrahedron* **1999**, *55*, 4287−4298.

(20) Sawyer, D. T.; Nanni, E. J., Jr.; Roberts, J. L., Jr. *Adv. Chem. Ser.* **1982**, *201*, 585−600.

(21) Ouannes, C.; Wilson, T. *J. Am. Chem. Soc.* **1968**, *90*, 6527−6528.

(22) (a) Scurlock, R. D.; Ogilby, P. R. *J. Phys. Chem.* **1987**, *91*, 4599−4602. (b) Clough, R. L.; Dillon, M. P.; Iu, K. K.; Ogilby, P. R. *Macromolecules* **1989**, *22*, 3620−3628.

(23) Schuler, R. H.; Albarran, G. *Radiat. Phys. Chem.* **2002**, *64*, 189−195.

(24) Albarran, G.; Bentley, J.; Schuler, R. H. *J. Phys. Chem. A* **2003**, *107*, 7770−7774.

(25) (a) Gibbs, H. D. *J. Biol. Chem.* **1927**, *72*, 649−664. (b) Ettinger, M. B.; Ruchhoft, C. C. *Anal. Chem.* **1948**, *20*, 1191−1196.

(26) (a) Gibian, M. J.; Ungermann, T. *J. Am. Chem. Soc.* **1979**, *101*, 1291−1293. (b) Afanas'ev, I. B.; Kuprianova, N. S. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1361−1364.

(27) Nanni, E. J., Jr.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481−4485.

(28) Maran, F.; Celadon, D.; Severin, M. G.; Vianello, E. *J. Am. Chem. Soc.* **1991**, *113*, 9320−9329.

(29) (a) Rigo, A.; Sytevanato, R.; Finazzi, A., A.; Rotilio, G. *FEBS Lett.* **1977**, *80*, 130−132. (b) Melhuish, W. H.; Sutton, H. C. *J. Chem. Soc., Chem. Commun.* **1978**, 970−971.

JA052045B