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Singlet Oxygen Production in the Reaction of Superoxide with Organic Peroxides

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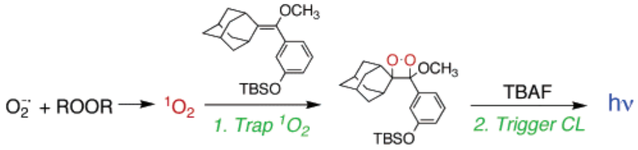
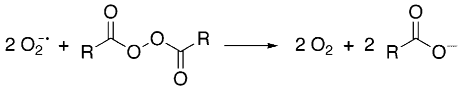


A selective chemiluminescent probe for singlet oxygen has been employed to detect and quantify singlet oxygen in the reactions of superoxide with organic peroxides. The production of singlet oxygen has been quantified in the reaction of superoxide with benzoyl peroxide (BP). No singlet oxygen was detected in the reactions of superoxide with cumyl peroxide, *tert*-butyl peroxide, or *tert*-butyl hydroperoxide. On the basis of these results and on the temperature dependence of the reaction, we proposed a mechanism for singlet oxygen formation in the reaction of superoxide with BP.

The reaction of acyl peroxides with superoxide ($O_2^{\bullet -}$) has been shown to generate $^1O_2$.[1−3] Interest in this reaction lies in its potential to be a source of $^1O_2$ in biological systems, where both $O_2^{\bullet -}$ [4−7] and peroxides[8] are formed. Singlet oxygen is a known cytotoxic species, oxidizing a variety of biological substrates, such as proteins,[9] amino acids,[10−12] and nucleic acids.[13−17] Danen and Arudi, using chemical $^1O_2$ traps, reported high yields of transformation products (ca. 100% combined yield) in the reactions of benzoyl and lauroyl peroxides with $O_2^{\bullet -}$, which were taken as evidence of efficient $^1O_2$ production, although they acknowledged the potential for false positives as a result of other oxidation pathways.[1] They also proposed a stoichiometry for the reaction, in which 2 equiv of $O_2^{\bullet -}$ react with an acyl peroxide, leading to 2 equiv of dioxygen (a fraction as $^1O_2$) and two carboxylate anions (Scheme 1).[1]

SCHEME 1



Khan obtained strong qualitative evidence of the production of $^1O_2$ in the reaction of benzoyl peroxide (BP) with $O_2^{\bullet -}$ from the direct detection of its characteristic 1270-nm phosphorescent signal.[2] We recently confirmed the production of $^1O_2$ in the $O_2^{\bullet -}$/BP system using a chemiluminescence-based detection method.[3] In this contribution, we quantify the yield of $^1O_2$ in this reaction and expand the scope of the study to include alkyl peroxides (*tert*-butyl and cumyl peroxides) and a hydroperoxide (*tert*-butyl hydroperoxide).

To quantify the production of $^1O_2$ in these reactions, we applied a recently developed method in which a stable dioxetane precursor is used to selectively detect $^1O_2$ in the presence of other reactive oxygen species (ROS). This detection method is based on thermally stable spiroadamantylidene-substituted dioxetanes, initially developed by Schaap et al.[18−20] and subsequently studied by others.[21−24] In this detection scheme, $^1O_2$ is trapped by a vinyl ether probe (**1**) in the form of a stable dioxetane (**2**) (Scheme 2). The trapped $^1O_2$ is quantified by the addition of a chemical trigger, tetra-*n*-butylammonium fluoride (TBAF), to induce a chemically initiated electron exchange luminescence (CIEEL) process by which dioxetane **2** decomposes to give a chemiluminescent (CL) signal.

We have reported the selectivity of this detection method for $^1O_2$ over $O_2^{\bullet -}$,[3] a problematic interferent in the chemiluminescence-based detection of $^1O_2$, and have also used this method

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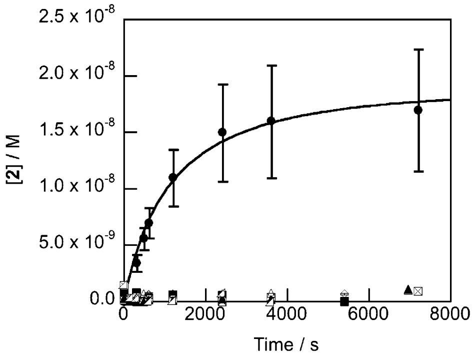
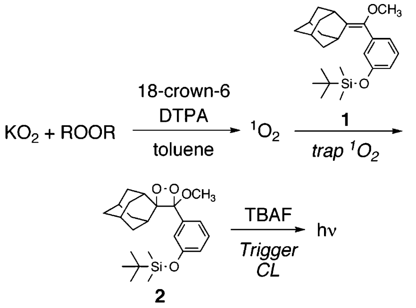


FIGURE 1. Formation of dioxetane **2** over time in the reaction of 2 mM superoxide with 0.71 mM benzoyl peroxide in toluene at 25 °C (circles). Error bars represent one standard deviation from the average of four measurements. Shown for comparison are the results of experiments in which benzoyl peroxide was replaced with 1, 5, and 10 mM cumyl peroxide (squares), 1, 5, and 10 mM *tert*-butyl peroxide (triangles), or 10 mM *tert*-butyl hydroperoxide (diamonds). Note that data at all concentrations are shown and are overlapping.

SCHEME 2



to quantify the production of $^1O_2$ in the reaction of $O_2^{\bullet-}$ with hydrogen peroxide.[25] Here we report that a significant yield of $^1O_2$ is only observed in the reaction of $O_2^{\bullet-}$ with BP and not with the alkyl peroxides or hydroperoxide tested. We also report the temperature-dependent reaction kinetics for the case of $O_2^{\bullet-}$ and BP.

Reaction of Organic Peroxides with Superoxide: General Observations. The reaction of $O_2^{\bullet-}$ (KO_2, 2 mM, solubilized with 18-crown-6 ether) with BP was carried out in toluene in the presence of **1** (100 μM) and diethylenetriaminepentaacetic acid (DTPA, 4 mM, added to bind trace metal impurities). Kinetic time points were obtained by sampling aliquots of the reaction mixture and quenching the reaction by dilution in acetonitrile. The formation of dioxetane **2** over time was monitored by measuring its CL signal, triggered by TBAF-induced desilylation (Figure 1).

The identity of $^1O_2$ as the ROS responsible for the formation of dioxetane **2** in this reaction was supported by the fact that neither $O_2^{\bullet-}$ nor BP alone elicited a signal from **1**. The $^1O_2$ yield was predictably enhanced in 85% deuterated toluene, in which the lifetime of $^1O_2$ is 10 times longer than in toluene, and completely depressed in the presence of 520 μM 1,4-diazabicyclo[2.2.2]octane (DABCO),[26] a known $^1O_2$ quencher.[3]

Two similar peroxides, cumyl peroxide (CP) and *tert*-butyl peroxide (TBP), were also investigated to explore the possibility of a general mechanism for the reaction of $O_2^{\bullet-}$ with organic peroxides. No evidence for the formation of $^1O_2$ in the reactions of $O_2^{\bullet-}$ with either CP or TBP was found (Figure 1), though the reactions were carried out under conditions ($O_2^{\bullet-}$ concentration, solvent, and temperature) similar to those in the BP reactions. Shown in Figure 1 are data from reactions in which either CP or TBP was present at 1, 5, and 10 mM. Similarly, no evidence of $^1O_2$ formation was found in the reaction of $O_2^{\bullet-}$ with *tert*-butyl hydroperoxide (TBHP) (Figure 1).

Benzoyl Peroxide: Kinetics and $^1O_2$ Yield. The formation of dioxetane **2** in the reaction of BP with $O_2^{\bullet-}$ in the presence of probe **1** was fit well by a second-order kinetic model incorporating the stoichiometry shown in Scheme 1 (eq 1).

$$[\mathbf{2}]_t = \alpha \times f \times \left([\mathrm{BP}]_0 - \frac{\Delta_0[\mathrm{BP}]_0}{[\mathrm{O}_2^{\bullet -}]_0 e^{\Delta_0 kt} - 2[\mathrm{BP}]_0}\right) \qquad (1)$$

Here $[\mathbf{2}]_t$ is the concentration of dioxetane **2** at time t, $\Delta_0 = [O_2^{\bullet-}]_0 - 2[\mathrm{BP}]_0$, α is the yield of $^1O_2$, and f is the fraction of the $^1O_2$ that is trapped by probe **1** (eq 2):

$$f = \frac{k_{\mathrm{rxn},1}[\mathbf{1}]}{k_{\mathrm{solv}} + k_{\mathrm{DTPA}}[\mathrm{DTPA}] + k_{\mathrm{rxn},1}[\mathbf{1}]} \qquad (2)$$

where $k_{\mathrm{rxn},1}$ is the rate constant for reaction of **1** with $^1O_2$, while k_{solv} and k_{DTPA} are the rate constants for quenching of $^1O_2$ by the solvent and DTPA, respectively.

The second-order kinetics observed in the case of the reaction of $O_2^{\bullet-}$ with BP enabled the estimation of the initial $O_2^{\bullet-}$ concentration, $[O_2^{\bullet-}]_0$, from kinetic data obtained in this study. Using global fitting software, we simultaneously fit several sets of data obtained under identical conditions to eq 1, with the value for $[O_2^{\bullet-}]_0$ shared among the data sets. Using this method, we obtained a value of 2.0 (± 0.2) mM for $[O_2^{\bullet-}]_0$. Because stock solutions of KO_2 in toluene were always prepared identically, this value for $[O_2^{\bullet-}]_0$ was used throughout the studies described here.

The yield of $^1O_2$ from the reaction of 0.71 mM BP with 2 mM $O_2^{\bullet-}$ was calculated from the kinetic data fit and the known $^1O_2$ trapping fraction, f. Given a rate constant for the reaction of probe **1** with $^1O_2$ (4.5×10^5 M^{-1} s^{-1}, measured in methylene chloride),[3] the rate constant for deactivation of $^1O_2$ by the solvent ($k_{\mathrm{solv,toluene}} = 3.4 \times 10^4$ s^{-1}),[27] the rate constant for quenching of $^1O_2$ by DTPA (1.0×10^7 M^{-1} s^{-1}),[3] and with 100 μM **1** and 4 mM DTPA, the quantity of $^1O_2$ produced that is trapped under these conditions is 0.06%. Despite the small trapping yield, the sensitivity of CL detection enables the use of this detection scheme. On the basis of the kinetic data fit and the known $^1O_2$ trapping fraction, the average yield of $^1O_2$ at 25 °C is 5 (± 1)%. The observed second-order rate constant for the reaction is 0.3 (± 0.2) M^{-1} s^{-1}.

The second-order kinetics in the reaction of BP with $O_2^{\bullet-}$ were confirmed by following the disappearance of BP in the presence of $O_2^{\bullet-}$ in toluene at 22 °C over 75 min (Figure 2). Over this time period, there was a 14% decrease in the BP concentration. Under these conditions, a bimolecular rate constant of 0.8 (± 0.2) M^{-1} s^{-1} was observed.

Benzoyl Peroxide: Temperature Dependence. To probe the mechanism of $^1O_2$ production, the temperature dependence of the reaction of $O_2^{\bullet-}$ with BP was examined. The reaction

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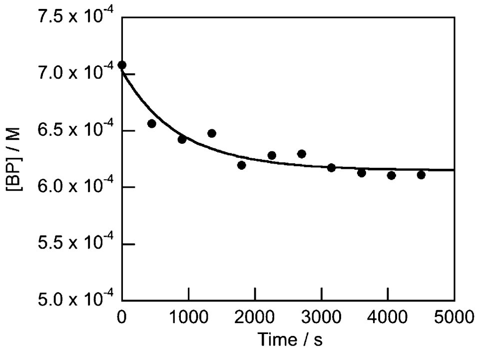


FIGURE 2. Disappearance of benzoyl peroxide over time in the reaction of 0.2 mM superoxide with 0.71 mM BP in toluene at 22 °C. The data are fit to a second-order exponential decay ($R^2 = 0.93$).

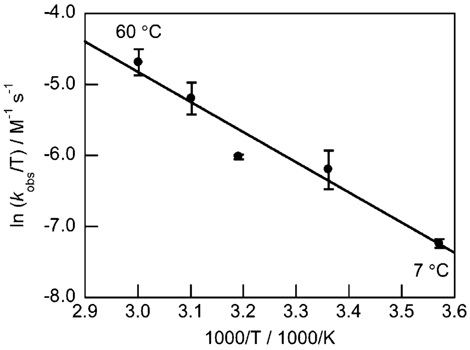
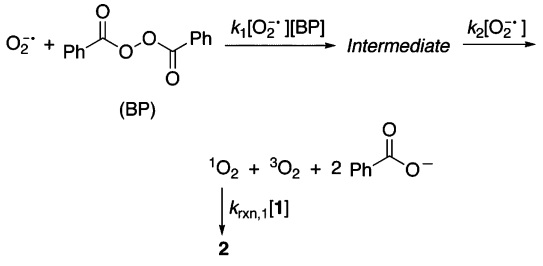


FIGURE 3. Temperature dependence of the observed second-order rate constant, k_{obs}, for the reaction of superoxide with benzoyl peroxide. The reaction rate constant is plotted as $\ln(k_{obs}/T)$ according to the Eyring equation. The equation corresponding to the best-fit line is $y = (-4.3 \pm 0.5)x + (8 \pm 2)$. Errors in the slope and intercept represent a 95% confidence level. The R^2 value is 0.9537. Error bars represent one standard deviation from the average of three measurements.

SCHEME 3

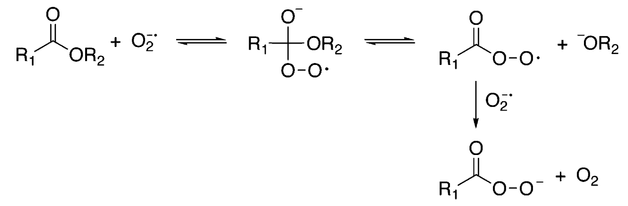


was carried out over a temperature range from 7 to 60 °C (280 to 333 K), and the observed second-order rate constant, k_{obs}, was determined at each temperature. This temperature dependence is illustrated in the form of an Eyring plot in Figure 3.

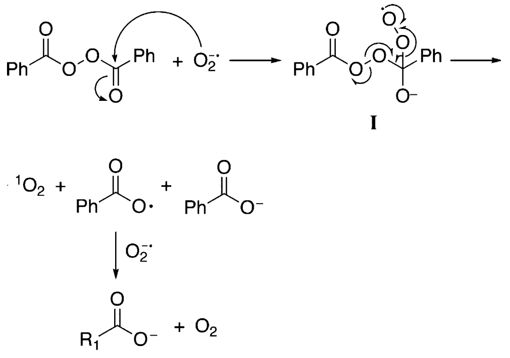
As illustrated, the reaction of $O_2^{\bullet-}$ with BP exhibits a normal temperature dependence with an increasing reaction rate at elevated temperatures. The following activation parameters were obtained from the temperature dependence data: $\Delta H^{\ddagger}$, 8.6 kcal mol^{-1} (36 kJ mol^{-1}); $\Delta S^{\ddagger}$, -31 cal mol^{-1} K^{-1} (-131 J mol^{-1} K^{-1}); activation energy, E_a, 9.1 kcal mol^{-1} (38 kJ mol^{-1}). At 25 °C (298 K), $\Delta G^{\ddagger}$ is 18 kcal mol^{-1} (75 kJ mol^{-1}).

Mechanistic Implications. The kinetic data obtained for the reaction of $O_2^{\bullet-}$ with BP are consistent with a two-step mechanism, in which 1 equiv of $O_2^{\bullet-}$ is involved in each step (Scheme 3). The rate of the final step of the reaction, trapping of $^1O_2$ by probe **1**, is known (45 s^{-1} for [**1**] = 100 μM).[3] The first step is assumed to be the rate-determining step because the data are fit well by a mixed second-order kinetic model incorporating first-order dependence on each of the individual reactants. If the first step were not the rate-determining step, kinetics indicative of a formal termolecular reaction should be observed. Thus, it is proposed that the calculated activation parameters correspond to the initial step of the reaction of $O_2^{\bullet-}$ with BP. The large negative $\Delta S^{\ddagger}$ value suggests a highly ordered transition state with respect to the ground state. A plausible mechanism may be found in the known cleavage of esters by nucleophilic attack of $O_2^{\bullet-}$ at the carbonyl carbon.[28-30] The reduction of an ester by $O_2^{\bullet-}$ is proposed to proceed through nucleophilic addition and elimination.[30] This initial step may then be followed by reduction of the peroxy radical by $O_2^{\bullet-}$ (Scheme 4). Thus, two superoxide ions are consumed in this mechanism, one as a nucleophile and one as a reductant.

SCHEME 4



SCHEME 5



If $O_2^{\bullet-}$ attacks one of the carbonyl carbons in BP in a similar manner, a peroxy intermediate (**I**) would be formed, which could decompose as shown in Scheme 5 to yield $^1O_2$, benzoate anion, and a benzoyloxy radical. Reduction of the benzoyloxy radical by $O_2^{\bullet-}$ would result in the proposed reaction products.[1]

A key feature of the proposed mechanism for $^1O_2$ formation is attack by $O_2^{\bullet-}$ at the carbonyl carbon of BP. The fact that CP, TBP, and TBHP lack carbonyl functionalities and that $^1O_2$ is not observed upon their exposure to $O_2^{\bullet-}$ supports this mechanistic hypothesis.

A remaining possibility for the reaction of an organic peroxide with $O_2^{\bullet-}$ is addition to one of the peroxide oxygen atoms, yielding Haber−Weiss-type products, RO^-, $RO^{\bullet}$, and O_2 (Scheme 6); however, our results suggest that this pathway, if it occurs, is not the source of $^1O_2$ in this reaction.

Because $^1O_2$ is not observed in the cases of CP or TBP, if such a reaction occurs, either it generates ground-state O_2 or

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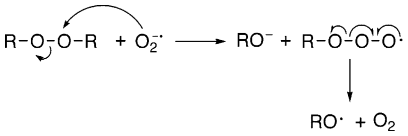
SCHEME 6



the yield of $^1O_2$ is too low to be trapped and observed by this detection method (<0.1%). Gibian and Ungermann also noted that TBP is apparently inert toward $O_2^{\bullet -}$.[31] Peters and Foote confirmed this result.[32] Thus, it appears that $^1O_2$ formation may be limited to the reaction of $O_2^{\bullet -}$ with acyl peroxides.

A trap-and-trigger detection method was used to quantify the production of $^1O_2$ in the reactions of $O_2^{\bullet -}$ with three organic peroxides and one hydroperoxide. Only in the case of BP was a significant yield of $^1O_2$ observed (5%). No $^1O_2$ was observed in the reactions of $O_2^{\bullet -}$ with alkyl peroxides/hydroperoxides (cumyl and *tert*-butyl), indicating the potential importance of the carbonyl functionality in the reaction. This observation, taken together with the observed bimolecular reaction kinetics, indicates that the rate-determining process may be the attack of $O_2^{\bullet -}$ at the BP carbonyl. The formation of $^1O_2$ in the reactions of $O_2^{\bullet -}$ with acyl peroxides with yields as high as 5%, as measured here, represents a potentially significant source of $^1O_2$ via nonphotochemical reactions in both biological and environmental systems.

Experimental Section

Materials. Potassium superoxide (KO_2), benzoyl peroxide (purum, moistened (25%) with water), cumyl peroxide (98%), *tert*-butyl peroxide (98%), *tert*-butyl hydroperoxide (anhydrous solution in decane (5.27 M) over 4 Å molecular sieves), 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6), 1,4-diazabicyclo-[2.2.2]-octane (DABCO), diethylenetriaminepentaacetic acid (DTPA), toluene-d_8 (99%), acetonitrile (CH_3CN), toluene, and tetrahydrofuran (THF, without stabilizer) were purchased from commercial suppliers and used as received. The synthesis and purification of vinyl ether probe **1** have been previously described.[3]

Software. OriginPro (v. 7.5) global fitting software was used to simultaneously fit several sets of data obtained in the reaction of $O_2^{\bullet -}$ with benzoyl peroxide to estimate the concentration of dissolved $O_2^{\bullet -}$ in saturated solutions of KO_2 in toluene.

Chemiluminescence Measurements. Chemiluminescence (CL) measurements were made as described previously.[3] For all assays, CL samples were prepared by 1:1000 dilution in CH_3CN. The luminometer sensitivity was set to 60.1%, the default setting. The CL signal from **2** was triggered by adding 20 μL of 10 mM TBAF, via syringe through a light-tight manual injection port, to 300 μL of the reaction solution in CH_3CN. The CL signals obtained were calibrated by comparison to external standards prepared from an independently synthesized sample of **2**.[3]

Reactions of Superoxide with Benzoyl Peroxide. All glassware was acid-washed, rinsed thoroughly with Milli-Q water, and dried in an oven (110 °C) prior to use. Saturated solutions of KO_2 containing 18-crown-6 (47 mM) and diethylenetriaminepentaacetic acid (DTPA, 4 mM) were prepared in toluene and 99% toluene-d_8. The solutions were mixed on a vortex mixer for 1 min, followed by centrifugation at 3300 rpm for 2 min to allow settling of any undissolved KO_2. Solutions of dissolved KO_2 were used immediately upon preparation. Stock solutions of BP (0.035 M) and DABCO (0.026 M) were prepared in toluene. A stock solution of **1** (430 μM) was prepared in a 50% toluene, 50% THF mixture. For each trial, the following were combined in an amber 2-mL autosampler vial containing a micro stirbar: 363 μL of KO_2 solution, 117 μL of stock solution of **1** (100 μM final concentration), and an aliquot of stock BP solution (0.71 mM final concentration). The total reaction volume was 500 μL. For all trials, the BP was added last and marked time zero for the reaction. Reaction vials were thermostated at 25 °C for the duration of the kinetic assay. Time points were obtained by sampling aliquots of the reaction mixture and quenching the reaction by 1:1000 dilution in CH_3CN. The CL signal was then measured for each time point as described above.

In the deuterated toluene experiment, the final composition of the reaction was 85% toluene-d_8. In the DABCO quenching experiment, the reaction contents were changed as follows: 363 μL of KO_2 solution (toluene), 117 μL of stock solution of **1** (100 μM final concentration), 10 μL of stock BP solution (0.71 mM final concentration), and 10 μL of stock DABCO solution (520 μM final concentration). In the trial without DABCO, the 10-μL aliquot of stock DABCO solution was replaced with 10 μL of toluene. The reaction of BP (0.71 mM) with KO_2 in toluene was also carried out at various temperatures, ranging from 7 to 60 °C, using a thermostated water bath. All other conditions were the same as the reactions at 25 °C.

In a separate experiment, the reaction of BP with KO_2 was carried out in a manner similar to that described above, and the disappearance of BP was monitored by high-performance liquid chromatography with UV absorbance detection (HPLC−UV). For this kinetics experiment, the following were combined in a 2-mL autosampler vial: 726 μL of KO_2 solution, 117 μL of THF, 137 μL of toluene, and 20 μL of stock BP solution (0.71 mM final concentration). The total reaction volume was 1000 μL. The BP was added last, and analysis by HPLC−UV commenced immediately after this addition. The HPLC analyses were performed on an 1100 series Hewlett-Packard liquid chromatograph equipped with a UV-absorbance detector. The chromatographic column used was a Supelco C18, 150 × 4.6 mm, 5-μm particle size column. Benzoyl peroxide was analyzed by an isocratic method with a mobile phase of 75:25 methanol/water, a flow rate of 1.0 mL/min, an injection volume of 10 μL, and a detection wavelength of 238 nm. Time points were obtained every 7.5 min. The reaction was carried out at 22 °C. Chromatographic peak areas were converted to BP concentration using an external calibration curve.

Reactions of Superoxide with Cumyl- and *tert*-Butyl Peroxides and *tert*-Butyl Hydroperoxide. All glassware was treated in the same manner as that for the BP reactions, and the KO_2 solutions were prepared identically. Stock solutions of CP, TBP, and TBHP were prepared in toluene. Reactions of CP (1, 5, and 10 mM), TBP (1, 5, and 10 mM), and TBHP (10 mM) with KO_2 were each carried out in a manner similar to that for the BP reactions. Time points were obtained and analyzed as in the BP reactions. All reactions were carried out at 25 °C.

Determination of the Dissolved $O_2^{\bullet -}$ Concentration. The concentration of dissolved $O_2^{\bullet -}$ in the saturated solutions of KO_2 (solubilized with 18-crown-6) in toluene prepared for this study was estimated from kinetic data obtained for the reaction of $O_2^{\bullet -}$ with BP using OriginPro global fitting software. Several sets of data obtained under identical conditions were simultaneously fit to eq 1, with the value for the initial $O_2^{\bullet -}$ concentration, $[O_2^{\bullet -}]_0$, shared among the data sets. The value for $[BP]_0$ was input, and all other variables were solved by OriginPro. Using this method, we obtained a value of 2.0 (± 0.2) mM for $[O_2^{\bullet -}]_0$.

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