Synergism between Airborne Singlet Oxygen and a Trisubstituted Olefin Sulfonate for the Inactivation of Bacteria

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Supporting Information

ABSTRACT: The reactivity of a trisubstituted alkene surfactant (8-methylene-7-ene-1 sulfonate, 1) to airborne singlet oxygen in a solution containing E. coli was examined. Surfactant 1 was prepared by a Strecker-type reaction of 9-bromo-2-methylene-2-ene with sodium sulfitel. Submicellar concentrations of 1 were used that reacted with singlet oxygen by an “ene” reaction to yield two hydroperoxides (7-hydroperoxy-8-methylene-8-ene-1 sulfonate and (E)-8-hydroperoxy-8-methylene-6-ene-1 sulfonate) in a 4:1 ratio. Replacing the H2O solution for D2O where the lifetime of solution-phase singlet oxygen increases by 20-fold led to an ~2-fold increase in the yield of hydroperoxides pointing to surface activity of singlet oxygen with the surfactant in a partially solvated state. In this airborne singlet oxygen reaction, E. coli inactivation was monitored in the presence and absence of 1 and by a LIVE/DEAD cell permeabilization assay. It was shown that the surfactant has low dark toxicity with respect to the bacteria, but in the presence of airborne singlet oxygen, it produces a synergistic enhancement of the bacterial inactivation. How the ene-derived surfactant hydroperoxides can provoke 1O2 toxicity and be of general utility is discussed.

INTRODUCTION

Although singlet oxygen [1O2 (1Δg)] is an effective toxin for inactivating bacteria,1,2 methods to generate it suffer from photosensitizer problems including solubilization,3–5 degradation, and bleaching.6 Turbid solutions7,8 can also present problem because light can be blocked from reaching the sensitizer. Because of these issues, there is a need to develop methods for killing bacteria without the physical contact of photosensitizer with the solution. Airborne 1O2 offers some promise in this regard.9–15

Figure 1 shows the three-phase apparatus that we used in this study for the delivery of 1O2 to the air/water interface of a bacterial solution. By virtue of how the apparatus works, the solution is devoid of any photosensitizers, where gas-phase singlet oxygen diffuses to the solution surface. By analogy, Majima et al.9,10 carried out experiments using a sensitizing TiO2 surface and a terrylenediimide oxygen acceptor adsorbed on another surface that was separated by 1 mm, indicating the formation of a diffusible 1O2 species (similar to the Kautsky three-phase test of 80 years ago).14,15

That the apparatus in Figure 1 leads to 1O2 at the air/water interface for E. coli inactivation is not surprising because its design is similar to that of an apparatus invented by Midden.13 What is new and better (we regard our innovation as an offshoot of the Midden and Majima systems) is the unique function of surfactant 1 in E. coli inactivation by airborne 1O2.

Our hypothesis was that a 1O2-active surfactant (1) would synergistically enhance bacterial inactivation. Synergy has been found in other branches of singlet oxygen research. It has been found in the photodynamic inactivation of bacteria with biofilm dispersions of a 2-aminoimidazole-triazole conjugate,16 in photodynamic therapy (PDT) with drug additives such as carboplatin,17 and with the simultaneous reaction of nitric oxide18 or SO3•−,19 among other 1O2 topics. Similar to surfactant 1, there was a report on a 2,5-disubstituted furan surfactant with a cationic tetraalkylammonium headgroup that was oxidized by 1O2 to an endoperoxide in a liposome study,20 but the reaction was not examined for antibacterial activity.

Here we show that an 1O2-active surfactant can synergistically enhance microbe inactivation from airborne 1O2 through hydroperoxide formation. Our work serves as a starting point where in-situ-generated surfactant hydroperoxides function as secondary toxins to pure 1O2 for enhanced bactericidal action. Following the Experimental Section, our results will be presented in four parts: first, the rationale for the selection of surfactant 1; second, measured surfactant photoperoxide formation via airborne 1O2; third, measured E. coli killing by 1O2 with and without surfactant 1; and fourth, measured E. coli killing by 1O2, followed by the addition of hydroperoxides 2 and 3 in the dark.

EXPERIMENTAL SECTION

Reagents and Instrumentation. Porous Vycor glass (Corning 7930) was purchased from Advanced Glass and Ceramics (Holden, MA) and stored at room temperature. Sodium sulfate (99.9% pure) was purchased from Sigma-Aldrich and used as received. 9-Bromo-2-methylene-2-ene was prepared by a Strecker-type reaction of 9-bromoacetone and acrylamide in the presence of NaOH to produce a 70% yield of the ene derivative. 8-Methylene-7-ene-1 sulfonate was prepared by a Strecker-type reaction of 9-bromo-2-methylene-2-ene and sodium sulfite to produce a 65% yield of the ene derivative. 8-Hydroperoxy-8-methylene-6-ene-1 sulfonate and (E)-8-hydroperoxy-8-methylene-6-ene-1 sulfonate were prepared by the reaction of 8-methylene-7-ene-1 sulfonate and singlet oxygen generated from a gas-phase photosensitizer in aqueous solution.REFEREE COPY

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Silicon phthalocyanine dichloride, aluminum(III) phthalocyanine chloride tetrasulfonic acid, 9-bromo-2-methylnon-2-ene, sodium sulfate, triphenyl phosphine (PPh₃), benzoic acid, dimethylsulfone, DMF, CH₂Cl₂, ethanol, D₂O, and DMSO-d₆ were purchased from commercial suppliers and were used as received. Dichloromethane was distilled over phosphorus pentoxide prior to use. Deionized water was purified with a U.S. Filter Corporation deionization system (Vineland, NJ). Nuclear magnetic resonance (NMR) data were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for ¹H NMR and at 100.6 MHz for ¹³C NMR. UV−vis data were collected on a Hitachi UV−vis U-2001 instrument. FAB-mass spectrometry data were collected on a JEOL JMS-HX110 spectrometer using a m-nitrobenzyl alcohol matrix, a 10 kV acceleration voltage, and a Xe beam FAB gun (6 kV) on the MS-1 ion source. Infrared spectra were recorded on a Nicolet iS10 FT-IR spectrometer. Solution temperatures were measured with a digital pyrometer (Thermo Scientific). An Olympus Fluoview FV10i confocal fluorescence microscope was used to analyze stained E. coli and assess membrane permeability following singlet oxygen exposure.

**Sensitizing Glass Plate.** Using a Pasteur pipet, 50 μL of methanol containing 8 × 10⁻⁴ M aluminum(III) phthalocyanine chloride tetrasulfonic acid (Pc) was deposited onto one side of PVG (disk shape 14.0 mm × 1.0 mm or square shape 2.25 cm² and 1.0−1.5 mm). Most of the methanol had evaporated after 12−24 h at 26 °C, at which point the sample was used. The result was PVG sensitizing glass loaded on one side with 1.1 × 10⁻⁵ mols of Pc/g of PVG with the penetration of the sensitizer into the glass core and edges.

**Apparatus.** A three-phase apparatus was constructed for airborne ¹O₂ delivery to the air/water interface of a solution (Figures 2). The sensitizing glass plate was placed sensitizer face down, above a short quartz cuvette (1.0 × 1.0 × 0.7 cm³) containing 0.60 mL of water (from a micropipet, precision ±0.005 mL) and illuminated perpendicularly from a 3.0 cm distance with 669 nm light (383 mW) from a diode laser (model 7404, Intense, North Brunswick, NJ). The light from the laser overlapped well with the Pc absorption. The 669 nm light was passed through an FT-400-EMT optical fiber (Thorlabs, Newton NJ), which produced a Gaussian distribution of incident photons on the sensitizing glass plate (total dose ≈ 1700 J/cm²). The diameter of the laser spot on the sensitizing glass plate was 0.95 cm (area = 0.71 cm²). The sensitizing glass plate was not in contact with the water. The sensitizing glass plate sat atop the short cuvette above the water interface by 0.4 mm situated at the sides of the cuvette. Moving laterally from the cuvette side to the midpoint of the meniscus, the distance between the sensitizing plate and water was 1.5 mm. These distances were measured with a miniature ruler and a 10X magnifying glass with an uncertainty of ±0.04 mm. Water evaporation was negligible and did not measurably change the volume over the

![Figure 1](image1.png)

Figure 1. (a) Red 669 nm light is directed in from above to a glass plate whose bottom side is coated with aluminum(III) phthalocyanine chloride tetrasulfonic acid (Pc). (b) O₂ is sensitized by excited Pc sites on the plate where ’¹O₂ traverses a ~0.4−1.5 mm distance to reach the E. coli solution of 0.1 mM surfactant 1, where (c) hydroperoxides 2 and 3 are produced.

![Figure 2](image2.png)

Figure 2. Apparatus for generating airborne ’¹O₂ where it travels a short distance to a solution containing surfactant 1 and E. coli. (a) A sensitizer glass plate covers but does not contact the water solution in the quartz cuvette. (b) Red 669 nm light is directed in from above via an optical fiber connected to a diode laser. A piece of white paper was placed in front of the beam and moved downward to capture the approximate path of the beam contacting the sensitizer plate (Nikon digital camera settings: ISO 100, F20, and 1/50 s flash burst, 3 s total exposure).
course of a 1 h experiment. The water temperature was increased by 3.5 ± 0.3 °C in 1 h, which slightly reduces the lifetime of singlet oxygen (by ~10 ns). An analysis of the water samples after photolysis indicated that no Pc molecules had dissolved from the sensitizing glass nor had any relocated from the glass to the water.

Synthesis of Sodium 8-Methylnon-7-ene-1-sulfonate (1). Yield 38 mg (70%), purity >98%. A Streeker reaction between 2-bromo-2-methylnon-2-ene (0.05g, 0.22 mmol) and Na₂SO₃ (0.057g, 0.45 mmol) took place in 4 mL of refluxing DMSO-water (1:1) under a nitrogen atmosphere in 12 h. After the mixture was cooled to room temperature, 2 mL of deionized H₂O and 2 mL of ethanol were added in succession. The filtrate was partitioned with CH₂Cl₂ (4 × 4 mL), and the CH₂Cl₂ fraction was discarded. The aqueous fraction was evaporated to dryness, leaving an off-white solid product that was recrystallized in ethanol-water (8:2). ¹H NMR (D₂O, 400 MHz): δ 5.1 (t, J = 14.8 Hz, 1H), 2.8 (s, J = 16 Hz, 2H), 1.93 (m, 2H, 1.68 (m, 2H), 1.64 (s, 1H), 1.56 (s, 1H), 1.36 (m, 2H), 1.29 (m, 4H). ¹³C NMR (D₂O, 100.6 MHz): δ 133.2, 125.2, 51.1, 28.8, 27.9, 27.6, 27.1, 24.8, 24.0, 16.9. IR (neat) ν 2967, 2916, 2851, 1465, 1453, 1155 cm⁻¹. HRMS (FAB) m/z calcd for [C₁₀H₁₉O₃SNa₂]⁺, 265.0854; found for [C₁₀H₁₉O₃SNa₂]⁺, 265.0854. The solubility of surfactant 1 was 210 ± 20 g/L in deionized H₂O, and the critical micellar concentration (cmc) was 9.7 mM on the basis of an NMR titration method similar to that of Wang and Feng.³⁹ (Figure S9, Supporting Information).

Generation of Sodium 7-Hydroperoxy-8-methylnon-8-ene-1-sulfonate (2) and Sodium (É)-8-Hydroperoxy-8-methylnon-6-ene-1-sulfonate (3). Yield 1.4 mg (85%) as a 4:1 mixture of 2/3. After the reaction of 1 with O₂ in aerated water was removed under a stream of dry N₂. The residue was partitioned with chloroform (10 × 1 mL). The ratio of 2 and 3 was determined by ¹H NMR analysis of the 4.8 and 5.5 ppm protons and comparison with benzoic acid as an internal standard in DMSO-d₆. Hydroperoxides 2 and 3 were stable enough for characterization as a mixture, but they began to decompose after 1 to 2 days in D₂O. ¹H NMR (D₂O, 400 MHz): δ 11.2 (s, 1H), 10.8 (s, 1H), 5.5 (m, 2H), 4.8 (s, 2H), 4.1 (t, J = 14 Hz, 1H). ¹³C NMR (D₂O-d₆, 100.6 MHz): δ 145.1, 135.2, 129.6, 113.3, 88.2, 80.8, 52.0, 32.3, 30.7, 29.4, 28.8, 25.5, 17.2. Control reactions showed that 669 nm irradiation of a piece of native PVC that had no Pc coating did not yield 2 and 3. The addition of PPh₃ to the postreaction mixture led to corresponding allylic alcohols that were also detected in the absence of oxygen. We monitored the disappearance of 1 and the appearance of surfactant peroxides 2 and 3 (mass balance 91%), where 2 and 3 were stable enough for characterization as a mixture but began to decompose after 1 to 2 days at 26 °C.

Figure 3 shows that the airborne O₂ oxidation of 1 led to hydroperoxides (2 and 3) at double the efficiency in D₂O as in H₂O (the yield was 18% in H₂O and 33% in D₂O). Because O₂ is not transferred deep into bulk water, it is not subject to the 20-fold Δ increase in D₂O (69 µs at 20 °C) as in H₂O (3.5 µs at 20 °C).²³,³⁶ Air moisture was found to introduce a small (~0.2%) amount of H₂O into the D₂O solution during the 1 h reaction period on the basis of the NMR integration of the HOD signal, but this was not an explanation of the modest product increase in D₂O. In D₂O, airborne O₂ reactions carried out with 1 above its cmc led a 2% yield of hydroperoxides 2 and 3 (far less than the 33% yield of a surfactant that can readily form a hydroperoxide product. Thus, we selected terminally branched-chain olefin sulfonate 1 with an eye toward the ease of formation of allylic hydroperoxides. Trisubstituted olefins²⁵⁻²⁷ are much more reactive with O₃ (~20–500-fold) than are di- and mono-substituted olefins.²⁸ For example, the chemical quenching rate constant (kₚ) of O₃ with 2-methyl-2-pentene is reasonably high (6 × 10⁻³ M⁻¹ s⁻¹).²⁹

In the case of the detergent concentration, we selected a relatively low 1 mM concentration of 1 so the hydrophobic group would preferably point away from the surface. Our results show that the cmc of 1 (C₁₀H₁₉O₃SNa⁺) (9.7 mM at 26 °C) is lower than that of straight-chain C₁₀H₂₀SO₃Na⁺ (43 mM at 25 °C)³⁰ but similar to that of straight-chain C₁₂H₂₅SO₃Na⁺ (9.8 mM or 12 mM at 25 °C).³⁰ The cmc for branched hydrophobic groups, this mainly applies to internal rather than terminal unsaturated sites of olefin sulfonates. By running experiments below the micellar concentrations of 1, the surfactant tends not to aggregate into environments away from the air/water interface.

Airborne Singlet Oxygen Attack on a Partially Solvated Surfactant. The apparatus brought airborne O₂ in from above onto the H₂O or D₂O solution for an ene reaction³³,³⁴ with 1 mM detergent 1. The two hydroperoxides that were formed (2 and 3) have a shift of the double bond relative to that of 1, which is a fingerprint reaction for singlet oxygen. We monitored the disappearance of 1 and the appearance of surfactant peroxides 2 and 3 (mass balance 91%), where 2 and 3 were stable enough for characterization as a mixture but began to decompose after 1 to 2 days at 26 °C.

Figure 3 shows that the airborne O₂ oxidation of 1 led to hydroperoxides (2 and 3) at double the efficiency in
with 1 below its cmc) likely as a result of the micellar protection of the alkene site from incoming airborne \(^{1}\text{O}_2\) at the air/water interface. Below the cmc, the results point to surface activity where airborne singlet oxygen attacks 1 in a partially solvated state.

Partially solvated 1 may relate to the observed stereo-selectivity of hydroperoxides 2 and 3 because the ratio was 4:1 (Table 1). Hydrogen abstraction proceeds mostly from the secondary hydroperoxide in polar solvents.\(^{29}\) Some control of steric, the ene reaction of \(^{1}\text{O}_2\) with trisubstituted alkenes, such as 2-methyl-2-pentene, usually yields the secondary and tertiary hydroperoxides in an \(\sim 1:1\) ratio with a slight favoring of the secondary hydroperoxide in polar solvents.\(^{35,36}\) Some control of hydroperoxide product selectivity has been found for photo-oxidations if the alkene is contained in Nafton\(^{40}\) and zeolites.\(^{41,42}\) Secondary and tertiary hydroperoxides have been seen to decompose at different rates when encapsulated within zeolites,\(^{43,44}\) but the obvious explanation that one of the hydroperoxides decomposes more rapidly prior to quantitation is not the case for 2 and 3.

We tentatively attribute the 4:1 ratio of 2/3 to \(^{1}\text{O}_2\) coming top down on the interface, where the methyl protons are surface “exposed” and more easily rotated than the methylene protons, with the latter being more wetted or anchored at the solution/air interface. In a transition-state model drawing (Scheme 2), we propose that the distal oxygen of the perepoxide transition structure preferably abstracts a methyl hydrogen prior to surface H bonding. Facile rotation\(^{45,46}\) may be key, where the methylene allylic hydrogens of the hexyl sulfonate chain are more restricted to rotation and thus less conformationally accessible (higher barrier to rotation) than the methyl groups. We do not think that electronic repulsion\(^{47}\) takes place between the distal perepoxide oxygen and the sulfonate anion to explain methyl rather than methylene H-abstraction regioselectivity.

We now turn our attention to the bacterial killing results.

**Top-Down Approach to Bacterial Killing with Airborne \(^{1}\text{O}_2\) and Surfactant 1.** Here, we make a case that detergent 1 synergistically enhances the bactericidal action of incoming \(^{1}\text{O}_2\). Table 2 shows that the apparatus produces airborne \(^{1}\text{O}_2\) at levels toxic to bacteria (entries 1–3). Samples containing 50, 30, and 15 \(\mu\text{g/mL}\) \(E.\text{coli}\) were inactivated by 25, 38, and 41%, respectively, after 1 h. Table 3 (entry 5) shows that the inactivation of 50 \(\mu\text{g/mL}\) \(E.\text{coli}\) when followed in 10 min increments led to 27% killing after 1 h.

However, synergistic \(E.\text{coli}\) inactivation was seen when combining airborne \(^{1}\text{O}_2\) and surfactant 1 (Table 2, entries 4–6). That is, the number of \(E.\text{coli}\) killed increased by 1.7- to 2-fold compared to \(^{1}\text{O}_2\) treatment without 1. The inactivation by 1 was 2.6% (entry 7) and by airborne \(^{1}\text{O}_2\), was 25% (entry 1), which adds up to 27.6%, not the 50% seen with airborne \(^{1}\text{O}_2\) in the presence of surfactant 1 (entry 4). The synergism was not restricted to the 50 \(\mu\text{g/mL}\) \(E.\text{coli}\) concentration but was also seen at 30 and 15 \(\mu\text{g/mL}\).

Table 2 shows that the surfactant 1 toxicity in the dark is low. For example, for 50 \(\mu\text{g/mL}\) \(E.\text{coli}\), 2.6% was killed by 1 \(\mu\text{M}\) 1, and for 15 \(\mu\text{g/mL}\) \(E.\text{coli}\), 7.3% was killed (entries 7–9). The addition of a 4:1 mixture of 2 (0.144 mM) and 3 (0.036 mM) (similar to the amount generated in situ in Figure 3) in the dark was also relatively nontoxic, and the mixture led to 5–8% \(E.\text{coli}\) inactivation (Table 2, entries 10–12). Entry 13 shows a control reaction of the \(E.\text{coli}\) viability of 1.5% in the dark without surfactant 1 or hydroperoxides 2 and 3. The red light emitted from the device was also mostly nontoxic to \(E.\text{coli}\), and the inactivation ranged from 3.7 to 8% for \(E.\text{coli}\) concentrations of 50 to 15 \(\mu\text{g/mL}\) (Table 2, entries 14–16). These observations point to low levels of 3–8% \(E.\text{coli}\) inactivation based on additives 1–3 in the dark or in red light alone. Next, we explored the effects of incubating hydroperoxides 2 and 3 with \(^{1}\text{O}_2\)-pretreated cells.

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**Table 1. Reaction of Methyl Noneone Sulfonate 1 with Airborne \(^{1}\text{O}_2\) at or near the Air–Water Interface**\(^{4}\)

| entry | medium of \(^{1}\text{O}_2\) solution | % conversion after 1 h | product ratio 2/3 |
|-------|----------------------------------|-----------------------|-------------------|
| 1     | airborne \(\text{H}_2\text{O}\)    | 18 ± 2                | 75:25 (±3)        |
| 2     | airborne \(\text{D}_2\text{O}\)    | 33 ± 3                | 79:21 (±2)        |

\(^{a}\)Samples were illuminated at 669 nm. Airborne \(^{1}\text{O}_2\) is generated and crosses an intervening gap to the \(\text{H}_2\text{O}\) or \(\text{D}_2\text{O}\) solution of 1 (1.0 mM).

\(^{b}\)Ratio of product calculated from the integration of the \(^1\text{H}\) NMR 4.8 and 5.5 ppm signals. Error bounds were obtained from three measurements.

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**Scheme 1**

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**Scheme 2**
Table 2. *E. coli* Inactivation by the Airborne Singlet Oxygen Treatment as a Function of Additives and Other Conditions

| Entry | Condition | *E. coli* (μg/mL) | Surfactant 1 added (mM) | 4:1 mixture of hydroperoxides 2 and 3 added (mM) | % Killed after 1 h | Number of Cells Killed |
|-------|-----------|-------------------|-------------------------|-----------------------------------------------|-----------------|------------------------|
| 1     | Airborne 1^O_2 | 50                |                        |                                               | 25 ± 5          | 7.5 × 10^6             |
| 2     | 30         |                   |                        |                                               | 38 ± 5          | 6.8 × 10^6             |
| 3     | 15         |                   |                        |                                               | 41 ± 4          | 3.7 × 10^6             |
| 4     | Airborne 1^O_2 | 50                | 1.0                    |                                               | 50 ± 6          | 1.5 × 10^7             |
| 5     | 30         |                   | 1.0                    |                                               | 71 ± 3          | 1.3 × 10^7             |
| 6     | 15         |                   | 1.0                    |                                               | 70 ± 3          | 6.3 × 10^6             |
| 7     | Dark       | 50                | 1.0                    |                                               | 2.6 ± 0.5       | 5.2 × 10^4             |
| 8     | 30         |                   | 1.0                    |                                               | 6.3 ± 1.1       | 1.2 × 10^5             |
| 9     | 15         |                   | 1.0                    |                                               | 7.3 ± 2.0       | 1.4 × 10^5             |
| 10    | Dark       | 50                |                        | 0.2                                           | 5 ± 1           | 1.0 × 10^5             |
| 11    | 30         |                   |                        | 0.2                                           | 7 ± 3           | 1.4 × 10^5             |
| 12    | 15         |                   |                        | 0.2                                           | 8 ± 3           | 1.6 × 10^5             |
| 13    | Dark       | 50                |                        |                                               | 1.5 ± 0.5       | 3.0 × 10^5             |
| 14    | 669 nm light (no 1^O_2) | 50            |                        |                                               | 3.7 ± 0.5       | 7.4 × 10^4             |
| 15    | 30         |                   |                        |                                               | 6.3 ± 0.6       | 1.2 × 10^5             |
| 16    | 15         |                   |                        |                                               | 8 ± 2           | 1.6 × 10^5             |

*Airborne 1^O_2 is generated and crosses an intervening gap to the H_2O solution. *b*Error bounds were obtained from three or more measurements.

Table 3. Percent of *E. coli* Killed after Treatment with Airborne 1^O_2 in the Presence and Absence of Hydroperoxides 2 and 3

| Entry | Irradiation time (min) | % E. coli killed by airborne 1^O_2 | Surfactant 1 (mM) | 4:1 mixture of hydroperoxides 2 and 3 (mM) | % E. coli killed |
|-------|------------------------|-----------------------------------|-------------------|---------------------------------------------|----------------|
| 1     | 10                     | 10 ± 2                            | 0.01              | 15 ± 2                                      |                |
| 2     | 20                     | 16 ± 3                            | 0.03              | 27 ± 3                                      |                |
| 3     | 30                     | 21 ± 2                            | 0.08              | 30 ± 3                                      |                |
| 4     | 45                     | 26 ± 3                            | 0.12              | 42 ± 2                                      |                |
| 5     | 60                     | 27 ± 5                            | 0.15              | 46 ± 3                                      |                |
| 6     | 60                     | 28 ± 3                            | 1.0               | 27 ± 4                                      |                |

*Airborne 1^O_2 is generated and crosses an intervening gap to the H_2O solution. *b*Error bounds were obtained from three measurements. *E. coli* cells were treated with airborne 1^O_2 for 1 h. Hydroperoxides 2 and 3 in a 4:1 ratio were added to the cells in the dark for 2 min. *E. coli* cells were treated with airborne 1^O_2 for 1 h. Surfactant 1 was then added to the cells in the dark for 2 min.

**Effect of Added Hydroperoxides.** The above data suggest that airborne 1^O_2 with surfactant 1 enhanced singlet oxygen toxicity by an increase in oxidative stress (e.g., partial loss of cell membrane integrity). Evidence supporting this idea is shown in Table 3. Airborne 1^O_2 exposure was followed with the postreaction addition of a 4:1 mixture of 2 and 3 in the dark (entries 1–5). Entries 1–5 ranged from 0.01 to 0.15 mM to mimic the hydroperoxide concentrations that form in situ for the reaction of 1 with airborne 1^O_2 in H_2O in Figure 3.

Airborne 1^O_2 treatment for 1 h followed by the addition of 0.15 mM hydroperoxides 2 and 3 in the dark produced a similar inactivation of 50 μg/mL *E. coli* (46%, Table 3, entry 5) compared to that of airborne 1^O_2 with surfactant 1 (50%, Table 2, entry 4). The measured inactivation by hydroperoxides 2 and 3 was 5%, and by airborne 1^O_2 it was 25%, whereas the amount from the combination of airborne 1^O_2 and surfactant 1 was 50%, fully 20% greater inactivation. Pre-exposure to airborne 1^O_2 with the postreaction addition of 1 in the dark did not enhance the *E. coli* inactivation (Table 3, entry 6). We believe that this enhanced inactivation is relevant to synergy, where 1^O_2-predamaged cells in the presence of 2 and 3 provoke cell killing. Thus, we sought to gain insight into whether membrane damage was significant in 1^O_2-treated cells.

We find evidence for cell permeabilization after 1^O_2 treatment in the presence or absence of 1 based on fluorescent labeling with a commercially available LIVE/DEAD BacLight bacterial viability kit (Figure S17, Supporting Information). With SYTO-9 and propidium iodide stains added to 50 μg/mL *E. coli* samples after treatment and centrifugation, the propidium iodide staining of cells indicated damaged membranes. Consequently, we propose that airborne 1^O_2 causes permeabilization but that some cells can recover. However, the presence of hydroperoxides 2 and 3 may impede such a recovery by further destabilizing the cell. In a similar vein, Redmond et al. attributes signaling and bystander effects to diffusing species such as H_2O_2 for the killing of neighboring cells adjacent to those photodynamically damaged.

The results of this work show a heightened *E. coli* sensitivity to hydroperoxides produced in situ or added after airborne 1^O_2 treatment. We know that 1^O_2 exposure in the presence or absence of 1 leads to compromised cell membranes. We do not know the relative toxicities of 2 and 3, for example, whether one hydroperoxide will cause greater membrane damage after the initial 1^O_2 reaction. Our work also does not resolve whether 1 interacts with the cell membrane of the bacterium by adsorption or intercalation of its hydrophobic chain, but we believe that such sorption processes play a minor role as a result of the submicellar requirement mentioned earlier for hydroperoxide 2 and 3 formation. Our interest in a relatively low 1 mM detergent 1 concentration was to potentially aim the hydrophobic group toward the surface, rather than aggregated it into a micelle away from the air/water interface. It turns out that reactive species preceding hydroperoxide formation are not likely to contribute to the toxicity because intermediates in the 1^O_2 ene reaction are usually not thought to form. In the absence of *E. coli*, we find no NMR evidence for facile hydroperoxide self-degradation, such as through hydroperoxide pair Russell reactions, although we have not scrutinized
hydroperoxide samples after 2 days when decomposition takes place.

In summary, E. coli oxidation was carried out with airborne $^{1}$O$_{2}$ and with the addition of I or hydroperoxides before and after $^{1}$O$_{2}$ exposure to examine the mechanistic aspects. A Majima–Menden-like apparatus, as used here, exposes $^{1}$O$_{2}$ to bacteria free from the effects of sensitizer pigmentation, bleaching, and degradation. Here, the sensitizer glass plate was physically isolated from water as a means to inactivate bacteria. Offering an innovative feature, the combination of airborne $^{1}$O$_{2}$ with an oxidizable surfactant is promising. A $\sim$2-fold $^{1}$O$_{2}$ toxicity enhancement was found in the presence of surfactant I.

### CONCLUSIONS

The arrival of airborne $^{1}$O$_{2}$ to a water interface was used instead of its generation by a solvated photosensitizer. The apparatus has the advantage of being a source of gaseous singlet oxygen, of its generation by a solvated photosensitizer. The apparatus for the graphic arts work.

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