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OH-Radical Specific Addition to Glutathione S-Atom at the Air–Water Interface: Relevance to the Redox Balance of the Lung Epithelial Lining Fluid

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Supporting Information

ABSTRACT: Antioxidants in epithelial lining fluids (ELF) prevent inhaled air pollutants from reaching lung tissue. This process, however, may upset ELF’s redox balance, which is deemed to be expressed by the ratio of the major antioxidant glutathione (GSH) to its putative oxidation product GSSG. Previously, we found that at physiological pH O3(g) rapidly oxidizes GS−(aq) (but not GSH−) to GSO− rather than GSSG. Here, we report that in moderately acidic pH ≤ 5 media ·OH oxidizes GSH−(aq) to sulfenic GSOH−, sulfenic GSO2−, and sulfonic GSO3− acids via ·OH specific additions to reduced S-atoms. The remarkable specificity of ·OH on water versus its lack of selectivity in bulk water implicates an unprecedented steering process during [OH•--GSH−] interfacial encounters. Thus, both O3 and ·OH oxidize GSH to GSOH− under most conditions, and since GSOH− is reduced back to GSH in vivo by NADPH, redox balance may be in fact signaled by GSH/GSOH ratios.

How to cope with the detrimental effects of air pollution on human health and quality of life in ever-expanding megacities is a pressing, complex issue.1–4 Decades after the implementation of environmental controls, O3, and PM2.5 (≤2.5 μm diameter particulate matter) concentrations significantly exceed standard limits in cities worldwide.5–8 Numerous studies have shown that premature mortality9,10 and all-cause (AC)2,2 but particularly cardiorespiratory11 acute and chronic health effects display statistically significant positive correlations with ambient O3, and PM2.5 concentrations.4,10,12,13 A rigorous statistical analysis of air pollution data and age-specific cardiovascular (CV) and AC premature mortality rates for 483 counties in 15 US states from 2000 to 2010 confirmed positive associations between PM2.5 and O3 and between the levels of both pollutants with CV and AC mortality rates. However, it revealed that the ∼30% decrease of PM2.5 and O3 levels in that period did not translate into statistically significant changes in premature mortality rates.14 It is apparent that socially optimal levels of control, those that balance marginal benefits versus the marginal costs of abatement, cannot be designed solely on the basis of epidemiological data but will require information on the chemical and biological mechanisms of the adverse health effects induced by specific air pollutants.15–18

Inhaled pollutants (O3, NO2, and PM2.5) are prevented from coming in contact with lung tissue by an epithelial lining fluid (ELF, 0.2−0.5 μm thick) exuded from underlying lung and resident immune cells.19 ELF contains a suite of endogenous antioxidants (AO) such as glutathione (GSH), ascorbic and uric acids and α-tocopherol, of which GSH (~100−500 μM) is the most abundant.19–26 Inhaled oxidants, mainly but not exclusively O3, may react as such with GSH or be converted into more reactive OH-radicals (−OH)27,28 upon colliding with the ELF via Fenton-type chemistry.23,29–32 Inhaled particulates are known to induce ·OH generation in ELF surrogates.16,33–40 Much of the damage inflicted by superoxide O2− and H2O2 in vivo is in fact due to their conversion into the more reactive ·OH in reactions catalyzed by transition metal ions.41 Glutathione, in addition to ascorbate,29 by being the most abundant and highly reactive ELF antioxidant both toward O3 and ·OH at physiological pH, may be the main scavenger of polluted air oxidants and pro-oxidants.

It has long been considered that the main function of GSH is to scavenge exogenous oxidants into “ox-GSH” innocuous species. It was further believed that “ox-GSH” was the disulfide GSSG that would be produced from the recombination of the thyl G5− radicals ensuing (S)−H atom abstraction by ·OH.32–45 The current view is that scavenging has a dual
function in the sense that it not only deactivates powerful oxidants, but the \([\text{GSH}] / [\text{ox-GSH}]\) ratios generated in the ELF relay the intensity of oxidative stress to the immune system,\(^{46−48}\) thereby unleashing systemic responses to external injury. A nonspecific systemic immune response to inhaled oxidants would be expected if they were converted into a common ·OH intermediate in the ELF,\(^{49,50}\) and oxidative stress signaled by \([\text{GSH}] / [\text{ox-GSH}]\) ratios.\(^{21,46,51−53}\) We have previously shown that the products of GSH\(^{23}\) and cysteine\(^{31}\) reactions with \(\text{O}_3(\text{g})\) at the air−water interface are GSH-sulfonic, and Cys-sulfenic, Cys-sulfinic, and Cys-sulfonic acids rather than GSSG or CySSCy, respectively (Scheme 1).

We now report the results of experiments that identify, for the first time, the products of GSH oxidation by ·OH on the surface of aqueous solutions in air at 1 atm.

In our experiments, we investigate the initial stages of the chemical reactions taking place on the surface of aqueous GSH (γ-L-glutamyl-L-cysteinyl-glycine) and GSSG solutions briefly (≤50 μs) exposed to gas-phase OH-radicals. Reagents and products are simultaneously and unambiguously detected online via electrospray ionization mass spectrometry (ES-MS) of continuously flowing, fresh, dilute GSH and GSSG aqueous microjets collided with ·OH(g) pulses generated in the 266 nm laser photolysis of \(\text{O}_3\) (into \(\text{O}_2 + \text{O}^{(1D)}\)) in \(\text{O}_3/\text{O}_2/\text{H}_2\text{O}/\text{N}_2\) gas beams. In these events, ·OH(g) thermally accumulates on the surface of water, and then reacts with available substrates or terminates as \(\text{H}_2\text{O}_2\).\(^{54−57}\)

\[
\begin{align*}
\text{GSH} + \cdot \text{OH} &\rightarrow \text{products} \quad (R1) \\
\cdot \text{OH} + \cdot \text{OH} &\rightarrow \text{H}_2\text{O}_2 \quad (R2)
\end{align*}
\]

The high reactivity of ·OH and its preference for the surface over bulk water\(^{58,59}\) ensure that these processes take place in the outermost interfacial layers.\(^{55,56}\) See the experimental section below and Supporting Information (SI) for details.

Figure 1 shows negative ion ES mass spectra of 100 μM GSH(aq) (pH 4.4) microjets alternatively exposed to \(\text{O}_2/\text{H}_2\text{O}/\text{N}_2\) and \(\text{O}_3/\text{O}_2/\text{H}_2\text{O}/\text{N}_2\) gas beams both in the dark and under laser pulses.

At pH 4.4, GSH is mostly present as the monoanion GSH\(^−\) (m/z = 306) of its glycine carboxylic group (pK\(_a\) = 3.7); the cysteine thiol −SH group (pK\(_a\) = 8.8) and the α-ammonium-glutamyl zwitterion moiety remain neutral (Scheme 1).\(^{23,43}\) We verified that the addition of \(\text{O}_3(\text{g})\) does not generate new signals, in accordance with our previous report on GSH\(^−\) inertness toward \(\text{O}_3\) (cf. with GS\(^{2−}\) + \(\text{O}_3\)).\(^{23}\) We also verified that GSH\(^−\) and GSSG\(^−\)/GSSG\(^2−\) signals were not affected, nor

Scheme 1
did new signals appear under 266 nm pulses in the absence of O$_3$(g) (see SI, Figures S2 and S3).

Mass spectra change upon 266 nm irradiation of O$_3$/O$_2$/H$_2$O/N$_2$ gas beams: GSH$^+$ signals decay as a function of laser energy (i.e., [·OH]) and new signals appear, which are therefore ascribed to products of (GSH$^+$ + ·OH) reactions. The reaction GSH$^+$ + ·OH in bulk water is diffusionally controlled: $k_1 \geq 3.5 \times 10^{13}$ M$^{-1}$ s$^{-1}$.\textsuperscript{45,69} We estimate $\left[\text{·OH}(\text{g})\right]_0 \sim 8$ ppmv at the spot where ·OH(g) are generated (see [·OH(g)] estimates in SI). We consider that 8 ppmv is an upper limit to $\left[\text{·OH}(\text{g})\right]_0$.

Exposures to 1 ppmv = 2.5 × 10$^{13}$ molecules cm$^{-3}$ (at 1 atm, 298 K) for $\tau \leq 10$ µs: $E = [\cdot\text{OH}(\text{g})] \times \tau < 2.5 \times 10^8$ molecules cm$^{-3}$ s$^{-1}$, are much smaller than those in typical flow reactor experiments on ·OH(g) reactions: $E \geq 2 \times 10^{16}$ molecules cm$^{-3}$ s$^{-1}$.\textsuperscript{55} The molecular formulas of products can be unambiguously inferred from their mass-to-charge ratios. Thus, the $m/z = 322 = 306 + 16$ signal is assigned to a sulfenic acid (GSOH$^-$), $m/z = 338 = 306 + 32$ to a sulfonic acid (GSO$_2$H$^-$), and $m/z = 354 = 306 + 48$ to a sulfonic acid (GSO$_2$H$^+$). We have previously found that the $m/z = 336 = 354 - 18$ signal results from collisionally induced loss of neutral H$_2$O from GSO$_2$H$^-$.$\textsuperscript{55}$ The GSO$_2$H$_2$ (n = 1–3) acids correspond to 1, 2, and 3 O atom (2-electron) transfers to GSH$^-$ (see Scheme 1), which seem to ensue from initial ·OH additions to reduced S-atoms followed by H-abstractions by O$_3$: G(HO)(O)$_{n-1}$S–H$^- + O_3 = GSO$_2$(O)$_{n-1}$OH$^+$ + HO$_2^*$. (Scheme 2) and/or by ·OH: G(HO)(O)$_{n-1}$S–H$^- + ·OH = GSO$_2$(O)$_{n-1}$OH$^+$ + H$_2$O.

$\text{GSH}^+ + ·\text{OH} \rightarrow \text{GSHOH}^-$ (R3)

$\text{GSHOH}^- + O_2 \rightarrow \text{GSHSO}^- + \text{HO}_2^*$ (R4a)

$\text{GSHOH}^- + O_2 \rightarrow \text{GSOH}^- + \text{HO}_2^* + \text{H}_2\text{O}$ (R4b)

Sulfenic acids are weak acids ($pK_a \approx 7$–8)$\textsuperscript{-1}$ and should remain protonated at pH 4. The stronger sulfonic acid ($pK_a \sim 2$) and sulfonic acid ($pK_a < 1$), however, would replace the carboxylic acid in the glutamyl zwitterion (Scheme 1). To our knowledge, this is the first report on the direct detection of glutathionen sulfenic and sulfonic acids at the air–water interface.

That the products we observe stem from ·OH-addition to S atom rather than H-abstraction from S–H (or the pool of available N–H and C–H bonds),$\textsuperscript{61,62}$ is substantiated by the conspicuous absence of the species that should have appeared if the glutathionen radical GS$^-$ (m/z = 305) had been produced via H-atom abstraction in the initial attack by ·OH.$\textsuperscript{33,63–66}$ If GS$^-$ had been present, $k(\text{GS}^- + \text{GSH}^+) = 6.6 \times 10^7$ M$^{-1}$ s$^{-1}$.\textsuperscript{43} GS$^-$ could have added to GSH$^+$ into GSS(H)G$^-$: GS$^-$ + GSH$^+$ = GSS(H)G$^-$, at [GSH$^+$] > 0.2 mM in $\tau_{1/2} \leq 0.69$ s.\textsuperscript{143,60} We estimate $\left[\text{GSH}(\text{g})\right]_0 \sim 6.6 \times 10^7$ M$^{-1}$. We consider that 8 ppmv is an upper limit to $\left[\text{GSH}(\text{g})\right]_0$.

Figure 2 shows ES mass spectral signals from aqueous 100 µM GSH microjets exposed to irradiated O$_3$/O$_2$/H$_2$O/N$_2$ mixtures as a function of pulse energy (i.e., [·OH]). In Figure 2, laser energies at 1, 5, 10, 20, 30, and 40 mJ pulse$^{-1}$ correspond to $\left[\text{·OH}(\text{g})\right]_0 \approx 0.5, 2.7, 5.1, 9.4, 13.1,$ and 16.2 ppmv, which, from the mean ·OH speed $c = 6.09 \times 10^4$ cm s$^{-1}$ at 298 K, correspond to $0.2 \times 10^{18}, 1.0 \times 10^{18}, 1.9 \times 10^{18}, 3.5 \times 10^{18}, 4.9 \times 10^{18}$, and $6.1 \times 10^{18}$ molecules cm$^{-2}$ s$^{-1}$ fluxes on the surface of the microjets, respectively. It is apparent that above a certain ·OH dose, GSH$^+$ is depleted in the outermost interfacial layers, whereupon excess ·OH recombines into relatively unreactive H$_2$O$_2$ toward the GSH$^-$ remaining in underlying layers (R2).$\textsuperscript{55,56}$ We have observed the same behavior in the oxidaion of mono- and dicarboxylic acids initiated by ·OH at the air–water interface under similar conditions.$\textsuperscript{55,56}$ The limited depletion of GSH$^-$ under excess ·OH represents direct evidence that we observe a truly interfacial reaction taking place in the outermost water layers.

We also performed experiments in which aqueous GSH solutions containing Fe$^{2+}$ at pH $\sim 4$ were exposed to O$_3$(g) in the absence of 266 nm irradiation. Recall that at pH 4, GSH is present as the inert monooxidation GSH$^+$ toward O$_3$(g).$\textsuperscript{23}$ Previous experiments in our laboratory have shown that the exposure of Fe$^{2+}$ solutions to O$_3$(g) generates reactive mono-
and poly nuclear O≡Fe(IV) oxo-ferryl species and, possibly, some -OH. Figure 3 shows that in the presence of Fe²⁺ as catalyst, O₃(g) can oxidize GSH⁻ to sulfenic, sulfinic, and sulfonic acids even in acidic media via the reactive intermediates generated in fast Fenton-type chemistry at the air–water interface.

These experiments show that the presence of transition metals in ELF’s, possibly carried by inhaled particulates, extends the reactivity of O₃(g) towards GSH into media that are more acidic than the normal circumneutral physiological range. This finding is relevant to certain pathologies, such as asthma, which are known to acidify the respiratory tract, and to the mechanism of the synergistic adverse health effects of O₃ and particulates. The inability of glutathione to scavenge O₃ in acidic media in the absence of Fe²⁺ is compensated by ascorbic acid. However, at pH < 5 ascorbic acid scavenges O₃(g) into a toxic ozonide rather than innocuous dehydroascorbic acid as it does in neutral media.

The question of whether GSSG could have been formed in the oxidation of GSH but went undetected because it might be rapidly consumed under present conditions is now addressed. Figure 4 shows negative ion ES mass spectra of 100 μM GSH.
GSSG(aq) microjets exposed to O₂(g)/H₂O(g)/N₂(g), and to O₃(g)/O₂(g)/H₂O(g)/N₂(g) with the 266 nm laser on and off.

Figure 5 shows mass spectral signals of reactants and products as functions of pulse energy. At pH 4.3, GSSG is present both as monoanion GSSG⁻ (m/z = 611) and dianion GSSG²⁻ (m/z² = 305), which are inert toward O₃(g) (cyan traces in Figure 4A,B) in accordance with our previous study.²³ In the presence of ·OH, however, product signals appear at m/z = 322, 338 and 354, i.e., the same as those observed in the GSH⁻ + ·OH reaction (Figure 1). It is key to note, however, that relative product signal intensities GSO₃H⁻ > GSO₂H⁻ > GSOH⁻ in the GSSG⁻ + ·OH reaction (Figure 5C) differ from those GSO₂H⁻ > GSO₃H⁻ ≫ GSOH⁻ in the GSH⁻ + ·OH reaction (Figure 2C). These findings are consistent with the rapid, sequential oxidation of GSH⁻ by ·OH into GSOH⁻ and GSO₂H⁻, and the fact that GSOH⁻ is a primary product of the GSSG⁻ + ·OH reaction (Scheme 3) rather than a second-generation species, as is the case from GSH⁻ + ·OH (Scheme 2).

The above observations are consistent with a mechanism involving the addition of ·OH to S atoms into a discrete radical adduct HO−S(·)−H, which reacts with O₂/OH leading to sulfoacids −SOH (+ HO₂⁻/H₂O) or, in its absence, may decompose into thyl S· radicals (+ H₂O).⁷⁵ Note that the fraction of GS⁻ (generated in the decomposition of the initial GSSG−–OH⁻ adduct) in equilibrium with its peroxyl GS-OO⁻ radical (m/z = 337) in water saturated with air ([O₂(aq)] = 2.6 × 10⁻⁴ M) is given by [GS-OO⁻]/[GS⁻] = K₆₅−O⁻ × [O₂(aq)] = 3.2 × 10⁻⁸ M⁻¹ × 2.6 × 10⁻⁴ M = 0.₈₆. The absence of m/z = 337 signals in Figure 4B therefore indicates that GS⁻ is rapidly converted into the sulfenic acid GSOH⁻ by excess ·OH. Again, there is no evidence of the formation of products initiated by H-atom abstraction from C−H/N−H groups.

The extraordinary specificity of ·OH for adding to the glutathione thiol −SH sulfur atom at the air–water interface, bypassing exothermic (and fast, both in gas-phase or bulk
evokes a recent theoretical radical recognition and steering mechanism in which ·OH is captured by the host GSH anionic carboxylic groups and directed toward the reactive −SH group by a concerted process involving multiple H-bonded interactions within a flexible GSH framework.76,78 The issue of whether the ·OH so positioned would directly H-abstract from S – H, thereby producing the GS− thyl radical in one step, or add to the S-atom into a discrete, long-lived intermediate GS(H)[OH]− that could react with other molecules has recently been addressed by high-level MP4 ab initio calculations for the CH3SH + ·OH adduct that lies about 3.5 kcal mol−1 below the reactants.27 This study, although predicting a significantly smaller stabilization than the 13 kcal mol−1 value derived from gas-phase kinetic experiments on CH3SH + ·OH,82 supports the existence of the discrete intermediate implied by our experiments. We wish to point out that previous experiments have shown that O3 reacts with various substrates at the air–water interface via O-atom (two-electron) rather than thermodynamically allowed one-electron transfers, and at much faster rates than those estimated from reaction rate constants in bulk water and [O3(aq)] deduced from its Henry’s law constant (H = 0.01 M atm−1).27,29,32 The suggestion was made that the steep water density gradient and the peculiar structure of interfacial water modifies the course of reactions by enabling oxidants (such as ·OH in the present case, and O3 in Fenton’s reaction83) to reach emerging functional groups, such as the glutathione thiol, relatively unencumbered by solvation water molecules.27,83

Summing up, in moderately acidic (pH ≤ 5) media, such as those created by diverse pathologies, which include asthma71,84 and the systemic immune response to inhaled particulates,85 glutathione GSH− is found to be oxidized by ·OH into sulfenic acid GSOH−, sulfenic GSO2H− and sulfonic GSO3H− acids86–89 with remarkable specificity. This is the first report on the direct detection of glutathione sulfenic and sulfonic acids at the air–water interface. The exceptional specificity of ·OH on the surface of water versus its lack of selectivity in bulk water implicates an unprecedented molecular recognition process during [OH−−GSH] interfacial encounters. The ·OH implicated in these events may be generated in situ from inhaled O3 in the presence of transition metal ions such as Fe2+, in addition to endogenous sources. Since both the cysteine sulfenic and sulfinic acid functionalities are reduced enzymatically back to the thiol by NADPH in vivo,47,87,89 our results suggest that redox balance and signal transduction by ELF glutathione involve sulfur oxoacids rather than a disulfide.

■ EXPERIMENTAL SECTION

The experimental setup has been described in a previous publication.55 Here we summarize specific features of the setup used in the experiments reported herein. The charged product species generated on the surface of GSH(aq) or GSSG(aq) microjets during τ > 10–50 μs contact times (τ is the lifetime of the microjets before they are pneumatically nebulized into smaller droplets) with O3(g) or ·OH(g) beams are monitored in situ by an ES-MS (Agilent 6130 Quadrupole LC/MS Electro spray System, Kyoto University).55 Samples are injected at 100 μL min−1 into the spraying chamber of the mass spectrometer through a grounded stainless steel needle (100 μm bore) coaxial with a sheath issuing nebulizer N2(g) at subsonic velocities (v ≤ 160 m/s).90 The surface specificity of our experiments had been demonstrated previously.27,90 Note that the products we observe are formed when gaseous reactants collide with the intact aqueous jets as they emerge from the nozzle, i.e., before jets are broken up into submicron charged droplets by the nebulizer gas.27 Since 266 nm pulses flash every 100 ms, and microjets break up within 10–50 μs after being ejected from the nozzle, we assume that the observed phenomena take place in fresh solutions.55 See SI for further details.

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b01819.

Additional data and experimental details (PDF)

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Notes
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