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GAPDH inhibition mediated by thiol oxidation in human airway epithelial cells exposed to an environmental peroxide

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ABSTRACT

Intracellular redox homeostasis in the airway epithelium is closely regulated through adaptive signaling and metabolic pathways. However, inhalational exposure to xenobiotic stressors such as secondary organic aerosols (SOA) can alter intracellular redox homeostasis. Isoprene hydroxy hydroperoxide (ISOPOOH), a ubiquitous volatile organic compound derived from the atmospheric photooxidation of biogenic isoprene, is a major contributor to SOA. We have previously demonstrated that exposure of human airway epithelial cells (HAEC) to ISOPOOH induces oxidative stress through multiple mechanisms including lipid peroxidation, glutathione oxidation, and alterations of glycolytic metabolism. Using dimedone-based reagents and copper catalyzed azo-alkynyl cycloaddition to tag intracellular protein thiol oxidation, we demonstrate that exposure of HAEC to micromolar levels of ISOPOOH induces reversible oxidation of cysteinyl thiols in multiple intracellular proteins, including GAPDH, that was accompanied by a dose-dependent loss of GAPDH enzymatic activity. These results demonstrate that ISOPOOH induces an oxidative modification of intracellular proteins that results in loss of GAPDH activity, which ultimately impacts the dynamic regulation of the intracellular redox homeostatic landscape in HAEC.

1. Introduction

Anthropogenic and biogenic emissions are major contributors to atmospheric air pollution [1]. These pollutants give rise to the formation of secondary organic aerosols (SOA) which are important constituents of ambient fine particulate matter ($PM_{2.5}$) [2,3]. Isoprene is a biogenic volatile organic compound that represents the most abundant non-methane hydrocarbon in the atmosphere [1,4]. Atmospheric oxidation of isoprene by ambient hydroxyl radical leads to the rapid formation of oxidized species, including an array of organic peroxides and hydroperoxides [1]. While interest in isoprene oxidation products has been based on their environmental relevance as components of ambient $PM_{2.5}$, recent studies have demonstrated direct biological effects of HAEC exposure to downstream isoprene oxidation products [4,5]. Specifically, exposure to isoprene hydroxy hydroperoxide (ISOPOOH), a first generation oxidation product of isoprene, has been shown to strongly induce oxidative stress, inflammation, and bioenergetic adaptations in human airway epithelial cells (HAEC) [6–8].

Post-translational reversible thiol oxidation of cysteinyl residues modulates the function of key regulatory proteins [9,10]. Examples include phosphatases and kinases involved in cellular signaling, transcription factors, and metabolic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [11–14]. The oxidation of cysteinyl thiols to protein sulfenic acids is highly regulated by the glutathione and the thioredoxin systems [15,16]. These systems, specifically the glutaredoxin and the peroxiredoxin family of enzymes, maintain a reducing intracellular redox environment at the expense of glutathione and thioredoxin, respectively [17,18]. Oxidized glutathione and thioredoxin are then reduced by glutathione reductase and

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Abbreviations

2-thio-DMD	2-thio-5,5-dimehtyl-1,3-cyclohexanedione
DAz-2	4-(3-aziodoprogpyl)-1,3-cyclohexanedione
DyN-2	4-(pent-4-yn-1-yl)cyclohexane-1,3-dione
DMD	5,5-dimehtyl-1,3-cyclohexanedione
9-HpODE	9-hydroperoxyoctadecadienoic acid
9-HODE	9-hydroxyoctadecadienoic acid
$PM_{2.5}$	Airborne Fine Particulate Matter
BSA	Bovine Serum Albumin
CuAAC	Copper-Catalyzed Azide Alkynyl Cycloaddition
DTT	Dithiothreitol
GPX4	Glutathione Peroxidase 4
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
HAEC	Human Airway Epithelial Cells
Iso-SOA	Isoprene-derived Secondary Organic Aerosols
ISOPOOH	Isoprene Hydroxyhydroperoxide
NEM	*N*-ethylmaleimide
PBS	Phosphate Buffered Saline
rmGAPDH	Rabbit Muscle Glyceraldehyde-3-Phosphate Dehydrogenase
SOA	Secondary Organic Aerosols
SDS	Sodium Dodecyl Sulfate
t-BOOH	*tert*-Butyl Hydroperoxide
TBS	Tris Buffered Saline

thioredoxin reductase using NADPH that is primarily produced by the pentose phosphate pathway [19]. We have previously shown that knocking down the expression of glucose-6-phosphate dehydrogenase in HAEC sensitizes cells to ISOPOOH-induced oxidative stress, consistent with pentose phosphate pathway-derived NADPH as a key regulator of ISOPOOH-induced oxidative stress [7].

The diversion of glucose from glycolysis to the pentose phosphate pathway involves the apparent sulfenylation of GAPDH [20]. The glycolytic dehydrogenase activity of GAPDH is mediated through a hemithioacetal intermediate formed between glucose-3-phosphate and the C152 thiolate of GAPDH [12,21]. Cysteine oxidation of GAPDH inhibits its activity by inhibiting the formation of the reaction intermediate [21]. Furthermore, the cysteine oxidation of GAPDH thiol residues can occur by reaction with various peroxides, lipid hydroperoxides, and other oxidizing agents like peroxynitrites [22,23]. Therefore, the oxidation state of GAPDH thiol residues plays a pivotal role in determining the function of GAPDH as a molecular switch and may be relevant to the redox toxicology of environmental oxidants.

We recently reported that ISOPOOH rapidly induces oxidation of glutathione, an early event in the onset of intracellular oxidative stress, through a mechanism involving glutathione peroxidase 4 (*GPX4*) [6]. ISOPOOH is a potent initiator of lipid peroxidation, which can be modulated by supplementation with unsaturated fatty acids, and we further showed that ISOPOOH treatment of HAEC causes an adaptive response leading to compensatory NADPH synthesis [6,7]. This response implicates alteration of cellular bioenergetics, specifically the shifting of glucose metabolism from glycolysis to the pentose phosphate pathway, as a compensatory event in the toxicology of ISOPOOH. This led us to postulate that exposure to ISOPOOH inhibits GAPDH activity through cysteinyl oxidation in HAEC either directly or through the formation of lipid peroxides.

In the present study, we investigate the role of oxidative protein modifications in the cellular bioenergetic responses to ISOPOOH-induced oxidative stress in HAEC. We show that exposure of HAEC to ISOPOOH induces the thiol oxidation of a wide range of proteins, including GAPDH, and report that ISOPOOH-induced thiol oxidation of GAPDH is accompanied by a profound loss of GAPDH enzymatic activity in HAEC. These findings support the postulate that GAPDH acts as a molecular switch between glycolysis and the pentose phosphate pathway in HAEC exposed to ISOPOOH.

2. Methods

2.1. Material and reagents

All reagents for cell culture including: fetal bovine serum (FBS), minimum essential media with glutamax (MEM), Dulbecco's phosphate buffered saline (PBS), and Restore western blot stripping buffer were purchased from Gibco, Thermo Fisher Scientific (Waltham, MA, USA). Bovine type 1 collagen, ammonium bicarbonate, tris base, sodium dodecyl sulfate (SDS), dimethyl sulfoxide (DMSO), streptavidin sepharose beads, and Corning Spin-X 0.24 μm cellulose acetate spin filter columns were obtained from Fisher Scientific, Thermo Fisher Scientific (Waltham, MA, USA). Laboratory reagents and chemicals including: dithiothreitol (DTT), hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (*t*-BOOH), D-(+)-glucose, human fibronectin, fatty acid free fraction V bovine serum albumin (BSA), tween-20, urea, glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (rmGAPDH), D-glyceraldehyde 3-phosphate solution, NAD grade I free acid, potassium phosphate dibasic, sodium L-ascorbate, copper (II) sulfate, diazo biotin-azide, *N*-ethylmaleimide (NEM), and 5,5-dimethyl-1,3-cyclohexanedione (dimedone) were purchased from Sigma-Aldrich (St Louis, MO, USA). 9-hydroxyoctadecadienoic acid (9-HODE), 9-hydroperoxyoctadecadienoic acid (9-HpODE), linolein hydroperoxides (an isomeric mixture of mono-, di-, and tri-hydroperoxides formed from the autoxidation of trilinolein), 4-(3-azidopropyl)-1,3-cyclohexanedione (DAz-2), phosphine-biotin, and 4-(pent-4-yn-1-yl)cyclohexane-1,3-dione (DyN-2) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Glyceraldehyde-3-phosphate dehydrogenase activity assay kits were purchased from Abcam (Cambridge, United Kingdom). All western blot supplies including: 40 % acrylamide/bis solution, TEMED, 10x Tris-buffered saline (TBS) solution, clarity western ECL solution, 0.45 μM nitrocellulose membranes, 4x Laemmli sample buffer, and micro bio-spin columns were purchased from Bio-Rad Laboratories (Hercules, CA, USA). The following antibodies were used: monoclonal mouse anti-GAPDH (Cell Signaling Technology, Danvers, MA, USA, cat# 97166S; 1:1000), polyclonal rabbit anti-GAPDH-SO_3 (AbFrontier, Seoul, South Korea, cat# LF-PA0006; 1:10,000), polyclonal rabbit anti-cysteine-sulfenic acid-dimedone (MilliporeSigma, Burlington, MA, USA, cat# ABS30; 1:1000), HRP-linked horse anti-mouse (Cell Signaling Technology, cat# 7076S; 1:10,000), HRP-linked goat anti-rabbit (Jackson ImmunoResearch, West Grove, PA, USA, cat# 111-035-003; 1:10,000), and HRP-linked avidin (Thermo Fisher Scientific, cat# 29994; 1:5000). Antibody dilutions were prepared in 5 % BSA in Tris-buffered saline with 0.1 % Tween-20 (TBST).

2.2. Synthesis of 2-hydroperoxy-2-methylbut-3-en-1-ol (ISOPOOH)

ISOPOOH was synthesized by perhydrolysis of 2-methyl-vinyloxirane with hydrogen peroxides, as previously described [24]. A freshly prepared solution of ethereal hydrogen peroxide (20 mL), was added to a round bottom flask equipped with a stir bar, and 2-methyl-2-vinyloxirane (1 g) and phosphomolybdic acid (2–3 mg) were added and the reaction was stirred at ambient temperature [25,26]. Reaction progress was monitored by ^{1}H NMR. Ether was removed under vacuum and the product isolated by silica gel chromatography to afford pure 1, 2-ISOPOOH.

2.3. Cell culture

SV-40 transformed human bronchial epithelial cell line 16HBE14 (16-HBE) was gifted from the laboratory of Dr. Ilona Jaspers (UNC Chapel Hill, NC, USA) [27]. 16-HBE cells were cultured with complete media consisting of MEM with Glutamax with 10 % FBS and 1 % penicillin-streptomycin. All cell culture plastics were coated with a matrix of: 30 μg/mL bovine type 1 collagen, 1 % human fibronectin, and 0.01 % BSA in LHC Basal Medium. Cells were cultured at 37 °C in a humidified environment containing 95 % air/5 % carbon dioxide (CO_2). For sulfenylation experiments involving copper-catalyzed azide alkynyl cycloaddition reaction (CuAAC reaction), cells were cultured either on uncoated plastic 10 cm dishes or in uncoated 6-well plates.

2.4. Cell exposure conditions

All exposures were conducted on naive 16-HBE cells at 80 % confluency. Prior to exposure, 16-HBE cells were washed with 1x PBS and equilibrated for 30 min in Locke's Buffer [17,28]. HAEC were exposed to a variety of hydroperoxides for 10 min in an incubated environment maintaining 37 °C with 95 % air/5 % CO_2 and ≥95 % relative humidity. Cells were then labeled with 1 mM dimedone analog (DyN-2 or DAz-2) in Locke's Buffer with 1:200 dilution of DMSO for 1 h.

2.5. Staudinger ligation

Cells labeled with DAz-2 underwent the Staudinger ligation for fluorescent detection (Sulfenylated Protein Cell-Based Detection Kit, cat# 600320; Cayman Chemicals, Ann Arbor, MI, USA) [11]. Labeled cells were then detected by confocal fluorescent microscopy using the Nikon A1R-HD25 confocal imaging system and 488 nm primary laser excitation (Nikon Instruments; Melville, NY, USA). Cells were visualized using a S Plan Fluor ELWD 40 × 0.6 NA WD 3.6 -2.8 air objective. Images were processed using ImageJ (National Institutes of Health, Bethesda, MD, USA). To enhance the visual determination of fluorescence, images were converted to 32-bit monochromatic images and background subtracted using a 50.0 pixel rolling ball radius. A green lookup table was applied followed by ROF denoising using a 25-theta threshold and despeckling.

2.6. Copper-catalyzed azide alkyne cycloaddition (CuAAC)

Cells labeled with DyN-2 were washed with ice-cold PBS, then lysed with buffer containing: 150 mM NaCl, 150 mM Tris-HCl (pH 7.4) 1 % (v/v) NP40, 1 mM DTT, with Calbiochem protease inhibitor cocktail set III (MilliporeSigma, Burlington, MA, USA, cat# ABS30, 1:1000) [29]. Cells were lysed for 30 min on ice followed by centrifugation at 12,000 g for 10 min at 4 °C. The protein content of the samples was normalized following detection of protein concentration via the Bradford assay. Normalized protein samples were then labeled with either azo-biotin or photo-cleavable azo-biotin using the CuAAC reaction by agitation for 1 h in a buffer adding the click reagents: 0.2 mM biotin azide, 0.2 mM tris (3-hydroxypropyltriazolylmethyl)amine (THPTA), 1 mM Cu_2SO_4, and 1 mM sodium ascorbate [30]. An aliquot of each sample was prepared for Streptavidin blotting with 4x Laemmli sample buffer and boiled for 5 min. Samples were kept at −80 °C for long term storage.

2.7. Avidin immunoprecipitation

For immunoprecipitation experiments, biotin-labeled samples were diluted 1:3 with cold methanol and kept for 20 min on ice. Samples were centrifuged at 3000 g for 5 min at 4°C. The supernatant was removed, and the samples were resuspended in 1 mL of cold methanol and centrifuged at 6000 g for 5 min at 4°C. The resulting pellet was resuspended in 200 μL of 1 % SDS followed by low pulse sonication until the solution was clear. Following sonication, 1 mL of PBS and 50 μL of streptavidin beads were added. Samples were rotated at room temperature for 2 h in the dark, and then centrifuged at 2000 g for 2 min at 4 °C. Following the removal of the supernatant, the streptavidin beads were transferred to 0.24 μm cellulose acetate spin columns then the beads were washed twice with 1 % SDS, 4 M urea in PBS, 1 M NaCl, and 25 mM ammonium bicarbonate. The beads were then resuspended in 400 μL of 25 mM ammonium bicarbonate and underwent photolysis by exposure to 370 nm UV light for 2 h at room temperature. Following photolysis, samples were centrifuged at 2000 g for 1 min at 4 °C and the eluate was collected. Beads were washed two additional times with 400 μL of 25 mM ammonium bicarbonate and were combined and dried. Dried samples were resuspended in 50 μL of 1x Laemmli Sample Buffer and were stored at −80 °C for anti-GAPDH western blot.

2.8. In vitro oxidation of purified GAPDH

Lyophilized GAPDH was resuspended at a concentration of 25 μM in 10 mM Tris-HCl at pH 7.6. GAPDH was reduced by the addition of 1 mM DTT, and the solution was desalinated using micro-spin columns. Columns were centrifuged at 1000 g for 10 min at 4°C. Protein amounts were determined using the Bradford assay. 25 μM GAPDH was exposed to varying concentrations of hydroperoxides for 5 min at room temperature. Following hydroperoxide exposure, 1 mM dimedone and 1 mM NEM were added to the sample for 5 min at room temperature. An aliquot of each sample was prepared for western blotting with 4x Laemmli sample buffer and boiled for 5 min. Samples were stored at −80 °C.

2.9. Western blotting

Protein samples were separated using 12 % acrylamide: bis-acrylamide gel and transferred to a nitrocellulose membrane. The nitrocellulose membrane was blocked with 5 % BSA in Tris-buffered saline with 0.1 % Tween-20 (TBST) for 1 h at room temperature. Primary antibody incubation was performed in 5 % BSA in TBST overnight at 4 °C, and secondary antibody incubation was performed in 5 % BSA in TBST for 1 h at room temperature. Avidin-HRP incubation was performed immediately after blocking in 5 % BSA in TBST for 1 h at room temperature. Blots were visualized using Bio-Rad clarity western ECL solution using Bio-Rad ChemiDoc MP Imaging System. Blots were stripped by incubation with Restore western blot stripping buffer for 1 h at room temperature, blocked with 5 % BSA in TBST, then re-probed for GAPDH loading control. Densitometry was used to measure the density of all bands detected for each sample in ImageJ.

The following primary antibodies were used: monoclonal mouse anti-GAPDH (Cell Signaling Technology, Danvers, MA, USA, cat# 97166S; 1:1000), polyclonal rabbit anti-GAPDH-SO_3 (AbFrontier, Seoul, South Korea, cat# LF-PA0006; 1:10,000), polyclonal rabbit anti-cysteine-sulfenic acid-dimedone (MilliporeSigma, Burlington, MA, USA, cat# ABS30; 1:1000). The following secondary antibodies were used: HRP-linked horse anti-mouse (Cell Signaling Technology, cat# 7076S; 1:10,000), and HRP-linked goat anti-rabbit (Jackson ImmunoResearch, West Grove, PA, USA, cat# 111-035-003; 1:10,000). Blots with HRP-linked avidin (Thermo Fisher Scientific, cat# 29994; 1:5000) were incubated at room temperature for 1 h. Antibody dilutions were prepared in 5 % BSA in Tris-buffered saline with 0.1 % Tween-20 (TBST).

2.10. GAPDH activity assay

Extracellular GAPDH was reduced and exposed to hydroperoxides as previously described. Exposed GAPDH samples (not labeled with dimedone or NEM) were diluted to 5 ng/μL in a solution containing: 10 mM K_2HPO_4 and 1 mM EDTA. Assay used 500 ng of GAPDH, 1 mM of NAD+, 1 mM of glyceraldehyde-3-phosphate, 50 mM K_2HPO_4, and 1 mM EDTA at pH 7.5. Initial rates were determined by measuring the

absorbance at 340 nm for 30 min using a microplate reader (CLARIOstar Plus, BMG Labtech, Cary, NC, USA).

For cellular GAPDH activity assays, 16-HBE cells were exposed to varying concentrations of hydroperoxides for 10 min. Cell lysates and activity solutions were prepared following the recommended protocol for GAPDH activity assay kit (Abcam, Cambridge, United Kingdom, cat# ab204732). Initial rates were determined by measuring the absorbance at 450 nm for 30 min 1 mM DTT was used as a reducing control at the end of the experiment.

2.11. Statistical analysis

All microscopy images were quantified using the NIS-Elements AR software (Nikon Instruments Corporation, Melville, NY, USA). Fluorescent intensities were normalized to background. ImageJ was used to quantify relative intensities of all bands detected for each sample, intensity values were normalized to background and loading control. All statistical analyses were performed using Graphpad PRISM 9.1 (Graphpad Software, La Jolla, CA, USA). Maximum oxidation responses were analyzed using a one-way ANOVA with multiple comparisons to vehicle control with Dunnett correction. A repeated measures ANOVA analysis with multiple treatment groups with post-hoc Tukey's multiple comparisons test was conducted for GAPDH activity assays. A threshold of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Exposure to ISOPOOH induces protein thiol oxidation in HAEC

We reported previously that exposure to concentrations of the environmental peroxide ISOPOOH as low as 100 μM, results in a rapid increase in intracellular glutathione oxidation through mechanisms involving lipid peroxidation in HAEC [6]. To characterize the oxidative effects of ISOPOOH exposure in HAEC on intracellular protein thiols, we used dimedone and dimedone analogs. While these reagents have been used extensively in the literature to detect protein thiol sulfenic acids, it has been suggested that they detect protein thiol sulfenyl amides [31,32]. DAz-2 was used to biotinylate oxidized protein thiols and allow them to be detected fluorometrically in HAEC exposed to 50 μM–1 mM ISOPOOH or H_2O_2. Relative to the vehicle-treated control, exposure of HAEC to 50 μM ISOPOOH for 10 min resulted in a marked increase in intracellular protein thiol oxidation (Fig. 1). Increase in protein thiol oxidation were induced by exposure to ISOPOOH concentrations ranging from 100 μM to 1 mM were similar in magnitude to those induced by the same concentration of H_2O_2 (Fig. 1B and C).

3.2. ISOPOOH exposure induces intracellular GAPDH thiol oxidation in HAEC

We next assessed the range of proteins thiol oxidation in HAEC exposed to ISOPOOH using the Copper-catalyzed azide alkyne cycloaddition (CuAAC) reaction to biotinylate oxidized protein thiols, allowing them to be quantified by western blotting using avidin conjugated horseradish peroxidase [29]. As shown in Fig. 2A, B and 2D, exposure of HAEC to 1 mM ISOPOOH induces an increase in protein thiol oxidation varying in molecular weight between 15 and 150 kDa. Exposure of HAEC to concentrations as low as 10 μM ISOPOOH induced a detectable increase in intracellular protein thiol oxidation (Fig. S1).

Given its role as a molecular switch that leads to the production of NADPH, we next focused on the effect of ISOPOOH on GAPDH thiol

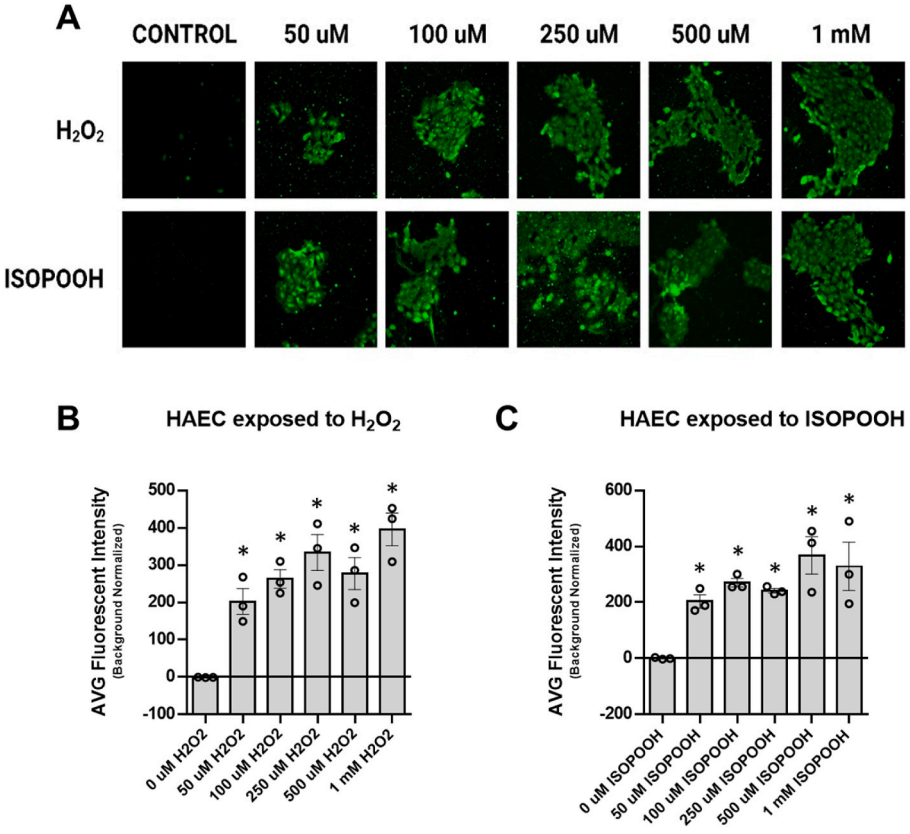

Fig. 1. ISOPOOH induces intracellular protein thiol oxidation. HAEC were exposed to 0 (Vehicle Control) – 1 mM ISOPOOH or H_2O_2 for 10 min, followed by labeling with DAz-2 and conjugation to Avidin-FITC using the Staudinger ligation reaction. Fluorescent intensities were background corrected, and comparisons were performed using a one-way ANOVA with multiple comparisons to vehicle control with Dunnett correction, *$p \leq 0.05$. Data shown are representative images of three independent experiments.

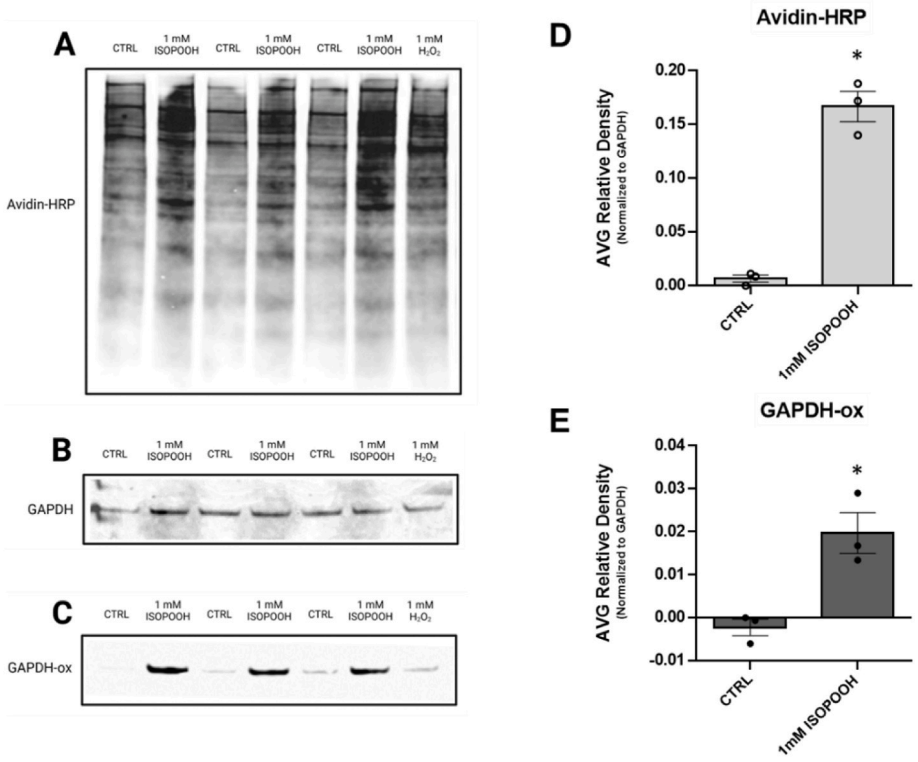

Fig. 2. HAEC exposure to ISOPOOH induces intracellular GAPDH thiol oxidation. HAEC were exposed to Vehicle Control, 1 mM ISOPOOH, or 1 mM H_2O_2 for 10 min and labeled with DYn-2 followed by conjugation to biotin by CuAAC. (A) Western blot with three different biological replicates showing protein thiol oxidation between 15 and 150 kD in HAEC exposed to ISOPOOH. (B) The same blot was stripped and reblotted for GAPDH. (C) The same lysates were then subjected to avidin immunoprecipitation and blotted against GAPDH. (D) Densitometry measurements of total protein thiol oxidation shown in A. (E) Densitometry of intracellular GAPDH thiol oxidation shown in C. Quantitations were normalized to background and loading control and compared using a two-tailed unpaired *t*-test with Welch's correction, *p $\leq$ 0.05. Data shown are western blots of three independent experiments.

oxidation [20]. Exposure of HAEC to ISOPOOH for 10 min resulted in a marked increase in intracellular GAPDH thiol oxidation (Fig. 2C and E). Intracellular GAPDH thiol oxidation in HAEC was detectable with exposure to a concentration of ISOPOOH as low as a 10 μM (Fig. S1). Protein sulfenic acids in high oxidizing environments can undergo further oxidation to form protein sulfinic and sulfonic acids [13,33]. We therefore investigated the possibility that ISOPOOH exposure of HAEC leads to hyperoxidation of intracellular GAPDH. As shown in Fig. 3, increasing concentrations of ISOPOOH ranging from 10 μM to 1 mM, resulted in a dose-dependent increase in sulfonylation of intracellular GAPDH.

3.3. Direct sulfenylation of GAPDH induced by ISOPOOH in vitro

Organic peroxides and hydroperoxides have been shown to induce protein thiol oxidation directly [22]. We therefore determined whether ISOPOOH sulfenylates GAPDH directly or it involves secondary reactants such as lipid hydroperoxides. As shown in Fig. 4, ISOPOOH induced a dose-dependent increase in extracellular GAPDH sulfenylation at concentrations ranging from 6.25 μM to 100 μM. ISOPOOH-induced sulfenylation of extracellular GAPDH showed potency approximately equimolar to that of other known strong oxidants such as H_2O_2 and *t*-BOOH and long chain lipid hydroperoxides like 9-HpODE (Figs. S2, S3, S4). These findings show that ISOPOOH, a low molecular weight organic hydroperoxide, can oxidize GAPDH directly in an acellular system.

3.4. ISOPOOH exposure induces loss of GAPDH activity

Oxidation-mediated GAPDH inactivation is believed to be a pivotal event that diverts glucose from glycolysis to the pentose phosphate pathway [7]. We therefore examined the effect of ISOPOOH exposure on the activity of GAPDH purified from rabbit muscle (rmGAPDH) as a potential measure of this bioenergetic adaptation. rmGAPDH was exposed to 1 mM DTT, a reducing control, which had no discernible effect on GAPDH activity (Fig. 5). In contrast, treatment of GAPDH with increasing concentrations of ISOPOOH ranging from 10 μM to 1 mM resulted in a pronounced, dose-dependent decrease in GAPDH enzymatic activity that was evidenced by slower kinetics and a reduced maximal concentration of NADH production (Fig. 5). At the maximal concentration of 1 mM ISOPOOH, the ablation of rmGAPDH activity was similar in magnitude to that effected by the 1 mM H_2O_2 positive control. 1 mM H_2O_2 was selected as the positive control due to its oxidative potency to induce intracellular GAPDH thiol oxidation as previously reported [12]. Treatment of rmGAPDH with a mixture of linolein hydroperoxides markedly inhibited its activity, while 9-HODE, a linoleic metabolite lacking a peroxide group, had no effect (Fig. S5).

We then determined whether the loss of intracellular GAPDH dehydrogenase activity underlies the bioenergetic adaptation in HAEC exposed to ISOPOOH. In a pattern similar to that observed in the rmGAPDH experiments, GAPDH in HAEC exposed to 10 μM to 1 mM ISOPOOH, showed a dose-dependent decrease in GAPDH activity when compared to the vehicle treated control (Fig. 6). Exposure of HAEC to the maximal 1 mM ISOPOOH exposure caused a complete loss of intracellular GAPDH enzymatic activity, similar to that induced by the 1 mM H_2O_2 positive control. The introduction of 1 mM DTT produced a rapid and robust reversal of ISOPOOH-induced loss of GAPDH activity, which is consistent with a reversible oxidative modification of the HAEC GAPDH cysteinyl thiols.

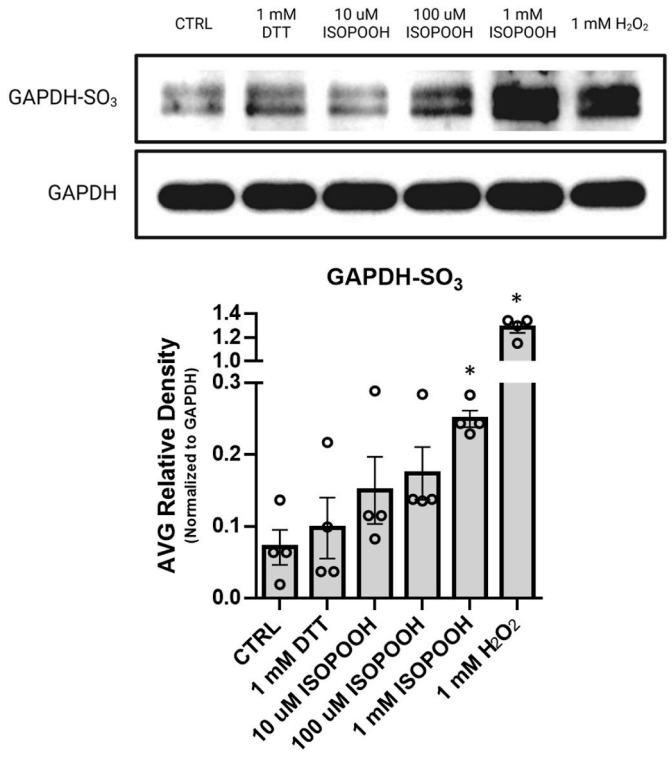

Fig. 3. HAEC exposure to ISOPOOH induces intracellular GAPDH sulfonylation. Proteins from HAEC exposed to 0–1 mM ISOPOOH and 1 mM H_2O_2 for 10 min were blotted for GAPDH-SO_3 and total GAPDH. Densitometry measurements were normalized to background and loading control. Comparisons were performed using a one-way ANOVA with multiple comparisons to vehicle control with Dunnett correction, *p ≤ 0.05. Data shown as representative images of western blots of four independent experiments.

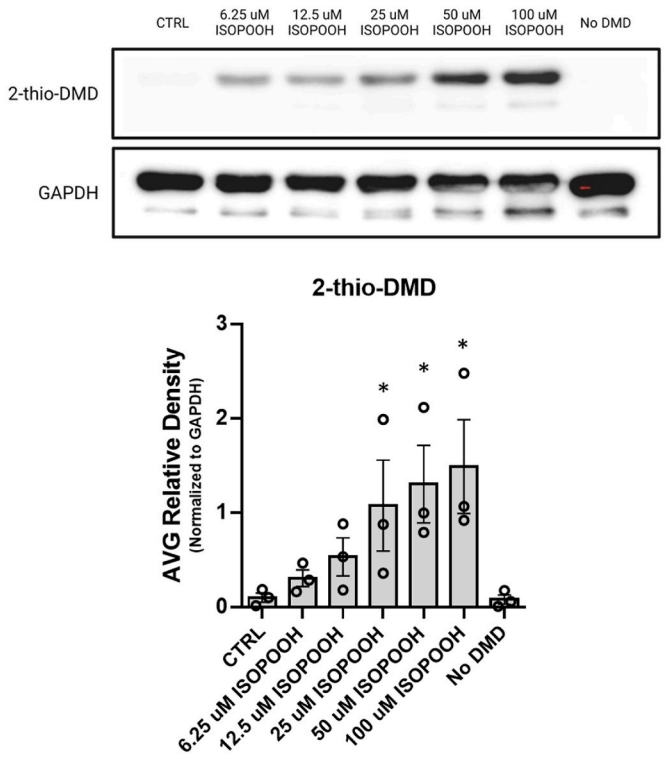

Fig. 4. ISOPOOH induces dose-dependent GAPDH sulfenylation. Rabbit muscle GAPDH was treated with 0 (Vehicle Control) – 100 μM ISOPOOH for 5 min and labeled with dimedone (DMD). Blot was probed with antibodies specific for 2-thiodimedone (2-thio-DMD) and GAPDH. Densitometry measurements were normalized to background and loading control. Comparisons were performed using a one-way ANOVA with multiple comparisons to vehicle control with Dunnett correction, *p ≤ 0.05. Data shown as representative images of western blots of three independent experiments.

4. Discussion

With an estimated release of 500 Tg/year, isoprene is the most abundant non-methane hydrocarbon in the atmosphere [1]. Isoprene is a known precursor of a variety of photochemical oxidation products including hydroperoxides that contribute to fine airborne particulates pollution ($PM_{2.5}$) associated with excess morbidity and mortality worldwide [34–36]. Given the ubiquitous nature of ISOPOOH in the atmosphere, a plausible exposure concentration of HAEC as reported previously would be 40–90 μM ISOPOOH [6]. We previously reported that direct exposure of HAEC to ISOPOOH induces intracellular glutathione oxidation, initiation of lipid peroxidation, and compensatory bioenergetic changes [6,7]. We have previously demonstrated that the pentose phosphate pathway is a critical source of NADPH that supports the glutathione antioxidant pathway in HAEC exposed to ISOPOOH [7]. Furthermore, key proteins involved in multiple cellular functions ranging from signaling to energy metabolism are subject to regulation through reversible oxidation of specific cysteinyl thiols [19]. In the present communication, we have established that exposure of HAEC to ISOPOOH induces cysteinyl thiol oxidation of intracellular proteins, including the pivotal regulatory protein GAPDH, which results in its inactivation.

This dynamic interplay of glucose metabolism between cellular bioenergetic pathways such as glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation on the one hand and cellular antioxidant pathways such as the pentose phosphate pathway in the other, has been well characterized, especially in the context of oxidative stress [21,37]. Peroxide treatment induces sulfenylation of GAPDH shifts glucose metabolism away from glycolysis and diverts it towards pentose phosphate pathway in a variety of cell types [38]. Since the inactivation of

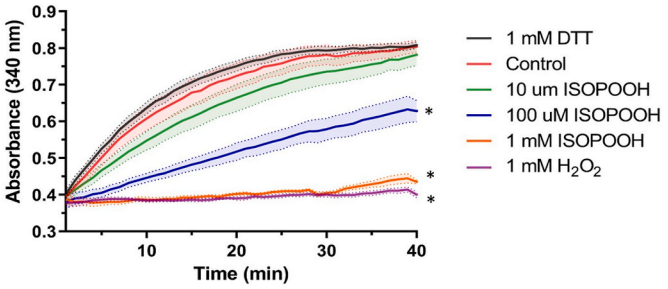

Fig. 5. ISOPOOH exposure dose-dependently inhibits GAPDH dehydrogenase activity. Rabbit muscle GAPDH was treated with 0 (Vehicle Control) – 1 mM ISOPOOH, 1 mM DTT (reducing control), or 1 mM H_2O_2 (positive control) for 5 min. GAPDH was diluted in a phosphate buffered solution containing glyceraldehyde-3-phosphate and NAD+. GAPDH activity was measured in a reaction monitored at 340 nm absorbance which marked the formation of NADH. All values are presented as mean ± SEM, n = 4. Comparisons were performed using a repeated measures ANOVA with multiple treatment groups and post-hoc Tukey's multiple comparisons test, *p ≤ 0.05.

GAPDH is reversible, this post-translational modification allows the cell to maintain intracellular equilibrium between meeting its energy needs through glycolysis and maintaining a homeostatic redox balance through the production of NADPH [37]. Therefore, the duality of GAPDH functions depending on its oxidation state, which inherently links cellular bioenergetics to redox homeostasis, and is the basis of the role of GAPDH as a molecular switch [20].

Our previous report of a bioenergetic adaptation of HAEC induced by

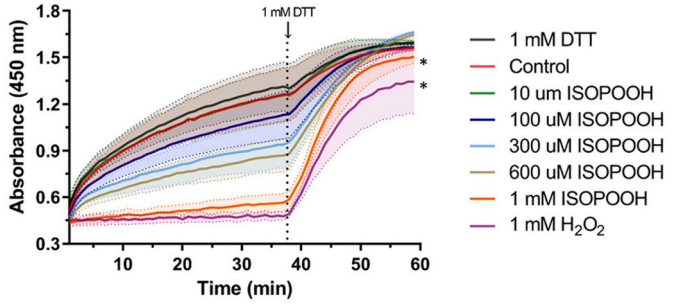

Fig. 6. Exposure to ISOPOOH induces dose-dependent inhibition of GAPDH activity in HAEC. GAPDH glycolytic activity was measured at 450 nM absorbance. Lysates prepared from HAEC exposed to 0 (Vehicle Control) – 1 mM ISOPOOH, 1 mM DTT (reducing control), or 1 mM H_2O_2 (positive control) for 10 min were assayed for GAPDH activity, measured as the reduction of NAD + to NADH. GAPDH activity during the linear region of the curves (initial 5 min) was statically lower in HAEC exposed to 1 mM ISOPOOH (0.11 nmol NADH/min) compared to control (0.869 nmol NADH/min). During acquisition, 1 mM DTT was added at the indicated time. All values are presented as mean ± SEM, n = 3. Comparisons were performed using a repeated measures ANOVA with multiple treatment groups and post-hoc Tukey's multiple comparisons test, *$p \leq 0.05$.

ISOPOOH exposure in the form of increased synthesis of NADPH, suggests shunting of glucose from glycolysis to the pentose phosphate pathway [7]. We also have shown that limiting glucose availability increases HAEC sensitivity to ISOPOOH induced oxidative stress, thus further supporting the requirement of glucose metabolism as a means of forming cellular reducing equivalents like NAPDH. Peralta et al. showed that GAPDH oxidation occurs through a proton relay mechanism within the GAPDH catalytic domain [12]. The relay network is geared specifically to the molecular structure of H_2O_2; thus, the mechanism by which organic peroxides and hydroperoxides act is still unclear.

The finding that ISOPOOH exposure of HAEC induces a reversible oxidation of GAPDH protein that inhibits its glycolytic activity is consistent with GAPDH oxidation acting as a molecular redox switch [38]. Furthermore, the inability of the hydroxy analog of 9-HpODE to elicit a similar effect to that of ISOPOOH on GAPDH activity indicates the essentiality of the peroxide moiety in this effect. Supporting this conclusion, it has been previously shown that inorganic and long chain lipid hydroperoxides can induce protein sulfenylation [22]. In this study, we demonstrate that ISOPOOH, an environmentally relevant, short chain hydroperoxide is also capable of oxidizing and inhibiting the enzymatic activity of intracellular GAPDH. We have previously shown that ISOPOOH is a potent initiator of lipid peroxidation [6]. We also report here that long chain lipid hydroperoxides such as 9-HpODE can sulfenylate GAPDH and that linolein hydroperoxides inhibit its glycolytic activity. Therefore, in a cellular system, the involvement of secondary lipid hydroperoxides formed by the reaction of ISOPOOH with unsaturated membrane fatty acids in oxidizing and inhibiting GAPDH activity cannot be ruled out. Therefore, the generation of secondary and tertiary hydroperoxide species from ISOPOOH exposure of HAEC is an area of future investigation that may lead to the elucidation of the mechanisms through which oxidative modifications of key regulatory proteins such as GAPDH regulate cellular homeostasis and cellular bioenergetics in HAEC undergoing redox stress from environmental exposures.

The observation that ISOPOOH exposure of HAEC leads to sulfonylation of intracellular GAPDH further emphasizes the concept that redox systems are dynamic. Cysteinyl thiol oxidation is an initiating step in the regulation of many redox regulatory proteins [19,33]. One such example is the peroxiredoxin family of enzymes, where oxidation of the peroxidatic cysteine to a sulfenic acid is essential for the formation of a disulfide with a vicinal resolving cysteine [13,16]. Sulfenic acid formation serves as a branching point for multiple competing post-translational modifications such as gluthionylation, persulfidation and sulfhydration, as well as sulfinylation of intracellular proteins [33,39–41]. Some of these modifications appear to serve to protect reactive sulfhydryl groups from being overoxidized to sulfinic and sulfonic derivatives which can result in the irreversible loss of protein functionality [39]. While peroxides of various types have been shown to induce protein thiol oxidation, it has been shown recently that redox-regulated proteins are not the direct targets of hydroperoxides, but rather that oxidation of critical thiols is mediated by thiol peroxidases such as peroxiredoxins. For instance, it was reported that peroxiredoxin-2 transfers oxidative equivalents to the redox-regulated transcription factor STAT3, suggesting that antioxidant pathways and redox signaling pathways are interconnected through peroxidase-mediated thiol oxidation [14,16].

In summary, we investigated protein thiol oxidation in HAEC as a mechanism of redox stress imposed by an acute exposure to the environmental hydroperoxide, ISOPOOH, derived from biogenic isoprene. Our findings show that exposure of HAEC to ISOPOOH induces intracellular protein thiol oxidation, hyper-oxidation of intracellular GAPDH, and the loss of GAPDH enzymatic activity. Taken together with our previous report [7], these findings highlight the notion that ISOPOOH exposure of HAEC leads to alterations in homeostatic cellular bioenergetics and demonstrates that ISOPOOH is a redox-active environmental hydroperoxide that contributes to the oxidative burden imposed by direct exposure of the human airway epithelium to secondary organic aerosols.

Disclaimer

The research described in this article has been reviewed by the Center for Public Health and Environmental Assessment, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does the mention of trade names of commercial products constitute endorsement or recommendation for use.

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CRediT authorship contribution statement

Syed Masood: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hye-Young H. Kim:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis, Conceptualization. **Edward R. Pennington:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis. **Keri A. Tallman:** Writing – review & editing, Validation, Resources, Methodology. **Ned A. Porter:** Writing – review & editing, Resources, Conceptualization. **Philip A. Bromberg:** Writing – review & editing, Supervision, Conceptualization. **Rebecca L. Rice:** Writing – review & editing, Resources. **Avram Gold:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Zhenfa Zhang:** Writing – review & editing, Resources. **James M. Samet:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2024.103199.

References

[1] A.P. Teng, J.D. Crounse, P.O. Wennberg, Isoprene peroxy radical dynamics, J. Am. Chem. Soc. 139 (2017) 5367–5377, https://doi.org/10.1021/jacs.6b12838.
[2] S.N. Pandis, R.A. Harley, G.R. Cass, J.H. Seinfeld, Secondary organic aerosol formation and transport, Atmos. Environ., Part A 26 (1992) 2269–2282, https://doi.org/10.1016/0960-1686(92)90358-R.
[3] A.J. Kramer, W. Rattanavaraha, Z. Zhang, A. Gold, J.D. Surratt, Y.-H. Lin, Assessing the oxidative potential of isoprene-derived epoxides and secondary organic aerosol | Enhanced Reader, Atmos. Environ. 130 (2016) 211–218.
[4] M. Arashiro, Y.H. Lin, K.G. Sexton, Z. Zhang, I. Jaspers, R.C. Fry, W.G. Vizuete, A. Gold, J.D. Surratt, In vitro exposure to isoprene-derived secondary organic aerosol by direct deposition and its effects on COX-2 and IL-8 gene expression, Atmos. Chem. Phys. 16 (2016) 14079–14090, https://doi.org/10.5194/acp-16-14079-2016.
[5] Y.-H.H. Lin, M. Arashiro, P.W. Clapp, T. Cui, K.G. Sexton, W. Vizuete, A. Gold, I. Jaspers, R.C. Fry, J.D. Surratt, Gene expression profiling in human lung cells exposed to isoprene-derived secondary organic aerosol, Environ. Sci. Technol. 51 (2017) 8166–8175, https://doi.org/10.1021/acs.est.7b01967.
[6] S. Masood, E.R. Pennington, S.O. Simmons, P.A. Bromberg, S.R. Shaikh, R.L. Rice, A. Gold, Z. Zhang, J.M. Samet, Live cell imaging of oxidative stress in human airway epithelial cells exposed to isoprene hydroxyhydroperoxide, Redox Biol. 51 (2022) 102281, https://doi.org/10.1016/j.redox.2022.102281.
[7] E.R. Pennington, S. Masood, S.O. Simmons, L. Dailey, P.A. Bromberg, R.L. Rice, A. Gold, Z. Zhang, W. Wu, Y. Yang, J.M. Samet, Real-time redox adaptations in human airway epithelial cells exposed to isoprene hydroxy hydroperoxide, Redox Biol. 61 (2023) 102646, https://doi.org/10.1016/j.redox.2023.102646.
[8] Y.H. Lin, M. Arashiro, E. Martin, Y. Chen, Z. Zhang, K.G. Sexton, A. Gold, I. Jaspers, R.C. Fry, J.D. Surratt, Isoprene-derived secondary organic aerosol induces the expression of oxidative stress response genes in human lung cells, Environ. Sci. Technol. Lett. 3 (2016) 250–254, https://doi.org/10.1021/acs.estlett.6b00151.
[9] A.E.M. Beedle, S. Lynham, S. Garcia-Manyes, Protein S-sulfenylation is a fleeting molecular switch that regulates non-enzymatic oxidative folding, Nat. Commun. 7 (2016), https://doi.org/10.1038/ncomms12490.
[10] J.D. Keyes, D. Parsonage, R.D. Yammani, L.C. Rogers, C. Kesty, C.M. Furdui, K. J. Nelson, L.B. Poole, Endogenous, regulatory cysteine sulfenylation of ERK kinases in response to proliferative signals, Free Radic. Biol. Med. 112 (2017) 534–543, https://doi.org/10.1016/j.freeradbiomed.2017.08.018.
[11] P.A. Wages, K.S. Lavrich, Z. Zhang, W.-Y.Y. Cheng, E. Corteselli, A. Gold, P. Bromberg, S.O. Simmons, J.M. Samet, Protein sulfenylation: a novel readout of environmental oxidant stress, Chem. Res. Toxicol. 28 (2015) 2411–2418, https://doi.org/10.1021/acs.chemrestox.5b00424.
[12] D. Peralta, A.K. Bronowska, B. Morgan, É. Dóka, K. Van Laer, P. Nagy, F. Gräter, T. P. Dick, A proton relay enhances H2O2 sensitivity of GAPDH to facilitate metabolic adaptation, Nat. Chem. Biol. 11 (2015) 156–163, https://doi.org/10.1038/nchembio.1720.
[13] M. Lo Conte, K.S. Carroll, The redox biochemistry of protein sulfenylation and sulfinylation, J. Biol. Chem. 288 (2013) 26480–26488, https://doi.org/10.1074/jbc.R113.467738.
[14] M.C. Sobotta, W. Liou, S. Stöcker, D. Talwar, M. Oehler, T. Ruppert, A.N.D. Scharf, T.P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling, Nat. Chem. Biol. 11 (2015) 64–70, https://doi.org/10.1038/nchembio.1695.
[15] L. Flohé, S. Toppo, L. Orian, The glutathione peroxidase family: discoveries and mechanism, Free Radic. Biol. Med. 187 (2022) 113–122, https://doi.org/10.1016/J.FREERADBIOMED.2022.05.003.
[16] S.G. Rhee, S.W. Kang, T.-S.S. Chang, W. Jeong, K. Kim, Peroxiredoxin, a novel family of peroxidases, IUBMB life (international union biochem, Mol. Biol. Life). 52 (2001) 35–41, https://doi.org/10.1080/15216540252774748.
[17] E.A. Gibbs-Flournoy, S.O. Simmons, P.A. Bromberg, T.P. Dick, J.M. Samet, Monitoring intracellular redox changes in ozone-exposed airway epithelial cells, Environ. Health Perspect. 121 (2013) 312–317, https://doi.org/10.1016/B978-0-323-08593-9.00020-6.
[18] S.G. Rhee, Overview on peroxiredoxin, Mol. Cells. 39 (2016) 1, https://doi.org/10.14348/MOLCELLS.2016.2368.
[19] C. Espinosa-Diez, V. Miguel, D. Mennerich, T. Kietzmann, P. Sánchez-Pérez, S. Cadenas, S. Lamas, Antioxidant responses and cellular adjustments to oxidative stress, Redox Biol. 6 (2015) 183–197, https://doi.org/10.1016/j.redox.2015.07.008.
[20] L. Torrente, G.M. DeNicola, GAPDH redox redux—rewiring pentose phosphate flux, Nat. Metab. 54 (5) (2023) 538–539, https://doi.org/10.1038/s42255-021-00523-3, 2023.
[21] N. Seidler, GAPDH: Biological Properties and Diversity, 2013.
[22] K.J. Nelson, C. Klomsiri, S.G. Codreanu, L. Soito, D.C. Liebler, L.C. Rogers, L. W. Daniel, L.B. Poole, Chapter 4 - use of dimedone-based chemical probes for sulfenic acid detection: methods to visualize and identify labeled proteins, Methods Enzymol. 473 (2010), https://doi.org/10.1016/S0076-6879(10)73004-4.
[23] C. Klomsiri, K.J. Nelson, E. Bechtold, L. Soito, L.C. Johnson, W.T. Lowther, S. E. Ryu, S.B. King, C.M. Furdui, L.B. Poole, Use of dimedone-based chemical probes for sulfenic acid detection: evaluation of conditions affecting probe incorporation into redox-sensitive proteins, Methods Enzymol. 473 (2010) 77–94, https://doi.org/10.1016/S0076-6879(10)73003-2.
[24] M. Riva, S.H. Budisulistiorini, Y. Chen, Z. Zhang, E.L.D. Ambro, X. Zhang, A. Gold, B.J. Turpin, J.A. Thornton, M.R. Canagaratna, J.D. Surratt, Chemical characterization of secondary organic aerosol from oxidation of isoprene hydroxyhydroperoxides, Environ. Sci. Technol. 50 (2016) 9889–9899, https://doi.org/10.1021/acs.est.6b02511.
[25] Y. Li, H.-D.D. Hao, Q. Zhang, Y. Wu, A broadly applicable mild method for the synthesis of gem-diperoxides from corresponding ketones or 1,3-dioxolanes, Org. Lett. 11 (2009) 1615–1618, https://doi.org/10.1021/ol900262t.
[26] Y. Li, H.-D.D. Hao, Y. Wu, Facile ring-opening of oxiranes by H2O2 catalyzed by phosphomolybdic acid, Org. Lett. 11 (2009) 2691–2694, https://doi.org/10.1021/ol900811m.
[27] A.L. Cozens, M.J. Yezzi, K. Kunzelmann, T. Ohrui, L. Chin, K. Eng, W.E. Finkbeiner, J.H. Widdicombe, D.C. Gruenert, CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells, Am. J. Respir. Cell Mol. Biol. 10 (1994) 38–47, https://doi.org/10.1165/ajrcmb.10.1.7507342.
[28] T.E. Taylor-Clark, B.J. Undem, Ozone activates airway nerves via the selective stimulation of TRPA1 ion channels, J. Physiol. 588 (2010) 423–433, https://doi.org/10.1113/jphysiol.2009.183301.
[29] P.A. Wages, Detecting protein sulfenylation in cells exposed to a toxicant, Curr. Protoc. Toxicol. 2017 (2017) 1–12, https://doi.org/10.1002/cptx.16.
[30] K. Windsor, T.C. Genaro-Mattos, H.Y.H. Kim, W. Liu, K.A. Tallman, S. Miyamoto, Z. Korade, N.A. Porter, Probing lipid-protein adduction with alkynyl surrogates: application to Smith-Lemli-Opitz syndrome, J. Lipid Res. 54 (2013) 2842–2850, https://doi.org/10.1194/jlr.M041061.
[31] C.E. Paulsen, T.H. Truong, F.J. Garcia, A. Homann, V. Gupta, S.E. Leonard, K. S. Carroll, Peroxide-dependent sulfenylation of the EGFR catalytic site enhances kinase activity, Nat. Chem. Biol. 8 (2012) 57–64, https://doi.org/10.1038/nchembio.736.
[32] H.J. Forman, M.J. Davies, A.C. Krämer, G. Miotto, M. Zaccarin, H. Zhang, F. Ursini, Protein cysteine oxidation in redox signaling: caveats on sulfenic acid detection and quantification, Arch. Biochem. Biophys. 617 (2017) 26–37, https://doi.org/10.1016/j.abb.2016.09.013.
[33] L.B. Poole, P.A. Karplus, A. Claiborne, Protein sulfenic acids in redox signaling, Annu. Rev. Pharmacol. Toxicol. 44 (2004) 325–372, https://doi.org/10.1146/annurev.pharmtox.44.101802.121735.
[34] Y. Liu, J. Brito, M.R. Dorris, J.C. Rivera-Rios, R. Seco, K.H. Bates, P. Artaxo, S. Duvoisin, F.N. Keutsch, S. Kim, A.H. Goldstein, A.B. Guenther, A.O. Manzi, R. A. Souza, S.R. Springston, T.B. Watson, K.A. McKinney, S.T. Martin, Isoprene photochemistry over the Amazon rainforest, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 6125–6130, https://doi.org/10.1073/pnas.1524136113.
[35] M. Arashiro, Y.-H.H. Lin, Z. Zhang, K.G. Sexton, A. Gold, I. Jaspers, R.C. Fry, J. D. Surratt, Effect of secondary organic aerosol from isoprene-derived hydroxyhydroperoxides on the expression of oxidative stress response genes in human bronchial epithelial cells, Environ. Sci. Process. Impacts. 20 (2018) 332–339, https://doi.org/10.1039/c7em00439g.
[36] J.M. Samet, S.L. Zeger, F. Dominici, F. Curriero, I. Coursac, D.W. Dockery, The National Morbidity, Mortality, Part II: Morbidity and Mortality from Air Pollution in the United States Final Version Includes a Commentary by the Institute's Health Review Committee, 2000.
[37] A. Stincone, A. Prigione, T. Cramer, M.M.C. Wamelink, K. Campbell, E. Cheung, V. Olin-Sandoval, N.M. Grüning, A. Krüger, M. Tauqeer Alam, M.A. Keller, M. Breitenbach, K.M. Brindle, J.D. Rabinowitz, M. Ralser, The return of metabolism: biochemistry and physiology of the pentose phosphate pathway, Biol. Rev. 90 (2015) 927–963, https://doi.org/10.1111/BRV.12140.
[38] C. Tristan, N. Shahani, T.W. Sedlak, A. Sawa, The diverse functions of GAPDH: views from different subcellular compartments, Cell. Signal. 23 (2011) 317–323, https://doi.org/10.1016/j.cellsig.2010.08.003.
[39] T. Ida Dóka, M. Dagnell, Y. Abiko, N.C. Luong, N. Balog, T. Takata, B. Espinosa, A. Nishimura, Q. Cheng, Y. Funato, H. Miki, J.M. Fukuto, J.R. Prigge, E.E. Schmidt, E.S.J. Arnér, Y. Kumagai, T. Akaike, P. Nagy, Control of protein function through oxidation and reduction of persulfidated states, Sci. Adv. 6 (2020), https://doi.org/10.1126/SCIADV.AAX8358/SUPPL_FILE/AAX8358_SM.PDF.
[40] M.M. Gallogly, J.J. Mieyal, Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress, Curr. Opin. Pharmacol. 7 (2007) 381–391, https://doi.org/10.1016/J.COPH.2007.06.003.
[41] C.W. Huang, P.K. Moore, H2S synthesizing enzymes: biochemistry and molecular aspects. https://doi.org/10.1007/978-3-319-18144-8_1, 2015.