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FORUM REVIEW ARTICLE

A Metabolic Paradigm for Hydrogen Sulfide Signaling *via* Electron Transport Chain Plasticity

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Abstract

Significance: A burgeoning literature has attributed varied physiological effects to hydrogen sulfide (H_2S), which is a product of eukaryotic sulfur amino acid metabolism. Protein persulfidation represents a major focus of studies elucidating the mechanism underlying H_2S signaling. On the contrary, the capacity of H_2S to induce reductive stress by targeting the electron transport chain (ETC) and signal by reprogramming redox metabolism has only recently begun to be elucidated.
Recent Advances: In contrast to the nonspecific reaction of H_2S with oxidized cysteines to form protein persulfides, its inhibition of complex IV represents a specific mechanism of action. Studies on the dual impact of H_2S as an ETC substrate and an inhibitor have led to the exciting discovery of ETC plasticity and the use of fumarate as a terminal electron acceptor. H_2S oxidation combined with complex IV targeting generates mitochondrial reductive stress, which is signaled through the metabolic network, leading to increased aerobic glycolysis, glutamine-dependent reductive carboxylation, and lipogenesis.
Critical Issues: Insights into H_2S-induced metabolic reprogramming are ushering in a paradigm shift for understanding the mechanism of its cellular action. It will be critical to reevaluate the physiological effects of H_2S, for example, cytoprotection against ischemia-reperfusion injury, through the framework of metabolic reprogramming and ETC remodeling by H_2S.
Future Directions: The metabolic ramifications of H_2S in other cellular compartments, for example, the endoplasmic reticulum and the nucleus, as well as the intersections between hypoxia and H_2S signaling are important future directions that merit elucidation. *Antioxid. Redox Signal.* 38, 57–67.

Keywords: metabolism, mitochondria, redox metabolism, electron transport chain, reductive stress

A MYRIAD OF signaling modules maintain metabolic homeostasis and orchestrate cellular responses to environmental cues such as O_2 and nutrient fluctuations. Mitochondria, as hubs of carbon and energy metabolism, are important constituents of signaling networks. Central to oxidative metabolism, mitochondria recycle the reducing equivalents in NADH and $FADH_2$ that are captured during catabolism, and funnel them to the lipidic electron acceptor, coenzyme Q (CoQ or ubiquinone), generating $CoQH_2$ (or ubiquinol). Several mitochondrial dehydrogenases intersect at the CoQ pool, a redox node that influences amino acid, lipid, nucleotide, choline, and sulfide metabolic pathways (Fig. 1). Of the various CoQ users, complex I (NADH:ubiquinone oxidoreductase) and complex II (succinate dehydrogenase) are components of the electron transport chain (ETC). $CoQH_2$ transfers electrons to complex III (cytochrome bc_1), which in turn reduces cytochrome c, and subsequently, complex IV (cytochrome c oxidase).

In the canonical operation of the ETC, O_2 serves as a terminal electron acceptor and is converted to H_2O at complex IV. In a recently described noncanonical route, $CoQH_2$ transfers electrons to complex II, and fumarate serves as the terminal electron acceptor (39, 78). Electron transfer at complexes I, III, and IV is coupled to proton translocation

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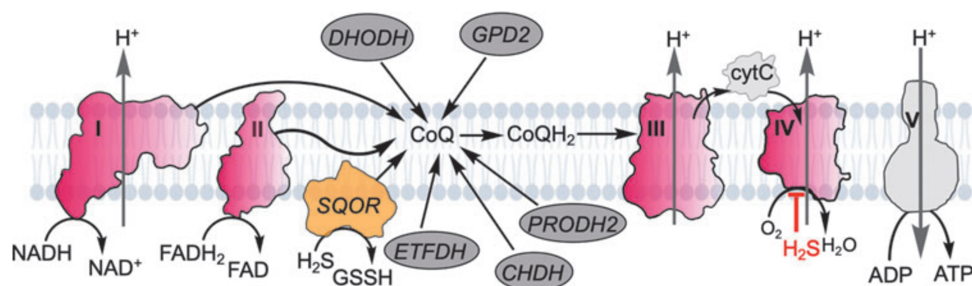

FIG. 1. Intersection of H_2S and mitochondrial energy metabolism. CoQ is an electron acceptor that is used by several enzymes including DHODH, GPD2, ETFDH, CHDH, PRODH2, and SQOR, which oxidizes H_2S to GSSH. In the canonical operation of the ETC, $CoQH_2$ is recycled by complex III. Both H_2S oxidation and its inhibition of complex IV lead to a reductive shift in the CoQ pool that is propagated upstream to the NADH/NAD^+ and $FADH_2$/FAD pools. CHDH, choline dehydrogenase; CoQ, coenzyme Q; cytC, cytochrome C; DHODH, dihydroorotate dehydrogenase; ETC, electron transport chain; ETFDH, electron transfer flavoprotein quinone oxidoreductase; FAD, flavin adenine dinucleotide; GPD2, glycerol 3-phosphate dehydrogenase; GSSH, glutathione persulfide; H_2S, hydrogen sulfide; PRODH2, proline dehydrogenase 2; SQOR, sulfide quinone oxidoreductase.

across the inner mitochondrial membrane, generating a proton motive force that drives ATP synthesis by complex V and powers other functions such as transport.

A decrease in the O_2 concentration triggers a major signaling response that is initiated by stabilization of HIF1, the hypoxia inducible factor, which regulates the expression of numerous target genes (72). Hydrogen sulfide (H_2S) is another modulator of redox metabolism whose primary target is the ETC (38). H_2S is both an ETC substrate (22) and an inhibitor (60), and recent studies have revealed that H_2S induces reductive stress with wide-ranging metabolic effects that fan out from the mitochondrion (38). At H_2S concentrations that inhibit complex IV and therefore access to O_2 as a terminal electron acceptor, cells exploit a fork in the ETC, using fumarate as an alternate electron acceptor (39).

In this noncanonical operation of the ETC, electron flow is redirected away from complex III to complex II, leading to CoQ regeneration, which is supported by fumarate that is sourced from different pathways. A similar use of the fork in the ETC is observed under hypoxic conditions (78). Some of the metabolic consequences of H_2S-induced reductive stress include enhanced aerobic glycolysis (84), reductive carboxylation of glutamine (48), and lipid biogenesis (8). The emerging metabolic paradigm for H_2S signaling, which ripples out from the ETC, is the focus of this review.

Enzymology of H_2S Homeostasis: Production and Oxidation

Sulfide production

H_2S is a product of mammalian sulfur metabolism and is derived from the amino acids, cysteine and homocysteine (Fig. 2). While the canonical reactions catalyzed by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE)

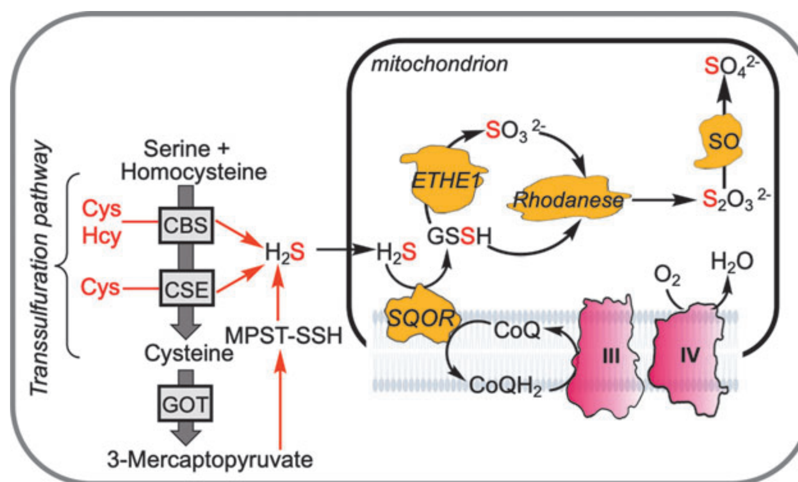

FIG. 2. Sulfide production and oxidation pathways. H_2S is derived (*red arrows*) from cysteine and/or homocysteine in reactions catalyzed by CBS and CSE, or from 3-mercaptopyuvate in a reaction catalyzed by MPST, which releases H_2S from a persulfide intermediate (MPST-SSH). The cysteine-generating canonical reactions catalyzed by CBS and CSE constitute the transsulfuration pathway. H_2S is oxidized in the mitochondrion to either thiosulfate or sulfate. The oxidation pathway comprises SQOR, ETHE1 (a persulfide dioxygenase), rhodanese (a thiosulfate sulfur transferase), and SO. CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; GOT, glutamate-oxaloacetate transaminase or cysteine aminotransferase; MPST, mercaptopyruvate sulfurtransferase; SO, sulfite oxidase.

in the transsulfuration pathway generate cysteine, substrate promiscuity and lax substrate specificity also support H_2S synthesis by both enzymes (3, 30, 31). At cellular concentrations of substrates, the dominant route for H_2S synthesis by CBS is *via* the β-elimination of cysteine and homocysteine, and by CSE, *via* the α,β-elimination of cysteine (10, 76). Both CBS and CSE are PLP enzymes (74), while CBS additionally harbors a heme cofactor (75), which makes it susceptible to gas regulation by $^{\bullet}$NO and CO (66, 80). The transsulfuration pathway enzymes also utilize cysteine (CBS, CSE) (25, 90) and homocysteine (CSE) (90) as substrates, generating the corresponding persulfides.

Mercaptopyruvate sulfurtransferase (MPST) uses 3-mercaptopyruvate, which is a product of the transamination of cysteine catalyzed by glutamate-oxaloacetate transaminase (GOT), a component of the malate–aspartate shuttle (59, 92). The immediate product of the MPST reaction is an enzyme-bound persulfide, which can be transferred to a thiophilic acceptor such as thioredoxin or cysteine, from which H_2S is subsequently released. Interestingly, reduced thioredoxin at physiologically relevant concentrations increases the K_M for 3-mercaptopyruvate, effectively inhibiting MPST activity (91). Under oxidizing conditions, this inhibition is alleviated (91), which might represent a mechanism for regulating MPST-dependent persulfidation of alternate targets. While CBS and CSE reside in the cytoplasm, the MPST1 and MPST2 isoforms are located in the cytoplasm and mitochondrion, respectively (91).

Given the multitude of reactions catalyzed by CBS (76) and CSE (10), it is important to understand how the transsulfuration pathway meets cellular demands for cysteine *versus* H_2S. CBS is a major regulatory hub; it is inhibited by sumoylation (1, 34), CO (7, 66, 81), $^{\bullet}$NO (80), and nitrite (21), but is activated by AdoMet (18) and by glutathionylation (63). Furthermore, some of the allosteric effectors interact as exemplified by the observation that AdoMet increases the affinity of ferrous CBS for $^{\bullet}$NO (twofold) and CO (fivefold) and thereby sensitizes the enzyme to inhibition by these gas regulators (83).

A novel heme-dependent switch in CBS can flip the operating preference of the transsulfuration pathway from cysteine to H_2S synthesis (33). Under basal conditions or upon AdoMet activation of ferric CBS, the predominant flux is toward cysteine synthesis. On the contrary, conditions that induce CO or $^{\bullet}$NO synthesis and trigger H_2S-signaling such as endoplasmic reticulum stress (15) inhibit CBS. With reduced competition from the CBS product, cystathionine, CSE switches to cysteine-dependent H_2S synthesis (33). In addition, elevated homocysteine due to CBS deficiency or inhibition can stimulate CSE-dependent H_2S biogenesis (10). The implications of metabolic track switching by the transsulfuration pathway enzymes are potentially significant. Since compromised endoplasmic reticulum function is a significant contributing factor in the development of cardiovascular, neurodegenerative, neoplastic, and metabolic diseases (6), the track switching model suggests that H_2S-dependent signaling cascades might be perturbed in these complex diseases.

Global suppression of protein synthesis *via* the integrated stress response or due to mTORC1 inhibition induces translation of the activating transcription factor ATF4, eliciting a defensive response. CSE expression is upregulated by ATF4, which in turn increases H_2S production and is a component of the ATF4-dependent stress and antiaging response (15, 79). On the contrary, 1,25-dihydroxyvitamin D3 regulates CBS and increases transsulfuration pathway activity in pre-osteoblastic cells; the effects on H_2S production were not studied (37).

Sulfide oxidation

A mitochondrial resident sulfide oxidation pathway clears H_2S (23, 49) and shields the ETC from respiratory poisoning (Fig. 2) (48). Sulfide quinone oxidoreductase (SQOR) catalyzes the committing step in this pathway converting H_2S to glutathione persulfide (GSSH) (42, 56) and transfers electrons to CoQ *via* an flavin adenine dinucleotide cofactor (43). The next step in the pathway results in the conversion of GSSH to sulfite in a reaction catalyzed by the persulfide dioxygenase, ETHE1 (29, 32). Rhodanese catalyzes a sulfur transfer reaction from GSSH to sulfite forming thiosulfate (47), or sulfite can be further oxidized to sulfate in cells harboring sulfite oxidase (36). SQOR has an unusual cysteine trisulfide redox cofactor (27, 45) that confers an $\sim 10^5$-fold rate enhancement of the sulfide addition reaction over a cysteine disulfide (44).

The sulfane sulfur can be transferred to a variety of acceptors other than GSH, including coenzyme A and methanethiol, forming the corresponding persulfides or it can be transferred to sulfite forming thiosulfate (28, 42). GSSH is, however, predicted to be the dominant product at physiologically relevant concentrations of acceptors (41, 42).

The sulfide oxidation pathway enzymes exhibit apical localization in colonic crypts (48), likely representing a host adaptation to high H_2S exposure from microbial metabolism at the host–microbiota interface (53). Colorectal cancer tissues exhibit elevated levels of the sulfide oxidation pathway enzymes compared with normal margins (48). Sulfide preconditioning increases hypoxia tolerance in male mice *via* upregulation of SQOR in the brain; the higher expression level of SQOR in the brain of female mice is correlated with increased tolerance to hypoxia (54). A neuron-specific increase in SQOR expression confers resistance to hypoxia and to ischemic brain injury (54). The mechanism underlying the hypoxic increase in sulfide, that is, through increased synthesis and/or decreased oxidation, remains to be established. An SQOR inhibitor ST1, with an IC_{50} value of 29 n*M*, is competitive with respect to CoQ, and cardioprotective in a mouse model of heart failure with reduced ejection fraction (26).

In addition to the sulfide oxidation pathway, H_2S can also be oxidized by ferric heme proteins, including hemoglobin (86, 87), myoglobin (5), neuroglobin (70), and cytochrome c (85). Under aerobic conditions, the initially formed ferric sulfide is oxidized to a mixture of thiosulfate and ferrous heme bound polysulfides (Fig. 3). This mode of H_2S clearance might be important in red blood cells, which lack mitochondria and in cells with low SQOR expression.

Complex I and SQOR compete for the CoQ pool

With its dependence on CoQ as an electron acceptor, SQOR is potentially impacted by and influences other CoQ users including complex I. Complex I is a quantitatively significant competitor for the CoQ pool, feeding electrons from NADH oxidation to the ETC. Thus, pharmacological inhibition by rotenone or genetic ablation of complex I (by

FIG. 3. Scheme outlining the mechanism of sulfide oxidation to thiosulfate and polysulfides catalyzed by ferric hemeproteins.

NDUFS3 knockdown) increases the cellular efficiency of H_2S clearance (39). Consistent with a competitive relationship, ectopic expression of the water-forming NADH oxidase *Lb*NOX (82) in the mitochondria, but not in the cytoplasm, accelerates H_2S oxidation by dissipating the NADH/NAD^+ pool (39). Similarly, depletion of the mitochondrial NADPH pool by TPNOX (12) enhances H_2S clearance, while the nicotinamide nucleotide transhydrogenase (NNT) inhibitor, 4-chloro-7-nitrobenzofurazan chloride, attenuates the effect of TPNOX (39).

These effects can be explained by the activity of NNT, an electrogenic mitochondrial inner membrane transhydrogenase that converts NADH and $NADP^+$ to NAD^+ and NADPH, respectively. Under pathophysiological conditions, NNT catalyzes the reverse reaction, using NADPH to support NADH and ATP generation, but in so doing depletes cellular antioxidant capacity (62).

Complex II reversal supports fumarate-dependent H_2S oxidation

In addition to their capacity for endogenous H_2S synthesis, colonic epithelial cells are exposed to inhibitory concentrations of exogenous H_2S that is derived from gut microbial metabolism and can range from $\sim$0.2 to 2.4 m*M* (14, 53). We have recently discovered that when complex IV is inhibited, H_2S oxidation is sustained *via* a redox cycle between SQOR and complex II, operating in reverse (39). Under these conditions, fumarate is utilized as a terminal electron acceptor, leading to succinate accumulation, while $CoQH_2$ is oxidized to CoQ, enabling H_2S oxidation (39). When CoQ is limiting, an alternative route for electron transfer from SQOR is to O_2, generating H_2O_2 (39). The electron transfer rate from reduced SQOR to O_2 is, however, $\sim$1000-fold lower than to CoQ, and therefore unlikely to be significant if complex II is available to recycle $CoQH_2$.

On the contrary, in cells where complex II and/or fumarate are limiting, electron transfer from SQOR to O_2 could represent a route for sulfide oxidation and a source of reactive oxygen species (ROS) production.

Fumarate is derived from both the purine nucleotide cycle and the malate–aspartate shuttle (Fig. 4) (39). The enzymes in the purine nucleotide cycle, adenylosuccinate synthetase, adenylosuccinate lyase, and adenosine monophosphate (AMP) deaminase, are found in most tissues. AMP deaminase hydrolyzes AMP into inosine monophosphate and ammonia. Adenylosuccinate synthetase catalyzes the condensation of inosine 5′-monophosphate and aspartate to adenylosuccinate in the presence of guanosine triphosphate. Adenylosuccinate lyase cleaves the C-N bond of adenylosuccinate, yielding AMP and fumarate, and it also catalyzes the cleavage of 1-(5-phosphoribosyl)-4-(*N*-succinocarboxamide)-5-aminoimidazole to 1-(5-phosphoribosyl)-5-amino-4-imidazolecarboxiamide and fumarate, in the *de novo* purine biosynthetic pathway. The purine nucleotide cycle is important for ATP generation during intense exercise in muscles and the regeneration of Krebs cycle intermediates (51).

The malate–aspartate shuttle translocates reducing equivalents from the cytoplasm to the mitochondrion for oxidative

FIG. 4. Fumarate serves as a terminal electron acceptor for H_2S oxidation. When complex IV is inhibited (*e.g.,* by high H_2S concentrations), O_2 cannot be used as a terminal electron acceptor and a new redox cycle between SQOR and complex II recycles $CoQH_2$. The fumarate used to support complex II reversal is derived from the MAS and the PNC. Fumarate hydratase, adenylosuccinate synthetase, adenylosuccinate lyase, and adenosine monophosphate deaminase are fumarate hydratase, adenylosuccinate synthetase, adenylosuccinate lyase, and AMP deaminase, respectively. The *up* and *down red arrows* indicate metabolites that increase and decrease, respectively, in H_2S-treated cells. AMP, adenosine monophosphate; MAS, malate–aspartate shuttle; PNC, purine nucleotide cycle.

phosphorylation (Fig. 4). The cytoplasmic malate dehydrogenase (MDH)1 oxidizes NADH to NAD^+ as it reduces oxaloacetate to malate, which is transferred across the inner mitochondrial membrane using the electroneutral and reversible α-ketoglutarate/malate carrier. The mitochondrial isoform of MDH2 catalyzes the reverse reaction, generating NADH and oxaloacetate, which is converted *via* GOT2 to aspartate. The latter enters the cytoplasm *via* the electrogenic aspartate/glutamate carrier that powers aspartate efflux to the cytoplasm and glutamate influx to the mitochondrion by proton translocation from the inner mitochondrial membrane space.

Finally, cytoplasmic GOT1 converts aspartate to oxaloacetate, completing the shuttle. Knockdown of GOT1 but not GOT2 increased the efficiency of sulfide clearance, suggesting that oxaloacetate in the cytosolic arm of the malate–aspartate shuttle is diverted toward fumarate synthesis (39, 48). In addition to its generation by GOT1, cytosolic oxaloacetate is also derived from citrate cleavage catalyzed by ATP-citrate lyase, as described below.

Metabolomic analysis of the human colorectal adenocarcinoma HT29 cells, reveal that 1 h after (100 μM) H_2S treatment, malate, aspartate, glutamate, and α-ketoglutarate levels are down, while succinate levels are up compared with untreated controls (48). Reduced malate levels could reflect its conversion to fumarate catalyzed by fumarate hydratase (Fig. 4), which in turn supports reverse complex II activity and leads to the observed accumulation of succinate. Since succinate acts as competitive inhibitor of α-ketoglutarate-dependent dioxygenases, H_2S-induced succinate accumulation could impact histone and DNA methylation linking H_2S metabolism to epigenetic regulation (77). Succinate accumulation could also promote protein succinylation, a posttranslational modification, which reportedly increases complex II activity and could further promote H_2S clearance (64).

Physiological Implications of H_2S-Mediated Complex II Reversal

Upregulation of endogenous H_2S synthesis or provision of exogenous H_2S is cytoprotective against ischemic injury if administered during the reperfusion phase [reviewed in Nicholson and Calvert (61)]. We posit that complex II reversal by H_2S is the underlying mechanism of the cytoprotective effect. The twin effects of H_2S on complex II reversal and complex IV inhibition would be predicted to lower the mitochondrial membrane potential and $CoQH_2$ buildup, which are drivers of complex I reversal and ROS formation and injury during reperfusion (11, 67). The relevance of this proposed mechanism for the cardioprotective effects of H_2S warrants testing.

Complex II reversal and the use of fumarate as the terminal electron acceptor are also observed under hypoxic conditions (78). Metabolic labeling studies reveal that organs such as the kidney, liver, and brain constitutively use fumarate as a terminal electron acceptor, while other organs such as the white adipose tissue and heart resort to fumarate oxidation under conditions of exercise stress. In tissues such as the lung, gastrocnemius muscle, pancreas, and thymus on the contrray, succinate oxidation appears to be the dominant reaction catalyzed by complex II.

In addition to H_2S oxidation, $CoQH_2$ recycling by fumarate reduction also supports the activity of other CoQ users, notably dihydroorotate dehydrogenase, thus supporting *de novo* pyrimidine synthesis (78). The mechanisms by which $CoQH_2$ oxidation at complex III *versus* complex II is regulated are not understood. Modulation of the O_2 affinity and/or the O_2 reduction rate at complex IV by the gas regulators H_2S and $^\bullet NO$ has been postulated to be an important mechanism for partitioning electron flow at the ETC fork, and remains to be more completely elucidated (4).

Colonocytes are adapted to tolerate exposure to toxic levels of H_2S and avert its build up by expressing high levels of the sulfide oxidation pathway enzymes (40, 48). Butyrate is a major energy source for colonocytes, and acyl-CoA dehydrogenase (ACADS) catalyzes the oxidation of butyryl-CoA to crotonyl-CoA, transferring electrons to CoQ *via* the ETF/ETF-QO redox protein pair (Fig. 5). In the backdrop of high luminal H_2S exposure, the CoQ pool can become limiting and the observed increase in butyryl-CoA and decrease in crotonyl-CoA levels are consistent with ACADS inhibition (2, 68). We have demonstrated that SQOR catalyzes the synthesis of coenzyme A persulfide (CoA-SSH) (45), which is a tight-binding inhibitor of ACADS (73). We posit that inhibition of ACADS by SQOR-derived CoA-SSH represents a strategy for prioritizing sulfide detoxification. Whether this strategy is additionally facilitated by complex II reversal (Fig. 5) remains to be tested.

Increased colonization of sulfate reducing bacteria and higher fecal sulfide is reportedly associated with ulcerative colitis (65, 69), suggesting the involvement of H_2S in intestinal bowel disease pathogenesis possibly *via* dysregulated butyrate oxidation.

A Metabolic Paradigm for H_2S Signaling

The prevalent view of how H_2S signals is through reactive persulfides and polysulfides, leading to posttranslational modifications on accessible cysteines (35, 58). The issues with the lack of specificity of this mechanism and the challenges

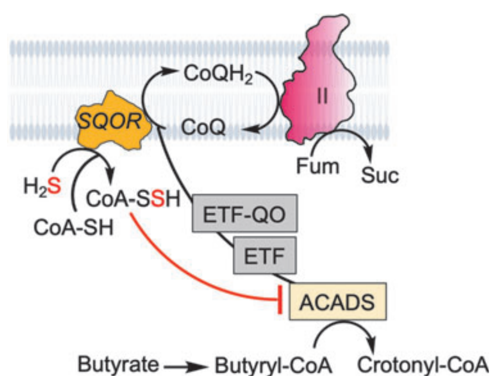

FIG. 5. Model showing interactions between H_2S and butyrate oxidation and complex II reversal. SQOR is promiscuous and can use CoA as a sulfane sulfur acceptor, forming CoA-SSH, which inhibits ACADS. The latter oxidizes butyryl-CoA, which is a major fuel source for colonocytes. Inhibition of ACADS by CoA-SSH relieves competition for the CoQ pool and allows prioritization of H_2S oxidation. CoQ recycling through complex II reversal further supports SQOR-mediated H_2S oxidation. Fum and Suc denote fumarate and succinate, respectively. ACADS, acyl-CoA dehydrogenase.

with rigorously establishing a physiologically relevant and quantitatively significant change in the persulfidation status of target proteins have been reviewed (38). In contrast to protein per- and polysulfidation, complex IV inhibition is a specific and a physiologically established target of H_2S action. Its consequent impact on mitochondrial bioenergetics and cell metabolism has recently begun to be elucidated. Pleiotropic effects ensue upstream of complex IV in the ETC following inhibition by H_2S, which includes a reductive shift in the electron carriers (*i.e.,* $CoQH_2$/CoQ and NADH/NAD^+) (Fig. 6A), which in turn affect redox reactions that are dependent on them (Fig. 6B).

Consistent with this model, pharmacological inhibition or genetic ablation of complex I increases the efficacy of H_2S oxidation (39), presumably by decreasing competition for CoQ. Genetic ablation of SQOR, on the contrary, sensitizes cells to sulfide-dependent inhibition of respiration (48), enhances the reductive shift in the NAD^+ pool in response to H_2S (48, 54), and creates an electron acceptor insufficiency (48). These changes in turn lead to uridine and aspartate becoming limiting for cell proliferation, which can be relieved by exogenous uridine and either aspartate or pyruvate (48), which upon reduction to lactate increases NAD^+ availability (Fig. 6B).

H_2S enhances aerobic glycolysis

Glycolysis and oxidative phosphorylation are tightly regulated to meet cellular demands for NAD^+ to support oxidative metabolism, ATP, and macromolecular precursor synthesis (52). While complex II reversal recycles $CoQH_2$, the contributions of complexes III and IV to proton motive force generation are precluded by this noncanonical mode of ETC operation, impacting the transmembrane potential and ATP synthesis. A decrease in ATP and an increase in ADP levels are observed in H_2S-treated cells (Fig. 6A), which are associated with an increase in aerobic glycolysis (84). Thus, glucose carbons are predominantly diverted to lactate, which sets up a redox neutral cycle between glyceraldehyde 3-phosphate dehydrogenase in the glycolytic pathway and lactate dehydrogenase, and leads to NADH recycling that is independent of the malate–aspartate cycle and the ETC (Fig. 6B).

Since SQOR knockdown enhances the sensitivity of cells to respiratory inhibition by H_2S, upregulation of aerobic glycolysis occurs at a lower concentration (50 μM) of sulfide compared with scrambled controls (100 μM). In contrast, ETHE1 knockdown does not sensitize cells to H_2S-triggered upregulation of aerobic glycolysis (84).

Although the cytoplasmic and mitochondrial NADH pools are interconnected *via* the malate–aspartate shuttle, the effect of H_2S on aerobic glycolysis is sensitive only to the mitochondrial NADH/NAD^+ ratio (84). Thus, while dissipation of the mitochondrial NADH pool increases basal aerobic glycolysis, these cells do not show a further increase in glucose consumption in response to H_2S. GOT1 or GOT2 knockdowns have no effect on H_2S-stimulated aerobic glycolysis, which is consistent with an H_2S-induced disabling of the intercompartmental malate–aspartate shuttle as a carrier of reducing equivalents (48, 84). Specifically, H_2S-induced reductive stress with a consequent shortage of mitochondrial NAD^+ would limit the MDH2-dependent conversion of malate to oxaloacetate. Furthermore, the reductive redirection

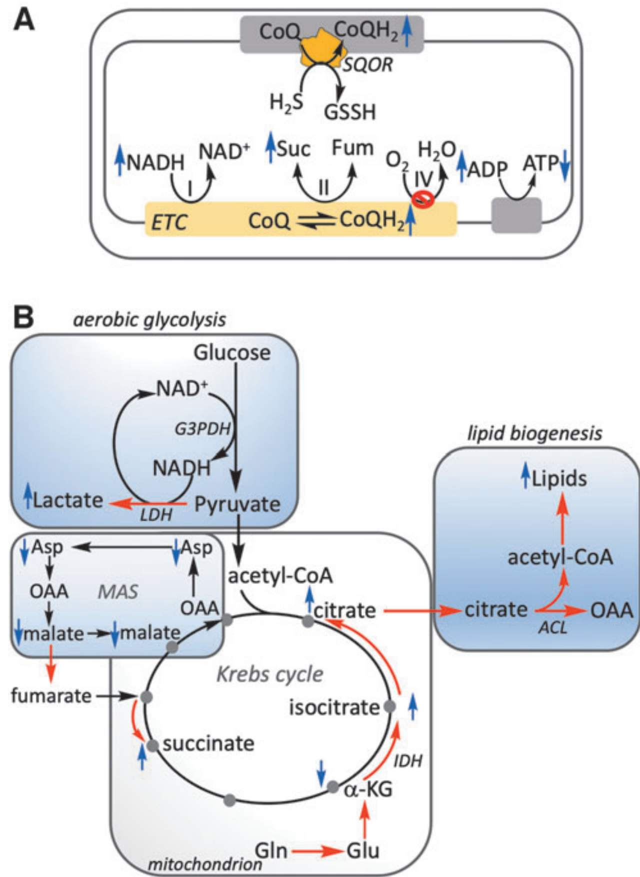

FIG. 6. Metabolic effects of H_2S. (A) H_2S oxidation by SQOR leads to $CoQH_2$ generation, which combined with H_2S inhibition of complex IV results in a reductive shift upstream in the NADH/NAD^+ pool, reversal of complex II, and decreased ATP synthesis. The observed and predicted changes (*up* or *down*) in metabolites are depicted by the *blue arrows*. **(B)** Perturbations in mitochondrial bioenergetics by H_2S have pleiotropic effects on central carbon and energy metabolism that are depicted by the *red arrows*. The metabolic adaptations include enhanced aerobic glycolysis (leading to glucose carbon being secreted as lactate), glutamine-dependent reductive carboxylation, and increased lipid biogenesis. H_2S-induced reductive stress also affects the MAS, which furnishes fumarate to support complex II reversal and leads to aspartate insufficiency, limiting proliferation. The *blue up* and *down arrows* indicate metabolites whose levels increase and decrease, respectively, in response to H_2S treatment. ACL, ATP-citrate lyase; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase.

within the Krebs cycle leading to increased conversion of α-ketoglutarate to citrate would also decrease oxaloacetate availability for the GOT2-dependent synthesis of mitochondrial aspartate.

Finally, a drop in the transmembrane potential would adversely impact aspartate export, which is coupled to glutamate and proton import into the mitochondrion. These changes in the mitochondrial arm of the shuttle are predicted to fan out to the cytoplasm *via* the decreased availability of aspartate for GOT1-dependent synthesis of oxaloacetate and its diversion to fumarate *via* MDH1 and fumarate hydratase (Fig. 4).

Several physiological effects have been associated with H_2S-mediated enhancement of aerobic glycolysis, including the proangiogenic migratory behavior of endothelial cells (50). In this, and a previous study (24), endogenous H_2S biogenesis was enhanced by dietary, specifically sulfur amino acid, restriction, which led to the ATF4-dependent upregulation of CSE and to H_2S synthesis, and conferred protection against hepatic ischemia-reperfusion injury. Increased H_2S-induced glucose consumption has also been associated with inhibition of cardiomyocyte hypertrophy (46). CSE knockdown in human umbilical vein endothelial cells reportedly enhanced glycolysis and increased the release of antiangiogenic factors, soluble Fms-like tyrosine kinase-1 and endoglin that are associated with preeclampsia (71).

However, the possibility that a compensatory increase in CBS expression in CSE knockdown cells contributed to increased rather than decreased H_2S production was not examined. Increased glycolysis at low concentrations of H_2S was concluded to underlie the observed increase in ATP concentrations in murine red blood cells; however, glucose consumption was not measured, and the mechanism underlying the ETC-independent upregulation of glycolysis was not addressed in this study (89).

H_2S stimulates glutamine-dependent reductive carboxylation and lipogenesis

Mitochondrial electron acceptor insufficiency induced by H_2S promotes reductive carboxylation of glutamine-derived α-ketoglutarate to citrate (Fig. 6B). Sulfide exposure of HT29 cells labeled with [U-^{13}C]-labeled glutamine increased the fraction of the $m+5$ isotopolog of citrate, consistent with its generation *via* reductive carboxylation, which leads to the retention of all five glutamine carbons (48). Reductive carboxylation is upregulated during growth factor-dependent (nonmalignant) and growth factor-independent (malignant) cell proliferation (13, 57) when the ETC operation is impaired due to disease-associated mitochondrial DNA mutations (9), or hypoxia (55, 88).

H_2S promoted the utilization of glutamine-derived carbon for lipid biogenesis in a panel of malignant and nonmalignant cell lines (HT29, HCEC, HCT116, J774, $143B^{WT}$, and 143^{CytB}), but not in the human hepatocellular carcinoma cell line HepG2 (8). The magnitude of the H_2S-dependent increase in [^{14}C]-glutamine incorporation into lipids varied from $\sim$1.1- to 5-fold under normoxic (20% O_2) conditions (8). Hypoxic culture (2% O_2) also increased [^{14}C]-glutamine incorporation into lipids in these cell lines except for HepG2, which exhibited no change, and J774, which exhibited significantly reduced radiolabel incorporation. Hypoxia, however, blunted the H_2S-stimulated incorporation of glutamine into lipids except in the human osteosarcoma cell line 143B, which exhibited an $\sim$15-fold increase at 2% *versus* an $\sim$5-fold increase in lipid labeling at 20% O_2 (8).

A possible rationale for increased lipid biogenesis is that it counters H_2S-induced reductive stress by consuming reducing equivalents. Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) uses NADPH to carboxylate α-ketoglutarate, while 14 NADPH equivalents are used for the synthesis of a single equivalent of palmitate, a precursor of many lipids. Lipidomic analysis revealed that H_2S elicits numerous changes in lipid profiles including an increase in triacylglycerides and a decrease in phosphatidylcholine. Sulfide did not increase the flux of glucose into the pentose phosphate pathway in malignant (HT29) or nonmalignant (HCEC) colonocytes. Thus, the NADPH pool that fuels lipid biosynthesis could be derived from mitochondrial NADH *via* the activity of NNT (19). This is consistent with the observation that targeted dissipation of the mitochondrial but not the cytoplasmic NAD(P)H pool significantly decreased the level of H_2S-stimulated labeling of lipids from glutamine (8).

Summary

To understand the chemical biology underlying the varied physiological effects attributed to H_2S, it is imperative to identify plausible and specific biological targets that can mediate specific cellular responses. While the field has been focused on persulfide modifications of cysteine thiols [reviewed in Filipovic *et al.* (17)] and while these posttranslational modifications undoubtedly exist in the proteome (16, 20), a quantitatively significant change in persulfidation levels in a target protein that is associated with a functional change in the cellular milieu remains to be demonstrated [reviewed in Kumar and Banerjee (38)]. In contrast, the ability of H_2S to target the ETC, a central bioenergetic highway that has widespread ramifications on redox metabolism, represents a plausible molecular mechanism by which sulfide signals. Recent studies on the metabolic reprogramming elicited by H_2S, which originates in the mitochondrion, are leading to a paradigmatic shift in our understanding of how H_2S signals. These studies are revealing a central role for reductive stress signaling and have led to the exciting discovery of plasticity in the eukaryotic ETC, allowing the use of fumarate to support oxidative metabolism when complex IV activity is stalled (39). Additional metabolic impacts of H_2S in other cellular compartments, particularly the endoplasmic reticulum and the nucleus, represent areas that are ripe for investigation.

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Abbreviations Used

ACADS = acyl-CoA dehydrogenase
ACL = ATP-citrate lyase
AdoMet = *S*-adenosylmethionine
AMP = adenosine monophosphate
ATF4 = activating transcription factor 4
CBS = cystathionine β-synthase
CHDH = choline dehydrogenase
CoA-SSH = coenzyme A persulfide
CoQ = coenzyme Q
CSE = cystathionine γ-lyase
cytC = cytochrome C
DHODH = dihydroorotate dehydrogenase
ETC = electron transport chain
ETFDH = electron transfer flavoprotein quinone oxidoreductase
ETHE1 = persulfide dioxygenase
FAD = flavin adenine dinucleotide
G3PDH = glyceraldehyde 3-phosphate dehydrogenase
GOT1/2 = glutamate-oxaloacetate transaminase 1 or 2
GPD2 = glycerol 3-phosphate dehydrogenase
GSH = glutathione
GSSH = glutathione persulfide
H_2S = hydrogen sulfide
IDH1/2 = isocitrate dehydrogenase 1 or 2
LDH = lactate dehydrogenase
MDH1/2 = malate dehydrogenase 1 or 2
MPST = mercaptopyruvate sulfurtransferase
NNT = nicotinamide nucleotide transhydrogenase
PRODH2 = proline dehydrogenase 2
ROS = reactive oxygen species
SQOR = sulfide quinone oxidoreductase