



Review

Impact of Hydrogen Sulfide on Mitochondrial and Bacterial Bioenergetics

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Abstract: This review focuses on the effects of hydrogen sulfide (H₂S) on the unique bioenergetic molecular machines in mitochondria and bacteria—the protein complexes of electron transport chains and associated enzymes. H₂S, along with nitric oxide and carbon monoxide, belongs to the class of endogenous gaseous signaling molecules. This compound plays critical roles in physiology and pathophysiology. Enzymes implicated in H₂S metabolism and physiological actions are promising targets for novel pharmaceutical agents. The biological effects of H₂S are biphasic, changing from cytoprotection to cytotoxicity through increasing the compound concentration. In mammals, H₂S enhances the activity of F₀F₁-ATP (adenosine triphosphate) synthase and lactate dehydrogenase via their S-sulphydration, thereby stimulating mitochondrial electron transport. H₂S serves as an electron donor for the mitochondrial respiratory chain via sulfide quinone oxidoreductase and cytochrome *c* oxidase at low H₂S levels. The latter enzyme is inhibited by high H₂S concentrations, resulting in the reversible inhibition of electron transport and ATP production in mitochondria. In the branched respiratory chain of *Escherichia coli*, H₂S inhibits the *bo*₃ terminal oxidase but does not affect the alternative *bd*-type oxidases. Thus, in *E. coli* and presumably other bacteria, cytochrome *bd* permits respiration and cell growth in H₂S-rich environments. A complete picture of the impact of H₂S on bioenergetics is lacking, but this field is fast-moving, and active ongoing research on this topic will likely shed light on additional, yet unknown biological effects.

Keywords: hydrogen sulfide; donors; gasotransmitters; molecular bioenergetics; inhibition; electron transport chain



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1. Introduction

For a long time, hydrogen sulfide (H₂S) had been considered merely as a highly toxic and occasionally lethal gas. However, it was discovered that in mammals H₂S has physiological relevance and is endogenously generated [1,2]. Currently, H₂S is considered to be a member of the class of gasotransmitters or, in other words, endogenous gaseous signaling molecules, along with nitric oxide and carbon monoxide [3–7]. Although still debated, cyanide has also recently been proposed to be part of this class [7,8]. H₂S contributes to the regulation of important physiological processes in the cardiovascular, gastrointestinal, nervous, and respiratory systems. It shows various physiological effects in mammalian cells, but in a biphasic, concentration-dependent manner. At low, nanomolar concentrations, H₂S exhibits cytoprotective effects. At higher levels the compound induces cytotoxicity. For instance, H₂S exerts vasorelaxant effects through the opening of K_{ATP} (adenosine triphosphate) channels in vascular smooth muscle cells [9]. It also functions as a neuromodulator in the brain [1] and serves as a stimulator of angiogenesis [10]. At concentrations of 200 μM and higher, H₂S induces apoptosis of aorta smooth muscle cells through the activation of mitogen-activated protein kinases and caspase-3 [11]. In mammalian systems, H₂S is

proposed to signal via four different mechanisms: (i) serving as an antioxidant that detoxifies reactive oxygen species (ROS) and/or reactive nitrogen species (RNS); (ii) blocking and/or reducing metal active sites in metalloproteins, e.g., heme Fe sites in heme proteins; (iii) performing protein S-persulfidation, also known as S-sulphydration (post-translational modification of a cysteine residue by adding a thiol group); (iv) performing the chemical reduction of disulfide bonds in proteins (see [12,13] and references therein) (Figure 1).

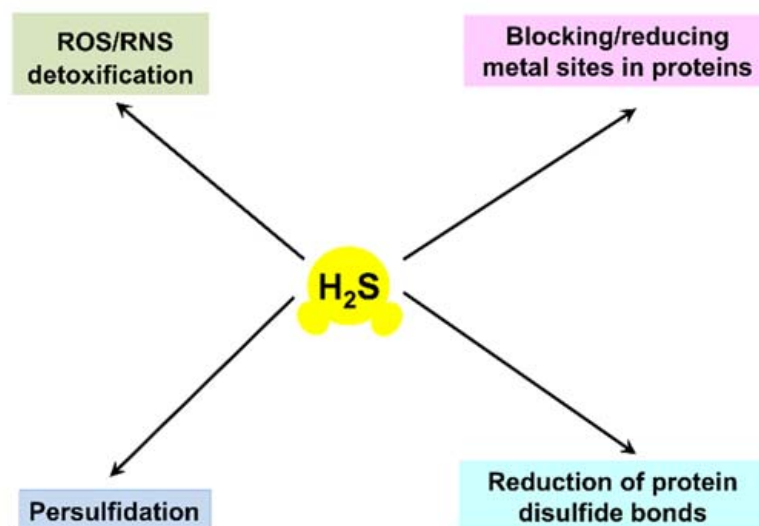
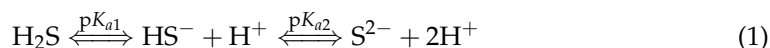


Figure 1. Proposed H₂S signaling mechanisms in mammalian systems.

The physiological roles of H₂S are not limited to mammals but appear to be inherent in all kingdoms of life. In higher plants, H₂S signaling functions seem to occur mainly through a persulfidation-based mechanism [14]. It regulates important physiological functions, including fruit ripening; stomatal movement; senescence in flowers, leaves, and fruits; photosynthesis via promotion of photosynthetic enzyme expression and chloroplast biogenesis; and the promotion of root organogenesis, seed germination, nodulation, and N₂ fixation. H₂S can also activate antioxidant systems in plant cells, thereby contributing to defense against adverse environment situations, such as drought-induced oxidative stress, salinity stress, temperature stress (high and low temperatures), toxic heavy metal stress, as well as different biotic stresses (see [14] and references therein).

Although most bacteria can generate and sense H₂S, the exact physiological roles of this compound in the prokaryotes are not yet wholly understood. In particular, whether endogenous H₂S is a true signaling effector molecule that induces a response in the same microbial cell, or whether the H₂S-mediated effect is more a response to environmental and/or host-derived H₂S, remains to be established [6]. Meanwhile, Shatalin et al. reported that inhibition of H₂S production through inactivation of H₂S-generating enzymes in *Bacillus anthracis* Sterne, *Pseudomonas aeruginosa* PA14, *Staphylococcus aureus* (MSSA RN4220 and MRSA MW2), and *Escherichia coli* MG1655 makes these pathogenic species very sensitive to different classes of antibiotics [15]. The addition of exogenous H₂S reverses this effect. The authors concluded that endogenously generated H₂S increases bacterial resistance to oxidative stress imposed by antibiotics. The antibiotic-induced ROS cause DNA damage via the Fenton reaction. It is suggested that H₂S prevents oxidative DNA damage in bacteria via the following cytoprotective mechanisms: (i) direct reduction of H₂O₂ into H₂O; (ii) depletion of Fe²⁺, a catalyst of the Fenton reaction; (iii) transient depletion of free cysteine, a reducing agent that fuels the Fenton reaction; and (iv) stimulation of the activities of superoxide dismutase (SOD) and catalase [15]. The latter two enzymes are the well-known ROS scavenging systems [16]. It should be noted, however, that Shatalin et al. [15] did not suggest a mechanism for the depletion of Fe²⁺ and free cysteine by H₂S.

H₂S is a colorless flammable gas, soluble in water (100 mM at 25 °C [17]), and with high cell membrane permeability [18]. It is a weak acid; therefore in aqueous solutions, it equilibrates with hydrosulfide (HS[−]) and sulfide (S^{2−}) according to Equation (1):



According to the reported pK_{a1} values that varied from 6.97 to 7.06 at 25 °C (see [19] and references therein), at physiological pH 7.4, 69–73% of the total hydrogen sulfide pool in the solution is in the form of HS[−], and 27–31% exists as H₂S. Taking into account the reported pK_{a2} values (from 12.2 to 19), the concentration of S^{2−} in the solution at pH 7.4 is negligible. The mitochondrial matrix in eukaryotic cells is usually alkaline, with a pH value of about 8 [20,21]. Accordingly, in the matrix, the concentration of HS[−] is higher, 90–91%, and the remainder is in the form of H₂S. On the contrary, intralysosomal pH in living cells is highly acidic, 4.7–4.8 [22]. Therefore, inside lysosomes, the undissociated form of hydrogen sulfide, H₂S, dominates (>99%). H₂S acts as a reducing agent. Respectively, the standard redox potential (vs. the standard hydrogen electrode, pH 7) E^{0'}(S⁰/H₂S) is −230 mV (E^{0'}(S⁰/HS[−]) = −270 mV) [19]. Herein, unless otherwise stated, we use the term “H₂S” to designate the total hydrogen sulfide pool (H₂S + HS[−] + S^{2−}).

This review focuses on the effects of H₂S on the respiratory chains of mammalian mitochondria and bacteria, mammalian F₀F₁-ATP synthase, and mammalian lactate dehydrogenase (LDH) in light of recent findings.

2. Endogenous Production of H₂S

In mammalian tissues, H₂S can be endogenously produced via both non-enzymatic and enzymatic pathways.

2.1. Non-Enzymatic Production of H₂S

Non-enzymatic formation of H₂S usually takes place in the reactions of thiols or thiol derivatives with other molecules [12,13,23]. Inorganic polysulfides, persulfides, and thiosulfate can be reduced with reduced glutathione (GSH) to yield H₂S (Figure 2). This demands the presence of reducing equivalents, such as NADPH (reduced nicotinamide adenine dinucleotide phosphate), because glutathione disulfide (GSSG), also produced in the reaction, needs to be converted back to GSH by NADPH-dependent glutathione reductase. Inorganic sulfide salts, such as Na₂S or NaHS, can undergo hydrolysis. Yang et al. also reported that cysteine is the preferred substrate for the non-enzymatic pathway, and that the reaction required coordinated catalysis by Fe³⁺ and pyridoxal phosphate (PLP) [24] (Figure 2).

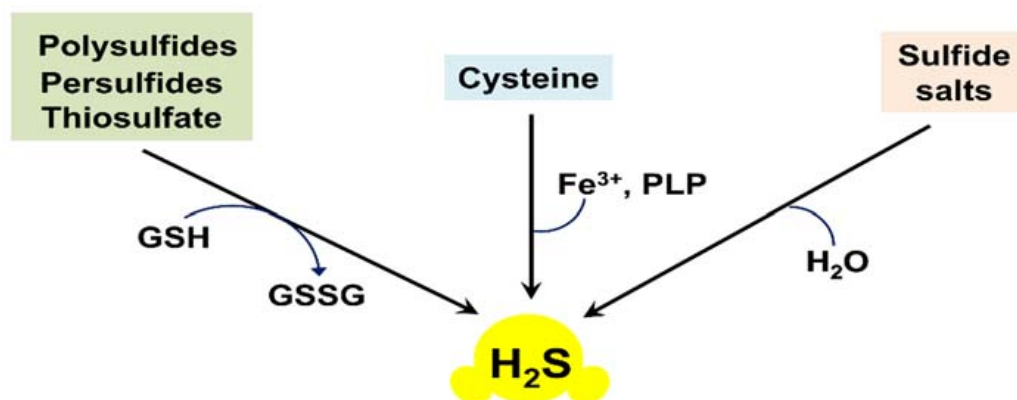


Figure 2. Non-enzymatic endogenous production of H₂S in mammalian tissues.

2.2. Enzymatic Production of H₂S

Enzymatic production of H₂S in mammalian systems is carried out primarily by cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate-sulfurtransferase (3MST) [12,13,23,25,26]. The main enzymatic reactions resulting in the biosynthesis of H₂S are shown in Figure 3. CBS can catalyze the condensation of homocysteine and L-cysteine to produce L-cystathionine and H₂S (Figure 3, reaction 1). In the presence of L-cysteine, CBS can also produce H₂S, with the formation of L-serine as a byproduct (Figure 3, reaction 2). CSE can decompose homocysteine to yield H₂S, α-ketobutyrate, and ammonia (Figure 3, reaction 3). Furthermore, CSE can catalyze the conversion of L-cysteine into H₂S, pyruvate, and ammonia (Figure 3, reaction 4). Both CBS and CSE can generate H₂S through β-replacement of cysteine by a second cysteine, with the formation of lanthionine as a byproduct (Figure 3, reaction 5). Additionally, CSE can catalyze the γ-replacement reaction between two homocysteine molecules, with the production of H₂S and homolanthionine as a by-product (Figure 3, reaction 6). 3MST generates H₂S from 3-mercaptopyruvate coupled with either of the two enzymes, mitochondrial cysteine aminotransferase (CAT) or peroxisomal D-amino acid oxidase (DAO). 3MST transfers a sulfur atom from 3-mercaptopyruvate onto itself, resulting in the formation of the enzyme-bound persulfide (3MST-SS) and pyruvate (Figure 3, reaction 7). H₂S is then released from the persulfide in the presence of a reductant, e.g., thioredoxin (Trx). CAT and DAO in turn produce 3-mercaptopyruvate from L-cysteine and D-cysteine, respectively [13].

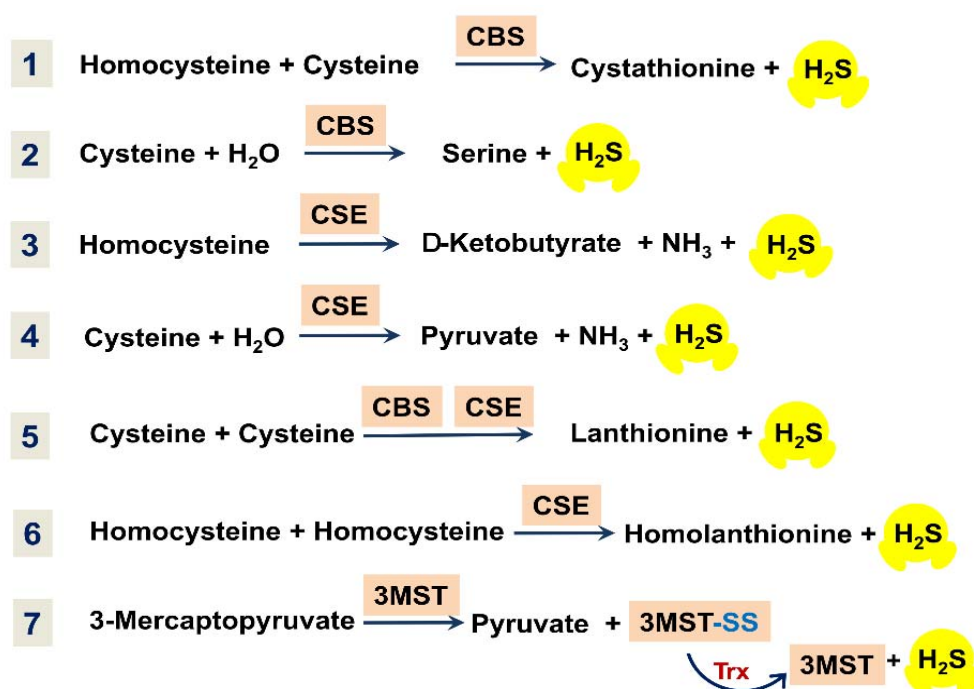


Figure 3. Overview of main reactions for enzymatic production of H₂S in mammalian tissues.

The majority of bacterial species whose genomes were completely sequenced have the orthologs of mammalian genes encoding CBS, CSE, or 3MST [15]. Since H₂S provides defense against modern antibiotics in bacteria, suppression of H₂S-producing enzymes in pathogens by new drugs would be a promising antimicrobial treatment strategy [15,27]. The use of H₂S biogenesis as a target for versatile antibiotic potentiators may have therapeutic potential for the fight against difficult-to-treat infections based on bacterial antibiotic tolerance.

3. S-Sulfhydration of ATP Synthase

In eukaryotes, one of the main targets of H₂S signaling is the mitochondria. These cell organelles are known to be the power plants of the eukaryotic cell. Energy transduction events in mitochondria occur in the O₂-dependent respiratory electron transport chain. The mammalian respiratory chain is unbranched [28,29]. It consists of four membrane-bound multi-subunit complexes: I, (reduced nicotinamide adenine dinucleotide) NADH: ubiquinone reductase or type I NADH dehydrogenase; II, succinate dehydrogenase; III, ubiquinol: cytochrome *c* reductase or cytochrome *bc*₁ complex; and IV, cytochrome *c* oxidase or cytochrome *aa*₃. The chain transfers electrons from NADH and succinate to O₂. The electron transfer reactions catalyzed by complexes I, III, and IV are coupled to the generation of the proton motive force. The latter is used by ATP synthase (F₀F₁-ATP synthase or complex V) to produce ATP.

Modis et al. observed S-sulfhydration of subunit α of the synthase (ATP5A1) in HepG2 and HEK293 cell lysates in response to exposure to H₂S, using a biotin switch assay [30]. Sulfhydration of subunit α of the synthase increases with increasing the H₂S concentration, 50–300 μ M. H₂S at low concentrations (10–100 nM) stimulates the specific activity of ATP synthase, while at higher concentrations (1–10 μ M) a tendency for inhibition of the activity is detected (Figure 4). Such a bell-shaped concentration–response curve is quite typical for the effects of H₂S. Sulfhydration occurs at two highly conserved cysteine residues in subunit α of the synthase, Cys244, or Cys294. Mutation of either of the two cysteines (C244S or C294S) leads to a slight reduction in the catalytic activity of ATP synthase. The double mutant (C244S/C294S) exhibits more than 50% inhibition of the enzyme activity. In vivo, subunit α of the synthase is basally sulfhydrated. The basal sulfhydration is mostly due to CSE-derived endogenous H₂S generation because it is suppressed in liver homogenates harvested from CSE^{−/−} mice. Burn injuries that upregulate CSE and increase H₂S generation result in an increase in S-sulfhydration of subunit α of the synthase. Thus, S-sulfhydration of subunit α of the synthase could be a physiological mechanism to maintain ATP synthase in a physiologically activated state, thereby supporting mitochondrial bioenergetics [30].

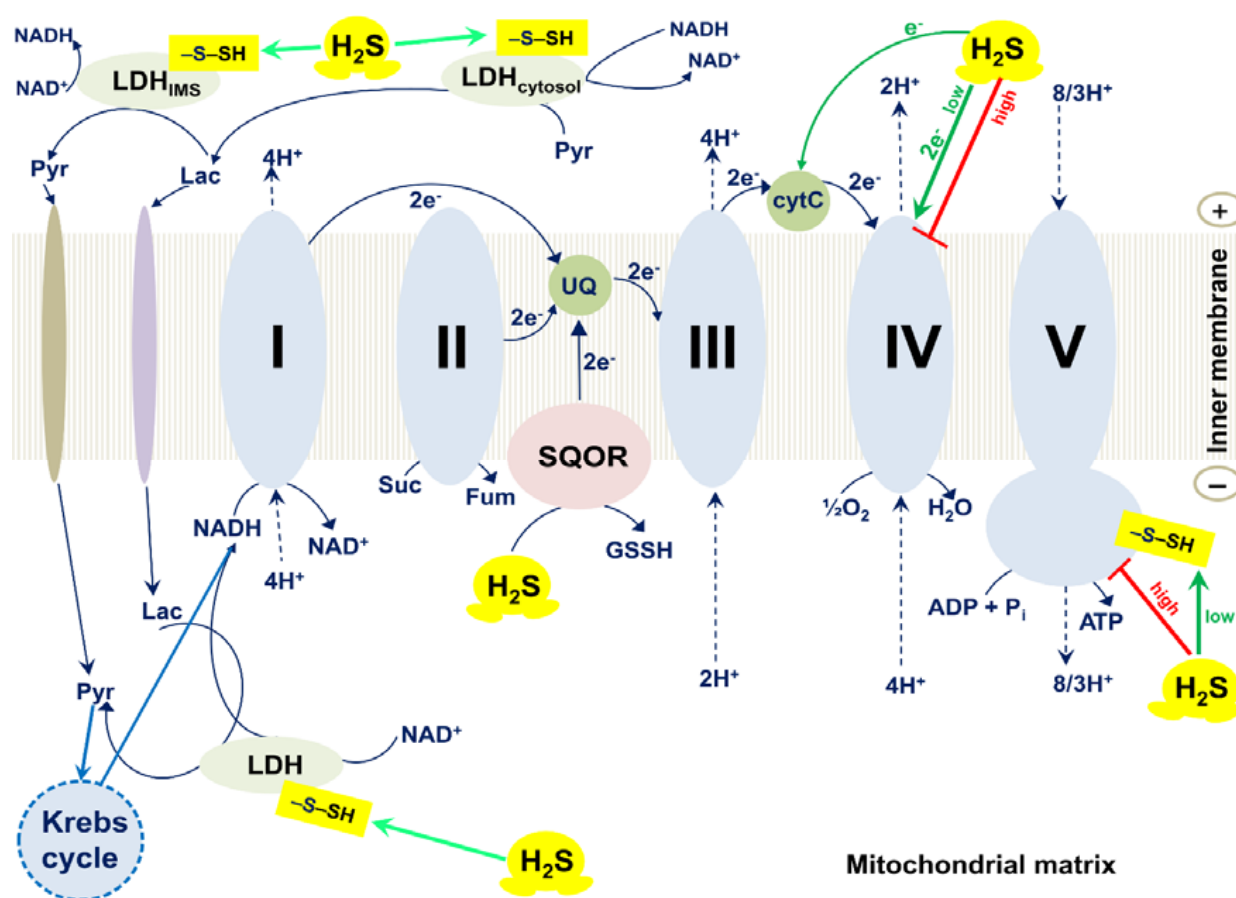


Figure 4. Effects of H₂S on mammalian mitochondrial electron transport chain, ATP synthase, and lactate dehydrogenase. The mammalian respiratory chain includes four different membrane-bound complexes: I, II, III, and IV. H₂S at low concentrations stimulates the activity of F₀F₁-ATP synthase (also known as complex V) via S-sulphydration of Cys244 or Cys294 of its α subunit [30]. H₂S increases the activity of lactate dehydrogenase (LDH) via S-sulphydration of its Cys163 that, in turn, stimulates mitochondrial electron transport [31]. H₂S can also donate electrons to the respiratory chain via sulfide quinone oxidoreductase (SQOR) by directly reducing ubiquinone (UQ) [32]. At high (toxic) concentrations H₂S inhibits complex IV (cytochrome *c* oxidase) and F₀F₁-ATP synthase that leads to reversible inhibition of mitochondrial electron transport and ATP production [30,33–35]. At low concentrations H₂S serves as an electron donor for complex IV, either directly [36–38] or indirectly, via reduction of its native substrate cytochrome *c* [39]. The outer mitochondrial membrane is not shown for simplicity.

4. S-Sulphydration of Lactate Dehydrogenase (LDH)

LDH catalyzes the reversible conversion of lactate to pyruvate with the reduction of NAD⁺ to NADH, and vice versa. The enzyme is a tetramer that is usually composed of the two most common types of subunits, LDHA and LDHB [40]. LDHA and LDHB can assemble into five different isoenzymes: LDH1, LDH2, LDH3, LDH4, and LDH5. Isoenzymes that are rich in LDHA catabolize pyruvate to lactate with the concomitant production of NAD⁺ from NADH. Conversely, isoenzymes rich in LDHB facilitate lactate-to-pyruvate conversion with the concomitant formation of NADH from NAD⁺.

Untereiner et al. reported that in the colon cancer cell line HCT116, LDHA catalyzes the conversion of pyruvate to lactate, and H₂S donation increases the total cellular lactate levels [31]. Notably, H₂S enhances the catalytic activity of LDHA, which is primary cytosolic, doing so via S-sulphydration of its Cys163 (Figure 4). Experiments with whole HCT116 cell extracts showed that H₂S also stimulates the LDHB activity, although to a smaller extent. Importantly, H₂S stimulates oxidative phosphorylation in HCT116 cells in an LDHA-dependent manner. Thus, in colon cancer cells, H₂S-induced stimulation of the catalytic activity of LDHA leads to the stimulation of mitochondrial electron transport.

The authors hypothesize that the increase in the LDHA activity causes an increase in cytosolic lactate. This enhances the flux of lactate into the mitochondria through the intracellular lactate shuttle. Lactate enters the mitochondrial intermembrane space. Lactate and pyruvate also enter the mitochondrial matrix. Lactate is converted to pyruvate via the mitochondrial LDH that is primarily made up of LDHB and also activated by H_2S . Pyruvate in the matrix is oxidized via the Krebs cycle to generate NADH that stimulates the activity of the mitochondrial respiratory chain. Additionally, the oxidation of lactate to pyruvate by the mitochondrial LDH is coupled to the reduction of NAD^+ to NADH. This NADH, in turn, is utilized by the mitochondrial respiratory chain to further support electron transport.

5. H_2S Donates Electrons to the Mitochondrial Respiratory Chain via Sulfide Quinone Oxidoreductase (SQOR)

Powell and Somero first reported that the oxidation of H_2S can occur in mitochondria, and that the process is coupled to oxidative phosphorylation [41]. The mitochondrial oxidation of H_2S was observed in the gill and foot tissue of *Solemya reidi*, a gutless clam living in sulfide-rich habitats. Later, Goubern et al., using permeabilized human colon adenocarcinoma HT29 cells, showed that H_2S at low micromolar concentrations can serve as an electron donor for the mammalian respiratory chain [32]. This is accompanied by mitochondrial energization. The latter is extremely sensitive to the amount of H_2S delivered instantaneously to mitochondria. H_2S donates electrons at the respiratory chain at the level of UQ.

The enzyme that catalyzes the electron transfer from H_2S to UQ is SQOR [42]. SQOR belongs to group four of the flavin disulfide reductase (FDR) superfamily. The enzyme contains a tightly bound FAD (flavin adenine dinucleotide) and two spatially proximal redox-active cysteines. A peculiar feature of human SQOR is the fact that the two cysteines are linked via a bridging sulfur atom which forms the redox-active cysteine trisulfide configuration. The trisulfide appears to be the active form of SQOR, being present at the start and end of the catalytic cycle [43].

When H_2S is oxidized in the mitochondrial matrix by the action of SQOR under physiological conditions, GSH serves as the primary sulfur acceptor (Figure 4). In the reaction that is coupled to the reduction of UQ to UQH_2 , glutathione persulfide (GSSH) is produced. GSSH is then oxidized by persulfide dioxygenase (ETHE1) to yield sulfite (SO_3^{2-}) and regenerate GSH. GSSH can also be a substrate for rhodanese or thiosulfate sulfurtransferase (TST). TST catalyzes the reaction of GSSH with SO_3^{2-} to generate GSH and thiosulfate ($\text{S}_2\text{O}_3^{2-}$). Alternatively, SO_3^{2-} can be oxidized to sulfate (SO_4^{2-}) by sulfite oxidase (SUOX) located in the intermembrane space, with the concomitant reduction of cytochrome *c*. Thiosulfate and sulfate can be excreted with urine (see [42] and references therein). Thus, electrons from the sulfide oxidation pathway can enter the mitochondrial respiratory chain at the level of the cytochrome bc_1 complex (from SQOR) and cytochrome *c* oxidase (from SUOX).

6. H_2S at Toxic Concentrations Inhibits Mitochondrial Cytochrome *c* Oxidase

H_2S exposure, at high concentrations and high rates, is extremely toxic to mammals, including humans [35]. The acute toxicity of H_2S is generally attributed to the suppression of the mitochondrial cytochrome *c* oxidase (complex IV). Complex IV couples the transfer of electrons from cytochrome *c* to O_2 with the generation of the transmembrane proton gradient using the mechanism of proton pumping [16,44–65]. The enzyme carries four redox-active metal sites: Cu_A , heme *a*, heme a_3 , and Cu_B . Electrons from cytochrome *c* primarily accepted by Cu_A are transferred to heme *a* and then to the catalytic binuclear center composed of heme a_3 and Cu_B in which the reduction of oxygen to water occurs [66–69]. There are two forms of cytochrome *c* oxidase: slow (resting oxidase as obtained from the preparation) and fast (the completely reduced enzyme exposed to a “pulse” of O_2) [70]. The fast form is also called pulsed or unrelaxed. The slow form differs from the fast form in lower reactivity for inhibitors [71] and slower intramolecular electron transfer [72].

H₂S at high concentrations was reported to inhibit the O₂ consumption by the beef heart cytochrome *c* oxidase in the isolated form and in submitochondrial particles (Figure 4) [33,34,73]. The inhibition by H₂S appears to be non-competitive with respect to both substrates, cytochrome *c* and O₂ [34]. The inhibition efficiency increases with decreasing pH: the *K_i* values measured at pH 8.05, 7.48, and 6.28, are 2.6, 0.55, and 0.07 μM, respectively [37]. The initial rate of inactivation of the fast form of the isolated cytochrome *c* oxidase is proportional to total H₂S concentration, with an initial rate constant, *k_{on}*, of $2.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.47 [38]. The inhibition of complex IV leads to blockage of the mitochondrial respiratory chain and, as a consequence, to the dissipation of the membrane potential, the cessation of oxidative phosphorylation, and enhanced ROS production [35]. Consistently, examination of the cytotoxic effects of inhalational H₂S exposure showed that sublethal (>50 ppm) concentrations of the inhaled gas caused inhibition of cytochrome *c* oxidase activity in the lung and heart, which can be observed *ex vivo* and is associated with pathological alterations [74–79]. That complex IV is suppressed by high (usually 10–100 μM) concentrations of H₂S *in vitro* in various cell lines is well documented [75,80–85]. The H₂S-induced inhibition is reversible, e.g., 10–30 min after the exposure of tissue homogenates to H₂S, the activity of complex IV gets back to its initial level, simultaneously with the decomposition of the inhibitor [86].

H₂S, presumably in the form of HS[−], binds to the ferric heme *a*₃ and Cu_B in either state (either cupric or cuprous) [38]. The final enzyme product of H₂S inhibition is a mixed-valence state of the oxidase in which Cu_A and heme *a* are reduced and heme *a*₃ is in the ferric H₂S-bound form [33,87]. Cu_B in the final inhibited enzyme remains reduced even after reoxidation of the H₂S-bound oxidase, as evidenced by an electron paramagnetic resonance spectroscopy study [88]. The fact that Cu_B remains cuprous in the presence of ferricyanide suggests that H₂S binding to Cu_B¹⁺ increases its redox potential and makes re-oxidation difficult. In other words, Cu_B¹⁺ in this state may also be H₂S-bound [88]. Nicholls et al. [38] suggested a model for the H₂S-mediated inhibition of the mitochondrial cytochrome *c* oxidase (Figure 5). According to this model, during the steady-state turnover of complex IV, the first molecule of HS[−] transiently binds to cupric or cuprous Cu_B. Then, it is transferred to the ferric heme *a*₃ that blocks the catalytic reaction of the heme with O₂. In the final protein-inhibitor adduct, Cu_B is likely reduced and presumably bound to the second molecule of HS[−]. Heme *a* and Cu_A are most likely reduced in the adduct.

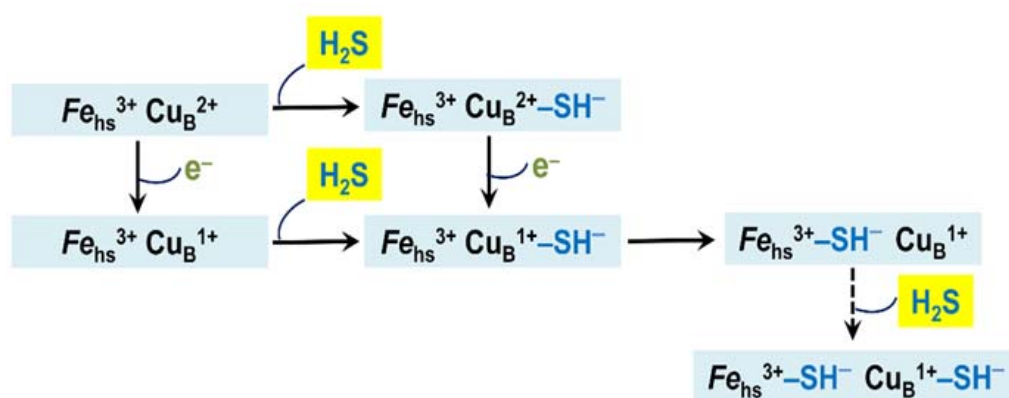


Figure 5. Proposed mechanism of inhibition of mitochondrial cytochrome *c* oxidase and *Escherichia coli* cytochrome *bo*₃ by H₂S. Shown is the catalytic binuclear center in different redox and ligation states. The center consists of the copper ion Cu_B and the high-spin heme (*Fe_{hs}*). The latter is heme *a*₃ in cytochrome *c* oxidase and heme *o*₃ in cytochrome *bo*₃. The metal redox groups which are not part of the center are not shown.

7. H₂S at Low Concentrations Serves as Electron Donor for Mitochondrial Cytochrome *c* Oxidase

H₂S at low concentrations can act as a substrate for mitochondrial complex IV (Figure 4) [36,37]. The fast form of the oxidized cytochrome *c* oxidase reacts aerobically with low H₂S levels in the absence of reduced substrates. However, the initial product of this reaction is not the inhibited enzyme but a catalytic intermediate, compound 'P' (Figure 6) [38]. Compound 'P' then decays into another catalytic intermediate, compound 'F'. It is proposed that in this reaction two H₂S molecules donate two electrons to the fully oxidized binuclear center, $a_3^{3+}\text{Cu}_B^{2+}$, converting it to the fully reduced state, $a_3^{2+}\text{Cu}_B^+$. The concomitant H₂S oxidation product is possibly hydrogen persulfide (HSSH), although a form of free sulfur (S⁰ or S₈) cannot be excluded [38]. Then the fully reduced binuclear center reacts with O₂, producing compound 'P'. It remains unclear whether cytochrome *c* oxidase contributes significantly to a dissimilatory mechanism for the endogenously generated H₂S, or if SQOR is the major contributor in vivo. Notably, H₂S can also reduce complex IV indirectly, via reduction of its native substrate cytochrome *c* (Figure 4). The reaction between H₂S and cytochrome *c* presumably leads to the initial formation of the HS[•]/S^{•−} radical. HS[•]/S^{•−} could then be trapped by proteins producing protein persulfides and superoxide, or be trapped by O₂ yielding HSO₂[•] [39].

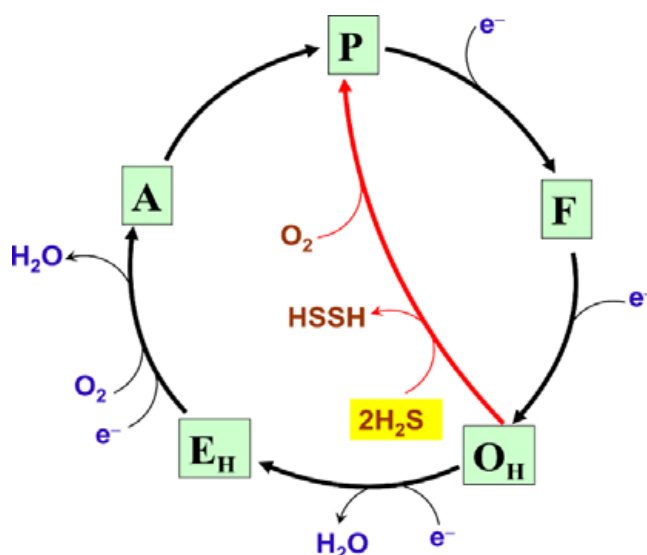


Figure 6. Proposed molecular mechanism of the reaction of the fast form of mitochondrial cytochrome *c* oxidase with low H₂S levels. O_H, E_H, A, P, and F are unrelaxed oxidized, unrelaxed one-electron-reduced, compound 'A', compound 'P', and compound 'F' catalytic intermediates, respectively. Chemical and pumped protons involved in the catalytic cycle are not shown for clarity.

8. Effect of H₂S on the Operation of the Branched Respiratory Chain of *E. coli* and Bacterial Growth

Under steady-state conditions, the mammalian tissue concentration of H₂S is usually in the low nanomolar range, e.g., it is around 15 nM in mouse brain and liver tissues [89]. The mammalian gut, in this sense, is unique among other body compartments. Millimolar concentrations (1.0–2.4 mM) of H₂S are commonly present in the gut [90]. The reason is that in the gastrointestinal tract, unlike other compartments, H₂S is generated by both the mammalian CBS, CSE, and 3MST, and by the microbial communities inhabiting the gut, of which sulfate-reducing bacteria are the key H₂S-producing species [90,91]. At such extremely high H₂S levels, bacterial respiratory chains which terminate in cytochrome *c* oxidase or other heme-copper oxidases should be inhibited. The question then arises as to how under these conditions bacteria inhabiting the mammalian gut can maintain aerobic respiration. It turned out that *E. coli*, and possibly other bacteria inhabiting H₂S-rich

environments, has a unique *bd*-type terminal oxidase that is not inhibited by H₂S, even at toxic concentrations [92–95].

E. coli is an essential member of the intestinal microbiome of humans and warm-blooded animals. The large intestine of humans normally harbors several *E. coli* strains at a given point in time [96]. In contrast to the electron transport chain of mammals that is unbranched, *E. coli*, like many other prokaryotes, possesses the branched aerobic respiratory chain (Figure 7) [45,97–102]. The *E. coli* chain comprises of type I and type II NADH dehydrogenases which transfer electrons from NADH to ubiquinol-8 (UQ8) or menaquinol-8 (MQ8); succinate dehydrogenase transferring electrons from succinate to UQ8; and three terminal oxidases, cytochromes *bo*₃, *bd*-I, and *bd*-II which transfer electrons from UQ8 or MQ8 to O₂ producing H₂O [103–112]. The proton motive force generated by type I NADH dehydrogenase and the terminal oxidases is used by F₀F₁-ATP synthase to make ATP [113–115]. Being a true proton pump, cytochrome *bo*₃ produces the proton motive force with higher efficiency as compared to the evolutionarily unrelated *bd*-type oxidases [116–121]. The three-dimensional structures of cytochrome *bo*₃ and cytochrome *bd*-I were determined [122–125]. The *bo*₃ oxidase is a member of type A-1 heme-copper oxidase superfamily [46,48,49,51,54,56–59,61–63]. Cytochrome *bo*₃ contains the UQ8 binding site, heme *b*, and the catalytic binuclear center formed by heme *o*₃ and Cu_B [101,126]. Cytochrome *bd*-I and cytochrome *bd*-II are members of the L subfamily of the cytochrome *bd* oxygen reductase family [127,128]. According to recent work by Murali et al. [129], the latter family should be expanded into a huge superfamily. The *bd* oxidase has the UQ8/MQ8 binding site, and three heme prosthetic groups, *b*₅₅₈, *b*₅₉₅, and *d* but lacks any copper site [97,98,108,127,130–133]. Hemes *b*₅₉₅ and *d* may form a di-heme active site for oxygen chemistry, as evidenced by a number of studies [134–147]. These hemes are in van der Waals contacts [124,125], enabling fast electron transfers between them [119,145,146]. Thus, the functional di-heme active site in the *bd* oxidase implies that heme *b*₅₉₅ is able to rapidly donate an electron and a proton to heme *d* to perform a concerted four-electron reduction of oxygen to water. This role of heme *b*₅₉₅ in cytochrome *bd* is similar to that of Cu_B in the catalytic binuclear center of cytochrome *bo*₃. Cytochromes *bo*₃ and *bd* have low and high O₂ affinity, respectively [148–153]. As a consequence, the *bo*₃ enzyme dominates in *E. coli* under conditions of high O₂ concentration, whereas the *bd* oxidase is expressed at low aeration [154–158]. Cytochrome *bd* performs vital physiological functions in *E. coli* and other prokaryotes [16,131,159–163]. In particular, the enzyme endows the microbes with resistance to nitric oxide [164–173], peroxynitrite [160,174], hydrogen peroxide [16,175–180], cyanide [92,181,182], and ammonia [183]. The *bd*-type oxidases are present in the respiratory chains of bacteria and archaea but not in humans and animals. For this reason, they could serve as suitable protein targets for next-generation antibiotics [133,171,184–190].

Forte et al. studied the effect of H₂S on the oxygen consumption by the purified terminal oxidases *bo*₃, *bd*-I, and *bd*-II from *E. coli* [92]. It turned out that the activity of cytochrome *bo*₃ is quickly inhibited with an apparent half-maximal inhibitory concentration *IC*₅₀ of 1.1 μM H₂S (pH 7.4). This value is similar to the *K*_i of 0.55 μM H₂S obtained by Nicholls and Kim for the beef heart cytochrome *c* oxidase at pH 7.48 [37]. The inhibition of the *bo*₃ oxidase appeared to be fully reversible. The rapid and total recovery of the enzymatic activity occurs following the removal of H₂S from the solution by the H₂S scavenger, the *Entamoeba histolytica* O-acetylserine sulphydrylase (*Eh*OASS), in the presence of excess O-acetyl-L-serine (OAS). In contrast, neither the *bd*-I oxidase nor the *bd*-II oxidase is inhibited under identical conditions, even at a high concentration of H₂S (58 μM) [92]. Similar results were obtained when examining the effect of H₂S on the oxygen consumption by cell suspensions of the *E. coli* mutant strains which possess *bo*₃, *bd*-I, and *bd*-II as the only terminal oxidase. The oxygen uptake by cells having *bo*₃ as the sole oxidase is rapidly inhibited by 50 μM H₂S. As in the case of the purified enzyme, the inhibition is promptly and completely restored after H₂S depletion by the *Eh*OASS/OAS system. Conversely, H₂S at the same concentration does not affect the oxygen uptake by mutant cells having either

bd-I or *bd*-II as the only oxidase [92]. Similar data on the *E. coli* membrane vesicles were reported by Korshunov et al. [93].

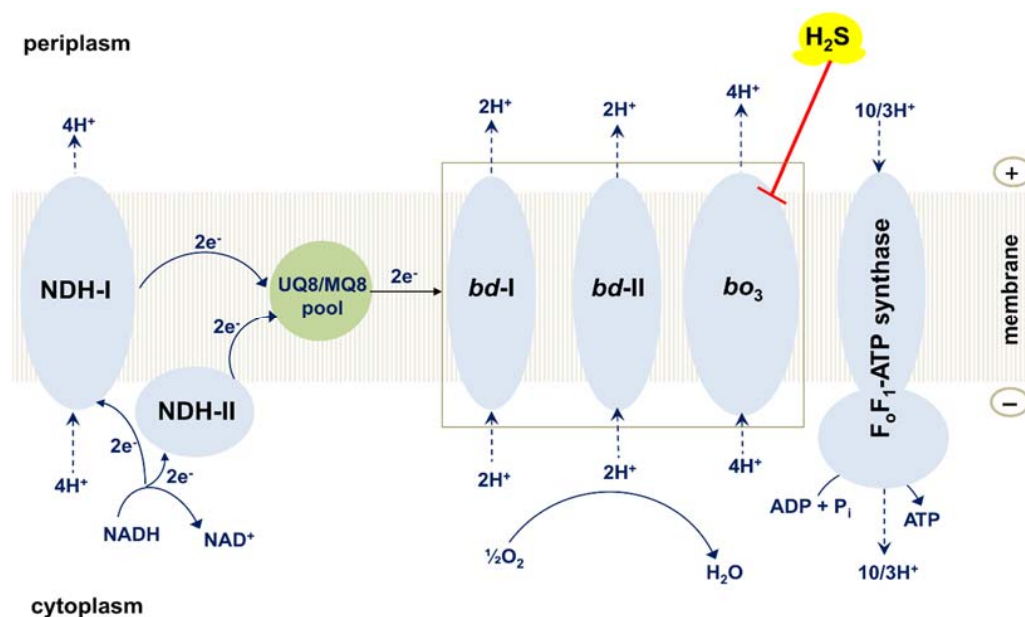


Figure 7. Effect of H₂S on the operation of the branched respiratory chain of *E. coli*. Shown is the schematic view of the branched aerobic respiratory chain of *E. coli*. H₂S inhibits cytochrome *bo*₃ but does not affect cytochrome *bd*-I and cytochrome *bd*-II. Succinate dehydrogenase and other substrate dehydrogenases are not shown for clarity.

Forte et al. also tested if *bd*-I and/or *bd*-II oxidases, besides enabling aerobic respiration, promote the growth of *E. coli* cells in the presence of H₂S [92]. A quantity of 200 μ M H₂S appeared to severely inhibit the growth of mutant cells with only cytochrome *bo*₃. On the contrary, virtually no effect on cell growth was detected following the addition of H₂S at the same concentration to the strains which express either *bd*-I or *bd*-II as the sole oxidase. Thus, in contrast with cytochrome *bo*₃, which is potently and reversibly inhibited by H₂S, both cytochrome *bd*-I and cytochrome *bd*-II are H₂S-insensitive, and therefore, able to sustain respiration and cell growth in the presence of high levels of H₂S. We assume that the mechanism of the inhibition of the *E. coli* cytochrome *bo*₃ by H₂S is very similar to that suggested for the H₂S-mediated inhibition of another heme-copper terminal oxidase, mitochondrial complex IV (Figure 5).

The H₂S resistance of cytochrome *bd* is probably a key trait not only found in *E. coli*. Saini et al. [191] reported that H₂S promotes the respiration and growth of *Mycobacterium tuberculosis*, *Mycobacterium smegmatis*, and *Mycobacterium bovis* BCG. The authors concluded that the suppression of the cytochrome *bcc-aa*₃ supercomplex by H₂S leads to the switching of the electron flow from MQ to cytochrome *bd*. The latter, in turn, stimulates respiration and ATP production, leading to the increased growth of the mycobacteria [191]. Furthermore, Kunota et al. [192] showed that multidrug-resistant and drug-susceptible clinical *M. tuberculosis* strains (but not non-pathogenic *M. smegmatis*) generate H₂S endogenously, maintaining bioenergetic homeostasis by stimulating respiration primarily via the *bd*-type terminal oxidase. These findings are in agreement with the fact that the *bd* oxidases of the *E. coli* respiratory chain are H₂S-insensitive [92,93].

9. Concluding Remarks

Accumulated evidence has shown that H₂S is an effector molecule that controls energy metabolism. Depending on the concentration, H₂S can either stimulate or inhibit the mammalian mitochondrial respiratory chain and F₀F₁-ATP synthase. An involvement of LDHA in the stimulatory effects of H₂S on mitochondrial respiration has also been suggested. High H₂S inhibits the bacterial respiratory chain terminating in cytochrome *bo*₃

but not cytochrome *bd*. The effects of H₂S on a bacterial ATP synthase are still unknown. Although the role of H₂S in microorganisms has been investigated less, there is currently an explosion of interest in the link between H₂S and bacterial energy metabolism as a result of the recognition that this gaseous signaling molecule is critically important for the growth and colonization potential of pathogens, such as *M. tuberculosis*. A clearer picture of the effects of H₂S on energy metabolism would represent a significant advance for the development of novel therapeutics.

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