Biosynthesis of H_2S in Organisms

Subjects: Plant Sciences

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Hydrogen sulfide (H_2S), which is a gasotransmitter, can be biosynthesized and participates in various physiological and biochemical processes in plants. H_2S also positively affects plants' adaptation to abiotic stresses.

hydrogen sulfide | resistance | cysteine residues | plant growth regulator

1. Biosynthesis Pathway of H_2S

The biosynthesis of H_2S includes both non-enzymatic and enzymatic pathways.

1.1. Non-Enzymatic Pathway

The non-enzymatic pathway of H_2S biosynthesis occurs due to the reaction of thiols or thiol-containing compounds with other molecules [1][2][3][4]. Glutathione (GSH) reduces inorganic polysulfides or hydrolyzed inorganic sulfide salts, i.e., sodium sulfide (Na_2S) or sodium hydrosulfide (NaHS) with water to produce H_2S [2][3][4]. Cysteine is the preferred substrate for the non-enzymatic production of H_2S, and the process is catalyzed by iron and vitamin B_6 [5].

1.2. Enzymatic Pathway

The trans-sulfuration pathway is the primary source of endogenous H_2S and is the only way to produce endogenous cysteine, involving cellular sulfur metabolism and redox regulation [6] (**Figure 1**). Methionine, acting as a substrate, is catalyzed to produce cysteine eventually, with H_2S as the byproduct. Cystathionine-β-synthase (CBS, EC 4.2.1.22), cystathionine-γ-lyase (CSE, EC 4.4.1.1), cysteine aminotransferase (CAT, EC 2.6.1.3), and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2) are all involved in this pathway. CBS and CSE are only located in the cytoplasm, while CAT and 3-MST can be present in the cytoplasm and mitochondria [7]. In the first irreversible step of converting methionine to cysteine, CBS catalyzes the condensation of homocysteine with serine (Ser) or cysteine to form cystathionine. The main role of CSE in the trans-sulfuration pathway is in the conversion of cystathionine to cysteine and α-ketobutyrate. CBS can produce H_2S through β-substitution reactions [7][8][9]. Similarly, CSE can produce H_2S via the β-elimination reaction with cysteine or the γ-replacement reaction between two homocysteine molecules. There is also a mechanism for H_2S production, mediated by CAT and 3-MST in the transsulfuration pathway. CAT catalyzes l-cysteine and α-ketoglutarate to form 3-mercaptopyruvate (3-MP) and glutamate. The sulfur group of 3-MP is then transferred to 3-MST-accepting nucleophilic Cys247 in the presence of 3-MST, to produce 3-MST-bound persulfide and pyruvate. After this stage, the MST-persulfide reacts with thiols or is reduced by thioredoxin (Trx) to form H_2S [10][11]. In addition, 3-MST transfers the sulfur group from 3-MP to

cyanide, to form thiocyanate [12]. Similar to the CAT/3-MST pathway, there is also a d-amino acid oxidase (DAO, EC 1.4.3.3)/3-MST pathway to generate H_2S (**Figure 1**). DAO metabolizes d-cysteine into 3-MP, which is metabolized into H_2S by 3-MST [13].

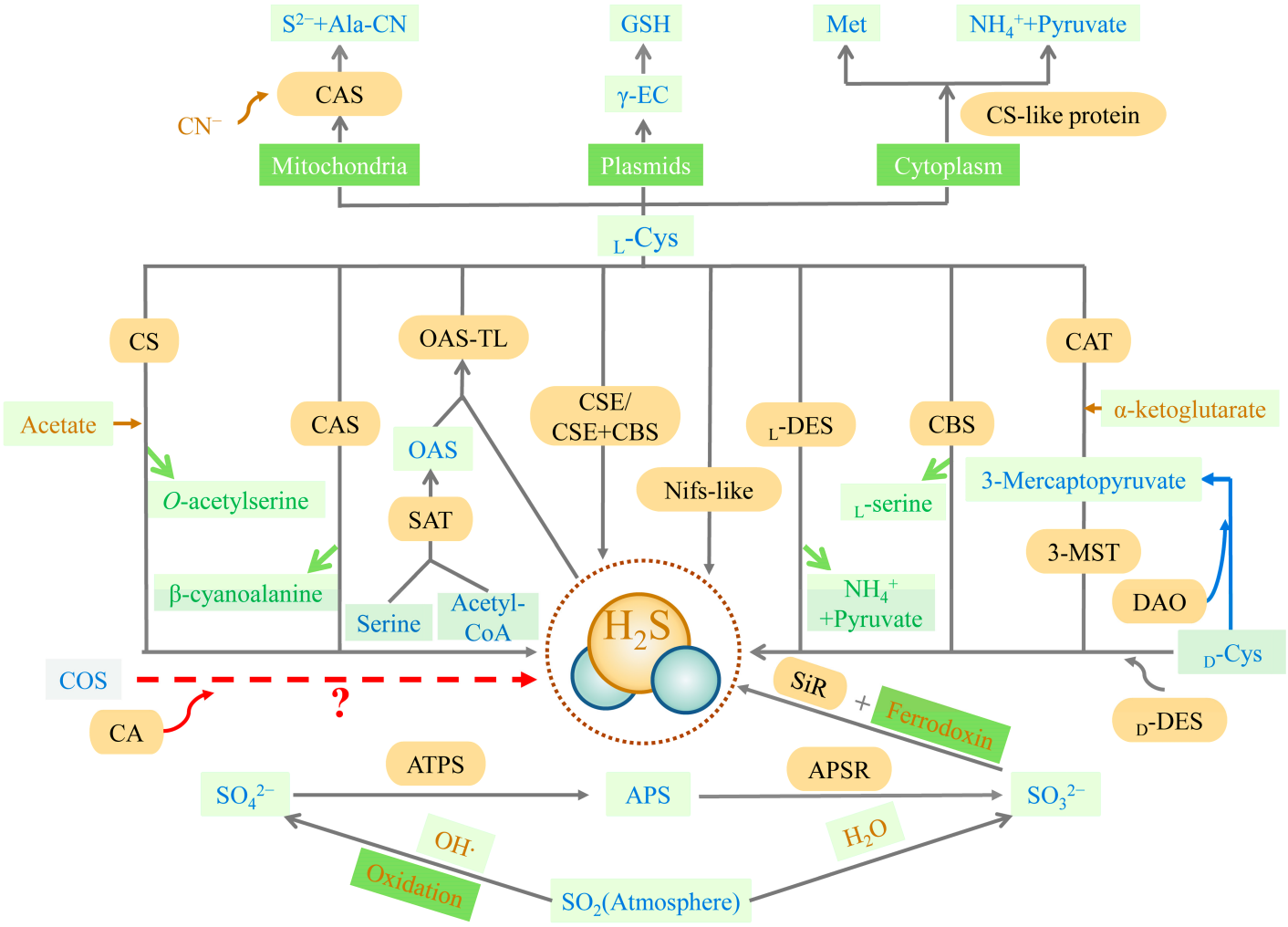

Figure 1. Synthesis of H_2S. APS (adenosine 5′-phosphosulfate), APSR (APS reductase, EC 1.8.99.2), ATPS (ATP sulfurylase, EC 2.7.7.4), CA (carbonic anhydrase, EC 4.2.1.1), CAS (l-3-cyanoalanine synthase, EC 4.4.1.9), CAT (cysteine aminotransferase, EC 2.6.1.3), CBS (cystathionine-β-synthase, EC 4.2.1.22), COS (carbonyl sulfide), CN^- (cyanide), CS (*O*-acetyl-l-serine via cysteine synthase, EC 4.2.99.8), CSC (hetero-oligomeric cysteine synthase complex), CSE (cystathionine-γ-lyase, EC 4.4.1.1), DAO (d-amino acid oxidase, EC 1.4.3.3), d-Cys (d-cysteine), d-DES (d-cysteine desulfhydrase, EC 4.4.1.15), γ-EC (γ-glutamylcysteine), GSH (glutathione), HO· (hydroxyl radicals), H_2O (water), H_2S (hydrogen sulfide), l-Cys (l-cysteine), l-DES (l-cysteine desulfhydrase, EC 4.4.1.1), Met (methionine), 3-MST (3-mercaptopyruvate sulfurtransferase, EC 2.8.1.2), NH_4^+ (ammonium), Nifs-like (nitrogenase Fe-S cluster-like), OAS (*O*-acetyl-l-serine), OSATL (*O*-acetylserine(thiol)lyase, EC 2.5.1.47), S^{2-} (Sulfide), SAT (serine acetyltransferase, EC 2.2.1.30), SiR (sulfite reductase, EC 1.8.7.1), SO_2 (sulfur dioxide), SO_3^{2-} (sulfite), SO_4^{2-} (sulfate). The closed lines filled with yellow represent enzymes. Rectangles represent substance/organelle reaction conditions. Rectangles filled with light green represent substances (wherein the blue-lettered cells are pivotal substances, yellow-lettered cells are involved in the synthesis of substrates, and green-

lettered cells are reaction synthesis byproducts). Rectangles filled with dark green represent substance organelles/reaction conditions (wherein the white-lettered cells are organelles and the yellow-lettered cells are reaction conditions). Yellow arrows represent the substrates involved in the reaction, green arrows represent byproducts of the reaction, and red arrows represent the need for verification by further research. The reactions involved in the blue arrows are not currently found in plants.

In plants, the synthesis pathway of H_2S can be divided into five types, according to the different substrates. These are cysteine degradation and sulfite reduction, cyanide detoxification, iron-sulfur cluster turnover, and carbonyl sulfide (COS) conversion [14] (**Figure 1**). Similar to animals, the metabolism of cysteine is the main source of endogenous H_2S production in plants. The plants also have a characteristic sulfate reduction assimilation of H_2S production. H_2S synthesis occurs in chloroplasts, cytoplasm, and mitochondria [15]. First, H_2S is mainly derived from cysteine degradation in the plant, catalyzed by different cysteine-degrading enzymes, including l-cysteine desulfhydrase (l-DES, EC 4.4.1.1), d-cysteine desulfhydrase (d-DES, EC 4.4.1.15), and l-3-cyanoalanine synthase (CAS, EC 4.4.1.9) [16]. Second, H_2S is derived from the reductive assimilation of sulfite (SO_3^{2-}) in the plants (**Figure 1**). These two pathways of H_2S synthesis are closely linked. Sulfate or atmospheric sulfur dioxide (SO_2) is the source of SO_3^{2-} production in the plant in the presence of adenosine 5′-phosphosulfate (APS) reductase (APSR, EC 1.8.99.2). Atmospheric SO_2 can also produce SO_3^{2-} spontaneously via non-enzymatic interaction with water. Sulfite reductase (SiR, EC 1.8.7.1) reduces SO_3^{2-} to H_2S in the presence of chloroplast enzymes and ferredoxin [17][18]. Under alkaline conditions in the chloroplast stroma, plants spontaneously transport HS^- (a dissociated form of H_2S) into the cytoplasm (cytosol). With pyridoxal phosphate as a cofactor, l/d-cysteine is catalyzed by l/d-DES to produce pyruvate, NH_4^+, and H_2S in the cytoplasm, chloroplasts, and mitochondria. Third, the nitrogenase Fe-S cluster-like (NFS/NifS-like) protein, which has similar activity to l-cysteine desulfurases, also catalyzes the conversion of cysteine to alanine and sulfur or sulfide using l-Cys as a substrate. It is also a possible source of H_2S in plants [19][20]. The cyanide detoxification mechanism is also an important source of H_2S in the plant (**Figure 1**). Ser and Acetyl-CoA are used to synthesize the intermediate reaction of *O*-acetyl-l-serine (OAS), catalyzed by Ser acetyltransferase (SAT, EC 2.2.1.30). *O*-acetyl-serine(thiol)lyase (OASTL, EC 2.5.1.47), also known as cysteine synthase, catalyzes the insertion of a particular sulfide (in this case, H_2S) into the carbon skeleton via an elimination reaction, and produces cysteine [21]. CAS involves cyanide detoxification and regulates the production of H_2S in mitochondria. H_2S is both an intermediate reduction product of sulfate assimilation and a substrate for the synthesis of cysteine. The biosynthesis of cysteine in plastids implies a transition between a reduction in the assimilated sulfate-reducing pathway and actual metabolism [21]. The iron-sulfur cluster located in *Arabidopsis* mitochondria is capable of assembling the NIF system and presents cysteine desulfurase activity, which may also offer a potential source of H_2S [22]. Besides the four H_2S synthesis pathways described above, carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the hydrolysis of COS to produce carbon dioxide (CO_2) and H_2S. The plants absorb COS from the air and achieve the efficient use of sulfur assimilation via CA [23][24], which may also be a source of endogenous H_2S in the plant.

2. Metabolic Pathways of H_2S

H_2S exhibits diverse physiological and signaling roles, mainly in four distinct biochemical ways (**Figure 2**): (1) reacting with reactive molecule species, such as ROS, reactive nitrogen species (RNS), hypochlorite (HOCl), and reactive carbonyl species (RCS) [25][26][27]; (2) binding to the metal center of metalloproteins or the reduction of the hemoglobin center [28][29][30]; (3) post-translationally modifying proteins with specific structures (e.g., proteins containing cysteine residues (-SH)), which are mainly via *S*-sulfhydration [1][31][32][33]. The other PTMs are described in detail below [1]; (4) activities involving the oxidative and methylation pathways [6][34].

H_2S reacts with the reactive molecule species, including ROS and RNS [1] (**Figure 2**). H_2S can react with several biological oxidants, including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO\cdot$), nitrogen dioxide (NO_2), peroxynitrite (ONOOH), and many others. H_2S reacts readily with HOCl to form polysulfides (-S-S_n-S-) [25]. Excessive ROS and RNS levels lead to oxidative stress when plants are exposed to adversity. In turn, H_2S significantly impacts the products of the plant's defensive system. Nitric oxide (NO) is an important signaling molecule. H_2S can react with NO, leading to the formation of various nitrogen (nitrous oxide (N_2O), nitroxyl (HNO), *S*-nitrosothiols (RS-NO/SNO), and sulfur derivatives (e.g., S^0, S^-), which are thus involved in physiological signaling. NO converts the adversity-induced $O_2^{\bullet-}$ to the less toxic $ONOO^-$. H_2S further reacts with $ONOO^-$ to form thionitrate ($HSNO_2$) [27]. This property of H_2S to actively scavenge $ONOO^-$ provides strong support for the inference that it synergizes with NO to reduce ROS oxidative stress. In addition, H_2S can react directly with NO to produce HNO and also react with RS-NO to form thionitrous acid (HSNO) [27]. HSNO can be metabolized to provide NO^+, NO, and NO species, thus acting as a transportable NO reservoir in the organism that is involved in NO signaling.

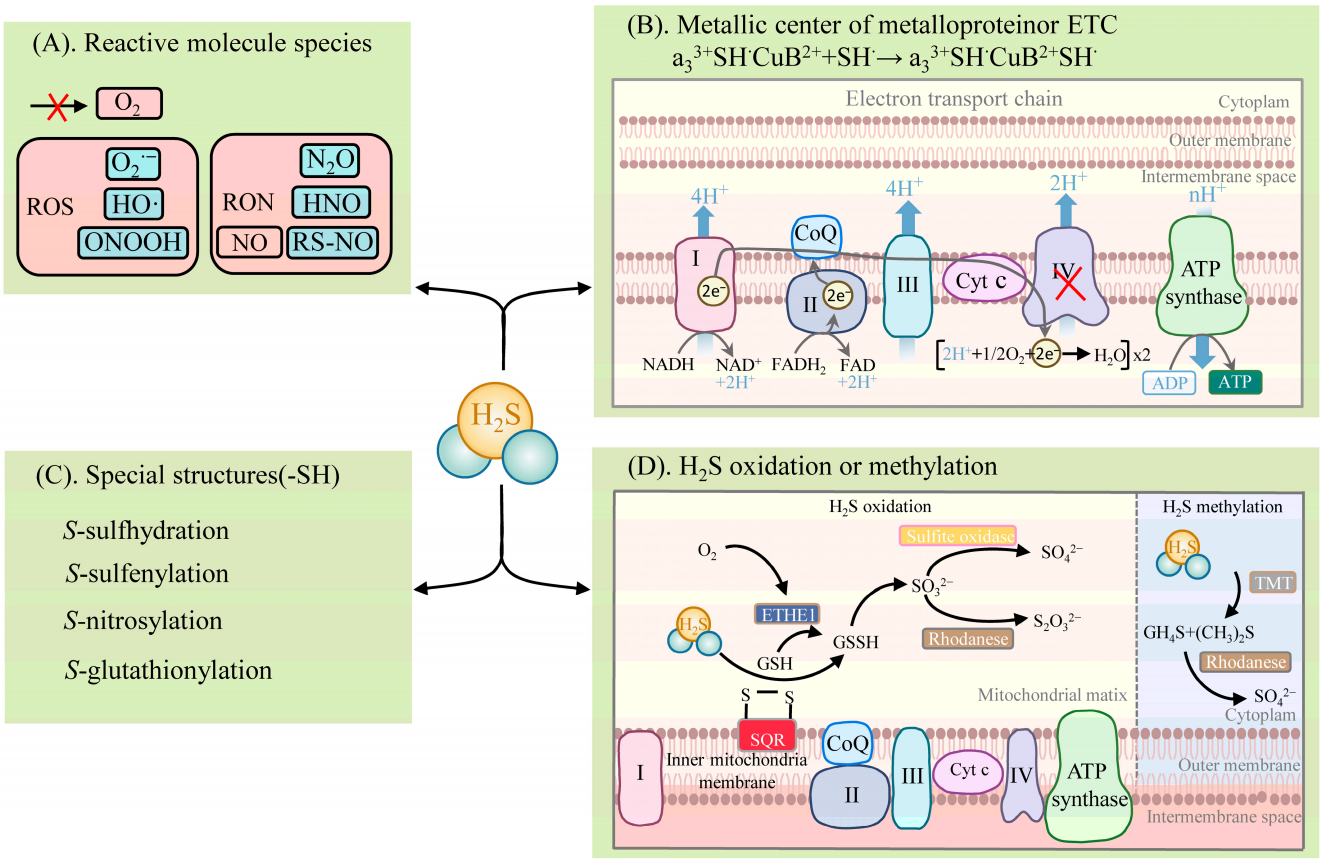

Figure 2. Metabolism of H_2S: (**A**) reaction of H_2S with reactive molecule species; (**B**) binding or electron transfer of H_2S to the metal center of a metalloprotein; (**C**) reaction of H_2S with proteins with specific structures (involved in post-translational modifications); (**D**) metabolic pathways of H_2S oxidation and methylation. Note: I (mitochondrial complex I), II (mitochondrial complex II), III (mitochondrial complex III), IV (mitochondrial complex IV), Cyt c (Cytochrome c), CoQ (coenzyme Q/ubiquinone), CH_4S (methanethiol), $(CH_3)_2S$ (dimethyl sulfide), ETHE1 (ethylmalonic encephalopathy 1 protein), GSH (glutathione), GSSG (glutathiol), HNO (nitroxyl), H_2S (hydrogen sulfide), HO· (hydroxyl radicals), N_2O (nitrous oxide), NO (nitric oxide), O_2 (oxygen), $O_2^{\bullet-}$ (superoxide radical), ONOOH (peroxynitrite), RON (reactive nitrogen species), ROS (reactive oxygen species), RS-NO (*S*-nitrosothiols), SO_4^{2-} (sulfate), $S_2O_3^{2-}$ (thiosulfuric acid), SO_3^{2-} (sulfite), SQR (sulfide quinone reductase), TMT (thiol *S*-methyl-transferase), TST (thiosulfate sulfurtransferase). A red cross indicates an inhibitory effect on enzyme activity or the production of a substance.

H_2S binds to the metal centers of metalloproteins or participates in electron transfer [1] (**Figure 2**). H_2S involves the physiological regulation of the oxidative phosphorylation of the electron transfer chain (ETC) by means of binding to components of the ETC. It is mainly the direct binding of H_2S to COX that affects ETC function, and the reduction of COX by H_2S leads to the formation of $HS^{\bullet}$ or $S^{\bullet-}$ (which can interact with protein sulfhydryl groups (thiol)), affecting other components of the ETC. ETC complex IV, also known as COX, consists of two redox centers, Cyt a, Cu_A, and Cyt a_3, Cu_B. H_2S associates with the COX component, hematoxylin a_3 (heme a_3), and the Cu_B center, thus participating in the electron transfer of the ETC [28][29]. H_2S favors the formation of a polar environment (tyrosine (Tyr) residues and Cu_B centers) around the heme a_3 subunit, while H_2S promotes heme a_3 reduction to achieve an increase in COX enzyme activity at low concentrations. At high concentrations, H_2S can bind directly to the component a_3 and Cu_B centers of COX, resulting in the formation of the stable H_2S-Cu_B and unstable hemoglobin H_2S-Fe^{2+} inhibitory groups. In this case, the stability of the H_2S-Fe^{2+} group is dependent on H_2S concentration. However, the inhibition of COX by H_2S can behave differently, depending on the concentration. Unlike medium concentrations, a high concentration of H_2S is accompanied by the formation of stable hemoglobin a_3 H_2S-Fe^{3+} inhibitory groups, the inhibitory effect of which is irreversible [28][29][30]. In addition, the reduction of COX by H_2S promotes increased ATP synthesis (which can bypass complex III to promote ETC activity) and the accumulation of reactive sulfur [6].

H_2S can post-translationally modify proteins by converting the thiol group of cysteine residues to the persulfide group (-SSH) [1][31][32][33] (**Figure 2**). This modification is named *S*-sulfhydration [1]. The increased nucleophilicity of the converted persulfides, compared to the thiol group, highlights the highly reactive nature of *S*-sulfhydration. This also explains the potential for persulfides to act as mediators of sulfide signaling.

The metabolic pathways of H_2S include oxidation and methylation [34] (**Figure 2**). The oxidation of H_2S occurs in the mitochondria and involves several enzymes, such as sulfide quinone reductase (SQR) and the ethylmalonic encephalopathy 1 protein (ETHE1, also known as persulfide dioxygenase), thiosulfate sulfurtransferase (TST, also known as rhodanese), and mitochondrial sulfite oxidase. SQR oxidizes H_2S in the inner mitochondrial membrane to produce persulfide species (e.g., glutathiol (GSSG)). At the same time, electrons released by SQR are captured by ubiquinone and transferred from H_2S to coenzyme Q and to ETC at complex III. The persulfide is further

oxidized by ETHE1 to produce SO_3^{2-}, which is further oxidized by sulfite oxidase to SO_4^{2-}, or by TST to $S_2O_3^{2-}$ [34]. The metabolism of H_2S by methylation occurs more as a complementary mechanism to oxidation and takes place in the cytoplasm. Then, thiol *S*-methyltransferase converts H_2S to methanethiol (CH_4S) and dimethyl sulfide $(CH_3)_2S$, which is further oxidized by rhodanese to produce thiocynate and SO_4^{2-} [6].

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