FORUM EDITORIAL

Signaling of Hydrogen Sulfide and Polysulfides

Hideo Kimura

Abstract

It has been almost two decades since the first demonstration of hydrogen sulfide (H_2S) as a physiological mediator of cognitive function and vascular tone. H_2S is physiologically important because it protects various organs from ischemia–reperfusion injury besides regulating inflammation, oxygen sensing, cell growth, and senescence. The production, metabolism, and regulation of H_2S have been studied extensively. H_2S modulates target proteins through sulfhydration (or sulfuration) or by the reduction of cysteine disulfide bonds. A large number of novel H_2S -donating compounds are being developed owing to the therapeutic potential of H_2S . Recently, polysulfides, rather than H_2S , have been identified as molecules that sulfhydrate (or sulfurate) their target proteins. *Antioxid. Redox Signal.* 22, 347–349.

THREE ENZYMES, CYSTATHIONINE β -SYNTHASE (CBS), cystathionine γ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST), together with cysteine aminotransferase (CAT), are known to produce hydrogen sulfide (H_2S) (2). In recent years, many studies have been undertaken to delineate the roles of H_2S as a signaling molecule. To achieve this objective, the process for regulating H_2S levels (through the regulation of enzyme activity) and the metabolic turnover of H_2S have been extensively studied. The CBS activity is enhanced by S-adenosylmethionine. The activity of CAT and CSE is regulated by Ca^{2+} in a calmodulin-independent manner. Controversial observations have been reported for CSE, which is activated by Ca^{2+} in a calmodulin-dependent manner. The 3MST produces H_2S in the presence of thioredoxin. A novel pathway for generating H_2S from D-cysteine was recently identified (2). This pathway is active only in some specific organs and exhibits a much higher efficiency than that exhibited by the L-cysteine pathway. It also shows therapeutic potential by protecting these organs against ischemia–reperfusion injury.

One of the modes through which H_2S carries out signaling is by the addition of sulfur to the cysteine residues of target proteins, thereby producing bound sulfane sulfur. This elicits a conformational change in the proteins and modifies their activities. The above process is called sulfhydration or sulfuration, and it is analogous to the nitrosylation reaction carried out by NO (5). Recently, however, polysulfides, but not H_2S , have been shown to induce sulfhydration (or sulfuration). Molecules with the same oxidation state do not

react with each other. Because the oxidation state of S in both H_2S and cysteine residues is -2 , H_2S and cysteine residues are unable to react with each other. In contrast, because the oxidation state of S in polysulfides is 0, it easily reacts with cysteine by adding bound sulfane sulfur to this residue (sulfhydration). Polysulfides activate the transient receptor potential ankyrin 1 (TRPA1) channels by sulfhydrating (sulfurating) two cysteine residues at the amino terminus (2). They also facilitate the translocation of Nrf2 (which upregulates the transcription of antioxidant genes) to the nucleus by sulfhydrating an accompanying protein called Keap1. The activity of the phosphatase and tensin homolog (PTEN), a lipid phosphatase, which regulates the growth of cancer cells, is also regulated by polysulfides (2). The physiological roles of polysulfides are therefore quite robust (Fig. 1).

H_2S has been reported to inhibit cancer development at various stages (6). H_2S donors are cytotoxic to human cancer cells. They also induce cell cycle arrest in the G2/M phase and promote apoptosis. However, they do not affect the survival of normal human cells. Several recent studies have shown that H_2S -releasing NSAIDs, which were initially developed by Wallace *et al.* as anti-inflammatory drugs having no side effects on the gastrointestinal tract, exhibit enhanced chemopreventive effects in cancer cells (7). These compounds inhibit cancer cell proliferation and promote apoptosis, as well as cell cycle arrest. The parental NSAIDs alone do not confer any beneficial effects in cancer cells. H_2S donors and H_2S -releasing NSAIDs show great promise as therapeutic agents against several forms of cancer. Contrary to these observations, CBS is

FIG. 1. Regulation of H₂S production, modification of target proteins, and cancer therapy. Under oxidative stress, a cysteine residue 346 in CBS is oxidized to sulfenic acid, which is modified by glutathionylation. Glutathionylated CBS increases the production of cysteine, H₂S, and glutathione, resulting in the decrease in cellular oxidative stress (3). Modification of the activity by the addition of sulfur to cysteine residues of target proteins is called sulfhydrylation or sulfuration, which has been proposed as a mode of action of H₂S (5). However, recently, polysulfides but not H₂S were found to take the reaction (2). It resolved the problem that sulfur in H₂S and cysteine residues cannot react with each other due to the same oxidation state, whereas that in polysulfides is in a different oxidation state and easily reacts with cysteine residues. H₂S as well as H₂S-releasing NSAIDs suppress cancer growth (7). In contrast, in ovarian and colon cancer, growth is enhanced by H₂S produced by upregulated CBS (1). In these cancer cells, the suppression of CBS activity can be used as a potential therapy. CBS, cystathionine β -synthase; H₂S, hydrogen sulfide.

upregulated in colorectal and ovarian cancer cells, where H₂S is involved in promoting cellular bioenergetics, proliferation, and migration (1). In these cancer cells, the suppression of CBS activity can be used as a potential therapy (Fig. 1).

Banerjee and colleagues, in their original article, demonstrated that the CBS activity is enhanced by the S-glutathionylation of a cysteine residue of CBS in cells under oxidative stress (3). Cells experiencing oxidative stress decrease their glutathione levels to prevent further damage. S-glutathionylation increases the CBS activity, which in turn generates cysteine (a substrate for the production of glutathione) as well as H₂S. The generation of these two products makes cells resistant to oxidative stress (Fig. 1).

In their review article, Paul and Snyder discussed the physiological role of sulfhydrylation (sulfuration), a process that involves the modification of protein activities and produces effects opposite to those elicited by nitrosylation (5). Sulfhydrylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) increases its catalytic activity, whereas nitrosylation suppresses it. Parkin, an E3 ubiquitin ligase, is a protein that is affected during Parkinson's disease. It is less sulfhydrylated and shows a reduced catalytic activity in patients suffering from Parkinson's disease. However, the same protein is nitrosylated and inactivated to a higher degree after the

onset of this neurodegenerative disease (5). Paul and Snyder also gave one more example of the involvement of H₂S in neurodegenerative disorders: the levels of H₂S and CSE are lower in patients suffering from Huntington's disease than in normal individuals. H₂S thus plays an important role in the pathophysiology of this disease (5).

H₂S induces conditions similar to hypoxia and acts on effector molecules known to mediate hypoxic responses. The metabolism of H₂S is coupled to that of O₂, and the concentrations of H₂S are inversely related to those of O₂ in tissues. Based on these observations, H₂S has been proposed as an O₂ sensor (4). In his review article, Olson discussed that the O₂-dependent metabolism of H₂S is an O₂ sensor (4). The highly reactive sulfur is able to form bonds with its own species as well as with nitrogen and oxygen. It regulates protein functions through sulfhydrylation (sulfuration) and nitrosylation, as described in the Forum article by Paul and Snyder (5). O₂-dependent H₂S inactivation can be effectively used in accurately determining O₂ availability and for coordinating the supply and demand at O₂-sensing tissues and cells. The development of specific inhibitors of H₂S metabolism and the development of H₂S donors that release H₂S in a controlled manner will enhance the understanding of the O₂-sensing process of this molecule.

The effects of H₂S and related compounds on various types of cancers and their ability to suppress cancer development have been described previously (6, 7). According to Szabo and colleagues, the production of H₂S by CBS is increased in colorectal and ovarian cancers and both play an important role in promoting cellular bioenergetics, proliferation, and migration (1). H₂S induces angiogenesis and local vascular relaxation, which increases the blood flow and nutrient supply to the tumor. It also enhances oxygen consumption, ATP production, and glutathione production to support tumor growth. The therapeutic potential of CBS inhibitors in cancer therapy has been discussed previously (1). The unavoidable side effects of these inhibitors include the accumulation of cellular homocysteine, a cytotoxic molecule and a cardiovascular risk factor.

H₂S exerts regulatory effects on inflammatory processes and promotes their resolution. According to Wallace *et al.*, several H₂S-releasing anti-inflammatory drugs, which are being developed for the treatment of a wide range of diseases, show considerable promise in relevant animal models. Many of these drugs are undergoing human trials (7). H₂S-releasing NSAIDs show therapeutic potential because they can be used as chemopreventive agents in several forms of cancer.

In his review article, Kimura discussed the regulation of H₂S-producing enzymes, described a novel pathway that produces H₂S from D-cysteine, and highlighted some of the recent findings on polysulfides as potential signaling molecules (2). Polysulfides (but not H₂S) add sulfur to the cysteine residues of target proteins, thereby generating bound sulfane sulfur (sulfhydrylation or sulfuration). Examples include the activation of the TRPA1 channels and the facilitation of Keap1 to release Nrf2, which then translocates to the nucleus (2). Reactions, in which the induction is attributed to H₂S, are classified into two groups: the first group includes H₂S, which is involved in the reduction of the cysteine disulfide bond, and the second group includes polysulfides, which are involved in sulfhydrylation or sulfuration.

Some of these Forum articles were presented and discussed in the Third International Conference on H₂S in Biology and

Medicine, held in Kyoto and organized by Hideo Kimura in June, 2014 (Nitric Oxide 39: Supplement 1, 2014).

Acknowledgments

This work was supported by a grant from the National Institute of Neuroscience, KAKENHI (23659089) Grant-in-Aid for Challenging Exploratory Research, and KAKENHI (26460115) Grant-in-Aid for Scientific Research to H.K.

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Address correspondence to:

Dr. Hideo Kimura

Department of Molecular Pharmacology

National Institute of Neuroscience

4-1-1 Ogawahigashi

Kodaira, Tokyo 187-8502

Japan

E-mail: kimura@ncnp.go.jp

Date of first submission to ARS Central, August 17, 2014;
date of acceptance, August 31, 2014.

Abbreviations Used

3MST = 3-mercaptopyruvate sulfurtransferase

CAT = cysteine aminotransferase

CBS = cystathionine β -synthase

CSE = cystathionine γ -lyase

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

H₂S = hydrogen sulfide

TRPA1 = transient receptor potential ankyrin 1