Atomic Sulfur: An Element for Adaptation to an Oxidative Environment

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During the period of rising oxygen concentration in the Earth's atmosphere (Figure 1), sulfur atoms were incorporated into proteins as redox-active cysteine residues [1] and antioxidant molecules such as thioredoxin, glutathione, and glutaredoxin appeared.

Cysteine residues in proteins form intra- and inter-molecular disulfides that maintain protein and peptide structure and also regulate protein function. In addition, the redox-active cysteine residues are known to regulate the redox state of proteins and function by reversible oxidation to cysteine sulfinic or cysteine sulfenic residues, or by -SH group sulfuration to persulfides. Finally, cysteine residues of the catalytic sites of such enzymes as sulfurtransferases and sulfotransferases, contributing to the transfer of elemental sulfur and sulenate, respectively, are involved in sulfur metabolism. Recently it has been reported that four sulfurtransferases, cystathionine β-synthase (EC 4.2.1.22, CBS) [2], cystathionine γ-lyase (EC 4.4.1.1, CSE) [3], thiosulfate sulfurtransferase (rhodanese, TST) [4,5], and 3-mercaptopyruvate sulfurtransferase (MST) [4–9] produce hydrogen sulfide and polysulfides. The physiological functions of these sulfurtransferases have been elucidated.

Two years after the publication of the first special issue in 2014, a large number of investigations have demonstrated the extensive regulatory involvement of the endogenous pool of sulfide and, to date, research in this area has progressed significantly. In this issue, we present the findings and review work related to hydrogen sulfide and polysulfide, hydrogen sulfide and polysulfide-producing enzymes, antioxidative function and antioxidants (thioredoxin, glutathione, enzymes), cysteine persulfide and protein polysulfidation, sulfane sulfur, elemental sulfur, sulfur amino acids, hydrosulfide and tyrosine sulfation, including that from previous special issues.
The physiological actions of hydrogen sulfide and polysulfides have been widely reported [10–16]. Kimura reviewed the functions of H₂S and polysulfides as biological mediators [10]. The concentration of H₂S, determined by the activity of enzymes involved in its formation and clearance, depends on S-adenosyl methionine and CBS glutathionylation (both enhance the activity of CBS), NO and CO levels (suppress CBS activity), and Ca²⁺ concentrations (regulation of CSE and cysteine aminotransferase activity). However, the regulation of H₂S degrading enzymes (Sulfide-Quinone Reductase and sulfur dioxygenase) is currently poorly understood. Polysulfides, found in tissues, modify the activities of channels, enzymes, and transcription factors through the mechanism of sulfuration. Polysulfide production, degradation, and regulation of these processes remain to be fully investigated and understood. The reaction of H₂S with NO can result in the formation of highly reactive substances (HSNO, GSNO, HNO, or HSSNO); elucidation of the mechanisms controlling their production and physiological effects will help to unravel the multiple roles of H₂S and related molecules.

Hydrogen sulfide is synthesized in adipose tissue and is involved in the regulation of adipogenesis [11]. Deficiency of H₂S may contribute to adipose tissue inflammation associated with obesity/metabolic syndrome. Experimental obesity induced by high calorie diets results in increased adipose tissue inflammation associated with obesity/metabolic syndrome.
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obesity/metabolic syndrome. Experimental obesity induced by high calorie diets results in increased or decreased H\textsubscript{2}S in perivascular adipose tissue depending on duration time. Hyperglycemia suppresses the CSE-H\textsubscript{2}S pathway in various adipose tissue depots. Hence, it was proposed that augmentation of H\textsubscript{2}S signaling could diminish adipose tissue inflammation and correct related metabolic abnormalities.

Gastroprotective properties of H\textsubscript{2}S and its role in the defense mechanism against stress were confirmed by stimulation of H\textsubscript{2}S production and the observed beneficial effect of H\textsubscript{2}S on healing of gastric ulcers [12]. The results presented point to the possible role of rhodanese in H\textsubscript{2}S production in the gastric mucosa of rats.

The mechanism of H\textsubscript{2}S-mediated gastroprotection against ischemia/reperfusion (I/R) lesions has been studied [13]. I/R-induced gastric mucosa damage was reduced by pretreatment with H\textsubscript{2}S precursors (L-cysteine, GYY4137, NaHS) and this effect was accompanied by restoration of gastric microcirculation due to the vasodilatory activity of H\textsubscript{2}S. The mechanism was shown to involve the antioxidative properties of H\textsubscript{2}S, in the sense of increased expression of antioxidative enzymes (SOD-2 and GPx-1) in response to pretreatment with NaHS.

H\textsubscript{2}S produced in vascular smooth muscle cells can directly regulate the vascular tone in an autocrine manner. H\textsubscript{2}S synthesized in endothelial cells may have specific roles and specific targets e.g., Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, endothelial NO synthase, and may be regulated independently of smooth muscle cells i.e., by mediators such as acetylcholine or leptin. In some pathological conditions, including obesity and I/R injury, endothelial H\textsubscript{2}S signaling is upregulated, which can be regarded as a specific protective mechanism, maintaining the regulation of vascular tone [14]. Challenges for future research include the regulation of endothelial H\textsubscript{2}S generation in pathological conditions, and possible therapeutic approaches to modulate its synthesis, metabolism, and signaling.

H\textsubscript{2}S is produced by the gut flora. H\textsubscript{2}S released in the colon (sulfate reducing bacteria are ubiquitous in mammalian colon) may contribute to the control of arterial blood pressure. Studies suggest the possibility of modifying the flora for therapeutic benefit.

H\textsubscript{2}S and sulfane sulfur are formed from lipoic acid (LA) non-enzymatically but LA cannot be a direct donor of H\textsubscript{2}S/sulfane sulfur in tissues [16]. Under physiological conditions, an increased level of H\textsubscript{2}S in samples containing LA and dihydrolipoic acid (DHLA) was accompanied by a decrease in sulfane sulfur level. This suggests that DHLA acts as a reducing agent that releases H\textsubscript{2}S from sulfane sulfur-containing compounds, indicating the possible mechanism of pharmacological action of LA.

As for hydrogen sulfide and polysulfide related enzymes, the tissue and cellular distribution of MST in the mouse was further investigated by Tomita and colleagues [17]. Interestingly MST was extensively distributed in the endocrine organs of the mouse in addition to other tissues. They also confirmed the findings in rat tissues that Nagahara and colleagues previously reported [18]. Most and Papenbrock [19] reviewed the TST family of plant enzymes, which were shown to protect against cyanide toxicity and oxidative stress.

In relation to the antioxidative functions of sulfur, Mukwevho and colleagues [20] reviewed its integration into proteins as the redox-active cysteine residue and in species such as glutathione, thioredoxin, and glutaredoxin, which served as antioxidant molecules. Guevara-Flores and colleagues [21] reviewed unique thiol-based antioxidant systems in invertebrate parasites to understand their antioxidant defense mechanisms. This information could help to design drugs targeting these organisms. Bhuiyan and colleagues [22] reported that natural organosulfur compounds served as antioxidants and chemo-sensitizers and were implicated in the in vitro inhibition of tumor cell proliferation through the induction of apoptosis. TST catalyzes the H\textsubscript{2}S release reaction from garlic organosulfur compounds and Cys/GSH mixed disulfide conjugates that were spontaneously produced. Thus, a water-soluble, glutathione-garlic extract, serving as a slowly releasing hydrogen sulfide precursor was postulated to have potential therapeutic applications. Lin and colleagues [23] reviewed the evidence that gram-negative bacteria principally prevent cysteine overoxidation via the formation of mixed protein disulfides with low molecular weight thiols such as glutathione and glutathionyl
spermidine. Cysteine was identified as highly susceptible to reactive oxygen species. Campos-Acevedo and Rudiño-Piñera [24] reported the optimum energy to perform X-ray of a crystal structure with an interface disulfide bond. The interface disulfide bond of thioredoxin 1 from Litopenaeus vannamei was very stable (less susceptible to being reduced by X-rays).

Cysteine persulfide and protein polysulfidation were reviewed by Kasamatsu and colleagues [25] who concluded that reactive persulfide species were physiologically important. Species such as cysteine persulfide and glutathione persulfide had higher nucleophilicity than the parents, cysteine (Cys) and glutathione. These reactive species protected against oxidants and contributed to redox signaling regulation. Protein polysulfidation also protected against oxidants.

Toohey and Cooper [26] reviewed the nature of thiosulfoxide (sulfane) sulfur, the history of its regulatory role, its generation in biological systems, and its functions, including synthesis of cofactors such as molybdenum cofactor and iron-sulfur clusters, sulfuration of tRNA, modulation of enzyme activities, and regulation of the redox environment.

Sulfane sulfur, the reactive sulfur atom in a zero to divalent (0 to $-2$) oxidation state can be converted to hydrogen sulfide on reduction with thiol-containing reducing agents [27]. Examples of this type of bound sulfur are the polysulfides involved in modification of protein function through persulfidation of cysteine residues (difficult to achieve with $H_2S$ directly), scavenging reactive carbonyl compounds in the central nervous system, and which also function in the redox regulation system in addition to other possible physiological roles.

Further studies [28] demonstrated high levels of sulfane sulfur (cyanolysable bound sulfur) in high grade gliomas (III/IV and IV grades) in comparison to various human brain regions which correlated with a decreased activity of CSE, MST, and TST. These results indicated the importance of sulfane sulfur for malignant cell proliferation and tumor growth and also suggest the possible application of dietary cysteine restriction in the treatment of glioma. These observations suggest that further investigations should be carried out to confirm whether precursors of sulfane sulfur might inhibit the proliferation of gliomas. Sulfane sulfur dependency of the proliferation of cancer cells has been reported previously [29,30]. Opposing effects in malignant and normal cells opens up interesting opportunities for nutritional strategies in anticancer therapy.

In the case of the sulfur amino acids, excessive dietary intake of cystine and methionine can promote liver pathology such as diet-induced fatty liver [31]. The lipotropic effect of methionine may be mediated by sulfane sulfur. The hepatosteatogenic effect of cystine may be related to the removal of sulfane sulfur by cysteine catabolites. This form of sulfur is recognized as a regulatory agent in many physiological processes but the mechanism of control remains to be explained. Possible preventive and therapeutic strategies are discussed in relation to the avoidance of lifestyle induced health problems.

Toohey [32] has discussed the roles of hydrosulfide and hypothesized that the sulfur atom was involved in vitamin $B_{12}$-dependent methyl group transfer. He further proposed that sulfane sulfur/hydrogen sulfide may be beneficial in treating Alzheimer’s disease. Finally, Yang and colleagues [33] reviewed the activity and function of tyrosylprotein sulfotransferase, which catalyzes the sulfation reaction, whereby sulfonate is transferred from $3′$-phosphoadenosine-$5′$-phosphosulfate to tyrosine resulting in protein tyrosine sulfation and inducing protein-protein interactions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nagahara, N. Regulation of mercaptopyruvate sulfurtransferase activity via intrasubunit and intersubunit redox-sensing switches. *Antioxid. Redox Signal.* 2013, 19, 1792–1802. [CrossRef] [PubMed]
2. Abe, K.; Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 1996, 16, 1066–1071. [PubMed]
3. Hosoki, R.; Matsu, K.; Kimura, H. The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. *Biochem. Biophys. Res. Commun.* 1997, 237, 527–531. [CrossRef] [PubMed]
4. Kimura, H. Hydrogen sulfide and polysulfides as signaling molecules. Proc. Jpn. Acad. Ser. B 2015, 91, 131–159. [CrossRef] [PubMed]
5. Mikami, Y.; Shibuya, N.; Kimura, Y.; Nagahara, N.; Yamada, M.; Kimura, H. Hydrogen sulfide protects the retina from light-induced degeneration by the modulation of Ca\(^{2+}\) influx. J. Biol. Chem. 2011, 286, 39379–39386. [CrossRef] [PubMed]
6. Mikami, Y.; Shibuya, N.; Kimura, Y.; Nagahara, N.; Ogasawara, Y.; Kimura, H. Thioredoxin and dihydrolipoic acid are required for 3-mercaptoppyruvate sulfurtransferase to produce hydrogen sulfide. Biochem. J. 2011, 439, 479–485. [CrossRef] [PubMed]
7. Shibuya, N.; Mikami, Y.; Kimura, Y.; Nagahara, N.; Kimura, H. Vascular endothelium expresses 3-mercaptoppyruvate sulfurtransferase and produces hydrogen sulfide. J. Biochem. 2009, 146, 623–626. [CrossRef] [PubMed]
8. Shibuya, N.; Tanaka, M.; Yoshida, M.; Ogasawara, Y.; Togawa, T.; Ishii, K.; Kimura, H. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. Antioxid. Redox Signal. 2008, 11, 703–714. [CrossRef] [PubMed]
9. Yadav, P.K.; Yamada, K.; Chiku, T.; Koutmos, M.; Banerjee, R. Structure and kinetic analysis of H\(_2\)S production by human mercaptoppyruvate sulfurtransferase. J. Biol. Chem. 2013, 288, 20002–20013. [CrossRef] [PubMed]
10. Kimura, H. Hydrogen sulfide and polysulfides as biological mediators. Molecules 2014, 19, 16146–16157. [CrossRef] [PubMed]
11. Beltowski, J.; Jamroz-Wiśniewska, A. Hydrogen sulfide in the adipose tissue—Physiology, pathology and a target for Ppharmacotherapy. Molecules 2017, 22, 63. [CrossRef] [PubMed]
12. Bronowicka-Adamska, P.; Wróbel, M.; Magierowski, M.; Magierowska, K.; Kwiecień, S.; Brzozowski, T. Hydrogen sulphide production in healthy and ulcerated gastric mucosa of rats. Molecules 2017, 22, 530. [CrossRef] [PubMed]
13. Magierowski, M.; Magierowska, K.; Hubalewska-Mazgaj, M.; Sliwowski, Z.; Papajani, V.T.; Paci, M.; Melino, S. Glutathione-garlic sulfur conjugates: Slow hydrogen sulfide releasing agents for therapeutic applications. Molecules 2015, 20, 1731–1750. [CrossRef] [PubMed]
14. Most, P.; Papenbrock, J. Possible roles of plant sulfurtransferases in detoxification of cyanide, reactive oxygen species, selected heavy metals and arsenate. Molecules 2015, 20, 1410–1423. [CrossRef] [PubMed]
15. Molecules 2017, 22, 388. [CrossRef] [PubMed]
16. Tomita, M.; Nagahara, N.; Ito, T. Expression of 3-mercaptoppyruvate sulfurtransferase in the mouse. Molecules 2016, 21, 1707. [CrossRef] [PubMed]
17. Nagahara, N.; Ito, T.; Kitamura, H. Tissue and subcellular distribution of mercaptoppyruvate sulfurtransferase in the rat: Confocal laser fluorescence and immunoelectron microscopic studies combined with biochemical analysis. Histochem. Cell Biol. 1998, 110, 243–250. [CrossRef] [PubMed]
18. Most, P.; Papenbrock, J. Possible roles of plant sulfurtransferases in detoxification of cyanide, reactive oxygen species, selected heavy metals and arsenate. Molecules 2015, 20, 1410–1423. [CrossRef] [PubMed]
19. Mukwewho, E.; Ferreira, Z.; Ayelos, A. Potential role of sulfur-containing antioxidant systems in highly oxidative environments. Molecules 2014, 19, 19376–19389. [CrossRef] [PubMed]
20. Guevara-Flores, A.; Martinez-González, J.J.; Rendón, J.L.; Arenas, I.P. The architecture of thiol antioxidant systems among invertebrate parasites. Molecules 2017, 22, 259. [CrossRef] [PubMed]
21. Bhuiyan, A.I.; Papajani, V.T.; Paci, M.; Melino, S. Glutathione-garlic sulfur conjugates: Slow hydrogen sulfide releasing agents for therapeutic applications. Molecules 2015, 20, 1731–1750. [CrossRef] [PubMed]
22. Lin, J.C.-Y.; Chiang, B.-Y.; Chou, C.-C.; Chen, T.-C.; Chen, Y.-J.; Lin, C.-H. Glutathionylspermidine in the modification of protein SH groups: The enzymology and its application to study protein glutathionylation. Molecules 2015, 20, 1452–1474. [CrossRef] [PubMed]
23. Campos-Acevedo, A.A.; Rudinño-Piñera, E. Crystallographic studies evidencing the high energy tolerance to disrupting the interface disulfide bond of thioredoxin 1 from white leg shrimp Litopenaeus vannamei. Molecules 2014, 19, 21113–21126. [CrossRef] [PubMed]
25. Kasamatsu, S.; Nishimura, A.; Morita, M.; Matsunaga, T.; Hamid, H.A.; Akaike, T. Redox signaling regulated by cysteine persulfide and protein polysulfidation. *Molecules* 2016, 21, 1721. [CrossRef] [PubMed]

26. Toohey, J.I.; Cooper, A.J.L. Thiosulfoxide (sulfane) sulfur: New chemistry and new regulatory roles in biology. *Molecules* 2014, 19, 12789–12813. [CrossRef] [PubMed]

27. Koike, S.; Ogasawara, Y. Sulfur atom in its bound state is a unique element involved in physiological functions in mammals. *Molecules* 2016, 21, 1753. [CrossRef] [PubMed]

28. Wróbel, M.; Czubak, J.; Bronowicka-Adamska, P.; Jurkowska, H.; Adamek, D.; Papla, B. Is development of high-grade gliomas sulfur-dependent? *Molecules* 2014, 19, 21350–21362. [CrossRef] [PubMed]

29. Jurkowska, H.; Wróbel, M. N-acetyl-L-cysteine as a source of sulfane sulfur in astrocytoma and astrocyte cultures: Correlations with cell proliferation. *Amino Acids* 2008, 34, 231–237. [CrossRef] [PubMed]

30. Jurkowska, H.; Uchacz, T.; Roberts, J.; Wróbel, M. Potential therapeutic advantage of ribose-cysteine in the inhibition of astrocytoma cell proliferation. *Amino Acids* 2011, 41, 131–139. [CrossRef] [PubMed]

31. Toohey, J. Sulfur amino acids in diet-induced fatty liver: A new perspective based on recent findings. *Molecules* 2014, 19, 8334–8349. [CrossRef] [PubMed]

32. Toohey, J.I. Possible involvement of hydrosulfide in B12-dependent methyl group transfer. *Molecules* 2017, 22, 582. [CrossRef] [PubMed]

33. Yang, Y.-S.; Wang, C.-C.; Chen, B.-H.; Hou, T.-H.; Hung, K.-S.; Mao, Y.-C. Tyrosine sulfation as a protein post-translational modification. *Molecules* 2015, 20, 2138–2164. [CrossRef] [PubMed]