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RESEARCH HIGHLIGHT

# Protein *S*-sulfhydration as a major sources of $H_2S$ bioactivity

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**The physiological and biomedical importance of hydrogen sulfide ($H_2S$) has been extensively studied in our body. $H_2S$ can be endogenously produced in a variety of cells and tissues by cystathionine γ-lyase, cystathionine β-synthase, and/or 3-mercaptopyruvate sulfurtransferase, and is involved in the regulation of vascular function, cell growth, insulin secretion, neurotransmission, myocardial contractility, inflammation, and nociception, etc.$H_2S$ post-translation ally modifies proteins by yielding a hydropersulfide moiety (–SSH) in specific cysteine residue(s), termed as *S*-sulfhydration. It is becoming increasingly recognized that *S*-sulfhydration is a major sources of $H_2S$ bioactivity. In this research highlight, we discuss our latest published findings which demonstrate the *S*-sulfhydration regulation of proteins by $H_2S$ and their importance in aging and cancer protection.**

***Keywords:*** $H_2S$; Cysteine; *S*-sulfhydration; Post-translational modification

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In 2002, Wang first created and defined the word "gasotransmitter", and described the criteria for being a gasotransmitter [1]. Hydrogen sulfide ($H_2S$), joining with nitric oxide (NO) and carbon monoxide, is important member in gas transmitter family [1, 2]. Not only from environment and industry activity, $H_2S$ can also be endogenously produced in our body by specific enzymes, including cystathionine γ-lyase, cystathionine β-synthase, and/or 3-mercaptopyruvate sulfurtransferase, which are expressed or can be induced in most of cell types [3-5]. $H_2S$ can freely penetrate into cell membrane independent of any cognate membrane receptors or other transportation machineries[1].Accumulated evidence demonstrated that $H_2S$ is almost involved in all life functions, including cell growth, glucose metabolism, insulin secretion, neurotransmission, myocardial contractility, inflammation, energy generation, and redox balance, etc [2]. Despite a wealth of recent publications suggest the important path physiological roles for $H_2S$, its molecular mechanisms of action remains poorly defined. It is proposed that the major signaling mechanism of $H_2S$ is through the *S*-sulfhdyration of reactive cysteine residues on target proteins by yielding a hydropersulfide moiety (-SSH), with the potential to confer a functional changes, which is analogous to *S*-nitrosylation of proteins by NO [5].

Multiple cellular functions are controlled through the post-translational modification of proteins, including phosphorylation, glycosylation, ubiquitination, methylation, and *S*-nitrosylation, etc. Post-translational

**Table 1. *S*-sulfhydrated proteins and their functional changes**

| S-sulfhydrated proteins/ electrophiles | Tissue/cell types | Outcome | Targeted cysteine residues | Detection methods | Reference |
|---|---|---|---|---|---|
| GAPDH | Liver/HEK-293 | NADH generation | 150 | BSA/TSA/MS | 5,7 |
| Actin | Liver | Actin polymerization | N/A | BSA | 5 |
| β-tubulin | Liver | N/A | N/A | BSA | 5 |
| Kir6.1 | Aorta/HEK-293 | Opening of $K_{ATP}$ channel/vasorelaxation | 43 | BSA | 3 |
| NF-κB/p65 | Liver/HEK293 /Macrophage | Anti-apoptosis | 38 | BSA/MS | 8,9 |
| PTP1B | Hela/HEK-293 | Restoration of ER homeostasis | 215 | BSA/MS | 10 |
| 8-nitro-cGMP | Fibroblasts | Inhibition of cardiac cell senescence and oxidative stress | N/A | BSA | 11 |
| Keap1 | Fibroblasts | Nrf2 activation and inhibition of oxidative stress | 151 | BSA | 12,13 |
| Parkin | Brain/SH-SY5Y cells | Higher parkin E3 ligase activity/neuroprotective action | 95 | BSA/MS | 14 |
| Platelet proteins | Platelet | Anti-thromogenesis | N/A | BSA | 15 |
| Androgen receptor | Prostate cancer cells | Androgen receptor dimerization | 611/614 | BSA | 16 |
| MEK1 | Endothelial cells/fibroblasts | PARP-1 activation/DNA damage repair | 341 | BSA | 17 |
| eNOS | Endothelial cells | NO generation and endothelial cell growth | 443 | BSA | 18 |
| P66Shc | Cortex/SH-SY5Y cells | Anti-oxidant | 59 | BSA | 19 |
| $Ca^{2+}$ TRP channels | Bone marrow mesenchymal stem cells | $Ca^{2+}$ influx and osteogenic differentiation | N/A | BSA | 4 |
| Membrane proteins | Heart | Cardioprotection | N/A | BSA | 20 |

Note: BSA, biotin switch assay; TSA, tag-switch assay; MS, mass spectrometry

modifications are key mechanisms to increase proteomic diversity and serve to sense and transduce cellular signals in a precisely coordinated manner **[6]**. The amino acid cysteine in protein is quite reactive, and **o**xidative modification of cysteine residues is an important mechanism that regulates protein structure and ultimately functions, including *S*-nitrosylation, *S*-glutathionylation, and *S*-sulfenylation, etc [6]. Since the first paper describing $H_2S$ *S*-sulfhyration of protein in 2009 [5], there have been a dozen of proteins observed to be modified by $H_2S$ through *S*-sulfhydration, as demonstrated by modified biotin switch assay and/or liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Table 1) [3-5]. It is predicted that, by change in electrostatic environment, hydrophobicity, contiguity and orientation of aromatic side chains, and proximity of target thiols to transition metals or redox centres, *S*-sulfhydration can alter protein conformation and the final function and activity of target proteins [16, 21].

It is well known that $H_2S$ at physiological relevant concentrations suppresses oxidative stress and protects from cell death. The relative low concentration and the small molecular weight of $H_2S$ make it hard to directly scavenge reactive oxygen species. Our recent paper discovered that $H_2S$*S*-sulfhydrates Keap1, which subsequently stimulates Nrf2 nuclear translocation and induces anti-oxidant gene transcription and glutathione production. Deficiency of $H_2S$ enhanced oxidative stress and promoted cellular aging [12]. Nrf2 is a master transcription factor that regulates the expressions of a group of antioxidant genes. Nrf2 activation can be attributed to the dissociation from Keap1 through posttranslational modulation of crucial cysteine residues in Keap1 protein. In normal condition, Keap1 is basically *S*-sulfhydated, and exogenously applied $H_2S$increases but removal of endogenous $H_2S$ reduces Keap1 *S*-sulfhydration. The cysteine residues 151 in Keap1 BTB domain is required for $H_2S$-mediated *S*-sulfhydration. $H_2S$ interacted with cysteine residues 151 leading Keap1 conformational change, which triggerNrf2 release from Keap1 following its nuclear translocation and anti-oxidant gene transcription. $H_2S$-induced activation of Nrf2 is not caused by increased Nrf2 protein expression, Nrf2 phosphorylation, or Nrf2 *S*-sulfhydration.

More recently, we made another discovery that $H_2S$ effectively inhibits androgen receptor (AR) transactivation by *S*-sulfhydrating both cysteine 611 and 614 located in the DNA binding domain (DBD) of AR [16]. AR signaling is essential for the normal development and functions of prostate as well as the initiation and progression of prostate cancer.AR is a nuclear receptor, which is often activated as DBDs bind as dimmers to two hexameric sequences orientated as direct or inverted repeats. The interaction of $H_2S$ and AR through both cysteine611 and 614 may destroy zinc-sulfur cluster and cause a structural change in AR-DBD, triggering abnormal AR dimerization and DNA binding ability. It is still unclear how $H_2S$ *S*-sulfhydration of cysteine-611/614 alters AR zinc-finger structure and

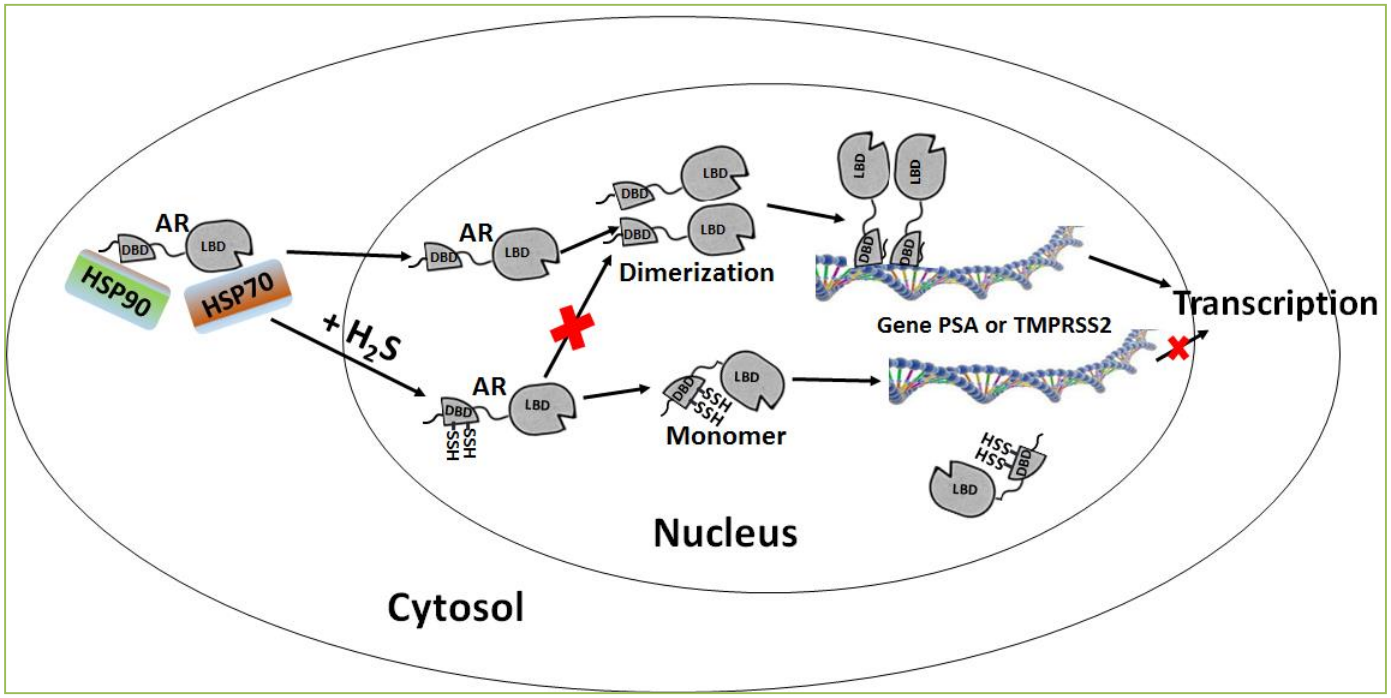


**Figure 1. *S*-sulfhydration regulation of androgen receptor (AR) by $H_2S$.** $H_2S$ represses AR transactivation by *S*-sulfhydrating cysteine residues 611/614 located in the DNA binding domain (DBD). AR S-sulfhydration leads to reduced AR dimerization following lower AR binding with the promoters of prostate-specific antigen (PSA) and TMPRSS2, two AR-targeted genes, while AR *S*-sulfhydration does not affect heat shock protein (HSP) 70 and 90 interaction with AR and AR nuclear translocation.

dimerization. $H_2S$ suppressing AR downstream gene expression were not due to the alteration of AR interaction with heat shock proteins and AR nuclear localization (Figure 1). We further observed that glucocorticoid receptor and estrogen receptor α, another two hormone receptors containinghighly identical DBD as AR, are not *S*-sulfhydrated by $H_2S$, indicating the specificity of cysteine *S*-sulfhydration by $H_2S$ in target proteins. Maintenance of sufficient level of $H_2S$ could effectively inhibit ant androgen-resistant growth of prostate cancer cells. Based on these discoveries, $H_2S$ can not only serve as a valuable prognosis indicator but also an effective therapeutic target for treatment of both early state of prostate cancer and castration-resistant prostate cancer.

In summary, our studies reveal that protein *S*-sulfhydration serves an important role in a wide range of $H_2S$-mediated signaling pathways, including protein activity, localization, stability, and interaction, and stress response. Compared with the large number of *S*-nitrosylated proteins which have been identified, the observed *S*-sulfhydrated proteins and the specificity of target cysteine are incompletely understood. Protein de-sulfhydration/trans-sulfhydration, the removal or transfer of SH group from cysteine thiol side in proteins, is very important but never reported so far. It is also not clear whether *S*-sulfhydration or de-sulfhydration/ trans-sulfhydration is spontaneous and unregulated, or catalyzed by enzymes and other cellular constituents [21]. Similar to *S*-nitrosylation, the formation or removal of an individual *S*-sulfhydration can depend on many factors including the reactivity of the individual cysteine residues, its surrounding environment, and the composition of the local redox-environment, etc. Advances in the detection of *S*-sulfhydration will facilitate research directed at the identification of *S*-sulfhydrated proteins in both health and diseases. There is a long way to fully understand the stories of *S*-sulfhydrationand de-sulfhydration/trans-sulfhydration, but I believe we do have a bright future.

## Conflict of interests

The authors declare no conflict of interests.

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### References

1. Wang R. Two's company, three's a crowd: can $H_2S$ be the third endogenous gaseous transmitter? FASEB J 2002; 16:1792-1798.

2. Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 2012; 92:791-896.
3. Mustafa AK, Sikka G, Gazi SK, Steppan J, Jung SM, Bhunia AK, *et al*. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. Circ Res 2011; 109:1259-1268.
4. Liu Y, Yang R, Liu X, Zhou Y, Qu C, Kikuiri T, *et al*. Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca(2+) channel sulfhydration. Cell Stem Cell 2014; 15:66-78
5. Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, *et al*. $H_2S$ signals through protein S-sulfhydration. Sci Signal 2009; 2:ra72.
6. Chung HS, Wang SB, Venkatraman V, Murray CI, Van Eyk JE. Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. Circ Res 2013; 112:382-192.
7. Zhang D, Macinkovic I, Devarie-Baez NO, Pan J, Park CM, Carroll KS, *et al*. Detection of protein S-sulfhydration by a tag-switch technique. Angew ChemInt Ed Engl 2014; 53:575-581.
8. Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, *et al*. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. Mol Cell 2012; 45:13-24.
9. Du J, Huang Y, Yan H, Zhang Q, Zhao M, Zhu M, *et al*. Hydrogen sulfide suppresses oxidized low-density lipoprotein (ox-LDL)-stimulated monocyte chemoattractant protein 1 generation from macrophages via the nuclear factor κB (NF-κB) pathway. J Biol Chem 2014; 289:9741-9753.
10. Krishnan N, Fu C, Pappin DJ, Tonks NK. $H_2S$-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. Sci Signal 2011;4:ra86.
11. Terzić V, Padovani D, Balland V, Artaud I, Galardon E. Electrophilic sulfhydration of 8-nitro-cGMP involves sulfane sulfur. Org Biomol Chem 2014; 12:5360-5364.
12. Yang G, Zhao K, Ju Y, Mani S, Cao Q, Puukila S, *et al*. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. Antioxid Redox Signal 2013;18:1906-1919.
13. Guo C, Liang F, Shah Masood W, Yan X. Hydrogen sulfide protected gastric epithelial cell from ischemia/reperfusion injury by Keap1 S-sulfhydration, MAPK dependent anti-apoptosis and NF-κB dependent anti-inflammation pathway. Eur J Pharmacol 2014; 725:70-78.
14. Vandiver MS, Paul BD, Xu R, Karuppagounder S, Rao F, Snowman AM, *et al*. Sulfhydration mediates neuroprotective actions of parkin. Nat Commun 2013; 4:1626.
15. Grambow E, Mueller-Graf F, Delyagina E, Frank M, Kuhla A, Vollmar B. Effect of the hydrogen sulfide donor GYY4137 on platelet activation and microvascular thrombus formation in mice. Platelets 2014; 25:166-174.
16. Zhao K, Li S, Wu L, Lai C, Yang G. Hydrogen sulfide represses androgen receptor transactivation by targeting at the second zinc finger module. J Biol Chem 2014; 289:20824-20835.
17. Zhao K, Ju Y, Li S, Altaany Z, Wang R, Yang G. S-sulfhydration of MEK1 leads to PARP-1 activation and DNA damage repair. EMBO Rep 2014; 15:792-800.
18. Altaany Z, Ju Y, Yang G, Wang R. The coordination of S-sulfhydration, S-nitrosylation, and phosphorylation of endothelial nitric oxide synthase by hydrogen sulfide. Sci Signal 2014; 7:ra87.
19. Xie ZZ, Shi MM, Xie L, Wu ZY, Li G, Hua F, *et al*. Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. Antioxid Redox Signal 2014 Jun 26. [Epub ahead of print]
20. Mazza R, Pasqua T, Cerra MC, Angelone T, Gattuso A. Akt/eNOS signaling and PLN S-sulfhydration are involved in $H_2S$-dependent cardiac effects in frog and rat. Am J Physiol RegulIntegr Comp Physiol 2013; 305:R443-451.
21. Lu C, Kavalier A, Lukyanov E, Gross SS. S-sulfhydration/desulfhydration and S-nitrosylation/ denitrosylation: a common paradigm for gasotransmitter signaling by $H_2S$ and NO. Methods 2013; 62:177-181.