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Mini Review

The Signaling Roles of Sulfane Sulfur in Bacteria

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ABSTRACT

Hydrogen sulfide has been proposed as a signaling molecule; however, the convincing examples were rarely reported. It has been reported that H2S is first oxidized to sulfane sulfur that then induces sulfur-metabolizing genes in several bacteria. Recently, the findings that sulfane sulfur rather than H2S activate MexR-regulated antibiotic resistance and LasR-mediated quorum sensing are direct examples that sulfane sulfur act as signaling molecules regulating bacterial physiology beyond sulfur-metabolism. Since the intracellular level of sulfane sulfur is always associated with growth phases, sulfane sulfur is likely the common signaling molecules in bacteria. This mini review focuses on the key findings on sulfane sulfur signaling in bacteria.

Keywords: Hydrogen sulfide; Sulfane sulfur; MexR; LasR

INTRODUCTION

Hydrogen sulfide (H2S) is well known as a signaling molecule in mammalian cells, playing important roles in anti-inflammatory activities and angiogenesis [1]. In microbes, endogenous production of H2S was reported to protect bacteria against oxidative and antibiotic stress [2]. Though sulfur species have important physiological roles, their participation in signaling pathways involving in bacterial physiology are still unclear.

H2S and sulfane sulfur often coexist, making it difficult to distinguish their functions. Many microorganisms generate H2S during normal growth [3], and H2S produced *in vivo* can be chemically [4] and enzymatically [5] converted to sulfane sulfur. The direct involvement of H2S in protein persulfidation, which alters protein activity and influence diverse biological processes, has been questioned, as it cannot directly modify cysteine thiols [6]. Compared with H2S, sulfane sulfur is constantly present inside cells and can directly modify protein thiols. There are appreciable amounts (>100 μM) of sulfane sulfur in the plasma, cells, and tissues of mammals [7]. Therefore, it is generally accepted that cellular sulfane sulfur is the real effector molecule of H2S signaling.

Sulfane sulfur signaling

Sulfane sulfur generally refers to a sulfur atom with zero valence linked to one or two sulfur atoms, including inorganic persulfide or polysulfide (H2Sn, n ≥ 2) and organic persulfide or polysulfide (RSnH, ≥ 2) [8]. Several pathways have been reported to generate sulfane sulfur. Cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) produce sulfane sulfur from cysteine [7], 3-mercaptopyruvate sulfurtransferase (3-MST) [9] and cysteinyl-tRNA synthetase 2 (CARS2) [10] produce sulfane sulfur from cysteine. Sulfide/quinone oxidoreductase (SQR) can also oxidize H2S to sulfane sulfur [11]. Since sulfane sulfur with high concentrations is toxic, the excessive sulfane sulfur can be oxidized by persulfide dioxygenase (PDO) [12] or be reduced by cellular thiols, thioredoxin and glutaredoxin to H2S [13]. Has quantified total sulfane sulfur in biological samples and found sulfane sulfur content changed with the growth phase [14]. This finding indicated sulfane sulfur may serve as important signals, mediating many physiological and pathological processes.

Sulfur metabolism regulation by sulfane sulfur in bacteria

Previous work has reported the signaling roles of sulfane sulfur in activating sulfur-oxidizing genes. Six types of gene regulators (FisR, SqrR, CstR, OxyR and CsoR) have been identified that

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could directly respond to sulfane sulfur. SqrR from *Rhodobacter capsulatus* [15], CstR from *Staphylococcus aureus* [16], and CsoR from *Streptomyces coelicolor* [5] are transcription repressors that bind to the upstream sequence of sulfur-oxidizing genes promoter. When reacted with sulfane sulfur, the transcription factors detached from promoter and activated transcription. FisR from *Cupriavidus pinatubonensis* [9] and OxyR from *Escherichia coli* [8] are the typical transcription activators. They are also reported to be sensitive to high levels of sulfane sulfur, which can turn on their regulated genes.

Other natural behaviors regulated by sulfane sulfur in bacteria

In new work published in the issue of Molecular Microbiology, Xuan, Xun, and coworkers describe the *Pseudomonas aeruginosa* MexR, a transcriptional repressor of the mexAB-oprM operon, could respond to sulfane sulfur, derepressing the mexAB-oprM operon and increasing the bacterium's resistance to antibiotics [17]. MexR is shown to sense different sulfane sulfur species, including H2Sn, S8, GSSH, and Cys-SSH. The sulfane sulfur level in *P. aeruginosa* is growth phase-dependent. Since MexR also represses its own gene expression [18], high levels of sulfane sulfur of the stationary phase induced the co-increase of mexR and mexA expression. Finally, sulfane sulfur mediated the intrinsic resistance, which is quite different from the oxidative stress-regulated adaptive resistance, though both oxidative stress and sulfane sulfur could deactivate MexR [19].

Another study by Xuan, Xun and colleagues showed sulfane sulfur was able to modify the regulator LasR, regulating LasR-mediated quorum sensing and virulence in *P. aeruginosa* PAO1 [20]. The LasR activity requires a threshold level of 3O-C12-HSL, which readily diffuses out of the cell and equilibrates with the external concentration [21]. However, the 3O-C12-HSL alone failed to fully activate LasR. The LasR mediated quorum sensing is also modulated by cellular sulfane sulfur. The work revealed LasR is significantly more active after sulfane sulfur modification. Cellular sulfane sulfur level corresponded well with the LasR activity, and lower cellular sulfane sulfur may slow down the LasR activity even in the presence of high levels of the quorum sensing molecule. Sulfane sulfur acts a "brake" on quorum sensing autoinduction. This finding reveals a new level of control of LasR activity in *P. aeruginosa.*

The two examples that connect and contrast the previous work are twofold. First, the striking contrast: unlike the sulfane sulfur-sensing transcriptional regulators already characterized that mediated the activation of sulfur-metabolizing genes, sulfane sulfur regulates MexR and LasR activity involving in virulence and antibiotic resistance regulation beyond sulfur metabolism in bacteria. The striking similarity to previous work is that the transcription factors MexR, LasR, CstR, SqrR, FisR and OxyR all use their cysteine residues to react with sulfane sulfur directly, which further determined sulfane sulfur is the real effector molecule of H2S signaling.

Signaling by sulfane sulfur through cysteine modification

Sulfane sulfur regulates the activity of gene regulators by modifying the key cysteine residues. For example, FisR reacts with sulfane sulfur, forming a mixture of disulfide and tetrasulfide crosslinked species between Cys 53 and Cys 64, this in turn activates transcription [22]. CstR reacts directly with low molecular weight thiol persulfides and sodium tetrasulfide (S4), forming a mixture of di-, tri- and tetrasulfide crosslinked species between two conserved cysteines [16]. OxyR and CsoR respond to sulfane sulfur *via* persulfidation [23]. For MexR, it forms di- and trisulfide crosslinks between Cys30 and Cys 62 after sulfane sulfur treatment [24,25]. The LasR modification by sulfane sulfur is similar but different with persulfidation and trisulfidation on Cys 188 and a pentasulfur link between Cys 201 and Cys 203 [26,27,28]. The modification by sulfane sulfur on cysteine is specific to different proteins, which indicated that sulfane sulfur may act as a common signaling and function in various bacterial activities [29].

CONCLUSION

In this Mini-review, we have briefly touched on recent progress in sulfane sulfur signaling in bacteria. Sulfane sulfur is the real effector molecule of H2S signaling. It alters protein activity by modifying proteins thiols. Besides activating sulfide-oxidizing genes, sulfane sulfur is also involved in pathogenicity and antibiotic resistance regulation. Since sulfane sulfur is a normal cellular component, changing with growth phases, sulfane sulfur is likely a common signal molecule in bacteria, influencing diverse biological processes.

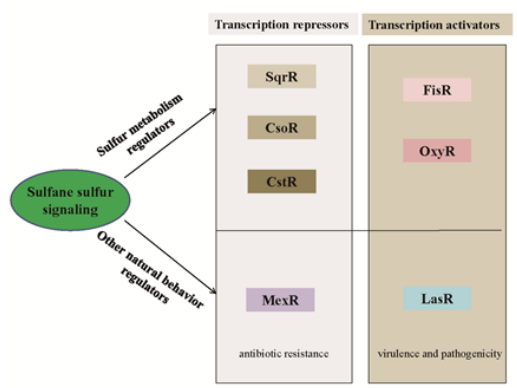

Figure 1: Transcription factors that have been identified to sense sulfane sulfur signaling and their mediated bacteria behaviors.

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