
Persulfidation (S-sulphydration) and H₂S

Milos R. Filipovic

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Abstract

The past decade has witnessed the discovery of hydrogen sulfide (H_2S) as a new signalling molecule. Its ability to act as a neurotransmitter, regulator of blood pressure, immunomodulator or anti-apoptotic agent, together with its great pharmacological potential, is now well established. Notwithstanding the growing body of evidence showing the biological roles of H_2S , the gap between the macroscopic descriptions and the actual mechanism(s) behind these processes is getting larger. The reactivity towards reactive oxygen and nitrogen species and/or metal centres cannot explain this plethora of biological effects. Therefore, a mechanism involving modification of protein cysteine residues to form protein persulfides is proposed. It is alternatively called S-sulphydration. Persulfides are not particularly stable and show increased reactivity when compared to free thiols. Detection of protein persulfides is still facing methodological limitations, and mechanisms by which H_2S causes this modification are still largely scarce. Persulfidation of protein such as K_{ATP} could contribute to H_2S -induced vasodilation, while S-sulphydration of GAPDH and NF- κB inhibits apoptosis. H_2S regulates endoplasmic reticulum stress by causing persulfidation of PTP-1B. Several other proteins have been found to be regulated by this posttranslational modification of cysteine. This review article provides a critical overview of the current state of the literature addressing protein S-sulphydration, with particular emphasis on the challenges and future research directions in this particular field.

Keywords

Hydrogen sulfide • Polysulfides • Sulfenic acids • Persulfidation • S-sulphydration • S-nitrosation

1 H_2S as a Signalling Molecule

In order to maintain life, nature actually uses a limited number of chemical reactions, one of which is sulfur-based chemistry, mainly exploited for the control of intracellular redox homeostasis and redox-based signalling. Hydrogen sulfide (H_2S) is the simplest of the thiols found in the cells, and ever since the first report of its potential physiological role (Abe and Kimura 1996), there has been a growing literature on the subject of H_2S signalling. Very fast, H_2S joined the other two gases, nitric oxide (NO) and carbon monoxide (CO), as the third gasotransmitter (Wang 2002; Li et al. 2009; Mustafa et al. 2009a).

Numerous are the physiological functions assigned to be exclusively or partly regulated by H_2S , some of which are vasodilation (Yang et al. 2008; Mustafa et al. 2011), neurotransmission (Abe and Kimura 1996; Kimura et al. 2005), angiogenesis (Papapetropoulos et al. 2009; Szabó and Papapetropoulos 2011), inflammation (Li et al. 2005; Whiteman and Winyard 2011), hypoxia sensing

(Olson et al. 2008; Peng et al. 2010), etc. In addition, H₂S showed a tremendous pharmacological potential in preventing ischemia–reperfusion injury (Calvert et al. 2009, 2010). Furthermore, H₂S is able to induce suspended animation-like state in mice (Blackstone et al. 2005). Several pharmacological donors of H₂S have been developed with hope of their eventual use in disease treatment (Sparatore et al. 2008; Zhou et al. 2012; Szczesny et al. 2014; Zhao et al. 2013).

H₂S is produced by the action of at least three enzymes, cystathionine beta synthase (CBS), cystathionine gamma lyase (CSE) and mercaptopyruvate sulfurtransferase (MST) (Kabil et al. 2014; Kabil and Banerjee 2014). Differently expressed in different tissues (and even cellular compartments), these enzymes control H₂S production with different efficiencies. How are these enzymes regulated to maintain spatio-temporal production/distribution of H₂S is still unclear.

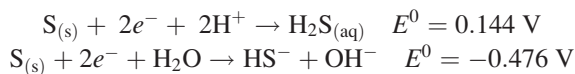
The intracellular levels of H₂S are also a matter of debate, with values spanning from nondetectable to >100 μM, although it seems more probable that the steady-state levels are at low micromolar and/or submicromolar levels, depending on the tissue (Olson 2012; Olson et al. 2014; Kabil et al. 2014). Conversely, the flux of H₂S production is huge, almost as that of glutathione, suggesting that the removal of H₂S is an efficient and tightly regulated process (Vitvitsky et al. 2012; Kabil et al. 2014; Kabil and Banerjee 2014).

Hydrogen sulfide is a weak acid and immediately ionizes in aqueous solution reaching the equilibrium between H₂S/HS[−]/S^{2−} species:



The pK_{a1} is around 6.9, while pK_{a2} is estimated to be >12, which means that under physiological conditions, approximately two thirds of H₂S are in the form of bisulfide (hydrogen(sulfide)(1-)), with negligible amounts of sulfide anion (sulfide(2-)). H₂S diffuses freely through the membranes (Mathai et al. 2009; Cuevasanta et al. 2012). Although the diffusion coefficient profile of H₂S is systematically lower than that of H₂O, the differences in the transmembrane Gibbs energy profiles are more dominant. Because of its hydrophobicity, H₂S experiences no barrier to permeation, so it can partition into the interior of the membrane readily (Riahi and Rowley 2014).

Sulfur atom in H₂S is in −2 oxidation state, but sulfur is very versatile in its ability to accept or donate electrons. It can cycle between −2 and +6 oxidation state, due to the six valence electrons and completely empty 3d orbital. Although the standard redox potential often cited in the literature for the two electron oxidation of H₂S to sulfur is 0.144 V (which makes H₂S a weaker reducing agent than cysteine or glutathione), another standard redox potential could be found for the alkaline conditions, which suggests that H₂S is a stronger reducing agent:



H₂S does not react readily with oxygen; however, the solutions of H₂S undergo oxidation, just like the solutions of other thiols (such as cysteine and glutathione).

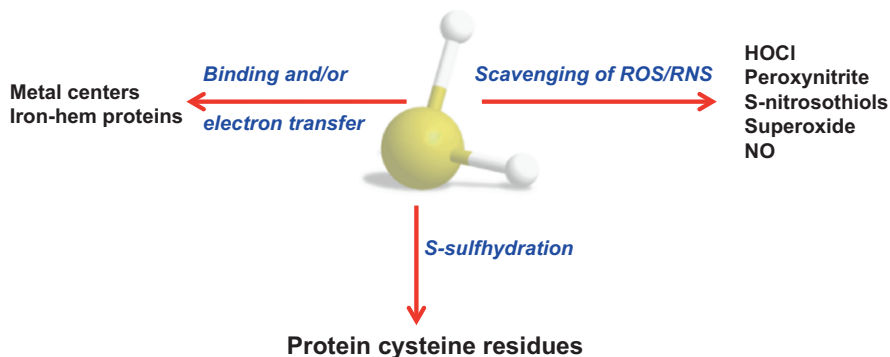


Fig. 1 Three main biochemical ways for direct H_2S signalling. Hydrogen sulfide can either react directly with reactive oxygen and nitrogen species (*arrow to the right side*) or react with metal centres by binding and/or electron transfer (*arrow to the left side*), or it can modulate cysteine residues of the proteins in a reaction called S-sulphydration (*central arrow*)

This process is believed to be catalysed by the traces of metal ions present in the solution (Kotronarou and Hoffmann 1991); therefore, the thorough cleaning of solutions from heavy metals and removal of oxygen can keep H_2S solution stable for a while (Wedmann et al. 2014). Oxidation products of H_2S in solution are polysulfides, sulfites, thiosulfites and eventually elemental sulfur.

In biological systems, reactivity of H_2S could be divided in three groups of reactions: (1) reaction with/scavenging of reactive oxygen (ROS) and reactive nitrogen species (RNS), (2) binding to and/or subsequent redox reactions with metal centres and (3) reaction with proteins, herein called persulfidation (alternatively S-sulphydration) (Fig. 1).

1.1 Reactions with ROS and RNS

H_2S reacts readily with hypochloric acid (HClO), produced by neutrophils, leading to the formation of polysulfides (Nagy and Winterbourn 2010). H_2S also reacts with peroxynitrite (Carballal et al. 2011; Filipovic et al. 2012a), in a reaction that generates thionitrate (HSNO_2) isomer, which can decompose and serve as an NO donor (Filipovic et al. 2012a). In addition, H_2S can also scavenge superoxide (Wedmann et al. 2014). Although the rates of these reactions are higher than those found for cysteine or glutathione, they are not so much higher that can overcome the difference in concentration (particularly when compared to glutathione which is present in millimolar steady-state levels) suggesting that H_2S cannot really serve as an antioxidant. Nonetheless, H_2S has been shown to have antioxidant and immediate protective effects in the cells exposed to ROS and RNS, something that cannot simply be explained by the modulation of proteins and gene expression. Since soluble macromolecules occupy a significant fraction of the total cell volume (Fulton 1982), within such crowded medium relative size and

shape of a molecule and probability of its successful diffusion, placement and effective contact with a potential target become crucial factors that significantly alter its reaction rates (Minton 1998). This speaks in favour of hydrogen sulfide, when compared to GSH, despite the difference in steady-state concentrations (Filipovic et al. 2012a).

H₂S reacts with NO signalling pathways as well (Whiteman et al. 2006; Ali et al. 2006; Yong et al. 2010, 2011; Filipovic et al. 2012b, 2013; Eberhardt et al. 2014). With protein S-nitrosothiols, H₂S reacts to form the smallest S-nitrosothiols, thionitrous acid (HSNO), which can freely diffuse through the membrane and serve as *trans*-nitrosating agent (Filipovic et al. 2012b). More importantly, H₂S can react directly with NO giving nitroxyl (HNO), the one-electron-reduced sibling of NO which possesses the signalling properties of its own (Eberhardt et al. 2014). 2 μ M combination of NO and H₂S gives the same rate of HNO generation as \sim 1 mM Angeli's salt, the commonly used pharmacological source of nitroxyl. Eberhardt et al. (2014) recently showed that co-localization of H₂S and NO production facilitate intracellular generation of HNO which then activates transient receptor potential A1 channel (TRPA1) leading to the Ca²⁺ influx and to the release of calcitonin gene-related peptide (CGRP), the strongest known vasodilator.

1.2 Reactions with Metal Centres

One of the main biological targets for H₂S would be metal centres. H₂S can coordinate and then additionally reduce the metal centre. In haemproteins, polar active site favours the reduction, while nonpolar centres favour coordination only (Pietri et al. 2009). Indeed, the presence of positive charges around the haem centres leads to a complete change in the haems' reactivity, allowing the design of efficient catalysts for H₂S removal (Ivanovic and Filipovic 2012).

H₂S binds to both haem a_3 and Cu_B centres of cytochrome c oxidase, with K_i being 0.2 μ M for the purified enzyme (Hill et al. 1984; Nicholls et al. 2013). This suggests that cytochrome c oxidase should be permanently inhibited under physiological conditions (considering the micromolar steady-state concentrations of H₂S), which is obviously not the case. In fact H₂S shows biphasic effects on whole cell respiration, stimulating it at low doses and then completely inhibiting it at higher concentrations (Koenitzer et al. 2007). Binding of H₂S to haem has an important function in mollusc, *Lucina pectinata*, where H₂S binds to haemoglobin I and is then transported to the symbiotic chemoautotrophic bacteria living in their gills (Ríos-González et al. 2014).

Reaction of oxyhaemoglobin with H₂S leads to the green pigment, sulfhaemoglobin, which is in fact a chemically modified porphyrin centre. This subsequently affects oxygen-binding capacity of that haemoglobin. H₂S also binds and reduces cytochrome c, a reaction that in the presence of oxygen leads to superoxide production (Wedmann et al. 2014). In addition, Pálkás et al. (2014) have recently investigated interactions of H₂S with human myeloperoxidase

(MPO), a major contributor to inflammatory oxidative stress, to show that H_2S inhibits the enzyme by reducing iron centre and by binding to the reduced Fe^{2+} .

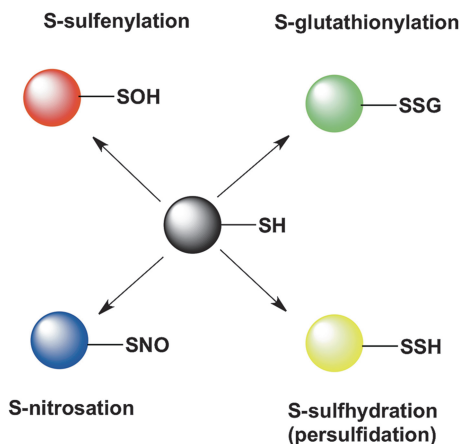
It is still, however, unclear to which extent is the coordination and/or reduction of metal centres involved in signalling by H_2S . Miljkovic et al. (2013) demonstrated that metal centres in mitochondria are responsible for the H_2S -stimulated haem centre-catalysed reduction of nitrite, a reaction which can explain the use of nitrite as an antidote for acute H_2S poisoning.

1.3 Protein Persulfides

The third way of direct H_2S signalling would be modulation of proteins by modification which is named S-sulfhydration, although the more correct term should be persulfidation. Protein persulfides add up to the list of oxidative posttranslational modifications (oxPTMs) of cysteine, such as S-nitrosation, S-sulfenylation and S-glutathionylation (Fig. 2). Modification of proteins by H_2S could explain the plethora of effects that H_2S exhibit and several proteins have been identified to be indeed controlled by this modification (Mustafa et al. 2009a, b, 2011; Paul and Snyder 2012). However, this field of research is at its beginning, and it is still facing difficulties/challenges such as the proper choice of detection method, understanding of the mechanism(s) by which persulfidation takes place and the actual impact it has on the cellular functions.

Based on the calculation of the bond energies of GSSG and GSSH, the latter has $\sim 18 \text{ kJ mol}^{-1}$ lower bond energy (Filipovic et al. 2012b) so due to their inherent instability, there is limited information about persulfide reactivity to date. Francoleon et al. (2011) were among the first who reported preparation of the glutathione and papain persulfides, while Pan and Carroll (2013) successfully prepared persulfide on glutathione peroxidase 3. Zhang et al. (2014) also reported

Fig. 2 Oxidative posttranslational modifications of protein cysteine residues known to regulate protein structure/function



facile preparation of persulfide of bovine serum albumin (BSA), which can be used as a model to study protein persulfide reactivity.

Electronegativity of sulfur is almost identical to that of carbon; therefore in R-S-SH, the sulfur atom covalently bound to carbon could be considered as sulfane sulfur. Sulfane sulfur is sulfur with six valence electrons and a formal charge 0, often represented by S⁰. This sulfane sulfur could be a good target for nucleophilic attack, while the other sulfur atom, which is formally -1, could react with electrophiles. The pK_a of persulfides is lower than that of corresponding thiols, suggesting that at physiological conditions, majority of persulfide would be in deprotonated form (R-S-S⁻), making the persulfide “super” nucleophilic. This dramatically increases persulfides’ reactivity when compared to the corresponding thiols, as recently demonstrated for the reaction with H₂O₂ (Ida et al. 2014) and previously for the reduction of cytochrome c³⁺ (Francoleon et al. 2011).

Artaud and Galardon (2014) synthesized a persulfide analogue of the nitrosothiol SNAP, which opens up new possibilities for examining metastable low molecular weight (LMW) persulfides. The authors clearly demonstrate that spontaneous decay of LMW persulfides does not lead to H₂S release, but when mixed with other thiols, such as glutathione, an immediate H₂S release could be observed. Additionally, Bailey et al. (2014) characterized tritylhydrosulfide (TrtSSH), another LMW persulfide, to show that protonated form does not react with nucleophiles, while it readily reacts with electrophiles and reducing agents. Protonation leads to no change in substance stability, while the deprotonation stimulates decomposition with the elimination of elemental sulfur.

Based on the current literature data, the following characteristics of persulfides could be described:

1. Persulfides are metastable species which decompose in solution in a complex manner, leading to the formation of elemental sulfur, among other molecules.
2. Persulfides are much better nucleophiles than corresponding thiols which can explain their better reactivity.
3. Persulfides are better reducing agents than corresponding thiols.
4. Persulfides readily react with electrophiles.
5. Persulfides could transfer sulfane sulfur to other thiols, leading to *trans*-persulfidation (vide infra).

2 Persulfide Formation

Although there is a growing interest for persulfidation of proteins, only a few studies actually addressed the issue of the mechanism(s) behind protein S-sulphydration. The original misconception (which tends to overtake this whole field) was that thiolate on the protein can react directly with H₂S to form protein persulfide (Fig. 3a). However, that reaction is impossible due to the thermodynamic constraints. Both sulfur atoms get oxidized in the reaction so the electrons will have to end up on protons, leading to elimination of hydrogen as a gas. Incubation of

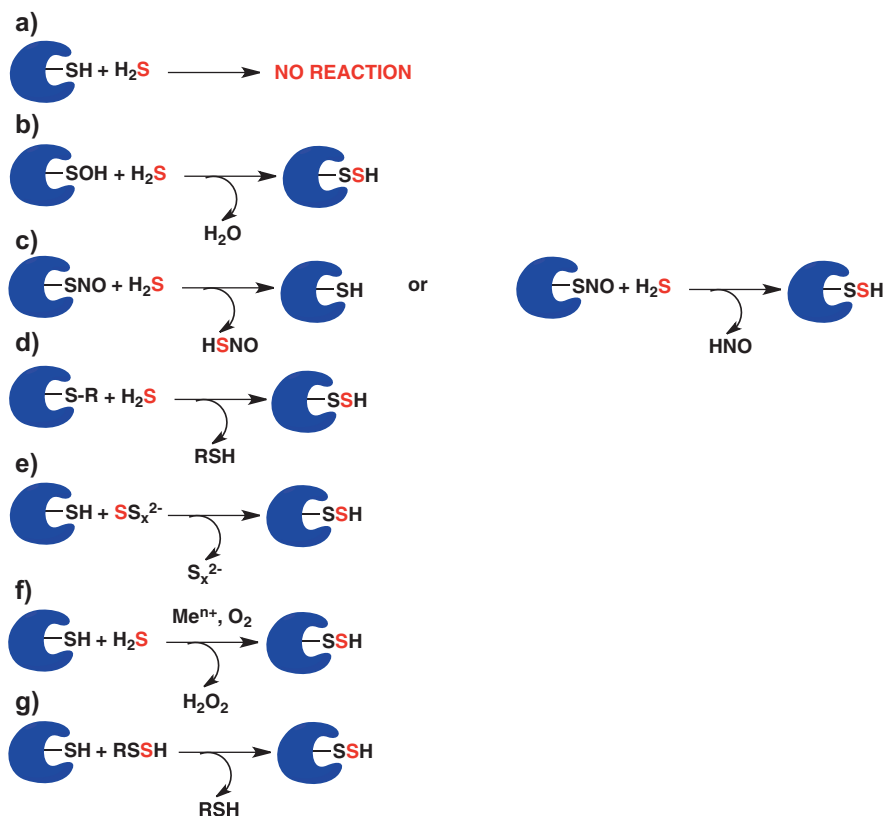


Fig. 3 Proposed reaction mechanisms for persulfide formation. (a) A direct reaction between protein thiols and H_2S is not possible, but H_2S can react with sulfenic acids (b). (c) Reaction of *S*-nitrosated cysteines with H_2S leads to the formation of HSNO, but depending on the protein environment surrounding the thiol, it is also possible to generate protein persulfides in this reaction. (d) H_2S could react with already existing inter- or intramolecular disulfides, while sulfane sulfur in polysulfides could react directly with protein thiols and give persulfide (e). (f) Metal centres could act as oxidants for the formation of protein persulfides from H_2S and protein thiols. (g) Persulfides could serve as carriers of sulfane sulfur and engage in “*trans*-*S*-sulfhydration” reaction

proteins, such as GAPDH, BSA or immunoglobulins with H_2S , led to no detectable protein *S*-sulfhydration (Zhang et al. 2014; Wedmann et al. 2014) confirming the theory.

So how are the proteins modified by H_2S ? Getting the answer(s) to this question is of the utmost importance for our understanding of H_2S signalling and also for the interpretation of the vast amount of data accumulated to date.

2.1 Enzymatic Generation of Persulfides

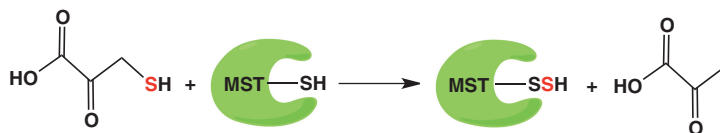
Despite the recent interest for protein persulfidation, sparked by the discovery of H₂S as a signalling molecule, protein persulfides formed as intermediates that facilitate sulfur delivery in several biosynthetic pathways have been known for a while (Mueller 2006). Persulfides are found to be formed in sulfurtransferases and cysteine desulfurases. Particularly interesting examples are the two enzymes involved in H₂S production and its oxidation, mercaptopyruvate sulfurtransferase (MST) and sulfide–quinone oxidoreductase (SQR), respectively.

MST is expressed in both mitochondria and cytoplasm and could be found in kidney cells, liver and cardiac cells, neuroglial cells, etc. (Kabil and Banerjee 2014; Kimura 2014). Recent studies suggest that MST is an important source of H₂S in some organisms and tissues (Mikami et al. 2011; Módos et al. 2013). Although it was known for a while that during the reaction protein persulfide is formed in the catalytic site (MST-Cys-S-SH), the mechanism of H₂S release has been only recently discovered. Yadav et al. (2013) reported the first crystal structure of MST with its cysteine residue in the form of persulfide. This allowed them to propose a detailed reaction mechanism, while kinetic analysis of the reaction led to conclusion that thioredoxin is likely to be the major physiological persulfide acceptor for MST (Fig. 4a).

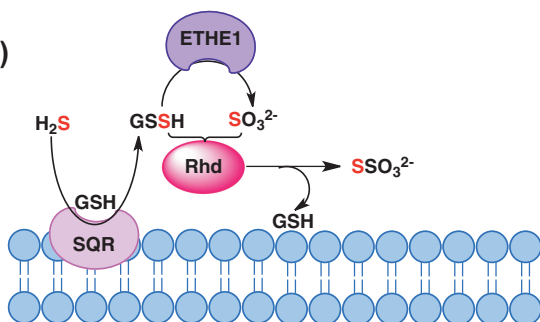
The same group, led by Ruma Banerjee, elucidated the full mechanism of SQR (Libiad et al. 2014). The sulfide oxidation pathway begins with SQR, and it also includes a sulfur dioxygenase, rhodanese and sulfite oxidase (Fig. 4b). By consuming H₂S and its persulfide products, SQR and sulfur dioxygenase are important switch-off regulators of sulfide signalling (Bouillaud and Blachier 2011). In the first step, SQR catalyses the oxidation of H₂S to sulfane sulfur, which remains covalently attached to the enzyme. In the second step, this sulfane sulfur could be transferred to sulfite, to form thiosulfate (Jackson et al. 2012), but recently, Libiad et al. (2014) showed that in fact glutathione is a more probable acceptor of sulfane sulfur. This leads to the generation of glutathione persulfide, which can be consumed by rhodanese to actually form thiosulfate rather than to use it as a substrate (Fig. 4b).

Recent work on reactive cysteine persulfides and S-polythiolation has shaken our understanding of the action of CBS and CSE (Ida et al. 2014). Namely, the authors demonstrated that persulfide formation by CSE and CBS-mediated CysSSCys metabolism are facile and more likely a source of biological persulfides (Fig. 4c). Therefore, the direct enzymatic production of per- and polysulfide that is highly prevalent has been suggested. In fact, the authors question the role of H₂S as a major signalling molecule suggesting that Cys-based persulfides may be the actual signalling species (Ono et al. 2014).

a)



b)



c)

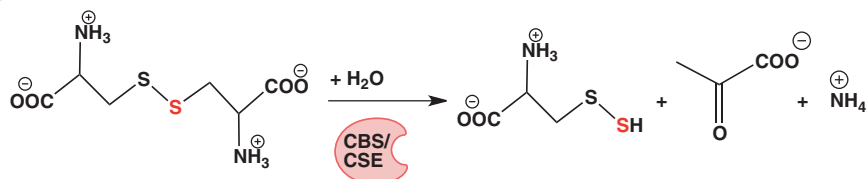


Fig. 4 Enzymatic production of protein persulfides. (a) Generation of protein persulfide on MST enzyme during its catalytic cycle. (b) Generation of glutathione persulfide during the enzymatic oxidation of H_2S . In the first step, SQR catalyses the oxidation of H_2S to sulfane sulfur with glutathione as probable acceptor of this sulfane sulfur. This leads to generation of glutathione persulfide, which can be consumed by rhodanese to actually form thiosulfate. (c) Cysteine persulfides are formed in the enzymatic cleavage of cystine by CBS or CSE

2.2 Direct Nonenzymatic Generation of Persulfides by H_2S

An obvious similarity between protein S-sulphydration and S-glutathionylation could be drawn. Based on known mechanisms for the generation of glutathionylated proteins, it is possible to assume that the same reactions could lead to generation of protein persulfides.

2.2.1 Reaction with Sulfenic Acids

Protein S-sulfonylation (Fig. 2) is the reversible oxidation of protein thiols to sulfenic acids (R-SOH) that has been recognized as an important oxidative post-translational modification of cysteines (Paulsen and Carroll 2013; Gupta and Carroll 2014). Most recently, an extensive study was published identifying the

whole cell sulfenylome, i.e. site-specific mapping and quantification of protein S-sulfenylation in the cells (Yang et al. 2014). More than 1000 proteins have been characterized as modified by sulfenylation. Peroxiredoxins are the most abundant antioxidant enzymes in the cytosol, and their catalytic cycle relies on sulfenic acid formation (Poynton and Hampton 2014). Sulfenic acid formation is shown to be involved in H₂O₂-mediated inactivation of protein tyrosine phosphatases (PTPs) (Paulsen et al. 2011). Also, reversible sulfenylation is shown to switch on or off the activity of transcription factors such as OxyR, OhrR or Orp1-Yap1 (for extensive review, see Paulsen and Carroll 2012). Epidermal growth factor receptor and the phosphatases SHP2, PTEN and PTP1B were all found to be sulfenylated upon regular signalling by epidermal growth factor (Paulsen et al. 2011).

The biochemistry of sulfenic acid formation, reactivity and functions has been extensively reviewed recently (Paulsen and Carroll 2013; Gupta and Carroll 2014). Sulfenic acids are known to react with thiols to form disulfides. The same could be said for the reaction of sulfenic acids with H₂S (Fig. 3b). Namely, in the recent study, Zhang et al. (2014) showed that formation of sulfenic acids on GAPDH and subsequent reaction with H₂S do indeed lead to the formation of protein persulfides. Furthermore, they used sulfenic acid of bovine serum albumin (BSA), known as an example of a relatively stable protein sulfenic acid (Carballal et al. 2003), to successfully generate S-sulphydrated BSA. Although the steady-state concentration of H₂S is orders of magnitude lower than that of glutathione (Olson et al. 2014; Kabil et al. 2014), which makes it difficult for H₂S to compete for the reaction with protein sulfenic acids, the high flux of H₂S generation (Vitvitsky et al. 2012), its free diffusion (Mathai et al. 2009; Cuevasanta et al. 2012) and therefore its ability to reach deeper parts of the proteins suggest that the reaction of H₂S with sulfenic acids could still be a major source of protein persulfide formation (Fig. 3b). Indeed, Zhang et al. (2014) find that intracellular persulfidation co-localizes with endoplasmic reticulum, an organelle rich with sulfenic acids.

It is worth noting that sulfenic acids can be formed from the thiols just by the presence of traces of metal ions in the buffers and oxygen (Paulsen and Carroll 2013). Considering that R-SOH could react with H₂S to form protein persulfides, these reaction steps could be an explanation for the misconception that H₂S reacts directly with thiols.

2.2.2 Reaction with S-Nitrosothiols

Protein S-nitrosation is considered by some to be the second most important posttranslational modification of proteins. The number of proteins found to be controlled by this modification is constantly increasing. To date, S-nitrosation has been implicated in the regulation of proteins involved in muscle contractility, neuronal transmission, host defence, cell trafficking, apoptosis, etc. (Hess and Stamler 2012; Seth and Stamler 2011; Foster et al. 2009; Lima et al. 2010). S-nitrosation of haemoglobin has been proposed to regulate its ability to release oxygen (Reynolds et al. 2013). S-nitrosation of ryanodine receptors regulates intracellular Ca²⁺ levels (Xu et al. 1998) as does S-nitrosation of transient receptor potential cation channels (TRPs) (Yoshida et al. 2006). The role of S-nitrosation in

controlling the protein function has been extensively reviewed elsewhere (Hess and Stamler 2012; Seth and Stamler 2011).

The mechanism of protein S-nitrosation is still the matter of debate (Broniowska and Hogg 2012). Like in the case of S-sulfhydration, direct reaction of NO with thiols to lead to the S-nitrosothiol formation is thermodynamically unfavourable. The mechanism usually involves either the reaction of thiols with higher nitrogen oxides, such as N_2O_3 , or reactions with metal centre-catalysed one-electron oxidation product of NO (Broniowska and Hogg 2012). *Trans*-nitrosation, transfer of NO^+ moiety, from one protein to another is also a subject of a lively debate. Cysteine and glutathione have been proposed as common carriers. However, H_2S also reacts with S-nitrosothiols leading to the formation of the smallest S-nitrosothiol, HSNO (Fig. 3c, Filipovic et al. 2012b). HSNO can freely diffuse through the membranes serving as a carrier of NO^+ and conveying further trans-nitrosation. In addition, HSNO is very unstable and highly reactive so it could react further with H_2S -forming polysulfides and/or sulfur, which could be a source of protein S-sulfhydration (Fig. 3c). The reaction of S-nitrosothiols with H_2S to directly give persulfides and nitroxyl (HNO) is thermodynamically unfavourable ($\Delta_{\text{rxn1}}G^\circ \approx +40 \text{ kJ mol}^{-1}$) (Filipovic et al. 2012b). Although it seems that *trans*-nitrosation is a generally favoured pathway, a recent computational study pointed out that the surrounding of S–NO bond within a protein could significantly affect the thermodynamic feasibility of the thiolation reaction, making it possible for certain proteins (depending on the surrounding of the cysteine residue) to get directly S-sulfhydrated (Fig. 3c, Talipov and Timerghazin 2013).

2.2.3 Reaction with Disulfides

Reaction of thiols with disulfides normally proceeds until the equilibrium is established (Moriarty-Craige and Jones 2004). Following the same logic, H_2S should be able to react with disulfides leading to persulfide formation (Fig. 3d). Protein disulfides are always formed between cysteine residues (of the same or different polypeptide chain), or they appear as mixed disulfides between the cysteine residues of the protein and glutathione (in S-glutathionylated proteins). Based on the calculation of the bond energies of GSSG and GSSH, the latter has $\sim 18 \text{ kJ mol}^{-1}$ lower bond energy (Filipovic et al. 2012b) which makes the reaction of H_2S with oxidized thiols very slow. Francoleon and colleagues demonstrated that glutathione persulfide could indeed be formed in a reaction mixture containing oxidized glutathione and H_2S , but the product was unstable (Francoleon et al. 2011). Formation of protein persulfides by the H_2S -induced reduction of intra- or intermolecular protein disulfides seems highly unlikely in the cells, due to the low levels of H_2S when compared to other thiols (such as glutathione or cysteine) and the very slow reaction rate. Zhang et al. (2014) did not observe any S-sulfhydration as a consequence of the reaction of H_2S with disulfides of BSA, and a study by Wedmann et al. (2014) confirmed this observation on immunoglobulins purified from human blood. The use of very high, non-physiological concentrations of H_2S or its donors in experimental setups could, nonetheless, overcome this limitation and lead to protein modification.

2.3 Nonenzymatic S-sulphydration by Species Originating from H₂S

2.3.1 Reaction with Polysulfides

Polysulfides (HS_x[−]) are the products of incomplete H₂S oxidation on its way to total oxidation to elemental sulfur. Contrary to a widely spread opinion in a biological community that H₂S easily oxidizes, H₂S is known as a very persistent and difficult-to-get-rid-off pollutant in industrial processes. The oxidation that indeed does occur on air is most probably caused by the impurities present in the solutions such as traces of metal ions, which are inevitable contaminants of all standard buffer solutions (Kotronarou and Hoffmann 1991).

The length of polysulfide chain can be from 2 to 7. It is worth noting that older chemical literature clearly points out that H₂S₂ is a molecule that can be prepared under extreme conditions (Parsons and Walton 1921). It is a substance of pungent smell that cannot even stand the humidity from air, and it immediately decomposes to give H₂S and elemental sulfur (Parsons and Walton 1921). Therefore, assuming that H₂S₂ is an important player in the biological system would be wrong. If formed, H₂S₂ would immediately decompose.

Longer polysulfides are more stable, with $x = 4$ and/or 5 being the most abundant species. They are formed even in biological milieu and could be responsible for the part of the effects assigned to H₂S (Fig. 3e). Indeed, Kimura et al. (2013) demonstrated that polysulfides have a 300 times higher potency in activating TRP channels. In addition, Greiner et al. (2013) linked polysulfides to protein thiol oxidation suggesting that all sources of H₂S (salts and H₂S donors) inevitably contain polysulfides, which in turn oxidize cysteine residues leading to persulfide formation. All inorganic polysulfides with more than two sulfur atoms contain sulfane sulfur atoms, which could undergo nucleophilic attack by free protein thiols leading to the formation of protein persulfides. However, polysulfides could also serve as reducing agents. Francoleon et al. (2011) suggested that persulfides are much more potent reducing agents than H₂S, while Wedmann et al. (2014) recently showed that polysulfides are capable of completely cleaving intramolecular disulfides present in immunoglobulins. H₂S solutions prepared with care, however, could not do the same. Only when mixed with traces of metal ions, they achieved the same effect (Wedmann et al. 2014).

Although there is very little doubt that polysulfides are formed in the cells, it is still unclear how they could serve as signalling molecules. Signalling molecules should be able to achieve the effect at low doses, but at the same time, their production should be tightly controlled, and their reactivity should be specific, just like in the case of NO or H₂O₂. Formation of polysulfides cannot be controlled; it is guided by stochasticity as the reactions that polysulfide undergo are highly unpredictable and will largely depend on the availability of oxygen and metal centres on the one hand and the protein thiols/disulfides on the other hand. In addition, polysulfides are charged, and it is almost impossible to achieve their fully protonated form under physiological condition, making the diffusion through the membrane impossible without a facilitator.

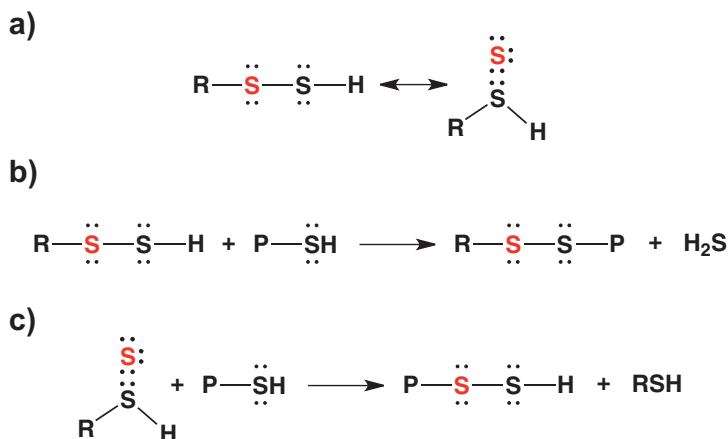
2.3.2 Reaction with Metal Centres and Generation of HS•

Although not studied into too much detail, metal centres could play an important role in catalysing formation of protein persulfides (Fig. 3f). Namely, some iron haem centres are able to oxidize hydrogen sulfide forming HS• (Miljkovic et al. 2013), which could in turn react with free thiols to finally generate protein persulfides (Zhang et al. 2014). Cytochrome c, for example, readily reacts with H₂S (Wedmann et al. 2014). Iron porphyrins in general could be a good source of HS•. HS• reacts further with H₂S, in a diffusion-controlled reaction, to give H₂S₂•⁻ (Das et al. 1999) which should be a powerful persulfidation agent when formed intracellularly, something that is yet to be confirmed. As demonstrated by Zhang et al. (2014), incubation of GAPDH with iron porphyrin and H₂S leads to the strongest generation of protein persulfides. This complemented with their observation that a large portion of intracellular S-sulfhydration co-localizes with mitochondria, the organelle richest in metallo-proteins.

2.3.3 “Trans-persulfidation” by Polythiolated Cysteine or Glutathione

A recent study by Ida et al. (2014) suggested very high levels of circulatory LMW persulfides. Francoleon et al. (2011) noticed that treatment of papain with GSSH leads to the enzyme inhibition in the same manner as it does the persulfidation of this enzyme. The authors recently went a step further suggesting that cysteine persulfide and/or glutathione persulfide could be the main persulfidating agents in the cells (Ono et al. 2014). Cysteine persulfides could even be transported through the membrane. S-nitrosocysteine does the same and is considered as one of the main carriers of “NO⁺” moiety in *trans*-nitrosation reactions (Broniowska and Hogg 2012). Making a parallel with S-nitrosothiol chemistry, the term “*trans*-persulfidation” could be coined to describe this process (Fig. 3g).

The mechanism of this process is still unclear. As mentioned above, the persulfides would react with electrophiles rather than with nucleophiles (Artaud and Galardon 2014; Bailey et al. 2014), and when they do react with nucleophiles (such as other free thiols), this reaction leads to the release of H₂S (which represents the basis of one of the methods for persulfide detection: reduction with DTT). The reaction with nucleophiles could only go as an attack to sulfane sulfur, but that leads to the formation of a mixed disulfide and elimination of H₂S. Some literature data exist suggesting that persulfides could exist in the tautomeric thiosulfoxide form (Scheme 1), which would then act as a perfect donor of sulfane sulfur (Kutney and Turnbull 1982; Steudel et al. 1997). However, it is worth noting that although this may be true for the extreme case of F₂S₂, neither experimental nor computational data support the existence of this tautomeric form (Steudel et al. 1997). Therefore, the elucidation of this mechanism is to be done in some future studies.



Scheme 1 Possible mechanism of *trans*-persulfidation. (a) The tautomeric forms of protein or LMW persulfides. Sulfane sulfur is marked *red*. (b) Reaction of LMW persulfides with protein thiols leads to the H₂S elimination and formation of mixed disulfide. (c) Reaction of thiosulfoxide tautomer, however, could lead to *trans*-persulfidation

3 Detection of Protein Persulfides

Detection of protein *S*-sulfhydration represents a certain challenge as the persulfide group exhibits the reactivity similar to other, free thiols (Flavin 1962; Heimer 1981; Mueller 2006; Pan and Carroll 2013; Zhang et al. 2014). Four distinctive approaches have been proposed (Fig. 5) for the persulfide detection, all of which are summarized here with a particular emphasis on the potential experimental problems that might occur.

The original method for protein persulfide detection was suggested by Mustafa et al. (2009b), and it was based on a premise that protein persulfides would not react with electrophilic thiol-blocking reagent *S*-methyl methanethiosulfonate (MTS). In the subsequent step, persulfides were labelled with N-[6-(biotinamido)hexyl]-3'-(2'-pyridyldithio)propionamide (biotin-HPDP) (Fig. 5a). This method allowed the simultaneous labelling of *S*-sulfhydration and *S*-nitrosation. Using this method, Mustafa and colleagues suggested that a large number of proteins were a target for H₂S signalling and that basal protein persulfidation is as high as 25 % (Mustafa et al. 2009b).

To date, this is the most used methodological approach in reporting protein *S*-sulfhydration of different proteins. However, certain methodological limitations arise. Although MTS has been widely used to study *S*-nitrosation (Forrester et al. 2009) and has been an efficient tool in trapping mixed disulfides *in vivo* (Peaper et al. 2005), Karala and Ruddock (2007) were able to show that *in vitro* MTS treatment of both peptides and proteins resulted in the artificial formation of intramolecular and intermolecular protein disulfide bonds which could lead to

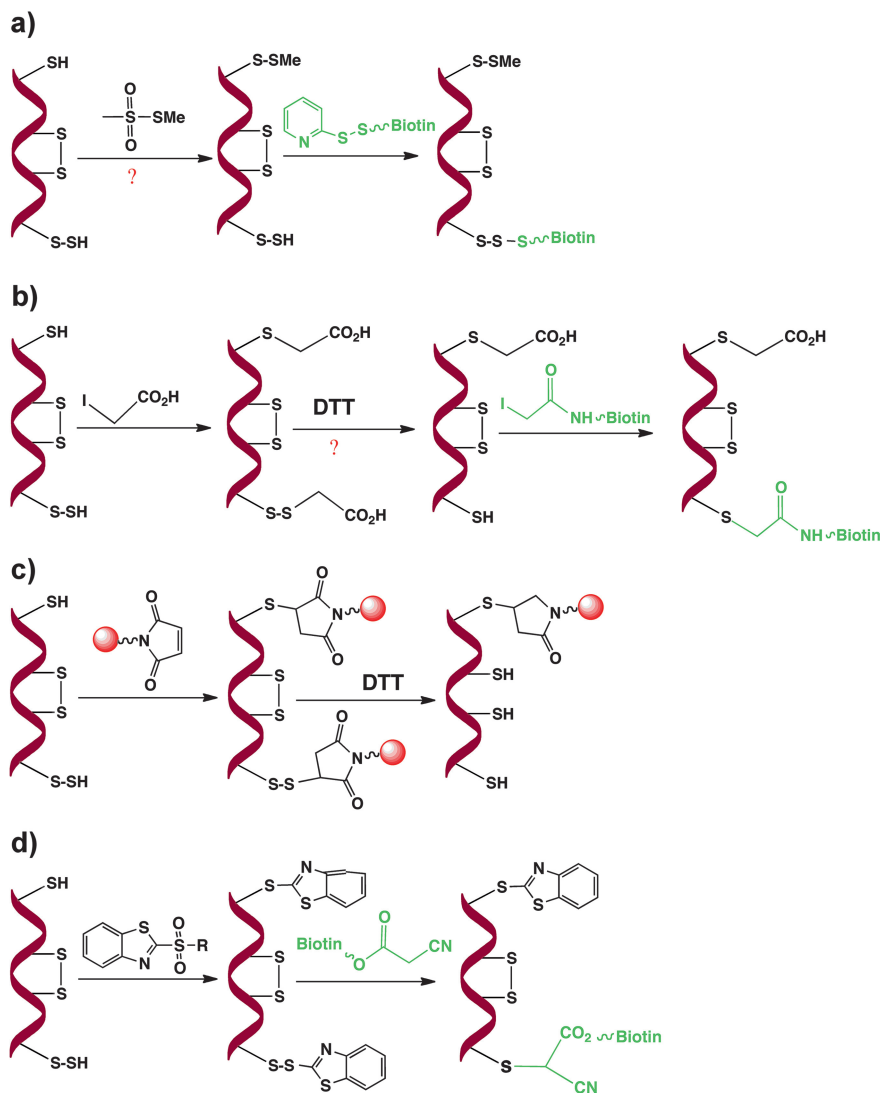


Fig. 5 An overview of the methods currently employed for the detection of protein persulfides. (a) The first method was based on a chemically wrong premise that protein persulfides would not react with electrophilic thiol-blocking reagent S-methyl methanethiosulfonate (MMTS). In the subsequent step, persulfides were labelled with N-[6-(biotinamido)hexyl]-3'-(2'-pyridyldithio) propionamide (biotin-HPDP). (b) In the second method, iodoacetic acid (IAA) is used to initially block both free thiols and protein persulfides. In the subsequent steps, alkylated persulfide is cleaved with DTT and then labelled with iodoacetamide-linked biotin (IAP). Although DTT would indeed cleave this adduct, it is unclear how this method distinguishes the persulfides from intra- and intermolecular disulfides and S-nitrosothiols, which would also be reduced by DTT. (c) In this method, both persulfide and free thiol would be blocked by the thiol fluorescently labelled N-ethyl maleimide (Cy5-conjugated maleimide). The adduct of persulfide and Cy5-maleimide is a disulfide that will be then cleaved by the DTT leading to a decrease of the in-gel fluorescence signal in the samples containing persulfides. (d) Finally, the use of the methylsulfonyl benzothiazole (MSBT) to block thiols and persulfides in the first step, followed by the tag switch with cyanoacetate derivatives in the second step, leads to the efficient labelling of persulfides, by the method called tag-switch technique

general data misinterpretation. In addition, Pan and Carroll (2013) studied how persulfides react with both electrophilic and nucleophilic species, reaffirming the nucleophilic properties of the persulfide sulfane sulfur. They unambiguously showed that persulfides react with MMTS (and its analogue S-4-bromobenzyl methanethiosulfonate) as readily as free thiols, questioning the interpretation of the data obtained by modified biotin-switch technique (MBST) approach. Two possible models were proposed to explain the data generated by MBST: (1) free thiols may be incompletely blocked in the first MMTS alkylation step and subsequently react with the pyridyldisulfide biotin reagent; (2) alternatively or in addition, labelling may be achieved via stepwise thiol-disulfide exchange in a reaction catalysed by trace free thiols (RSH).

In their attempt to identify persulfidation of protein tyrosine phosphatase 1B (PTP1B), Krishnan et al. (2011) proposed an approach for persulfide detection based on a completely opposite chemical premise. Namely, they proposed that thiol-blocking reagent, iodoacetic acid (IAA), will react with both free thiols and protein persulfides (Fig. 5b), which is in agreement with the persulfides' reactivity similar to that of free thiols. In the subsequent steps, however, they proposed the cleavage of the alkylated persulfide with DTT and then labelling of that particular cysteine with iodoacetamide-linked biotin (IAP). Although DTT would indeed cleave this adduct, it is unclear how this method distinguishes the persulfides from intra- and intermolecular disulfides and S-nitrosothiols, which would also be reduced by DTT.

Sen et al. (2012) proposed an alternative method for the persulfide detection. This method is based on the fact that both persulfide and free thiol would be blocked by the thiol-blocking reagent N-ethyl maleimide. The authors used Cy5-conjugated maleimide in the first step followed by the use of DTT in the second (Fig. 5c). The adduct of persulfide and Cy5-maleimide is a disulfide that will be cleaved by the DTT leading to a decrease of the in-gel fluorescence signal in the samples containing persulfides. This method has its advantage of being relatively simple and available to every researcher (because the reagents are commercially available). The only limitation of the method is that it does not offer actual persulfide labelling which would allow wide proteomic analysis. Particular care should be taken to ensure that all thiols and persulfides are indeed blocked in the initial step, as that is the crucial step for the subsequent data interpretation.

Most recently, Zhang et al. (2014) proposed a different approach for protein persulfide detection, named tag-switch assay (Fig. 5d). This method was based on an idea that thiol-blocking reagent should be introduced in the first step, which would tag both free thiols and persulfides. If an appropriate tag is employed, the disulfide bond in persulfide adducts might show much enhanced reactivity to certain nucleophiles than common disulfides in proteins. Therefore, a tag-switching reagent (containing both the nucleophile and a reporting molecule such as biotin) could be introduced to label only the persulfide adducts. It should be noted that thiol adducts from the first step are thioethers, which are not expected to

react with the nucleophile. A major challenge in this technology was for the newly generated disulfide linkage from persulfide moieties to display a unique reactivity for a suitable nucleophile to an extent that distinguishes them from common disulfides. Using the methylsulfonyl benzothiazole (MSBT) to block thiols in the first step in combination with the tagged-cyanoacetate derivatives in the second step, persulfides could be efficiently labelled. Neither free thiols, intramolecular disulfides, S-glutathionylated or sulfenylated proteins were tagged by this approach (using BSA as a model protein). This method was further adapted for visualization of intracellular S-sulfhydration by fluorescence microscopy. Despite the lack of reactivity of BSA sulfenic acid derivative with the method, it is expected that sulfenic acids should react with cyanoacetate derivatives. Protein sulfenic acids are, in general, very unstable and prone to further oxidation, so it is improbable that any of them will remain in the solution after 30–45 min of initial incubation with the MSBT. Nonetheless, it is better to treat the cell lysates with dimedone, prior to the incubation with the first component of tag-switch assay, in order to eliminate any doubts of potential cross-reactivity (Park et al. 2015).

3.1 Detection of Sulfane Sulfurs

Although the methods for sulfane sulfur detection detect more than just protein persulfides, they could serve as a useful tool for the fast and easy proof-of-concept type of experiments. With recent advances in making these methods quantitative, it is also possible to assume that the results suggesting an increase or decrease of the sulfane sulfur levels do suggest the same trends for protein persulfides.

The traditional method for sulfane sulfur detection is cyanolysis (Wood 1987). The method is based on the reaction between sulfane sulfurs and cyanide under alkaline conditions ($\text{pH} > 8.5$). In this reaction, thiocyanate (SCN^-) is formed, which in the reaction with Fe^{3+} generates the $\text{Fe}(\text{SCN})^{2+}$ complex with characteristic absorbance maximum at 460 nm.

Recently, two new methods have been proposed. The first one is designed to be used for the detection of sulfane sulfur levels in the cells. Chen et al. (2013) based their discovery on an assumption that sulfane sulfurs are likely to react with the nucleophile components of a fluorescent probe, which could then undergo spontaneous and fast cyclization to release fluorophore. They successfully produced two probes, named SSP1 and SSP2, which could be easily used for intracellular detection of sulfane sulfur. The same group improved the probes designing a new DSP-3 fluorescence sensor for hydrogen polysulfides (Liu et al. 2014a).

Most recently, the group led by Ming Xian established isotope dilution mass spectrometric method for the quantification of sulfane sulfurs (Liu et al. 2014b). Accurate and reliable measurements of sulfane sulfurs in biological samples are required in order to understand the impact of H_2S signalling. The method that Liu et al. (2014b) proposed employs a triphenylphosphine derivative to capture sulfane

sulfurs as a stable phosphine sulfide product. The concentration of this product can be determined by isotope dilution mass spectrometry using a ¹³C₃-labelled phosphine sulfide as an internal standard. Using this method, the authors found that average concentrations of sulfane sulfur were 57.0 (liver), 150.9 (kidney), 46.0 (brain), 61.8 (heart), 56.1 (spleen) and 20.8 nmol/g (lungs).

4 Persulfidation in Action

As already mentioned, H₂S regulates the plethora of biological functions from neurotransmission and blood pressure to cardioprotection. To date, several key protein targets have been identified which do undergo oxPTM of the cysteine residue, suggesting that this modification can be responsible for some of the H₂S effects such as vasodilation, prevention of cell death and senescence, cell differentiation, etc.

4.1 S-sulphydration of K_{ATP} Regulates Vasodilation

Seminal work by Yang et al. (2008) showed that CSE knockout mice develop hypertension, confirming previous assumptions about H₂S being the endogenous regulator of blood pressure. Several other studies have been published recently showing that majority of H₂S-induced vasodilation goes via its interaction with NO. This effect can be dual, via direct reaction with NO that leads to the nitroxyl (HNO) formation and subsequent activation of HNO–TRPA1–CGRP signalling cascade (Eberhardt et al. 2014) or by inhibiting the phosphodiesterase 5 and increasing the cGMP levels (Colleta et al. 2012). Nonetheless, the part of H₂S-induced vasodilation could be assigned to the activation of K_{ATP} channels. Persulfidation of C34 on the Kir6.1 subunit of K_{ATP} channel on smooth muscle cells prevents its association with ATP and promotes its binding to phosphatidylinositol-4,5-bisphosphate (PIP₂). This leads to the channel opening and K⁺ influx and subsequently to the smooth muscle cells' relaxation. Several other Ca²⁺ channels have been also implicated in the H₂S-induced vasodilation.

4.2 Persulfidation of Electrophilic Messengers and Cardioprotection

Recently, direct persulfidation of several electrophilic messengers (such as 8-nitro-cGMP) by sulfide has been reported offering additional mechanism for H₂S-mediated signalling (Nishida et al. 2012). Redox signalling by electrophilic by-products, such as nitrated cyclic nucleotides and nitro- or keto-derivatives of unsaturated fatty acids, all generated by the inflammation-related enzymes, ROS and/or NO, has attracted much interest lately (Nishida et al. 2014; Fujii and Akaike 2013). S-alkylation of cysteine residues by 8-nitroguanosine 3',5'-cyclic monophosphate mediates several redox signalling pathways. Nishida et al. (2012)

showed that HS^- could directly attack the above-mentioned electrophiles, forming derivatives that have biological effect of themselves. Namely, formation of 8-SH-cGMP blocks the S-guanylation of H-Ras, a modification which normally activates H-Ras to signal cell senescence as a response to stress (Fujii and Akaiki 2013). The authors link the protective cardiovascular effects of H_2S and its donors to increased formation of 8-SH-cGMP and inhibition of H-Ras signalling. It is worth mentioning, however, that Terzić et al. (2014) showed that nucleophilic attack of the hydrosulfide anion to 8-nitro-cGMP cannot take place, as previously proposed. Instead, the formation of reactive species containing sulfane sulfur, like persulfides, is required (Terzić et al. 2014).

4.3 S-sulphydration of GAPDH and NF- κ B Protects Against Apoptosis

GAPDH was the first protein characterized as S-sulphydrated in the study that sparked the whole research field (Mustafa et al. 2009a). GAPDH has been known for a while as regulator of a cell death cascade (Hara et al. 2005). S-nitrosation of catalytic C152 abolishes its catalytic activity but makes it able to bind to Siah1, an E3 ubiquitin ligase. Siah1, which possesses a nuclear localization tag, helps the translocation of GAPDH to a nucleus where it enables Siah1 to degrade nuclear proteins, leading to cell death (Hara et al. 2005). Mustafa et al. showed that GAPDH is endogenously S-sulphydrated at C150 which increases its enzymatic activity severalfold (Mustafa et al. 2009a). The authors also demonstrated that DTT treatment of GAPDH decreases its activity, suggesting that endogenous persulfidation regulates its function. CSE knockout mice showed ~35 % reduced activity of GAPDH when compared to control mice. Zhang and associates confirmed recently, using selective tag-switch assay, that GAPDH is indeed endogenously S-sulphydrated (Zhang et al. 2014). The fact that S-sulphydration of GAPDH increases its enzymatic activity means that it also prevents its interaction with Siah1 protecting the cells from apoptosis.

Nuclear factor- κ B (NF- κ B) is an anti-apoptotic transcription factor, which is under basal conditions kept in cytosol via interaction with the inhibitor- κ B α (Napetschnig and Wu 2013). During the inflammation, cells produce tumour necrosis factor- α (TNF- α), which could lead to cell death (Aggarwal et al. 2012). H_2S is known to have protective effects in inflammation but without distinctive mechanism that can explain it (Li et al. 2005; Whiteman and Winyard 2011). Sen and colleagues offered such mechanism in a study describing the persulfidation of NF- κ B (Sen et al. 2012). Namely, they showed that C38 of p65 subunit of NF- κ B is persulfidated which promotes its binding to the co-activator ribosomal protein S3, augmenting its binding to the promoters of anti-apoptotic genes (Fig. 6a). In addition, TNF- α stimulated transcription of CSE, increasing the total amount of S-sulphydrated NF- κ B. Conversely, Du et al. 2014 showed recently that hydrogen sulfide suppresses oxidized low-density lipoprotein-induced macrophage inflammation by inhibiting NF- κ B. This study suggests that persulfidation of C38 on p65

in fact prevents the NF- κ B from leaving the cytosol, therefore completely inhibiting its DNA binding activity. Further studies are obviously needed to give more conclusive answer about the role of H₂S in inflammation.

4.4 S-sulphydration of Parkin Protects Against Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disease caused by the death of dopamine-generating cells in the substantia nigra (Shulman et al. 2011). One of the proteins considered responsible for this cell death is parkin. Parkin is an E3 ubiquitin ligase that catalyses ubiquitination of diverse substrates. Mutations in parkin, which lead to the loss of its activity, are one of the causes of PD (Shulman et al. 2011; Moore et al. 2005). Parkin has reactive cysteine residues, which can be subjected to oxidative posttranslational modifications. S-nitrosation of parkin, for example, inhibits its E3 ubiquitin ligase activity contributing to the Parkinson's disease (Chung et al. 2004).

Recently, Solomon Snyder and colleagues demonstrated that cysteines C59, C95 and C182 could undergo S-sulphydration (Vandiver et al. 2013). This leads to the increase of parkin's activity (Fig. 6b). Persulfidation of parkin is markedly decreased in PD brains, whereas S-nitrosation is increased. Development of H₂S donors opens up a possibility of their use in the early treatment of PD. Increase of parkin's activity could salvage the neurons from the cell death by removing damaged proteins. Parkin is also an important regulator of mitophagy, leading to the removal of damaged mitochondria, particularly in ischemia–reperfusion injury. As H₂S is known to have great pharmacological potential in preventing ischemia–reperfusion injury, it is possible that part of this effect goes via persulfidation of parkin and increased removal of damaged mitochondria.

4.5 S-sulphydration of PTP-1B Regulates ER Stress

Protein tyrosine phosphatases (PTPs) in conjunction with protein tyrosine kinases are important controllers of various biological functions. PTPs are particularly sensitive to oxidative posttranslational modifications of cysteine, as cysteine is present, is in the active site and is important for their function (van Montfort et al. 2003). H₂O₂ is known to modulate function of these enzymes by forming an inactive adduct which contains sulfenic acid in the active site (van Montfort et al. 2003). PTP-1B is one of the members of this class of enzymes, located in the cytoplasmic face of the endoplasmic reticulum, where it plays an important role in ER stress signalling (Paulsen et al. 2012).

Persulfidation of C215 leads to the loss of enzymatic activity, which increases the phosphorylation of Y619 and therefore the activation of PERK in response to ER stress (Krishnan et al. 2011). PERK activation leads to global inhibition of protein translation. The mechanism by which H₂S causes persulfidation of PTP-1B,

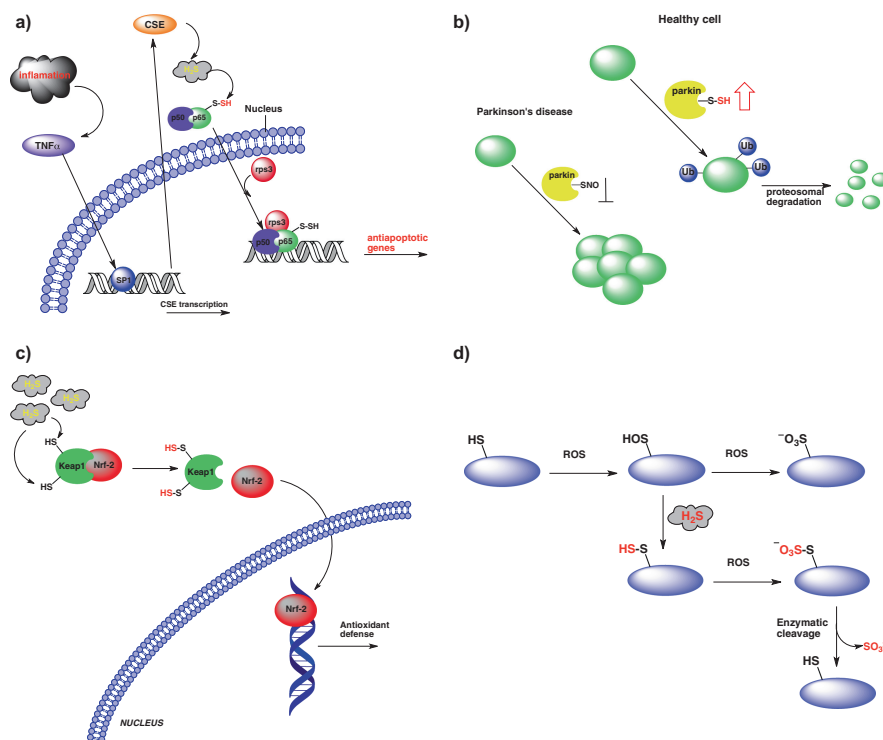


Fig. 6 Biological role of protein persulfidation. (a) As a response to inflammation, tumour necrosis factor alpha (TNF- α) migrates to nucleus where it binds to the transcription factor specificity protein 1 (SP1) causing the transcription and overexpression of CSE. H₂S produced by CSE induces persulfidation of p65 subunit of NF- κ B which translocates to nucleus where its interaction with co-activator ribosomal protein S3 (rps3) is enhanced, resulting in the transcription of anti-apoptotic genes. (b) S-nitrosation of parkin inhibits its E3 ubiquitin ligase activity which leads to accumulation of toxic proteins. This is found in Parkinson's disease where H₂S production is reduced. Persulfidation of parkin, on the other hand, increases parkin's enzymatic activity, which could be used as a therapeutic approach for Parkinson's disease treatment. (c) Keap-1 keeps Nrf-2 in the cytoplasm where it can be ubiquitinated and proteolytically degraded. Persulfidation of critical cysteines on Keap1 disturbs its interaction with Nrf-2 leading to the release of Nrf-2 which can now move into the nucleus where it binds to antioxidant response element (ARE) causing the transcription of various antioxidant defence genes. (d) The hypothetical role of persulfidation in protection of protein function during oxidative stress. When exposed to hydrogen peroxide, proteins undergo oxidation to form sulfenic acids (P-SOH), sulfinic acids (P-SO₂H) and sulphonic acids (P-SO₃H), which cause the irreversible inactivation of the protein. Sulfenic acids could react with H₂S to form persulfides. In addition, protein persulfides could be formed by other mechanisms, and when exposed to ROS, they will readily react with it, forming P-S-SO₃⁻, which can be enzymatically cleaved to restore free thiol on the protein

as in many other cases, is not clear. In the case of PTP-1B though, it is possible that ER stress in fact leads to PTP-1B inactivation by initial formation of sulfenic acid in the active site, which then reacts with H₂S to give persulfide. Although both

modifications are inhibitory, persulfide could be then removed easier and the enzyme's activity restored. Therefore, H₂S could serve to protect the enzyme from a longer-lasting and potentially irreversible inhibition.

4.6 S-sulphydration of Keap1 and p66Shc Increases Cellular Antioxidative Defence and Prevents Senescence

A major mechanism in which antioxidant enzymes are induced involves the activation of the antioxidant response element (ARE) by the oxidative-stress sensor protein Kelch-like ECH-associated protein 1 (Keap1) and the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) (Hybertson et al. 2011; Kaspar et al. 2009). Under basal conditions, Keap1 sequesters Nrf-2 in the cytoplasm by binding to its Neh2 domain (Hybertson et al. 2011; Kaspar et al. 2009; Wakabayashi et al. 2004). Chemical inducers, such as sulforaphane, are known to react with Keap1 cysteine residues, thereby promoting Nrf-2 nuclear accumulation and hence ARE activation (Wakabayashi et al. 2004). A widely accepted model for Nrf-2 nuclear accumulation describes that a modification of the Keap-1 cysteines leads directly to the dissociation of the Keap1–Nrf-2 complex and to the translocation of Nrf-2 into the nucleus.

H₂S cardioprotective effects in ischemia–reperfusion injury are partly caused by Nrf-2 nuclear translocation and activation of antioxidant defence enzymes (Calvert et al. 2009, 2010). Recently, two independent studies suggested that Keap-1 gets S-sulphydrated when cells were exposed to H₂S. Modification of C151 on Keap1 stimulates dissociation of Nrf-2 enabling its translocation to nucleus where it regulates expression of cytoprotective genes and delays cellular senescence (Fig. 6c; Yang et al. 2013). In second study, Hourihan et al. (2013) found that H₂S stabilizes Nrf2 via covalent modification of amino acids C226 and C613 in the Keap1. The authors show that H₂S leads to the production of H₂O₂, which inhibits Keap1 by stimulating the formation of an intramolecular disulfide bond between C226 and C613. The Keap1 C226 and C613 residues are also persulfidated. This may be explained either by the ability of H₂S to reduce C226–C613 disulfides originally formed by H₂O₂ or by direct reaction of H₂S with sulfenylated residues formed by H₂O₂. More importantly, the authors observed that Nrf-2 controls CBS, CSE and sulfide–quinone reductase-like enzyme, suggesting that a feedback loop exists between Nrf-2 and H₂S.

P66Shc belongs to the ShcA family of proteins whose members share three common functionally identical domains: the C-terminal Src homology 2 domain (SH2), the central collagen homology domain (CH1) and the N-terminal phosphotyrosine-binding domain (PTB) (Giorgio et al. 2005). In response to oxidative stress such as UV exposure or H₂O₂, p66Shc gets activated by phosphorylation at Ser-36. The activated p66Shc is then dephosphorylated and translocates to mitochondria, where it binds to cytochrome c helping in electron transport process (Giorgio et al. 2005). P66Shc^{−/−} mice show 30 % increase of the lifespan. It has been shown recently that sulphydration of p66Shc impaired the association of PKC_{βII}

and p66Shc and attenuated H₂O₂-induced p66Shc phosphorylation, a critical step in p66Shc-mediated mitochondrial ROS generation (Xie et al. 2014). H₂S is known to have dramatic effects on inhibiting oxidative stress, something that cannot be simply explained by its direct redox chemistry. A study by Xie and associates suggests that H₂S may inhibit mitochondrial reactive oxygen species production via a p66Shc-dependent mechanism.

4.7 MEK1/PARP-1 Activation and DNA Damage Repair

The salvage of DNA damage is essential for normal cell function. DNA damage stimulates a complex and highly concerted DNA damage repair response, which includes poly(ADP-ribose)ation catalysed by poly(ADP-ribose)ation polymerases (PARPs). Upon DNA damage, PARPs bind to DNA strand breaks and catalyse poly(ADP-ribose)ation which attracts other DNA damage repair proteins (D'Amours et al. 1999). The activation of PARPs is regulated by several kinases, of which MEK/ERK signalling cascade plays an important role (Cohen-Armon et al. 2007). Zhao et al. (2014) reported recently that H₂S attenuates DNA damage in human endothelial cells by causing S-sulfhydration of cysteine 341 on MEK1. This facilitates the translocation of phosphorylated ERK1/ERK2 into nucleus where it activates PARP-1 and increases the DNA damage repair yield, protecting cells from senescence.

4.8 TRP Channel S-sulfhydration Regulates Osteogenic Differentiation

Bone marrow mesenchymal stem cells (BMMSCs) are nonhaematopoietic multipotent stem cells and play an important role in the maintenance of the bone marrow homeostasis (Prockop 1997; Pittenge et al. 1999). BMMSCs and BMMSC-derived osteoblasts are responsible for bone formation and balancing osteoclast-mediated bone resorption in order to maintain bone mineral density (BMD) (Pittenge et al. 1999). CBS-deficient patients exhibit a variety of phenotypes, including osteoporosis. Osteoporosis is characterized by low bone mass and deterioration of osseous microarchitecture, resulting in decreased bone strength and increased risk of fragility fractures. This phenotype is often observed in patients with hyperhomocysteinemia (Herrmann et al. 2005; Melton 2003).

It has been demonstrated recently that H₂S deficiency causes aberrant intracellular Ca²⁺ influx because of reduced persulfidation of cysteine residues on multiple TRP channels (Liu et al. 2014c). Decreased Ca²⁺ influx downregulates PKC-/Erk-mediated Wnt/beta-catenin signalling which controls osteogenic differentiation of BMMSCs. Therefore, the authors suggest that bone marrow mesenchymal stem cells produce H₂S in order to regulate their self-renewal and

osteogenic differentiation and that H₂S deficiency results in defects in BMMSC differentiation. This study is in agreement with the previous observation that hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H₂O₂-induced oxidative damage (Xu et al. 2011) suggesting that development of new H₂S-releasing drugs could be a potential therapeutic route for the treatment of osteoporosis.

5 Placing Persulfidation in a Broader Biological Context and Future Directions

The biochemical properties of protein persulfides discussed here suggest a specific and enhanced reactivity, which can be used for the regulation of protein's function. As mentioned above, persulfidation increases the nucleophilicity of protein thiols, and, as shown in several examples of proteins such as GAPDH and parkin, it increases their enzymatic activity. Further, the increased reducing power of persulfides suggests that they could act as efficient antioxidants. For example, GSSH reacts rapidly with H₂O₂, neutralizing its toxic effects. In fact, one of the possible roles of protein persulfidation could be the protection of a particular protein from irreversible damage induced by ROS and/or RNS (Fig. 6d). Thiol oxidation, which initially starts with the formation of sulfenic acids (still reversible modification), could proceed further with the formation of irreversible sulfonic acids. H₂S could react with sulfenic acid preventing this oxidation. In addition, persulfidated protein, even when exposed to ROS/RNS, will form an adduct that could be cleaved by the action of certain enzymes restoring free thiol. Therefore, spatio-temporal distribution of sulfenylation vs. persulfidation could help us to understand the relative ratio and importance of these two oxPTMs of cysteine.

S-nitrosation of proteins is a very important posttranslational modification of proteins. S-nitrosation of parkin leads to its inactivation (Chung et al. 2004). Conversely, persulfidation of parkin stimulates the enzyme increasing its ligase activity, which prevents accumulation of toxic proteins (Vandiver et al. 2013). On the other hand, intracellular S-nitrosothiol levels were found to be lower in cells with lower H₂S production, suggesting its role in *trans*-nitrosation reactions as well (Filipovic et al. 2012a). Therefore, the understanding of the NO and H₂S crosstalk, with particular emphasis on S-nitrosation vs. persulfidation, should be one of the future tasks.

All these could be done with proper tools for persulfide detection. Further development of assays, which would allow easy labelling of protein persulfides and their subsequent proteomic analysis, would accelerate the progression of the field. It is worth mentioning that most of the protein persulfidation reports to date used the methodology, which has been shown to generate artefacts.

Very little is known about the mechanisms of protein persulfidation. *Trans*-persulfidation of proteins by low molecular weight persulfides is an exciting concept that warrants further exploring. The role of metal centres in facilitating

persulfidation is also of potential interest. Elucidation of all these mechanisms (the role of H₂S vs. polysulfides vs. LMW persulfide) could help us in getting a few steps closer to the understanding of actual contribution of different H₂S-producing enzymes in the regulation of the intracellular S-sulphydration levels.

Finally, the question that warrants an equally important attention is the extent to which this modification is regulatory and to which it is just a consequence of stochastic events. Answering this could lead to the identification of specific targets that could later on be pharmacologically tempered with. Therefore, this field of research will remain one of the hot topics for many years to come.

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References

- Abe K, Kimura H (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. *J Neurosci* 16:1066–1071
- Aggarwal BB, Gupta SC, Kim JH (2012) Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* 119:651–665
- Ali MY, Ping CY, Mok YY et al (2006) Regulation of vascular nitric oxide in vitro and in vivo: a new role for endogenous hydrogen sulphide? *Br J Pharmacol* 149:625–634
- Artaud I, Galardon E (2014) A persulfide analogue of the nitrosothiol SNAP: formation, characterization and reactivity. *Chembiochem* 15:2361–2364
- Bailey TS, Zakharov LN, Pluth MD (2014) Understanding hydrogen sulfide storage: probing conditions for sulfide release from hydrodisulfides. *J Am Chem Soc* 136:10573–10576
- Blackstone E, Morrison M, Roth MB (2005) H₂S induces a suspended animation-like state in mice. *Science* 308:518
- Bouillaud F, Blachier F (2011) Mitochondria and sulfide: a very old story of poisoning, feeding, and signaling? *Antioxid Redox Signal* 15:379–391
- Broniowska KA, Hogg N (2012) The chemical biology of S-nitrosothiols. *Antioxid Redox Signal* 17:969–980
- Calvert JW, Jha S, Gundewar S et al (2009) Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circ Res* 105:365–374
- Calvert JW, Elston M, Nicholson CK et al (2010) Genetic and pharmacologic hydrogen sulfide therapy attenuates ischemia-induced heart failure in mice. *Circulation* 122:11–19
- Carballal S, Radi R, Kirk MC, Barnes S, Freeman BA, Alvarez B (2003) Sulfenic acid formation in human serum albumin by hydrogen peroxide and peroxynitrite. *Biochemistry* 42:9906–9914
- Carballal S, Trujillo M, Cuevasanta E et al (2011) Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. *Free Radic Biol Med* 50:196–205
- Chen W, Liu C, Peng B, Zhao Y, Pacheco A, Xian M (2013) New fluorescent probes for sulfane sulfurs and the application in bioimaging. *Chem Sci* 4:2892–2896
- Chung KK, Thomas B, Li X et al (2004) S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* 304:1328–1331
- Cohen-Armon M, Visocek L, Rozensal D, Kalal A, Geistrikh I, Klein R, Bendetz-Nezer S, Yao Z, Seger R (2007) DNA-independent PARP-1 activation by phosphorylated ERK2 increases Elk1 activity: a link to histone acetylation. *Mol Cell* 25:297–308
- Coletta C, Papapetropoulos A, Erdelyi K et al (2012) Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc Natl Acad Sci USA* 109(23):9161–9166

- Cuevasanta E, Denicola A, Alvarez B, Moller MN (2012) Solubility and permeation of hydrogen sulfide in lipid membranes. *PLoS ONE* 7:e34562
- D'Amours D, Desnoyers S, D'Silva I, Poirier GG (1999) Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* 342:249–268
- Das TN, Huie RE, Neta P, Padmaja S (1999) Reduction potential of the sulfhydryl radical: pulse radiolysis and laser flash photolysis studies of the formation and reactions of center dot SH and HSSH center dot(-) in aqueous solutions. *J Phys Chem A* 103:5221–5226
- Du J, Huang Y, Yan H et al (2014) Hydrogen sulfide suppresses oxidized low-density lipoprotein (ox-LDL)-stimulated monocyte chemoattractant protein 1 generation from macrophages via the nuclear factor κ B (NF- κ B) pathway. *J Biol Chem* 289:9741–9753
- Eberhardt M, Dux M, Namer B et al (2014) H₂S and NO cooperatively regulate vascular tone by activating a neuroendocrine HNO-TRPA1-CGRP signalling pathway. *Nat Commun* 5:4381
- Filipovic MR, Miljkovic J, Allgäuer A et al (2012a) Biochemical insight into physiological effects of H₂S: reaction with peroxynitrite and formation of a new nitric oxide donor, sulfinyl nitrite. *Biochem J* 441:609–621
- Filipovic MR, Miljkovic J, Nauser T et al (2012b) Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H₂S and S-nitrosothiols. *J Am Chem Soc* 134:12016–12027
- Filipovic MR, Eberhardt M, Prokopovic V et al (2013) Beyond H₂S and NO interplay: hydrogen sulfide and nitroprusside react directly to give nitroxyl (HNO). A new pharmacological source of HNO. *J Med Chem* 56:1499–1508
- Flavin M (1962) Microbial transsulfuration: the mechanism of an enzymatic disulfide elimination reaction. *J Biol Chem* 237:768–777
- Forrester MT, Foster MW, Benhar M, Stamler JS (2009) Detection of protein S-nitrosylation with the biotin-switch technique. *Free Radic Biol Med* 46:119–126
- Foster MW, Hess DT, Stamler JS (2009) Protein S-nitrosylation in health and disease: a current perspective. *Trends Mol Med* 15:391–404
- Francoleon NE, Carrington SJ, Fukuto JM (2011) The reaction of H₂S with oxidized thiols: generation of persulfides and implications to H₂S biology. *Arch Biochem Biophys* 516:146–153
- Fujii S, Akaike T (2013) Redox signaling by 8-nitro-cyclic guanosine monophosphate: nitric oxide- and reactive oxygen species-derived electrophilic messenger. *Antioxid Redox Signal* 19:1236–1246
- Fulton AB (1982) How crowded is the cytoplasm? *Cell* 30:345–347
- Giorgio M, Migliaccio E, Orsini F et al (2005) Electron transfer between cytochrome *c* and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* 122:221–233
- Greiner R, Palinkas Z, Basell K et al (2013) Polysulfides link H₂S to protein thiol oxidation. *Antioxid Redox Signal* 19:1749–1765
- Gupta V, Carroll KS (2014) Sulfenic acid chemistry, detection and cellular lifetime. *Biochim Biophys Acta* 1840:847–875
- Hara MR, Agrawal N, Kim SF et al (2005) S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol* 7:665–674
- Heimer NE (1981) Biologically oriented organic sulfur chemistry. 21. Hydrodisulfide of a penicillamine derivative and related compounds. *J Org Chem* 46:1374–1377
- Herrmann M, Widmann T, Colaianni G, Colucci S, Zallone A, Herrmann W (2005) Increased osteoclast activity in the presence of increased homocysteine concentrations. *Clin Chem* 51:2348–2353
- Hess DT, Stamler JS (2012) Regulation by S-nitrosylation of protein post-translational modification. *J Biol Chem* 287:4411–4418
- Hill BC, Woon TC, Nicholls P, Peterson J, Greenwood C, Thomson AJ (1984) Interactions of sulphide and other ligands with cytochrome *c* oxidase. An electron-paramagnetic-resonance study. *Biochem J* 224:591–600

- Hourihan JM, Kenna JG, Hayes JD (2013) The gasotransmitter hydrogen sulfide induces nrf2-target genes by inactivating the keap1 ubiquitin ligase substrate adaptor through formation of a disulfide bond between cys-226 and cys-613. *Antioxid Redox Signal* 19:465–481
- Hybertson BM, Gao B, Bose SK, McCord JM (2011) Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol Aspects Med* 32:234–246
- Ida T, Sawa T, Ihara H et al (2014) Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc Natl Acad Sci USA* 111:7606–7611
- Ivanovic-Burmazovic I, Filipovic MR (2012) WO2012/175630
- Jackson MR, Melideo SL, Jorns MS (2012) Human sulfide:quinone oxidoreductase catalyzes the first step in hydrogen sulfide metabolism and produces a sulfane sulfur metabolite. *Biochemistry* 51:6804–6815
- Kabil O, Banerjee R (2014) Enzymology of H₂S biogenesis, decay and signaling. *Antioxid Redox Signal* 20:770–782
- Kabil O, Motl N, Banerjee R (2014) H₂S and its role in redox signaling. *Biochim Biophys Acta*. doi:10.1016/j.bbapap.2014.01.002
- Karala AR, Ruddock LW (2007) Does s-methyl methanethiosulfonate trap the thiol-disulfide state of proteins? *Antioxid Redox Signal* 9:527–531
- Kaspar JW, Niture SK, Jaiswal AK (2009) Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med* 47:1304–1309
- Kimura H (2014) Hydrogen sulfide and polysulfides as biological mediators. *Molecules* 19:16146–16157
- Kimura H, Nagai Y, Umemura K, Kimura Y (2005) Physiological roles of hydrogen sulfide: synaptic modulation, neuroprotection, and smooth muscle relaxation. *Antioxid Redox Signal* 7:795–803
- Kimura Y, Mikami Y, Osumi K, Tsugane M, Oka J, Kimura H (2013) Polysulfides are possible H₂S-derived signaling molecules in rat brain. *FASEB J* 27:2451–2457
- Koenitzer JR, Isbell TS, Patel HD et al (2007) Hydrogen sulfide mediates vasoactivity in an O₂-dependent manner. *Am J Physiol Heart Circ Physiol* 292:H1953–H1960
- Kotronarou A, Hoffmann MR (1991) Catalytic autooxidation of hydrogen sulfide in wastewater. *Environ Sci Technol* 25:1153–1160
- Krishnan N, Fu C, Pappin DJ, Tonks NK (2011) H₂S-induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci Signal* 4(203):ra86
- Kutney GW, Turnbull K (1982) Compounds containing the sulfur-sulfur double bond. *Chem Rev* 82:333–357
- Li L, Bhatia M, Zhu YZ et al (2005) Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. *FASEB J* 19:1196–1198
- Li L, Hsu A, Moore PK (2009) Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation—a tale of three gases! *Pharmacol Ther* 123:386–400
- Libiad M, Yadav PK, Vitvitsky V, Martinov M, Banerjee R (2014) Organization of the human mitochondrial H₂S oxidation pathway. *J Biol Chem pii: jbc.M114.602664*
- Lima B, Forrester MT, Hess DT, Stamler JS (2010) S-nitrosylation in cardiovascular signaling. *Circ Res* 106:633–646
- Liu C, Chen W, Shi W et al (2014a) Rational design and bioimaging applications of highly selective fluorescence probes for hydrogen polysulfides. *J Am Chem Soc* 136:7257–7260
- Liu C, Zhang F, Munske G, Zhang H, Xian M (2014b) Isotope dilution mass spectrometry for the quantification of sulfane sulfurs. *Free Radic Biol Med* 76C:200–207
- Liu Y, Yang R, Liu X et al (2014c) Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca(2+) channel sulfhydration. *Cell Stem Cell* 15:66–78
- Mathai JC, Missner A, Kugler P et al (2009) No facilitator required for membrane transport of hydrogen sulfide. *Proc Natl Acad Sci USA* 106:16633–16638
- Melton LJ 3rd (2003) Adverse outcomes of osteoporotic fractures in the general population. *J Bone Miner Res* 18:1139–1141

- Mikami Y, Shibuya N, Kimura Y, Nagahara N, Ogasawara Y, Kimura H (2011) Thioredoxin and dihydrolipoic acid are required for 3-mercaptopyruvate sulfurtransferase to produce hydrogen sulfide. *Biochem J* 439:479–485
- Miljkovic JL, Kenkel I, Ivanovic-Burmazovic I, Filipovic MR (2013) Generation of HNO and HSNO from nitrite by heme-iron-catalyzed metabolism with H₂S. *Angew Chem Int Ed Engl* 52:12061–12064
- Minton AP (1998) Molecular crowding: analysis of effects of high concentrations of inert cosolutes on biochemical equilibria and rates in terms of volume exclusion. *Methods Enzymol* 295:27–149
- Módos K, Coletta C, Erdélyi K, Papapetropoulos A, Szabo C (2013) Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J* 27:601–611
- Moore DJ, West AB, Dawson VL, Dawson TM (2005) Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci* 28:57–87
- Moriarty-Craige SE, Jones DP (2004) Extracellular thiols and thiol/disulfide redox in metabolism. *Annu Rev Nutr* 24:481–509
- Mueller EG (2006) Trafficking in persulfides: delivering sulfur in biosynthetic pathways. *Nat Chem Biol* 2:185–194
- Mustafa AK, Gadalla MM, Sen N et al (2009a) H₂S signals through protein S-sulphydration. *Sci Signal* 2:ra72
- Mustafa AK, Gadalla MM, Snyder SH (2009b) Signaling by gasotransmitters. *Sci Signal* 2:re2
- Mustafa AK, Sikka G, Gazi SK et al (2011) Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulphydrates potassium channels. *Circ Res* 109:1259–1268
- Nagy P, Winterbourn CC (2010) Rapid reaction of hydrogen sulfide with the neutrophil oxidant hypochlorous acid to generate polysulfides. *Chem Res Toxicol* 23:1541–1543
- Napetschnig J, Wu H (2013) Molecular basis of NF-κB signaling. *Annu Rev Biophys* 42:443–468
- Nicholls P, Marshall DC, Cooper CE, Wilson MT (2013) Sulfide inhibition of and metabolism by cytochrome c oxidase. *Biochem Soc Trans* 41:1312–1316
- Nishida M, Sawa T, Kitajima N et al (2012) Hydrogen sulfide anion regulates redox signaling via electrophile sulphydration. *Nat Chem Biol* 8:714–724
- Nishida M, Toyama T, Akaike T (2014) Role of 8-nitro-cGMP and its redox regulation in cardiovascular electrophilic signaling. *J Mol Cell Cardiol* 73:10–17
- Olson KR (2012) A practical look at the chemistry and biology of hydrogen sulfide. *Antioxid Redox Signal* 17:32–44
- Olson KR, Healy MJ, Qin Z et al (2008) Hydrogen sulfide as an oxygen sensor in trout gill chemoreceptors. *Am J Physiol Regul Integr Comp Physiol* 295:R669–R680
- Olson KR, DeLeon ER, Liu F (2014) Controversies and conundrums in hydrogen sulfide biology. *Nitric Oxide* 41:11–26
- Ono K, Akaike T, Sawa T et al (2014) Redox chemistry and chemical biology of H₂S, hydro-persulfides, and derived species: implications of their possible biological activity and utility. *Free Radic Biol Med*. doi:10.1016/j.freeradbiomed.2014.09.007
- Pálinkás Z, Furtmüller PG, Nagy A et al (2014) Interactions of hydrogen sulfide with myeloperoxidase. *Br J Pharmacol*. doi:10.1111/bph.12769
- Pan J, Carroll KS (2013) Persulfide reactivity in the detection of protein S-sulphydration. *ACS Chem Biol* 8:1110–1116
- Papapetropoulos A, Pyriochou A, Altaany Z et al (2009) Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc Natl Acad Sci USA* 106:21972–21977
- Park CM, Macinkovic I, Filipovic MR, Xian M (2015) Use of the “Tag-Switch” method for the detection of protein S-Sulphydration. *Methods Enzymol*. doi:10.1016/bs.mie.2014.11.033
- Parsons LB, Walton JH (1921) Preparation and properties of the persulfides of hydrogen. *J Am Chem Soc* 43:2539–2548
- Paul BD, Snyder SH (2012) H₂S signalling through protein sulphydration and beyond. *Nat Rev Mol Cell Biol* 13:499–507

- Paulsen CE, Carroll KS (2013) Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. *Chem Rev* 113:4633–4679
- Paulsen CE, Truong TH, Garcia FJ et al (2011) Peroxide-dependent sulfenylation of the EGFR catalytic site enhances kinase activity. *Nat Chem Biol* 8:57–64
- Peaper DR, Wearsch PA, Cresswell P (2005) Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. *EMBO J* 24:3613–3623
- Peng YJ, Nanduri J, Raghuraman G et al (2010) H₂S mediates O₂ sensing in the carotid body. *Proc Natl Acad Sci USA* 107:10719–10724
- Pietri R, Lewis A, León RG et al (2009) Factors controlling the reactivity of hydrogen sulfide with hemeproteins. *Biochemistry* 48:4881–4894
- Pittenge MF, Mackay AM, Beck SC et al (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147
- Poynton RA, Hampton MB (2014) Peroxiredoxins as biomarkers of oxidative stress. *Biochim Biophys Acta* 1840:906–912
- Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 276:71–74
- Reynolds JD, Bennett KM, Cina AJ et al (2013) S-nitrosylation therapy to improve oxygen delivery of banked blood. *Proc Natl Acad Sci USA* 110:11529–11534
- Riahi S, Rowley CN (2014) Why can hydrogen sulfide permeate cell membranes? *J Am Chem Soc* 136:15111–15113
- Ríos-González BB, Román-Morales EM, Pietri R, López-Garriga J (2014) Hydrogen sulfide activation in hemeproteins: the sulfheme scenario. *J Inorg Biochem* 133:78–86
- Sen N, Paul BD, Gadalla MM et al (2012) Hydrogen sulfide-linked sulphydration of NF- κ B mediates its antiapoptotic actions. *Mol Cell* 45:13–24
- Seth D, Stamler JS (2011) The SNO-proteome: causation and classifications. *Curr Opin Chem Biol* 15:129–136
- Shulman JM, De Jager PL, Feany MB (2011) Parkinson's disease: genetics and pathogenesis. *Annu Rev Pathol* 6:193–222
- Sparatore A, Perrino E, Tazzari V et al (2008) Pharmacological profile of a novel H₂S-releasing aspirin. *Free Radic Biol Med* 46:586–592
- Stedel R, Drozdova Y, Miaskiewicz K, Hertwig RH, Koch W (1997) How unstable are thiosulfoxides? An ab initio MO study of various disulfanes RSSR (R = H, Me, Pr, All), their branched isomers R₂SS, and the related transition states. *J Am Chem Soc* 119:1990–1996
- Szabó C, Papapetropoulos A (2011) Hydrogen sulphide and angiogenesis: mechanisms and applications. *Br J Pharmacol* 164:853–865
- Szczesny B, Módos K, Yanagi K et al (2014) AP39, a novel mitochondria-targeted hydrogen sulfide donor, stimulates cellular bioenergetics, exerts cytoprotective effects and protects against the loss of mitochondrial DNA integrity in oxidatively stressed endothelial cells in vitro. *Nitric Oxide* 41:120–130
- Talipov MR, Timerghazin QK (2013) Protein control of S-nitrosothiol reactivity: interplay of antagonistic resonance structures. *J Phys Chem B* 117:1827–1837
- Terzić V, Padovani D, Balland V, Artauda I, Galardon E (2014) Electrophilic sulphydration of 8-nitro-cGMP involves sulfane sulfur. *Org Biomol Chem* 12:5360–5364
- van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H (2003) Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. *Nature* 423:773–777
- Vandiver MS, Paul BD, Xu R et al (2013) Sulphydration mediates neuroprotective actions of parkin. *Nat Commun* 4:1626
- Vitvitsky V, Kabil O, Banerjee R (2012) High turnover rates for hydrogen sulfide allow for rapid regulation of its tissue concentrations. *Antioxid Redox Signal* 17:22–31
- Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, Yamamoto M, Kensler TW, Talalay P (2004) Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc Natl Acad Sci U S A* 101:2040–2045

- Wang R (2002) Two's company, three's a crowd: can H₂S be the third endogenous gaseous transmitter? *FASEB J* 16:1792–1808
- Wedmann R, Bertlein S, Macinkovic I, Boeltz S, Miljkovic J, Munoz L, Herrmann M, Filipovic MR (2014) Working with “H₂S”: facts and apparent artifacts. *Nitric Oxide* 41:85–96
- Whiteman M, Winyard PG (2011) Hydrogen sulfide and inflammation: the good, the bad, the ugly and the promising. *Expert Rev Clin Pharmacol* 4:13–32
- Whiteman M, Li L, Kostetski I et al (2006) Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. *Biochem Biophys Res Commun* 343:303–310
- Wood JL (1987) Sulfane sulfur. *Methods Enzymol* 143:25–29
- Xie ZZ, Shi MM, Xie L et al (2014) Sulphydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. *Antioxid Redox Signal*. doi:10.1089/ars.2013.5604
- Xu L, Eu JP, Meissner G, Stamler JS (1998) Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. *Science* 279:234–237
- Xu ZS, Wang XY, Xiao DM, Hu LF, Lu M, Wu ZY, Bian JS (2011) Hydrogen sulfide protects MC3T3-E1 osteoblastic cells against H₂O₂-induced oxidative damage-implications for the treatment of osteoporosis. *Free Radic Biol Med* 50(10):1314–23
- Yadav PK, Yamada K, Chiku T, Koutmos M, Banerjee R (2013) Structure and kinetic analysis of H₂S production by human mercaptopyruvate sulfurtransferase. *J Biol Chem* 288: 20002–200013
- Yang G, Wu L, Jiang B et al (2008) H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322:587–590
- Yang G, Zhao K, Ju Y et al (2013) Hydrogen sulfide protects against cellular senescence via S-sulphydration of Keap1 and activation of Nrf2. *Antioxid Redox Signal* 18:1906–1919
- Yang J, Gupta V, Carroll KS, Liebler DC (2014) Site-specific mapping and quantification of protein S-sulphenylation in cells. *Nat Commun* 5:4776
- Yong QC, Hu LF, Wang S, Huang D, Bian JS (2010) Hydrogen sulfide interacts with nitric oxide in the heart: possible involvement of nitroxyl. *Cardiovasc Res* 88:482–491
- Yong QC, Cheong JL, Hua F et al (2011) Regulation of heart function by endogenous gaseous mediators-crosstalk between nitric oxide and hydrogen sulfide. *Antioxid Redox Signal* 14: 2081–2091
- Yoshida T, Inoue R, Morii T et al (2006) Nitric oxide activates TRP channels by cysteine S-nitrosylation. *Nat Chem Biol* 2:596–607
- Zhang D, Macinkovic I, Devarie-Baez NO et al (2014) Detection of protein S-sulphydration by a tag-switch technique. *Angew Chem Int Ed Engl* 53:575–581
- Zhao Y, Bhushan S, Yang C et al (2013) Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury. *ACS Chem Biol* 8:1283–1290
- Zhao K, Ju Y, Li S, Altaany Z, Wang R, Yang G (2014) S-sulphydration of MEK1 leads to PARP-1 activation and DNA damage repair. *EMBO Rep* 15:792–800
- Zhou Z, von Wantoch Rekowski M, Coletta C et al (2012) Thioglycine and L-thiovaline: biologically active H₂S-donors. *Bioorg Med Chem* 20:2675–2678

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