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Recent development of polysulfides: Chemistry and biological applications

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Abstract

Polysulfides (RSS_nSR, $n \geq 1$) are a class of sulfane sulfur compounds that have gained significant recent attention due to their connections to hydrogen sulfide (H_2S) and hydropersulfides (RSSH), which are known to play important roles in redox signaling. While the potential regulatory functions of polysulfides in biological systems have been recognized for a long time, understanding their interactions with H_2S/RSSH have only recently begun. In this Mini Review, some of the most recent discoveries of polysulfides within biological contexts are summarized and these include their biological formation pathways, detection methods for animal and plant samples, properties, and unique functions. These studies have set up a solid foundation for understanding polysulfide biology, and more mechanistic details are expected to be discovered in the coming years.

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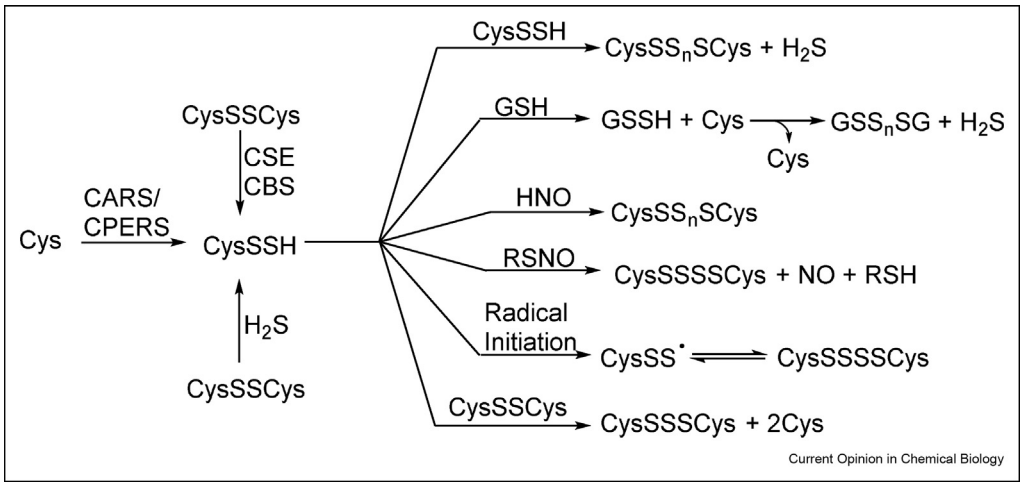
Introduction

Polysulfides have been used for medicinal purposes by humans for thousands of years, with some of their first uses seen throughout ancient cultures in the form of garlic [1]. Diallyl trisulfide (DATS) is a compound found in garlic and other members of the *Allium* genus, and its characteristic polysulfide structure contributes to its antioxidant, antibacterial, and insect-repelling properties. Interestingly, recent studies have revealed that polysulfides exist in varying micromolar concentrations in human biological fluids including plasma, seminal fluid, tears, etc. suggesting the potential roles of polysulfides in life [2]. The 'Renaissance' of polysulfides in redox biology is closely related to the growing appreciation of hydrogen sulfide (H_2S) and hydropersulfides (RSSH) as important cellular regulating molecules. Polysulfides can serve as their precursors. For this reason, cysteine trisulfide (CysSSSCys) and glutathione trisulfide (GSSSG) are often used as the equivalents of persulfides in many recent studies. In this Mini Review, we summarize some recent discoveries of polysulfides within biological contexts, including their formation, detection, properties/reactions, and unique functions. The chemical properties of polysulfides appear to be closely related to their reduced persulfide forms, which have been demonstrated to be strongly associated with regulating inflammation, oxidative stress, and mitochondrial function.

Formation of polysulfides under biologically relevant conditions

There are several ways in which polysulfides can be endogenously produced and most pathways require the initial generation of persulfides (Scheme 1). These persulfides can then cross-react to form polysulfides of various sulfur bridge lengths. Alternatively, they can undergo sulfur transfer reactions with other thiols (RSH) such as glutathione (GSH) to form glutathione persulfide (GSSH) and subsequently glutathione polysulfides. In addition, persulfides can react with nitroxyl (HNO) to form polysulfides. Toscano et al. studied the reaction mechanism and proved that *N*-hydroxylperthiosulfenamide (RSS−NH−OH) was the key intermediate [3]. Compared to thiols, persulfides are more potent traps for HNO. This reaction may be a new pathway for HNO signaling. Another interesting reaction of RSSH is transnitrosation with *S*-nitrosothiols (RSNO). RSNO compounds like GSNO or CysSNO are important nitric oxide (NO) reservoirs in cells. Fukuto et al. studied persulfide *trans*-nitrosation (with GSNO) and found that the reaction led to the formation of NO, GSH, and polysulfides [4]. It was believed that *S*-nitrosated persulfides (RSSNO) are much more unstable than RSNO, due to the enhanced stability of perthiyl radicals ($RSS^{\bullet}$) vs thiyl radicals ($RS^{\bullet}$). Once RSSNO is formed via *trans*-nitrosation, it readily degrades to form NO and $RSS^{\bullet}$, which then dimerizes to form polysulfides. Vasorelaxation was detected upon the incubation of vascular tissues with either persulfides or cysteine trisulfide with *S*-nitroso-N-acetylpenicillamine (SNAP), indicating that NO was released by this

Scheme 1



Possible routes for endogenous formation of polysulfides.

reaction [5,6]. This further demonstrated the ability for persulfides and polysulfides (as persulfide precursors) to endogenously liberate NO from RSNO.

Since persulfides are the primary precursors of endogenous polysulfides, it is worth noting that the majority of persulfides are synthesized through enzymatic reactions with the formation of cysteine persulfides. This was suggested in 2014 when Ida et al. demonstrated that cystathionine-γ-lyase (CSE) or cystathionine-β-synthase (CBS) could produce persulfides as major products when incubated with cystine (CysSSCys) [7]. In 2017, Akaike et al. found that cysteinyl-tRNA synthetase (CARS) acts as a cysteine persulfide synthase (CPERS), which can directly generate persulfides and indirectly polysulfides through a co-translational process [8]. Other polysulfidation methods also exist, including radical cross-reactions between two equivalents of per/polypersulfides, which was demonstrated in 1962 by Nakabayashi and Tsurugi who found that the radical initiation of persulfides resulted in polysulfide products and H_2S [9]. This claim has been furthered by recent postulations that tetrasulfides are the product of perthiyl radical reactions ($RS_nS\bullet$, $n \geq 1$), in which radical initiation is caused by lipid peroxidation in cells [10].

While polysulfides can be formed from persulfides, they can also be considered the precursors of persulfides. Bianco et al. utilized standard analytical techniques (NMR, MS, etc.) to study the reactions between H_2S, disulfides, persulfides, polysulfides, and thiols. It was found that these species exist simultaneously in a dynamic equilibrium [11]. Therefore, it is difficult to discern which species is the actual biological effector. Since polysulfides are most efficient in inducing persulfide formation, biologically relevant polysulfides such as cysteine trisulfide (CysSSSCys) or glutathione trisulfides (GSSSG) are now commonly used as 'persulfide donors' in studies. For example, HEK293T cells treated with CysSSSCys led to a dose-dependent increase of intracellular persulfides (in the form of CysSSH and GSSH) [12]. This treatment also resulted in strong protection against *N*-ethylmaleimide (NEM)-mediated cytotoxicity (e.g. electrophilic stress) [13]. On the other hand, polysulfides are also thiol-oxidized products and can directly react with thiols to cause thiol-oxidation. Eaton et al. studied the effects of polysulfides (using CysSSSCys as the model) on purified proteins and on cellular stress response [14]. CysSSSCys was found to induce disulfide formation on thiol-containing proteins including cGMP-dependent protein kinase-1 (PKGIα), phosphatase and tensin homolog (PTEN), and Kelch-like ECH-associated protein 1 (KEAP1). In cellular response studies with HEK293 cells, CysSSSCys induced global protein thiol oxidation, cellular and ER-stress induced apoptosis, and inhibited cellular metabolism and proliferation. Interestingly, pre-reacting CysSSSCys with H_2S before cell treatment did not induce ER-stress. Similar inhibition of cellular growth was seen in other cancer cells upon incubation with dibenzyl trisulfide and DATS, suggesting that polysulfides may be used as anti-cancer compounds.

Stability of polysulfides under biologically relevant conditions

Polysulfides are known to be relatively unstable in aqueous solutions, which consequently adds a layer of difficulty to their study within biological contexts. Bowden et al. recently studied the stabilities of polysulfides (trisulfides and tetrasulfides) derived from

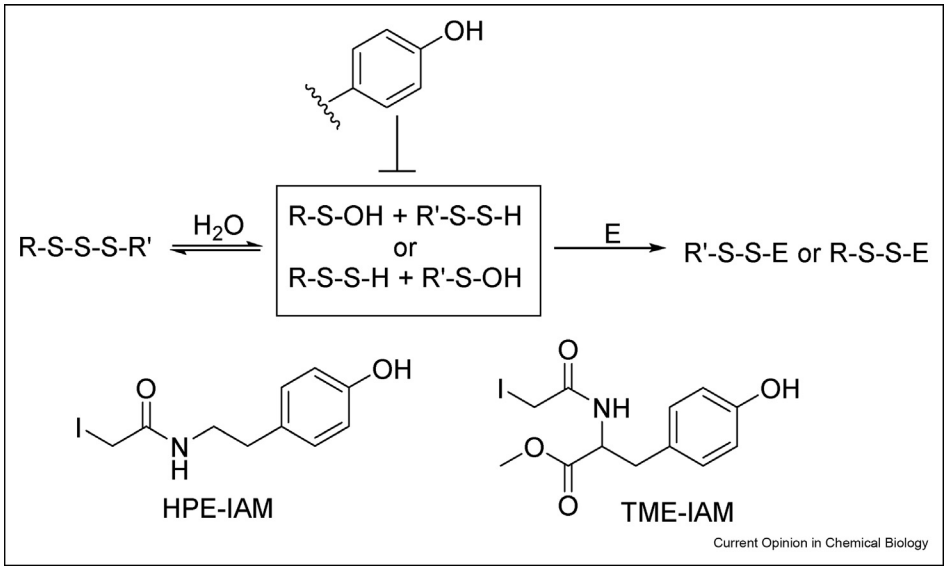
cysteine, glutathione, and *N*-acetylcysteine (NAC) under biologically relevant conditions [15]. It was found that tetrasulfides were more susceptible to degradation than trisulfides, and higher pH would cause faster degradation. Additionally, glutathione-derived polysulfides were more unstable than cysteine-derived polysulfides. For example, CysSSSCys and GSSSG exhibited degradation at pH 9 with half-lives of 11.4 days and 19 h, respectively. The final degradation products were disulfides and elemental sulfur. It was also found that the presence of amines accelerated degradation. Interestingly, NAC-derived polysulfides showed much enhanced stability (NAC trisulfide was stable for 8 days between the pH ranges of 5.8–9.0), presumably due to the lack of free amine in their structure. The authors further found that elevated temperatures (60 °C) could promote degradation while the presence of metal ions (zinc or iron) had little effects. These results suggest that the instability of polysulfides within biological contexts does not stem from metal interactions, but rather from other nucleophilic factors such as excess amines.

The stability issue of polysulfides in aqueous buffers could be a problem in analyzing sulfur metabolism in biological systems. It is suggested polysulfides undergo hydrolysis under weak alkaline and physiological conditions to form hydropersulfides (-SSH) and sulfenic acids (-SOH) (Scheme 2). Usually, this is an equilibrium that favors the starting materials. The studies from Akaike et al. found that thiol-blocking reagents such as iodoacetamide (IAM), NEM, monobromobimane (mBB), etc. could trap the hydrolysis products and thus promote hydrolysis or electrophilic degradation [16,17]. Since these thiol-blocking reagents are commonly used to trap and characterize −SH containing molecules (including H_2S, H_2S_2, RSH, RSSH), polysulfide hydrolysis and subsequent derivatization by the thiol-blocking reagents could generate false positive results. Interestingly, hydroxyphenyl-containing molecules such as tyrosine were found to stabilize polysulfides and prevent their hydrolysis, although the mechanism remains unclear. Based on these results, Akaike et al. conjugated iodoacetamide with hydroxyphenyl moieties and created two new −SH blocking reagents: 4-hydroxyphenyl)ethyl iodoacetamide (HPE-IAM) and *N*-iodoacetyl L-tyrosine methyl ester (TME-IAM) [8,18]. These compounds showed excellent −SH trapping ability, especially for persulfides, while minimizing the hydrolysis of polysulfides. In a related study by Dick et al. [19], it was found that IAM and NEM were not suitable for persulfide labeling as the resulting adducts (R-S-S-Alk) could be converted into the corresponding thioethers (R-S-Alk) by cellular nucleophiles. However, other reagents, such as mBB and *N*-t-butyl-iodoacetamide, did not have this problem and could conserve persulfide characteristics.

Detection of polysulfides in biological systems

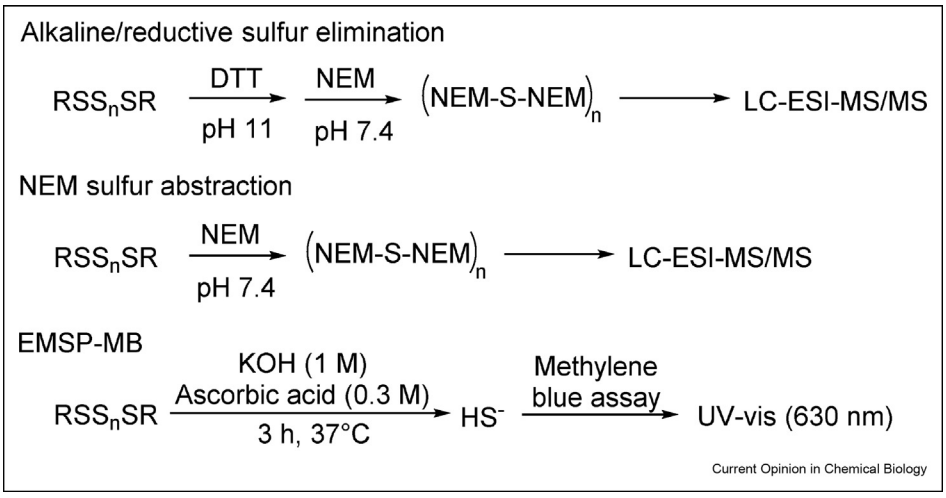
Alliaceous and cruciferous vegetables are rich with polysulfide compounds including dimethyl trisulfide, dipropyl trisulfide, diallyl trisulfide, and diallyl tetrasulfide, thereby contributing to some of their health benefits. To quantify polysulfide contents in these species, Kasamatsu et al. recently explored two methods

Scheme 2



Potential hydrolysis of polysulfides and subsequent reactions.

Scheme 3



Polysulfide detection methods.

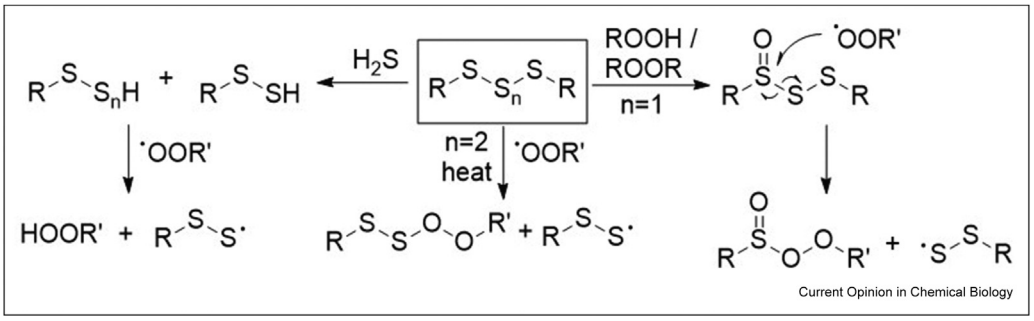
based on the use of liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (Scheme 3) [20]. The first method, alkaline/reductive sulfur elimination, was to treat the samples with dithioreitol (DTT) at pH 11. This would liberate all sulfane sulfur atoms from polysulfides to form HS^-. Then, NEM was used to capture HS^-, forming bis-alkylated product NEM-S-NEM, which was quantified by LC-ESI-MS/MS. This method worked well for both hydrophilic polysulfides (such as GSSSG and protein polysulfides) and lipophilic polysulfides (such as dimethyl- and diallyl-polysulfides). The second method, NEM sulfur abstraction, was based on the recent discovery that strong electrophilic thiol-blocking reagents can react with the midchain sulfane sulfur atoms in polysulfides, and this reaction occurs at neutral pH. As such, when NEM was directly treated with polysulfides under pH 7.4, it should abstract sulfane sulfurs from polysulfides to form NEM-S-NEM, which can then be quantified by LC-ESI-MS/MS. While NEM sulfur abstraction is simpler than alkaline/reductive sulfur elimination, it appeared to be less generic as the structures of the polysulfides could affect their reactivity significantly. These methods were used to analyze 22 vegetables. The highest polysulfide contents were found in onion, broccoli, Chinese chive, garlic, and komatsuna, while the lowest polysulfide levels were found in eggplant, carrot, lettuce, and tomato.

In order to quantify polysulfide contents in human fluid samples, Ikeda et al. developed a method named "elimination method of sulfide from polysulfide" or (EMSP) [21]. They found the use of sulfide antioxidant buffer (SAOB), a strong redox buffer consisting of a cocktail of sodium salicylate (0.5 M), ascorbic acid (0.12 M), and NaOH (2.2M), could eliminate sulfane sulfurs from polysulfides to form sulfide (HS^-). This buffer was then optimized by removing salicylate and using KOH instead of NaOH. The mixture included only L-ascorbic acid (0.3 M) and KOH (1 M) and was found to be more efficient than SAOB when reacted for 3 h at 37 °C. After removing the sulfane sulfur atoms from the polysulfides, a standard methylene blue assay (MB) was used to quantify the liberated sulfides. They used this method to quantify the concentrations of polysulfides in human biological fluids including plasma, tears, saliva, nasal discharge, and seminal fluids [2]. These concentrations were found to be an average of 7469 μM, 954 μM, 41 μM, 398 μM, and 595 μM, respectively. Furthermore, they found that saliva and seminal fluid polysulfide levels were respectively correlated with amylase and sperm activities, suggesting the possible association between polysulfide levels with amylase and sperm activity. Since physical or psychosocial stress can increase amylase activities and are also known to cause oxidative stress, perhaps polysulfides are the products of persulfide antioxidant activity.

Properties and biological functions of polysulfides

The full extent of the biological functions of polysulfides is not yet fully known, although there has been significant progress toward this in recent years. It is becoming widely accepted that persulfide formation via reactions with H_2S contributes to cellular signaling to a greater extent than H_2S alone [3]. Polysulfides are known to have antioxidant effects, but only recently have the mechanisms of this activity been studied. Pratt and coworkers found that polysulfides likely contribute to their protective effects through radical interactions

Scheme 4



The reactions of polysulfides with peroxyl radicals.

with oxidative species. To investigate this, fluorescent studies were carried out with the fluorescent probes PBD-BODIPY and a phosphine-coumarin conjugate, which were used to quantify autooxidation reactions through fluorescence turn-on upon radical oxidation. It was found that at high temperatures (≥100 °C) cumyl tetrasulfides were able to slow oxidation. However, at 37 °C, these polysulfides were unable to contribute to antioxidant activity unless they were first oxidized [22]. This study also found that tetrasulfide bonds were much more scissile than trisulfides due to the higher stability of the resulting perthiyl radicals whereas trisulfides and disulfides would each result in less stable thiyl radicals. The center S—S bond of tetrasulfides is considerably weaker (36.3 kcal/mol) than the S—S bond in trisulfides (53.4 kcal/mol). Furthermore, it was found that polysulfides are more efficient antioxidants than H_2S alone, but their ability to prevent lipid peroxidation increases when combined [10]. This antioxidant activity is likely attributed to persulfide formation rather than polysulfides themselves as persulfides were found to be the most efficient inhibitors of lipid peroxidation. Similarly, Kaneko et al. found that tetrasulfides are modestly capable of slowing lipid peroxidation, whereas disulfides and trisulfides are less able to do so, which is again suggested to be due to the stability of the perthiyl radical species [23]. Altogether, these results suggest that polysulfides can act as *pseudo*-catalytic reservoirs that can be drawn upon through intermolecular interactions to form persulfide and perthiyl radicals. Scheme 4 summarizes the possible reactions of polysulfides with peroxyl radicals.

Polysulfides can act as mediators for cellular signaling and may be anti-inflammatory agents. Kloesch et al. studied the effects of polysulfides on interleukin (IL) IL-1β induced inducible nitric oxide synthase (iNOS) [24]. A persulfide donor (P*) derived from penicillamine was used in this study and would decompose to form polysulfides. It was found that decomposed P* could cause significant decrease of iNOS expression and nitrite formation (up to 85% and 75%, respectively) in ATDC5 cells and RAW 264.7 macrophages, and these effects were independent of H_2S. The authors suggested that both persulfides and polysulfides were able to influence enzymatic NO production, although the exact form of polysulfide form of P* remained unknown. In a similar study, Nakazawa et al. studied how GSSSG impacted inflammation responses in retinal pigment epithelial (RPE) cells. They found that 200 μM GSSSG could inhibit LPS-induced expression of IL-6, IL-1β, and C—C motif chemokine ligand 2 (CCL2) in ARPE-19/primary RPE cells. Extracellular signal-regulated kinase 1/2 (ERK1/2) activity was also enhanced in the presence of GSSSG. It was thus suggested that polysulfides attenuated LPS-induced inflammatory gene expression via ERK signaling hyperactivation and this was independent of the NRF2/HMOX1 pathway [25]. Polysulfides have also been recently tested as potential treatments for cardio and neural-related diseases. For example, the administration of 100 μM CysSSSCys to cardiac tissue could reduce lipid peroxidation and prevent myocardial ischemia-reperfusion injury, and the intranasal administration of GSSSG (50 mg/kg) to mice significantly reduced the risk of paraplegia induced spinal cord ischemia [26,27]. The oral co-administration of GSSSG (50 mg/kg/day) with Paclitaxel (4 mg/kg/day) reduced Paclitaxel-Induced Peripheral Neuropathy (PIPN) in mice [28]. As PIPN is a dose-limiting side effect and is common in patients taking Paclitaxel, there is much need to alleviate some of these side effects.

Conclusion

The chemistry of polysulfides within biological contexts is a complex and dynamic area of study. In this review, we covered the recent discoveries related to biosynthetic pathways of polysulfides, the stability and detection of polysulfides within biologically relevant conditions, and how the chemical properties of polysulfides affect their biological functions. The precise mechanisms by which polysulfides exert their effects within biological systems can be difficult to interpret as

polysulfides are known to form a highly complicated equilibrium network with other sulfur-containing molecules including thiols, persulfides, disulfides, and H_2S. The disruption of any of these species would shift the equilibrium. Nevertheless, each of these sulfur species has distinct chemistries and properties. The substitution groups on the sulfur atoms would also affect their chemistry. Polysulfides, as the relatively stable yet reactive member of this sulfur network, have the potential to modulate a variety of functions. This calls for a better understanding of the structural-activity relationship of polysulfides, as well as their direct and indirect reactions with biomolecules such as enzymes. We look forward to seeing more interesting studies of polysulfides coming out in the next few years.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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