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Review

Sulfane sulfur – new findings on an old topic*

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This work is dedicated to Prof. Lidia Włodek, who introduced us into the fascinating sulfane sulfur world

Sulfane sulfur is a divalent sulfur atom bonded to another sulfur which is very reactive and labile. Compounds containing this reactive sulfur include persulfides, polysulfides, thiosulfate, thiosulfinates, polythionates, and elemental sulfur. Sulfane sulfur appears in a number of biologically important compounds, including thiocysteine, thiocystine and thiotaurine, products of the cysteine metabolism, as well as glutathione persulfide. Sulfane sulfur compounds can modify cysteine residues in proteins *via* an S-sulfhydration reaction to produce protein persulfides. It has been also postulated that cysteine persulfides can be incorporated into proteins during translation. Recently, the sulfane sulfur compounds, especially the persulfides and polysulfides, have attracted increasing interest due to their regulatory and antioxidant properties. Compounds containing sulfane sulfur are also regarded as a form of H_2S storage, which can easily release this gasotransmitter in response to biological signals. Both reactive sulfur species (H_2S and sulfane sulfur) always coexist in biological systems. This review is focused on new findings in the field of sulfane sulfur's biological role, and disruption of its level in some patho/physiological conditions. A few sulfane sulfur donors with potential applications are presented. In recent years, in parallel to increasing interest in biological importance of sulfane sulfur, new analytical methods have been developed for sensitive and reliable determination of its level in the cells and tissues.

Key words: Sulfane sulfur, hydrogen sulfide, persulfides, polysulfides, S-sulfhydration

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Abbreviations: ASA, aspirin; ACEI, angiotensin-converting enzyme inhibitor; ALDH, aldehyde dehydrogenase CAPD, continuous ambulatory peritoneal dialysis; CAT, cysteine aminotransferase; CARS, cysteinyl tRNA synthetase; CKD, chronic kidney disease; CMS, chronic mild stress; CSE, cystathionine γ-lyase; CVD, cardiovascular disease; DTT, dithiothreitol; DADS, diallyl disulfide; DATS, diallyl trisulfide; DAS, diallyl sulfide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione; HD, hemodialysis; GPCG, Gomori-positive cytoplasmic granulations; HBITC, 4-hydroxybenzyl isothiocyanate; IMI, imipramine; MST, 3-mercaptopyruvate sulfurtransferase; LA, lipoic acid; NAC, N-acetylcysteine; PTP1B, protein tyrosine phosphatase; PTEN, lipid phosphatase; RSS, reactive sulfur species; ROS, reactive oxygen species; RS_2, resonance synchronous spectroscopy; SP, sulfane sulfur probe; SQR, quinine sulfide oxidoreductase; STST, rhodanese; TNFα, tumor necrosis factor

SULFANE SULFUR – WHAT IS IT, HOW IS IT FORMED AND WHAT WAS KNOWN ABOUT ITS BIOLOGICAL ROLE IN THE PAST

Presence of compounds containing a reactive form of sulfur in the cells was discovered in the late 1950s (Sorbo 1957; Cavallini *et al.,* 1959), while the term sulfane sulfur appeared in the scientific literature in the 1970s (Abdolrasulnia & Wood, 1979). A greater interest in this topic developed in the 1980–1990s (Wood 1987; Toohey 1989; Westley & Westley, 1991). Sulfane sulfur means a reactive, labile sulfur atom covalently bonded to another sulfur atom. This form of sulfur, with 6 valence electrons, occurs in the oxidation state of 0 (often denoted as S^0) or –1, and easily leaves the compound being transferred to various acceptors, of which the cyanide anion (CN^-) is the best known, hence it is also called "cyanolysable sulfur". Other nucleophilic compounds, such as sulfates (IV) (SO_3^{2-}), thiolate anions (RS^-), and triphenylphosphine (PhP:) have similar acceptor properties for sulfane sulfur as CN^-. Compounds containing sulfane sulfur found in biological systems include: elemental sulfur (S_8), persulfides (RSS^-), thiosulfate (SSO_3^{2-}), and thiosulfonates ($RS_2O_2^-$) (Table 1). Polysulfides (RS_nR), containing 3 or more sulfur atoms ($n \geq 3$), also belong to this pool. Presence of these reactive sulfur forms in animal organisms was confirmed by using the sulfur isotope [^{35}S] (Schneider & Westley, 1969). Sulfane sulfur does not exist in a free form, as it is always bound to another sulfur atom.

Besides the term "sulfane sulfur" referring to the whole pool of compounds containing this reactive form of sulfur, the concepts of acid-labile and bound sulfur can be also found in the literature. "Acid-labile sulfur" was introduced by Ogasawara and others (Ogasawara *et al.,* 1994; Ogasawara *et al.,* 1995) to describe sulfur that is released, in the form of H_2S, from iron-sulfur proteins following addition of hydrochloric acid. This type of sulfur is mostly located in the mitochondrial fraction. In turn, "bound sulfur" refers to the form of sulfur which can be released as H_2S by reduction with dithiothreitol (DTT) (Ogasawara *et al.,* 1993; Ogasawara *et al.,* 1994). The literature suggests that the bound sulfane sulfur mainly alludes to persulfides (RSSH) and polysulfides (RS_nR) (Koike & Ogasawara, 2016).

Sulfane sulfur is transported in a form that is bound with proteins, such as the persulfides or trisulfides. The cystathionine γ-lyase (E.C. 4.4.1.1; CSE) and 3-mercaptopyruvate sulfurtransferase (E.C. 2.8.1.2; MST) are responsible for endogenous synthesis of compounds containing sulfane sulfur, and also play an important role

Table 1. Sulfur compounds with their oxidation states.

Sulfur compound	Formula	Biologically-relevant example	Oxidation state of sulfur atom
Thiols	RSH	Cysteine (CysSH), Glutathione reduced (GSH)	–2
Disulfides	RSSR	Cystine (CysSSCys), Glutatione oxidized (GSSG)	–1, –1
Persulfides	RSSH	Thiocysteine, (CysSSH), Glutathione persulfide (GSSH), ProteinSSH	–1, –1
Hydrogen sulfide	H_2S	H_2S	–2
Elemental sulfur	S_8	S_8	0
Polysulfides	RSS_nSR	Thiocystine (CysSSSCys) Diallyltrisulfide (DATS)	–1, 0, –1
Sulfenic acid	RSOH	Cysteinesulfenic acid	0
Sulfinic acid	RS(O)OH	Cysteinesulfinic acid, Hypotaurine	+2
Sulfinic acid	$RS(O)_2OH$	Cysteine sulfonic acid, Taurine	+4
Sulfate (IV)	SO_3^{2-}		+4
Sulfate (VI)	SO_4^{2-}		+6
Thiosulfate	SSO_3^{2-}		+4, 0 +5, –1
Thiosulfinates	RS(O)SH	Allicin (diallyl thiosulfinate)	+1, –1
Thiosulfonates	$RS(O)_2SH$	Thiotaurine	+3, –1
S-ntrosothiols	RSNO	S-nitrosoglutathione (GSNO)	0

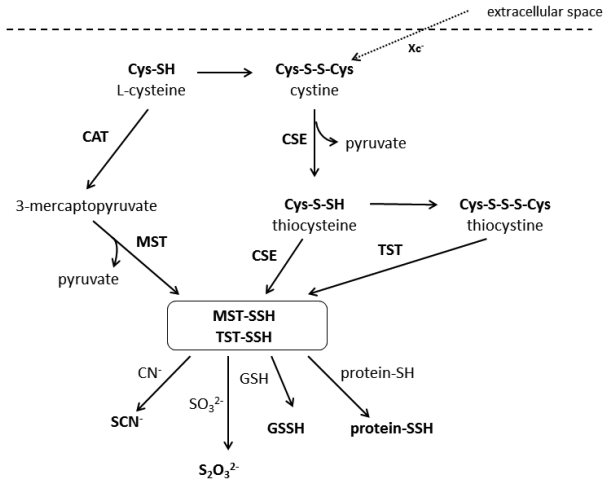
as sulfurtransferases, transferring the sulfane sulfur atom in the form of trisulfide (CSE) or persulfide (MST) (Table 2). Rhodanese (E.C. 2.8.1.1; TST) plays a special role in transport of the labile sulfur, namely, it transfers the sulfur atom from anionic donors to thiophilic acceptors (Koj & Frendo, 1962). The plasma albumin also transports sulfane sulfur in the form of persulfides (Toohey, 1989; Iciek & Wlodek, 2001).

It is worth to emphasize at this point that the sulfane sulfur transporting enzymes (MST, TST) are called sulfurtransferases and belong to the E.C. 2.8.1 subclass, as opposed to sulfotransferases (E.C. 2.8.2). The latter family of enzymes catalyzes sulfonation/sulfation (addition of sulfate) and is essential for the metabolism of endogenous substances and exogenous compounds (Coughtrie *et al.*, 1998).

Compounds containing sulfane sulfur are formed endogenously during anaerobic cysteine transformations in reactions catalyzed by the mentioned sulfurtransferases: CSE and MST. The anaerobic cysteine pathway can be initiated by cysteine aminotransferase (E.C. 2. 6.1.3; CAT) catalyzing the transamination of cysteine to 3-mercaptopyruvic acid, which is a sulfur donor transferred by MST to various nucleophilic acceptors, such as CN^- and SO_3^{2-}, as well as to sulfhydryl groups of proteins. Alternatively, cystine (resulting from oxidation of cysteine) may be cleaved by CSE to form sulfane sulfur-containing cysteine persulfide (thiocysteine) (CysSSH). Thiocysteine is easily converted into a more stable form of cysteine trisulfide, i.e. thiocystine (Cys-SSS-Cys), which also contains a sulfane sulfur atom and can be a substrate for rhodanese (Scheme 1). TST is mainly located in cellular mitochondria and catalyzes transfer of a sulfane sulfur atom in the form of persulfide to nucleophilic acceptors. The sulfane sulfur atom can also be transferred from thiocysteine or thiocystine to glutathione (GSH), present in cells at high concentrations, leading to the formation of glutathione persulfide (GSSH), which is another physiological compound containing sulfane sulfur. Cysteine and glutathione persulfides are widespread low molecular weight persulfides, which were found in both, prokaryotic and eukaryotic organisms (Ida *et al.*, 2014; Sawa *et al.*, 2018). It has been found that quite high concentrations of these species were endogenously produced by CSE and maintained in the plasma and inside the cells. Availability of CysSSCys as the major source of biological persulfides can be an important factor for formation

Table 2. Enzymes involved in the sulfane sulfur formation and transport.

Enzyme	Abbreviation	E.C. number
cystathionine γ-lyase (γ-cystationase)	CSE (CST, CTH)	E.C. 4.4.1.1
3-mercaptopyruvate sulfurtransferase	MST (MpST)	E.C. 2.8.1.2
rhodanese	TST (Rhd)	E.C. 2.8.1.2
cysteine aminotransferase	CAT	E.C. 2. 6.1.3



Scheme 1. Anaerobic cysteine transformations leading to formation of the sulfane sulfur compounds.
CAT, cysteine transaminase; CSE, cystathionine γ-lyase; MST, 3-mercaptopyruvate sulfurtransferase; TST, rhodanese

of sulfane sulfur-containing compounds. It is known that CysSSCys is a dominant form of cysteine in extracellular fluids and it is taken up by the cells by a Na^+-independent transporter Xc^- in exchange for glutamate (Ono *et al.,* 2014).

Compounds with sulfane sulfur also include disulfides where the C-S bond is adjacent to an unsaturated bond, i.e. C=C or C=O, e.g. mercaptopyruvate disulfides (R-S-S-CH_2-CO-COOH), 2-mercaptoacetaldehyde (R-S-S-CH_2-CHO) or allyl disulfides (R-S-S-CH_2-CH=CH_2). Presence of double bonds in the vicinity of the C-S bond allows the above compounds to tautomerize to a thiosulfoxide form containing a sulfane sulfur atom (Toohey, 1989; Iciek & Włodek, 2001).

Due to the aforementioned susceptibility of sulfane sulfur to the nucleophilic attack of cyanide ions, the role of sulfane sulfur was initially mainly associated with the process of cyanide detoxification with formation of safe rhodanates (SCN^-) (Baskin *et al.*, 1999). To date, thiosulfate has been used as an antidote for cyanide poisoning (Parker-Cote *et al.*, 2018). As demonstrated in the 1990s, sulfane sulfur-containing persulfides (RSSH) have stronger antioxidant properties than analogous thiols (RSH), which allows sulfane sulfur to be considered as an important component of the antioxidant defense (Everett *et al.*, 1994). Toohey (Toohey, 1989) pointed to participation of sulfane sulfur compounds in sulfuration, which is a post-transcriptional modification of tRNA, in the synthesis of iron-sulfur centers and some vitamins. The absence or deficiency of sulfane sulfur compounds found in many cancer cells suggested a role of this reactive sulfur in the processes of proliferation and apoptosis. This was also confirmed by numerous studies documenting the anti-cancer effects of sulfane sulfur-containing compounds from garlic (Iciek *et al.*, 2001, Seki *et al.*, 2008). Disorders in sulfur metabolism have been also found in patients with the acquired immune deficiency syndrome (AIDS). A sulfane sulfur generating system has been shown to inhibit HIV replication in the lymphocyte and macrophage cultures (Toohey, 2009).

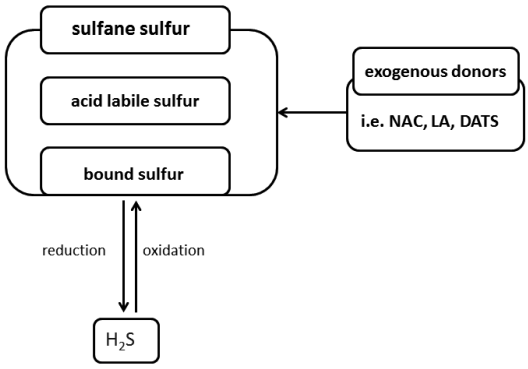
CURRENT TRENDS IN RESEARCH ON THE SULFANE SULFUR POTENTIAL

In the next two decades following the interesting works of Wood (Wood, 1987), Toohey (Toohey, 1989) and Westley & Westley (Westley & Westley, 1991), the sulfane sulfur topic was marginalized, and then at the beginning of the new millennium there was an abrupt return to the seemingly forgotten subject. In recent years, sulfane sulfur-containing compounds, especially persulfides and polysulfides, have been experiencing a renaissance. This was again sparked by Toohey (Toohey, 2011), who suggested that the sulfane sulfur compounds could be largely responsible for the biological activity attributed to hydrogen sulfide (H_2S). Hence, after about 15 years of fascination with H_2S and its physiological role, the interest of researchers was extended to the sulfane sulfur compounds.

H_2S, regarded as the third gasotransmitter (besides the nitric oxide and carbon oxide), regulates many important biological processes, such as the cell cycle (Baskar & Bian, 2011; Yang *et al.*, 2006), neurological function (Abe & Kimura, 1996), angiogenesis (Katsouda *et al.*, 2016), and inflammatory processes (Zhang *et al.*, 2016). It was shown to influence the blood pressure (Yang *et al.*, 2008; Drapala *et al,.* 2017), and was found to be protective in the digestive tract (Linden, 2014), erectile dysfunction (Srilatha *et al.*, 2007), and to possess cardioprotective (Donnarumma *et al.*, 2017) and anticoagulant properties (Olas & Kontek, 2014). It also plays an important role in the kidney disease (Feliers *et al.,* 2016). In addition, H_2S has been demonstrated to regulate the insulin sensitivity and adipose tissue lipolysis (Beltowski *et al.,* 2018). H_2S is synthesized by the same enzymes that are involved in the formation of sulfane sulfur. H_2S concentration initially determined in the tissues at the level of μM, turned out to be in fact significantly lower, i.e. in the order of nM (Furne *et al.*, 2008). Sulfane sulfur is easily released in the form of H_2S by the action of reducing agents or under the influence of acids (bound and acid-labile sulfur). Therefore, the determined H_2S level is often incorrect, because during the assessment procedure H_2S is released from the sulfane sulfur compounds. As it is known, H_2S is a toxic compound, hence its physiological concentrations are kept low. In turn, sulfane sulfur has low toxicity and a high biological potential. In addition, Na_2S preparations (used in many studies as exogenous sources of H_2S) are contaminated with elemental sulfur, so the effects attributed to H_2S are also caused by sulfane sulfur.

Toohey's remarks emphasizing the importance of sulfane sulfur in the effects attributed to H_2S, had triggered almost an avalanche of research on the biological role of this reactive form of sulfur (Toohey & Cooper, 2014; Kimura, 2015). Redox active sulfur-containing molecules, by analogy to reactive oxygen species (ROS), are called reactive sulfur species (RSS). Compounds with sulfane sulfur and H_2S belong to RSS, which are formed physiologically under non-oxidative conditions. We know today that there is a close relationship between H_2S and sulfane sulfur, and that these two RSS always coexist (Scheme 2). The latest studies suggest that it is rather the sulfane sulfur, and not the H_2S itself, that acts as a signaling molecule and is responsible for biological actions of RSS.

Compounds with sulfane sulfur have the ability to modify the thiol groups (-SH) to the corresponding persulfides (-SSH) by transferring the labile sulfane sulfur atom. This reaction, called S-sulfhydration in the literature (a better name would be persulfidation or sulfura-

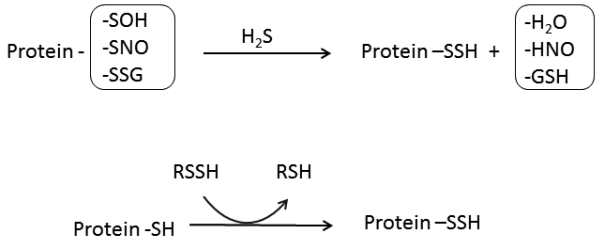


Scheme 2. A relationship between sulfane sulfur compounds and H_2S.
NAC, N-acetylcysteine; LA, lipoic acid; DATS, diallyl trisulfide

tion), plays an important role in the thiol redox signaling and is a reversible, covalent modification of thiols, including the cysteine residues of proteins called "redox switches". Persulfides (RSSH) are formed as a result of oxidation of thiols (RSH) (sulfur changes its oxidation state from –2 to –1), and therefore persulfidation cannot be the result of direct reaction of thiol residues with H_2S. In this reaction, the compounds containing sulfane sulfur, which is at a –1 or 0 oxidation state, play a role of the oxidizing agents (Iciek *et al.*, 2016b). Persulfides can be also formed by reaction with the HS^- anion resulting from dissociation of H_2S, but only if the thiol group is reversibly oxidized to the form of sulfenic acid (-SOH), S-nitrosothiol (-SNO) or disulfide (-SSR) (Scheme 3). The former mechanism seems to be preferred because the reducing environment dominates in the cells, where the -SH groups appear in the reduced form.

Not only enzymes involved in the metabolism and transport of sulfane sulfur (MST, TST, quinone sulfide oxidoreductase (E.C. 1.3.5.1; SQR)) are subject to persulfidation, but also many other proteins. Formation of persulfides was demonstrated, among others, for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), protein tyrosine phosphatase (PTP1B), lipid phosphatase (PTEN), albumin, tubulin, actin, pyruvate carboxylase, ATP synthase, lactic dehydrogenase and aldehyde dehydrogenase (ALDH) (Mustafa *et al.*, 2009; Greiner *et al.*, 2013; Krishnan *et al.*, 2011; Ju *et al.*, 2015; Módis *et al.*, 2016; Untereiner *et al.*, 2017; Iciek *et al.*, 2018). In these proteins, cysteine residues function as specific sensors capable of binding the sulfane sulfur and forming persulfides, which directly affects their biological activity.

ATP-sensitive potassium channels (K_{ATP}) are also activated *via* persulfidation (Peers *et al.*, 2012; Mustafa *et al.*, 2011). Activation of K_{ATP} channels in the vascular smooth muscle cells involves formation of a persulfide form of the protein, which reduces its ATP binding affinity and



Scheme 3. Possible mechanisms of persulfide formation.

results in vasorelaxation (Mustafa *et al.*, 2011). Another example of a biologically important molecule that is regulated in this way is the NFκB transcription factor. Under basic conditions it is inactive because it is bound to the IκB inhibitor (IκBNFκB). A multifunctional proinflammatory cytokine, tumor necrosis factor alpha (TNFα), activates the IκB kinase which leads to the release of IκB and the translocation of NFκB to the nucleus. Sen and co-workers had shown that TNFα stimulated transcription of CSE and has led to S-sulfhydration of NFκB on the p65 subunit (Sen *et al.*, 2012). Generally, the IκB kinase is an important target for the redox regulation of NFκB. In a previous study, it was found that NO exerted an inhibitory effect on NFκB through S-nitrosylation of the IκB kinase (Reynaert *et al.*, 2004). It is believed that this inhibitory effect of NO on NFκB plays an important role in a negative feedback regulation of NO production.

Most of the covalent protein -SH group modifications (e.g. S-nitrosylation, S-glutathionylation, oxidation to sulfenic acids) cause a decrease in the protein catalytic activity. Formation of protein persulfides in many cases leads to an increase in their catalytic activity, although cases of inhibition in protein activity as a result of modification by RSS have been also described (e.g. PTP1B, PTEN, ALDH).

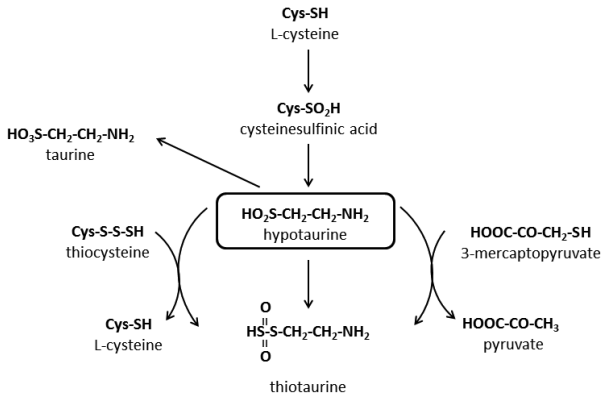
S-sulfhydration reactions are a source of persulfides also during a mitochondrial H_2S oxidation. This process allows for the removal of toxic H_2S in the form of thiosulfate or sulfate (VI), but is also a source of persulfide generation (i.e. GSSH and SQR-SSH), which can then modify target proteins. Some authors had compared H_2S oxidation to the mitochondrial electron transport chain, stressing that both mitochondrial processes generate reactive species, either RSS or ROS (Mishanina *et al.*, 2015).

As previously mentioned, formation of persulfides is regarded as a post-translational modification of existing cysteine residues in the proteins. However, research by Akaike and others (Akaike *et al.*, 2017) provided a strong evidence that cysteine persulfides can be incorporated into proteins at the translation stage. These authors have proven that cysteinyl tRNA synthetases (CARS) are able to convert CysSH to CysSSH *via* a pyridoxal phosphate -dependent process using the second Cys as a sulfur donor. The CysSSH synthesized by CARS can then form a tRNA-bound CysSSH adduct resulting in its incorporation into proteins. The authors even suggested to consider CysSSH as a 22nd amino acid, very much like selenocysteine. This is a new aspect of protein persulfidation, and the possibility of CySSH incorporation during translation additionally highlights the importance of this process in redox regulation.

THIOTAURINE AS A SULFANE SULFUR COMPOUND

Thiotaurine (2-aminoethane thiosulfonate) is a cysteine-derived metabolite characterized by the presence of a thiosulfonate group. It was first discovered by Sorbo (Sorbo, 1958) and then its biological occurrence was described by Cavallini and others (Cavallini *et al.*, 1959).

Aerobic cysteine catabolism proceeds through the cysteinesulfinic acid and hypotaurine to taurine (Frendo *et al.*, 1959). Thiotaurine is generated by a sulfurtransferase through sulfur transfer from the 3-mercaptopyruvate to hypotaurine (Scheme 4). Thiotaurine, as a thiosulfonate, contains one sulfur atom bound to another sulfur which is the highly reactive sulfane sulfur. As mentioned in the first paragraph, generally,



Scheme 4. Formation of thiotaurine.

compounds containing sulfane sulfur are formed endogenously during anaerobic cysteine transformation. As thiotaurine is generated by the reaction of hypotaurine with 3-mercaptopyruvate, it can be regarded as a link between the aerobic and anaerobic cysteine metabolism (Baseggio *et al.*, 2019). Another possibility of thiotaurine generation relies on the transsulfuration between thiocysteine (CysSSH) formed *via* the anaerobic cysteine route and hypotaurine (Scheme 4). Thiotaurine possesses antioxidant and regulatory properties. Interestingly, a previous study had shown that the protective, antioxidant action of thiotaurine was stronger than that of taurine (Acharya & Lau-Cam, 2013). It was probably connected with presence of a thiosulfonate group in thiotaurine. Thiotaurine can be considered as an RSS and a kind of H_2S store (thiotaurine has an ability to release H_2S under the influence of thiols (RSH)). This reaction's mechanism involves formation of persulfide (RSSH) and hypotaurine (Baseggio *et al.*, 2019). Recently, a study presenting the anti-inflammatory properties of thiotaurine has shown that the effects of thiotaurine on human neutrophil responses were significantly higher than those of taurine or related compounds (Capuozzo *et al.*, 2015). It has been suggested that this thiosulfoxide modulates human leukocyte activation by persulfidation of some target proteins (Capuozzo *et al.*, 2017).

SULFANE SULFUR DONORS

As the metabolism of sulfane sulfur is disturbed in some pathophysiological conditions, precursors which can affect its level are being searched for.

As mentioned in the first paragraph, L-cysteine is the basic substrate for the sulfane sulfur biosynthesis, however, because of its toxicity, it cannot be directly introduced into cells. L-cysteine, in its reduced form, is especially toxic to the central nervous system and produces neuronal damage *via* different neurotoxic mechanisms (Janaky *et al.*, 2000). Toxicity of L-cysteine can be connected with overexpression of the NMDA receptor, increased glutamate level and interaction with carbonyl compounds. Generation of cysteine-S-conjugates in reactions with catecholamines seems to be another cause of L-cysteine-related neurotoxicity (Wlodek, 2002).

Methionine, as a precursor for L-cysteine, can be directly administered to the cells, but conversion of methionine to L-cysteine requires energy in the form of ATP.

Thiazolidine derivatives

In the context of safe L-cysteine precursors, the so-called thiazolidine derivatives are an interesting group of compounds. They are formed in a non-enzymatic process of condensation of cysteine with carbonyl compounds. These compounds release cysteine when introduced into cells (Wlodek *et al.*, 1993a). Of all the thiazolidine derivatives studied, 2-methylthiazolidine-2,4-dicarboxylic acid (a product of cysteine and pyruvic acid condensation), deserves special attention. This compound not only has the hepatoprotective and immunomodulatory properties, but is the only one of the whole series of known products of cysteine condensation with carbonyl compounds (e.g. with formaldehyde, acetaldehyde or ribose), that increases the activity of enzymes associated with anaerobic metabolism of cysteine *in vivo* (Wlodek *et al.*, 1993b; Wlodek & Rommelspacher, 1997).

N-acetylcysteine

N-acetylcysteine (NAC) is a well-known drug and a popular antioxidant. Despite its wide use, the molecular mechanism of its action is not fully elucidated, as yet. It is generally accepted that NAC is a precursor of Cys and GSH, and in this way exerts an antioxidant action. However, some studies have shown that NAC protected against the oxidative stress but did not affect the GSH levels (Patriarca *et al.*, 2005). The effect of NAC on the level of sulfane sulfur was studied by Jurkowska and Wróbel (Jurkowska & Wróbel, 2008) in astrocytes and astrocytoma cultures. They found that inhibition of astrocytoma U373 proliferation was connected with elevation of the sulfane sulfur level in these cells. However, in murine astrocytes, NAC lowered the level of sulfane sulfur and decreased cell proliferation. This suggested that normal and cancer cells respond to NAC differently. A similar effect, showing elevation of the sulfane sulfur level, was observed by the same authors in a study conducted on a human neuroblastoma SH-SY5Y cell line (Jurkowska & Wróbel, 2018).

Recently, Ezerina and others (Ezerina *et al.*, 2018) demonstrated in their excellent paper that NAC treatment had increased the sulfane sulfur production *via* intermediate H_2S generation in the human lung H838 cell line. H_2S is then oxidized to sulfane sulfur species, mostly in mitochondria. The authors had provided evidence suggesting that sulfane sulfur was a key mediator of antioxidant and cytoprotective effects of NAC.

Another study, carried out on the SW480 colon cancer cells, had shown again that NAC had increased expression and activity of MST, as well as SQR in these cells. Those authors suggested that NAC could also act as a direct substrate for MST, being likely persulfidated to NACysSSH (Zuhra *et al.*, 2019).

Summing up, it seems that the mechanism of an antioxidant and protective action of NAC is connected with the sulfane sulfur formation. NAC affects the RSS metabolism through promotion of expression of enzymes involved in RSS biosynthesis and mitochondrial H_2S oxidation.

Garlic-derived allyl sulfides

Garlic (*Allium sativum*) is rich in various sulfur-containing compounds, of which allicin is the most prominent. This unstable compound is quickly converted into various allyl sulfides, including the diallyl disulfide (DADS) and diallyl trisulfide (DATS). DATS (as a trisulfide) contains a sulfane sulfur atom, while DADS

(being an allyl disulfide) can tautomerize to the thiosulfoxide form and in this way it can be a source of the sulfane sulfur.

It was demonstrated in previous *in vitro* and *in vivo* studies that the garlic-derived DADS and DATS can be precursors of the reactive sulfane sulfur. Our study, conducted on mice, suggested that DADS can constitute a source of sulfane sulfur for the liver, thereby activating the anaerobic sulfur metabolism. DADS administration also leads to an increase in the number of Gomori-positive cytoplasmic granulations (GPCG) in the brain of mice (Iciek *et al.*, 2005). This suggests that sulfane sulfur created in the liver is transported in the form of albumin hydropersulfides in the plasma and can be stored in the glial cells as GPCG.

In another *in vivo* study, it was shown that DADS corrected the hepatic sulfane sulfur level and activities of sulfurtransferases involved in its biosynthesis that had been lowered in the liver of Ehrlich ascites tumor-bearing mice due to the tumor development. Interestingly, DADS did not alter these parameters in the cancer cells themselves (Iciek *et al.*, 2007). This indicates that this compound is capable of acting selectively.

Similarly, studies comparing biological activity of various garlic-derived allyl sulfides (allyl sulfide DAS, disulfide DADS and trisulfide DATS) performed on healthy mice had revealed that DADS and DATS elevated the total sulfane sulfur pool and activity of the sulfane sulfur biosynthetic enzymes in the mouse liver (Iciek *et al.*, 2012a). The same results were obtained for the mouse kidney (Iciek *et al.*, 2016a). Thus, it can be concluded that DADS and DATS displayed a beneficial action in the liver and in the kidney, which can be used for protection of normal tissues during chemotherapy or for reduction of damage to these organs.

The effect of DADS and DATS on the level of sulfane sulfur has been also studied in some cancer cell lines. Our previous study investigated the effect of DAS, DADS and DATS on sulfane sulfur and activity of sulfurtransferases in relation to cell proliferation, viability, caspase 3 activity and H_2O_2 production in the HepG2 cells. Among the compounds under study, DATS showed the highest biological activity inhibiting cell proliferation, inducing caspase 3 activity and increasing H_2O_2 production. Interestingly, this compound lowered the sulfane sulfur level in the HepG2 cells and did not affect the activity of sulfurtransferases (Iciek *et al.*, 2012b). It appears that in that case DATS is reduced in cancer cells to persulfide which then reacts with molecular oxygen in the presence of transition metal ions, leading to H_2O_2 generation and thereby influencing transmission of signals regulating cell proliferation and apoptosis (Iciek *et al.*, 2009).

On the other hand, Jurkowska *et al.* (2017) have shown an increased level of sulfane sulfur and an increased activity of MST and TST in the human glioblastoma U87MG cells under the influence of DATS. In neuroblastoma SH-SY5Y, a second cell line studied by these authors, this effect was not observed (Jurkowska *et al.*, 2017).

Summing up, garlic-derived DADS, and especially DATS, can be good precursors of sulfane sulfur for normal hepatic and kidney cells. In the case of cancer cells, these compounds act more selectively and their effect depends on specificity of the cell line.

Isothiocyanates from the Brassicaceae family

The first report indicating that RSS, namely H_2S, can be released from isothiocyanates naturally occurring in the *Brassicaceae* family was published by Citi and others (Citi *et al.*, 2014). H_2S releasing capacity of these compounds can be a reliable explanation for the multiple biological effects of brassicas.

One study investigated the effect of 4-hydroxybenzyl isothiocyanate (HBITC; a natural compound from white mustard seeds) on cell proliferation, as well as on H_2S and thiosulfate level in the human neuroblastoma (SH-SY5Y) and glioblastoma (U87MG) cells (Jurkowska *et al.*, 2018). The obtained results demonstrated an increase in the H_2S and thiosulfate levels. Unfortunately, the whole pool of sulfane sulfur was not assayed in that study, however, it can be speculated that it would be increased under the influence of HBITC, similarly to thiosulfate which is one of the compounds containing sulfane sulfur. On the other hand, thiosulfate is also a product of mitochondrial oxidation of H_2S.

Lipoic acid

Literature data indicate that the mechanism of biological activity of lipoic acid (1,2-dithiolane-3-pentanoic acid, LA) can be also associated with the sulfane sulfur metabolism. The study of Bilska and others (Bilska *et al.*, 2008) revealed a significant increase in the sulfane sulfur level in the heart, liver and kidney of rats that had been previously intraperitoneally treated with LA. It has been also shown that both, H_2S and sulfane sulfur, are formed from LA non-enzymatically in the presence of environmental light. It has been suggested that H_2S is the first product of non-enzymatic light-dependent decomposition of LA that is then probably oxidized to the sulfane sulfur-containing compounds (Bilska-Wilkosz *et al.*, 2017).

Another study also revealed a statistically significant increase in the sulfane sulfur level in the kidney and heart of rats which were sacrificed 90 min after the second dose of LA (two doses of LA administered intraperitoneally at 50 mg of LA per kg of body weight each) (Iciek *et al.*, 2011, Sokołowska *et al.*, 2014).

An increase in the sulfane sulfur levels and MST activity was also found in erythrocytes of patients undergoing continuous ambulatory peritoneal dialysis due to end-stage renal disease, who received oral LA supplementation at a dose of 600 mg daily for 30 days (Iciek *et al.*, 2014).

Moreover, some studies demonstrating the anti-inflammatory properties of LA link this activity with RSS formation. Dudek and others (Dudek *et al.*, 2013) demonstrated that intraperitoneal pretreatment with LA reduced paw edema formation in mice. In addition, those authors showed that LA did not produce an antiedematous effect in the presence of glibenclamide, a K_{ATP} channel blocker, which indirectly provides evidence that endogenous H_2S acts as an anti-inflammatory agent. This suggests that LA affects the sulfane-sulfur metabolism, leading to H_2S release, and this is one of putative mechanisms of biological action of LA.

Another study on a mouse model of zymosan-induced peritonitis, indicated that LA (administered at a dose 50 mg per kg of body weight) increased the sulfane sulfur level in the peritoneal exudates by 11.5 times when compared to the control group (Zygmunt *et al.*, 2013).

All of the above-cited papers prove that NAC, LA, some thiazolidine derivatives, HBITC, and garlic-derived allyl polysulfides affect the level of sulfane sulfur and can be regarded as its potential donors.

CHANGES IN THE SULFANE SULFUR CONTENT DURING AGING

In our previous study, plasma levels of sulfane sulfur, as well as the level of total, free and protein-bound thiols, were studied in different age groups of animals (Iciek *et al*., 2004). The determination was conducted in plasma of 3 months old (young), 19 months old (middle) and 31 months old (old) rats. This study revealed that the plasma sulfane sulfur declined with age (to 84% in the middle-age group and 78% in the old group, in comparison with the young group). It was also observed that in general, the level of thiols decreased with age. It means that aging is accompanied by a decrease in the most important thiol antioxidants, i.e. GSH and cysteine. A decrease in plasma sulfane sulfur means that inhibition of the anaerobic sulfur metabolism progresses with age. The consequence of this is the weakening of the antioxidant defense and disturbances in the regulatory mechanisms implicating sulfane sulfur.

An interesting report presenting the sulfane sulfur contents at different growth phases in various organisms has been published recently by Ran and others (Ran *et al*., 2019). The authors have developed a sensitive method of reliable quantification of sulfane sulfur in bacteria, yeast, mammalian cells and zebrafish. They have found that all studied species contain sulfane sulfur but the contents are different among the tested organisms. Moreover, their results revealed that the total sulfane sulfur content in all of the studied organisms increased in the early developmental stage, then was maintained at a nearly constant level and finally declined in the late stage of growth. This was documented for microorganisms (*Escherichia coli, Staphylococus sciuri* and *Saccharomycies cerevisiae*), as well as for the mammalian cells (normal colonic musosal cell line (FHC) and colorectal cancer cell line (HCT116)), and in zebrafish (Ran *et al*., 2019).

Both of the above-cited studies were performed on miscellaneous organisms and using different methods, but they led to the same conclusion that the sulfane sulfur contents vary with growth phase and during aging. This means that the regulatory and antioxidant properties of sulfane sulfur also possibly vary throughout the life of the organism.

SULFANE SULFUR IN THE CHRONIC KIDNEY DISEASE

Disorders of sulfane sulfur and H_2S homeostasis have been observed in kidney diseases. Previous studies revealed a decreased level of sulfane sulfur in plasma of patients with a chronic kidney disease (CKD) undergoing hemodialysis (HD) (Wlodek *et al*., 2001), and a continuous ambulatory peritoneal dialysis (CAPD) (Wlodek *et al*., 2010). On the other hand, studies performed with erythrocytes of CKD patients showed a lower level of sulfane sulfur in the CKD predialysis group, while in the CAPD group the level of sulfane sulfur remained at the level observed in healthy controls (Wlodek *et al*., 2010). This suggests that CAPD (as a replacement therapy) helps to preserve the anaerobic sulfur metabolism in erythrocytes.

In line with the above-presented studies, other groups of researchers have reported a decrease in the H_2S level in plasma of HD patients, which also reflects a decreased sulfhemoglobin level (Perna *et al*., 2010). After a single session of hemodialysis, the plasma H_2S level had increased in these patients (Perna *et al*., 2011). In another study, a lower H_2S level in plasma of CKD patients was also documented, which was positively correlated with an estimated glomerular filtration rate (eGFR) (Kuang *et al*., 2018).

The CKD patients suffer from a decreased glomerular filtration and defective metabolism, which leads to accumulation of different uremic toxins. One of these toxins, namely cyanate, is formed during urea degradation. Sokołowska and co-workers (Sokołowska *et al*., 2011, Sokołowska *et al*., 2013, Sokołowska *et al*., 2014) conducted a comprehensive research evaluating the effect of cyanate alone or in combination with LA, on the anaerobic cysteine metabolism level in different rat tissues. These studies had shown that cyanate decreased the level of sulfane sulfur and reduced the activity of sulfurtransferases, but increased the ROS level in the rat liver, heart and cerebral cortex. LA, administered to rats together with cyanate, restored the control level of sulfane sulfur and decreased the ROS level in theses tissues. This means that cyanate exhibits a toxic action in the studied tissues, while LA acts as a protectant against the cyanate toxicity. The same authors performed a similar study in the rat kidney that revealed changes similar to those observed in other tissues, however, the kidney level of sulfane sulfur was not affected by cyanate. Interestingly, the kidney is the tissue with the highest level of sulfane sulfur and cysteine compared to other tissues. So far, this phenomenon has not been sufficiently explained.

SULFANE SULFUR IN ADDICTIONS

Asevedo and others (Asevedo *et al*., 2014) suggested that NAC might offer some therapeutic benefits in the treatment of addiction. Echevarria and others (Echevarria *et al*., 2017) published an extensive review of currently available literature dealing with NAC efficacy in the treatment of cocaine addiction, based on which it can be concluded that NAC may be suitable for reducing the relapse rate in the already abstinent subjects. Similar review about using NAC in the treatment of psychiatric disorders has been recently published by other researchers (Ooi *et al*., 2018). These authors support the use of NAC as an adjunctive treatment to reduce the total and negative symptoms of schizophrenia, as well as a medication helpful in reducing the cocaine and cannabis use.

The research of Kowalczyk-Pachel and others (Kowalczyk-Pachel *et al*., 2016) indicated that cocaine modulated metabolism of the sulfur-containing compounds in the liver and kidney of rats. The authors showed that acute cocaine administration significantly increased the whole pool of sulfane sulfur in the rat kidney, while repeated cocaine treatment enhanced the content of the whole pool of sulfane sulfur in both organs. These results suggest that at a relatively low dose, cocaine shifts the cysteine metabolism towards formation of the sulfane sulfur compounds which possess antioxidant and redox regulatory properties and are a source of H_2S which can support mitochondrial bioenergetics.

In another paper, the same authors studied the effect of acute and subchronic administration of cocaine, as well as cocaine self-administration using the yoked procedure on the level of sulfane sulfur in the rat plasma (Kowalczyk-Pachel *et al*., 2013). The results revealed that the level of sulfane sulfur markedly decreased after acute cocaine treatment, while chronic drug administration did not evoke changes in its level. During maintenance, the plasma sulfane sulfur content was decreased both, in the cocaine self-administering and yoked groups. This could be a result of the sulfane sulfur compounds' contribu-

tion to the antioxidant defense, since it is believed that cocaine evokes oxidative stress (Cisneros *et al.*, 2018).

SULFANE SULFUR IN THE CARDIOVASCULAR DISEASE IN RELATION TO ETHNICITY AND GENDER

Recently, Rajpal and others (Rajpal *et al.*, 2018) reported results of a clinical case-control study aimed to measure biochemical pools of sulfane sulfur (total, acid-labile and bound) in the plasma of subjects with cardiovascular diseases (CVD), in comparison to healthy controls. Interestingly, they analyzed the obtained results in relation to gender and ethnicity. The authors revealed a significant reduction in the plasma total, acid-labile and bound sulfur in CVD patients compared to controls among the Caucasian subjects, but this was not observed in African Americans. On the other hand, comparison of controls between African Americans and Caucasians revealed a significant reduction in plasma total sulfane sulfur in African Americans. Regarding the gender, this study suggested that women with CVD displayed a significant reduction in the total and acid-labile sulfane sulfur. These changes were mainly observed in the Caucasian race. In case of men, the CVD patients showed a significant reduction in plasma total and bound sulfur compared to healthy controls. Considering the race, this reduction was again observed only in the Caucasian males with CVD. Summing up, these observations suggest that ethnicity and gender are significant factors influencing the total, acid-labile or bound sulfane sulfur in diagnosis of CVD.

SEASONAL AND CIRCADIAN CHANGES IN THE SULFANE SULFUR LEVEL

Ikeda and coworkers (Ikeda *et al.*, 2019) studied distribution of sulfane sulfur in the form of polysulfides in human biological fluids, including the plasma, saliva, tears, nasal discharge and semen, and these authors looked for their association with the amylase and sperm activities. They did not observe a statistically significant association between the plasma polysulfides and age, gender or body mass index (BMI). The data related to age do not agree with a previously presented tendency towards a decrease in sulfane sulfur level with age, however, in that study the range of the investigated patients was rather narrow (22–43 years old). The circadian rhythm of plasma polysulfides has been also studied, however, the obtained results were inconclusive. Depending on the assay method, the highest level of plasma polysulfides was at 15.30 or at 21.30. The authors explain this discrepancy by different reactivity of reagents used in these methods. The most interesting results obtained in this study indicated that the level of seminal polysulfides was positively correlated with the plasma polysulfide and with the amount of alive sperm (Ikeda *et al.*, 2019). It can be speculated that polysulfides in the seminal fluids can play an important protective role against oxidative stress and in this way affect the sperm activity.

On the other hand, Wróbel and others (Wróbel *et al.*, 2000) examined L-cysteine desulfuration in tissues of *Rana temporaria*, in relation to the season (in October and January). For each of the investigated tissues, changes in the sulfane sulfur compounds and in the enzymatic activities of sulfurtransferases were dependent on the month in which the determination was performed and on the character of the tissue. This study indicated a high content of sulfane sulfur in the frog liver in the autumn (October). The authors suggested that this could be connected with preparation for protein synthesis carried out in the liver during hibernation.

EFFECT OF SOME DRUGS ON THE SULFANE SULFUR LEVEL

The effect of aspirin (ASA) on anaerobic cysteine metabolism was studied in mouse liver, brain and kidney. The studies indicated an ASA-induced decrease in the number of GPGC in the brain, but the sulfane sulfur level in this organ was not affected. Conversely, the sulfane sulfur content in the liver dropped. ASA did not change the CSE and TST activity in either organ (Bilska *et al.*, 2010). On the other hand, the sulfane sulfur increase was observed in the kidneys of mice receiving ASA (Bilska-Wilkosz *et al.*, 2013). These results revealed that ASA was able to influence the anaerobic cysteine metabolism, leading to formation of sulfane sulfur and affecting the numbers of GPGC. This is a new aspect of the well-known and widely used drug.

Some studies strongly suggested that oxidative stress played a significant role in a stress-induced depressive illness. In this aspect, it can be expected that a chronic antidepressant treatment will alleviate the effects of oxidative stress, in parallel with normalization of depression-like symptoms. Duda and others (Duda *et al.*, 2016) used an animal chronic mild stress (CMS) model to study the effect of one of the well-known antidepressants, imipramine (IMI), on GSH antioxidant status and sulfane sulfur level in the rat liver. The obtained results indicated that CMS induced a marked increase in the sulfane sulfur content *vs.* the control, while the chronic imipramine treatment had statistically significantly decreased its content in the IMI non-responding CMS animal group and statistically non-significantly in the IMI responding CMS group.

Recently, Donnarumma and others (Donnarumma *et al.*, 2016) examined the effects of zofenopril, a sulfhydrylated angiotensin-converting enzyme inhibitor (ACEI), on RSS and NO bioavailability and cardiac damage, in murine and swine models of myocardial ischemia/reperfusion (I/R) injury. The results demonstrated that zofenoprilat significantly augmented both, the plasma and myocardial H_2S and NO levels in mice, while CSE and MST activities were unaltered. The sulfane sulfur, which is considered as a storage reservoir of H_2S, was increased in a statistically significant manner in the plasma of pigs pretreated with zofenopril for 7 days when compared to the placebo group. These results suggest that zofenopril, which reduces mortality and morbidity in infarct patients to a greater extent than other ACEIs, is a unique ACEI drug and the mechanism of its action is connected with RSS.

As documented earlier in a study conducted on rats, physiological saline (0.9% NaCl) widely used in medicine, can also influence the level of RSS. Repeated intravenous administration of saline had decreased the sulfane sulfur and H_2S level, as well as the CSE activity, in the rat liver (Iciek *et al.*, 2017). This was associated with an increase in the blood pressure. It suggests that prolonged saline injections lead to disturbances in the RSS formation.

SULFANE SULFUR AND CANCER

Compared to normal cells, the tumor cells are characterized by low TST and MST activity and trace CSE activity (Toohey, 1989; Włodek *et al.*, 1993c; Iciek & Włodek, 2001), which results in disturbed synthesis and transport of compounds from the sulfane sulfur pool.

Toohey suggested that the uncontrolled proliferation of cancer cells might be a consequence of sulfane sulfur deficiency and excessive activity of the enzymes, which in normal cells would be inactivated by this reactive form of sulfur (Toohey, 1989). Therefore, numerous studies on different cell lines were performed to check whether the sulfane sulfur precursors can inhibit the uncontrolled proliferation of selected cancer cells.

The *in vitro* studies performed on cancer cell lines, mentioned earlier in the context of sulfane sulfur donors, showed that NAC inhibited proliferation of the astrocytoma U373 and human neuroblastoma SH-SY5Y cell lines (Jurkowska & Wróbel, 2008; Jurkowska & Wróbel, 2018). Garlic-derived DADS and DATS inhibited cell proliferation, induced caspase 3 activity and increased H_2O_2 production in the HepG2 cells (Iciek *et al*., 2001; Iciek *et al*., 2012b). Another study revealed inhibition of cell proliferation of both, the human glioblastoma U87MG and neuroblastoma SH-SY5Y cells, after administration of DATS (Jurkowska *et al*., 2017). Similar study performed by the same authors on these two cell lines (U87MG and SH-SY5Y) but using HBITC also demonstrated inhibition of proliferation of these cells (Jurkowska *et al*., 2018).

Although in all likelihood, the supply of sulfane sulfur has an inhibitory effect on proliferation of cancer cells, the mechanisms of action of sulfane sulfur compounds can vary. For example, it has been shown that the garlic-derived organosulfur compounds, including DADS and DATS, cause cell cycle arrest, stimulate the mitochondrial apoptotic pathway, increase histones' acetylation, inhibit carcinogen activation, influence gap-junctional intercellular communication, and participate in development of multidrug resistance (Iciek *et al*., 2009).

On the other hand, some aspects of the sulfane sulfur activity were investigated in *in vivo* studies. Ramalho *et al*. (Ramalho *et al*., 2013) showed the irreversible poisoning action of the acetone cyanohydrin in Ehrlich tumor cells. The use of acetone cyanohydrin was motivated by the fact that its action in the organism was similar to the molar equivalent of cyanide. It is known that the defense of living organisms against the toxic effects of cyanide involves its transformation with sulfane sulfur and TST into a less toxic thiocyanate, excreted with urine. Cibin *et al*. (Cibin *et al*., 2010) have suggested that one of the characteristics of cancer cells, namely residual activity of TST due to a sulfane sulfur deficiency, makes these cells susceptible to the toxic effects of cyanide.

Interestingly, the post-mortem studies of human grade II to IV glioma samples have shown that the level of sulfane sulfur in the highest grade gliomas was high in comparison to the other human brain regions, and it was correlated with a decreased activity of CSE, MST and TST. This can suggest sulfane sulfur accumulation and points to its importance for malignant cell proliferation and tumor growth (Wróbel *et al*., 2014). This surprising result suggests similarity to a sulfane sulfur overproduction in some virus-infected cells, which contain supraoptimal concentrations of this reactive sulfur (Toohey, 1989).

SULFANE SULFUR QUANTIFICATION IN BIOLOGICAL SAMPLES

Because of high reactivity of most sulfane sulfur-containing compounds, their detection in biological samples is still a challenge, and new and sensitive methods are needed.

For many years, a procedure described by Wood (Wood, 1987) has been the most popular method of sulfane sulfur determination. It is based on the reaction of sulfane sulfur with cyanide, yielding thiocyanate that reacts with Fe^{3+} ions to produce a red complex which is assayed spectrophotometrically at 460 nm. The second analytical method for sulfane sulfur detection that is worth mentioning is based on HPLC analysis with fluorometric detection in combination with flow gas dialysis for bound sulfur, developed by Ogasawara and others (Ogasawara *et al*., 1993, 1994). In this method, sulfur bound to proteins is released by DTT as sulfide, which can be converted in a reaction with p-phenylenediamine and Fe^{3+} ions into a fluorescent derivative thionine.

In recent years, due to the increased interest in the biological role of sulfane sulfur, several new methods of its determination have been developed, which are characterized by a much higher sensitivity compared to the cyanolysis method. It seems that fluorescent probes offer particular advantages as chemical tools to develop sensitive, selective and easy to use methods for the sulfane sulfur quantification.

Chen and others (Chen *et al*., 2013) prepared two fluorescent Sulfane Sulfur Probes (SSP) named SSP1 and SSP2 for the detection of persulfides, polysulfides, and elemental sulfur. SSP2 was successfully applied for sulfane sulfur bioimaging in living cells. Moreover, a few fluorescent probes selective for hydrogen persulfide (H_2S_2) were developed (Shimamoto & Hanaoka, 2015; Takano *et al*., 2016). Bibli and others (Bibli *et al*., 2018) have described a LC-MS/MS-based method that uses another probe, SSP4, to detect endogenously generated polysulfides in biological samples. Takano and others (Takano *et al*., 2017a) have designed a reversible off/on fluorescent probe SSip-1, which is weakly fluorescent, however, after reaction with sulfane sulfur it becomes strongly fluorescent. A review of chemical tools for the study of RSS, covering fluorescent probes based on various design strategies and their applications in biological studies, was presented by Takano and others (Takano *et al*., 2016; Takano *et al*., 2017b).

Han and others (Han *et al*., 2018) synthesized a near-infrared fluorescent probe BD-diSH for sulfane sulfur detection and imaging. This probe, composed of two moieties, displayed high sensitivity and selectivity for the sulfane sulfur present in living cells. The authors applied this method to image sulfane sulfur in the cytoplasm of living cells, as well as to evaluate the level of sulfane sulfur in the *ex-vivo*-dissected organs. Moreover, they imaged the sulfane sulfur level *in vivo* after intraperitoneal injection of BD-diSH in mice.

Interestingly, a new fluorescent probe (SSP5) has been designed recently to detect sulfane sulfurs using a Point-of-care sulfane sulfur smartphone spectrum apparatus (S4A). The authors suggest that the proposed system (SSP5+S4A) has the potential for high accuracy and rapid detection of sulfane sulfur with relatively low costs and results comparable to those obtained with standard laboratory equipment (Neil *et al*., 2019).

Furthermore, just a few months ago other authors described another sensitive method for reliable quantification of sulfane sulfur in biological samples (Ran *et al*., 2019). Based on the fact that all sulfane sulfur compounds react with sulfite yielding thiosulfate under appropriate conditions, they developed a method of thiosulfate determination in reaction with monobromobimane using HPLC.

Also very recently, it has been reported that sulfane sulfur, including that in polysulfides and persulfides, could be detected by using resonance synchronous spectroscopy (RS_2) (Li *et al.*, 2019). Sulfane sulfur displayed species-specific RS_2 spectra. The protonated form of persulfide (RSSH) was electrophilic and produced a RS_2 signal, while RSS^- was nucleophilic with no RS_2 signal. These data supported the idea that RSS, such as RSSH/RSS^-, may act as antioxidants inside cells.

It is worth recalling that proteins modified by S-sulfhydration (persulfidated proteins) contain cysteine residues in the form of cysteine persulfides (CysSSH), bearing the sulfane sulfur atom. Researches interested in this topic also lately developed several selective methods for detection of Cys residues present in the S-sulfhydrated proteins. They include a biotin-switch assay, cysteinyl labeling assay, the N-maleimide assay, Biotin-Thiol Assay, protein persulfide detection protocol (ProPerDP), tag-switch assay and a mass spectrometry assay. A good summary of the above-mentioned methods is presented in the review by Zhang and others (Zhang *et al.*, 2017).

CONCLUSIONS

Sulfane sulfur currently attracts an increasing interest, mainly due to its regulatory and protective properties. It is endogenously produced during anaerobic cysteine transformations, moreover, some of its exogenous donors have been identified. It is well documented that many proteins exist as protein persulfides due to posttranslational modification of some of their Cys groups by RSS (persulfidation). However, recently a possibility of CySSH incorporation during translation has been also postulated. Sulfane sulfur and H_2S are redox partners, and always coexist in biological systems. As presented in this review, disturbances in the sulfane sulfur level may reflect some pathological symptoms, and in addition this level changes with age or other factors. In consideration of these facts, a sensitive, reliable and easy to use method of the sulfane sulfur level determination is needed. Several of such methods have been recently developed. In spite of many studies concerning the biological importance of sulfane sulfur, it seems that a lot still remains to be discovered.

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