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Carbon disulfide. Just toxic or also bioregulatory and/or therapeutic?

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Abstract

The overview presented here has the goal of examining whether carbon disulfide (CS_2) may play a role as an endogenously generated bioregulator *and/or* has therapeutic value. The neuro- and reproductive system toxicity of CS_2 has been documented from its long-term use in the viscose rayon industry. CS_2 is also used in the production of dithiocarbamates (DTCs), which are potent fungicides and pesticides, thus raising concern that CS_2 may be an environmental toxin. However, DTCs also have recognized medicinal use in the treatment of heavy metal poisonings as well as having potency for reducing inflammation. Three known small molecule bioregulators (SMBs) nitric oxide, carbon monoxide, and hydrogen sulfide were initially viewed as environmental toxins. Yet each is now recognized as having intricate, though not fully elucidated, biological functions at concentration regimes far lower than the toxic doses. The literature also implies that the mammalian chemical biology of CS_2 has broader implications from inflammatory states to the gut microbiome. On these bases, we suggest that the very nature of CS_2 poisoning may be related to interrupting or overwhelming relevant regulatory or signaling process(es), much like other SMBs.

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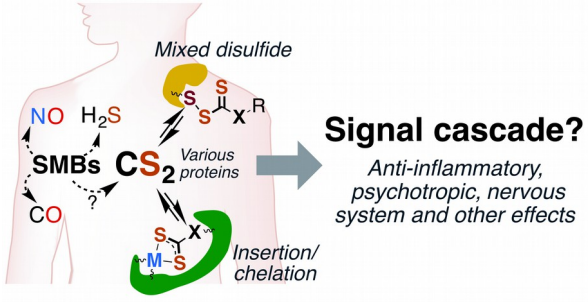

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1. Introduction

Small molecule bioregulators (SMBs) are physiological signaling agents with interconnected pathways based on their unique chemistry and interactions with specific biological targets.[1] This cellular signaling paradigm is a burgeoning field in mammalian chemical biology, and over the past several decades, SMBs central to this network have been identified. Three key SMBs, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S) have been called "gasotransmitters" although they are solubilized at the physiologically pertinent concentrations. A number of specific functions have been elucidated; for example, NO plays a key role in regulating blood pressure via the activation of soluble-guanylyl cyclase (sGC).[2] Other known physiological functions are not yet as well understood, and the biological roles of these SMBs and of respective derivatives such as nitroxyl (HNO), peroxynitrite ($ONOO^-$) and persulfides ($R\text{-}SS^-$) continue to be the subjects of extensive biomedical research efforts.[1-11]

If one were to envision an unidentified (so far) SMB in mammalian chemical biology, it may be appropriate to consider the shared properties of NO, CO, and H_2S. All are relatively nonpolar but are partially soluble in aqueous and lipid systems; thus, they readily diffuse in physiological media via spatial concentration gradients.[12] The actions of these SMBs involve chemical reactions with targets such as metal centers or redox active amino acids.[7] Furthermore, all were initially considered to be environmental toxins; yet each was found to be formed by endogenous processes. In this context, carbon disulfide (CS_2) is a small, nonpolar, readily diffusible molecule long recognized for its toxicity that may be generated endogenously. These similarities led us to contemplate whether CS_2 might be an unrecognized SMB and/or have therapeutic potential.[13]

The purpose of this review is to explore the possibility of such roles for carbon disulfide. We will first outline possible modes of toxicity and then will survey the chemical properties of CS_2 in the context of physiologically relevant conditions. Next, we will discuss known mechanisms for CS_2 metabolism in order to visualize a framework by which CS_2 may be managed endogenously. Finally, some therapeutic targets are suggested.

2. General Physical and Chemical Properties of CS_2

2.1 Physical properties and known sources

Pure CS_2 is a colorless, pleasant-smelling liquid displaying a high vapor pressure (48.21 kPa at 25°C), moderate water solubility (2.3 g/L at 22°C), and high lipophilicity.[14] It is a linear, nonpolar molecule like its homolog carbon dioxide (CO_2) with carbon- sulfur bonds 155.6 pm in length.[15] CS_2 is slightly more soluble than CO_2 in aqueous solutions; the Henry's law constants at 25 °C in pure water are 0.054 M atm^{-1} and 0.034 M atm^{-1} respectively.[16]

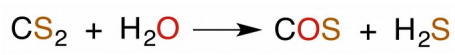
Although CS_2 is a listed environmental pollutant, it is produced naturally by soil-based microorganisms and is formed in vegetation fires and volcanoes. These sources account for 40-50% of atmospheric release, and the rest is anthropogenic.[17] CS_2 is produced industrially from heating a carbon source such as charcoal or natural gas with sulfur.[18] The annual industrial production is ~75 million kilograms per year (ca. 2010) for use in the rayon and cellophane, oil and gas, agricultural, and metal ore processing industries.[18, 19] It has been used in production of viscose rayon since the nineteenth century,[20] when the deleterious effects on exposed workers in that industry were first described.[21]

2.2. Toxicity

As noted above, CS_2 toxicity was first described in the 1850s[21] and is the subject of numerous subsequent studies.[22-26] Of particular interest are the neurotoxic effects.[27-33] Acute CS_2 poisoning causes toxic psychosis and narcotic somnolence. Long-term sub-acute exposures are linked with memory, behavioral and cognitive disturbances, and also concomitant nerve demyelination, cytostructural damage, and progressive paralysis of the extremities.[33,34] The mechanism of CS_2 neurotoxicity, however, is currently unknown. Epidemiological studies of chronic CS_2 exposure are somewhat contradictory, particularly regarding reproductive[35-37] and cardiovascular issues.[38,39] From a brief overview of the literature, the described toxicity, pathology, and/or metabolism of CS_2 indicate a breadth of targeted biochemical effects.

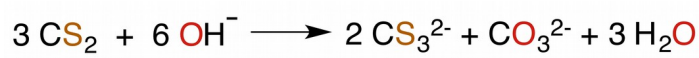
2.3 Some key chemical reactions

CS_2 is stable to hydration and at biologically relevant pH values is relatively unreactive toward hydrolysis to carbonyl sulfide (COS) in aqueous solutions (eq. 1).



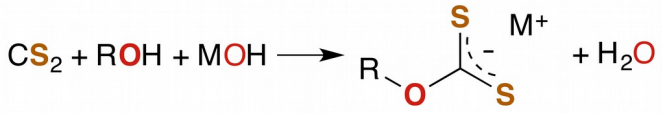
(1)

The approximate half-life of this reaction is 1.1 years in pH 9 aqueous solution.[19,40] CS_2 does react with a variety of stronger nucleophiles. For example in strongly alkaline solution, it forms trithiocarbonate and carbonate (eq. 2).[41]



(2)

The industrially important reaction of CS_2 with primary and secondary alcohols and a metal hydroxide to form xanthates (eq. 3) was reported as early as 1822.[42]

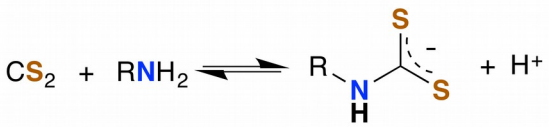


(3)

Acid catalyzes xanthate decomposition to the alcohol and CS_2, while in neutral or slightly alkaline pH xanthates undergo hydrolytic disproportionation (eq. 4).[41] Hydrolytic disproportionation is slow with half-lives on the order of several hours.[43]

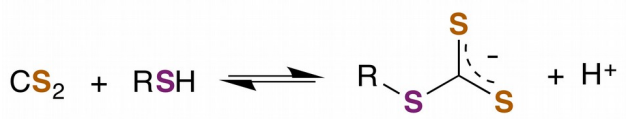
(4)

The analogous reactions of CS_2 with primary and secondary amines lead to the formation of dithiocarbamates (DTCs) (eq. 5). This equilibrium is dependent on the nature of the amine (e.g., sterics and electronics) and on the solution pH. In acidic solutions, dithiocarbamates readily decompose to the constituent amine plus CS_2.[44-46] At pH 7.4 and 35 °C, primary and most secondary alkyl-DTCs are relatively stable, whereas under mild acidic conditions, secondary alkyl-DTCs revert to the amine and CS_2 with rate constants on the order of 10^{-3} s^{-1} at pH 5.9.[47]



(5)

A CS_2 reaction that might have particular significance with regard to biological properties is the reversible formation of trithiocarbonates (TTCs, also known as thioxanthates) as illustrated in eq. 6.[48-50] We will illustrate possible roles of this reaction in greater detail below.



(6)

2.4 Interactions with metal centers

Carbon disulfide and its adducts (xanthates, DTCs, and TTCs) can serve as ligands in metal complexes. Typically, CS_2 coordinates more strongly to metal centers than does either CO_2 or COS.[51,52] There are a number of examples of η^1- and η^2- CS_2 complexes[53-55] as well as polynuclear complexes[56] where the CS_2 moiety is a bridging ligand (Figure 1).

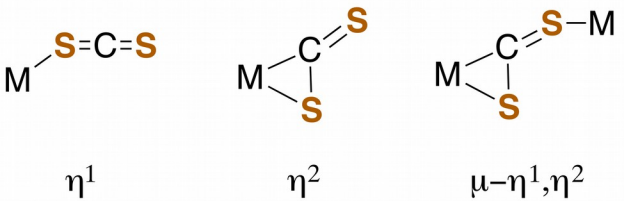
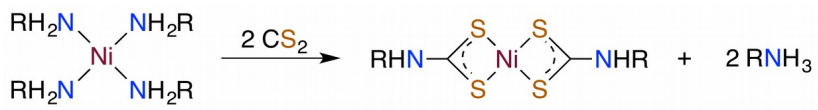


Figure 1. Binding modes of CS_2 with metal centers.

Such coordination can activate CS_2 toward reaction with nucleophiles. For example, reversible CS_2 insertion into a metal-ligand bond is a common reaction (eq. 7), presumably because of the stronger bonding of the 1,1-dithiolate ($-CS_2^-$) functionality.

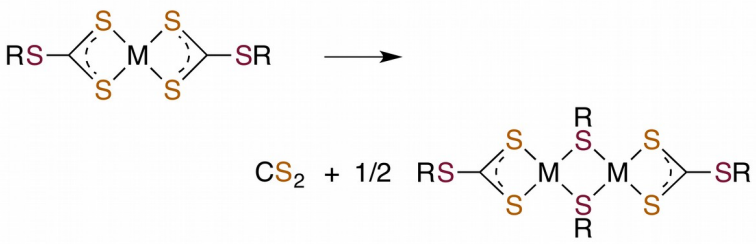
(7)

This has been seen for metal-bound thiolates[57] and alkoxides[58] and with thiols or amines with loss of H^+.[59] Analogous reactions of CS_2 with coordinated nucleophilic carbon centers result in C-C bond formation via the generation of a dithiocarboxylate ligand.[60] The analogous reactions with coordinated phosphines lead to a zwitterion (R_3P^+-CS_2^-) adducts as a bidentate ligand bound to the metal.[61] Similar CS_2 insertions have been reported for Cu(I)-amidinate complexes[62] and for nickel(II)–amine bonds in polar protic or aprotic solvents (eq. 8).[63] For example, although the trithiocarbonate zwitterion [$^+H_3NCH_2CH_2SCS_2^-$] forms in the direct CS_2 reaction with 2-aminoethanethiol,[48] the reaction with bis(2-mercapto-ethylamine)nickel(II) favors insertion between the metal and the amine to give a pendant thiol on the DTC complex.[63]



(8)

Metal centers also react with various $RXCS_2^-$ (where X = NR' or S) ligands to form stable complexes under a variety of conditions. Although generally stable in neutral aqueous solutions, some $M(S_2CXR)$ complexes eliminate CS_2.[64-69] For example, spontaneous release of CS_2 from the Ni(II) S-benzyl- and tert-butyl-trithiocarbonate complexes (eq. 9) occurs with first order rate constants on the order of 10^{-4} s^{-1} at 35 °C in lipophilic media.[64]



(9)

Studies of such ligand-metal interactions involving 1,1-dithiolate functional groups ($-CS_2^-$) have received relatively low attention in recent years but have been the subject of several reviews.[70-73]

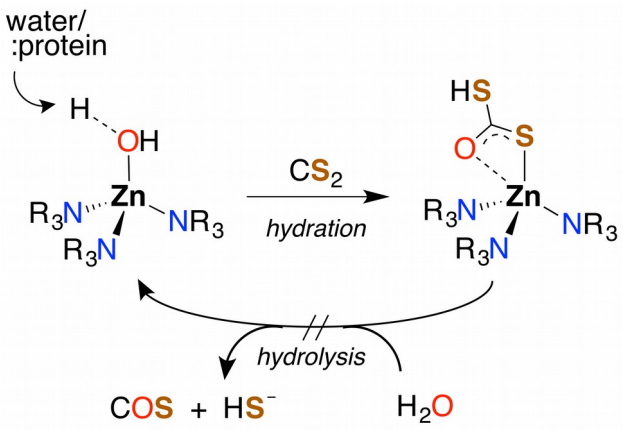
3. Biological Chemistry

3.1 Hydrolysis (or not)

Like CO_2, carbon disulfide is rather unreactive toward spontaneous hydration and hydrolysis at biological pH values, so a significant proportion of the dissolved compound is present in the solvated form, CS_2 (*aq*). Furthermore, carbonic anhydrase (CA), the enzyme that catalyzes the hydration of CO_2, is ineffective in activating the analogous reaction of CS_2 (eq. 1). For example, with excess CS_2, the turnover frequency of cytosolic CA II, the most efficient of the fourteen CA isomers,[74] is about 9 orders of magnitude slower ($\sim 5 \times 10^{-4}$ s^{-1}) than the analogous reaction with CO_2 ($\sim 10^6$ s^{-1}).[75]

The first step of CS_2 hydrolysis, like CO_2, is hydration. Using a model for the Zn-active site of CA II, Anders and coworkers computationally probed the formation of dithiocarbonate, the hydrated intermediate of CS_2 (Scheme 1) by considering a mechanism analogous to CO_2 hydration.[76-78] It is argued that dithiocarbonate formation has a slightly higher barrier of activation owing to a decreased electrophilicity of CS_2.[76] In addition, the intermediate dithiobicarbonate (HCS_2O^-) is a thermodynamic sink, which further slows

hydrolysis.[51] The calculations with this active site model suggest that CA does not complete a



Scheme 1 Hydration of CS_2 in Zn-based CA model active site. Anders and coworkers calculated a mechanism analogous to that for CO_2, with hydration leading to a stable dithiobicarbonate adduct. The subsequent step (hydrolysis) is slow.

catalytic cycle with CS_2. Rather, CS_2 is proposed to inhibit CA. Although CS_2 does not appear to interact with CA II,[75] xanthates and TTCs are effective inhibitors.[79] Nevertheless, there are bacterial-evolved, archaeal CS_2 hydrolases that do convert CS_2 to CO_2 and H_2S through intermediate COS (eqs. 1 and 10).[80] In spite of the high homology with carbonic anhydrases, these CS_2 hydrolases apparently do not use CO_2 as a substrate,[80] just as CA does not efficiently catalyze CS_2 hydrolysis. Notably, COS is readily hydrolyzed by both types.[75,77,78]

$$COS + H_2O \longrightarrow CO_2 + H_2S \qquad (10)$$

The presence of CS_2 hydrolases in some microorganisms and clear divergence in evolution imply an evolutionary need for a CS_2-specific enzyme. *Thiobacillus thioparus* and *Thiobacillus denitrificans* are microbes that use reduced sulfur species (dimethyl sulfide, methyl mercaptan, and H_2S) for energy production.[81] These may need such hydrolases to function in CS_2-rich environments. *T. thioparus* has been shown to consume CS_2.[82]

3.2 *Methane monooxygenase (MMO) and ammonia monooxygenase (AMO)*

Carbon disulfide has been shown to be an inhibitor of nitrification in *Nitrosomonas* and *Nitrosococcus* bacterial strains[83,84] and reported to inhibit methane monooxygenase (MMO).[85] From a detoxification viewpoint, MMO is evolutionarily and mechanistically similar to ammonium monooxygenase (AMO),[85] and methanotrophs that depend on MMO

and live in high sulfur environments (e.g., *Methylomicrobium alcaliphilium*) have active CS_2 hydrolases. The underlying mechanism proposed for CS_2 inhibition of nitrification involves reaction at a nucleophilic amine (lysine) or thiol (cysteine) to form a DTC or TTC near the active site of AMO.[83,85] The resulting 1,1-dithiolate may then chelate and sequester the active site copper or modify the activity owing to structural or electronic changes. An alternative mechanism would be reversible CS_2 insertion into metal–N or –S bonds as discussed above. Thus, one can envision CS_2 diffusing to a reactive site and either inserting in a metal-ligand bond motif or binding a proximal amine or thiolate to form a protein bound TTC or DTC as illustrated in pathways i and ii, respectively, in Figure 2. In either case, the resulting changes in structure, electronics, metal chelation, etc. would impact protein function.

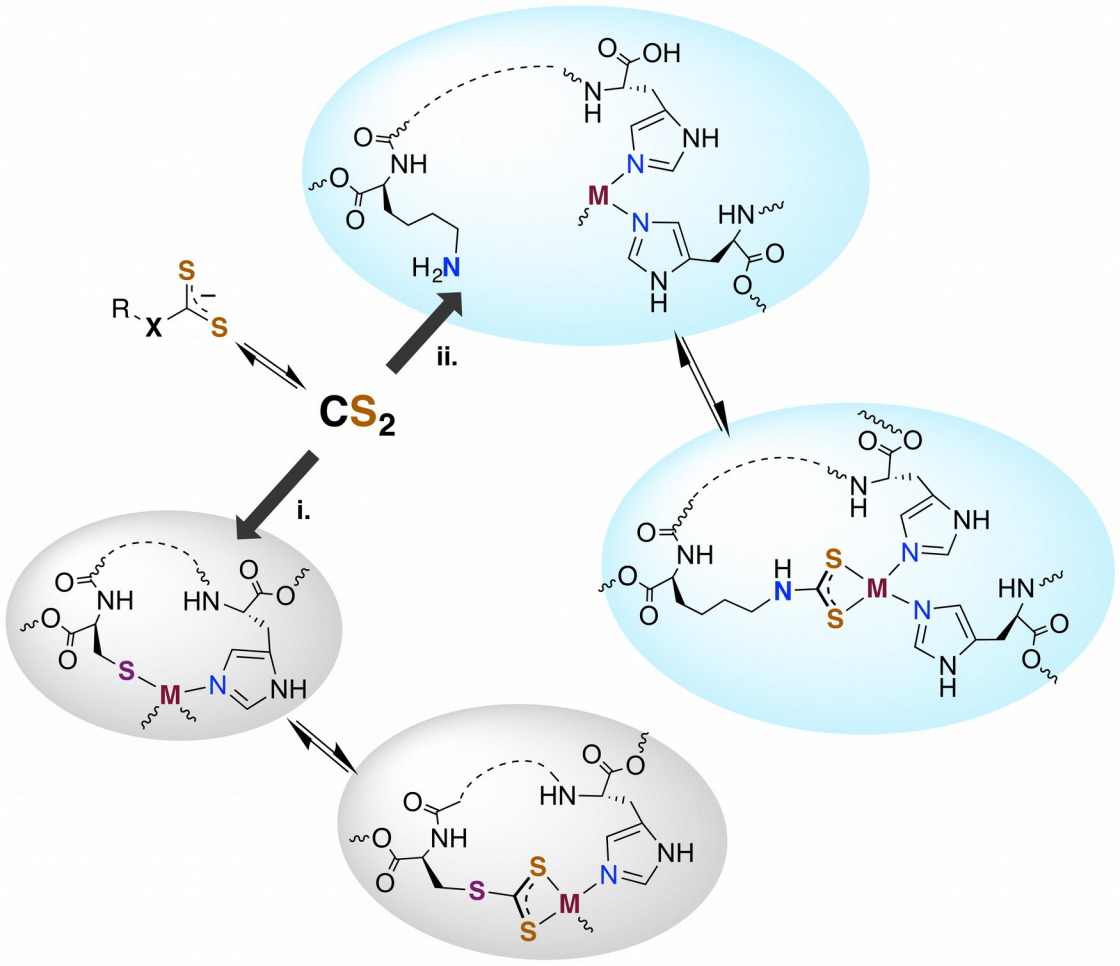


Figure 2 Possible interactions of CS_2 with biological metals. Since it is lipophilic, CS_2 could readily transport to the active sites of cellular proteins and (**i**) insert into a metal–cysteine bond to form a trithiocarbonate chelate or (**ii**) bind a protein amine or thiol proximal to a metal center where the resulting dithiocarbamate or trithiocarbonate can bind. X = NR' or S

3.3 Carbon monoxide dehydrogenase (CODH)

CODH interconverts CO to CO_2 in anaerobic bacteria and is responsible for the final step of the production of acetyl coenzyme A (acetyl-CoA). Ragsdale and coworkers[87] have reported that CS_2 is a rapid binding, reversible inhibitor of this Ni/Fe-S cluster protein. Consistent with its interaction with CA, CS_2 does not bind or interact with the CO_2-binding site. Instead it serves as a competitive inhibitor to the terminal binding site of CO at an iron center of the cluster, the site of acetyl-CoA synthesis. Two proposals were offered regarding CS_2 binding at this site: insertion into a ligand-iron bond or coordination as CS_2 to the metal. An alternative possibility would be CS_2 binding via electrophilic reaction at a protein thiol or amine near the coordination site. Equally likely is that CS_2 binds the thiol of acetyl-CoA itself. Since CODH catalyzes the acetylation of the homocysteine thiol moiety of CoA,[88] CS_2 binding at this thiol would generate a TTC that inhibits CODH via chelation of the iron.

3.4 Potential targets in mammalian physiology

In general, CS_2 exposure in humans and assorted mammals has been assessed via detection of the metabolites thiazolidine-2-thione-4-carboxylic acid (TTCA), 2-thio-5-thiazolidinones, and occasionally 2-oxothiozolidine-4-carboxylic acid (Figure 3) in the urine.[27,30,32,89-91] TTCA is the product of CS_2 reaction with cysteine or cysteine-containing poly/oligopeptides, and its urinary release has been shown to increase linearly with CS_2 oral intake by rats.[92] Other major metabolites, such as the 2-thio-5-thiazolidinones, likely result from terminal amines in polypeptides and other biological amines acting as nucleophiles at the CS_2 carbon to form (initially) DTCs followed by eventual cyclization. These metabolic scenarios are considered below.

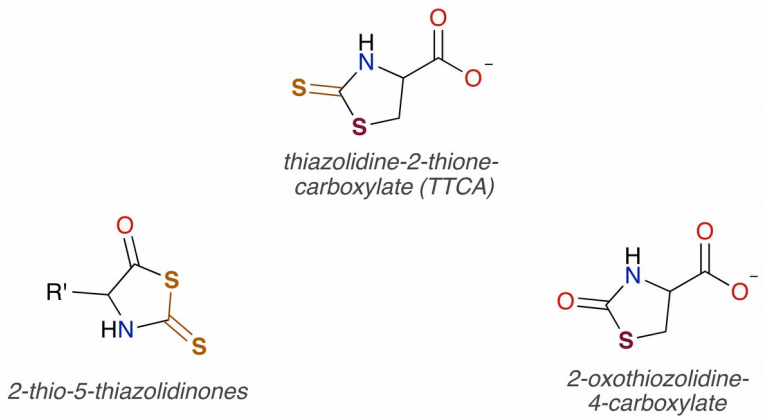


Figure 3 Cyclic metabolites found in the urine of mammals after exposure to CS_2.

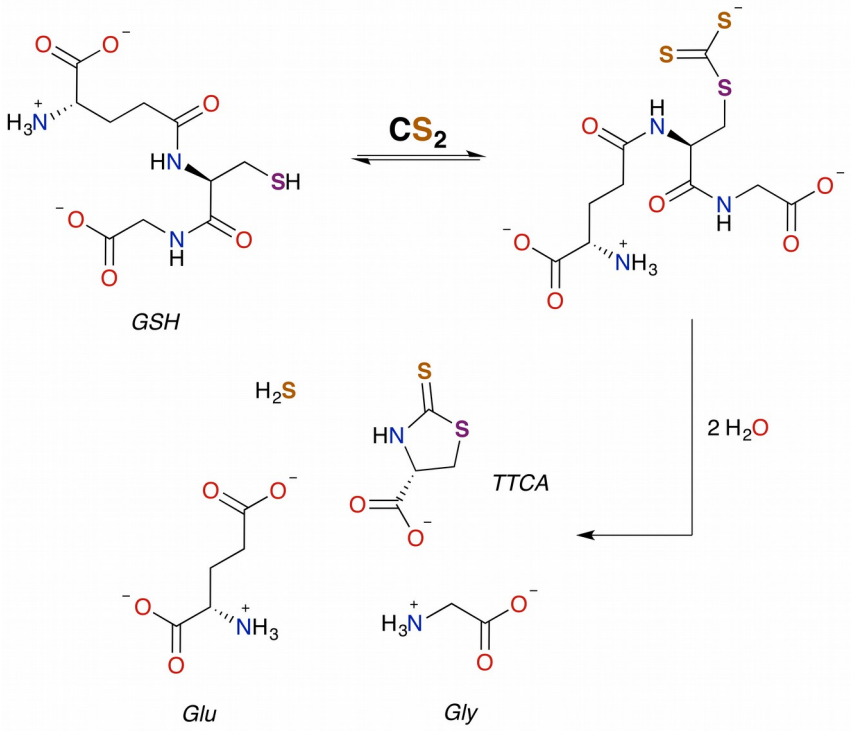
3.4.1 Thiols and trithiocarbonates (TTCs)

Biological thiols such as glutathione (GSH) and cysteine are essential for maintaining oxidative homeostasis in mammalian cells and serum. GSH and its oxidized partner GSSG constitute a redox buffer system that helps mitigate cellular damage from reactive radicals. GSH is present in cytosols at millimolar concentrations,[93] and while in plasma these lower weight thiols are present in very low concentrations (0.1 to 20 μM), plasma-based protein sulfhydryls are as high as 0.5 mM.[94,95] Owing partially to their high concentrations and CS_2's electrophilicity, such thiols must be considered likely targets or biological sinks for CS_2. This interaction will strongly depend on the pK_a of the specific thiol and on the local pH. Biological sulfhydryls (R-SH) can display reasonably low pK_a values, though these are medium dependent. The pK_a of GSH can be as high as 9.2 (extrapolated to zero ionic strength)[96-98] and as low as around 8.66.[99] Similarly, the pK_a of free cysteine ranges from 8.2[100] to 8.6[97] and varies considerably within proteins. Peptide amines are somewhat more basic ($pK_a \geq 9$) than these thiolates and when protonated will not be nucleophiles for CS_2. Thiols and thiolates ($R\text{-}S^-$), therefore, remain the predominant nucleophiles in biological media.[101] This should lead to the reversible formation of TTCs as illustrated in the first step of Scheme 2 and in eq. 6.

As aforementioned, TTCA release through the urinary tract is a signature of external exposure to CS_2. This thiazolidine is apparently derived from the reaction of CS_2 with the thiolate of cysteine or GSH, in the latter case resulting also in liberation of glycine and L-

glutamic acid.[89,102,103] However, it is not known whether TTCA formation is enzyme assisted. While reaction with a secondary amine of GSH to give a DTC is a mechanistic possibility, such sites are much less nucleophilic than the thiolate.[104] In either case, intramolecular cyclization and liberation of H_2S would result in TTCA as roughly outlined in Scheme 2.

The formation of alkyl TTCs in aqueous solution is reversible (eq. 6),[104] although the equilibria are dependent on the pH and on the pK_a of the parent thiol. Such equilibria are not well characterized with biological thiols, but these are especially interesting given that TTCs may be a means to sequester or transport CS_2 in serum or to provide a pathway for the interactions of CS_2 with metalloproteins. In addition, TTC formation as post-translational protein modification at cysteine residues likely leads to important structural



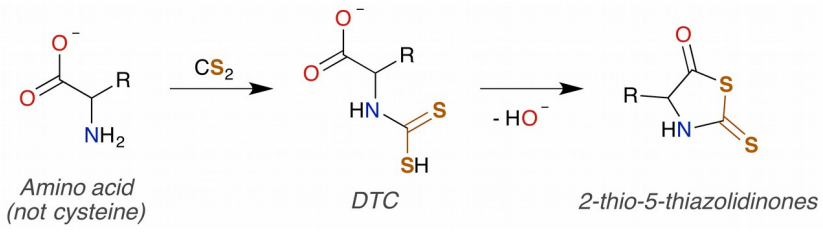
Scheme 2 Pathway for the formation of TTCA from the reaction of GSH with CS_2.[30,49]

and/or functional effects that also may be important signaling pathways, even if such reactions are reversible. Protein microenvironments can markedly affect the acidity of a thiol through interactions with charged amino acid side chains and/or hydrogen bonding.[99-101] Such interactions with protein thiols are potential targets and switches for CS_2 signaling.

Another signaling mechanism may involve the direct reaction of TTCs with transition metal centers or by reversible CS_2 insertion into metal thiolate bonds to form TTC metal complexes (Figure 2). The latter pathway was demonstrated with a variety of metal-thiolate complexes under ambient conditions[57,68,69] including the biologically relevant zinc(II) and copper(I) centers (see above). Thus, one can envision metal thiolate centers such as zinc fingers or iron redox centers as targets for the reversible formation of CS_2 adducts. Although the above examples were in non-aqueous media, similar reactivity may be relevant in the cores of particular proteins or other lipophilic areas.

3.4.2 Amines and dithiocarbamates (DTCs)

DTCs and their derivatives (e.g., disulfides of the type $R_2NC(S)SSC(S)NR_2$) already have a number of medical applications. CS_2 spontaneously reacts with endogenous amines to form DTCs[30,106-108] as the first step in a sequence that converts free and terminal amino acids to thiazolidine-type cyclic moieties (2-thio-5-thiazolidinones)[89,109] (Scheme 3). An important feature of DTCs is the ability to chelate metal ions such as copper or zinc, often binding to them in the enzyme pocket[110-112] or potentially even stripping them from proteins.[110] The high binding affinity to heavy metal ions finds medicinal application in chelation therapy through this latter route.[113,114] DTCs may also sequester metals essential for certain protein functions, and, thus have been explored as possible drugs targeting HIV/AIDS via inhibition of reverse transcriptase activity.[115-118]



Scheme 3 Conversion of an amino acid to a DTC, followed by subsequent cyclization to 2-thio-5-thiazolidinone.

DTCs are commonly used fungicides and pesticides, and thus their toxicity has been reviewed[119,120] most recently by Rodriques-Lima and coworkers.[121] Many of the pathologies of DTC toxicity mirror those documented for CS_2, including potent central nervous system (CNS) effects under acute exposure as well as links to Parkinson's disease via induction of

severe CNS depression.[122-124] DTC disulfides also have neuropathies very similar to that of those produced by CS_2 exposure,[125-129] although a different mechanism of toxicity has been proposed.[129]

The similar symptoms of DTCs and CS_2 toxicity suggest a shared mechanism. Under acidic conditions, *N,N*-diethyldithiocarbamate (DEDTC) releases CS_2, but it is generally assumed that DEDTC is stable at or above neutral pH.[130,131] Valentine and coworkers[103] investigated possible *in vivo* CS_2 release from disulfiram and DTCs such as DEDTC to establish whether such release induced symptoms attributed to toxicity characteristic of CS_2 exposure. Using radiolabeling, they found a marked increase in urinary TTCA attributed to the presence of free CS_2 regardless of the compound tested (disulfiram or a DTC). Not surprisingly, oral exposure to DEDTC gave much higher fluxes of TTCA owing to the acidic environment of the dietary tract. Incorporation of the labeled carbon at the thiocarbonyl of TTCA indicates that CS_2 is generated from the various DTCs. This result may answer a question raised by Cvek and Dvorak in a review of DTC cellular interactions,[132] namely, that it is unclear how polar DTCs might enter a cell through lipid bilayers. The *in vivo* study by Valentine *et al*[102] supports the view that DTCs and CS_2 are in labile equilibria. This lability under physiological conditions thereby provides a mechanism to transport this functionality through hydrophobic membranes by passive diffusion of CS_2.

The reaction between neuronal amines and CS_2 has often been hypothesized to be the source of carbon disulfide-induced neuropathological effects.[30,106,107] Catecholamine neurotransmitters, including epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine have amine-bearing side chains sufficiently nucleophilic[133] to react with CS_2 *in vivo*. This reaction is a likely cause of the significant norepinephrine depletion in the adrenal glands and storage granules of rodents exposed to CS_2 vapor (64 ppb).[106] Norepinephrine is produced from dopamine via dopamine β-hydroxylase (DBH), a monooxygenase with copper in the active site,[107] and it has been suggested[106] that DBH is inhibited by DTC formation and chelation of that copper. Experiments *in vitro* showed a neuronal amine is needed to inhibit DBH in the presence of CS_2 and that this effect is reversible.[107] Notably, CS_2 proved to be more effective inhibitor of DBH than an added glycine-based DTC.[107] This suggests that lipophilic CS_2 enters chatecholamine storage

granules where it reacts to form DTCs and inhibits DBH (Figure 4). Such a process could play a regulatory role by controlling dopamine/norepinephrine levels.[134,135]

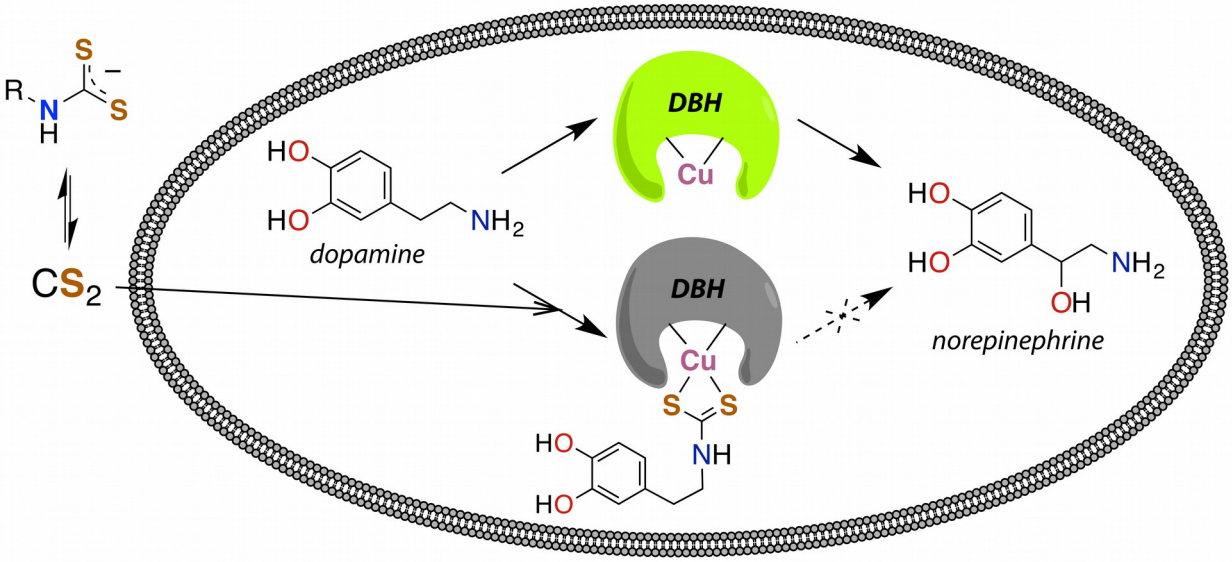


Figure 4 Release of CS_2 from DTCs allows for diffusion across membranes, like the storage granules of catecholamines. Dopamine β-hydroxylase (DBH), a copper-centered enzyme, converts dopamine to norepinephrine and is deactivated by CS_2 via the DTC of catecholamines.[107]

Disulfiram (also called "antabuse"), the disulfide of DEDTC, has long been used in alcohol aversion therapy.[131] It functions by inhibiting acetaldehyde dehydrogenase (ALDH), thereby greatly enhancing the severity of hangovers due to alcohol consumption. The molecular mechanism of ALDH inhibition is mediated by the metabolic products of disulfiram, the first of which is DEDTC formed by reduction of the disulfide bond (Figure 5). Metabolites of DEDTC are irreversible inhibitors of ALDH.[121] Covalent addition of one of these metabolites to an essential cysteine residue inhibits rodent ALDH *in vivo* by initiating disulfide bond formation. The current thinking is that oxidation by microsomes forms methyl diethylthiocarbamoyl sulfoxides (Figure 5) as the active agent[130,136] Direct reactions with DTCs or their disulfides have also been proposed.[119,135] Either of these mechanisms provide a route for CS_2 to interact with specific proteins having ideally placed cysteines and could be essential in bio-signaling chemistry (Figure 6).[113,136,138]

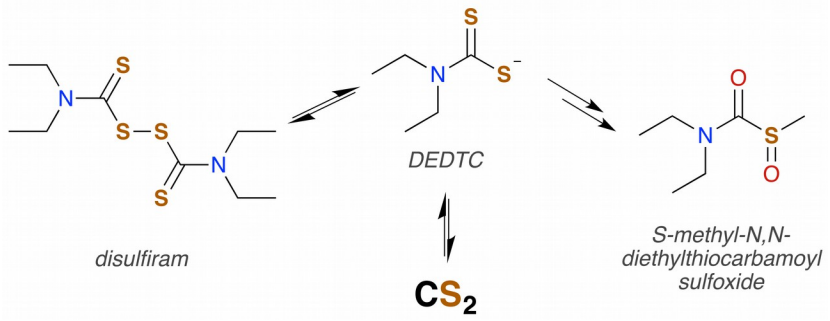


Figure 5 Disulfiram is readily reduced *in vivo* to form DEDTC, which generates carbon disulfide or is eventually converted to a thiocarbamoyl. All have been implicated in inhibiting ALDH via mixed disulfide formation with protein thiols.

Disulfiram is also finding therapeutic use in treating cocaine addiction via an ALDH-independent mechanism.[137,139] This may function by inhibiting DBH, the copper- dependent enzyme required for metabolism of dopamine to norepinephrine. These inhibitory effects have been attributed to the copper-chelating activity of DEDTC formed *in vivo*. Such copper-chelation is also thought to induce proteasome inhibition leading to the proposal that disulfiram could serve in anticancer therapy.[140]

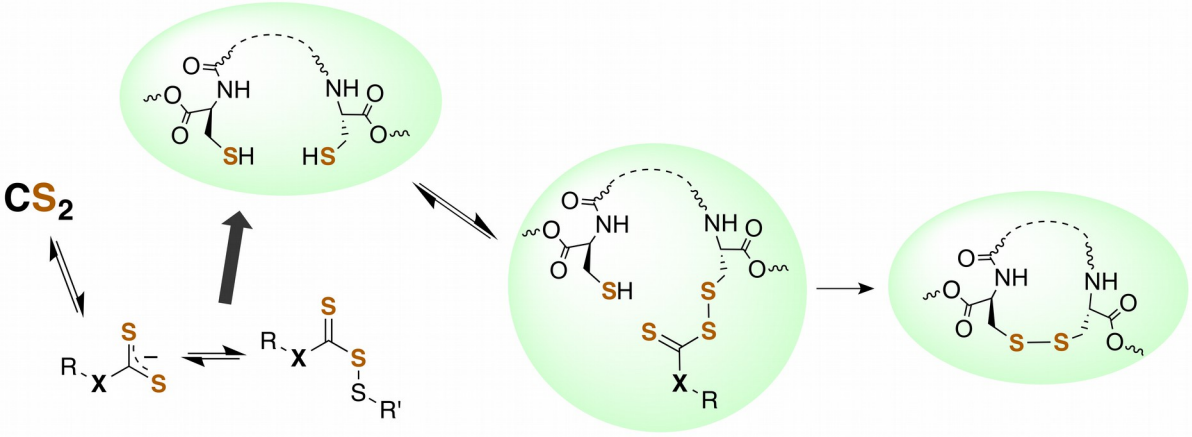
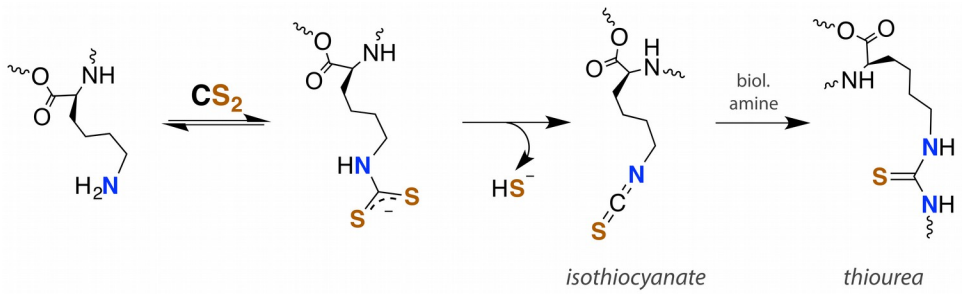


Figure 6 Possible interactions of CS_2-adducts with proteins suggested by the inhibitory action of disulfiram and its metabolites with ALDH. Formation of a mixed disulfide on a critical protein thiol leads to oxidation and intra-protein disulfide formation with a nearby thiol. X = NR' or S

Although there is strong evidence implicating CS_2 generation in DTC toxicity,[130] it is important to consider concentration regimes. One proposed mechanism for CS_2 neuropathy involves the induction of intra- and inter-protein cross-linking. The proteins β-

lactoglobulin, porcine neurofilaments, bovine serum albumin, and hemoglobin were each observed to react with CS_2 to form thiourea based crosslinks and dimers. This involved predominately lysine ε-amines,[129] but also lysine and the *N*-termini of certain proteins.[141,142] DTC formation on an exposed amine, followed by loss of HS^- would give isothiocyanate intermediates that couple with another free amine (Scheme 4).[133] However, it should be noted that these *in vivo* processes were demonstrated in rats exposed to 50 ppm CS_2 (5 times the occupational threshold)[19] in inhaled air for 2 weeks.



Scheme 4 Transformation of CS_2 derived lysine dithiocarbamates into dimers responsible for CS_2-induced protein cross-linking via thioureas moieties.[141]

3.4.3 Nuclear factor □B (NF-□B)

DTCs are known inhibitors of NF-□B,[132,144] a class of closely related, pro-inflammatory factors.[146-154] NF-□B is a crucial mediator in inflammation-based tumor growth and progression[146] and triggers the transcription of genes that regulate cellular proliferation and differentiation,[150] angiogenesis, and proinflammatory cytokines and chemokines. NF-□B directly and indirectly affects a wide range of functions from the vascular endothelium to immune response,[152] thus making it an attractive therapeutic target.

What stimulates the constitutive expression of NF-□B in cancer cells is not fully understood.[155] An extracellular signal triggers a cytosolic-based cascade to release NF-□B from an inhibitor (I-□B). Following translocation to the nucleus, NF-□B tunes the regulation of diverse genes. To activate NF-□B, the I-□B must be phosphorylated by an I-□B kinase (IKK) resulting in its conjugation to ubiquitin (ubiquitination) and subsequent degradation.[132,156-159] DTCs can act as thiol antioxidants, which are also known to inhibit NF-

□B.[152] However, the inhibitory activity of DTCs appears to be isolated from its antioxidant abilities[160] and relies on a mechanism that has not been fully elucidated.

A potential target for CS_2 is a cysteine (Cys38) in the DNA binding site of NF-□B (Figure 7, pathway 1).[161] Treatment with thiol-reactive compounds such as N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) prevents DNA binding.[152] TPCK reacts far more freely at this residue than it does with the cellular GSH, suggesting that the Cys38 thiol is more nucleophilic or has a lower pK_a,[161] thereby making it a likely target for CS_2 via the reversible formation of a TTC.[150] Such adduct formation could also provide a mechanism for DTC inhibition of NF-□B, given that dithiocarbamates release CS_2 *in vivo* (see above).

Thiolate-reactive compounds also inhibit the IKK complex further upstream in the signaling cascade (Figure 7, pathway 2). The presumed target is again a critical cysteine (Cys179), located between two serine residues (Ser177/181) in the activation loop of the protein β-subunit.[164] Ser177/181 are each phosphorylated by another kinase to activate IKK (Figure 7).[165] Replacing Cys179 with alanine prevents phosphorylation, indicating that this cysteine is non-innocent in the activation step.[166] Further, Cys179 is implicated as a participant in the catalytic domain of activated IKK[164,167] due to significant (albeit reversible) enzyme deactivation occurring with DTC treatment of pre-phosphorylated IKK□. The

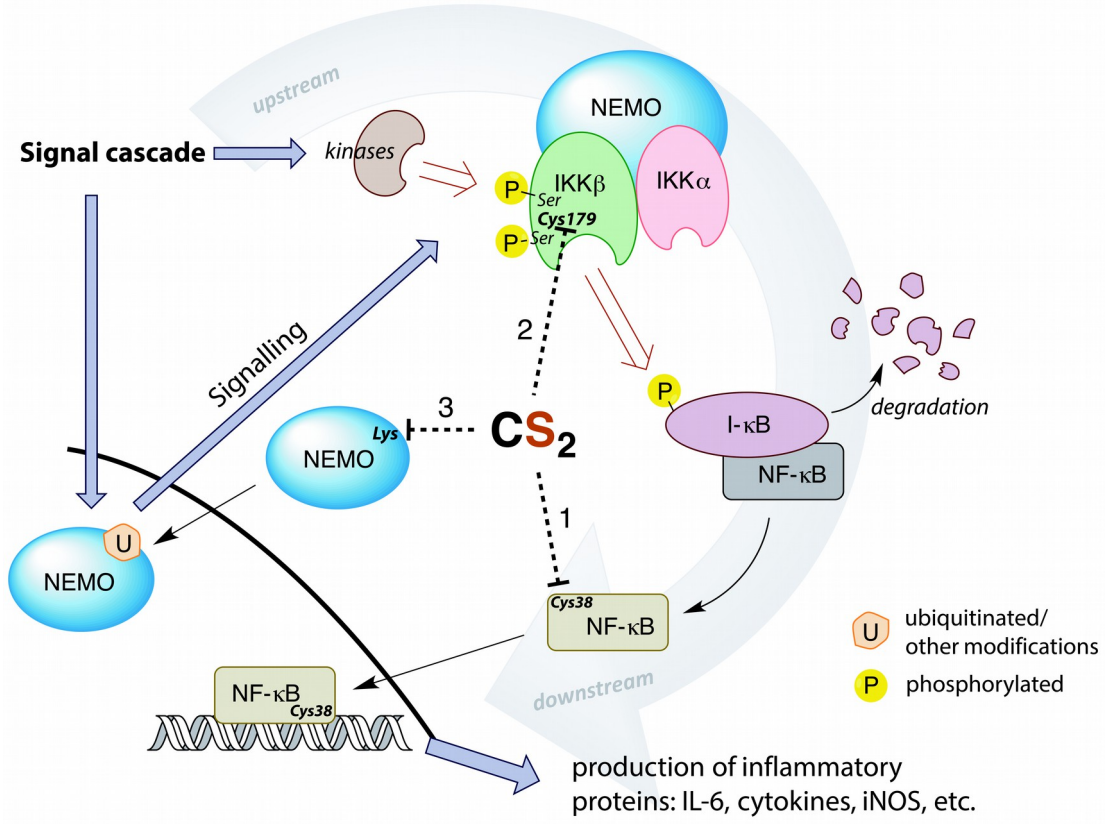


Figure 7 Simplified depiction of NF-□B activation and gene up-regulation to produce pro-inflammatory proteins. The IKK complex (top right) is activated through a signal cascade and in turn activates NF-□B. Possible targets for CS_2 inhibition are the Cys38 in the DNA binding region of NF-□B (pathway 1), the Cys179 in IKKβ (pathway 2), or lysines in the zinc finger binding domains of the pre-ubiquitinated scaffold protein NEMO (pathway 3) . Cys179 appears to be the most ubiquitous and likely target. (IL-6 = interleukin-6, a proinflammatory cytokine, iNOS = inducible nitric oxide synthase, I-□B = inhibitor of NF-□B, NEMO = NF-□B essential modulator (also known as IKK□), IKK = I-□B kinase).[146,148,152,155,159,161-164]

fact that DTC inhibition is reversible suggests a mechanism distinct from the disulfide formation step that is responsible for ALDH inhibition. Indeed, CS_2 liberated from a dithiocarbamate may form a TTC at Cys179, thereby providing a reversible IKK inhibition pathway.

Another possible upstream target for CS_2 is the NF-□B essential modulator (NEMO), which recruits other IKK activators (Figure 7, pathway 3). NEMO can be activated by ubiquitination at lysine residues including those in a zinc finger binding domain.[159,162] This suggests another site for CS_2/DTC interference. DTCs have been shown to decrease the expression of NEMO[155] in addition to the IKKβ, consistent with this hypothesis.

NF-□B is an alluring therapeutic target due to the prevalence of heightened inflammation in a variety of disease states.[70,132,152] If CS_2 is indeed an SMB that plays a

critical role in the regulation of these important transcription factors, then it stands to reason that CS_2 delivery may have therapeutic value, notably in the context of inflammation reduction. Along this line of thought, delivery of CS_2 has been proposed in the patent literature for the treatment of inflammatory conditions and was claimed to directly inhibit NF-□B.[168]

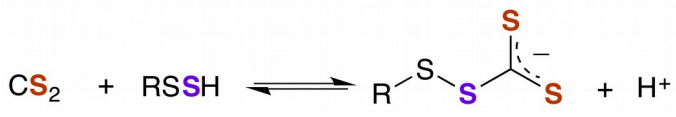
3.4.4 Interactions with cytochrome P450

The hindered ability to metabolize drugs oxidatively is a symptom also attributed to CS_2 toxicity at relatively high levels of exposure,[30] and this suggests inhibition of hepatic monooxygenases such as cytochrome P450. The interaction of $^{14}CS_2$ with P450, NADPH, and O_2 results in the expiration of some $^{14}CO_2$,[169] and since carbonic anhydrases are inactive for CS_2 hydrolysis (Sec. 3.1), another mechanism needs to be considered. Oxidative desulfuration[169,170] is proposed to occur via CS_2 oxidation to an unstable S-oxygenated intermediate[171] that fragments to elemental sulfur and to COS or monothiocarbonate.[90,172] Carbonic anhydrase can hydrolyze COS to H_2S and CO_2, while monothiocarbonate reacts with carbamyl phosphate synthetase to form thiourea.[31] The zero-valent byproduct S(0) can modify activities of the targeted proteins by forming persulfides with cysteine residues.[171,173,174] This modification may be one cause of hepatic and renal toxicity,[108] although such toxicity has also been attributed to the H_2S from CA catalyzed COS hydrolysis.[172] Given the bioregulatory properties of H_2S, such release may have other physiological implications.[163,175] Other potential CS_2-derived sources of H_2S include TTC cyclization and DTC conversion to an isothiocyanate.

3.4.5 Persulfides and selenoproteins

To this point we have largely focused on the reactions of CS_2 with the more common biological amine and thiol nucleophiles. Reactions with thiols (or more appropriately thiolates) are readily predicted, since these are among the most prevalent and potent nucleophiles present and are capable of reacting with both “hard” and “soft” electrophiles.[176] However, the recent discovery of ubiquitous and significant levels of hydropersulfides (RSSH) in mammalian cells, tissue and plasma[177,178] presents other likely targets for CS_2 reactivity/biological activity. Numerous protein persulfides have been

detected and the concentration of glutathione hydropersulfide (GSSH) has been found to be as high as 150 μM in mouse brain and 50 μM in mouse liver and heart, although their biological functions are still being elucidated. These levels make GSSH the second most prevalent sulfur species in cells, behind only GSH itself. The possible reaction between CS_2 and hydropersulfides seems especially relevant, since hydropersulfides are much more reducing and nucleophilic than the analogous thiols.[179,180] These properties predict a more efficient and favorable reaction with persulfides compared to thiols. Moreover, hydropersulfides are more acidic than the corresponding thiols by about 1-2 pK_a units, indicating a higher percentage of the nucleophilic persulfide anion compared to thiolates at physiological pH. To our knowledge, the reaction between hydropersulfides and CS_2 has not been reported, but it is not difficult to envision formation of perthioxanthates ($RSSCS_2^-$) (eq. 11).



(11)

Another potent class of biological nucleophiles is represented by selenoproteins. Like hydropersulfides, selenocysteine is more acidic than cysteine by 2-3 pK_a units. Thus, selenocysteines exist predominantly as the anionic selenide at physiological pH. In general, selenols are stronger nucleophiles than are thiols under conditions of equal ionization and even more so under physiological conditions where a higher percentage of the selenide anion would be present compared to thiolate.[181] Of course, selenoproteins are far less prevalent compared to thiol proteins. To date, only about 25 human selenoproteins have been identified with functions ranging from thyroid hormone regulation to antioxidant function.[182] For comparison, the human genome encodes for over 200,000 cysteines that are widely distributed among proteins.[183] The reaction of a selenide with CS_2 can be envisioned to be analogous to that shown for the thiol/thiolate with the generation of the selenoxanthate ($RSeC(S)S^-$). Therefore, due to the nucleophilicities of persulfides and selenols, future studies into the chemical biology of CS_2 should consider the possible interactions with persulfides and/or selenoproteins.

3.5 Endogenous production?

A hallmark of the SMBs NO, CO, and H_2S is that each is generated endogenously by enzymatic processes.[1] While at present there is no direct evidence for mammalian CS_2 production by a specific mammalian enzyme, there is ancillary evidence for endogenous production.[184,185] For example, humans (controlled for exposure, medication, etc.) exhale CS_2. While healthy individuals do not exhale it at levels where there is a significant difference in alveolar gradient,[186] elevated CS_2 levels on the breath are associated with a variety of disease states.[185] For instance, patients with schizophrenia have high positive CS_2 gradients, indicating higher CS_2 levels in their blood steams.[187] Given CS_2's interaction with catecholamines resulting in increased dopamine levels,[107] and the association of high levels of dopamine with schizophrenia,[134,135] an abundance of CS_2 may play a role. Exhaled CS_2 is also associated with gastric cancers, especially those induced by *Helicobacter pylori*.[184] A significant increase in CS_2 is found in human feces after eating, suggesting a role of the gut microbiome in its production.[188,189] Moreover, it is found in all samples of healthy donors and missing in many patients with foodborne bacterial infections like *Campylobacter jejuni*,[188] implicating a positive association of CS_2 production with a healthy gut microbiome.

Exhaled CS_2 is also claimed to be a marker for organ rejection after lung transplantation,[184,185] although COS has also been claimed as such a marker.[190] It is notable that CS_2 or COS would be associated with organ transplantation, as it is well-established that CO has protective effects in organ transplantation, reducing probability of rejection.[4]

What are the sources of exhaled CS_2? It is possible that, like other sulfur containing volatiles, it is a by-product of metabolic pathways of methionine.[184,185] Alternatively, it has been inferred that it derives from a collection of gut bacteria and micro-organisms of the human microbiome that produces other sulfur-containing small metabolites.[187-189] However, one should also consider the possibility of a dedicated, enzymatic source. This is most apparent in certain rodents that exhale CS_2 in micromolar concentrations.[191] The olfactory system of such rodents appears to have sensory neurons sensitized to CS_2 through an isoform of membrane-bound receptor guanylyl cyclase, type D (GC-D).[192] This receptor triggers a cyclic guanosine monophosphate (cGMP)-signaling cascade, much like the response of sGC to NO. This CS_2-initiated cascade, when combined with an odor, promotes in rodents a preference for the food associated with that odor, an important instinctual

survival mechanism.[191] Knockouts for the gene encoding the olfactory GC-D resulted in loss of the ability to form these preferences.[192,193] This result implies that, at least with these rodents, CS_2 has a signaling-inducing target rather than being simply a metabolic byproduct. A form of CA II has also been reported to play a role in such signal transduction,[192] even though other CAs generally do not interact strongly with CS_2.[75] The murine olfactory system is also sensitive to CO_2, but by several orders of magnitude less than to CS_2. These observations suggest that CS_2 is endogenously produced in mammals at concentration regimes appropriate for an SMB.

3.5.1 Bodily inventory of CS_2

A quantitative relationship between the CS_2 present in humans (generally assumed to be from external exposure) and temporal parameters is challenging to establish.[30,194,195] Few studies account for chronic exposure[196] or the limits of quantification. CS_2 exposure has largely been assessed via detection of the urinary metabolite TTCA (Scheme 4). Occupational atmospheric exposure limits are set as low as 5 ppm, a level that generates detectable quantities of TTCA[38] but does not show conspicuous negative health effects. Various sources have found that TTCA is not detected without external exposure, and therefore have argued against endogenous production.[197-199] The TTCA detection limit is ~1 μM in urine, which correlates approximately with atmospheric exposure of 0.2–0.4 ppm CS_2.[195,197] Other studies have estimated that only 2-6% of CS_2 ingested is metabolized to TTCA[89,197-202] owing to alternative metabolic pathways as well as loss through respiration. Thus, one can argue that low endogenous levels of CS_2 may go undetected. For comparison, NO has dynamic and contrasting functions over concentrations from as low as a few nanomolar for vascular functions, to 100 nM for angiogenic and anti-apoptotic effects, to 300 nM for apoptotic conditions, to a few micromolar under nitrosative stress.[203]

Notably, TTCA is detected in the urine of individuals not directly exposed to CS_2.[204] Specifically, consumption of brassica vegetables such as broccoli, cauliflower, and cabbage results in urine excretions of 10 μmol TTCA per liter of urine. This observation suggests that these vegetables may be a natural source of CS_2 given the nature of TTCA formation.[17] While TTCA is also found in the vegetables themselves, far greater quantities are found when they are boiled.[205] Given that TTCA is derived from the reaction of GSH or cysteine

with CS_2, it is reasonable that heat simply increases the rate of this reaction and thus of TTCA production. Hence, it stands to reason that these food sources may contain trace amounts of CS_2 or a precursor thereof. Moreover, brassica vegetables are promoted as healthy and beneficial foods due to their association with lower cancer risk in a multitude of studies.[206] The bioactive compounds are the sulfur containing phytochemicals glucosinolates (Figure 8). They have anti-inflammatory, genetic, and epigenetic properties once metabolized and have been probed for therapeutic potential. Glucosinolates undergo enzymatic conversion to form isothiocyanates and DTCs, which in turn can generate CS_2 and TTCA.[205,207]

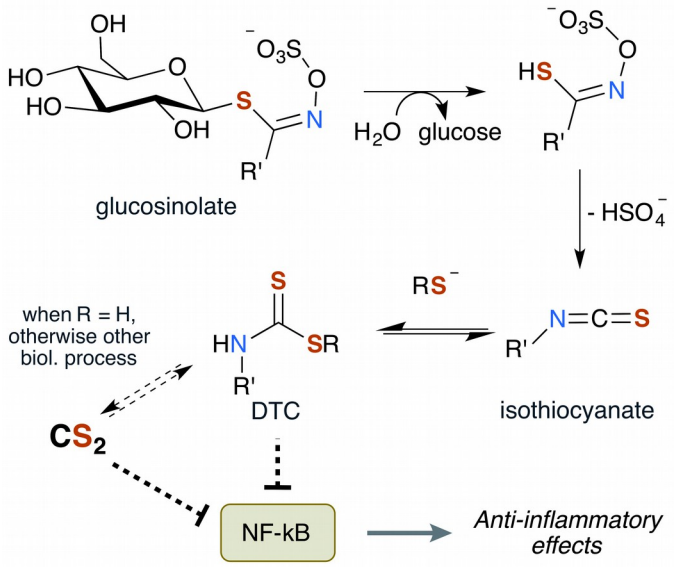


Figure 8. Brassica vegetables contain glucosinolates, which undergo enzymatic conversion to a variety of metabolites, with various effects, including anti-inflammatory action. Since DTCs are generated, these compounds may also yield CS_2.

4. Summary and Outlook

In this review, we've attempted to draw together literature evidence indicating that carbon disulfide may have roles in mammalian biology beyond its toxicity. The various chemical pathways of CS_2 that might contribute to such properties are summarized in Figure 9. We have posed the questions: Does CS_2, its precursors, or the resulting metabolites have therapeutic potential? and/or Is CS_2 an unrecognized small molecule bioregulator? Like NO, CO, and H_2S, carbon disulfide is a small molecule that is nonpolar and diffusible and is also known to be toxic at relatively high concentrations. Furthermore, there is fragmentary evidence that CS_2 is produced endogenously, even in humans, although

it is possible that this production is at least in part from the closely associated microbiome. The biological chemistry and metabolism of CS_2 suggest a commonality in biochemical targets with other SMBs. This includes reactivity with amino acids like cysteine and lysine and the formation of adducts in cytosol, serum, and proteins. Metal centers are also likely targets via the insertion of CS_2 into metal ligand bonds to form chelating ligands. These reactions readily occur at ambient temperatures and in aqueous media. Such pathways may initiate interconnected signaling cascades. For examples, reactions of CS_2 with biological nucleophiles in some cases release H_2S, and the inhibition of NF-□B is critically involved in inducible nitric oxide synthase activation. Furthermore, it is entirely likely that CS_2 generated by a specific enzyme, system, etc. would be ultimately consumed and metabolized in a specific manner when functioning normally, e.g., through oxidative desulfurization in the liver.

Major advances in the investigation of the bioregulatory, therapeutic, etc. roles of SMBs involved the development of strategies for controlled delivery. For example, researchers into the chemical biology of NO have long drawn on the availability of water-soluble salts of various "NONOate" anions, $R_2NN(O)NO^-$, that allow for *in vitro* and *in vivo* NO generation with well-defined half-lives ranging from minutes to hours.[208] Ultimately, analogous donors with predictable kinetics for CS_2 release under physiological conditions will need to be utilized to evince CS_2 interactions with specific biological targets. In this context, one can envision designing DTC or TTC compounds (or their metal complexes) that, given their reversible equilibria with amines or thiols, would release controlled quantities of CS_2 over time to *in vitro* or *in vivo* targets. An alternative strategy would be to use photochemical methods to uncage CS_2 as is being explored extensively with NO and CO.[209] The advantage of the latter strategy would be the ability to control location, timing, and dosage of such release.

The observations discussed herein by no means establish an unambiguous case for the bioregulatory or therapeutic roles of carbon disulfide. But they do point to the exciting potential represented by the chemical biology of CS_2 and the relatively unexplored research challenges and opportunities presented by these possibilities.

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6. Author contributions:

AWD, DFZ, and PCF each contributed to the preparation and editing of the full manuscript. AWD prepared the initial draft and all the figures and PCF completed the final draft. JMF critically read the manuscript and contributed Sec. 3.4.5.

7. Financial Interest:

None of the authors have competing financial interests relevant to the contents of this article.

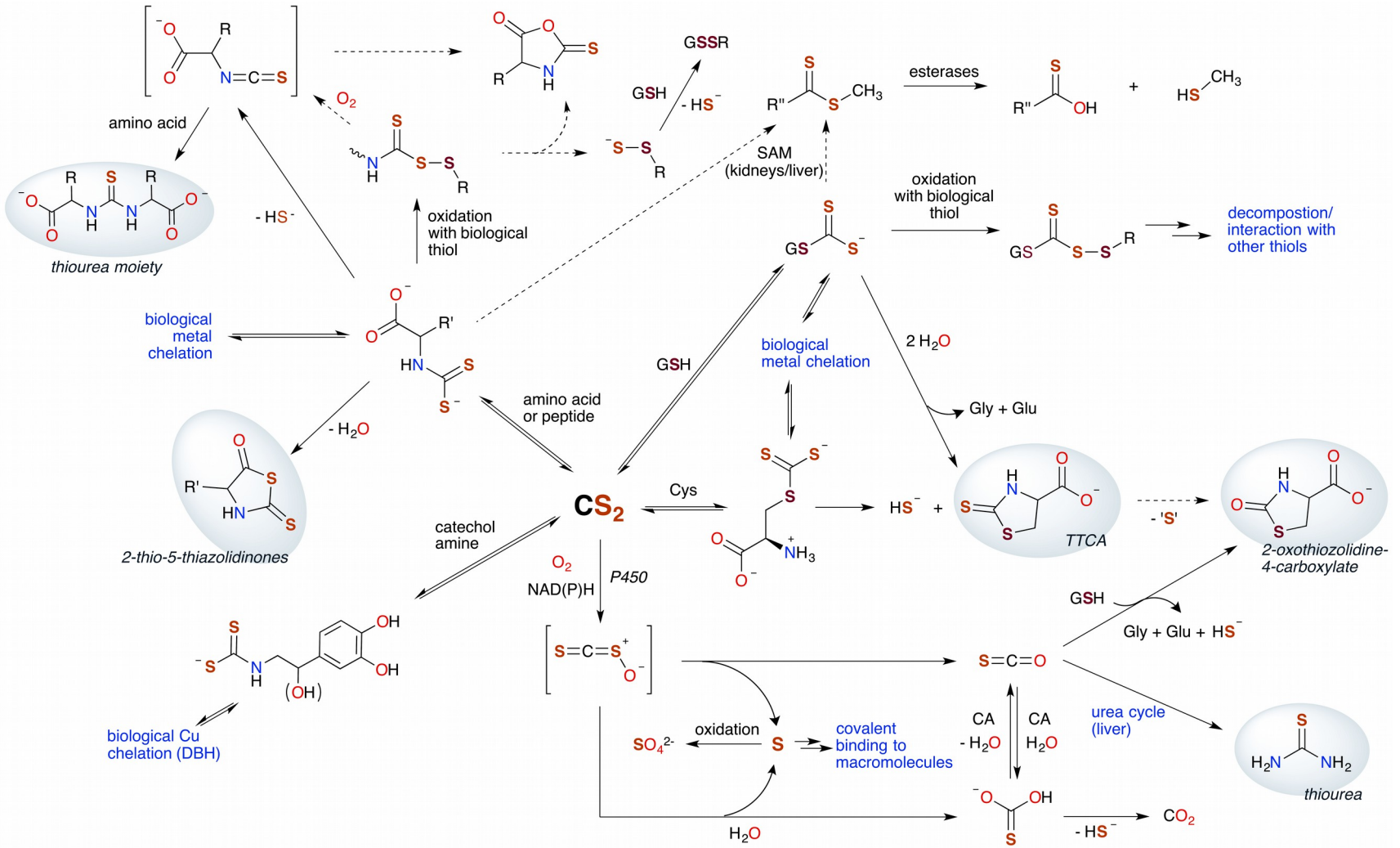

Figure 9. Hypothetical metabolic map for CS_2. Species highlighted in grey are known human urinary metabolites of CS_2..[30,90,171-175] The signaling chemistry is proposed to be through CS_2 reactions with biological nucleophiles. Dotted arrows represent possible, albeit minor, pathways. H_2S (HS^- under physiological conditions) is produced at several sites, suggesting an interplay between CS_2 and this SMB. (SAM = S-adenosyl methionine methyltransferases, CA = carbonic anhydrases, P450 = cytochrome P450 monooxygenase, DBH = dopamine β-hydroxylase, TTCA = thiazolidine-2-thione-4-carboxylate.)

8. Abbreviations used

AIDS	acquired immunodeficiency syndrome
ALDH	acetaldehyde dehydrogenase
AMO	ammonium monooxygenase
CA	carbonic anhydrase
cGMP	cyclic guanosine monophosphate
CNS	central nervous system
CO	carbon monoxide
CO_2	carbon dioxide
CoA	coenzyme A
CODH	carbon monoxide dehydrogenase
COS	carbonyl sulfide
CS_2	carbon disulfide
Cys	cysteine
DBH	dopamine β-hydroxylase
DEDTC	*N,N*-diethyldithiocarbamate
DTC	dithiocarbamate
GC-D	guanylyl cyclase, isoform D
Glu	L-glutamic acid
Gly	glycine
GSH	reduced glutathione
GSSG	oxidized glutathione dimer
H_2S	hydrogen sulfide
HIV	human immunodeficiency virus
I-κB	inhibitor NF-κB
IKK (α,)	I-κB kinase (α,-subunits)
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
Lys	L-lysine
MMO	methane monooxygenase
NAD(P)H	reduced nicotinamide adenine dinucleotide (phosphate)
NEMO	NF-κB essential modulator (also IKK)
NF–κB	nuclear factor κB
NO	nitric oxide
P450	cytochrome P450
SAM	S-adenosyl methionine transmethylase
Ser	L-serine
sGC	soluble guanylyl cyclase
SMB	small molecule bioregulator
TPCK	*N*-tosyl-L-phenylalanine chloromethyl ketone
TTC	trithiocarbonate
TTCA	thiazolidine-2-thione-4-carboxylic acid

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