Biological Chemistry of Naturally Occurring Thiols of Microbial and Marine Origin

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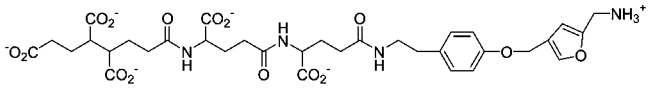
The presence of thiols in living systems is critical for the maintenance of cellular redox potentials and protein thiol-disulfide ratios, as well as for the protection of cells from reactive oxygen species. In addition to the well-studied tripeptide glutathione (γ-Glu-Cys-Gly), a number of compounds have been identified that contribute to these essential cellular roles. This review provides a survey of the chemistry and biochemistry of several critically important and naturally occurring intracellular thiols such as coenzyme M, trypanothione, mycothiol, ergothioneine, and the ovothiols. Coenzyme M is a key thiol required for methane production in methogenic bacteria. Trypanothione and mycothiol are very important to the biochemistry of a number of human pathogens, and the enzymes utilizing these thiols have been recognized as important novel drug targets. Ergothioneine, although synthesized by fungi and the Actinomycetales bacteria, is present at significant physiological levels in humans and may contribute to single electron redox reactions in cells. The ovothiols appear to function as important modulators of reactive oxygen toxicity and appear to serve as small molecule mimics of glutathione peroxidase, a key enzyme in the detoxification of reactive oxygen species.

Overview

In an era in which the importance of genomics and proteomics to the life sciences is emphasized, it can sometimes be easy to overlook the underpinning that chemistry plays in the elucidation of various biochemical processes in nature. For example, the importance of natural product isolation and characterization to our understanding of cellular biochemistry and physiology should not be underestimated. This review will survey the impact that the identification of various intracellular thiols (Figure 1), present at millimolar concentrations in various cells, has had on our expanding biochemical knowledge of complex life processes. As will become evident, the initial chemical characterizations of these particular thiols have led to an explosion of information on complex cellular biochemistry, having implications beyond the fundamental, in such areas as parasitology and drug design.

Coenzyme M (HSCoM)

Methanogenesis. The intracellular thiol 2-mercaptoethanesulfonate, or coenzyme M [HSCoM; (Figure 1, **1**)], was first characterized as a contributing cofactor to methane production in methanogenic microorganisms by McBride and Wolfe;[1] its structure, as the disulfide, was solved shortly thereafter.[2] The microorganisms that undertake methane production, the methanogens, belong to a separate phylogenetic domain, termed the *Archaea*.[3,4] Depending upon the specific methanogen, substrates as diverse as acetate, CO_2/H_2, formate, CO, methanol, methylamines, and methylthiols can be converted into methane gas in a thermodynamically favorable exergonic overall process beneficial to the growth of the particular microorganism (Table 1). It has been estimated that the methanogens may be the biogenic source of approximately 10^9 tons of atmospheric methane per year.[5,6] The concentration of HSCoM in methanogens has been determined to be in the millimolar range.[7] Disulfide reductases have been characterized in methanogens, such as *Methanothermobacter thermautotrophicus*, that maintain HSCoM in the reduced state.[8] In all cases, the metabolism of the above substrates in methanogens eventually leads to the formation of CH_3-SCoM, the methylated form of HSCoM. This molecule has been found to be the direct precursor to methane (see below).



8 Methanofuran (MFR)

Methane production from CO_2 results from a controlled stepwise reduction of CO_2 [initially bound to the cofactor methanofuran (MFR, **8**) as a carbamic acid] by six electrons to eventually produce methyl-tetrahydromethanopterin [CH_3-H_4MPT (Scheme 1, **9A**)], or equivalent, which is the source for methyl group transfer to HSCoM. There is some analogy here to the well-known C_1 chemistry of the tetrahydrofolates (THF).[10,11] The enzyme that catalyzes the transfer of the methyl group to HSCoM, methyl-tetrahydromethanopterin:coenzyme M methyltransferase, is a multienzyme complex that is membrane-associated and contains a cobalt corrinoid prosthetic group.[12−16] The activity of the enzyme complex is tightly linked to the formation of a sodium gradient across the membrane.[10,12,17,18] The cobalt corrinoid participates in the reaction mechanism for this enzyme.[19] The chemical mechanism of the transferase is believed to involve the reduction of cobalt to its enhanced nucleophilic Co(I) form followed by subsequent nucleophilic attack of the Co(I) on the methyl group of CH_3-H_4MPT. This is followed by transfer of the methyl group from the cobalt to the sulfur atom of HSCoM to produce CH_3-SCoM. An analogous reaction is that of the remethylation of homocysteine by 5-CH_3-THF to produce methionine by the cobalt-B_{12}-containing enzyme methionine synthase.[20,21]

The first step of methane production from other substrates such as methanol, mono-, di-, and trimethylated amines, methanethiol, and dimethyl sulfide occurs in a

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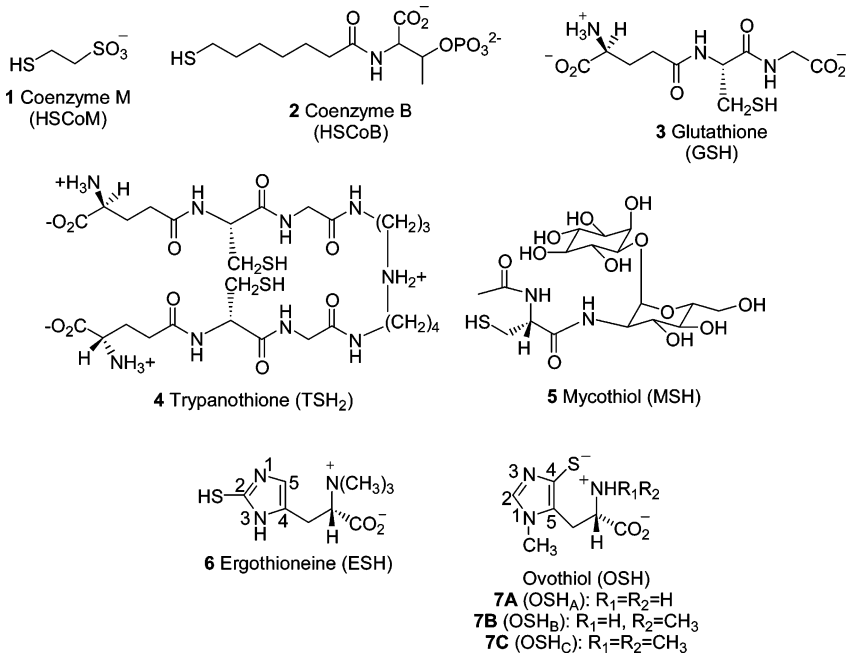


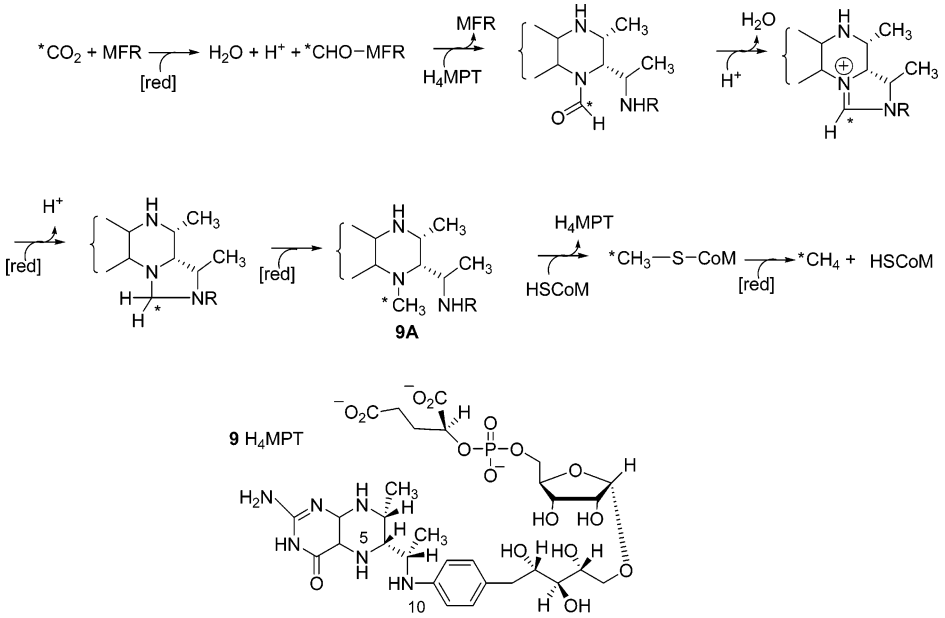
Figure 1. Major intracellular thiols found in microbial systems.

Table 1. Reactions and Gibbs Free Energies for Major Methane-Producing Pathways of Methanogens[a]

substrate	sample reaction	$\Delta G°$, kJ/mol CH_4
carbon dioxide[6]	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135
formate[6]	$4HCO_2^- + H^+ + H_2O \rightarrow CH_4 + 3HCO_3^-$	−145
carbon monoxide[6]	$4CO + 5H_2O \rightarrow CH_4 + 3HCO_3^- + 3H^+$	−196
ethanol[6]	$2CH_3CH_2OH + HCO_3^- \rightarrow 2CH_3COO^- + H^+ + CH_4 + H_2O$	−116
methanol[6]	$4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H_2O + H^+$	−105
	$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	−113
acetate[6]	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31
dimethyl sulfide[6]	$2(CH_3)_2S + 3H_2O \rightarrow 3CH_4 + HCO_3^- + 2H_2S + H^+$	−49
monomethylamine[9]	$4\ CH_3NH_3^+ + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ NH_4^+$	−77
dimethylamine[9]	$2(CH_3)_2NH_2^+ + 2H_2O \rightarrow 3CH_4 + CO_2 + 2NH_4^+$	−75
trimethylamine[6]	$4(CH_3)_3NH^+ + 9H_2O \rightarrow 9CH_4 + 3HCO_3^- + 4NH_4^+ + 3H^+$	−76

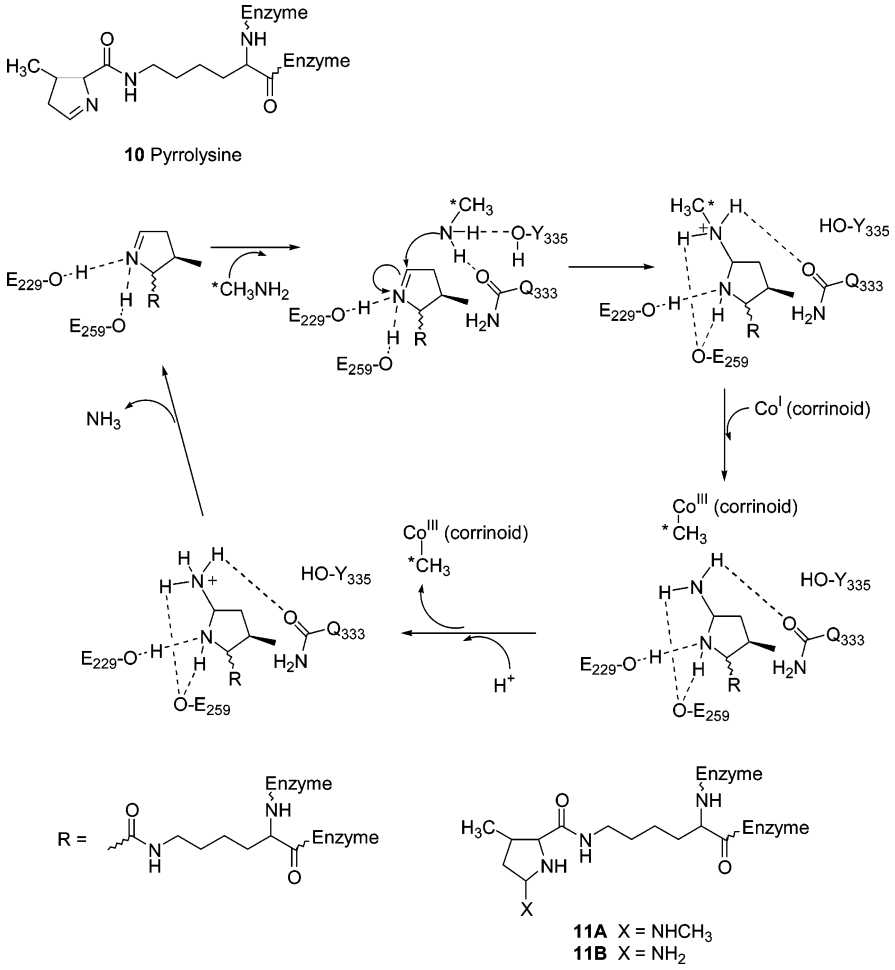
[a] Based on Zinder[6] and Deppenmeier.[9]

Scheme 1. Conversion of CO_2 to Methane Utilizing MFR, H_4MPT, and HSCoM

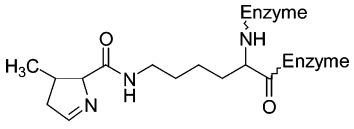


much different fashion.[22−27] Each substrate reacts with its specific methyltransferase (substrate:coenzyme M methyltransferase), which catalyzes methyl group transfer from that substrate to HSCoM to produce CH_3-SCoM and the demethylated substrate. To perform this transformation, *Methanosarcina barkeri* utilizes an unusual but naturally occurring amino acid in the active site of its monomethyltransferase. This very recently discovered amino acid, pyrrolysine (**10**), has been termed the 22nd "natural" protein amino acid[28−31] (the 21st having been identified as

Scheme 2. Proposed Mechanism for the Role of Pyrrolysine in the Transfer of the Methyl Group of Monomethylamine to HSCoM by Monomethylamine Methyltransferase from *Methanosarcina barkeri*[28]

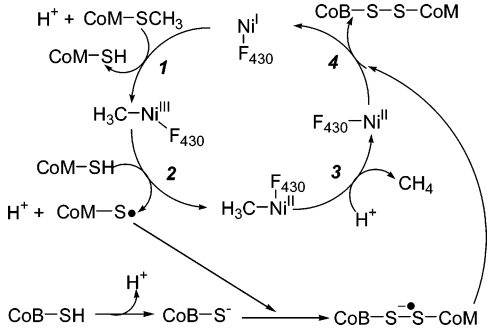


selenocysteine[32]). The presence of pyrrolysine in the active site of this enzyme may help in explaining the required chemistry involved in the transfer of a methyl group from monomethylamine to HSCoM, a rather difficult reaction to imagine mechanistically under the mild conditions present in the cell. On the basis of X-ray structural data on the enzyme and chemical models, it has been suggested that pyrrolysine directly takes part in the enzyme mechanism by virtue of its electrophilic nature (Scheme 2). The pyrrolysine is proposed to react with methylamine to produce the methylaminopyrrolysine adduct (Scheme 2, **11A**), allowing for a favorable attack of a Co(I) corrinoid on the properly oriented methyl group, yielding the aminopyrrolysine (Scheme 2, **11B**). Once transferred to the cobalt, the methyl group can undergo attack by HSCoM, in a step potentially similar to that of the latter half of the reaction for the above-mentioned methyl-tetrahydromethanopterin:coenzyme M methyltransferase.



The resulting CH_3-SCoM produced by any of the above reactions is then the substrate for reductive cleavage to methane and HSCoM, catalyzed by CH_3-SCoM reductase present in all methanogens.[33−36] The overall reaction

Scheme 3. Proposed Enzymatic Pathway for CH_3-CoM Reductase[a]



[a] ***1***: CH_3-SCoM is bound to the enzyme followed by the transfer of CH_3 to Ni(I) resulting in the formation of a Ni(III) species and free HSCoM. ***2***: HSCoM is oxidized to the thiyl radical, reducing CH_3-Ni(III) to CH_3-Ni(II). ***3***: CH_3-Ni(II) is converted to CH_4 and Ni(II) by protonation, concomitantly, a proton is removed from HSCoB, which then reacts with the •SCoM radical to give the mixed disulfide anion radical. ***4***: This radical returns Ni(II) to Ni(I), and the mixed disulfide is released. This mechanism is based upon that of Grabarse et al.[33]

catalyzed by this multienzyme complex is shown in Scheme 3.[33] This reaction utilizes yet another thiol, 7-mercaptoheptanoylthreonine phosphate (coenzyme B, HSCoB, **2**), which was first identified in 1980[37] and its structure elucidated in 1986.[38] The enzyme also utilizes a nickel porphinoid coenzyme (F_{430}) which cycles through Ni(I) to Ni(III) during the reaction mechanism. The final two-electron reductive cleavage of the methyl−sulfur bond in CH_3-SCoM produces a mixed disulfide (CoMS-SCoB) with the release of the disulfide and the methyl group as

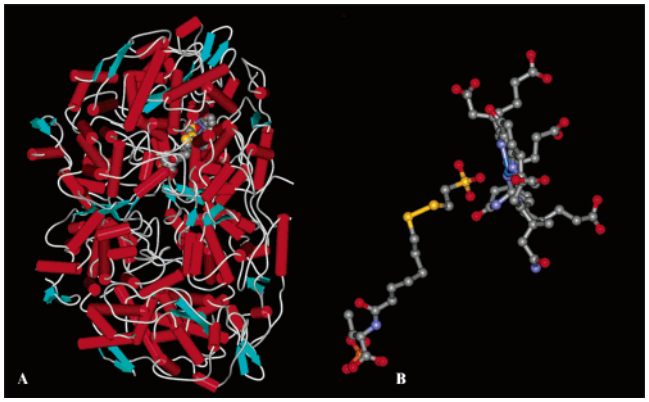


Figure 2. Schematic of the X-ray structure of CH_3-SCoM reductase from *Methanothermobacter thermautotrophicus*. CoMS-SCoB and F_{430} are shown in space-fill in the active site (A) and in ball-and-stick form in isolated view (B). HSCoM is close to the nickel porphinoid F_{430} cofactor in this enzyme−product complex based on coordinates (PDB 1HBM).[33]

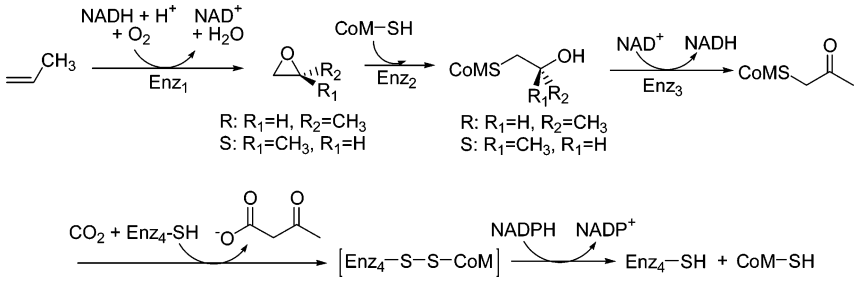
methane. The recent determination of the crystal structure of the CH_3-SCoM reductase has added immensely to our knowledge of the enzyme mechanism (Figure 2). Finally, the re-formation of the free thiol forms of HSCoM and HSCoB is accomplished by the enzyme CoMS-SCoB disulfide reductase. These disulfide reductases appear to be either flavoproteins or hemoproteins.[39−42]

It is particularly clear from the above discussion that the chemical identification and the utilization of HSCoM, CH_3-SCoM, and HSCoB as substrates and products to study the details of the process of methanogenesis have led to great strides in our understanding of this remarkable process.

Epoxide Biochemistry. Surprisingly, HSCoM has also recently been isolated from the Gram-negative bacterium *Xanthobacter* strain Py2[43,44] and the Gram-positive bacterium *Rhodococcus rhodochrous* B276.[45] In a nonmethanogenesis role, HSCoM has been linked to the bacterial catabolism of short-chain aliphatic alkenes as shown in Scheme 4. A monooxygenase epoxidation of the alkene substrate produces a mixture of enantiomeric epoxides, which are then conjugated to HSCoM by an epoxyalkane:coenzyme M transferase.[45] This enzyme is Zn^{2+}-dependent and appears to play a key role in activation of the thiol(ate) of HSCoM for nucleophilic addition to the epoxides.[46] The resulting adducts are oxidized to the corresponding ketones by stereoselective NAD^+-dependent dehydrogenases.[47,48] The ketones are carboxylated to acetoacetate by the enzyme NADPH:2-ketopropyl-CoM oxidoreductase/carboxylase with the intermediate formation of a CoM-*S*-protein mixed disulfide, which is subsequently reduced to the free HSCoM by this flavoenzyme.[49] The recent determination of the crystal structure of this enzyme has led to further insight into the mechanistic aspects of the carbon−sulfur cleavage reaction.[50] There has been recent evidence for the existence of a linear megaplasmid in *Xanthobacter* strain Py2 that codes for not only the enzymes for aliphatic alkene metabolism but also at least one of the genes for HSCoM biosynthesis.[51]

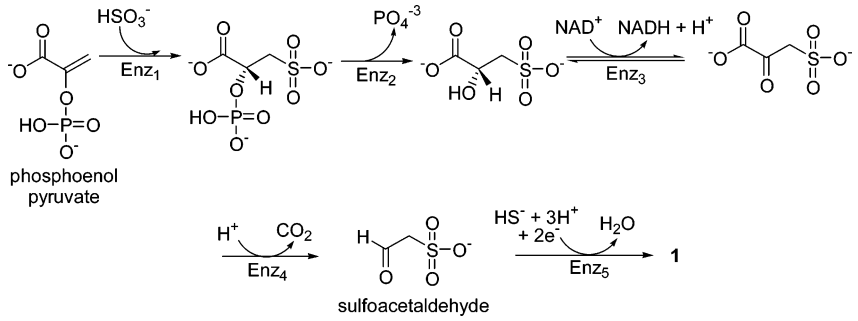
Biosynthesis. The simplicity of the chemical structure of HSCoM belies the complexity found for its biosynthesis in methanogens. White and co-workers have made impressive contributions in this area.[52] Several of the enzymes involved in the biosynthesis have recently been isolated and fully characterized. The pathway (Scheme 5) begins with the stereospecific addition of sulfite to phosphoenolpyruvate by phosphosulfolactate synthase.[53,54] The protein structure of this enzyme has recently been determined and suggests that a Mg^{2+}-stabilized enolate intermediate may be involved in the mechanism once adduct formation has proceeded.[54] Dephosphorylation by the Mg^{2+}-dependent 2-phosphosulfolactate phosphatase[55] followed by hydroxyl group oxidation by the enzyme (*R*)-sulfolactate (NAD^+-dependent) dehydrogenase[56] produces sulfopyruvate. Oxygen-sensitive sulfopyruvate decarboxylase,[57] which utilizes thiamine pyrophosphate as cofactor, decarboxylates the

Scheme 4. Catabolism of Epoxides Formed in *Xanthobacter* Strain Py2 and *Rhodococcus rhodochrous* B276 Using HSCoM[a]



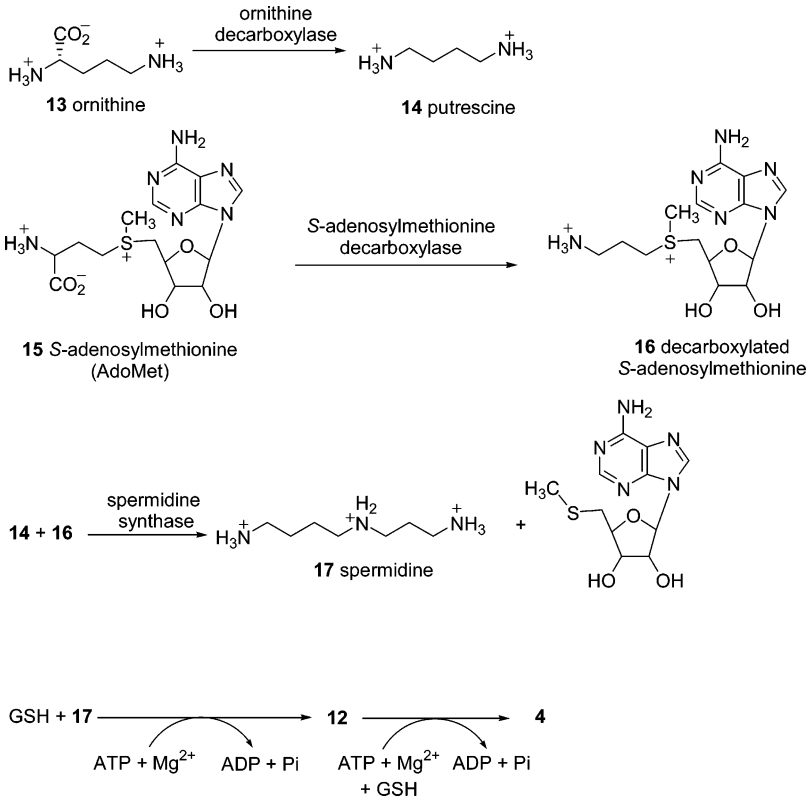
[a] Enz_1 = alkene monooxygenase; Enz_2 = epoxyalkane:HSCoM methyltransferase; Enz_3 = stereoselective NAD^+-dependent dehydrogenase; Enz_4 = NADPH-2-ketopropyl-HSCoM oxidoreductase/carboxylase.

Scheme 5. Biosynthesis of Coenzyme M[a]



[a] Enz_1 = phosphosulfolactate synthase; Enz_2 = 2-phosphosulfolactate phosphatase; Enz_3 = (*R*)-sulfolactate dehydrogenase; Enz_4 = sulfopyruvate decarboxylase; Enz_5 = unknown.

Scheme 6. Biosynthetic Pathway for the Formation of Trypanothione[a]

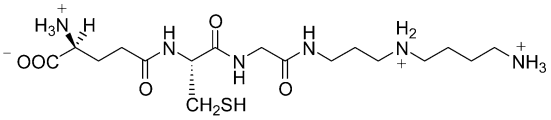


[a] The formation of trypanothione from two glutathione and one spermidine molecule appears to be catalyzed by trypanothione synthetase.

substrate to sulfoacetaldehyde and CO_2. The mechanism of this reaction has been suggested to be similar to the thiamine pyrophosphate-dependent decarboxylation of pyruvate by the enzyme pyruvate decarboxylase. The last enzyme in the pathway still remains an enigma, although the source of the sulfur for this reaction has been suggested to be the amino acid cysteine.[52,58] It is not known, however, if cysteine is directly involved in the enzyme mechanism or serves solely as the source of sulfide.

Intracellular Thiols Based on the Cysteine Nucleus

The presence of thiols in living systems is critical for the maintenance of cellular redox potentials and protein thiol-disulfide ratios, as well as for the protection of cells from reactive oxygen species (ROS). A number of important intracellular thiols containing the core amino acid cysteine (Figure 1: **3**, **4**, and **5**) appear to serve in these biochemical capacities. The best characterized thiol in this class is, of course, the tripeptide γ-L-glutamyl-L-cysteinylglycine (glutathione, γ-Glu-Cys-Gly, GSH, **3**). GSH is the predominant thiol in eukaryotic organisms (humans, animals, fungi, plants),[59] while homoglutathione (γ-Glu-Cys-β-Ala) is a major intracellular thiol in legumes.[60] GSH has also been detected in most Gram-negative bacteria (for example in the cyanobacteria and the purple bacteria).[59] Although GSH is present as the major intracellular thiol in the bacterium *Escherichia coli*, under stationary phase as well as anaerobic conditions, 80% of the GSH in *E. coli* actually exists as the conjugate N^1-monoglutathionylspermidine (**12**).[61] In halobacteria, the key intracellular thiols are thiosulfate ($-SSO_3^{2-}$) and the dipeptide γ-L-glutamyl-L-cysteine (γ-Glu-Cys).[62] GSH has not been detected in several of the major classes of Gram-positive bacteria.[63] Much has been determined with respect to GSH and its relationship to cellular reduction−oxidation (redox) processes, its activity as an enzyme cofactor, and its biosynthesis and degradation. Since GSH has an extensive scientific literature, readers are referred to several excellent reviews for a more detailed discussion.[64−69]



12 N^1-monoglutathionylspermidine

Trypanothione (TSH_2). The bis-glutathionyl conjugate of spermidine, termed trypanothione [N^1,N^8-bis(glutathionyl)spermidine, TSH_2, **4**], has been isolated from the pathogenic protozoa of the genera *Trypanosoma* and *Leishmania*.[70,71] Infection with these protozoa produces clinically and agriculturally important diseases such as African sleeping sickness (*Trypanosoma brucei;* 300 000 to 500 000 cases and 66 000 deaths annually),[72] Nagana cattle disease (*Trypanosoma congolense, Trypanosoma brucei brucei*; ~3 million cattle deaths with economic losses of U.S. $1 to 1.2 billion per year),[73] and Chagas disease in South and Central America (*Trypanosoma cruzi;* 16−18 million estimated cases with 300 000 new cases and 21 000 deaths per year).[74] Various forms of leishmaniasis (believed to affect 12 million people worldwide)[75] are caused by *Leishmania tropica*, *Leishmania donovani*, and *Leishmania brasiliensis*. First identified by Fairlamb and Cerami in 1985,[71] TSH_2 has been found to be a critical intracellular thiol in these protozoa. Over 70% of the intracellular GSH isolated from trypanosomatids is found in covalent linkage to spermidine (Scheme 6, **17**) as TSH_2.[76] Its isolation and characterization has been quintessential to our further understanding of the complex cellular biochemistry of these pathogenic microorganisms.

Biosynthesis. The biosynthesis of this natural product has been elucidated, and the pathway couples biosynthe-

sized GSH to spermidine, the latter itself originating from the amino acid ornithine (Scheme 6, **13**) and the propylamine moiety of decarboxylated *S*-adenosylmethionine (Scheme 6, **16**). It is interesting to note that *Trypanosoma granulosum* and *T. cruzi* epimastigotes are entirely dependent upon *exogenous* polyamines such as putrescine (Scheme 6, **14**) to supply components for spermidine biosynthesis, as they do not appear to have the enzyme ornithine decarboxylase required to produce putrescine.[77] Two enzymes responsible for the coupling of two molecules of GSH to the polyamine spermidine, glutathionylspermidine synthetase[78−81] and TSH_2 synthetase,[80−82] have been isolated from *Crithidia fasciculata*. Recently, the trypanothione synthase has been reported to accomplish the complete conversion of GSH to TSH_2;[83] this is similar to the activites of the TSH_2 synthetase enzymes found in *T. cruzi*[84] and *T. brucei*.[85] The deduced amino acid sequences of both these enzymes share ~50% sequence similarity to the *E. coli* N^1-monoglutathionylspermidine synthetase.[86,87] It is interesting to note that these enzymes from *C. fasciculata,*[78] as well as the *E. coli* enzyme,[87] also exhibit an amidase activity that can hydrolyze the glutathionyl−spermidine linkage. The enzyme mechanisms make use of acyl-phosphate intermediates to couple the amine functions to GSH.[79,87] The ratios of GSH, N^1-monoglutathionylspermidine, and TSH_2 can vary in these protozoa and are dependent on the growth phase of the particular microorganism as well as the levels of exogenous polyamines.[76,77] The availability of a synthetic scheme to prepare TSH_2 has allowed for its further study in cellular physiology, as well as in the design of inhibitors of TSH_2 biochemistry.[88,89]

Function. In many respects, the functions of TSH_2 parallel those found for GSH in other organisms.[90] Mirroring the chemistry first observed with GSH, TSH_2 biochemistry makes use of its thiol functions to control cellular redox[91] and to detoxify ROS[92−94] and reactive nitrogen species (RNS)[93,95,96] created by host cells to repel parasite invasion. At physiological pH, the thiol groups of TSH_2 are more reactive than that of GSH toward Ellman's reagent and 1-chloro-2,4-dinitrobenzene.[97] TSH_2 can reduce intracellular hydroperoxides with concomitant formation of the cyclic disulfide of TSH_2.[91]

The formation of the intramolecular disulfide of trypanothione (TS_2) during these processes requires the presence of the corresponding trypanothione (disulfide) reductase to reactivate this cofactor. This reductase has been characterized and crystallized from the nonpathogenic trypanosomatid *C. fasciculata*[98−100] as well as from *T. cruzi*.[101−103] Although not accepting GSSG as an alternate substrate,[103] the enzymes resemble in structure and in mechanism the enzyme glutathione (disulfide) reductase: trypanothione (disulfide) reductase is a flavoenzyme homologous to GSH reductase.[104] The reductase reaction begins with the reduction of the flavin with NADPH. Reduction of a cystine disulfide in the active site by the reduced flavin follows. One of the protein cysteines can then react with TS_2 to produce a mixed disulfide followed by attack of the second protein cysteine on the cysteine linked to one of the sulfurs of the trypanothione. This step releases the reduced TSH_2 and re-forms the cystine disulfide in the enzyme. Interestingly, trypanosomatids lack glutathione (disulfide) reductase,[71] and maintenance of reduced GSH levels is achieved both nonenzymatically and enzymatically, the latter through trypanothione-glutathione thioltransferase.[105,106] Trypanothione reductase is believed to be the target for arsenical[107,108] and antimonial[109−111] compounds, which exhibit antiparasitic activity. Cellular buildup of the trypanothione disulfide blocks critical cellular reactions dependent upon the reduced form (TSH_2).[112,113] In addition, synthetic compounds and several natural products, such as kukoamine A[114] (**18**), lunarine[103] (**19**), and (*E*,*Z*)-ajoene[115] (**20**) (Figure 3), have been found to be inhibitors of the reductase.[116]

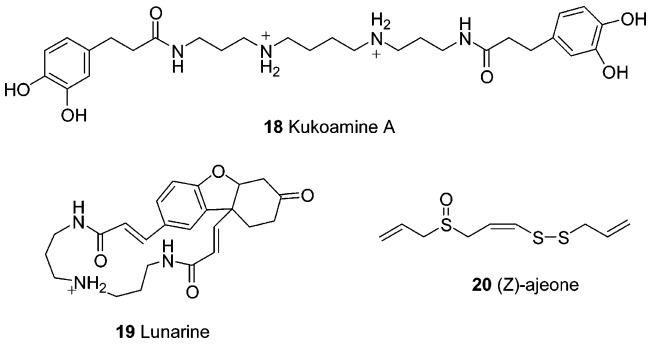
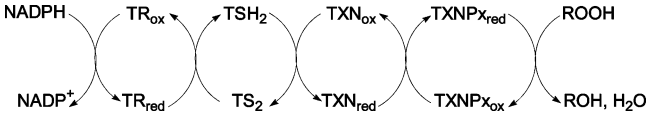


Figure 3. Selected natural products known to inhibit trypanothione reductase.

Scheme 7. TSH_2 Antioxidant System[a]



[a] TR: trypanothione reductase, TSH_2: reduced trypanothione, TS_2: trypanothione disulfide, TXN: tryparedoxin, TXNPx: tryparedoxin peroxidase. This figure is based upon that of Flohe et al.[92]

GSH protects cells against oxidative damage by nonenzymatic scavenging of free radicals and by enzymatic neutralization of toxic H_2O_2 and lipid hydroperoxides by GSH-dependent peroxidases.[66,117] A number of trypanosomatids also contain a TSH_2-dependent peroxidase system.[92,94] The hydroperoxide metabolism of *C. fasciculata* consists of trypanothione reductase, tryparedoxin (TXN),[118] and tryparedoxin peroxidase (TXNPx).[119] Evidence for the existence of this system in *T. cruzi* has also been reported.[94] TXN may act as the equivalent of thioredoxin in trypanosomatids. TXN can transfer reducing equivalents to TXNPx from TSH_2 (Scheme 7). It may also serve, in its reduced form, as a source of reducing equivalents for ribonucleotide reductase,[120] much like thioredoxin does in other organisms. The peroxidase belongs to the family of peroxiredoxins and contains two conserved cysteine residues, one of which appears to react with the hydroperoxide substrate. The resulting sulfenic acid is attacked by the second protein cysteine to produce the disulfide and water as a product.[121] The reduced form of TXN then reduces the oxidized TXNPx, reactivating the peroxidase for another catalytic reaction. The now oxidized TXN is reduced by interaction with reduced TSH_2.[92] The molecular structures of TXN and TXNPx have been recently determined.[122,123]

Another function of GSH in organisms is the enzyme-catalyzed conjugations of GSH with electrophiles, and hence GSH can be involved in xenobiotic metabolism and protection of the cell from reactive compounds.[124−129] GSH transferases couple GSH to a variety of electrophilic compounds to neutralize their toxic actions on proteins and nucleic acids.[66,125,126] Recently, TSH_2-*S*-transferase activity has been detected in a number of *Leishmania* and *Trypanosoma*. Surprisingly, the purified activity of this enzyme from *C. fasciculata* was found to be an activity of trypanosomatid ribosomal elongation factor 1B.[130,131] Another detoxification pathway that utilizes GSH in many organisms is the glyoxalase (Glx) system, which is composed of the enzymes GlxI[132,133] and GlxII.[134] GlxI converts cytotoxic pyruvaldehyde (methylglyoxal) to the thioester of GSH (*S*-

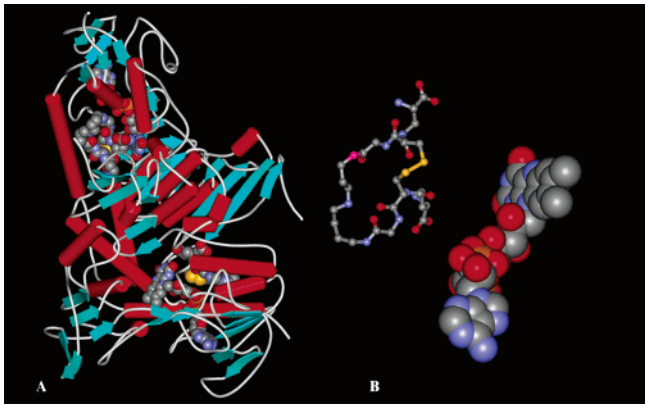


Figure 4. (A) Schematic of the X-ray structure of trypanothione reductase from *Trypanosoma cruzi*. Both FAD and trypanothione disulfide (space-fill) are shown in each of the two active sites in the dimeric form of the enzyme. (B) Isolated view of trypanothione disulfide (ball-and-stick) and FAD (space-fill) from one of these active sites based on coordinates (PDB 1BZL).[103]

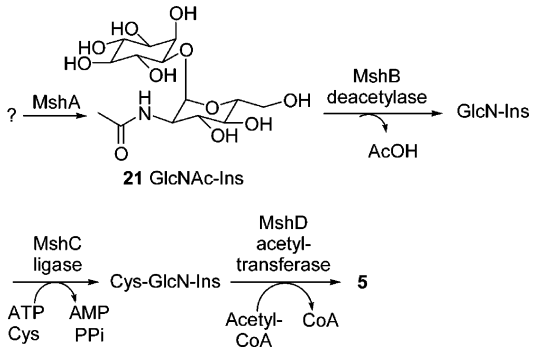
D-lactoylglutathione). This is followed by hydrolysis to D-lactate and GSH by GlxII. Recently, the presence of a related system utilizing TSH_2 instead of GSH as cosubstrate has been detected for GlxI from *Leishmania major*[135] and GlxII[136] from *T. brucei*. The GlxI enzyme from *L. major* appears to have close similarity to the nickel-activated GlxI enzymes from several bacterial species.[137,138]

Leishmania and some *Trypanosoma* are able to survive in macrophages by evading ROS and RNS produced by macrophages using an inducible nitric oxide synthase.[96] The metabolism of nitrosothiols, notably *S*-nitrosoglutathione, as well as mono- and di-*S*-nitrosotrypanothione, has been suggested to involve other intracellular thiols found in *Trypanosoma* such as ovothiols[95] (see below).

Drug Design. By genetic experiments, it has been determined that inactivation of TSH_2 function, by inhibition of either its biosynthesis or its intracellular levels by inactivation of trypanothione reductase (which directly controls active reduced TSH_2 levels), is detrimental to the viability of *L. donovani* and *T. brucei*.[112,113] Because TSH_2 is found in a number of human parasites, and clearly plays an important role in their cellular physiology, inhibitors of the biosynthesis or biochemistry of TSH_2 have been explored as potential antiparasitic agents.[116,139−146] Inhibition of GSH biosynthesis, such as with the γ-L-glutamyl-L-cysteine synthetase inhibitor buthionine sulfoximine,[147,148] causes GSH, and hence TSH_2, depletion in *Trypanosoma*. This condition is found to be toxic to these organisms. The crystal structures of the *T. cruzi* trypanothione reductase in complex with TSH_2 (Figure 4),[103] N^1-glutathionylspermidine disulfide,[99] mepacrine,[149] and quinacrine mustard[150] are serving as key leads for the design and synthesis of synthetic inhibitors of this enzyme.[90,142,151,152] Interestingly, on the basis of database searches and molecular modeling, a class of cyclic-polyamine natural products, notably lunarine (Figure 3, **19**), has been determined to act as novel trypanothione reductase inhibitors,[103] which should bode well for future developments in the medicinal chemistry of these pathogens.

Mycothiol (MSH). Another important intracellular thiol that has been identified in several microorganisms is the compound (1-D-*myo*-inosityl-2-(*N*-acetyl-L-cysteinyl)amino-2-deoxy-α-D-glucopyranoside), termed mycothiol (MSH, Figure 1, **5**). Although containing the cysteine nucleus as the active moiety in this cofactor, the molecular framework is that of a disaccharide containing *myo*-inositol (Ins) and glucosamine (GlcN). MSH was identified in its symmetrical disulfide form (MSSM) from *Streptomyces* sp. AJ 9463,[153]

Scheme 8. Steps in the Biosynthesis of Mycothiol



and as the free thiol (previously referred to as U17[154]), from *Mycobacterium bovis*[155] and *Streptomyces clavuligerus*;[156] the corrected structure[157] is shown in Figure 1.[158] A survey of the presence of MSH in various organisms, as detected by the formation of its bimane derivative, indicates that MSH biosynthesis appears to be exclusive to the Actinomycetales bacteria.[159] Organisms known to produce MSH (such as mycobacteria and streptomyces for example) have been shown to lack both GSH as well as TSH_2.[59,159] Hence MSH is the major intracellular thiol in these organisms.

Biosynthesis. A biosynthetic pathway for MSH in *Mycobacterium smegmatis*, and presumably occurring in all MSH-containing organisms, has been proposed on the basis of the isolation of various intermediates produced by wild type as well as mutants of *M. smegmatis* (Scheme 8).[160,161] MshA is believed to catalyze the linkage of inositol (or a close analogue) to *N*-acetylglucosamine to give GlcNAc-Ins (Scheme 8, **21**).[162] Although the enzyme has been cloned, enzymatic activity utilizing inositol and UDP-GlcNAc has not proved successful, although success might be achieved with other derivatives of inositol such as phosphoinositol.[162] The gene for MshB has also been cloned from *Mycobacterium tuberculosis*,[163] and the gene product has observed deacetylase activity, which produces the deacylated disaccharide GlcN-Ins.[164] It is believed that this enzyme may be important in regulating MSH biosynthesis.[164] Recent solutions of its molecular structure by crystallography, independently reported by two groups,[163,165,166] have indicated that this enzyme is a zinc hydrolase containing a lactate dehydrogenase fold. MshC is a ligase that couples cysteine to the amino group of the glucosamine in GlcN-Ins utilizing ATP. The products of this reaction are AMP, pyrophosphate, and Cys-GlcN-Ins. It has been reported to be related to Class I cysteinyl-tRNA synthetases, and the enzyme activates cysteine through formation of a cysteine-AMP derivative.[167] This activated intermediate is subsequently attacked by the amino group of GlcN-Ins. MshD, also known as MSH synthase, catalyzes the acetylation of the α-amino group on the cysteine in Cys-GlcN-Ins by acetylCoA.[168] The *M. tuberculosis* enzyme has just recently been crystallized and its molecular structure determined (Figure 5).[169] The structure of the enzyme exhibits homology to the Gcn5-related *N*-acetyltransferase enzymes.

Chemical syntheses have been reported for MSH;[170,171] unfortunately, none of these syntheses are particularly efficient modes for the production of large quantities of MSH. A recently reported protocol for the isolation of milligram quantities of MSH from *M. smegmatis* should aid in providing sufficient quantities for further studies of the cellular biochemistry of this unique thiol.[172]

Function. Pathogens[173,174] containing MSH appear to use this natural product as a protectant against electrophilic compounds as well as for the detoxification of ROS

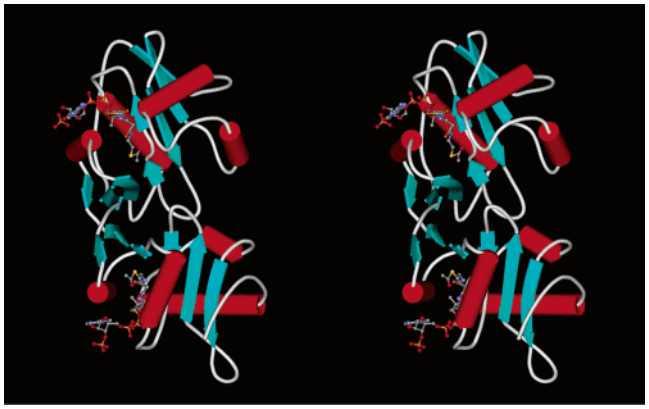


Figure 5. Stereoview (relaxed) of a schematic model of mycothiol synthase from *Mycobacterium tuberculosis* H37Rv. Two models of acetylSCoA are shown in ball-and-stick form. Based on coordinates (PDB 1OZP).[169]

and RNS. For example, it has been shown that *M. tuberculosis*, an organism that is increasingly detected in clinics due to multidrug resistance and is responsible for the deaths of 2 million people per year worldwide,[174] uses MSH to conjugate and detoxify antimycobacterial drugs in a manner similar to the formation of mercapturic acid derivatives in GSH detoxification mechanisms.[175] Evidence exists for the formation of MSH adducts of various antimycobacterial agents such as cerulenin (**22**) and rifamycin S (**23**) (Figure 6).[176] This is believed to occur by the action of a MSH-*S*-transferase (as yet unidentified), which produces the MSH-drug adduct. MSH-*S*-conjugate amidase (Mca), which has been isolated and characterized, catalyzes the hydrolysis of the *N*-acetylcysteine-*S*-adduct from the disaccharide.[175−177] This reaction produces GlcN-Ins and AcCysSR, where R is the drug molecule. The resulting AcCysSR is then excreted from the cell. The enzyme as isolated from *M. tuberculosis* contains a zinc ion. Studies have indicated that the enzyme is highly specific for the MSH structure but is promiscuous with respect to the structure of the attached drug molecule.[176]

MSH mirrors GSH and TSH_2 in its redox biochemistry as well.[178] In the process of peroxide reduction, the MSH disulfide is formed as a product. A corresponding MSH (disulfide) reductase has been identified, which recovers the disulfide (MSSM) to active MSH.[179−181] Although no structural data are yet available on the enzyme, MSH (disulfide) reductase is known to be a flavoenzyme and may therefore be mechanistically similar to the GSH and TSH_2 reductases.[180,181] It is interesting to note that a truncated version of MSH (des-*myo*-inositol MSH) has been shown to act as a substrate for this enzyme, allowing for the possible use of simpler structural analogues in the design of compounds targeting this pathway.[179]

MSH detoxification of reactive nitrogen species, such as NO•, results in the intermediate formation of *S*-nitrosomycothiol. This compound can then react further to form the unstable *N*-hydroxysulfenamide of MSH, which ultimately leads to the formation of nitrate. The enzyme that accomplishes this, surprisingly, has been identified as a formaldehyde dehydrogenase.[182]

Drug Design. MSH-deficient *M. smegmatis* mutants have been shown to be hypersensitive to a host of toxic agents such as alkylating agents, free radicals, and antibiotics,[183,184] and hence targeting the MSH biosynthetic and metabolic pathways with inhibitors may allow for the development of novel strategies against mycobacteria.[185]

For example, the inhibition of the MSH-*S*-transferase or the *S*-conjugate-amidase may allow increased sensitivity

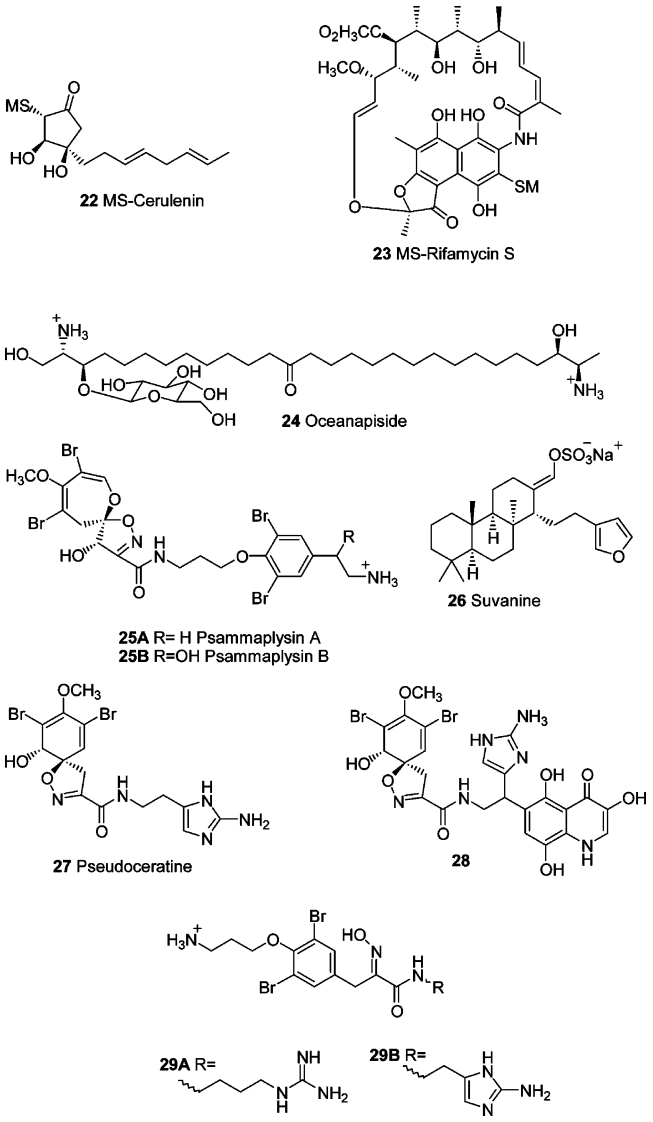


Figure 6. Compounds known to interact with mycothiol-*S*-conjugate amidase. Of these, **22** and **23** are known substrates, while **24**, **25A**, **25B**, **26**, **27, 28**, **29A**, and **29B** are inhibitory agents. MS = mycothiol.

to established antimycobacterial agents such as rifamycin S.[186] This blocked process would be expected to increase the intracellular levels of antimycobacterial agents in these cells. The *S*-conjugate amidase has been shown to be inhibited by a number of natural products including oceanapiside (**24**), psammaplysins A (**25A**) and B (**25B**), and suvanine (**26**)[187] and the bromotyrosine alkaloids pseudoceratine (**27**), **28**, **29A**, and **29B** (Figure 6).[188] The synthesis of novel alkaloids **28** and **29A**, isolated from the Australian nonverongid sponge *Oceanapia* sp.,[188] has recently been reported.[189] A study on the conformation of the *S*-conjugate bimane of MSH in solution has been reported,[190] which may aid in the design of inhibitors of *S*-conjugate amidase. It comes as no surprise, therefore, that the MSH biosynthetic and detoxification pathways are the subject of intense interest for the development of novel antimycobacterial agents to treat tuberculosis.[187,188,191,192]

Aromatic Intracellular Thiols (Thiolhistidines)

In addition to the aliphatic-based thiols presented above, naturally occurring aromatic thiols have been isolated and their properties investigated.

Ergothioneine (ESH). Ergothioneine (ESH) was initially isolated in 1909 by Tanret from ergot,[193] the fungal

infection of rye grain, and its structure was determined indicating ESH to be a betaine of 2-thiol-L-histidine (Figure 1, **6**).[194] ESH has since been detected in plants,[195−198] fungi,[199−201] bacteria,[201] animals,[202−204] and humans[205,206] in millimolar concentrations. In mammals, ESH has been detected in the liver,[207] kidney,[207] and blood,[205] notably the red blood cells.[208,209] Human red blood cells have been shown to contain 1.5−3.7 mg/100 mL of blood,[210] while human lenses contain 68−115 mg/100 g of lens.[206] To date, evidence for the biosynthesis of ESH has only been noted in certain fungi,[201] and in the Actinomycetales bacteria,[211] which includes the medically important bacterium *M. tuberculosis*. *Bacillus*, *Clostridium*, *Corynebacterium*, *Escherichia*, *Lactobacillus*, *Propionibacterium*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Vibrio* have been shown to be incapable of biosynthesizing ESH.[211]

It is believed that most organisms obtain ESH from their environment. Plants are thought to obtain ESH from the soil;[195] oat seedlings have been shown to incorporate radioactive ESH via their root system,[212] while animals and humans obtain ESH from dietary sources.[213] In animals, the amount of ESH present depends on tissue type,[214] diet,[197,198] and species.[204] Animals, and therefore humans, store ESH as the free form in tissues that are known to be exposed to oxidative stresses, including red blood cells,[203,208,209] seminal fluid,[215,216] the ocular lens,[204,206] liver,[207,217] and kidneys,[207] at levels that can reach millimolar. ESH is also found in the brain in micromolar amounts.[202]

ESH is sequestered by red blood cells at concentrations 2−9 times greater than that of blood plasma.[218] Early studies indicated that the incubation of red blood cells with ESH had no effect on the cell's intracellular ESH concentration.[217,219,220] This, combined with the knowledge that the concentration of ESH within red blood cells decreases with the age of the cell,[220,221] led to the conclusion that ESH was incorporated during red blood cell formation.[222] Recent work[218] using updated chromatographic techniques appears to demonstrate that red blood cells incubated with 1 mM ESH for 4 h do in fact gradually uptake ESH. These cells maintained their ESH levels against a concentration gradient in blood plasma. The uptake mechanism of red blood cells is unknown at this time but has been postulated to be transporter dependent.[218]

A study of bovine and porcine concentrations of ESH in ocular tissue indicated that ESH was present in all ocular tissues studied. The concentration of ESH in bovine lens was 17−25 times that of the cornea and retina.[204] It is believed that the high level of ESH may be important for lens transparency, as there appears to be an inverse relationship between cataracts and ESH concentration in the human lens.[206]

Biosynthesis. Initial studies of ESH biosynthesis in fungi have shown that histidine is incorporated intact into ESH (Scheme 9).[223,224] The three methyl groups of the trimethylammonium moiety are derived from *S*-adenosylmethionine via methylation,[224] and the sulfur atom appears to originate from cysteine.[223] There are two possible routes for the biosynthesis of ESH depending on the order of the methylation and sulfination: that is, the intermediacy of either thiolhistidine or hercynine (Scheme 9, **30**). Studies in *Neurospora crassa* using radiolabeled substrates suggested that methylation occurs first followed by sulfination, as radiolabels from hercynine were incorporated into ESH[225] while ^{14}C-thiolhistidine did not result in radiolabeled ESH.[223] Further work utilizing cell extracts of *N. crassa* indicated that O_2 and Fe^{2+} are required for the transfer of sulfur to hercynine. An intermediate in this process, *S*-(*β*-amino-*β*-carboxyethyl)ergothioneine sulfoxide (Scheme 9, **31**), was identified, which was then shown to be converted to ESH and pyruvate via a pyridoxal-requiring enzyme.[226] The use of ^{14}C and ^{35}S as precursors has indicated that the biosynthetic pathway of ESH in bacteria parallels that of fungi.[227]

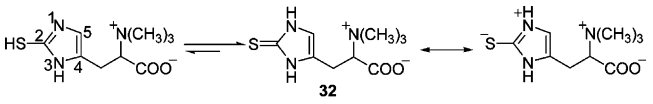


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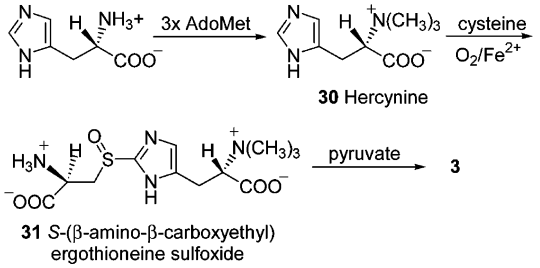
Figure 7. Ergothioneine tautomer structures.

Scheme 9. Proposed Steps in the Biosynthetic Pathway of Ergothioneine[a]



[a] The conversion of hercynine to ergothioneine is known to require the presence of oxygen and Fe^{2+};[226] however, no further details have been reported to date.

Chemical Properties. ESH differs from GSH, TSH_2, and MSH in that it exists primarily as a thione under physiological conditions (Figure 7, **32**).[228] The thione form was confirmed by the determination of the X-ray structure of the ESH dihydrate,[229] which exhibited a S−C bond length of 1.691 Å, a value intermediate between the S−C single and double bond lengths of 1.82 and 1.56 Å, respectively. ESH can exist in three different tautomeric forms (Figure 7).

Because of the thione/thiol tautomerism, ESH differs in reactivity from aliphatic thiols such as GSH. ESH has considerable nucleophilic character in both the neutral thiol and thione forms.[230] Like a typical sulfhydryl, ESH will react with iodoacetamide,[231] nitroprusside,[232] and bromobimanes.[200] Unlike sulfhydryls, however, ESH will not react with *N*-methyl-*N*′-nitro-*N*-nitroguanidine,[233] 4-nitroquinoline-*N*-oxide,[233] and metmyoblobin (metMb).[234] ESH has been shown to react with 2,2-diphenyl-1-picrylhydrazyl (DPPH),[235] a stable free radical that is known to react with antioxidants and will react with sulfhydryl groups by removing the hydrogen atom homolytically to form a sulfhydryl radical.[236] Unlike GSH, ESH and the model compound, 1-methyl-2-thiolimidazole, were determined not to react with carbon-centered or aliphatic peroxy radicals, due to the predominance of the thione form in neutral, aqueous solution.[237] At a higher pH, ESH ionizes to the thiolate, and although a better electron donor, still remains refractory to carbon-centered radicals.[237]

The most significant difference between ESH and other thiols is the instability of the ESH disulfide (ESSE) under physiological conditions. Most low molecular weight thiols, such as GSH, MSH, and TSH_2, are known to form disulfides upon exposure to aerated aqueous solutions, and organisms express reductase enzymes to maintain these thiols in their reduced form (see above). ESH, however, is stable in its reduced form in aerated aqueous solutions, and ESSE does not form under conditions that oxidize GSH.[232] ESH has an $E_o' = -0.06$ V[238] and is therefore more difficult to oxidize,[234] unlike other thiols, such as GSH, which has an $E_o' = -0.25$ V.[239] Only when the solution

containing ESH is strongly acidic is ESSE stable enough for the free and oxidized forms to exist simultaneously.[232] The instability of ESSE makes it impossible to detect in physiological systems, even if ESSE is formed, as the presence of large amounts of GSH in plant and animal systems would maintain ESH in the reduced state. At physiological conditions, the equilibrium between ESH and ESSE lies completely in the reduced state.

Function. ESH has been shown to have important antioxidative properties.[240] ESH has been shown to quench $^1O_2$ formation by several photosensitizers[241,242] and to have a strong quenching effect on indole fluorescence. The quenching of fluorescence and $^1O_2$ production indicates that ESH could be involved in protecting against photochemical damage caused by photosensitizing substances.[243]

$HO^\bullet$ is one of the most reactive biologically important free radicals, reacting at diffusion-controlled rates with cellular biomolecules.[244] Studies have determined that ESH can scavenge $HO^\bullet$ in a biologically relevant manner,[245] and the presence of a possible radical cation intermediate (in a thiol form) produced in the process was detected.[246] This intermediate appeared to decay to give a species that absorbed in the range 280−360 nm, where ESSE is believed to absorb. The similarities of these results with those for the model compound, 2-thioimidazole, suggest that the side chain of ESH has little effect on its oxidation reactions.

2-Imidazolethiones, such as ESH, protect ascorbic acid from copper(II)-catalyzed oxidation.[247] ESH may also be involved in a cooperative interaction with ascorbic acid to protect biological systems from ROS. The single-electron reactions of ascorbic acid and ESH have been investigated using an N_2O-saturated aqueous solution of 0.05 M NaN_3 with 1 mM ESH and 0.2 mM ascorbic acid.[246] It was estimated that 90% of the azide radicals reacted initially with ESH, which then decayed to give the semidehydroascorbate radical and repaired ESH. This result indicates that a cooperative interaction between ascorbic acid and ESH exists that could play an important role in the protection of biological systems from ROS.[246]

In addition to the stress caused by ROS, biological systems must also combat nitrosative stresses caused by RNS. Nitrogen can form a number of oxides of biological significance, including peroxynitrite ($ONOO^-$), which is formed by the reaction of nitric oxide ($NO^\bullet$) and superoxide at a near diffusion-controlled rate.[248] $ONOO^-$ is a nitrating agent and a powerful oxidant that can react with a number of biomolecules.[248] It has been implicated in the nitration of tyrosine residues in proteins, DNA oxidation, neuronal degradation, and enzyme inhibition,[244] the toxicity of which ESH has been shown to be effective in reducing. For example, the presence of ESH at low concentrations decreases nitration of tyrosine by $ONOO^-$ in a concentration-dependent manner; ESH was more effective than GSH or Trolox C, a vitamin E analogue.[249] ESH also decreases the oxidation of guanidine and adenine to xanthine and hypoxanthine in neuronal cell lines and in calf thymus.[249] These results may have implications for inflammatory conditions such as gout, which is characterized by an overproduction of uric acid, the oxidation product of xanthine, and other defects of purine metabolism.

$ONOO^-$ has been implicated in the neuronal degradation caused by Alzheimer's disease.[250,251] The generation of $ONOO^-$ and $NO^\bullet$ is believed to be the cause of apoptosis of cultured neurons exposed to β-amyloid peptide (βA), which is associated with senile plaques formed in the brains of Alzheimer's patients.[252] The neurons in the brain of a patient affected by Alzheimer's were found to display increased levels of inducible nitric oxide synthase (iNOS) and nitrosotyrosine, markers of $ONOO^-$ formation.[250,251] ESH was found to attenuate intracellular formation of $ONOO^-$ by βA, and it is believed that ESH may act through the scavenging of $ONOO^-$ rather than the control of its production. ESH has also been shown to protect against $ONOO^-$-mediated PC12 cell death in a concentration-dependent manner.[253] ESH did not block the production of $ONOO^-$ nor the induction of inducible nitric oxide synthase expression, which adds credence to the hypothesis that ESH acts as a scavenger of $ONOO^-$.[253] In support of ESH as a scavenger of $ONOO^-$, ESH has been shown to protect α_1-antiproteinase (αAP) from inactivation by $ONOO^-$.[254,255] ESH protects αAP from HOCl.[245] Few compounds can react with HOCl fast enough to inhibit this inactivation; however, ESH has been shown to protect αAP, indicating that it can scavenge HOCl at a biologically significant rate in addition to scavenging $ONOO^-$.

Both $NO^\bullet$ and $ONOO^-$ can react with thiols to form *S*-nitrosothiols.[248] *S*-Nitrosothiols have a fundamental role in many biological processes including vasodilation[256] and inhibition of platelet aggregation.[257] Transnitrosation can occur between thiols and *S*-nitrosothiols.[258] The decomposition of *S*-nitrosothiols is induced by UV radiation, metal ions, and other thiols. The major *S*-nitrosothiol found in the body is GSH based (GSNO). It has been found that ESH can accelerate GSNO decomposition in a concentration-dependent manner, to a rate faster than that of GSH itself.[230] In the presence of ESH, ammonia was determined to be the main nitrogen-containing end product in these reactions.[230] Another RNS of biological significance is sodium nitrite, which can oxidize oxyhemoglobin (oxyHb) to methemoglobin (metHb) through the formation of reactive intermediate species such as H_2O_2, $NO_2^\bullet$, and ferrylhemoglobin (ferHb).[259] Molecules containing the 2-thiolimidazole nucleus, such as ESH, have been found to inhibit the formation of metHb under these conditions.[259,260]

Reactions with Metals. Thiols, such as GSH, are known to be auto-oxidized in the presence of heavy metals such as copper and iron to form disulfides and peroxides.[261,262] These metals do not cause ESH oxidation to ESSE; instead, ESH chelates divalent metals.[263] It is most likely that ESH forms complexes with copper, nickel, cadmium, zinc, mercury, and cobalt through its sulfur atom. At physiological pH, copper(II) complexes with ESH are stable and do not decompose to give oxygen radicals, unlike with GSH or other thiols.[264,265] ESH binds to copper in such a way that the copper-catalyzed production of $HO^\bullet$ is inhibited.[245] Copper(II) can accelerate the single-electron reactions of ESH, as well as those of other 2-thiolimidazoles.[235] The ability of ESH to complex copper(II) may also be important in the protection of hemoglobin (Hb) and myoglobin (Mb) from oxidation. Copper(II) is known to stimulate the oxidation of oxyHb to metHb. Akanmu and co-workers found that the oxidation of oxyHb by copper could be prevented by the preincubation of oxyHb with ESH at concentrations equal to or greater than the concentration of copper.[245] In contrast, Smith and Reed[266] found no evidence of oxyHb protection by ESH and postulated that although ESH can bind copper(II), it does not block the site that is involved in hemoglobin (Hb) oxidation. The differences in ESH concentration and Hb preparation methods could explain the conflicting results.[266]

Mb, like Hb, is affected by oxidative stress due to H_2O_2 exposure, and as a result, the metMb iron is elevated to higher valance states, such as that of ferrylmyoglobin

(ferMb). MetMb can be oxidized to ferMb by H_2O_2 in 5 min. The addition of ESH can completely recover metMb.[245] When this is monitored by ultraviolet/visible spectroscopy, an almost immediate change in the spectrum is observed upon the addition of ESH to ferMb and within 10 min the original metMb spectrum was regenerated. It is believed that during the reduction of ferMb, ESH is oxidized to give ES$^{\bullet}$ or ESSE. The reactivity of ESH with ferMb is higher than that of GSH; it is closer to that of ascorbate.[267] ESH is not able to reduce metMb to oxymyoglobin[267] and is not believed to react with H_2O_2.[245,267] The ability of ESH to reduce ferMb is important for biological systems, as ferMb can oxidize various essential constituents through peroxidative activity, including membrane lipids and fatty acids such as arachidonic acid.[267] ESH can inhibit the peroxidation of arachidonic acid caused by mixtures of Mb/Hb of H_2O_2.[245]

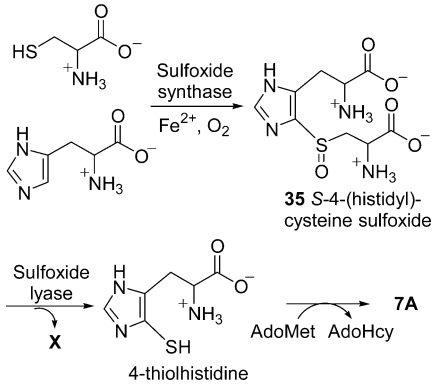
Bioactivity. ESH has been shown to inhibit both H_2O_2 and tumor necrosis factor-α (TNF-α) mediated activation of nuclear factor-κB (NF-κB) and interleukin-8 release, resulting in an overall anti-inflammatory effect in lung cells.[268] ESH is also believed to protect against oxidative damage in vivo caused by the Fenton reagent ferric-nitrilotriacetate (feNTA).[207] ESH has been shown to reduce kidney and liver damage in rats caused by feNTA when supplemented orally for 7 days prior to exposure. Though the molecular mechanisms of protection are unknown, oral ESH supplementation appears to protect against oxidative damage in vivo.[207]

ESH can block *N*-methyl-D-aspartate receptor overstimulation in neurons, a situation that would ordinarily lead to free radical production and cellular death. ESH serves as a neuroprotectant in these systems and may accomplish this by acting as an antioxidant.[269]

Ovothiols (OSH). In addition to ESH, a second class of naturally occurring thiolhistidines has been identified. The presence of the disulfide of 1-methyl-4-thiol-L-histidine [ovothiol A disulfide, $(OS_A)_2$] has been identified in echinoderms: in sea urchins, *Paracentrotus lividus*, *Sphaerechinus granularis,* and *Arabacia lixula*, the holothuroid, *Holothuria tubulosa*, the asteroids, *Astropecten aurantiacus* and *Marthasterias glacialis*,[270] and the mottled sea star, *Evasterias troschelii*.[271] It has been suggested that ovothiol A (OSH_A, Figure 1, **7A**) plays a critical role during cell division and growth of sea urchin eggs. The oxidative burst and the concomitant redox state of the eggs during fertilization require cyclic redox processes and protection against ROS (see below). Additional variants on this compound, that is, the methylated compounds denoted as ovothiols B and C (OSH_B, OSH_C, Figure 1, **7B**, **7C**),[271,272] have also been identified in marine invertebrates such as the spiny scallop, *Chlamys hastata*, and the purple sea urchin, *Strongylocentrotus purpuratus*, respectively.[271] There is evidence for the presence of 4-thiolhistidine (the unmethylated imidazole), as well as OSH_A, in the common octopus, *Octopus vulgaris*, and the european squid, *Loligo vulgaris*.[273] OSH_A has also been reported as the egg release pheromone of male *Platynereis dumerilii*, a species of marine invertebrates, to promote egg release in swarming females.[274]

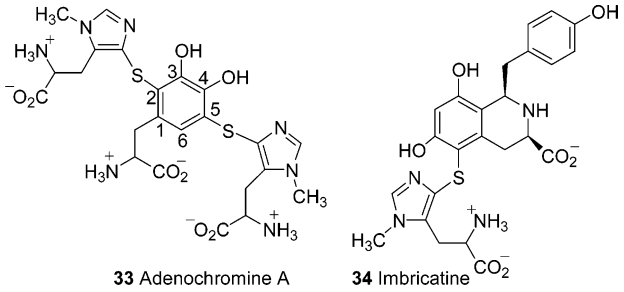
The presence of these amino acids in peptide linkage has been reported. Adenochrome, the purple iron(III)-sequestering pigment that occurs in the branchial hearts of the cephalopod *O. vulgaris*, has been found to be a mixture of peptides derived from glycine and three novel isomeric amino acids: adenochromines A (**33**), B, and C, which are responsible for the iron-binding properties of these pigments.[275] The adenochromines differ in the attachment

Scheme 10. Proposed Biosynthesis of Ovothiol A[a]



[a] Where **X** is a precursor that can be reduced by dithiothreitol to yield pyruvate and cysteine.[281]

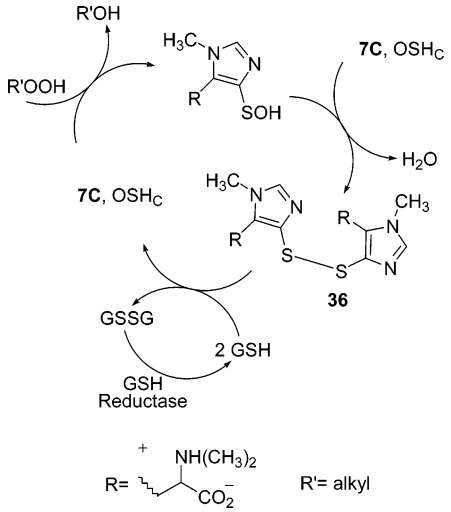
position of the sulfur atoms: in A (**33**) the 4-thiolhistidines are attached through their sulfur atoms to positions 2 and 5 of the dihydroxybenzene nucleus, while in B and C, the 4-thiolhistidines are attached at positions 5 and 6 and positions 2 and 6, respectively.[273,275] On the basis of degradation and labeling studies, it is believed that the 4-thiolhistidine, biosynthesized separately, is added to dopaquinone, which is itself formed by tyrosinase-like oxidation of Dopa.[276] A similar compound, imbricatine (**34**), produced by the starfish *Dermasterias imbricate,* is a derivative of OSH_A that, like the adenochromines, is linked via the sulfur atom to an aromatic ring. Imbricatine has been shown to elicit a "swimming response" from the sea anemone, *Stomphia coccinea*, upon contact with *D. imbricate*.[277] OSHs are not only found in marine invertebrates but have also been detected in the eggs of the rainbow trout, *Salmo gairdneri*, at levels of OSH_A and OSH_B of 1.7 and 0.34 nmol per milligram of protein, respectively, as well as in the eggs of the Coho salmon, *Oncorhynchus kisutch*.[278]



OSH_A also exists as an important intracellular thiol in *C. fasciculata*[279] and *L. donovani*.[155] OSH_A has been identified in all insect stages of the Kinetoplastida, most abundant in the *Leishmania* spp. promastigotes, where OSH_A concentrations can equal or exceed that of TSH_2.[93] Finally, OSH_A has been found in the halotolerant green alga *Dunaliella salina*.[280]

Biosynthesis. The biosynthetic origin of the 4-thiolhistidines is unknown, although it is clear that they originate from the insertion of the SH moiety into L-histidine at position 4 rather than position 2, as in the case of ESH and related metabolites. The biosynthetic steps appear to involve the modification of intact L-histidine. An identified intermediate in the biosynthetic pathway of OSH_A is *S*-4-(histidyl)cysteine sulfoxide (Scheme 10, **35**), produced by an enzyme termed the sulfoxide synthase. This enzyme appears to utilize Fe^{2+} and oxygen to produce a linkage between cysteine and L-histidine, resulting in the formation

Scheme 11. Redox Cycle for the Detoxification by Ovothiol C (OSH_c) of Peroxides Formed during Fertilization of Sea Urchin Eggs[a]



[a] Where GSH and GSSG represent glutathione and its disulfide, respectively. In addition, it is possible for a mixed GSH-OSHc disulfide to be formed.

of a sulfoxide.[281] Cleavage of the amino acid section of the cysteine from this intermediate makes use of the cofactor pyridoxal phosphate and a reductive step to convert the possible sulfenic acid intermediate into the thiol. Methylations utilizing *S*-adenosylmethionine and catalyzed by methyltransferase(s) follow this critical carbon−sulfur bond forming step.[281] The chemical syntheses of 4-thiolimidazoles and OSHs have been reported[282−284] and make these compounds available for further study.

Function. Despite its potential toxicity, *S. purpuratus* utilizes H_2O_2 to cross-link tyrosine residues in its fertilization envelope after fertilization via the enzyme ovoperoxidase.[285,286] The extracellular H_2O_2 is produced in a respiratory burst in the early part of embryonic activation. The presence of H_2O_2 would be a potential hazard to the embryo; however, the eggs and embryos contain approximately 5 mM intracellular OSH_C, which reacts chemically with the H_2O_2, forming OSH_C disulfide $(OS_C)_2$, Scheme 11, **36**), which could then be reduced back to free OSH_C by GSH[278] (Scheme 11).[287] The OSH system appears to be more effective than egg catalase in destroying H_2O_2 at the concentrations produced during fertilization. For example, in unfertilized as well as fertilized sea urchin eggs, metabolism of H_2O_2 was accounted for by the OSH pathway (73%) and by the enzyme catalase (27%), which disproportionates two molecules of H_2O_2 to two water molecules and one oxygen molecule.[278] Hence the OSH system may play a critical role in preventing oxidative damage during fertilization. The $(OS)_2$ produced in cellular redox reactions, such as reduction of organic peroxides, can be reduced to active OSH by reaction with GSH. A preliminary reduction/oxidation potential of OSH_A at physiological pH has been determined to be 0.17 V more positive than that of GSH.[288]

$$2\ GSH + OSSO \rightleftharpoons 2\ OSH + GSSG$$

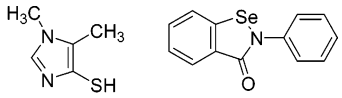
The equilibrium for this equation in the cell would be well to the right. Because of this, there is no obvious need for a separate ovothiol (disulfide) reductase, analogous to GSH reductase, and none has been found in any organism to date.

Eggs from *P. lividus* have been found to have a ratio of OSH_A to GSH of 60:1, indicating that OSH_A is the major thiol in this organism.[270] In *S. purpuratus* eggs, the intracellular levels of OSH_C reach 4.3 mM, while GSH levels lie at 3.3 mM.[271] Detailed kinetic studies on the redox properties of OSH and GSH indicate second-order rate constants of 3.18 s^{-1} M^{-1} for eq 1 and 1.60 s^{-1} M^{-1} for eq 2.[93]

$$2\ OSH + H_2O_2 \rightarrow OSSO + 2\ H_2O \quad (1)$$

$$2\ GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O \quad (2)$$

The pK_a for the thiol groups in the model compound, 1,5-dimethyl-4-thioimidazole (DMTI, **37**), and in GSH are 2.3[289] versus 8.7−9.2,[290,291] respectively, and hence at physiological pH, the related OSH will most likely exist in the more reactive thiolate form.[289] The nucleophilicity of DMTI, and by extension OSH, is enhanced by the aromaticity of the thiolate: the reaction of DMTI with the alkylating agent iodoacetamide occurs at a rate 9 times that of GSH.[289] The protonation state of the OSH nucleus has been shown to control some of the chemical properties of this molecule.



37 1,5-Dimethyl-4-thioimidazole (DMTI)

38 Ebselen

OSHs appear to function as low molecular weight mimics of the enzyme GSH peroxidase (and appear to replace the function of this enzyme in sea urchin eggs).[278] Peroxides in general have been implicated in a number of degenerative diseases such as inflammation, cancer, atherosclerosis,[292] and the aging process.[244,293] The protective aspects of the enzyme GSH peroxidase are critical: this enzyme catalyzes the reduction of harmful peroxides by GSH and protects cellular components from oxidative damage.[117] The enzyme's catalytic site contains a selenocysteine residue, and evidence indicates that the nucleophilic selenium atom (enzyme-SeH) directly attacks one of the oxygen atoms in a peroxide, forming the selenenic acid (enzyme Se-OH), which then reacts with GSH, forming a mixed selenyl sulfide adduct (enzyme-Se-SG). This adduct is subsequently attacked by an additional molecule of GSH, to yield the reactivated active site (enzyme-SeH)[117] and GSSG, which is then reduced by GSH reductase. Recently there has been intense interest in the development of small-molecule mimetics of GSH peroxidase[294−296] such that these compounds could aid in the overall control of peroxide levels in cells. In fact, the small molecule mimetic ebselen (**38**) has undergone clinical trials as an anti-inflammatory agent.[297] The isolation, characterization, and elucidation of the GSH peroxidase-like activities of the OSHs and related thiolhistidines might allow for these and other related but chemically prepared compounds to contribute to this area of medicinal chemistry. For example, the 4-thiolimidazoles have an ability to reduce oxygen-centered radicals as well. Since a number of these radicals have been implicated in various disease processes,[244,293] 4-thiolimidazoles, due to their apparent nontoxic properties, might contribute to the medicinal chemistry of the control of these ROS.[278] Analogues of OSH, such as those containing electron-withdrawing or electron-donating groups on the core imidazole nucleus, have been made successfully[298] in the hope of finding useful antioxidants. Some of these derivatives have been found to be potent scavengers of HOCl, $HO^{\bullet}$, and H_2O_2 and are able to inhibit copper-

induced low-density lipoprotein peroxidation.[299] The redox chemistry of OSH is also pertinent to a variety of parasites of medicinal and agricultural interest. In these cases it is suggested that the unique intracellular thiol in these organisms, TSH_2, plays the major role in the recycling of $(OS)_2$ to OSH. Unlike the other OSH-containing organisms discussed above, these parasites utilize an extremely active TSH_2 peroxidase, which relegates the nonenzymatic scavenging of H_2O_2 by OSH to a relatively minor role.[93]

Additional differences in chemical properties between OSH and GSH are similar to the differences between aromatic versus alkanethiols. The model compound, 1-methyl-5-ethyl-4-thioimidazole, is a better reductant than all naturally occurring aliphatic and aromatic thiols.[300] The aromatic nature of the thiol in OSH also allows for increased stability of the thiyl radical form of OSH[301] and broadens the biological chemistry of this molecule. For example, it has been determined that the reaction of OSH with H_2O_2 produces the OSH thiyl radical,[302] which is then converted to the disulfide.[302,303] The free radical stability of the thiyl radical is believed to be the result of the ability of the thiyl radical to share the electron with atoms of the aromatic imidazole nucleus.[301] AM1 semiempirical calculations on the model compound DMTI have indicated that the free radical density is found not only on the sulfur atom but also on carbons 4 and 5.[302] The presence of metal ions (for example copper ions) can affect changes in the chemistry of OSH, and it has been shown that OSH can form complexes with copper ions.[299]

Extensive experimentation has also shown the superiority of OSH over GSH for hydrogen atom abstraction or one-electron donation. The OSH model compound DMTI was compared to GSH for the one-electron donors Fremy's salt, Banfield's radical, galvinoxyl radical, and horse heart ferricytochrome *c*; in all cases, the model compound reacted at least 1 order of magnitude more rapidly than GSH,[289] with 2 orders of magnitude separating the model and GSH from Banfield's radical.[301] The model was also found to be superior to GSH as a scavenger of tyrosyl radicals.[301] Since the high respiratory burst of H_2O_2 and its breakdown with cellular components such as metals produces highly ROS, it is clear that the free radical chemistry of OSH might well be critical to cellular viability.

The interactions of RNS, such as $ONOO^-$, have been proposed to be critical components of the biochemistry of OSHs. RNS, including $NO^\bullet$, are used by host organisms as a defense against *Trypanosoma* parasites.[304] The formation of $ONOO^-$ from $NO^\bullet$ produces RNS that the parasite must be able to detoxify. OSH_A has been shown to decompose the *S*-nitroso groups of *S*-nitrosoglutathione and di-*S*-nitrosotrypanothione,[95] the intermediate products of nitric oxide detoxification. $NO^\bullet$ is also involved in the fertilization response in sea urchin eggs,[305] and its detoxification may provide yet another role for ovothiols in these organisms.

Further contributions of the OSHs to cellular physiology relate to their synergism with α-tocopherol (vitamin E). Some indications are that OSHs can reactivate α-tocopherol once α-tocopherol has been utilized to block lipid peroxidation.[300] The product from the interaction of α-tocopherol and lipid peroxide is then reconverted to vitamin E and $(OS)_2$. Redox cycling back to OSH by the GSH system can then occur.

Conclusions

As can be seen from the above discussions, the isolation, characterization, and utilization of various intracellular thiols and their disulfides as probes have led to a significant expansion in our knowledge of various aspects of sulfur biochemistry. Without the primary identification of each of these natural products, it would have been impossible to elucidate these extremely complex pathways. Further applications of natural product isolation and identification will ultimately lead to the elucidation of additional cellular pathways currently undetected and perhaps new inhibitors to target the above-described intracellular thiol pathways in microorganisms important in disease processes.

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References and Notes

(1) McBride, B. C.; Wolfe, R. S. *Biochemistry* **1971**, *10*, 2317−2324.
(2) Taylor, C. D.; Wolfe, R. S. *J. Biol. Chem.* **1974**, *249*, 4879−4885.
(3) Thauer, R. K. *Microbiology* **1998**, *144*, 2377−2406.
(4) Ferry, J. G. *Methanogenesis: Ecology, Physiology, Biochemistry, and Genetics*; Chapman and Hall: New York, 1993.
(5) Tyler, S. C. In *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*; Rogers, J. E., Whitman, W. B., Eds.; American Society for Microbiology: Washington, 1991; pp 7−38.
(6) Zinder, S. H. In *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*; Ferry, J. G., Ed.; Chapman & Hall: New York, 1993; pp 128−206.
(7) Balch, W. E.; Wolfe, R. S. *J. Bacteriol.* **1979**, *137*, 264−273.
(8) Smith, S. G.; Rouviere, P. E. *J. Bacteriol.* **1990**, *172*, 6435−6441.
(9) Deppenmeier, U. *Cell. Mol. Life Sci.* **2002**, *59*, 1513−1533.
(10) Hippler, B.; Thauer, R. K. *FEBS Lett.* **1999**, *449*, 165−168.
(11) Voet, D.; Voet, J. G. *Biochemistry*, 2nd ed.; John Wiley and Sons: Toronto, 1995.
(12) Gottschalk, G.; Thauer, R. K. *Biochim. Biophys. Acta* **2001**, *1505*, 28−36.
(13) Stupperich, E.; Juza, A.; Hoppert, M.; Mayer, F. *Eur. J. Biochem.* **1993**, *217*, 115−121.
(14) Harms, U.; Thauer, R. K. *Eur. J. Biochem.* **1996**, *241*, 149−154.
(15) Gartner, P.; Ecker, A.; Fischer, R.; Linder, D.; Fuchs, G.; Thauer, R. K. *Eur. J. Biochem.* **1993**, *213*, 537−545.
(16) van de Wijngaard, W. M. H.; Lugtigheid, R. L.; van der Drift, C. *Antonie van Leeuwenhoek* **1991**, *60*, 1−6.
(17) Lienard, T.; Becher, B.; Marschall, M.; Bowien, S.; Gottschalk, G. *Eur. J. Biochem.* **1996**, *239*, 857−864.
(18) Weiss, D. S.; Gartner, P.; Thauer, R. K. *Eur. J. Biochem.* **1994**, *226*, 799−809.
(19) Gartner, P.; Weiss, D. S.; Harms, U.; Thauer, R. K. *Eur. J. Biochem.* **1994**, *226*, 465−472.
(20) Banerjee, R. V.; Matthews, R. G. *FASEB J.* **1990**, *4*, 1450−1459.
(21) Guest, J. R.; Friendman, S.; Foster, M. A.; Tejerina, G.; Woods, D. D. *Biochem. J.* **1964**, *92*, 497−504.
(22) Ding, Y.-H. R.; Zhang, S.-P.; Tomb, J.-F.; Ferry, J. G. *FEMS Microbiol. Lett.* **2002**, *215*, 127−132.
(23) Gencic, S.; LeClerc, G. M.; Gorlatova, N.; Peariso, K.; Penner-Hahn, J. E.; Grahame, D. A. *Biochemistry* **2001**, *40*, 13068−13078.
(24) Sauer, K.; Thauer, R. K. *Eur. J. Biochem.* **2000**, *267*, 2498−2504.
(25) Krüer, M.; Haumann, M.; Meyer-Dlaucke, W.; Thauer, R. K.; Dau, H. *Eur. J. Biochem.* **2002**, *269*, 2117−2123.
(26) Tallant, T. C.; Paul, L.; Drzycki, J. A. *J. Biol. Chem.* **2001**, *276*, 4485−4493.
(27) Ferguson, D. J., Jr.; Gorlatova, N.; Grahame, D. A.; Krzycki, J. A. *J. Biol. Chem.* **2000**, *275*, 29053−29060.
(28) Hao, B.; Gong, W.; Ferguson, T. K.; James, C. M.; Krzycki, J. A.; Chan, M. K. *Science* **2002**, *296*, 1462−1465.
(29) Blight, S. K.; Larue, R. C.; Mahapatra, A.; Longstaff, D. G.; Chang, E.; Zhao, G.; Kang, P. T.; Green-Church, K. B.; Chan, M. K.; Krzycki, J. A. *Nature* **2004**, *431*, 333−335.
(30) Srinivasan, G.; James, C. M.; Krzycki, J. A. *Science* **2000**, *296*, 1459−1462.
(31) Hao, B.; Zhao, G.; Kang, P. T.; Soares, J. A.; Ferguson, T. K.; Gallucci, J.; Krzycki, J. A.; Chan, M. K. *Chem. Biol.* **2004**, *11*, 1317−24.
(32) Stadtman, T. *Annu. Rev. Biochem.* **1996**, *65*, 83−100.
(33) Grabarse, W.; Mahlert, F.; Duin, E. C.; Goubeaud, M.; Shima, S.; Thauer, R. K.; Lamzin, V.; Ermler, U. *J. Mol. Biol.* **2001**, *309*, 315−330.
(34) Horng, Y.-C.; Becker, D. F.; Ragsdale, S. W. *Biochemistry* **2001**, *40*, 12875−12885.
(35) Mahlert, F.; Grabarse, W.; Kahnt, J.; Thauer, R. K.; Duin, E. C. *J. Biol. Inorg. Chem.* **2002**, *7*, 101−112.
(36) Ermler, U.; Grabarse, W.; Shima, S.; Goubeaud, M.; Thauer, R. K. *Science* **1997**, *278*, 1457−1462.
(37) Gunsalus, R. P.; Wolfe, R. S. *J. Biol. Chem.* **1980**, *255*, 1891−1895.
(38) Noll, K. M.; Rinehart, K. L., Jr.; Tanner, R. S.; Wolfe, R. S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4238−4242.
(39) Kunkel, A.; Vaupel, M.; Heim, S.; Thauer, R. K.; Hedderich, R. *Eur. J. Biochem.* **1997**, *244*, 226−234.
(40) Madadi-Kahkesh, S.; Duin, E. C.; Heim, S.; Albracht, S. P. J.; Johnson, M. K.; Hedderich, R. *Eur. J. Biochem.* **2001**, *268*, 2566−2577.
(41) de Poorter, L. M. I.; Geerts, W. G.; Theuvenet, A. P. R.; Keltjens, J. T. *Eur. J. Biochem.* **2003**, *270*, 66−75.

(42) Duin, E. C.; Madadi-Kahkesh, S.; Hedderich, R.; Clay, M. D.; Johnson, M. K. *FEBS Lett.* **2002**, *512*, 263−268.
(43) Allen, J. R.; Clark, D. D.; Krum, J. G.; Ensign, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8432−8437.
(44) Small, F. J.; Ensign, S. A. *J. Biol. Chem.* **1997**, *272*, 24913−24920.
(45) Krum, J. G.; Ensign, S. A. *J. Bacteriol.* **2000**, *182*, 2629−2634.
(46) Krum, J. G.; Ellsworth, H.; Sargeant, R. R.; Rich, G.; Ensign, S. A. *Biochemistry* **2002**, *41*, 5005−5014.
(47) Clark, D. D.; Ensign, S. A. *Biochemistry* **2002**, *41*, 2727−2740.
(48) Clark, D. D.; Boyd, J. M.; Ensign, S. A. *Biochemistry* **2004**, *43*, 6763−6771.
(49) Clark, D. D.; Allen, J. R.; Ensign, S. A. *Biochemistry* **2000**, *39*, 1294−1304.
(50) Nocek, B.; Jang, S. B.; Jeong, M. S.; Clark, D. D.; Ensign, S. A.; Peters, J. W. *Biochemistry* **2002**, *41*, 12907−12913.
(51) Krum, J. G.; Ensign, S. A. *J. Bacteriol.* **2001**, *183*, 2172−2177.
(52) Graham, D. E.; White, R. H. *Nat. Prod. Rep.* **2002**, *19*, 133−147.
(53) Graham, D. E.; Xu, H.; White, R. H. *J. Biol. Chem.* **2002**, *277*, 13421−13429.
(54) Wise, E. L.; Graham, D. E.; White, R. H.; Rayment, I. *J. Biol. Chem.* **2003**, *278*, 45858−45863.
(55) Graham, D. E.; Graupner, M.; Xu, H.; White, R. H. *Eur. J. Biochem.* **2001**, *268*, 5176−5188.
(56) Graupner, M.; Xu, H.; White, R. H. *J. Bacteriol.* **2000**, *182*, 3688−3692.
(57) Graupner, M.; Xu, H.; White, R. H. *J. Bacteriol.* **2000**, *182*, 4862−4867.
(58) White, R. H. *Biochemistry* **1988**, *27*, 7458−7462.
(59) Newton, G. L.; Fahey, R. C. *Methods Enzymol.* **1995**, *251*, 148−166.
(60) Carnegie, P. R. *Biochem. J.* **1963**, *89*, 471−478.
(61) Smith, K.; Borges, A.; Ariyanayagam, M. R.; Fairlamb, A. H. *Biochem. J.* **1995**, *312*, 465−469.
(62) Newton, G. L.; Javor, B. *J. Bacteriol.* **1985**, *161*, 438−441.
(63) Fahey, R. C.; Brown, W. C.; Adams, W. B.; Worsham, M. B. *J. Bacteriol.* **1978**, *133*, 1126−1129.
(64) Wang, W.; Ballatori, N. *Pharmacol. Rev.* **1998**, *50*, 335−355.
(65) Meister, A. *Trends Biochem. Sci.* **1988**, *13*, 185−188.
(66) Pompella, A.; Visvikis, A.; Paolicchi, A.; De Tata, V.; Casini, A. F. *Biochem. Pharmacol.* **2003**, *66*, 1499−1503.
(67) Watson, W. H.; Chen, Y.; Jones, D. P. *Biofactors* **2003**, *17*, 307−314.
(68) Pastore, A.; Federici, G.; Bertini, E.; Piemonte, F. *Clin. Chim. Acta* **2003**, *333*, 19−39.
(69) Dolphin, D.; Avramovic, O.; Poulson, R. *Glutathione: Chemical, Biochemical, and Medical Aspects*; John Wiley & Sons: Toronto, 1989; Vol. IIIA.
(70) Fairlamb, A. H.; Blackburn, P.; Ulrich, P.; Chait, B. T.; Cerami, A. *Science* **1985**, *227*, 1485−1487.
(71) Fairlamb, A. H.; Cerami, A. *Mol. Biochem. Parasitol.* **1985**, *14*, 187−198.
(72) World Health Organization, African trypanosomiasis. www.who.int/tdr/diseases/tryp/files/tryp-poster.pdf.
(73) Food and Agriculture Organization of the United Nations, PAAT: The disease. www.fao.org/ag/againfo/programmes/en/paat/disease.html.
(74) World Health Organization, Chagas disease. www.who.int/tdr/diseases/chagas/files/chagas-poster.pdf.
(75) World Health Organization, The leishmaniases and Leishmania/HIV co-infections. www.who.int/mediacentre/factsheets/fs116/en/.
(76) Shim, H.; Fairlamb, A. H. *J. Gen. Microbiol.* **1988**, *134* (Part 3), 807−817.
(77) Mastri, C.; Thorborn, D. E.; Davies, A. J.; Ariyanayagam, M. R.; Hunter, K. J. *Biochem. Biophys. Res. Comm.* **2001**, *282*, 1177−1182.
(78) Oza, S. L.; Ariyanayagam, M. R.; Fairlamb, A. H. *Biochem. J.* **2002**, *364*, 679−686.
(79) Koenig, K.; Menge, U.; Kiess, M.; Wray, V.; Flohe, L. *J. Biol. Chem.* **1997**, *272*, 11908−11915.
(80) Smith, K.; Nadeau, K.; Bradley, M.; Walsh, C.; Fairlamb, A. H. *Protein Sci.* **1992**, *1*, 874−883.
(81) Tetaud, E.; Manai, F.; Barrett, M. P.; Nadeau, K.; Walsh, C. T.; Fairlamb, A. H. *J. Biol. Chem.* **1998**, *273*, 19383−19390.
(82) Henderson, G. B.; Yamaguchi, M.; Novoa, L.; Fairlamb, A. H.; Cerami, A. *Biochemistry* **1990**, *29*, 3924−9.
(83) Comini, M.; Menge, U.; Wissing, J.; Flohe, L. *J. Biol. Chem.* **2005**, in press.
(84) Oza, S. L.; Tetaud, E.; Ariyanayagam, M. R.; Warnon, S. S.; Fairlamb, A. H. *J. Biol. Chem.* **2002**, *277*, 35853−35861.
(85) Oza, S. L.; Ariyanayagam, M. R.; Aitcheson, N.; Fairlamb, A. H. *Mol. Biochem. Parasitol.* **2003**, *131*, 25−33.
(86) Lin, C. H.; Kwon, D. S.; Bollinger, J. M., Jr.; Walsh, C. T. *Biochemistry* **1997**, *36*, 14930−14938.
(87) Bollinger, J. M., Jr.; Kwon, D. S.; Huisman, G. W.; Kolter, R.; Walsh, C. T. *J. Biol. Chem.* **1995**, *270*, 14031−14041.
(88) Henderson, G. B.; Glushka, J.; Cowburn, D.; Cerami, A. *J. Chem. Soc., Perkin Trans. 1* **1990**, 911−914.
(89) Kellam, B.; Bycroft, B. W.; Chhabra, S. R. *Tetrahedron Lett.* **1997**, *38*, 4849−4852.
(90) Krauth-Siegel, R. L.; Inhoff, O. *Parasitol. Res.* **2003**, *90*, Suppl. 2, S77−S85.
(91) Muller, S.; Liebau, E.; Walter, R. D.; Krauth-Siegel, R. L. *Trends Parasitol.* **2003**, *19*, 320−328.
(92) Flohe, L.; Hecht, H. J.; Steinert, P. *Free Radical Biol. Med.* **1999**, *27*, 966−984.
(93) Ariyanayagam, M. R.; Fairlamb, A. H. *Mol. Biochem. Parasitol.* **2001**, *115*, 189−198.
(94) Lopez, J. A.; Carvalho, T. U.; de Souza, W.; Flohe, L.; Guerrero, S. A.; Montemartini, M.; Kalisz, H. M.; Nogoceke, E.; Singh, M.; Alves, M. J. M.; Colli, W. *Free Radical Biol. Med.* **2000**, *28*, 767−772.
(95) Vogt, R. N.; Steenkamp, D. J. *Biochem. J.* **2003**, *371*, 49−59.
(96) Thomson, L.; Denicola, A.; Radi, R. *Arch. Biochem. Biophys.* **2003**, *412*, 55−64.
(97) Moutiez, M.; Meziana-Cherif, D.; Aumercier, M.; Sergheraert, C.; Tartar, A. *Chem. Pharm. Bull.* **1994**, *42*, 2641−2644.
(98) Kuriyan, J.; Kong, X. P.; Krishna, T. S.; Sweet, R. M.; Murgolo, N. J.; Field, H.; Cerami, A.; Henderson, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8764−8768.
(99) Bailey, S.; Smith, K.; Fairlamb, A. H.; Hunter, W. N. *Eur. J. Biochem.* **1993**, *213*, 67−75.
(100) Hunter, W. N.; Bailey, S.; Habash, J.; Harrop, S. J.; Helliwell, J. R.; Aboagye-Kwarteng, T.; Smith, K.; Fairlamb, A. H. *J. Mol. Biol.* **1992**, *227*, 322−333.
(101) Zhang, Y.; Bailey, S.; Naismith, J. H.; Bond, C. S.; Habash, J.; McLaughlin, P.; Papiz, M. Z.; Borges, A.; Cunningham, M.; Fairlamb, A. H.; Hunter, W. N. *J. Mol. Biol.* **1993**, *232*, 1217−20.
(102) Zhang, Y.; Bond, C. S.; Bailey, S.; Cunningham, M. L.; Fairlamb, A. H.; Hunter, W. N. *Protein Sci.* **1996**, *5*, 52−61.
(103) Bond, C. S.; Zhang, Y.; Berriman, M.; Cunningham, M. L.; Fairlamb, A. H.; Hunter, W. N. *Struct. Fold. Des.* **1999**, *7*, 81−89.
(104) Shames, S. L.; Fairlamb, A. H.; Cerami, A.; Walsh, C. *Biochemistry* **1986**, *25*, 3519−3526.
(105) Moutiez, M.; Aumercier, M.; Schoneck, R.; Meziane-Cherif, D.; Lucas, V.; Aumercier, P.; Ouaissi, A.; Sergheraert, C.; Tartar, A. *Biochem. J.* **1995**, *310* (Part 2), 433−437.
(106) Moutiez, M.; Quemeneur, E.; Sergheraert, C.; Lucas, V.; Tartar, A.; Davioud-Charvet, E. *Biochem. J.* **1997**, *322* (Part 1), 43−48.
(107) Fairlamb, A. H.; Henderson, G. B.; Cerami, A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2607−2611.
(108) Cunningham, M. L.; Zvelebil, M. J.; Fairlamb, A. H. *Eur. J. Biochem.* **1994**, *221*, 285−295.
(109) Wyllie, S.; Cunningham, M. L.; Fairlamb, A. H. *J. Biol. Chem.* **2004**, *279*, 39925−39932.
(110) Ferreira, C. d. S.; Martins, P. S.; Demicheli, C.; Brochu, C.; Ouellette, M.; Frezard, F. *Biometals* **2003**, *16*, 441−446.
(111) Mukhopadhyay, R.; Dey, S.; Xu, N.; Gage, D.; Lightbody, J.; Ouellette, M.; Rosen, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10383−10387.
(112) Krieger, S.; Schwarz, W.; Ariyanayagam, M. R.; Fairlamb, A. H.; Krauth-Siegel, R. L.; Clayton, C. *Mol. Microbiol.* **2000**, *35*, 542−552.
(113) Dumas, C.; Ouelletee, M.; Tovar, J.; Cunningham, M. L.; Fairlamb, A. H.; Tamar, S.; Olivier, M.; Papadopoulou, B. *EMBO J.* **1997**, *16*, 2590−2598.
(114) Ponasik, J. A.; Strickland, C.; Faerman, C.; Savvides, S.; Karplus, P. A.; Ganem, B. *Biochem. J.* **1995**, *311* (Part 2), 371−375.
(115) Gallwitz, H.; Bonse, S.; Martinez-Cruz, A.; Schlichting, I.; Schumacher, K.; Krauth-Siegel, R. L. *J. Med. Chem.* **1999**, *42*, 364−372.
(116) Augustyns, K.; Amssoms, K.; Yamani, A.; Rajan, P. K.; Haemers, A. *Curr. Pharm. Des.* **2001**, *7*, 1117−1141.
(117) Flohe, L. In *Glutathione: Chemical, Biochemical, and Medical Aspects*; Dolphin, D., Avramovic, O., Poulson, R., Eds.; John Wiley and Sons: New York, 1989; Vol. IIIA, pp 643−731.
(118) Nogoceke, E.; Gommel, D. U.; Kiess, M.; Kalisz, H. M.; Flohe, L. *Biol. Chem.* **1997**, *378*, 827−836.
(119) Levick, M. P.; Tetaud, E.; Fairlamb, A. H.; Blackwell, J. M. *Mol. Biochem. Parasitol.* **1998**, *96*, 125−137.
(120) Ludemann, H.; Dormeyer, M.; Sticherling, C.; Stallmann, D.; Follmann, H.; Krauth-Siegel, R. L. *FEBS Lett.* **1998**, *431*, 381−385.
(121) Montemartini, M.; Kalisz, H. M.; Hecht, H. J.; Steinert, P.; Flohe, L. *Eur. J. Biochem.* **1999**, *26*, 516−524.
(122) Alphey, M. S.; Leonard, G. A.; Gourley, D. G.; Tetaud, E.; Fairlamb, A. H.; Hunter, W. N. *J. Biol. Chem.* **1999**, *274*, 25613−25622.
(123) Alphey, M. S.; Bond, C. S.; Tetaud, E.; Fairlamb, A. H.; Hunter, W. N. *J. Mol. Biol.* **2000**, *300*, 903−916.
(124) Armstrong, R. N. *Chem. Res. Toxicol.* **1997**, *10*, 2−18.
(125) Vuilleumier, S. *J. Bacteriol.* **1997**, *179*, 1431−1441.
(126) Eaton, D. L.; Bammler, T. K. *Toxicol. Sci.* **1999**, *49*, 156−164.
(127) Strange, R. S.; Jones, P. W.; Fryer, A. A. *Toxicol. Lett.* **2000**, *112−113*, 357−363.
(128) Strange, R. S.; Spiteri, M. A.; Ramachandran, S.; Fryer, A. A. *Mut. Res.* **2001**, *482*, 21−26.
(129) Pickett, C. B.; Lu, A. Y. H. *Annu. Rev. Biochem.* **1989**, *58*, 743−764.
(130) Vickers, T. J.; Fairlamb, A. H. *J. Biol. Chem.* **2004**, *279*, 27246−27256.
(131) Vickers, T. J.; Wyllie, S.; Fairlamb, A. H. *J. Biol. Chem.* **2004**, *279*, 49003−49009.
(132) Creighton, D. J.; Hamilton, D. S. *Arch. Biochem. Biophys.* **2001**, *387*, 1−10.
(133) Thornalley, P. J. *Biochem. Soc. Trans.* **2003**, *31*, 1343−1348.
(134) Vander Jagt, D. J. In *Glutathione: Chemical, Biochemical and Medicinal Aspects*; Dolphin, D., Avramovic, O., Poulson, R., Eds.; John Wiley and Sons: New York, 1989; Vol. III, pp 597−641.
(135) Vickers, T. J.; Greig, N.; Fairlamb, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13186−13191.
(136) Irsch, T.; Krauth-Siegel, R. L. *J. Biol. Chem.* **2004**, *279*, 22209−22217.
(137) Clugston, S. L.; Barnard, J. F.; Kinach, R.; Miedema, D.; Ruman, R.; Daub, E.; Honek, J. F. *Biochemistry* **1998**, *37*, 8754−8763.
(138) Sukdeo, N.; Clugston, S. L.; Daub, E.; Honek, J. F. *Biochem. J.* **2004**, *384*, 111−117.
(139) Steenkamp, D. J. *IUBMB Life* **2002**, *53*, 243−248.
(140) Schmidt, A.; Krauth-Siegel, R. L. *Curr. Top. Med. Chem.* **2002**, *2*, 1239−1259.

(141) Chan, C.; Yin, H.; McKie, J. H.; Fairlamb, A. H.; Douglas, K. T. *Amino Acids* **2002**, *22*, 297−308.
(142) Li, Z.; Fennie, M. W.; Ganem, B.; Hancock, M. T.; Kobaslija, M.; Rattendi, D.; Bacchi, C. J.; O'Sullivan, M. C. *Bioorg. Med. Chem.* **2001**, *11*, 251−254.
(143) Marsh, I. R.; Smith, H.; Bradley, M. *Chem. Commun.* **1996**, 941−942.
(144) Chitkul, B.; Bradley, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2367−2369.
(145) Huynh, T. T.; Huynh, V. T.; Harmon, M. A.; Phillips, M. A. *J. Biol. Chem.* **2003**, *278*, 39794−39800.
(146) Comini, M. A.; Guerrero, S. A.; Haile, S.; Menge, U.; Lunsdorf, H.; Flohe, L. *Free Radical Biol. Med.* **2004**, *36*, 1289−1302.
(147) Repetto, Y.; Opazo, E.; Maya, J. D.; Agosin, M. *Comp. Biochem. Physiol.* **1996**, *115B*, 281−285.
(148) Maya, J. D.; Rodriguez, A.; Pino, L.; Pabon, A.; Ferreira, J.; Pavani, M.; Repetto, Y.; Morello, A. *Biol. Res.* **2004**, *37*, 61−69.
(149) Jacoby, E. M.; Schlichting, I.; Lantwin, C. B.; Kabsch, W.; Krauth-Siegel, R. L. *Proteins* **1996**, *24*, 73−80.
(150) Saravanamuthu, A.; Vickers, T. J.; Bond, C. S.; Peterson, M. R.; Hunter, W. N.; Fairlamb, A. H. *J. Biol. Chem.* **2004**, *279*, 29493−29500.
(151) Salmon-Chemin, L.; Buisine, E.; Yardley, V.; Kohler, S.; Debreu, M.-A.; Landry, V.; Sergheraert, C.; Croft, S. L.; Krauth-Siegel, R. L.; Davioud-Charvet, E. *J. Med. Chem.* **2001**, *44*, 548−565.
(152) Garrard, E. A.; Borman, E. C.; Cook, B. N.; Pike, E. J.; Alberg, D. G. *Org. Lett.* **2000**, *2*, 3639−3642.
(153) Sakuda, S.; Zhou, Z. Y.; Yamada, Y. *Biosci. Biotech. Biochem.* **1994**, *58*, 1347−1348.
(154) Newton, G. L.; Fahey, R. C.; Cohen, G.; Aharonowitz, Y. *J. Bacteriol.* **1993**, *175*, 2734−2742.
(155) Spies, H. S.; Steenkamp, D. J. *Eur. J. Biochem.* **1994**, *224*, 203−213.
(156) Newton, G. L.; Bewley, C. A.; Dwyer, T. J.; Horn, R.; Aharonowitz, Y.; Cohen, G.; Davies, J.; Faulkner, D. J.; Fahey, R. C. *Eur. J. Biochem.* **1995**, *230*, 821−825.
(157) There was initial confusion as to the stereochemistry of the inosityl moiety, as MSH was often written as 1-D but drawn as 1-L; MSH has since been confirmed to be 1-D.
(158) Nicholas, G. M.; Kovác, P.; Bewley, C. A. *J. Am. Chem. Soc.* **2002**, *124*, 3492−3493.
(159) Newton, G. L.; Arnold, K.; Price, M. S.; Sherrill, C.; Delcardayre, S. B.; Aharonowitz, Y.; Cohen, G.; Davies, J.; Fahey, R. C.; Davis, C. *J. Bacteriol.* **1996**, *178*, 1990−1995.
(160) Anderberg, S. J.; Newton, G. L.; Fahey, R. C. *J. Biol. Chem.* **1998**, *273*, 30391−30397.
(161) Bornemann, C.; Jardine, M. A.; Spies, H. S. C.; Steenkamp, D. J. *Biochem. J.* **1997**, *325*, 623−629.
(162) Newton, G. L.; Koledin, T.; Gorovitz, B.; Rawat, M.; Fahey, R. C.; Av-Gay, Y. *J. Bacteriol.* **2003**, *185*, 3476−3479.
(163) Maynes, J. T.; Garen, C.; Cherney, M. M.; Newton, G.; Arad, D.; Av-Gay, Y.; Fahey, R. C.; James, M. N. *J. Biol. Chem.* **2003**, *278*, 47166−47170.
(164) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *J. Bacteriol.* **2000**, *182*, 6958−6963.
(165) McCarthy, A. A.; Knijff, R.; Peterson, N. A.; Baker, E. N. *Acta Crystallogr.* **2003**, *D59*, 2316−2318.
(166) McCarthy, A. A.; Peterson, N. A.; Knijff, R.; Baker, E. N. *J. Mol. Biol.* **2004**, *335*, 1131−1141.
(167) Sareen, D.; Steffek, M.; Newton, G. L.; Fahey, R. C. *Biochemistry* **2002**, *41*, 6885−6890.
(168) Koledin, T.; Newton, G. L.; Fahey, R. C. *Arch. Microbiol.* **2002**, *178*, 331−337.
(169) Vetting, M. W.; Roderick, S. L.; Yu, M.; Blanchard, J. S. *Protein Sci.* **2003**, *12*, 1954−1959.
(170) Jardine, M. A.; Spies, H. S.; Nkambule, C. M.; Gammon, D. W.; Steenkamp, D. J. *Bioorg. Med. Chem.* **2002**, *10*, 875−881.
(171) Lee, S.; Rosazza, J. P. *Org. Lett.* **2004**, *6*, 365−368.
(172) Steenkamp, D. J.; Vogt, R. N. *Anal. Biochem.* **2004**, *325*, 21−27.
(173) Wallace, R. J., Jr.; Nash, D. R.; Tsukamura, M.; Blacklock, Z. M.; Silcox, V. A. *J. Infect. Dis.* **1988**, *158*, 52−59.
(174) World Health Organization, Tuberculosis. http://www.who.int/mediacentre/factsheets/fs104/en/.
(175) Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2000**, *39*, 10739−10746.
(176) Steffek, M.; Newton, G. L.; Av-Gay, Y.; Fahey, R. C. *Biochemistry* **2003**, *42*, 12067−12076.
(177) Rawat, M.; Uppal, M.; Newton, G.; Steffek, M.; Fahey, R. C.; Av-Gay, Y. *J. Bacteriol.* **2004**, *186*, 6050−6058.
(178) Buchmeier, N. A.; Newton, G. L.; Koledin, T.; Fahey, R. C. *Mol. Microbiol.* **2003**, *47*, 1723−1732.
(179) Patel, M. P.; Blanchard, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 11538−11539.
(180) Patel, M. P.; Blanchard, J. S. *Biochemistry* **1999**, *38*, 11827−11833.
(181) Patel, M. P.; Blanchard, J. S. *Biochemistry* **2001**, *40*, 5119−5126.
(182) Vogt, R. N.; Steenkamp, D. J.; Zheng, R.; Blanchard, J. S. *Biochem. J.* **2003**, *2003*, 657−666.
(183) Rawat, M.; Newton, G. L.; Ko, M.; Martinez, G. J.; Fahey, R. C.; Av-Gay, Y. *Antimicrob. Agents Chemother.* **2002**, *46*, 3348−3355.
(184) Rawat, M.; Kovacevic, S.; Billman-Jacobe, H.; Av-Gay, Y. *Microbiology* **2003**, *149*, 1341−1349.
(185) Barry, C. E., III; Slayden, R. A.; Sampson, A. E.; Lee, R. E. *Biochem. Pharmacol.* **2000**, *59*, 221−231.
(186) Sareen, D.; Newton, G. L.; Fahey, R. C.; Buchmeier, N. A. *J. Bacteriol.* **2003**, *185*, 6736−6740.
(187) Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. *Bioorg. Med. Chem.* **2003**, *11*, 601−608.
(188) Nicholas, G. M.; Newton, G. L.; Fahey, R. C.; Bewley, C. A. *Org. Lett.* **2001**, *3*, 1543−1545.
(189) Kende, A. S.; Lan, J.; Fan, J. *Tetrahedron Lett.* **2004**, *45*, 133−135.
(190) Mahadevan, J.; Nicholas, G. M.; Bewley, C. A. *J. Org. Chem.* **2003**, *68*, 3380−3386.
(191) Fetterolf, B.; Bewley, C. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3785−3788.
(192) Nicholas, G. M.; Eckman, L. L.; Ray, S.; Hughes, R. O.; Pfefferkorn, J. A.; Barluenga, S.; Nicolaou, K. C.; Bewley, C. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2487−2490.
(193) Tanret, C. *Compt. Rend.* **1909**, *149*, 222−224.
(194) Barger, G.; Ewins, A. J. *J. Chem. Soc.* **1911**, 2336−2341.
(195) Audley, B. G.; Tan, C. H. *Phytochemistry* **1968**, *7*, 1999−2000.
(196) Melville, D. B.; Eich, S. *J. Biol. Chem.* **1956**, *218*, 647−651.
(197) Eagles, B. A.; Vars, H. M. *J. Biol. Chem.* **1928**, *80*, 615−622.
(198) Baldridge, R. C.; Lewis, H. B. *J. Biol. Chem.* **1953**, *202*, 169−176.
(199) Ban, R. W.; Stowell, E. C. *Arch. Biochem. Biophys.* **1956**, *63*, 259−260.
(200) Fahey, R. C.; Newton, G. L.; Dorian, R.; Kosower, E. M. *Anal. Biochem.* **1981**, *111*, 357−365.
(201) Melville, D. B.; Genghof, D. S.; Inamine, E.; Kovalenko, V. *J. Biol. Chem.* **1956**, *223*, 9−17.
(202) Briggs, I. *J. Neurochem.* **1972**, *19*, 27−35.
(203) Kawano, H.; Higuchi, F.; Mayumi, T.; Hama, T. *Chem. Pharm. Bull.* **1982**, *30*, 2611−2613.
(204) Shires, T. K.; Brummel, M. C.; Pulido, J. S.; Stegiink, L. D. *Comput. Biochem. Physiol.* **1997**, *117C*, 117−120.
(205) Epand, R. M.; Epand, R. F. *J. Clin. Chem. Clin. Biochem.* **1988**, *26*, 623−626.
(206) Shukla, Y.; Kulshrestha, O. P.; Khuteta, K. P. *Indian J. Med. Res.* **1981**, *73*, 472−473.
(207) Deiana, M.; Rosa, A.; Casu, V.; Piga, R.; Dessi, M. A.; Aruoma, O. I. *Clin. Nutr.* **2004**, *23*, 183−193.
(208) Muda, M.; Pelizzoni, F.; Sello, G.; Mussini, E. *J. Chromatogr.* **1988**, *434*, 191−195.
(209) Rae, C. D.; Sweeney, K. J. E.; Kuchel, P. W. *Magn. Reson. Med.* **1993**, *29*, 826−829.
(210) Kumosani, T. A. *Exp. Mol. Med.* **2001**, *33*, 20−2.
(211) Genghof, D. S. *J. Bacteriol.* **1970**, *103*, 475−478.
(212) Melville, D. B. *Vitam. Horm.* **1959**, *17*, 155−204.
(213) Kawano, H.; Otani, M.; Takeyama, K.; Kawai, Y.; Mayumi, T.; Hama, T. *Chem. Pharm. Bull.* **1982**, *30*, 1760−1765.
(214) Melville, D. B.; Horner, W. H.; Lubschez, R. *J. Biol. Chem.* **1954**, *206*, 221−228.
(215) Mann, T.; Leone, E. *Biochem. J.* **1953**, *53*, 140−148.
(216) Leone, E.; Mann, T. *Nature* **1951**, *168*, 205−206.
(217) Mayumi, T.; Kawano, H.; Sakamoto, Y.; Suehisa, E.; Kawai, Y.; Hama, T. *Chem. Pharm. Bull.* **1978**, *26*, 3772−3778.
(218) Mitsuyama, H.; May, J. M. *Clin. Sci.* **1999**, *97*, 407−411.
(219) Jocelyn, P. C. *Biochem. J.* **1958**, *70*, 656−660.
(220) Kuchel, P. W.; Chapman, B. E. *Biomed. Biochim. Acta* **1983**, *42*, 1143−1149.
(221) Touster, O. *J. Biol. Chem.* **1951**, *188*, 371−377.
(222) Hartman, P. E. *Methods Enzymol.* **1990**, *186*, 310−317.
(223) Melville, D. B.; Eich, S.; Ludwig, M. L. *J. Biol. Chem.* **1957**, *224*, 871−877.
(224) Melville, D. B.; Ludwig, M. L.; Inamine, E.; Rachele, J. R. *J. Biol. Chem.* **1959**, *234*, 1195−1198.
(225) Askari, A.; Melville, D. B. *J. Biol. Chem.* **1962**, *237*, 1615−1618.
(226) Ishikawa, Y.; Isreal, S. E.; Melville, D. B. *J. Biol. Chem.* **1974**, *249*, 4420−4427.
(227) Genghof, D. S.; van Damme, O. *J. Bacteriol.* **1964**, *87*, 852−862.
(228) Motohashi, N.; Mori, I.; Sugiura, Y. *Chem. Pharm. Bull.* **1976**, *24*, 1737−1741.
(229) Sugihara, A.; Uemura, K.; Matsuura, Y.; Tanaka, N.; Ashida, T.; Kakudo, M. *Acta Crystallogr.* **1976**, *B32*, 181−185.
(230) Misiti, F.; Castagnola, M.; Zuppi, C.; Giardina, B.; Messana, I. *Biochem. J.* **2001**, *356*, 799−804.
(231) Carlsson, J.; Kierstan, M. P.; Brocklehurst, K. *Biochem. J.* **1974**, *139*, 237−242.
(232) Heath, H.; Toennies, G. *Biochem. J.* **1958**, *68*, 204−210.
(233) Hartman, Z.; Hartman, P. E. *Envion. Mol. Mutagen.* **1987**, *10*, 3−15.
(234) Motohashi, N.; Mori, I. *Chem. Pharm. Bull.* **1983**, *31*, 1702−1707.
(235) Smith, R. C.; Gore, J. Z. *Phosphorus, Sulfur, Silicon* **1991**, *62*, 105−110.
(236) Russell, K. E. *J. Phys. Chem.* **1954**, *58*, 437−439.
(237) Jovanovic, S. V.; Simic, M. G. In *Anticarcinogenesis and Radiation Protection 2*; Nygaard, O. F., Upton, A. C., Eds.; Plenum Press: New York, 1991; pp 229−235.
(238) Jocelyn, P. C. *Biochemistry of the SH Group*; Academic Press: New York, 1972.
(239) Scott, E. M.; Duncan, I. W.; Ekstrand, V. *J. Biol. Chem.* **1963**, *238*, 3928−3933.
(240) Short, R. D. J.; Baskin, S. I. In *Oxidants, Antioxidants and Free Radicals*; Salem, H., Ed.; Taylor and Francis: Washington, D.C., 1997.
(241) Dahl, T. A.; Midden, W. R.; Hartman, P. E. *Photochem. Photobiol.* **1988**, *47*, 357−362.
(242) Hartman, P. E.; Dixon, W. J.; Dahl, T. A.; Daub, M. E. *Photochem. Photobiol.* **1988**, *47*, 699−703.
(243) Takeshima, S.; Inoue, T. *Chem. Pharm. Bull.* **1996**, *44*, 201−203.

(244) Baskin, S. I.; Salem, H. *Oxidants, Antioxidants, and Free Radicals*; Taylor & Francis: Washington, D.C., 1997.
(245) Akanmu, D.; Cecchini, R.; Aruoma, O. I.; Halliwell, B. *Arch. Biochem. Biophys.* **1991**, *288*, 10−16.
(246) Asmus, K.-D.; Bensasson, R. V.; Bernier, J.-L.; Houssin, R.; Land, E. J. *Biochem. J.* **1996**, *315*, 625−629.
(247) Smith, R. C.; Gore, J. Z. *Biochim. Biophys. Acta* **1990**, *1034*, 263−267.
(248) Acworth, I. N.; McCabe, D. R.; Maher, T. J. In *Oxidants, Antioxidants and Free Radicals*; Baskin, S. I., Salem, H., Eds.; Taylor and Francis: Washington, D.C., 1997; pp 22−77.
(249) Aruoma, O. I.; Spencer, J. P. E.; Mahmood, N. *Food Chem. Toxicol.* **1999**, *37*.
(250) Law, A.; Gautier, S.; Quirion, R. *Brain Res. Rev.* **2001**, *35*, 73−96.
(251) Smith, M. A.; Harris, P. L. R.; Sayre, L. M.; Beckman, J. S.; Perry, G. *J. Neurosci.* **1997**, *17*, 2653−2657.
(252) Butterfield, D. A.; Drake, J.; Pocernich, C.; Castegna, A. *Trends Mol. Med.* **2001**, *7*, 548−554.
(253) Jang, J.-H.; Aruoma, O. I.; Jen, L.-S.; Chung, H. Y.; Surh, Y.-J. *Free Radical Biol. Med.* **2004**, *36*, 288−299.
(254) Aruoma, O. I.; Whiteman, M.; England, T. G.; Halliwell, B. *Biochem. Biophys. Res. Commun.* **1997**, *231*, 389−391.
(255) Whiteman, M.; Halliwell, B. *FEBS Lett.* **1997**, *414*, 497−500.
(256) Mathews, W. R.; Kerr, S. W. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 1529−1537.
(257) Simon, D. I.; Stamler, J. S.; Jaraki, O.; Keaney, J. F.; Osborne, J. A.; Francis, S. A.; Singel, D. J.; Loscalzo, J. *Arteroscler. Thromb.* **1993**, *13*, 791−799.
(258) Jourd′heuil, D.; Hallen, K.; Feelisch, M.; Grisham, M. B. *Free Radical Biol. Med.* **2000**, *28*, 409−417.
(259) Arduini, A.; Mancinelli, G.; Radatti, G. L.; Hochsetein, P.; Cadenas, E. *Arch. Biochem. Biophys.* **1992**, *294*, 398−402.
(260) Smith, R. C.; Reeves, J. C.; Dage, R. C.; Schnettler, R. A. *Biochem. Pharmacol.* **1987**, *36*, 1457−1460.
(261) Sundquist, A. R.; Fahey, R. C. *J. Biol. Chem.* **1989**, *264*, 719−725.
(262) Tsen, C. C.; Tappel, A. L. *J. Biol. Chem.* **1958**, *233*, 1230−1232.
(263) Motohashi, N.; Mori, I.; Sugiura, Y.; Tanaka, N. *Chem. Pharm. Bull.* **1974**, *22*, 654−657.
(264) Rowley, D. A.; Halliwell, B. *FEBS Lett.* **1982**, *142*, 39−41.
(265) Rowley, D. A.; Halliwell, B. *FEBS Lett.* **1982**, *138*, 33−36.
(266) Smith, R. C.; Reed, V. D. *Chem.-Biol. Int.* **1992**, *82*, 209−217.
(267) Arduini, A.; Eddy, L.; Hochstein, P. *Arch. Biochem. Biophys.* **1990**, *281*, 41−43.
(268) Rahman, I.; Gilmour, P. S.; Jimenex, L. A.; Biswas, S. K.; Antonicelli, F.; Aruoma, O. I. *Biochem. Biophys. Res. Commun.* **2003**, *302*, 860−864.
(269) Moncaster, J. A.; Walsh, D. A.; Gentleman, S. M.; Jen, L.-S.; Aruoma, O. I. *Neurosci. Lett.* **2002**, *328*, 55−59.
(270) Palumbo, A.; Misuraca, G.; D′ischia, M.; Donaudy, F.; Prota, G. *Comput. Biochem. Physiol.* **1984**, *78B*, 81−83.
(271) Turner, E.; Klevit, R.; Hager, L. J.; Shapiro, B. M. *Biochemistry* **1987**, *26*, 4028−4036.
(272) Initial work assigned ovothiol as 1-methyl-5-thiol-L-histidine due to confusion regarding the nomenclature of substituted histidines. The corrected numbering system assigns ovothiol as 1-methyl-4-thiol-L-histidine.
(273) Rossi, F.; Nardi, G.; Palumbo, A.; Prota, G. *Comp. Biochem. Physiol.* **1985**, *80B*, 843−845.
(274) Rohl, I.; Scheider, B.; Schmidt, B.; Zeeck, E. *Z. Naturforsch.* **1999**, *54C*, 1145−1147.
(275) Ito, S.; Nardi, G.; Palumbo, A.; Prota, G. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2617−2623.
(276) Ito, S.; Nardi, G.; Palumbo, A.; Prota, G. *Experientia* **1979**, *35*, 14−15.
(277) Pathirana, C.; Andersen, R. J. *J. Am. Chem. Soc.* **1986**, *108*, 8288−8289.
(278) Turner, E.; Hager, L. J.; Shapiro, B. M. *Science* **1988**, *242*, 939−941.
(279) Steenkamp, D. J.; Spies, H. S. *Eur. J. Biochem.* **1994**, *223*, 43−50.
(280) Selman-Reimer, S.; Duhe, R. J.; Stockman, B. J.; Selman, B. R. *J. Biol. Chem.* **1991**, *266*, 182−188.
(281) Vogt, R. N.; Spies, H. S.; Steenkamp, D. J. *Eur. J. Biochem.* **2001**, *268*, 5229−5241.
(282) Spaltenstein, A.; Holler, T. P.; Hopkins, P. B. *J. Org. Chem.* **1987**, *52*, 2977−2979.
(283) Holler, T. P.; Ruan, F.; Spaltenstein, A.; Hopkins, P. B. *J. Org. Chem.* **1989**, *54*, 4570−4576.
(284) Holler, T. P.; Spaltenstein, A.; Turner, E.; Klevit, R. E.; Shapiro, B. M.; Hopkins, P. B. *J. Org. Chem.* **1987**, *52*, 4420−4421.
(285) Hall, H. G. *Cell* **1978**, *15*, 343−355.
(286) Foerder, C. A.; Shapiro, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4214−4218.
(287) Shapiro, B. M. *Science* **1991**, *252*, 533−536.
(288) Weaver, K. H.; Rabenstein, D. L. *J. Org. Chem.* **1995**, *60*, 1904−1907.
(289) Holler, T. P.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 4837−4838.
(290) Reuben, D. M. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 114−121.
(291) Kosower, E. M. In *Glutathione: Chemical, Biochemical and Medical Aspects*; Dolphin, D., Avramovic, O., Poulson, R., Eds.; John Wiley and Sons: New York, 1989; Vol. III, pp 103−146.
(292) Maytin, M.; Leopold, J.; Loscalzo, J. *Curr. Atheroscler. Rep.* **1999**, *1*, 156−164.
(293) Sies, H. *Oxidative Stress*; Academic Press Inc.: London, 1985.
(294) Bailly, F.; Zoete, V.; Catteau, J.-P.; Bernier, J.-L. *FEBS Lett.* **2000**, *486*, 19−22.
(295) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125−2179.
(296) Bailly, F.; Azaroual, N.; Bernier, J.-L. *Bioorg. Med. Chem.* **2003**, *11*, 4623−4630.
(297) Back, T. G.; Moussa, Z.; Parvez, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 1268−1270.
(298) Zoete, V.; Bailly, F.; Catteau, J.-P.; Bernier, J.-L. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2983−2988.
(299) Zoete, V.; Bailly, F.; Vezin, H.; Teissier, E.; Duriez, P.; Fruchart, J.-C.; Catteau, J.-P.; Bernier, J.-L. *Free Radical Res.* **2000**, *32*, 515−524.
(300) Marjanovic, B.; Simic, M. G.; Jovanovic, S. V. *Free Radical Biol. Med.* **1995**, *18*, 679−685.
(301) Holler, T. P.; Hopkins, P. B. *Biochemistry* **1990**, *29*, 1953−1961.
(302) Zoete, V.; Vezin, H.; Bailly, F.; Vergoten, G.; Catteau, J.-P.; Bernier, J.-L. *Free Radical Res.* **2000**, *32*, 525−533.
(303) Turner, E.; Klevit, R.; Hopkins, P. B.; Shapiro, B. M. *J. Biol. Chem.* **1986**, *261*, 13056−13063.
(304) James, S. L. *Microbiol. Rev.* **1995**, *59*, 533−547.
(305) Leckie, C.; Empson, R.; Becchetti, A.; Thomas, J.; Galione, A.; Whitaker, M. *J. Biol. Chem.* **2003**, *278*, 12247−12254.

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