Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function

ARTICLE *in* ANGEWANDTE CHEMIE INTERNATIONAL EDITION · OCTOBER 2003

Impact Factor: 11.26 · DOI: 10.1002/anie.200300573 · Source: PubMed

4 AUTHORS, INCLUDING:

Helmut Sies
Heinrich-Heine-Universität Düsseldorf
450 PUBLICATIONS **26,935** CITATIONS

SEE PROFILE

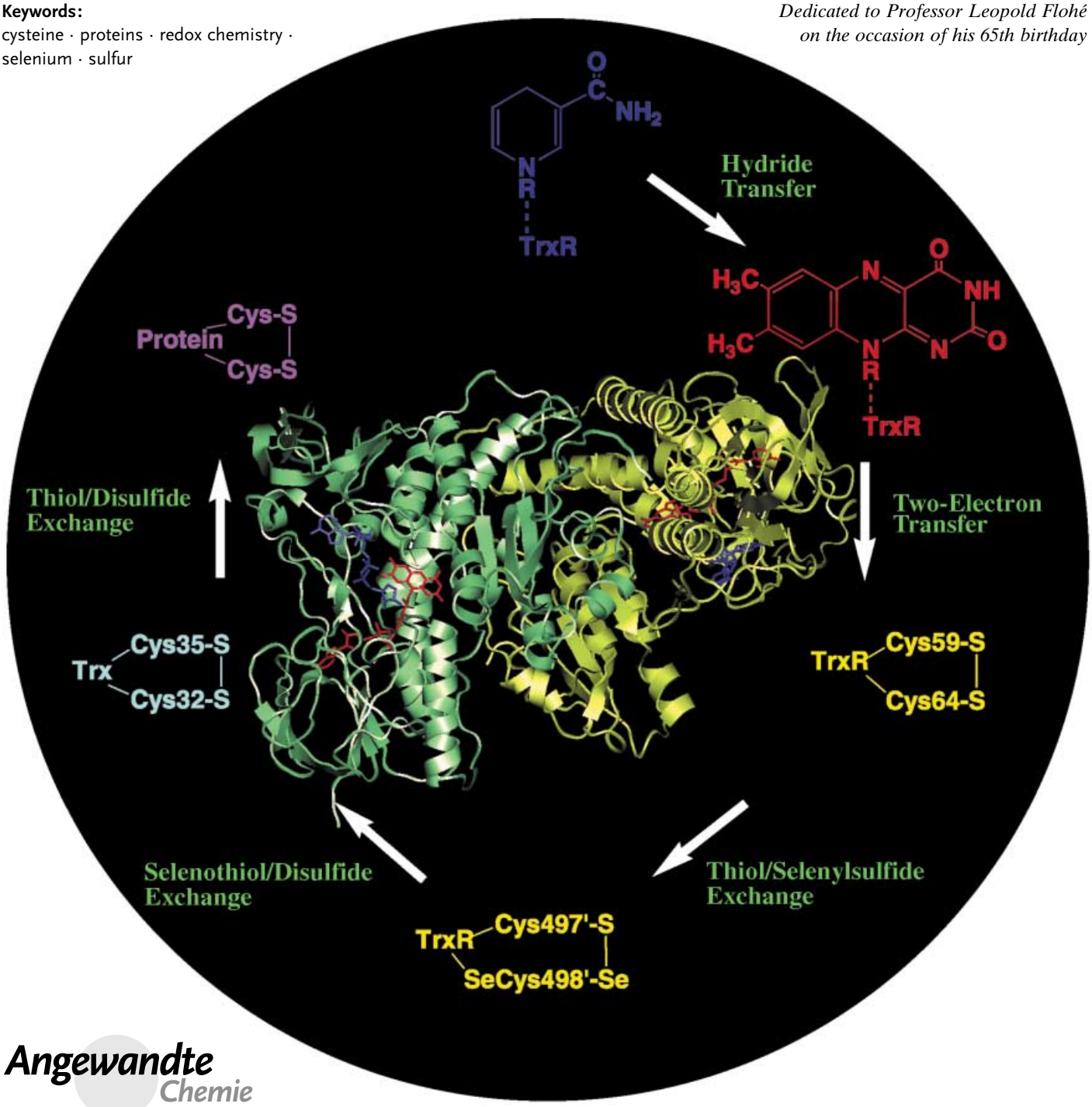
Chalcogen-Containing Proteins

Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function

Claus Jacob, Gregory I. Giles, Niroshini M. Giles, and Helmut Sies**

Keywords:
cysteine · proteins · redox chemistry · selenium · sulfur

Dedicated to Professor Leopold Flohé on the occasion of his 65th birthday



DOI: 10.1002/anie.200300573

Sulfur and selenium occur in proteins as constituents of the amino acids cysteine, methionine, selenocysteine, and selenomethionine. Recent research underscores that these amino acids are truly exceptional. Their redox activity under physiological conditions allows an amazing variety of posttranslational protein modifications, metal free redox pathways, and unusual chalcogen redox states that increasingly attract the attention of biological chemists. Unlike any other amino acid, the "redox chameleon" cysteine can participate in several distinct redox pathways, including exchange and radical reactions, as well as atom-, electron-, and hydride-transfer reactions. It occurs in various oxidation states in the human body, each of which exhibits distinctive chemical properties (e.g. redox activity, metal binding) and biological activity. The position of selenium in the periodic table between the metals and the nonmetals makes selenoproteins ideal catalysts for many biological redox transformations. It is therefore apparent that the chalcogen amino acids cysteine, methionine, selenocysteine, and selenomethionine exhibit a unique biological chemistry that is the source of exciting research opportunities.

From the Contents

1. Introduction

The chalcogen ("ore-generating") elements oxygen, sulfur, and selenium fulfill a wide range of essential biological functions. All three elements are constituents of functional groups in biomolecules that participate in redox reactions in vivo. The fundamental importance of chalcogen-based redox systems in biology becomes apparent when redox-active proteins as a whole are considered.[1–5] Most proteins rely on organic or inorganic cofactors (e.g. metal ions, NAD^+, FAD) for redox activity. In contrast, the chalcogen-containing amino acids cysteine and methionine (Cys and Met, both of which contain sulfur), selenocysteine and selenomethionine (SeCys and SeMet, which contain selenium), and tyrosine (Tyr, which contains redox-active oxygen) endow proteins with a powerful redox chemistry based on the side chains of the amino acids. Moreover, while most redox active metal cofactors occur in just two or three oxidation states in vivo, sulfur in cysteine has been found to occur in up to ten (i.e. from $+6$ to -2, including fractional oxidation states).[6] Figure 1 provides an overview of the most common cysteine, methionine, selenocysteine, and selenomethionine modifications that have thus far been identified in biological systems. Considering that each of them also represents a separate posttranslational protein modification with specific properties (e.g. redox activity, metal binding), the full complexity of this chalcogen biochemistry becomes apparent.

This Review will explore the field of chalcogen biochemistry from the perspective of different oxidation states with a focus on eukaryotic biochemistry. Section 2 deals with the ability of cysteine to undergo posttranslational redox modifications and considers its ability to react through different redox mechanisms, while Section 3 addresses the biological redox chemistry of disulfides and methionine, whose redox transformations during oxidative stress have recently received increased attention. Section 4 explores metal binding as an important property of these individual modifications. Paradoxically, not all redox enzymes have redox-active residues or metals at their active site, and Section 5 discusses dehydrogenase enzymes that contain redox-inactive cysteine or zinc–cysteine complexes. The distinctive redox behavior of selenocysteine and selenomethionine is briefly addressed in Section 6.

2. Cysteine-Based Redox Proteins

Any review of the biological redox chemistry of cysteine is confronted with the complexity of this subject.[7] To provide systematic guidance, it might be worthwhile to classify the redox behavior of cysteine along the lines of underlying redox mechanisms. Sulfur has an electron configuration of $3s^2 3p^4$, which allows oxidation states between $+6$ and -2. In cysteine the element is fully reduced (the oxidation state of -2 is assigned), and its thiol group can undergo a range of reactions under physiological conditions, some of which are illustrated in Scheme 1.

Cysteine in proteins participates in a range of different redox reactions that do not directly involve, but can be

[*] Dr. C. Jacob, Dr. G. I. Giles, Dr. N. M. Giles
School of Chemistry, University of Exeter
Stocker Road, Exeter EX4 4QD (UK)
Fax: (+44) 1392-26-3434
E-mail: C.Jacob@ex.ac.uk
Prof. Dr. H. Sies
Institute for Biochemistry and Molecular Biology I
Heinrich-Heine-Universität Düsseldorf
Postfach 101 007, 40001, Düsseldorf (Germany)
E-mail: sies@uni-duesseldorf.de

 DOI: 10.1002/anie.200300573

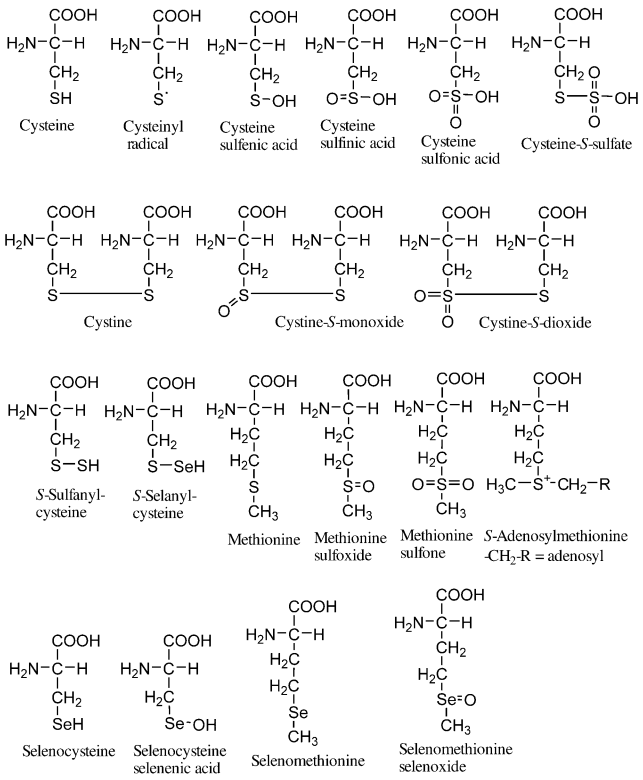
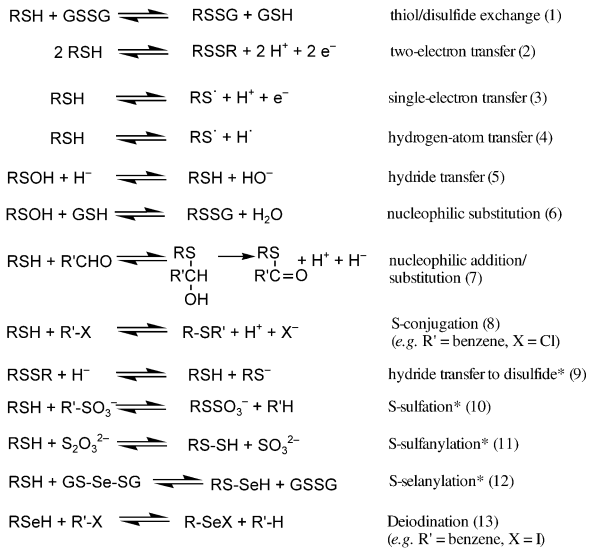


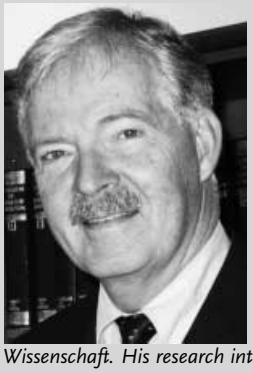
Figure 1. Common modifications of cysteine, selenocysteine, methionine, and selenomethionine in proteins. These modifications have been confirmed or postulated to occur in vivo.



Scheme 1. Redox mechanisms and reactions of cysteine. Details of these mechanisms and their occurrence in vivo are discussed in the text. The asterisk (*) marks reaction sequences theoretically possible in cysteine redox chemistry, but not yet confirmed in vivo. The protonation state of sulfur species in these reactions might vary.

coupled to, electron transfer. This has far-reaching implications for the redox activity of sulfur in vivo, exemplified by the tripeptide glutathione (γ-Glu-Cys-Gly, GSH). GSH is a vital intra- and extracellular mammalian peptide with many biological functions,[8–13] among them maintenance of the intracellular redox balance. GSH ($E^{o\prime}$(GSSG/2 GSH) ≈ −250 mV (NHE)) is readily oxidized to glutathione disulfide

Claus Jacob (born 1969) is a lecturer in inorganic chemistry at the University of Exeter. He received a BSc degree in Chemistry from University of Leicester in 1993, a DPhil in bioinorganic chemistry from the University of Oxford in 1997, and a Magister Artium degree in Philosophy, History, and Psychology from Hagen University in 1998. His research interests include the biological redox chemistry of the chalcogens, rational drug design, bioelectrochemistry, and philosophy of chemistry. He is Chairman and a Director of Exeter Antioxidant Therapeutics Ltd.



Dr. Helmut Sies (born 1942) is a Professor at Heinrich-Heine-Universität Düsseldorf (Germany) and Chairman of the Department of Biochemistry and Molecular Biology I at the Faculty of Medicine. He received an MD degree from the Ludwig-Maximilians-Universität in München (Germany) in 1967, where he completed his Habilitation for physiological chemistry and physical biochemistry in 1972. He was President of the International Society for Free Radical Research (1998–2000) and is President of the Nordrhein-Westfälischen Akademie der Wissenschaft. His research interests include oxidative stress (oxidants and antioxidants) as well as micronutrients and vitamins.

Dr. Gregory Giles (born 1974) is a research fellow at the University of Exeter. He received a BSc degree in Chemistry from the University of Durham in 1996 and a PhD in bioorganic chemistry from the University of Southampton in 2000. His research interests include redox biochemistry and the design of enzyme mimics. He is also a Director of Exeter Antioxidant Therapeutics Ltd.

Dr. Niroshini Giles, née Silva (born 1974) is a research fellow at the University of Exeter. She received a BSc (Hons) degree in Biochemistry from the Imperial College of Science, Technology and Medicine in 1996 and is an Associate of the Royal College of Science. She received a PhD from the University of Southampton in 1999 for her work on enzyme kinetics. Her research involves structural and mechanistic aspects of inflammation, antioxidants, redox signaling, and cancer therapy.

(GSSG) and the GSH/GSSG ratio adjusts the global intracellular thiol redox potential. Most healthy cells have a GSH/GSSG ratio in the range of 100:1.[10,11] This ratio decreases during oxidative stress (Sections 3 and 4) and disturbance of the GSH/GSSG ratio may affect cellular proteins, DNA, and membranes.

Mechanistically, GSH can participate in electron-transfer, atom-transfer, and thiol/disulfide-exchange reactions. As a consequence, it reacts with a wide spectrum of cellular oxidants, while its regeneration involves thiol/disulfide-exchange reactions. GSH and its associated redox proteins can therefore be seen as the "switchboard" of cellular redox chemistry that integrates various different cellular redox pathways.[12,13] These distinct redox mechanisms will now be discussed in more detail.

2.1. Thiol/Disulfide-Exchange Reactions

Thiol/disulfide-exchange reactions are nucleophilic substitutions of a thiol or thiolate nucleophile (RSH or RS^-) on a disulfide bond (RS–SR), which leads to the formal oxidation of the nucleophile and reduction of the "exchanged" leaving group (Reaction (1) in Scheme 1). These reactions are among the most common redox reactions of cysteine residues in vivo. They are, for example, used to form structural disulfides in peptides and proteins which are reversible to maintain the cellular redox balance, to regulate enzyme activity (*S*-thiolation or *S*-glutathionylation, see Section 2.2), to allow oxidative cell-signaling pathways, and to provide powerful redox catalysis. In proteins, the two-electron redox potential of the cysteine/cystine couple is influenced by adjacent amino acid residues and can vary from approximately -125 mV in the case of *Escherichia coli* (*E. coli*) disulfide-bond-promoting product of gene *dsbA* (DsbA) to -270 mV for thioredoxin Trx-1 (Table 1). This diversity in potentials is mirrored by the diverse role that these thiol/disulfide oxidoreductase proteins play in vivo, ranging from protein reduction to disulfide bond formation. Notably, although potentials can formally be measured for the thiol/disulfide couple of these proteins, this does not imply that electron transfer is necessarily part of their redox cycles in vivo.

The thioredoxin-motif Cys-Xaa1-Xaa2-Cys (Xaa = amino acid, see Table 1) is a typical thiol/disulfide redox motif present at the active site of thioredoxins (Trx), glutaredoxins (Grx), tryparedoxins (TXN), and DsbA and protein disulfide isomerases (PDI). Although these proteins fulfill different biological roles, their redox reactions follow a mechanism similar to that of Trx.

The ubiquitous 12 kDa thioredoxins are part of redox chains that play various roles in the reduction of proteins. Human Trx proteins, for example, may be involved in activation and binding of transcription factors and in the activity of redox enzymes and may also play a major role during oxidative stress and apoptosis.[14] They reduce disulfides in proteins through a thiol/disulfide-exchange mechanism, and in the process form an intramolecular disulfide at the active site. All Trx proteins have a similar tertiary structure, which consists of a central core of five strands forming one β sheet enclosed by four α helices. The active site of human Trx1 contains the conserved amino acid sequence Cys32-Gly-Pro-Cys35. The two cysteine residues are in close vicinity, thereby allowing the reversible oxidation of the SH to the S-S redox state. The thiol group of the N-terminal Cys32 has a low pK_a value (6.7) and acts as a nucleophile for the reduction of disulfides in target proteins. This reaction results in the intermediate formation of a mixed disulfide (Cys32-S-SR), followed by a rapid thiol/disulfide exchange with Cys35, which results in the thioredoxin forming a disulfide (Cys32-S-S-Cys35) and concomitantly generating two reduced cysteines in the target protein.[15] Oxidized Trx relies on thioredoxin reductase (TrxR) for regeneration of its reduced state in a process that consumes NADPH (see Section 2.3).

Thiol/disulfide-exchange reactions are not only used to break (i.e. reduce) disulfide bonds in proteins, but also to generate them. Disulfide bridges contribute significantly to protein folding and stability and this process is catalyzed by enzymes such as PDI and DsbA, which are located in the endoplasmic reticulum of eukaryotic cells or in the periplasm of prokaryotes, respectively. Although DsbA and PDI have an active-site motif similar to that of Trx (Table 1), their considerably more positive redox potentials (-125 mV and -127 mV, respectively) allow them to function as thiol/disulfide oxidoreductases and to facilitate correct disulfide bond formation.

2.2. Conjugation and S-Thiolation/S-Glutathionylation

The nucleophilic properties of the cysteine residue in glutathione (and proteins) are used in both conjugation and

Table 1: Thiol/disulfide oxidoreductases. The cysteine redox potential is fine-tuned in these proteins and ultimately influences their biological activity.

Protein	Motif in active site	Redox potential [mV versus NHE][a]	Biological function
Thioredoxin (12 kDa)	-Cys-Gly-Pro-Cys-	-270	protein reduction[14]
Glutaredoxin (9 kDa)	-Cys-Pro-Tyr-Cys-	-233 (Grx-1); -198 (Grx-3)	catalyzes reduction of ribonucleotide reductase by GSH[132]
Tryparedoxin (16 kDa)	-Cys-Pro-Pro-Cys-	-249	utilizes trypanothione in place of GSH[133,134]
Protein disulfide isomerases (57 kDa)	-Cys-Gly-His-Cys-	-127	catalyzes reduction and reformation of disulfide bonds[135]
DsbA (21 kDa)	-Cys-Pro-His-Cys-	-125	catalyzes formation/rearrangement of protein disulfide bonds from thiols/misformed disulfide bonds[136]

[a] Reaction: $RSSR + 2e^- + 2H^+ \rightarrow 2RSH$.

thiol/disulfide-exchange reactions. Reversible *S*-thiolation/*S*-glutathionylation is frequently observed in proteins; together with reversible *S*-nitrosylation and irreversible oxidation, this oxidative modification plays an important regulatory role.[16–19] Mechanistically, *S*-thiolation can occur by a range of different physiological processes, among them thiol/disulfide-exchange reactions (e.g. *S*-glutathionylation of a thiol group of a cysteinyl residue in a protein through slow reaction with GSSG), oxidation (e.g. reaction of a protein cysteinyl sulfenic acid with GSH), and reactions of nitrosylated species (e.g. reaction of a protein cysteinyl group with *S*-nitrosoglutathione (GSNO) leading to the formation of a mixed disulfide). Although resting levels of *S*-thiolated proteins in cells are only around 1%, oxidative stress (see Section 3) leads to a significant increase in *S*-thiolation, as observed for neutrophil and monocyte proteins, such as carbonic anhydrase III just minutes after the oxidative burst.[20] *S*-Thiolation modulates enzyme activity,[16–18] but is reversible and might ultimately protect proteins from irreversible oxidative damage.

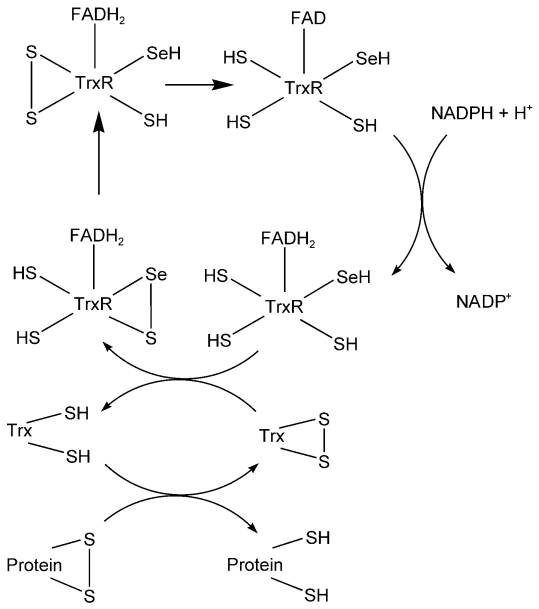
Cysteine takes part in related nucleophilic substitution reactions not considered as redox reactions. Such thioether-formation reactions (also called *S*-conjugation), for example, substitution of GSH for chloride in chlorobenzene, are irreversible processes that enhance excretion of toxic hydrophobic substances (such as halobenzenes) from the body by increasing their hydrophilicity.[8,21] These reactions are catalyzed by the glutathione *S*-transferase (GST) enzymes that contain cysteine residues in the active site (Reaction (7) in Scheme 1). Although GST enzymes do not catalyze redox reactions, they are redox-sensitive.[22–25]

2.3. Two-Electron Transfer Reactions

Although thiol/disulfide-exchange reactions are essential in maintaining the cellular redox balance and keeping proteins in their active reduced state, they do not lower the total number of disulfides in the cell. Reduction of disulfides without subsequent formation of other disulfides is achieved by the enzymes GSSG reductase ("glutathione reductase", GR), lipoamide dehydrogenase (LipDH), and thioredoxin reductase (TrxR). These proteins contain active-site cysteine residues that integrate the thiol/disulfide exchange with the electron-transfer mechanism and provide continuous reduction of disulfides by NADPH (Reaction (2) in Scheme 1).[26]

Human glutathione reductase regenerates GSH from GSSG, oxidizing NADPH in the process. This 104-kDa dimeric enzyme contains two redox systems per subunit, that is, a thiol/disulfide redox system based on two cysteine residues (Cys58 and Cys63) and a flavin cofactor. GSSG is reduced to two GSH units through thiol/disulfide exchange that initially leads to a mixed disulfide Cys58-S-SG and ultimately to an intramolecular disulfide Cys58-S-S-Cys63 in GR.[27] This disulfide is reduced by direct two-electron transfer from $FADH_2$ (Cys58-S-S-Cys63 + $FADH_2$ → Cys58-SH + Cys63-SH + FAD). FAD is subsequently reduced by hydride transfer from NADPH (FAD + NADPH + H^+ → $FADH_2$ + $NADP^+$). Glutathione reductase, therefore, integrates thiol/disulfide exchange, electron transfer, and hydride transfer, whereas Cys58 and Cys63 participate in two different types of redox reaction. In the yeast enzyme, Cys50 and FAD form a charge-transfer complex with an absorbance at 540 nm.[28] The relatively large difference in redox potential between the GSSG/2GSH ($E^{o\prime} = -241$ mV) and $NADP^+$/NADPH ($E^{o\prime} = -327$ mV) couples and the high ratio of NADPH/$NADP^+$ in healthy cells lead to extensive reduction of GSSG and the high GSH/GSSG ratio mentioned above.

Whereas glutathione reductase reduces GSSG to GSH, thioredoxin reductase enzymes form part of the cellular protein disulfide reduction machinery by reducing the disulfide in the active site of Trx in the presence of NADPH. Their catalytic mechanisms resemble that of GR with one major difference: Human TrxR is a 100–130-kDa dimeric flavoprotein that contains a Gly-Cys-SeCys-Gly sequence per subunit and—unlike GR—initially operates with a (thiol, selenol)/selenylsulfide (SH, SeH/S-Se) couple rather than a (thiol, thiol)/disulfide (2SH/S-S) redox couple (Scheme 2).[29] The selenocysteine and adjacent cysteine



Scheme 2. Reduction of protein disulfides by the thioredoxin–thioredoxin reductase redox chain. A chain of thiol/disulfide-exchange reactions drives the reduction of disulfides in proteins. TrxR couples thiol/disulfide exchange to intramolecular electron and hydride transfer.[15]

make up the redox-active site of the enzyme that reduces the Trx disulfide through a (thiol, selenol)/disulfide-exchange mechanism, forming a selenylsulfide in the process. The latter is re-reduced to the selenol and thiol through a (thiol, thiol)/selenylsulfide-exchange reaction involving two cysteine residues in an additional redox center located in the other subunit of the dimer. As in GR, electron transfer from $FADH_2$ then reduces the disulfide formed in the exchange reaction. Although the precise redox potentials of all redox couples involved (e.g. the (SH, SeH)/Se-S couple) have not yet been determined, reduction of Trx by NADPH seems to occur in a relatively small potential range from approximately

-327 mV ($NADP^+$/NADPH) to -283 mV (unbound FAD/$FADH_2$)[30] to -270 mV (for Trx, Cys-S-S-Cys/2 Cys-SH). The significant difference between the redox potentials of cysteine (-233 mV) and selenocysteine (-488 mV) is shown in Figure 2. In human TrxR, the selenylsulfide–disulfide redox

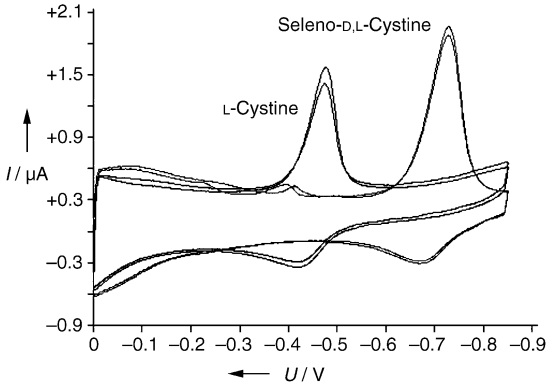


Figure 2. Cyclic voltammograms of cystine and selenocystine. Voltammograms of L-cystine (100 µM) and D,L-selenocystine (50 µM) were recorded in potassium phosphate buffer (pH 7.0; 200 mM) at 25 °C and at a scan rate of 500 mVs^{-1} with a dropping mercury working electrode, a standard silver reference electrode (SSE), and a platinum counterelectrode. The significant difference in redox potential of about 250 mV between selenocysteine/selenocystine (-710 mV versus SSE, i.e., -488 mV versus NHE) and cysteine/cystine (-455 mV versus SSE, -233 versus NHE) confers considerably more reducing properties to selenocysteine.

chain seems to play an essential role, which might become fully apparent once the potentials of all redox couples involved are determined.

Moreover, the activity of human TrxR can be reversed to reduce mediators of oxidative stress in the presence of reduced Trx, depending on the substrates. With peroxynitrite, TrxR acts as a peroxynitrite reductase, as was demonstrated by using the selenoorganic compound ebselen (2-phenyl-1,2-benzoisoselenazol-3[2*H*]-one) as a reductant.[31] Ebselen then acts as a "superfast" Trx oxidant.[32] Again in this case the presence of selenium might play a decisive role (see Section 6.).

Although known mammalian TrxR enzymes operate by direct two-electron transfer to the disulfide of the active site, two sequential one-electron transfers are theoretically possible. This reduction route is observed in plants and bacteria for ferredoxin-thioredoxin reductase (FTR). FTR is briefly discussed here, since it provides unique insight into the redox behavior of disulfides and thiyl radicals (other iron/sulfur proteins have been extensively reviewed elsewhere[33]). Spinach FTR is a 30-kDa heterodimeric enzyme that contains a redox-active disulfide (Cys 54-Cys 84) and an $[4Fe\text{-}4S]^{2+}$ ($S = 0$) iron–sulfur cluster in its 13-kDa catalytic β subunit.[34] This enzyme is unique in that it is the only known iron/sulfur protein that can reduce a disulfide bond in the presence of reduced [2Fe-2S] ferredoxin (the latter replaces the NADPH that is used as an electron source for mammalian TrxR enzymes). The reduced $[2Fe\text{-}2S]^{2+}$ cluster of ferredoxin acts as a one-electron donor that reduces Cys 54-S-S-Cys 84 to Cys 54-S^- and a thiyl radical (Cys 84-$S^\bullet$).[35] Cys 54-S^- then forms a heterodisulfide with Trx in a mechanistic step analogous to that of mammalian TrxR. The thiyl radical is deactivated and stabilized by coordinating to an iron center of the $[4Fe\text{-}4S]^{2+}$ ($S = 0$) cluster of FTR, forming an intermediate species with a formally oxidized $[4Fe\text{-}4S]^{3+}$ ($S = {}^1/_2$) cluster with one pentacoordinated iron center and a formally reduced Cys 84-S^- ligand.[36] A second ferredoxin molecule then supplies another electron to this intermediate species to generate Cys 84-S^- and $[4Fe\text{-}4S]^{2+}$ at the active site. Cys 84-S^- then attacks the mixed disulfide between FTR and Trx, resulting in fully reduced Trx and regeneration of the disulfide in the active site.

In general, the reduction potential for the one-electron reduction of a disulfide is experimentally inaccessible. By examination of the potentials for the available two-electron reduction, however, it is apparent that electron transfer from ferredoxin ($E^{o\prime} = -350$ mV) to Trx ($E^{o\prime} = -210$ mV) through the disulfide of the active site of FTR ($E^{o\prime} = -230$ mV) is thermodynamically favorable.[34] Unlike NADH, ferredoxin is only a one-electron donor, which prohibits the normal two-electron reduction pathway and requires the formation of a highly reactive thiyl radical as an intermediate. FTR uses the iron–sulfur cluster ($E^{o\prime} = -650$ mV for $[4Fe\text{-}4S]^{3+/2+}$) to stabilize this otherwise transient radical species. FTR therefore provides an elegant solution to a considerable mechanistic challenge (one- versus two-electron transfer) by amalgamating the redox and metal-binding properties of cysteine to enable its unique function.

As has been shown for spinach FTR, electron transfer to disulfides can result in the formation of thiyl radicals as intermediates. This leads us to consider the role of sulfur-based radicals in mammalian biology, in which the thiyl radical directly participates in electron- and atom-transfer reactions.

2.4. Radical Reactions

Radical-formation and -transfer mechanisms provide enzymes with additional cysteine-based redox pathways that involve redox processes distinctly different from exchange and electron-transfer reactions. The thiol/thiyl radical redox couple occurs in important enzymes such as the ribonucleotide reductase (RNRase) superfamily, pyruvate formate lyase (PFL), and benzylsuccinate synthase (BSS). In these enzymes the thiyl radical is formed by two alternative pathways: long-range one-electron transfer from the thiol (with subsequent loss of H^+ (Reaction (3) in Scheme 1) and short-range hydrogen-atom abstraction from the thiol (Reaction (4) in Scheme 1).

Long-range one-electron transfer is found in aerobic Fe-dependent (class I) RNRase from *E. coli*, which generates a thiyl radical by electron transfer from cysteine to an oxygen-centered tyrosyl radical.[37] In contrast, the formation of the thiyl radical in anaerobic formate-dependent (class III) RNRase (ARR) from bacteriophage T4 and in anaeorobic pyruvate formate lyase (PFL) from *E. coli* proceeds through a short-range hydrogen-atom transfer from cysteine to a (carbon-centered) glycyl radical.[38]

The formation of the glycyl radical in ARR requires a range of components, including a flavodoxin reductase system, NADPH, *S*-adenosylmethionine (SAM), and dithiothreitol (DTT).[39] The [4Fe-4S] iron/sulfur cluster of ARR reduces SAM to methionine and the 5′-deoxyadenosyl radical by one-electron transfer. This is followed by a hydrogen-abstraction step from glycine by the adenosyl radical, resulting in the formation of 5′-deoxyadenosine and the glycyl radical, which then forms the thiyl radical. Similarly, PFL is catalytically activated by PFL activase (which also contains an iron/sulfur center) utilizing SAM and generating a glycyl radical (Gly 734 in PFL from *E. coli*), methionine, and 5′-deoxyadenosine. A nucleophilic thiyl radical at Cys 418 in this enzyme is then formed by a sequential hydrogen abstraction from Cys 419 by the glycyl radical.

Once formed, these thiyl radicals are highly reactive (i.e. oxidizing) and are reduced to thiols by hydrogen-atom-abstraction reactions, the hallmark reaction of thiyl radicals. In RNRases the hydrogen atom is abstracted either from external dithiols or formate (in the case of ARR), which are ultimately oxidized to disulfides or CO_2, respectively. In *E. coli* PFL, the Cys 418 radical attacks the carbonyl carbon atom of the pyruvate substrate to form an unstable, covalent *S*-acetyl-PFL radical species that rapidly fragments to produce the (nonradical) acylthioester of Cys 418 and a formate radical, which then abstracts a hydrogen atom from Cys 419. The Cys 419 radical, in turn, abstracts a hydrogen atom from Gly 734 to recycle the glycyl radical. The *S*-acetyl-PFL form reacts with coenzyme A (CoA) to form acetyl-CoA and the recycled enzyme. Overall, this reaction generates acetyl-CoA and formate from CoA and pyruvate, all of which are nonradical species.[40]

A glycyl–thiyl radical chain is also found in benzylsuccinate synthase (BSS) from *Thauera aromatica* which catalyzes the initial step in the degradation of toluene to benzylsuccinate. This BSS contains an active-site glycyl radical at Gly 825. Abstraction of a hydrogen atom from the methyl group of toluene results in the formation of a benzyl radical, which attaches to the double bond of fumarate to form a benzylsuccinate radical intermediate. This species is then reduced to benzylsuccinate by hydrogen abstraction from the thiolate of Cys 489, which in turn abstracts the hydrogen atom from Gly 825 to regenerate the initial glycyl radical.[41]

Hydrogen-atom-transfer reactions represent one example of "atom-transfer reactions" that cysteine can undergo in vivo. Whereas hydrogen-atom transfer is a radical reaction, other atom-transfer reactions that involve cysteine (such as hydride transfer) proceed through nonradical mechanisms. These transfer reactions frequently occur as part of the same catalytic cycle and are therefore discussed together.

2.5. Atom-Transfer Reactions

Antioxidant enzymes such as glutathione reductase and thioredoxin reductase are frequently evaluated for additional catalytic activity. The peroxynitrite reductase activity of TrxR, for example, has already been mentioned. Peroxidation of thiols often leads to a distinct oxidation state of sulfur, with chemical properties that differ dramatically from those of the disulfide state: the sulfenic acid (Figure 1). This cysteine modification is only stable, however, in the absence of additional thiol groups (the latter would react with sulfenic acid to form a disulfide and water). In human GR, for example, oxidation with H_2O_2 results in the formation of the Cys 58-S-S-Cys 63 disulfide, an enzyme that exhibits poor peroxidase activity.[42]

In contrast, redox enzymes such as NADH peroxidase (Npx), NADH oxidase (Nox), and peroxiredoxins (Prx)[43] function through a cysteine (Cys-SH)/cysteine sulfenic acid (Cys-SOH) redox couple that allows effective peroxidation catalysis. These enzymes contain a catalytic cysteine residue that is oxidized to cysteine sulfenic acid, a highly reactive (i.e. oxidizing) cysteine species whose reduction can proceed through two distinct mechanisms both of which occur in vivo: either through an exchange reaction involving two thiol equivalents or through hydride transfer (Reaction (5) in Scheme 1).

Npx from *Streptococcus* (*Enterococcus*) *faecalis* is a tetramer of 46 kDa per subunit and belongs to the family of pyridine nucleotide–disulfide oxidoreductases. Unlike other cysteine-based oxidoreductases (e.g. GR), it contains just one cysteine residue in the active site of each subunit that catalyzes the reduction of H_2O_2 in the presence of NADH. The reduced thiolate nucleophile attacks H_2O_2 to form Cys 42-SOH and water. Hydride transfer from NADH to Cys 42-SOH then occurs through FAD. Structural data show that the flavin is located in the enzyme between the reducing NADH and the Cys 42-SH/Cys 42-SOH redox center to optimize hydride transfer.

This redox mechanism has been supported by mutagenesis experiments that were used to test if and how many cysteine residues are essential for this peroxidase activity. Mutagenesis of Cys 42 to serine or alanine results in a low turnover rate, emphasizing the importance of the cysteine redox center.[44,45] Introduction of an additional cysteine residue (Cys 40) at the active site, however, almost completely eliminates catalytic activity (k_{cat} of mutant is about 0.1 % of the value of that of wild-type Npx[42]). These results illustrate both the significance of the sulfenic acid state in peroxidases and the importance of avoiding the disulfide state in these enzymes as the formation of the Cys 40-S-S-Cys 42 disulfide from Cys 40-SH and Cys 42-SOH is detrimental to the activity.

Hydride transfer (e.g. from NADH or $FADH_2$) to disulfides—a mechanism possible in theory—does not seem (yet) to have been observed in proteins (Reaction (9) in Scheme 1); this is also true for electron transfer to sulfenic acids.

The redox mechanism of Nox from *Streptococcus* (*Enterococcus*) *faecalis* is closely related to that of Npx (Nox has 44 % sequence identity with Npx and the Cys-SH/Cys-SOH redox couple is conserved in both enzymes). Nox catalyzes the reduction of molecular oxygen to water with the consumption of four electrons (i.e. 2 mol equivalents of NADH). It is a dimeric enzyme (50 kDa per subunit) and each subunit contains one cysteine residue (Cys 42) and one FAD redox center. O_2 is first activated by reaction with $FADH_2$ to form peroxyflavin ($FADH_2O_2$). The latter reacts

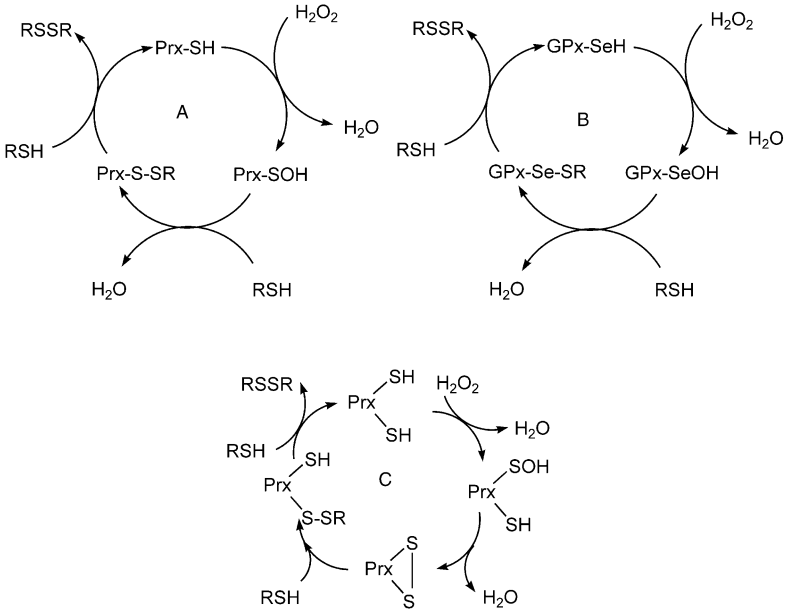
with the thiolate of Cys42[46] in a reaction that resembles the peroxidation step of the Npx cycle to form Cys42-SOH and FADHOH. The FADHOH then eliminates water to generate FAD, which is reduced by 1 equivalent of NADH to $FADH_2$. Cys42-SOH is re-reduced to the thiol by hydride transfer from $FADH_2$. The FAD formed in this reaction is reduced to $FADH_2$ by another equivalent NADH to regenerate the fully reduced thiol and $FADH_2$ for the next round of catalysis.

As in the case of Npx, mutagenesis experiments strongly support this redox mechanism. Replacement of Cys42 by serine (i.e. removal of one of the two redox centers) results in an enzyme that reduces O_2 to H_2O_2 by using 1 mol equivalent of NADH. Although the flavin center still maintains its function, the lack of the sulfur redox center prevents reduction of H_2O_2 to water.[46]

From a biochemical point of view, the sulfur-based redox mechanism of Nox is even more interesting when the overall reaction is considered from a different perspective. Nox catalyzes the reduction of oxygen by NADH, a reaction not dissimilar to processes that occur in the human mitochondrial respiratory chain. Whereas metal-based redox centers in proteins such as iron/sulfur proteins, the Rieske center, cytochrome *c*, and cytochrome *c* oxidase are responsible for the reduction of oxygen during respiration, Nox accomplishes a chemically related task by using exclusively nonmetal redox systems.

Although the catalytic cycles of Npx and Nox are closely related, the sulfenic acids contained in human peroxiredoxin enzymes exhibit a different redox mechanism that resembles that found in the well-known selenium enzyme glutathione peroxidase (GPx, see Section 6.). As is the case for GPx, Prx enzymes catalyze the reduction of H_2O_2 and alkyl hydroperoxides to water or the corresponding alcohol, oxidizing thiols to disulfides in the process. The cysteine residue (Cys47) in the active site of the human 1-Cys-Prx (hORF6) reacts with peroxide in a substitution reaction similar to those of Npx and Nox, in which the thiol(ate) forms a S–O bond and releases hydroxide. In marked contrast to Npx and Nox, however, Cys47-SOH is not reduced by hydride transfer (Prx is not a flavin protein) but reacts with 2 equivalents of thiols to regenerate the reduced form of Cys47 and a disulfide (Scheme 3). Reduction of the sulfenic acid therefore proceeds through exchange reactions that involve the substitution of OH^- by RS^- with subsequent formation of a mixed disulfide.

Although we have mentioned that the presence of two cysteine residues in close vicinity would prevent the formation of a stable sulfenic acid, 2-Cys-Prx enzymes use a redox pathway that involves disulfide formation from a sulfenic acid and a thiol (Scheme 3).[47] The disulfide formed is then reduced through a thiol/disulfide-exchange mechanism. This disulfide has been observed in the crystal structure of the 2-Cys-Prx heme-binding protein (HBP-23) as a disulfide bond that forms between Cys52 on one subunit and Cys173 on the second subunit.[48] The precise oxidation state of the cysteine residue in the active site has yet to be assigned unambiguously. The crystal structure of decameric peroxiredoxin TPx-B from human erythrocytes was recently determined with a resolution of 1.7 Å. Interestingly, this shows the Cys51 in the active site exists as a cysteine sulfinic acid (Cys-SO_2H), which is buried within the active-site pocket.[49] The presence of a sulfinic rather than a sulfenic acid in this enzyme and the precise meaning of this finding are still controversial. Unlike sulfenic acids, sulfinic acids cannot easily be reduced by GSH or other thiols, and as a consequence the presence of a second cysteine in close vicinity to the sulfinic acid might not lead to the formation of disulfide.[25]



Scheme 3. Catalytic cycles of 1-Cys and 2-Cys peroxiredoxins and glutathione peroxidase. Although they use different chalcogens for their catalysis, 1-Cys Prx (A) and GPx (B) have comparable redox cycles.[43,111] Peroxidation of the thiol (selenol) by H_2O_2 generates highly reactive sulfenic (selenenic) acids. Reduction occurs through exchange reactions in which 2 equivalents of substrate thiols (e.g. GSH, dithiothreitol) are consumed. The redox cycle of 2-Cys-Prx involves condensation of Cys-SOH and Cys-SH to form either an intramolecular disulfide or, as in case of the more common oligomeric Prxs, an intersubunit disulfide (C).[47] In vivo, these thiols and selenols are in equilibrium with corresponding thiolates and selenolates.

The growing interest in redox-active cysteine does not imply, however, that the biological redox chemistry of sulfur is limited to reactions of the thiol group. Less-well-known oxidized sulfur species in proteins (e.g. disulfide-*S*-oxides, methionine-*S*-oxides, persulfides) have only recently attracted attention. The next section discusses some of these biochemically more "exotic" and sometimes speculative species.

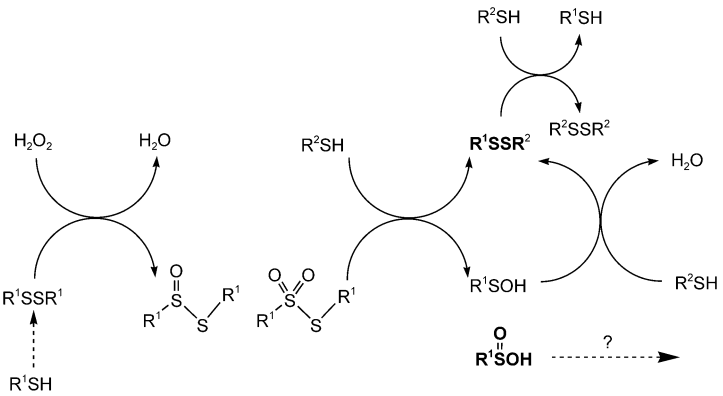
3. Beyond Cystine: Disulfide-S-Oxides, S-Sulfates, Sulfoxides, and Sulfones in Proteins

The ability of sulfur to occur in many oxidation states and to form sulfur–oxygen, sulfur–sulfur and sulfur–selenium bonds results in a range of less-well-known cysteine modifications. Whereas some of these species, such as cysteine *S*-sulfate (Cys-S-SO_3^-) are formed through transfer pathways, other species form as a result of oxidative stress.

Oxidative stress, a biochemical condition initially defined in 1985,[50–52] is associated with many human diseases ranging from neurodegenerative and autoinflammatory conditions such as Alzheimer's disease and rheumatoid arthritis to diabetes and cancer. This pathological condition is characterized by an imbalance between cellular oxidizing and reducing species and provides unusual oxidizing conditions in vivo characterized by the presence of reactive oxygen species that can cause oxidative damage to biomolecules. Owing to the accessibility of its oxidation states, cysteine is a prime target of many of these oxidants, and the harsh oxidizing conditions present during oxidative stress promote the formation of cysteine and methionine species that are found in considerably smaller amounts in healthy cells (Figure 1). It should be emphasized from the outset that many of the modifications discussed in this section have only recently been discovered in vivo and that their biochemistry (if any) is still controversial.

3.1. Disulfide-S-oxides

Disulfides are not necessarily the end products of the oxidation of cysteine under oxidative stress. Oxidation of disulfides can lead to highly reactive disulfide-*S*-monoxides (thiosulfinates, RS(O)SR) and disulfide-*S*-dioxides (thiosulfonates, $RS(O)_2SR$). Disulfides such as cystamine and GSSG are easily oxidized to disulfide-*S*-oxides.[53,54] These "activated" disulfides rapidly react with thiols in proteins and enzymes and deactivate the enzymes in the process. Disulfide-*S*-oxides provide a cysteine redox pathway leading to redox cascades that also involve sulfenic and sulfinic acids and glutathionylation of proteins. One such sulfur redox cascade involving disulfide-*S*-oxides is shown in Scheme 4. Although these species have primarily been discussed in the context of small reactive sulfur species and oxidative stress, new evidence also points to the presence of disulfide-*S*-oxides in proteins.[53–58] As these cysteine modifications are highly reactive and have virtually no distinct spectroscopic or chemical properties, their identification in proteins is difficult.



Scheme 4. Redox cascade of oxidized cysteine species. Oxidation of disulfides (RSSR) to disulfide-*S*-oxides with subsequent stepwise reduction reactions involving protein thiols (RSH) have been postulated to occur in vivo during oxidative stress.[55,83] Species in bold denote the likely end products of these cascades. The redox activity of sulfinic acids in vivo is still under investigation.[55]

Future developments in X-ray protein crystallography, mass spectrometry, and NMR spectroscopy might provide deeper insight into oxidative cystine modifications in proteins.

3.2. S-Sulfocysteine, Persulfides, and Selenopersulfides

S-sulfated cysteines, the so-called *S*-sulfocysteines (Cys-S-SO_3^-, Figure 1), are also found in vivo and these species can be considered a disulfide modification. Species such as *S*-sulfocysteine and *S*-sulfoglutathione ($GSSO_3H$) occur during enzyme-catalyzed detoxification of sulfite in humans. GSSG initially reacts with sulfite to form $GSSO_3H$ and GSH. The $GSSO_3H$ is then enzymatically hydrolyzed to sulfocysteinylglycine and, finally, to *S*-sulfocysteine.[59] These *S*-sulfated metabolites readily react with proteins. $GSSO_3H$, for example, is a competitive inhibitor of glutathione S-transferase (GST), which is active in the detoxification of carcinogens; this inhibition could thus be responsible for the carcinogenic properties of SO_2.[60] Although posttranslational modification of whole proteins by *S*-sulfation in humans has not yet been reported, *O*-sulfation (sulfate transfer from 3′-phosphoadenosine 5′-phosphosulfate to free hydroxy groups such as those of tyrosine residues in proteins) was recently described as a possible biochemical alternative to *O*-phosphorylation in signaling and regulatory pathways.[61,62]

Whereas *S*-sulfocysteine contains one highly oxidized sulfur atom, *S*-sulfanylcysteine (Cys-S-SH) is a persulfide species. This catenation event is found, for example, at the active site of sulfurtransferases such as bovine liver rhodanese.[63] This enzyme was recently used to generate an active site *S*-selanylcysteine (Cys-S-SeH) in vitro in a reaction involving selenite and GSH.[64] Although the relevance of this modification in vivo remains to be demonstrated, a chemically similar species, glutathione selenopersulfide (GSSeH), is an integral part of selenocysteine metabolism. An *S*-selanylcysteine residue was also postulated to exist in CO dehydrogenase from the aerobic bacterium *Oligotropha carboxidovorans*,[65] although this claim has now been withdrawn.[66] *S*-selanylation and its underlying mechanism (Reaction (12) in Scheme 1) are still highly controversial, but would provide an elegant pathway to introduce selenium into proteins that would not rely on the elaborate expression machinery required for the incorporation of selenocysteine (see Section 6.).

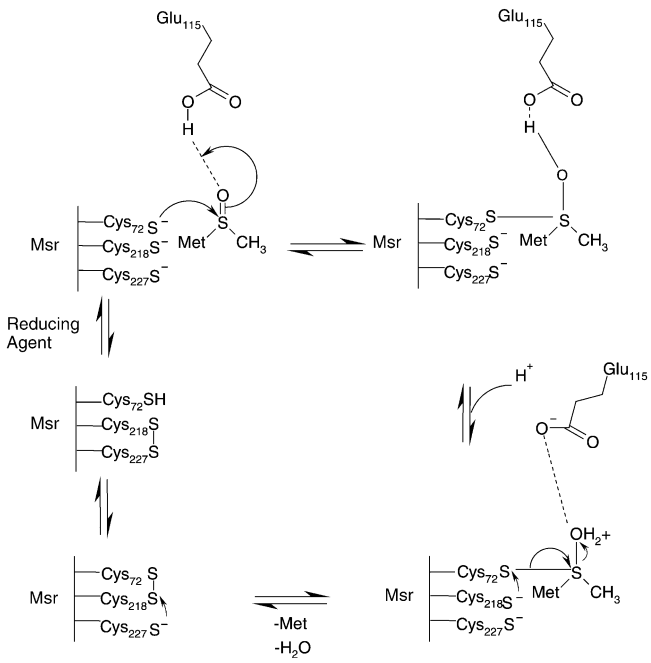
3.3. Sulfoxides and Sulfones—Biological Redox Chemistry of Methionine

Methionine is encoded by the start codon AUG, which sets the reading frame for protein synthesis and is therefore present in most proteins. Like disulfides, its dialkyl sulfide (thioether) group (Figure 1) is susceptible to oxidation with subsequent formation of sulfoxides and sulfones. The oxidation of methionine in proteins to methionine sulfoxide has a biochemical and physiological role.[67,68] Methionine exhibits antioxidant properties,[69,70] and their significance in vivo is presently under investigation.

Methionine residues on protein surfaces provide a high concentration of antioxidant residues that can act as protection against oxidation of other residues that are vital for activity. On the basis of a protein with just eight modifiable methionine residues and a spherical diameter of 60 Å, the concentration of methionine within that volume would be approximately 100 mM, about ten times the cytosolic concentration of GSH.[69] This protective role of methionine has been supported by recent experimental evidence. For example, oxidation of 8 of the 16 methionine residues in glutamine synthetase to the sulfoxide can occur per enzyme subunit without significantly affecting enzyme activity,[71] and methionine residues at or near the active site of this enzyme may prevent autoxidation by the substrate, products, or cofactors.

The discovery of the human enzyme methionine sulfoxide reductase (Msr) has added weight to the notion of redox-active methionine in proteins and enzymes. Whereas most sulfoxides do not spontaneously react with GSH, Msr ensures that sulfoxide formation is reversible in the presence of NADPH.

Reduction of the sulfoxide is also interesting from a mechanistic point of view (Scheme 5). Two possible reaction mechanisms have been proposed for Msr activity, proceeding either through a chain of thiol/disulfide-exchange steps or



Scheme 5. Mechanism of methionine sulfoxide reductase. Nucleophilic attack of cysteine at the oxidized sulfur atom leads to the formation of a tetravalent intermediate. Proton transfer and attack of Cys 218 on Cys 72 facilitate loss of water and collapse of the intermediate to release methionine. The active site is regenerated by thiol/disulfide exchange with Trx.[72]

utilizing a sulfenic acid intermediate. A catalytic mechanism has been reported for bovine MsrA (bMsrA), which has an 88% sequence similarity to that of human MsrA.[72] The Cys 72 residue in the active site of bMsrA forms a thiolate anion and its nucleophilic attack on the sulfoxide sulfur atom of Met(O) leads to the formation of a tetrahedral disulfide-*S*-oxide intermediate. Attack of Cys 218 on Cys 72, followed by proton transfer to the oxygen atom of the sulfoxide then leads to loss of water and to the formation of methionine and of a disulfide bond between Cys 72 and Cys 218. The reduced state is restored through thiol/disulfide exchange first between Cys 227 and Cys 218, followed by reduction by an external thiol substrate.[72]

An alternative mechanism has been proposed for *E. coli* MsrA catalysis. In this case the Cys 51 attacks the sulfur atom of the sulfoxide substrate with the formation of a tetrahedral intermediate that rearranges to form a sulfenic acid at Cys 51, releasing methionine in the process (i.e. a net oxygen-atom transfer from methionine sulfoxide to cysteine). Cys 198 then attacks the sulfur atom of the Cys 51 sulfenic acid to form a disulfide bond and water. The active site is fully reduced by thiol/disulfide exchange, initially through an adjacent cysteine residue (Cys 206) and then an external thiol.[73]

The biological role of methionine sulfone (Figure 1) is less apparent. The modified amino acid itself has been found to exhibit antiinflammatory properties. Interestingly, this highly oxidized form of methionine is more active than the sulfoxide but less active than methionine, thus showing no correlation between oxidation state of sulfur and this kind of antioxidant activity.[70] This result is comparable to recent investigations of disulfide-*S*-oxides that have also shown no direct correlation between the oxidation state of sulfur and in vitro reactivity.[53,55] As with these disulfide-*S*-oxides, methionine sulfoxide and sulfone might play a significant role during oxidative stress, either as antioxidants or as reactive sulfur species.[53–55]

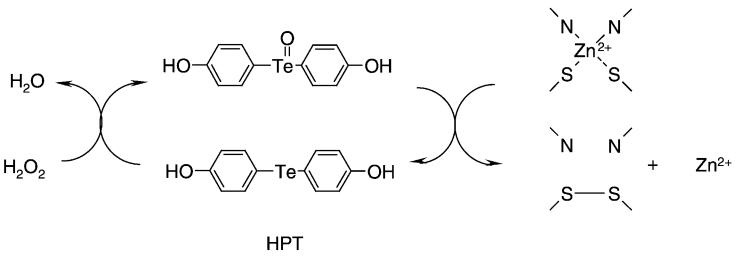
Apart from sulfoxide and sulfone modifications, methionine is also able to form a trivalent sulfonium species (Figure 1) found in the biological methylating agent *S*-adenosylmethionine (SAM). Sulfonium formation represents another modification of methionine that frequently occurs in vivo and exhibits its own, unique reactivity (the methyl group attached to the sulfur is highly electrophilic).

Oxidation of cysteine, cystine, and methionine in proteins has consequences reaching far beyond redox catalysis or antioxidant protection. For example, it was recently suggested that posttranslational cysteine modifications in proteins might be linked to enzyme regulation and signaling,[74] and sulfenic acid formation has been implied in the redox regulation of transcription factors such as OxyR and AP-1. A discussion of all of these redox-controlled processes is beyond the scope of this Review.

4. Metal-Binding Properties of Cysteine, Sulfinic Acids, and Sulfoxides

The thiolate group in cysteine is not only redox-active, but also forms coordinative bonds with a wide range of metal ions such as iron, zinc, cadmium, mercury, cobalt, and copper, but not group 1 or group 2 metal ions. Numerous proteins contain zinc/sulfur sites with structural (e.g. zinc finger proteins of transcription factors), catalytic (e.g. alcohol dehydrogenase and metallolactamases), and regulatory/inhibitory properties (e.g. glyceraldehyde 3-phosphate dehydrogenase).

Once the two main properties of the thiol group (i.e. metal binding and redox activity) are considered together, cysteine-linked reactions open the door to redox control of metal binding and metal control of sulfur redox activity in biological systems.



HPT

Scheme 6. Redox control of metal binding by organotellurium compounds in a catalytic cycle that mimics the function of GPx. The tellurium center of 4,4′-dihydroxydiphenyltelluride (HPT) is initially oxidized by hydrogen peroxide to form the telluroxide. This highly reactive species is then reduced by a zinc finger motif to regenerate the telluride. Oxidation of the zinc finger results in zinc release owing to the formation of the disulfide.[83]

4.1. Redox Control of the Cysteine Ligand

The interdependence between metal binding and redox activity of cysteine has been extensively studied for the zinc/sulfur protein metallothionein (MT).[75–78] MT is a major intracellular zinc-binding protein with a significant regulatory role in zinc uptake, distribution, storage, and release. Mammalian MT is a single polypeptide chain of approximately 60 amino acids, of which 20 are cysteine residues. Despite the lack of secondary structure, these cysteines together bind seven zinc ions with an exceptionally high metal-binding constant ($K(Zn^{2+}) = 3.2 \times 10^{13}\,M^{-1}$ at pH 7.4)[79] in two zinc/sulfur clusters (Zn_4Cys_{11} and Zn_3Cys_9).[79] MT readily participates in intermolecular metal-exchange and metal-transfer reactions.[75,77] Metal transfer from MT to proteins is greatly enhanced in the presence of other metal-binding agents such as GSH and citrate,[77] but also by oxidizing agents that release zinc from MT.[77,78,80] Oxidation of the thiol(ate) ligands in MT eliminates metal binding, which might be an important in vivo trigger for metal release and transfer to other proteins.[81]

A similar redox control exists for a number of transcription factors that contain a zinc finger motif. Transcription factors bind to DNA, initiating the initial stages of gene expression. The DNA-binding domains of these transcription factors are frequently built out of zinc fingers, motifs of approximately 30 amino acids in length containing a zinc ion. The zinc is ligated either by a pair of histidine and a pair of cysteine residues (CCHH), three cysteine groups and one histidine moiety (CCCH), or four cysteine residues (CCCC). The key feature of this structure is that the cysteine residue must be in its reduced form to allow coordination of zinc.[82] If the redox balance is altered such that the cysteine ligands are oxidized and can no longer bind zinc, the structural motif will collapse, leaving the transcription factor unable to bind DNA, thus modulating mRNA synthesis and gene expression.

Although the full in vivo implications of these findings are still under discussion, redox control of transcription factors in cancer cells has already attracted considerable interest among biochemists and might even serve as the target for catalytic redox drugs.[83] Whereas zinc finger motifs and MT only react slowly with oxidative stressors such as hydrogen peroxide, small quantities of sulfur-specific peroxidation catalysts that mimic GPx activity (Scheme 6) effectively destroy these proteins and significantly increase the stressor-induced cell damage in neuronal cell culture.

The reverse effect, that is, the use of metal ions to control the redox activity of sulfur, has equally important biochemical implications. Thionein, the metal-free (apo) form of MT, is considerably more reactive toward oxidizing agents than MT itself, a well-known fact that has recently been underlined.[76] A similar control mechanism is found in metal-poisoned cysteine enzymes (inhibitory or regulatory zinc/sulfur sites). Inhibition by zinc occurs in many enzymes, including mammalian liver fructose-1,6-bisphosphatase, muscle glyceraldehyde 3-phosphate dehydrogenase (GAPDH), muscle enolase, human caspase-3, human T cell protein tyrosine phosphatase, and yeast aldehyde dehydrogenase (ALDH).[84] In some instances, inhibition occurs at nanomolar concentrations of zinc in vitro, indicating the importance of metal-regulated cysteine residues. Notably, thionein is able to remove zinc from inhibitory (but not catalytic) protein sites, hinting at the existence of a complex feedback mechanism between cysteine redox activity and metal binding.[84]

There is, however, an interesting twist to redox control of metal binding: the recent discovery of sulfinic acid ligands bound to metal ions in enzymes has added another dimension to this issue.[43,85,86]

4.2. Sulfinic Acids and Sulfoxides as Ligands

Cysteine sulfinic acid has recently been found in metalloenzymes such as bacterial nitrile hydratase (NHase), in which the sulfinic acid binds to metals such as iron through the oxygen atom. These NHases catalyze the hydration of nitriles to the corresponding amides and can coordinate either a low-spin, non-heme Fe^{III} or non-corrinoid Co^{III}. Recent X-ray crystallographic analysis shows that iron-containing NHase from *Rhodococcus sp. N-771* has an unusual Cys109-Ser-Leu-Cys112-Ser-Cys114 metal-binding sequence.[85] It is suggested that in the active form of the enzyme, Cys112 and Cys114 are modified to the respective sulfinic ($Cys\text{-}SO_2H$) and sulfenic acids (Cys-SOH) and contribute to iron coordination. Other studies have confirmed that the sulfinic acid is present in both the active and inactive forms of the enzyme, whereas the existence of the sulfenic acid residue of the Cys114 in the active NHase is still controversial.[86] Further studies have shown that the Cys114 and Cys112 are modified together during posttranslational modification of the enzyme.[87] Oxidized cysteine acids that can act as ligands are also being investigated in some transcription factors such as BPV-1 E2 protein, activator protein-1,[43] and cobalt-containing NHase from *Pseudonacardia thermophila*

JCM 3095. The crystal structure of this NHase was recently resolved to 1.8 Å, and Cys 111 and Cys 113 were found to be modified to the sulfinic and sulfenic acids, respectively, and coordinated to Co^{III}.[88]

Metal binding of oxidized cysteine species is therefore very different from the metal binding of reduced cysteine. To date, bacterial NHases are the only examples of sulfinic acid ligands in metalloenzymes. However, these results are still controversial as the sulfenic and sulfinic acid might well be formed during the isolation and characterization of the enzyme. If posttranslational modification of cysteine to sulfenic and sulfinic acid is confirmed in NHase and other proteins, it might provide a mechanism to create types of metal-binding sites in proteins that have so far been mostly overlooked. A "redox switch" in metal preference from zinc (thiol) to iron (sulfenic/sulfinic acid) could also be important in oxidative stress, in which the antioxidant response of a protein to oxidation would be the release of the "antioxidant" metal zinc and subsequent binding of the "pro-oxidant" iron.

Methionine also plays a role in metal binding and there is evidence of redox-dependent metal binding to this amino acid. Reduced methionine readily coordinates to toxic metals such as lead, cadmium, and mercury, aiding in their excretion from the body.[89–91] It is also found as a metal ligand in many proteins such as ascorbate oxidase (copper)[92] and various cytochromes (iron).[93] As an example for oxidized methionine, the bacterial catalase from *Proteus mirablis PR* (a peroxide-resistant 56-kDa enzyme) contains a heme group found to interact with a methionine sulfone, which could prevent access of large substrates or inhibitors to the iron-containing active site.[94]

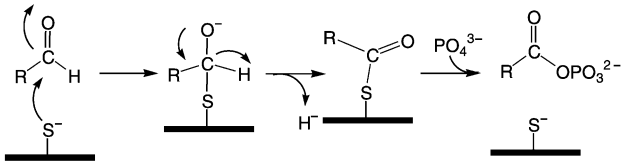
Metal binding to cysteine leads us to another group of redox enzymes that contain redox-inactive cysteine residues at the active site. The following section completes our discussion of cysteine-based redox catalysis with a look at dehydrogenases.

5. Cysteine Dehydrogenases and Proteases

Table 2 illustrates the mechanistic diversity available to cysteine in enzymes that catalyze the oxidation/reduction of NADPH. Whereas GR contains two redox-active cysteine residues that oxidize NADPH by hydride transfer to FAD with subsequent two-electron transfer to the disulfide, Npx oxidizes NADH by hydride transfer to a sulfenic acid through FAD. In contrast, the cysteine dehydrogenase GAPDH contains a cysteine that facilitates hydride transfer between aldehyde and NAD^+, whereas the human liver alcohol dehydrogenase (ADH) contains a zinc/cysteine coordination site that facilitates reversible hydride transfer between ethanol and NAD^+ without a change in the oxidation states of cysteine or zinc.

5.1. Thioether Formation and Breakage of C–H and C–N Bonds

Mammalian muscle GAPDH catalyzes the oxidation and subsequent phosphorylation of aldehydes to acyl phosphates, while reducing NAD^+ to NADH. Its catalytic cycle involves a crucial hydride-transfer step that requires an activated aldehyde substrate. The active site of the enzyme contains a cysteine residue (Cys 149) that attacks the aldehyde to form an activated, highly reactive, tetravalent thioether intermediate (Scheme 7). A hydride ion is transferred to NAD^+ to form



Scheme 7. Cysteine-catalyzed dehydrogenation. Dehydrogenases use cysteine to form a tetravalent thioether that facilitates hydride transfer. A similar mechanism is found in cysteine proteases.[84]

a thioester at the active site. The latter is cleaved by nucleophilic attack of phosphate, resulting in the formation of a phosphoester with regeneration of the cysteine residue in the active site. Importantly, cysteine itself acts as a nucleophile in this redox cycle and does not directly take part in the redox mechanism. It merely facilitates hydride abstraction from the aldehyde by forming the tetravalent thioether intermediate. Related enzymes such as ALDH follow a similar redox mechanism. These enzymes are inhibited by oxidizing agents such as reactive sulfur species,[55] peroxynitrite,[95] and zinc ions, emphasizing the importance of the

Table 2: Cysteine and NAD(P)H. Mechanistic diversity of hydride transfer involving cysteine in the active site.

Enzyme	Active site	Redox mechanism	Oxidation states
Glutathione disulfide reductase (GR)	cysteine	two-electron transfer from $FADH_2$ to disulfide, then hydride transfer from NADPH to FAD[27]	−2 (thiol), −1 (disulfide)
Thioredoxin reductase (TrxR)	cysteine, selenocysteine	selenylsulfide exchanges thiol to form disulfide, then as for GR[29,131]	−2 (thiol, selenol), −1 (disulfide, selenylsulfide)
NADH peroxidase (Npx)	cysteine	hydride transfer from NADH to cysteine sulfenic acid through FAD[43]	−2 (thiol), 0 (sulfenic acid)
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	cysteine	nucleophilic attack of thiolate on carbonyl group of aldehyde facilitates hydride transfer from aldehyde to $NADP^+$[84]	−2 (thiolate, thioether, thioester)
Alcohol dehydrogenase (ADH)	zinc/cysteine complex	coordination of ethanol to zinc facilitates (reversible) hydride transfer to $NAD(P)^+$[99]	−2 (thiol), +2 (zinc)

reduced and "metal-free" state of the cysteine residue in the active site.[84]

Interestingly, the thioether intermediate is not only a feature of dehydrogenases but is also found in another class of functionally entirely unrelated enzymes, the cysteine proteases such as papain, cathepsin B, and human caspases.[96] Rather than attacking the carbonyl group of an aldehyde, the nucleophilic thiolate function of the cysteine residue in the protease attacks the carbonyl group of its substrate amide. The tetrahedral thioether formed in this process then eliminates an amine RNH^- (compared to H^- in GAPDH), leading to the formation of a thioester acyl enzyme intermediate, which is subsequently hydrolyzed with release of the carboxylic acid and regeneration of free enzyme.

In cysteine proteases, cysteine does not directly take part in a redox process, and zinc ions strongly inhibit these enzymes.[84] In contrast, there are numerous proteases and dehydrogenases that are only active when zinc is tightly bound to their active-site cysteine residues.

5.2. The Role of Zinc/Sulfur Coordination in Breaking C–H and C–N Bonds

Mammalian ADH is an important dimeric 80-kDa enzyme that catalyzes the oxidation of ethanol to acetaldehyde under alkaline conditions, reducing NAD^+ to NADH in the process (at lower pH values, the enzyme catalyzes the oxidation of NADH in the presence of aldehyde).[97] It is essential for human alcohol tolerance and inhibition of the enzyme has been used in the treatment of alcoholism. Each of the two subunits of the enzyme has one binding site for NAD^+ and two binding sites for Zn^{2+}. Only one of the zinc ions is involved directly in catalysis. It is coordinated to Cys 46, His 67, Cys 174, and an exchangeable water molecule that forms a hydrogen bond to Ser 48. The other zinc ion plays a structural role as part of a $ZnCys_4$ site. The crystal structure of human liver ADH[98] has revealed that in the enzyme–NAD^+–alcohol active ternary complex, the OH group of ethanol binds to the zinc through an inner coordination sphere, with the side chains of Ser 48 and Phe 93 effectively locking the alcohol into a position that promotes hydride transfer from the alcohol to the NAD^+.[99] During hydride transfer (oxidizing ethanol and reducing NAD^+), neither the cysteine ligands in the active site nor the zinc ion change their oxidation states. ADH is, however, sensitive to cysteine oxidation, which releases the zinc ions and deactivates the enzyme.[53–55,80]

As is the case for the dehydrogenases, protease enzymes can utilize cysteine either directly as a catalytic residue or indirectly as a ligand to a catalytically active zinc ion. For example, matrix metalloproteases (MMPs) are zinc-dependent endopeptidases present in plants, invertebrates, and vertebrates. Cysteine acts in a regulatory role as ligand to the catalytically active zinc ion. In the active form of the enzyme, zinc is coordinated to three highly conserved histidine residues with a vacant coordination site for the peptide substrate. In the inactive propeptide, however, this fourth coordination site is occupied by a highly conserved cysteine residue in a sequence that caps the active site and is cleaved from the MMP upon proteolytic activation of the enzyme.[100]

Similarly, tissue inhibitors of metalloproteinases (TIMPs), peptides of approximately 20 kDa, contain several conserved cysteine residues (12 in the case of TIMP-1 and TIMP-2), which are essential not only for structural integrity but are directly involved in MMP inhibition. The crystal structure of the MMP3–TIMP-1 complex between human stromelysin-1 (MMP-3) and human TIMP-1 shows a cysteine residue near the active-site zinc of MMP-3, probably blocking its activity.[101]

6. From Sulfur to Selenium—Similarities and Differences

The role of selenium in biology is currently of considerable interest, and there are several recent reviews on this topic.[4,102–105] The aim of this section is not to provide a comprehensive review of the biological role of selenium, but rather to focus on the redox chemistry of the selenium-containing amino acids, their redox mechanisms, oxidation states, and metal-binding properties in proteins in comparison with cysteine and methionine.

6.1. Natural Occurrence of Selenium in Proteins

The element selenium is located below sulfur on the periodic table, and thus there are close similarities, but also striking differences, between these two elements in terms of their chemistry and biochemistry. The abundance of selenium in the earth's crust is about four orders of magnitude lower than that of sulfur, and this is also reflected in the natural abundance of these elements in biological systems. Whereas sulfur occurs in the human body in quantities similar to those of potassium (140 g in an adult), selenium is a trace element present in milligram amounts, primarily as selenocysteine, selenomethionine, and, at a much lower content, their metabolic precursors (e.g. GS-Se-SG, GSSeH, selenophosphate). Mammalian selenium-containing proteins can be divided into three groups: 1) specific selenocysteine-containing selenoproteins, 2) proteins that contain nonspecifically incorporated selenium, and 3) specific selenium-binding proteins. Selenoproteins with known functions identified so far include several glutathione peroxidases, two deiodinases, several thioredoxin reductases, and selenophosphate synthetase-2. A biological function of selenoprotein P (i.e. its important role in delivering hepatic selenium to specific tissues) was recently confirmed in gene knockout experiments in mice.[106,107] Selenoprotein W, a 15-kDa protein, an 18-kDa protein, and several selenoproteins identified in silico from nucleotide sequence databases contain selenocysteine, but their functions are not fully known. The current state of knowledge on these proteins has recently been reviewed.[4] Incorporation of selenocysteine into these proteins requires an elaborate translation machinery.[108] In contrast, selenomethionine is randomly incorporated into proteins in place of methionine.

6.2. Selenocysteine and Selenomethionine: Oxidation States, Redox Catalysis, and Antioxidant Activity

A major difference between selenocysteine and cysteine residues is a significantly lower pK_a value of the selenol group in selenocysteine (p$K_a = 5.2$) relative to that of the thiol group of cysteine (p$K_a = 8.3$).[109] As a consequence, selenols in selenocysteine-containing proteins are normally present as selenolates at physiological pH, whereas thiols in cysteine-containing proteins are usually present as undissociated thiols. There are, of course, notable exceptions, such as NADH oxidase and human thiol transferase. The dissociation states were found to be similar when cysteine replaced selenocysteine in the active site GPx mutant of phospholipid hydroperoxide GPx.[110] Furthermore, selenocysteine occurs mainly in the reactive selenol and selenenic acid oxidation states (Scheme 3) and, unlike disulfides, reactive diselenides have not yet been found in proteins. The redox cycle of GPx exemplifies the redox mechanism of these peroxidases: Selenocysteine is oxidized by oxygen-atom transfer, and reduction of selenenic acid occurs through an exchange reaction involving two GSH equivalents.[111]

Although diselenides have not been found in proteins yet, selenylsulfides are present in GPx and human TrxR. The redox behavior of selenocysteine in these proteins is rather interesting from a mechanistic point of view. Reduction of disulfides can proceed through exchange as well as electron-transfer reactions (see Section 2). Theoretically, the selenylsulfide in human TrxR would therefore also lend itself to reduction through electron transfer from $FADH_2$ (the disulfide subsequently formed in human TrxR is reduced by two-electron transfer). Electron transfer to the selenylsulfide does not occur in human TrxR and reduction follows a more elaborate pathway (Scheme 2): The selenylsulfide is first reduced by cysteine in an exchange reaction, and the resulting disulfide is then reduced by electron transfer. Common cysteine redox mechanisms such as electron transfer, hydride transfer, and radical reactions have not been observed for selenocysteine in proteins.

The occurrence of selenol, selenylsulfide, and selenenic acid in selenoproteins does not mean, however, that other selenium oxidation states are principally absent in vivo. The selenocysteine/selenocystine redox potential of -488 mV (Figure 2) indicates that diselenide formation is possible under physiological redox conditions. However, few proteins have two selenocysteine residues in close enough proximity to facilitate diselenide formation (perhaps with the exception of selenoprotein P, which contains up to 10 selenocysteine residues). A range of additional selenium oxidation states occurs during selenium metabolism: selenite (SeO_3^{2-}) is reduced stepwise by GSH to form H_2Se and ultimately selenophosphate. This process involves selenium species in various oxidation states ranging from $+4$ to -2. Excretion pathways of excess H_2Se also involve a trivalent selenonium species in the form of trimethylselenonium, which is excreted in urine. Although not yet known to take part in protein mechanisms, species such as seleninic acids could exist under physiological conditions and are known to be redox-active in the presence of zinc/thiolate coordination centers.[78]

Whereas selenocysteine is specifically incorporated into proteins,[112,113] selenomethionine occurs apparently randomly in proteins in place of methionine, depending on the supply of selenomethionine. Like methionine, selenomethionine is readily oxidized to the corresponding selenoxide.[114] Unlike methionine sulfoxide, which requires a sulfoxide reductase for reduction (see Section 3.3), selenomethionine selenoxide is nonenzymatically reduced by 2 equivalents of GSH.[115] Selenomethionine might, therefore, serve as a line of defense against permanent oxidative damage in proteins, because the funneling of oxidative challenge into a repairable site of damage can be advantageous over introducing nonrepairable damage sites, which require proteolytic degradation of the protein. Whether this strategy is utilized to a major extent in vivo is being debated.[116]

This distinctive feature of selenoxide is also used in effective synthetic antioxidants that mimic glutathione peroxidase activity, such as ebselen.[117] Whereas selenols are frequently oxidized to "dead end" diselenides, dialkyl, diaryl, and alkylaryl selenides cannot form diselenides and are among the best peroxidase redox catalysts; their activity is only surpassed by that of diaryl tellurides.[83,118–120] This rapidly growing field of research into selenoorganic compounds that carry out catalytic functions mimicking those of selenoproteins has recently been reviewed.[121]

Another interesting family of selenocysteine-containing enzymes are the deiodinases, which function in thyroxine metabolism.[122] The catalytic mechanism of these enzymes in vivo might be rather unusual and possibly involves nucleophilic substitution of an iodine atom of an aryl iodide (Reaction (13) in Scheme 1).[123] The selenolate of the selenocysteine (SeCys-Se^-) in the active site acts as a nucleophile that attacks an iodine atom of 3,3′,5,5′-tetraiodo-L-thyronine (prohormone L-thyroxine). An oxidized selenenyl iodide intermediate (SeCys-Se-I) is formed, and the reduced aromatic hormone 3,3′,5-triiodo-L-thyronine is protonated and released. The reduced form of selenocysteine is regenerated by reduction with dithiols (the latter are oxidized to disulfides in this process). A comparison with the S-conjugation reactions catalyzed by glutathione transferase enzymes is interesting: Whereas selenocysteine in the deiodinases attacks the halogen atom with the (reduced) aromatic ring as leaving group, cysteine in the GST enzymes attacks the ring with the reduced halide as leaving group.

6.3. Selenium and Metal Binding

Metal binding is a common feature of cysteine, and a range of metal–selenium sites might be expected in proteins. Surprisingly, this is not the case, as examples of selenocysteine–metal complexes have been observed in a few selenoproteins. Unexpectedly, in cases in which they have been observed, the selenocysteine is bound to the hard and intermediate rather than to the soft heavy-metal ions. A selenium–molybdenum bond was found in formate dehydrogenase from *E. coli*[124] and a selenium–tungsten bond was recently identified in formate dehydrogenase from *Desulfovibrio gigas*.[125] Although selenocysteine is essential for

formate dehydrogenase activity, the role of selenium in these proteins is unclear. The genus *Desulfovibrio* also contains an intermediate metal ligation in the form of a selenium–nickel coordination, found in the nickel–(iron/sulfur)–selenium-containing hydrogenases.[126] Genetic engineering has shown that it is possible for selenium to bind a soft heavy metal in a protein, by substituting selenocysteine for the cysteine ligand of copper in azurin.[127] A similar selenium–copper bond has yet to be observed in vivo. Metal binding to selenenic acids or selenomethionine, although chemically possible, has not yet been observed in proteins.

Metal binding to selenocysteine underlines the dichotomy between the chemically possible and biochemically realized. Selenium species in unusual oxidation states and metal–selenium interactions in proteins are areas for future investigation.

7. Summary and Outlook

This Review delineates the complex biological chemistry of cysteine, methionine, selenocysteine, and selenomethionine, discussing biological examples from the perspective of their redox chemistry. During protein synthesis, cysteine is incorporated as one of the 21 mammalian proteinogenic amino acids (with selenocysteine as the 21st amino acid).[108,128] A variety of posttranslational modifications ensure that this one amino acid is present in the form of about 10 different species, which exhibit unique chemical and biochemical properties. Thus, cysteine may be considered as the most diverse amino acid building block in proteins.

Whereas redox-active metals transfer electrons, NAD(P)H transfers hydride ions, oxoanions transfer oxygen atoms, FAD transfers hydride ions or electrons, and tyrosine forms radicals in biological systems, cysteine carries out all of these and other redox reactions, for example, long-range electron and short-range hydrogen-atom transfer and thiol/disulfide-exchange reactions. There are probably no other chemical species in biological systems that can occur in as many different oxidation states, allow for as many different posttranslational protein side-chain modifications, provide as many different redox pathways, generate such a variety of structurally distinct metal-binding sites, and fulfill a multitude of different biological functions as the "redox chameleon" sulfur in the amino acid cysteine.

With the identification of different oxidation states of sulfur in methionine, the discovery of a sulfoxide reductase, and the postulation of methionine–metal bonds in proteins, the biological redox chemistry of methionine is also becoming increasingly important.

Recent developments in genomics and bioinformatics have revealed genomic sequences that should lead to the identification of novel sulfur- and selenium-containing proteins. Similarly, the emerging discipline of proteomics will facilitate the discovery of new posttranslational modifications. Our knowledge of Nature's use of oxidation state to regulate protein structure and function is in its infancy; the recent developments outlined in this Review give an indication of possible unidentified pathways and reaction mechanisms that may be operating within the cell. For example, exciting new developments in the study of the oxidation states of sulfur have shown that disulfide-*S*-oxides can be formed under physiological conditions. In addition to their own reaction pathways, these oxidizing species can be "activated" by reduction, forming highly reactive sulfenic acids. Ongoing investigations into this redox metabolism of sulfur point to the existence of multiple redox sulfur cascades within the cell which influence protein activity and signaling.

A further topic of interest is the possibility of a metal-binding role of sulfenic/sulfinic acids and their potential importance in protein functions. Interestingly, the first cysteine persulfide coordinated to heme was recently identified in the active site of SoxAX, a *c*-type cytochrome from *Rhodovulum sulfidophilum*, which has provided insight into the bacterial sulfur cycle.[129] New posttranslational sulfur modifications are expected to be uncovered. At the same time, synthetic enzyme mimics may find applications in rational drug design, as shown by the use of organochalcogen GPx catalysts in therapy for cerebral ischemia,[130] and by promising leads from electrochemical and other in vitro and cell-culture studies with regard to cancer and dementia.[83,120]

This work was supported by the Wellcome Trust, the Leverhulme Trust, DAART, Exeter Antioxidant Therapeutics Ltd., and by the Deutsche Forschungsgemeinschaft (Schwerpunktsprogramm Selenoproteine Si 255/11-2). C.J. is Chairman of EAT Ltd. and H.S. is a Fellow of the National Foundation for Cancer Research. We also thank Professor G. Buettner (Iowa City), Dr. L.-O. Klotz (Düsseldorf), Prof. J. A. Littlechild (Exeter), Professor W. Stahl (Düsseldorf), and Professor J. Thomas (Ames) for helpful discussions.

Received: January 24, 2003 [A573]

[1] *Thiol Metabolism and Redox Regulation of Cellular Functions* (Eds.: A. Pompella, G. Bahegyi, M. Wellman-Rousseau), IOS Press, Amsterdam, **2002**, pp. 1–348.
[2] *Cellular Implications of Redox Signalling* (Eds.: C. Gitler, A. Danon), World Scientific, Singapore, **2003**.
[3] *Antioxidant and Redox Regulation of Genes* (Eds.: C. K. Sen, H. Sies, P. A. Baeuerle), Academic Press, San Diego, **2000**, pp. 1–556.
[4] *Protein Sensors and Reactive Oxygen Species, Part A: Selenoproteins and Thioredoxin* (Eds.: H. Sies, L. Packer), Academic Press, San Diego, **2002**, pp. 1–461.
[5] *Protein Sensors and Reactive Oxygen Species, Part B: Thiol Enzymes and Proteins* (Eds.: H. Sies, L. Packer), Academic Press, San Diego, **2002**, pp. 1–364.
[6] Y.M Torchinsky, *Sulfur in Proteins*, Pergamon, Oxford, **1981**, pp. 1–277.
[7] P. C. Jocelyn, *Biochemistry of the SH Group*, Academic Press, London, **1972**.
[8] *Glutathione Conjugation: Mechanisms and Biological Significance* (Eds.: H. Sies, B. Ketterer), Academic Press, London, **1988**, pp. 1–470.
[9] W. J. Lees, G. M. Whitesides, *J. Org. Chem.* **1993**, *58*, 642–647.
[10] T. P. Akerboom, M. Bilzer, H. Sies, *J. Biol. Chem.* **1982**, *257*, 4248–4252.
[11] R. C. Fahey, *Adv. Exp. Med. Biol.* **1977**, *86A*, 1–30.
[12] H. Sies, *Free Radical Biol. Med.* **1999**, *27*, 916–921.

[13] F. Q. Schäfer, G. R. Buettner, *Free Radical Biol. Med.* **2001**, *30*, 1191 – 1212.
[14] Y. Nishinaka, H. Masutani, H. Nakamura, J. Yodoi, *Redox Rep.* **2001**, *6*, 289 – 295.
[15] A. Holmgren, *Struct. Bonding (Berlin)* **1995**, *3*, 239 – 243.
[16] R. Brigelius, in *Oxidative Stress* (Ed.: H. Sies), Academic Press, London, **1985**, pp. 243 – 272.
[17] D. M. Ziegler, *Annu. Rev. Biochem.* **1985**, *54*, 305 – 329.
[18] J. A. Thomas, R. Mallis, H. Sies in *Cellular Implications of Redox Signalling* (Eds.: C. Gitler, A. Danon), World Scientific, Singapore, **2003**, pp. 141 – 174.
[19] G. I. Giles, C. Jacob, *Biol. Chem.* **2002**, *383*, 375 – 388.
[20] Y. C. Chai, S. Hendrich, J. A. Thomas, *Arch. Biochem. Biophys.* **1994**, *310*, 273 – 281.
[21] L. F. Chasseaud, *Adv Cancer Res.* **1979**, *29*, 175 – 274.
[22] J. P. Ploemen, M. L. van Iersel, L. W. Wormhoudt, J. N. Commandeur, N. P. Vermeulen, P. J. van Bladeren, *Biochem. Pharmacol.* **1996**, *52*, 197 – 204.
[23] R. Morgenstern, J. W. DePierre, L. Ernster, *Biochem. Biophys. Res. Commun.* **1979**, *87*, 657 – 663.
[24] R. Rinaldi, E. Eliasson, S. Swedmark, R. Morgenstern, *Drug Metab. Dispos.* **2002**, *30*, 1053 – 1058.
[25] Y. Ji, B. M. Bennett, *Mol. Pharmacol.* **2003**, *63*, 136 – 146.
[26] L. D. Arscott, S. Gromer, R. H. Schirmer, K. Becker, C. H. Williams, Jr., *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3621 – 3626.
[27] E. F. Pai, G. E. Schulz, *J. Biol. Chem.* **1983**, *258*, 1752 – 1757.
[28] L. D. Arscott, D. M. Veine, C. H. Williams, Jr., *Biochemistry* **2000**, *39*, 4711 – 4721.
[29] D. Mustacich, G. Powis, *Biochem. J.* **2000**, *346*, 1 – 8.
[30] J. T. Spence, M. J. Barber, L. P. Solomonson, *Biochem. J.* **1988**, *250*, 921 – 923.
[31] G. E. Arteel, K. Briviba, H. Sies, *Chem. Res. Toxicol.* **1999**, *12*, 264 – 269.
[32] R. Zhao, H. Masayasu, A. Holmgren, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 8579 – 8584.
[33] D. C. Rees, *Annu. Rev. Biochem.* **2002**, *71*, 221 – 246.
[34] C. R. Staples, E. Ameyibor, W. Fu, L. Gardet-Salvi, A. L. Stritt-Etter, P. Schurmann, D. B. Knaff, M. K. Johnson, *Biochemistry* **1996**, *35*, 11425 – 11434.
[35] S. Dai, C. Schwendtmayer, P. Schurmann, S. Ramaswamy, H. Eklund, *Science* **2000**, *287*, 655 – 658.
[36] G. N. Jameson, E. M. Walters, W. Manieri, P. Schurmann, M. K. Johnson, B. H. Huynh, *J. Am. Chem. Soc.* **2003**, *125*, 1146 – 1147.
[37] A. L. Persson, M. Sahlin, B.M Sjöberg, *J. Biol. Chem.* **1998**, *273*, 31016 – 31020.
[38] X. Sun, S. Ollagnier, P. P. Schmidt, M. Atta, E. Mulliez, L. Lepape, R. Eliasson, A Gräslund, M. Fontecave, P. Reichard, B. M. Sjöberg, *J. Biol. Chem.* **1996**, *271*, 6827 – 6831.
[39] S. Ollagnier, E. Mulliez, P. P. Schmidt, R. Eliasson, J. Gaillard, C. Deronzier, T. Bergman, A. Gräslund, P. Reichard, M. Fontecave, *J. Biol. Chem.* **1997**, *272*, 24216 – 24223.
[40] P. A. Frey, *Annu. Rev. Biochem.* **2001**, *70*, 121 – 148.
[41] J. A. Gerlt, P. C. Babbitt, *Annu. Rev. Biochem.* **2001**, *70*, 209 – 246.
[42] H. Miller, S. S. Mande, D. Parsonage, S. H. Sarfaty, W. G. Hol, A. Claiborne, *Biochemistry* **1995**, *34*, 5180 – 5190.
[43] A. L. Claiborne, T. C. Mallett, J. I. Yeh, J. Luba, D. Parsonage, *Adv. Protein Chem.* **2001**, *58*, 215 – 276.
[44] S. S. Mande, D. Parsonage, A. Claiborne, W. G. Hol, *Biochemistry* **1995**, *34*, 6985 – 6992.
[45] D. Parsonage, A. Claiborne, *Biochemistry* **1995**, *34*, 435 – 441.
[46] T. C. Mallett, A. Claiborne, *Biochemistry* **1998**, *37*, 8790 – 8802.
[47] B. Hofmann, H. J. Hecht, L. Flohé, *Biol. Chem.* **2002**, *383*, 347 – 364.
[48] S. Hirotsu, Y. Abe, K. Okada, N. Nagahara, H. Hori, T. Nishino, T. Hakoshima. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12333 – 12338.
[49] E. Schröder, J. Littlechild, A. Lebedev, N. Errington, A. Vagin, M. Isupov, *Struct. Bonding (Berlin)* **2000**, *8*, 605 – 615.
[50] H. Sies in *Oxidative Stress* (Ed.: H. Sies), Academic Press, London, UK, **1985**, pp. 1 – 8.
[51] H. Sies, *Angew. Chem.* **1986**, *98*, 1061 – 1075; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1058 – 1071.
[52] H. Sies in *Encyclopedia of Stress, Vol. III*, (Ed.: G. Fink), Academic Press, San Diego, **2000**, pp. 102 – 105.
[53] G. I. Giles, K. M. Tasker, C. Jacob, *Free Radical Biol. Med.* **2001**, *31*, 1279 – 1283.
[54] G. I. Giles, K. M. Tasker, C. Jacob, *Gen. Physiol. Biophys.* **2002**, *21*, 65 – 72.
[55] G. I. Giles, K. M. Tasker, C. Collins, N. M. Giles, E. O'Rourke, C. Jacob, *Biochem. J.* **2002**, *364*, 579 – 585.
[56] T. Okamoto, T. Akaike, T. Sawa, Y. Miyamoto, M. A. van der Vliet, H. Maeda, *J. Biol. Chem.* **2001**, *276*, 29596 – 29602.
[57] J. Li, F. L. Huang, K. P. Huang, *J. Biol. Chem.* **2001**, *276*, 3098 – 3105.
[58] G. Nöhammer in *Thiol Metabolism and Redox regulation of Cellular Functions* (Eds.: A. Pompella, G. Banhegyi, M. Wellman-Rousseau), IOS Press, Amsterdam, **2002**, pp. 48 – 60.
[59] B. Kagedal, M. Kallberg, B. Sorbo, *Biochem. Biophys. Res. Commun.* **1986**, *136*, 1036 – 1041.
[60] D. A. Keller, D. B. Menzel, *Chem.-Biol. Interact.* **1989**, *21*,145 – 156.
[61] C. D. Klaassen, J. W. Boles, *FASEB J.* **1997**, *11*, 404 – 418.
[62] M. Negishi, L. G. Pedersen, E. Petrochenko, S. Shevtsov, A. Gorokhov, Y. Kakuta, L. C. Pedersen, *Arch. Biochem. Biophys.* **2001**, *390*, 149 – 157.
[63] F. Gliubich, R. Berni, M. Colapietro, L. Barba, G. Zanotti, *Acta Crystallogr. Sect. D* **1998**, *54*, 481 – 486.
[64] Y. Ogasawara, G. Lacourciere, T. C. Stadtman, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9494 – 9498.
[65] H. Dobbek, L. Gremer, O. Meyer, R. Huber, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8884 – 8889.
[66] H. Dobbek, L. Gremer, R. Kiefersauer, R. Huber, O. Meyer, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15971 – 15976.
[67] N. Brot, H. Weissbach, *Arch. Biochem. Biophys.* **1983**, *223*, 271 – 281.
[68] W. Vogt, *Free Radical Biol. Med.* **1995**, *18*, 93 – 105.
[69] R. L. Levine, B. S. Berlett, J. Moskovitz, L. Mosoni, E. R. Stadtman, *Mech. Ageing Dev.* **1999**, *10*, 323 – 332.
[70] M. K. Unnikrishnan, M. N. Rao, *Agents Actions* **1990**, *31*, 110 – 112.
[71] R. L. Levine, L. Mosoni, B. S. Berlett, E. R. Stadtman, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 15036 – 15040.
[72] W. T. Lowther, N. Brot, H. Weissbach, B. W. Matthews, *Biochemistry* **2000**, *39*, 13307 – 13312.
[73] S. Boschi-Müller, S. Azza, S. Sanglier-Cianferani, F. Talfournier, A. Van Dorsselear, G. Branlant, *J. Biol. Chem.* **2000**, *275*, 35908 – 35913.
[74] T. Finkel, *FEBS Lett.* **2000**, *476*, 52 – 54.
[75] W. Maret, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 237 – 241.
[76] S. Zhang, J. Li, C.-C. Wang, C.-L. Tsou, *FEBS Lett.* **1999**, *462*, 383 – 386.
[77] C. Jacob, W. Maret, B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3489 – 3494.
[78] C. Jacob, W. Maret, B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1910 – 1914.
[79] J. D. Otvos, D. H. Petering, C. F. Shaw, *Comments Inorg. Chem.* **1989**, *1*, 1 – 35.
[80] C. Jacob, W. Maret, B. L. Vallee, *Biochem. Biophys. Res. Commun.* **1998**, *248*, 569 – 573.

[81] W. Maret, B. L. Vallee, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3478 – 3482.
[82] S. Lee, W. Maret, *Antioxid. Redox Signaling* **2001**, *3*, 531 – 534.
[83] N. M. Giles, N. J. Gutowski, G. I. Giles, C. Jacob, *FEBS Lett.* **2003**, *535*, 179 – 182.
[84] W. Maret, C. Jacob, B. L. Vallee, E. H. Fischer, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1936 – 1940.
[85] M. Tsujimura, N. Dohmae, M. Odaka, M. Chijimatsu, K. Takio, M. Yohda, M. Hoshino, S. Nagashima, I. Endo, *J. Biol. Chem.* **1997**, *272*, 29454 – 29459.
[86] D. Bonnet, J. M. Stevens, R. Alves de Sousa, M. Sari, D. Mansuy, I. Artuad, *J. Biochem.* **2001**, *130*, 227 – 233.
[87] T. Murakami, M. Nojiri, H. Nakayama, M. Odaka, M. Yohda, N. Dohmae, K. Takio, T. Nagamune, I. Endo, *Protein Sci.* **2000**, *9*, 1024 – 1030.
[88] A. Miyanaga, S. Fushinobu, K. Ito, T. Wakagi, *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1169 – 1174.
[89] D. N. Kachru, S. Khandelwal, S. K. Tandon, *Biomed. Environ. Sci.* **1989**, *2*, 265 – 270.
[90] A. C. Esteves, J. Felcman, *Biol. Trace Elem. Res.* **2000**, *76*, 19 – 30.
[91] Y. Kajiwara, A. Yasutake, T. Adachi, K. Hirayama, *Arch. Toxicol.* **1996**, *70*, 310 – 314.
[92] A. Messerschmidt, A. Rossi, R. Ladenstein, R. Huber, M. Bolognesi, G. Gatti, A. Marchesini, R. Petruzzelli, A. Finazzi-Agro, *J. Mol. Biol.* **1989**, *206*, 513 – 529.
[93] R. O. Louro, E. C. de Waal, M. Ubbink, D. L. Turner, *FEBS Lett* **2002**, *510*, 185 – 188.
[94] A. Buzy, V. Bracchi, R. Sterjiades, J. Chroboczek, P. Thibault, J. Gagnon, H. M. Jouve, G. Hudry-Clergeon, *J. Protein Chem.* **1995**, *14*, 59 – 72.
[95] D. P. Buchczyk, K. Briviba, F. U. Hartl, H. Sies, *Biol. Chem.* **2000**, *381*, 121 – 126.
[96] For a comprehensive review of these proteases, see: K. Brocklehurst, A. B. Watts, M. Patel, C. Verma, E. W. Thomas in *Comprehensive Biological Catalysis, Vol. 2* (Ed.: M. L. Sinnott), Academic Press, London, **1998**, pp. 381 – 423.
[97] J. D. Shore, H. Gutfreund, R. L. Brooks, D. Santiago, P. Santiago, *Biochemistry* **1974**, *13*, 4185 – 4191.
[98] H. Eklund, B. V. Plapp, J. P. Samama, C. I. Branden, *J. Biol. Chem.* **1982**, *257*, 14349 – 14358.
[99] Y. Pocker, *Chem.-Biol. Interact.* **2001**, *33*, 383 – 393.
[100] I. Massova, L. P. Kotra, R. Fridman, S. Mobashery, *FASEB J.* **1998**, *12*, 1075 – 1095.
[101] F. X. Gomis-Ruth, K. Maskos, M. Betz, A. Bergner, R. Huber, K. Suzuki, N. Yoshida, H. Nagase, K. Brew, G. P. Bourenkov, H. Bartunik, W. Bode, *Nature* **1997**, *389*, 77 – 81.
[102] M. P. Rayman, *Lancet* **2000**, *356*, 233 – 241.
[103] T. C. Stadtman, *Ann. N. Y. Acad. Sci.* **2000**, *899*, 399 – 402.
[104] M. Birringer, S. Pilawa, L. Flohé, *Nat. Prod. Rep.* **2002**, *19*, 693 – 718.
[105] G. E. Arteel, H. Sies, *Environ. Toxicol. Pharmacol.* **2001**, *10*, 153 – 158.
[106] L. Schomburg, U. Schweizer, B. Holtmann, L. Flohé, M. Sendtner, J. Köhrle, *Biochem. J.* **2003**, *370*, 397 – 402.
[107] K. E. Hill, J. Zhou, W. J. McMahan, A. K. Motley, J. F. Atkins, R. F. Gesteland, R. F. Burk, *J. Biol. Chem.* **2003**, *278*, 13640 – 13646.
[108] M. Thanbichler, A. Böck, *Methods Enzymol.* **2002**, 347, 3 – 16.
[109] R. E. Huber, R. S. Criddle, *Arch. Biochem. Biophys.* **1967**, *122*, 164 – 173.
[110] M. Maiorino, K. D. Aumann, R. Brigelius-Flohé, D. Doria, J. van den Heuvel, J. McCarthy, A. Roveri, F. Ursini, L. Flohé, *Biol. Chem. Hoppe-Seyler* **1995**, *376*, 651 – 660.
[111] P. Mauri, L. Benazzi, L. Flohé, M. Maiorino, P. G. Pietta, S. Pilawa, A. Roveri, F. Ursini, *Biol. Chem.* **2003**, *384*, in press.
[112] J. Köhrle, R. Brigelius- Flohé, A. Böck, R. Gartner, O. Meyer, L. Flohé, *Biol. Chem.* **2000**, *381*, 849 – 864.
[113] A. Böck, *BioFactors* **2000**, *11*, 77 – 78.
[114] A. Assmann, M. Bonifacic, K. Briviba, H. Sies, D. Asmus, *Free Radical Res.* **2000**, *32*, 371 – 376.
[115] A. Assmann, K. Briviba, H. Sies, *Arch. Biochem. Biophys.* **1998**, *349*, 201 – 203.
[116] R. J. Hondal, A. K. Motley, K. E. Hill, R. F. Burk, *Arch. Biochem. Biophys.* **1999**, *371*, 29 – 34.
[117] A. Müller, E. Cadenas, P. Graf, H. Sies, *Biochem. Pharmacol.* **1984**, *33*, 3235 – 3239.
[118] C. Jacob, G. E. Arteel, T. Kanda, L. Engman, H. Sies, *Chem. Res. Toxicol.* **2000**, *13*, 3 – 9.
[119] K. Briviba, R. Tamler, L. O. Klotz, L. Engman, I. A. Cotgreave, H. Sies, *Biochem. Pharmacol.* **1998**, *55*, 817 – 823.
[120] G. I. Giles, K. M. Tasker, R. J. Johnson, C. Jacob, C. Peers, K. N. Green, *Chem. Commun.* **2001**, *23*, 2490 – 2491.
[121] For a review, see: G. Mugesh, W.-W. du Mont, H. Sies, *Chem. Rev.* **2001**, *101*, 2125 – 2179.
[122] J. Köhrle, *Mol. Cell. Endocrinol.* **1999**, *151*, 103 – 119.
[123] J. Köhrle in *Protein Sensors and Reactive Oxygen Species, Part A: Selenoproteins and Thioredoxin* (Eds.: H. Sies, L. Packer), Academic Press, San Diego, **2002**, pp. 125 – 167.
[124] V. N. Gladyshev, S. V. Khangulov, M. J. Axley, T. C. Stadtman, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7708 – 7711.
[125] H. Raaijmakers, S. Macieira, J. M. Dias, S. Teixeira, S. Bursakov, R. Huber, J. J. Moura, I. Moura, M. J. Romão, *Struct. Bonding (Berlin)* **2002**, *10*, 1261 – 1272.
[126] G. Fauque, H. D. Peck, J. J. G. Moura, B. H. Huynh, Y. Berlier, D. V. Dervartanian, M. Teixeira, A. E. Przybyla, P. A. Lespinat, I. Moura, J. Legall, *FEMS Microbiol. Rev.* **1988**, *4*, 299 – 344.
[127] S. M. Berry, M. D. Gieselman, M. J. Nilges, W. A. van Der-Donk, Y. Lu, *J. Am. Chem. Soc.* **2002**, *124*, 2084 – 2085.
[128] J. F. Atkins, R. F. Gesteland, *Science* **2002**, *296*, 1409 – 1410.
[129] V. A. Bamford, S. Bruno, T. Rasmussen, C. Appia-Ayme, M. R. Cheesman, B. C. Berks, A. M. Hemmings, *EMBO J.* **2002**, *21*, 5599 – 5610.
[130] M. Parnham, H. Sies, *Expert Opin. Invest. Drugs* **2000**, *9*, 607 – 619.
[131] T. Sandalova, L. Zhong, Y. Lindqvist, A. Holmgren, G. Schneider, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9533 – 9538.
[132] F. Åslund, K. D. Berndt, A. Holmgren, *J. Biol. Chem.* **1997**, *272*, 30780 – 30786.
[133] E. Nogoceke, D. U. Gommel, M. Kiess, H. M. Kalisz, L. Flohe, *Biol. Chem.* **1997**, *378*, 827 – 836.
[134] N. Reckenfelderbaumer, R. L. Krauth-Siegel, *J. Biol. Chem.* **2002**, *277*, 17548 – 17555.
[135] J. Lundstrom, A. Holmgren, *Biochemistry* **1993**, *32*, 6649 – 6655.
[136] J. F. Collett, J. C. Bardwell, *Mol. Microbiol.* **2002**, *44*, 1 – 8.

