PART I

INTRODUCTION

CHAPTER 1

GLUTATHIONE AND THE SULFUR-CONTAINING AMINO ACIDS: AN OVERVIEW

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1.1 INTRODUCTION

Methionine and cysteine are two of the canonical amino acids that are incorporated into proteins, where they can be quite abundant. According to the Massachusetts Nutrient Data Bank, the sulfur amino acid content (methionine plus cysteine) for animal proteins, cereals, and nuts is between 37 and 41 mg/g protein. Legumes and fruits/vegetables average 25 and 23 mg/g protein, respectively [1]. Taurine, a non-protein β-amino sulfonic acid, is present in many animal tissues but is absent from most plants [2]. In addition, homocysteine is synthesized in vivo as an intermediate in methionine metabolism. Elevated plasma homocysteine has been associated with such chronic diseases as atherosclerosis, Alzheimer's disease, and osteoporosis [3–5]. Homocysteine thiolactone is synthesized by methionyl-tRNA synthetase when an elevated concentration of homocysteine leads to its selection for charging of $tRNA^{met}$ in place of methionine [6]. Structures of these amino acids are shown in Fig. 1.1.

The names of these amino acids recall their structure or discovery. Methionine reflects the fact that this amino acid contains a methyl group attached to a sulfur atom. The history of cysteine and cystine is particularly interesting. We first meet it on July 5, 1810 when William Hyde Wollaston, MD, secretary to the Royal Society, read a paper entitled "On Cystic Oxide, a New Species of Urinary Calculus," to that august body [7]. The bladder stone, which had been removed from a five-year-old boy, was named cystic oxide from the Greek word for bladder, *kystis*. We now know that such stones are largely comprised of cystine, which is quite insoluble, particularly at low pH. Subsequent work revealed that the substance was not an oxide and the terms cysteine (for the reduced form) and cystine (for the disulfide)

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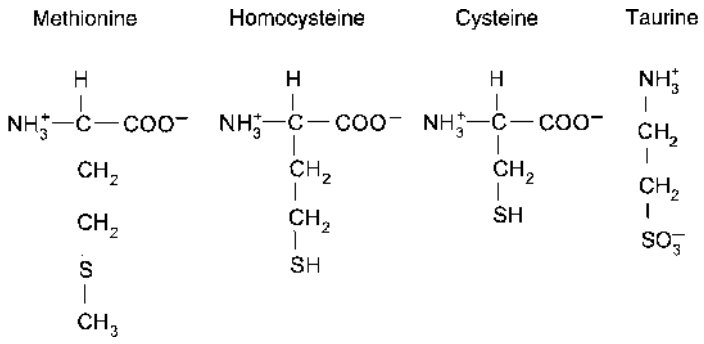

Figure 1.1 Structures of the common, sulfur-containing amino acids.

came into use. Cysteine (or more accurately, cystine) has the distinction of being our oldest known amino acid.

Homocysteine is a homolog of cysteine. Its discovery dates from 1932, when duVigneaud was examining the nature of the sulfur in insulin. He found that treatment of methionine with strong acid yielded homocysteine [8]. Subsequent work showed that homocysteine fed to animals could produce cysteine and that homocysteine could be produced after ingestion of methionine [9]. Taurine was discovered in 1824, just a few years after cystine, by Tiedemann and Gmelin [10]. Since it was originally isolated from ox bile, the name taurine reflects its bovine origin (*Bos taurus*).

Methionine cannot be produced, de novo, by animals and is therefore a dietary essential amino acid. Cysteine is not an essential amino acid, as it may be readily produced from methionine [11]. Taurine is an essential nutrient, during development, in some species [12].

1.2 WHY SULFUR-CONTAINING AMINO ACIDS?

Perhaps the most fundamental question that can be asked about these compounds is why they contain sulfur. The question may be better stated as: what properties of sulfur are fundamental to the functions of these amino acids? Methionine and cysteine are incorporated into proteins and also play important metabolic roles. However, we consider their roles in proteins to be primary and key to their selection; the metabolic roles are likely to have evolved subsequently. Sulfur belongs to group VIA of the periodic table. This group also includes oxygen and selenium. An appreciation of the importance of sulfur chemistry to the function of these amino acids is revealed by considering the roles of these amino acids in proteins and how these roles would be affected if the sulfur atom were replaced by an oxygen atom.

Cysteine's most distinctive role in proteins lies in its ability to form a disulfide linkage with another cysteine residue, thus providing a readily reversible covalent bond in vivo. Extracellular proteins are particularly rich in these disulfide linkages, which may be either intrachain or interchain and which play a fundamental role in determining the stability of proteins [13]. In fact, one of the earliest examples of

bioengineering involved cysteine. It occurred in 1906 when Karl Nessler designed a machine to curl a woman's hair by reducing the disulfide bonds in keratin. If the hair was then twisted around a series of rods and the disulfide bonds allowed to reform, the hair would be "curled." The original design was less than satisfactory (his wife lost considerable hair) but eventually the design was so improved that every woman could afford a "Toni" permanent at home if she wished.

The amino acid serine is a structural analogue of cysteine in which the sulfur atom is replaced by oxygen but serine shows no comparable tendency to form dioxides. This important difference may be explained by the acid dissociation of H_2O and H_2S since serine and cysteine may be regarded as derivatives of these compounds (Fig. 1.2). H_2S is a much stronger acid than is H_2O (pKa 7.04 and 15.74, respectively) (Fig. 1.2a), which means that, of the two conjugate bases, SH^- will be formed much more readily than OH^-. The reason for the difference in these dissociation constants is straightforward. Although both oxygen and sulfur share the same number of electrons in their outer orbitals ($2p^4$ and $3p^4$, respectively), because of oxygen's much smaller size these electrons are held much more closely and, therefore, more tightly to the positive nucleus in an oxygen atom than in a sulfur atom. Indeed, oxygen is much more electronegative than sulfur (3.44 and 2.58, respectively, on the Pauling scale). Applying these considerations to cysteine and serine, it is evident that cysteine will dissociate to H^+ and the corresponding thiolate anion much more readily than serine will dissociate to H^+ and the corresponding oxide (pK_{SH} of cysteine 8.3 and pK_{OH} of serine $\sim$13) (Fig. 1.2b). The pK values for these amino acids in proteins may vary somewhat but the principle remains. Since the formation of disulfide linkages first requires the dissociation of two cysteines, followed by the reaction of the two thiolate anions, we can appreciate that the formation of interchain linkages between two cysteine residues is feasible, whereas the formation of comparable interchain linkages between serine residues is highly unfavored. The same argument applies to other functions of cysteine, which require thiol dissociation. For example,

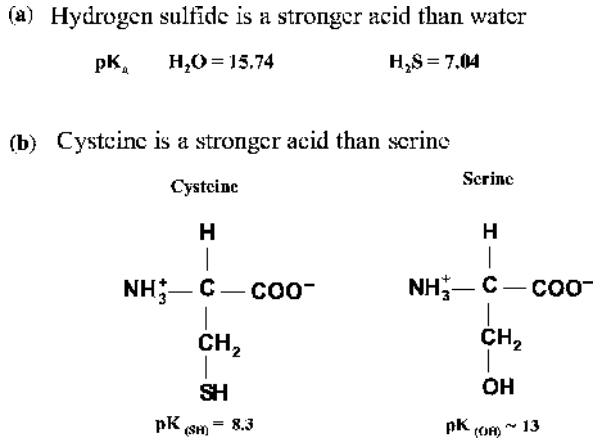

Figure 1.2 The importance of the sulfur atom to the chemistry of cysteine.

substitution of serine for cysteine in glutathione would provide a molecule that would essentially be incapable of becoming oxidized and unable to play a physiological role in oxidation-reduction reactions.

There are many other roles played by cysteine in proteins and they all rely on the unique chemistry of sulfur. Giles et al. [14] draw our attention to the multiple roles played by cysteine in biocatalysis, which include disulfide formation, metal binding, electron donation, and redox catalysis. Beinert and coworkers [15, 16] emphasize the role of iron-sulfur clusters in proteins, including their involvement in nitrogen fixation, electron transfer, the catalysis of homolytic reactions, and acting as sensors of iron and oxygen.

We may also enquire about the effects of substitution of methionine's sulfur with oxygen and how this would affect methionine's role in proteins. Methionine is among the most hydrophobic of amino acids; substitution of its sulfur with the much more electronegative oxygen would result in a δ^- charge at the oxygen atom, making the side chain much less hydrophobic. This would affect methionine's function in a number of ways. For example, methionine is the initiating amino acid in the synthesis of eukaryotic proteins. *N*-formylmethionine serves the same function in prokaryotes. As most of these methionine residues are subsequently removed, it is evident that their function lies in the initiation of translation rather than in the structure of the mature protein. In eukaryotic cells, the initiation of translation requires the association of the charged initiator tRNA (met-$tRNA^{met}$) with the initiation factor, eIF-2, and the 40S ribosomal subunit, together with a molecule of the mRNA that is to be translated. Drabkin and RajBhandary [17] have studied this reaction in detail and suggest that the hydrophobic nature of methionine is key to the binding of the initiator tRNA to eIF-2. Using appropriate double mutations (in codon and anticodon), they were able to show that the hydrophobic valine could be effective for initiation in mammalian cells but that the polar glutamine was very poor. The hydrophobicity of methionine also has an important effect on the role played by this amino acid in protein structure. Most of the methionine residues in globular proteins are found in the interior hydrophobic core; in membrane-spanning protein domains, methionine is often found to interact with the lipid bilayer [18]. The sulfur atom of methionine is key to its hydrophobicity and, therefore, to its functions in protein structure.

Not all methionine residues are buried in the interiors of proteins. In *Escherichia coli* glutamine synthetase, as much as one third of them are found on the protein surface, many clustered around the active site. These residues are susceptible to oxidation by certain reactive oxygen species (ROS), producing methionine sulfoxide. Figures 1.3a and 1.3b show the reaction of such a methionine residue with hydrogen peroxide. Levine et al. [19] view these methionine residues as playing the role of molecular lightning rods, in that they protect access of ROS to the active site. In line with this view is the fact that they report that oxidation of these residues has little effect on the catalytic activity of the enzyme. These oxidized methionine residues may be reduced to methionine by the enzyme methionine sulfoxide reductase. This is dealt with, in detail, in the chapters by Weissbach and by Moskovitz, including the roles played by this system in age-related diseases. What concerns us here, however, is the suitability of sulfur for this role. The production of the sulfoxide employs a

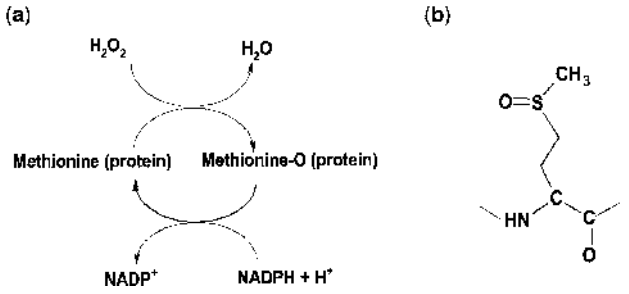

Figure 1.3 Methionine sulfoxide in proteins. (a) Oxidation of a methionine residue to methionine sulfoxide and its reduction back to methionine. (b) Structure of a methionine sulfoxide residue in a protein.

hybrid δ orbital that is available to sulfur. However, electrons in oxygen cannot access such orbitals, so that a methionine analog in which oxygen replaced the sulfur would be unable to employ this mechanism to protect against ROS.

1.3 *S*-ADENOSYLMETHIONINE, NATURE'S WONDER COFACTOR

S-adenosylmethionine (SAM) (Fig. 1.4) is an extraordinarily versatile cofactor; it has been said that it is second only to ATP in the number of reactions in which it is involved. Genomic and metabolic studies indicate that SAM is required by all organisms that have been studied. It may, therefore, be regarded as one of life's essential molecules. Nevertheless, not all organisms contain methionine adenosyltransferase

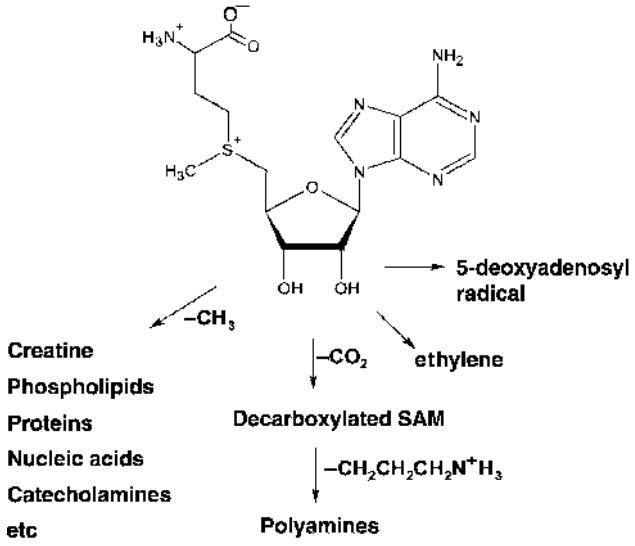

Figure 1.4 Some biosynthetic functions of *S*-adenosylmethionine.

and, therefore, not all organisms can synthesize SAM [20]. It appears that organisms without this enzyme can acquire SAM via transport from their surroundings. For example *Pneumocystis carinii*, an opportunistic fungal pathogen associated with AIDS, is a SAM auxotroph that actively transports this molecule [21].

SAM was discovered in the early 1950s by Cantoni [22] as the activated methyl donor required for a number of methylation reactions. It is known primarily for its role as a donor of methyl groups. No fewer than 60 different methyltransferases have been identified but the number is likely to be much higher. Analysis of seven completely sequenced genomes, including the human genome, suggests that class-1 SAM-dependent methyltransferases account for between 0.6% and 1.6% of open reading frames [23]. In mammals, this would indicate about 300 class-1 methyltransferases, to which must be added an unknown number of class-2 and class-3 enzymes. It is clear that we must expect the discovery of many new methyltransferases in the coming years.

The key to the function of SAM as a methyl donor lies in the sulfonium ion (Fig. 1.4). In particular, the electrophilic nature of the carbon atoms adjacent to the sulfur atom causes them to withdraw electrons and facilitate their transfer to suitable nucleophilic acceptors. In the case of methyltransferases, the methyl group is transferred, to produce the methylated product and *S*-adenosylhomocysteine (Fig. 1.5). In this context, it is of interest to enquire about the importance of sulfur. Why is sulfur so appropriate for these reactions? An answer becomes evident by considering the situation with two possible analogs of methionine, one in which an oxygen replaces the sulfur and one in which a methylene group replaces the sulfur. In the latter case, the molecule would be essentially inert in methyl transfer reactions as the methyl carbon would not be electrophilic. Considering the situation with oxygen, due to the very high electronegativity of this atom, the analogous oxonium compound would be so reactive that it would react promiscuously, without the need for an enzyme. The ideal biological methylating agent should be potentially reactive but not so highly reactive per se, that it would display an appreciable rate of uncatalyzed methylation reactions. With *S*-adenosylmethionine there is a very low level of background "noise" in the form of random, undesired methyl transfer reactions. A catalytic acceleration of 12 or so orders of magnitude by appropriate enzymes

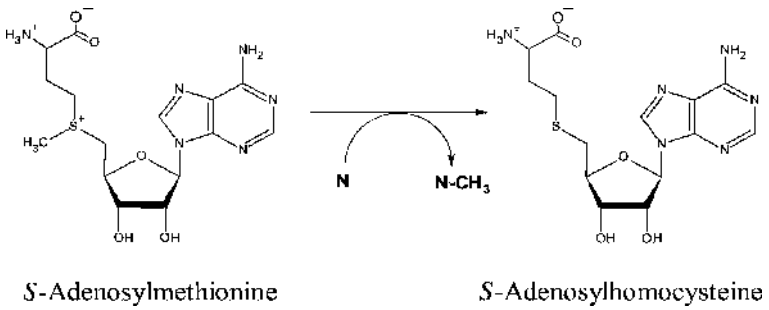

Figure 1.5 A typical methyltransferase reaction. N represents a suitable nucleophile.

enables the transfer of a methyl group to a specific nucleophile to occur at a rate that is physiologically relevant, and with a high signal-to-noise ratio (Richard Schowen, personal communication). We can compare *S*-adenosylmethionine to Goldilocks' sampling of the bowls of porridge. The oxonium compound is too hot, the compound in which sulfur is replaced by a methylene group is too cold but the sulfur-containing compound, *S*-adenosylmethionine, is just right.

S-adenosylmethionine is regarded as a high-energy molecule. More properly, it may be stated that SAM has a high methyl group-transfer potential. In fact, the ΔG^0 for SAM-dependent methylation reactions is greater than that for ATP hydrolysis [24]. Not surprisingly, therefore, the synthesis of SAM requires a considerable input of chemical energy, as is apparent from the reaction catalyzed by methionine adenosyltransferase,

$$\text{L-Methionine} + \text{ATP} + \text{H}_2\text{O} \longrightarrow \text{SAM} + \text{PPi} + \text{Pi}$$

The pyrophosphate produced is hydrolyzed to two molecules of inorganic phosphate, which tends to pull the reaction to the right. The synthesis of one molecule of SAM, therefore, requires the removal of all three phosphoryl groups from ATP.

Recent work has clarified quantitative aspects of methyl transfer in humans [25]. Application of stable isotope methodology which involves the constant infusion of [methyl-$^2H_3$] and [1-^{13}C] methionine permits the estimation of total methyl fluxes. Consensus values are 16.7 to 23.4 mmol/day per 70 kg body weight for young adults. These estimates are reduced by almost 10% in elderly subjects. Progress has also been made in delineating the quantitatively major methyltransferases. These are creatine synthesis, involving guanidinoacetate methyltransferase, and phosphatidylcholine synthesis, via phosphatidylethanolamine methyltransferase. Together, these may account for some 80% of total methyl transfer reactions in individuals who ingest customary North American diets, which may provide some 4 to 8 mmol creatine per day [26]. The other quantitatively major methyltransferase reaction is glycine *N*-methyl transferase, particularly in situations of high methionine intake. All three of these enzymes are either predominantly, or exclusively, found in the liver, which points to this organ as the major site of SAM utilization in mammals. In addition to these quantitatively major reactions, SAM can transfer its methyl group to a variety of nucleophilic acceptors, including amino acid residues in proteins, bases in DNA and RNA, small molecules, and even a metal oxide, arsenite [27, 28]. Methylation, therefore, plays a role in such diverse events as fetal development, hormone and phospholipid synthesis, protein repair, detoxification, and the regulation of gene expression.

SAM is, however, involved in a wide variety of reactions other than methylation. The arguments made for the importance of the sulfonium ion for facilitating methyl transfer are equally valid for the transfer of the other substituents to the sulfur. Polyamine synthesis involves the transfer of aminopropyl groups from decarboxylated SAM. For example, the addition of an aminopropyl group to putrescine results in the synthesis of spermidine and 5′-methylthioadenosine [29]. The 5′-deoxyadenosyl group of SAM may be transferred to the fluoride ion to produce the organofluorine,

5′-fluorodeoxyadenosine [30]. *S*-adenosylmethionine is also employed in such other alkylation reactions as the synthesis of the fruit-ripening hormone ethylene and the production of new methylene groups in cyclopropane fatty acids [29]. SAM can also be used as a source of ribosyl groups in the biosynthetic pathway for the synthesis of queuosine, a modified tRNA nucleoside [29].

Perhaps the most exciting recent finding related to SAM has been the identification of the superfamily of radical SAM enzymes. It has been predicted that this superfamily may contain more than 600 different enzymes, particularly in plants [31]. Radical SAM enzymes carry out the cleavage of relatively unreactive carbon-hydrogen bonds within alkyl groups [32]. Such reactions had hitherto been regarded as the province of hemoproteins or vitamin B12-containing enzymes. It seems that electron transfer from these clusters to SAM is critical to the formation of the SAM radical and is followed by its reductive cleavage to methionine and the 5′-deoxyadenosyl radical. The strongly oxidizing 5′-deoxyadenosyl radical can abstract a hydrogen atom from the carbon atom of various substrate molecules to produce 5′-deoxyadenosine and a radical substrate. The radical substrate may be converted to the final product of the enzyme-catalyzed reaction [31]. In some situations, SAM is not consumed in the overall reaction but is regenerated when the radical product abstracts a hydrogen from 5′-deoxyadenosine which facilitates its reaction with methionine, to yield SAM [31]. In most cases, however, 5′-deoxyadenosine is the final product so that these radical SAM enzymatic reactions reflect a net utilization of SAM.

SAM radical enzymes are involved in biotin synthesis, in which a sulfur atom is inserted across two carbon-hydrogen bonds to produce biotin's sulfur-containing ring. Lipoic acid synthesis also requires a SAM radical enzyme; in this case, two sulfur atoms are added to two poorly reactive C-H bonds, to produce the dihydrolipoyl group. Radical SAM enzymes are also involved in heme biosynthesis, thiamin biosynthesis, and bacterial lysine metabolism [32, 33]. The review by Wang and Frey should be consulted for a partial list of radical SAM enzymes [32].

S-adenosylmethionine is not the only sulfonium compound involved in methyl group metabolism. *S*-methylmethionine is a sulfonium compound in which an additional methyl group is linked to the sulfur atom of methionine; in effect, it is an analog of *S*-adenosylmethionine in which the adenosyl group is replaced by a methyl group. Plants contain significant quantities of this unusual amino acid, where it plays a role in their one-carbon metabolism [34]. *S*-methylmethionine can be metabolized in animal tissues. It has choline-sparing activity in chickens [35]. It has been suggested that this finding may result from the use of *S*-methylmethionine as a methyl-group donor in animals, either for betaine-homocysteine methyltransferase or phosphatidylethanolamine methyltransferase, or both [35]. Certainly, it is important to determine, at the enzymatic level, the role of this intriguing plant product in animals, particularly its potential role as a direct methyl donor.

1.4 GLUTATHIONE

Glutathione was discovered in 1922 by Sir Frederick Gowland Hopkins, the first professor of biochemistry at the University of Cambridge and future Nobelist.

To his considerable embarrassment, Hopkins proposed that glutathione was a dipeptide of glutamate and cysteine [36]. Nevertheless, from the very outset, Hopkins appreciated the key role of glutathione in oxidation-reduction reactions. His 1922 paper states: "When a tissue is washed until it has lost its power of reducing methylene blue the subsequent addition of glutathione to a buffer solution in which the tissue residue is suspended restores reducing power. This is the case when the dipeptide [sic] is added in its oxidized (disulfide) form. The tissue residue first reduces the sulfur group and a system is thus established which under anaerobic conditions continuously reduces methylene blue until an equilibrium" [37]. It is clear that Hopkins understood the role of glutathione as a biological reducing agent, and that there must be a means of reducing oxidized glutathione so as to regenerate the reduced form.

We have come a long way from Hopkins' era and now appreciate the very many functional roles played by glutathione. In addition to its role in oxidation-reduction reactions, glutathione plays a role in leukotriene synthesis, in the covalent modification of proteins, in the conjugation and excretion of lipophilic xenobiotics, and in the detoxification of methylglyoxal. These issues are dealt with, in depth, in this volume. Glutathione's most important function, however, lies in oxidation-reduction reactions and we will, therefore, highlight a few key features of its role in these reactions.

Schafer and Buettner have done considerable work to put our understanding of glutathione on a quantitative footing [38]. They emphasize the fact that glutathione is the major intracellular thiol-disulfide redox buffer. They point out that this depends both on the redox potential of the GSSG/2GSH redox pair:

$$\mathrm{GSSG} + 2\mathrm{H}^{+} + 2\mathrm{e}^{-} \longrightarrow 2\mathrm{GSH},$$

and on the total cellular concentrations of these compounds. Cytosolic GSH concentrations are in the region of 1 to 11 mM, which is much higher than most other redox-active compounds. In addition, they emphasize the fact that the redox potential of the glutathione system is quite dependent on the absolute concentrations of the redox species. This is so because in the calculation of the redox potential of the glutathione system, [GSH] enters as a squared term. Thus, the Nernst equation for glutathione half-cell is given by:

$$\mathrm{Ehc} = -240 - (59.1/2)\log\left(\frac{[\mathrm{GSH}]^2}{[\mathrm{GSSG}]}\right)\mathrm{mV}$$

These considerations both emphasize the importance of the total concentration of glutathione as well as the errors that can be introduced by employing the GSH/GSSG ratio, rather than $[\mathrm{GSH}]^2/[\mathrm{GSSG}]$ as the indicator of redox potential.

Schafer and Buettner [38] also emphasize the cellular compartmentation of glutathione. There are a number of compartments that are quite distinct from each other. The cytosol is the site of glutathione synthesis and usually contains the highest concentration of glutathione. Mitochondria have a separate pool, which, together with superoxide dismutase, plays an important role in protecting against oxidative damage brought about by the superoxide produced during oxidative phosphorylation.

A separate nuclear pool of glutathione is important for maintaining the redox state of crucial sulfhydryls in proteins required for DNA repair and gene expression. The glutathione pool within the lumen of the endoplasmic reticulum is relatively oxidized ($E_{hc} = -180$ mV, compared with -232 mV in the cytosol). This appears to be important for the formation of disulfide linkages in proteins that are destined for export into the extracellular space.

Finally, it is important to consider the constancy, or lack thereof, of the glutathione redox buffer. We are conditioned, of course, to regard a "buffer" as something to be functionally defended by cells. This is certainly true if we restrict ourselves to a particular functional or developmental window, but it is not necessarily the case when we have a broader perspective. Indeed, alterations in the functional properties of the "buffer" may have, or reflect, important and pleiotropic consequences. Schafer and Buettner [38] suggest that the glutathione system may be a useful indicator of the redox environment of cells. "Redox environment" is used as a general term for a linked set of cellular redox couples. It encompasses both the redox potentials and the concentrations of the different redox couples. It is apparent that, as reflected by the glutathione system, the cellular redox environment changes, in significant ways, during the life of a cell. Evidence for this idea comes from a variety of systems (slime mold, sea urchin eggs, NIH/3T3 cells), so that it appears to be a fundamental property. It appears that the cellular redox potential is relatively reduced during proliferative phases and becomes more oxidized with differentiation. Apoptosis occurs in a more oxidized cellular redox environment, which becomes even more oxidized during cell necrosis. These changes in the glutathione system are thought to reflect parallel changes in the redox state of critical protein sulfhydryl groups. Sulfhydryl groups, per se, are reduced but may become oxidized by virtue of forming

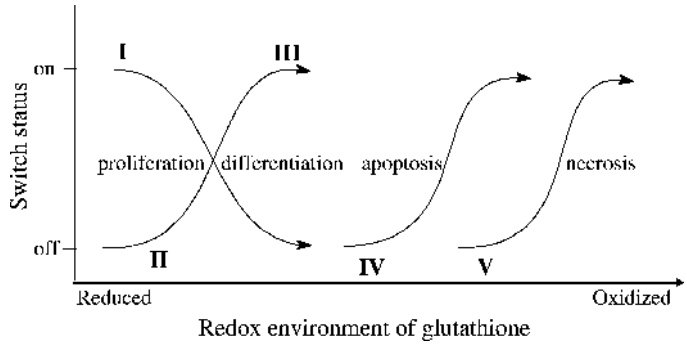

Figure 1.6 Protein nano-switches, in which protein sulfhydryl groups may be oxidized or reduced as a function of the redox potential of the cellular glutathione system. These switches may control regulatory programs that determine cells' fates. During the proliferative phase (**I**), the glutathione redox system is most reduced and the nano-switches that control this process are fully on. At **II**, the glutathione system becomes somewhat more oxidized, the proliferation switches are turned off and the differentiation switches are turned on, until most cells are differentiating (**III**). If the glutathione redox system becomes even more oxidized then death programs, such as apoptosis (**IV**) or necrosis (**V**) become activated. After Schafer and Buettner [38].

disulfide linkages with other cysteine residues or a mixed disulfide with other thiols, of which GSH is quantitatively the most important. The reduction potential of the various cellular redox potentials may be regarded as a means of activating or inhibiting broad programs of cellular activity. Modulation of the S-thiolation/dethiolation status of critical protein sulfhydryl groups can alter their activity with regard to signal transduction, nucleic acid synthesis, gene expression and cell cycle regulation. Schafer and Buettner [38] regard the redox states of certain critical sulfhydryl groups as acting as nano switches that can facilitate broad programs of cellular activity (Fig. 1.6). In this view, the cellular redox environment may determine whether a cell enters a proliferation or differentiation program or whether it enters a cell death program.

1.5 TAURINE—THE SECOND ESSENTIAL SULFUR-CONTAINING AMINO ACID?

Taurine is synthesized from cysteine by most mammals, but the ability to do so varies markedly. Although rodents have a large capacity for taurine synthesis, cats do not [39]; thus taurine is an essential nutrient for cats. The inability to synthesize the required amount of taurine was probably not a problem for feral cats since they are carnivores and their food supply would be expected to contain ample taurine but the same is not always true for the family pet, which may be fed a home-cooked diet [40]. Although most dogs do synthesize sufficient taurine, in some situations, especially with certain larger breeds, like Newfoundlands, taurine can become deficient, resulting in dilated cardiomyopathy [41]. Taurine synthetic activity is also very low in human liver [42] and there is evidence that taurine is a conditionally essential nutrient, particularly in premature infants [43], in patients who are on very long term parenteral nutrition [44], and possibly in those who are vegans [45], since fruits, vegetables, grains, legumes, and nuts do not contain measurable amounts of taurine [40]. In fact, taurine has been referred to as a "carninutrient," a nutrient that is provided in the diet primarily by animal products [46].

The amino acids we think of as essential are all incorporated into protein during its synthesis, but this is not the case for taurine. It does, however, play many roles and more are being recognized all the time. It is the most abundant free amino acid in animal tissues. For example, it represents more than 50% of free amino acids in muscle [18]; it has been reported to act as an antioxidant and a membrane stabilizer [42]. It has long been known to be essential for conjugation of bile acids to give taurocholic acid and taurochenodeoxycholic acid for excretion of cholesterol degradation products into the bile. Most of the bile salts are reabsorbed so this pathway does not normally represent much loss of taurine, but cystic fibrosis patients do have a marked decrease in recycling of bile salts [47] and thus may need a taurine supplement [48]. The cholesterol-lowering anion-exchange resin cholestyramine lowers reabsorption of bile salts and thus patients on this drug may also need supplementary taurine [2]. Taurine is also well known for its roles in immunomodulation [49] and in lowering of elevated blood pressure, possibly by an antisympathetic effect [50]. Recently, several new functions of taurine have been proposed.

One of the major classes of function recently ascribed to taurine is that of signaling molecule. In some cases, free taurine itself serves the role, for example as modulator of the glycine receptor in retinal progenitor cells to direct some of these cells to become rod photoreceptors [51]. Activation of glycine receptors in hypothalamus may also play a role in osmoregulation [52]. In other cases, taurine is conjugated to another small molecule, such as an amino acid or fatty acid. In 1985, Marnela et al. [53] reported on the isolation of γ-glutamyltaurine as a major taurine-containing peptide from calf's brain. This role of taurine will be discussed later in this volume (see Chapter 22). While looking for an endogenous ligand for the cannabinoid receptor, Devane et al. [54] isolated *N*-acylethanolamine, the first of a newly recognized family of lipid transmitters. In 2006, Saghatelian et al. [55] identified a new member of the family in mouse tissues, *N*-acyltaurine (NAT). In brain the acyl group is usually a long-chain saturated fatty acid (especially 24 : 0), whereas in liver and kidney it is a polyunsaturated fatty acid, such as 20 : 4 and 22 : 6. NATs were able to activate three different members of the transient receptor potential family of calcium channels [55], which may in part explain the known effects of taurine on calcium movement across cell membranes (as reviewed in [2]).

Although taurine has not been found as a constituent of proteins, it is found as a posttranscriptional modification of two mitochondrial tRNA molecules in humans [56]. Suzuki et al. found that the uridine at the anticodon first or wobble position of $tRNA^{lys}$ and $tRNA^{leu(UUR)}$ is modified by covalent addition of taurine through its amino group to a methyl at C5 of the uracil base, leaving a free sulfonic acid group. In $tRNA^{leu(UUR)}$, the modified uridine is 5-taurinomethyluridine and in $tRNA^{lys}$ it is 5-taurinomethyl 2-thiouridine [56] (Fig. 1.7). Suzuki and coworkers have shown that lack of modification with taurine causes defective translation in general for $tRNA^{lys}$ and specifically of UUG-rich genes for $tRNA^{leu(UUR)}$ [57]. In certain mitochondrial myopathies, the uridine modification is missing and this deficit has been suggested as the key factor responsible for the phenotypic features of these disorders [58]. Although there are no data at present, one could speculate that the cardiomyopathy observed in taurine-deficient dogs [59] is also due in part to incomplete modification of these mitochondrial tRNAs [56], due to lack of substrate, taurine.

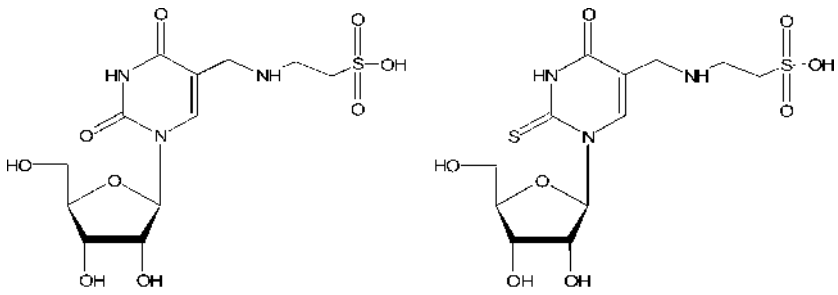

Figure 1.7 Chemical structures of the taurine-modified bases 5-taurinomethyl uridine and 5-taurinomethyl 2-thiouridine, in mitochondrial tRNA [56].

1.6 CONCLUSIONS

The fields of glutathione and the sulfur-containing amino acids continue to offer great opportunities for innovative research. The importance of reactive oxygen species, as both physiological and pathological agents, and the importance of defence against their deleterious effects are now apparent. Our understanding of the relative roles of the various intracellular glutathione pools, and how these different redox potentials are maintained, is in its infancy. The pleiotropic effects of changes in cellular redox environment are likely to become even more important to the fates of cells, including neoplastic cells. We know a great deal about the different taurine pools and their potential functions but our mechanistic understanding of these functions is very incomplete. However, we do appreciate that the roles of glutathione and the sulfur-containing amino acids are dependent on the singular chemical properties of sulfur. Much remains to be learned and we should recall the words of Hamlet,

> There are more things in heaven and earth, Horatio,
> Than are dreamt of in your philosophy.

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