5th Amino Acid Assessment Workshop

The Sulfur-Containing Amino Acids: An Overview[1,2]

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ABSTRACT Methionine, cysteine, homocysteine, and taurine are the 4 common sulfur-containing amino acids, but only the first 2 are incorporated into proteins. Sulfur belongs to the same group in the periodic table as oxygen but is much less electronegative. This difference accounts for some of the distinctive properties of the sulfur-containing amino acids. Methionine is the initiating amino acid in the synthesis of virtually all eukaryotic proteins; N-formylmethionine serves the same function in prokaryotes. Within proteins, many of the methionine residues are buried in the hydrophobic core, but some, which are exposed, are susceptible to oxidative damage. Cysteine, by virtue of its ability to form disulfide bonds, plays a crucial role in protein structure and in protein-folding pathways. Methionine metabolism begins with its activation to *S*-adenosylmethionine. This is a cofactor of extraordinary versatility, playing roles in methyl group transfer, 5′-deoxyadenosyl group transfer, polyamine synthesis, ethylene synthesis in plants, and many others. In animals, the great bulk of *S*-adenosylmethionine is used in methylation reactions. *S*-Adenosylhomocysteine, which is a product of these methyltransferases, gives rise to homocysteine. Homocysteine may be remethylated to methionine or converted to cysteine by the transsulfuration pathway. Methionine may also be metabolized by a transamination pathway. This pathway, which is significant only at high methionine concentrations, produces a number of toxic endproducts. Cysteine may be converted to such important products as glutathione and taurine. Taurine is present in many tissues at higher concentrations than any of the other amino acids. It is an essential nutrient for cats. J. Nutr. 136: 1636S–1640S, 2006.

KEY WORDS: • *methionine* • *cysteine* • *taurine* • *homocysteine* • *S-adenosylmethionine*

Methionine and cysteine may be considered to be the principal sulfur-containing amino acids because they are 2 of the canonical 20 amino acids that are incorporated into proteins. However, homocysteine and taurine also play important physiological roles (**Fig. 1**). Why does nature employ sulfur in her repertoire of amino acids? The other canonical amino acids are comprised only of carbon, hydrogen, oxygen, and nitrogen atoms. Because both sulfur and oxygen belong to the same group (Group 6) of the Periodic Table and, therefore, are capable of making similar covalent linkages, the question may be restated: why would methionine and cysteine analogs, in which the sulfur atom is replaced by oxygen, not serve the same functions? One of the critical differences between oxygen and sulfur is sulfur's lower electronegativity. Indeed, oxygen is the second most electronegative element in the periodic table. This accounts for the use of sulfur in methionine; replacement of the sulfur with oxygen would result in a much less hydrophobic amino acid. Cysteine readily forms disulfide linkages because of the ease with which it dissociates to form a thiolate anion. Serine, on the other hand, which differs from cysteine only in the substitution of an oxygen for the sulfur, does not readily make dioxide linkages. The difference results from the fact that thiols are much stronger acids than are alcohols, so that the alcohol group in serine does not dissociate at physiological pH. Substitution of oxygen for sulfur in *S*-adenosylmethionine would produce so powerful a methylating agent that it would promiscuously methylate cellular nucleophiles without the need for an enzyme.

Methionine and cysteine in proteins. Although both methionine and cysteine play critical roles in cell metabolism, we suggest that, in general, the 20 canonical amino acids were selected for the roles they play in proteins, not their roles in metabolism. It is important, therefore, to review the role played by these amino acids in proteins. Methionine is among the most hydrophobic of the amino acids. This means that 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. In some proteins a fraction of the methionine residues are somewhat surface exposed. These are susceptible to oxidation to methionine sulfoxide residues. Levine et al. (1) regard these methionine residues as endogenous antioxidants in proteins. In *E. coli* glutamine synthetase, they tend to be arrayed around the active site and may guard access to this site by reactive oxygen species. Oxidation of these methionine residues has little effect on the catalytic activity of the enzyme. These residues may be reduced to methionine by means of the enzyme methionine

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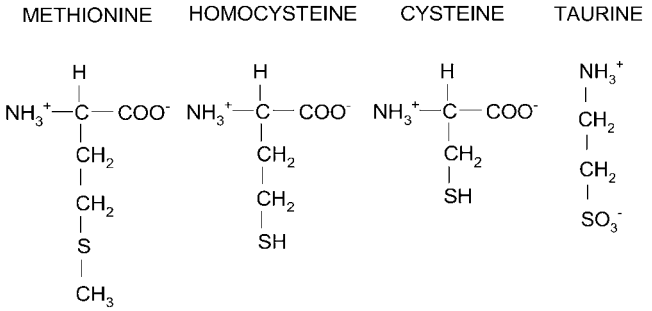

FIGURE 1 Structures of the sulfur-containing amino acids.

sulfoxide reductase (2). Thus, an oxidation–reduction cycle occurs in which exposed methionine residues are oxidized (e.g., by H_2O_2) to methionine sulfoxide residues, which are subsequently reduced:

$$\text{Methionine (protein)} + H_2O_2 \longrightarrow \text{Methionine sulfoxide (protein)} + H_2O$$

$$\text{Methionine sulfoxide (protein)} + \text{NADPH} + H^+ \longrightarrow \text{Methionine (protein)} + \text{NADP}^+ + H_2O.$$

It is considered that the impaired activity of methionine sulfoxide reductase and the subsequent accumulation of methionine sulfoxide residues are associated with age-related diseases, neurodegeneration, and shorter lifespan (2).

Methionine is the initiating amino acid in the synthesis of eukaryotic proteins; *N*-formyl methionine serves the same function in prokaryotes. Because most of these methionine residues are subsequently removed, it is apparent that their role lies in the initiation of translation, not in protein structure. In eukaryotes, translation initiation involves the association of the initiator tRNA (met-$tRNA_i^{met}$) with eIF-2 and the 40S ribosomal subunit together with a molecule of mRNA. Drabkin and Rajbhandary (3) 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 used for initiation in mammalian cells but that the polar glutamine was very poor.

Cysteine plays a critical role in protein structure by virtue of its ability to form inter- and intrachain disulfide bonds with other cysteine residues. Most disulfide linkages are found in proteins destined for export or residence on the plasma membrane. These disulfide bonds can be formed nonenzymatically; protein disulfide isomerase, an endoplasmic reticulum protein, can reshuffle any mismatched disulfides to ensure the correct protein folding (4).

S-Adenosylmethionine. *S*-Adenosylmethionine (SAM)[4] is a key intermediate in methionine metabolism. Discovered in 1953 by Cantoni (5) as the "active methionine" required for the methylation of guanidioacetate to creatine, it is now evident that SAM is a coenzyme of remarkable versatility (**Fig. 2**). In addition to its role as a methyl donor, SAM serves as a source of methylene groups (for the synthesis of cyclopropyl fatty acids), amino groups (in biotin synthesis), aminoisopropyl groups (in the synthesis of polyamines and, also, in the synthesis of ethylene, used by plants to promote plant ripening), and

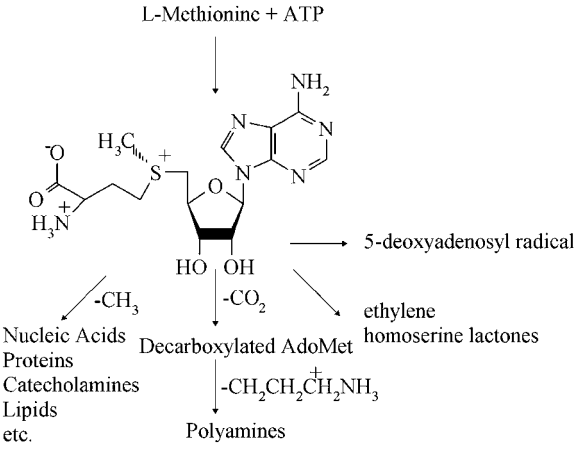

FIGURE 2 Metabolic versatility of S-adenosylmethionine.

5′-deoxyadenosyl radicals. SAM also serves as a source of sulfur atoms in the synthesis of biotin and lipoic acid (6). In mammals, however, the great bulk of SAM is used in methyltransferase reactions. The key to SAM's utility as a methyl donor lies in the sulfonium ion and in the electrophilic nature of the carbon atoms that are adjacent to the sulfur atom. The essence of these methyltransferase reactions is that the positively charged sulfonium renders the adjoining methyl group electron-poor, which facilitates its attack on electron-rich acceptors (nucleophiles).

SAM can donate its methyl group to a wide variety of acceptors, including amino acid residues in proteins, DNA, RNA, small molecules, and even to a metal, the methylation of arsenite (7,8). At present, about 60 methyltransferases have been identified in mammals. However, the number is almost certainly much larger. A bioinformatic analysis of a number of genomes, including the human genome, by Katz et al. (9) has suggested that Class-1 SAM-dependent methyltransferases account for 0.6–1.6% of open reading frames in these genomes. This would indicate about 300 Class 1 methyltransferases in humans, in addition to a smaller number of Class 2 and 3 enzymes. In humans, it appears that guanidinoacetate *N*-methyltransferase (responsible for creatine synthesis) and phosphatidylethanolamine *N*-methyltransferase (synthesis of phosphatidylcholine) are the major users of SAM (10). In addition, there is substantial flux through the glycine *N*-methyltransferase (GNMT) when methionine intakes are high (11). An important property of all known SAM-dependent methyltransferases is that they are inhibited by their product, *S*-adenosylhomocysteine (SAH).

Methionine metabolism. Methionine metabolism begins with its activation to SAM (**Fig. 3**) by methionine adenosyltransferase (MAT). The reaction is unusual in that all 3 phosphates are removed from ATP, an indication of the "high-energy" nature of this sulfonium ion. SAM then donates its methyl group to an acceptor to produce SAH. SAH is hydrolyzed to homocysteine and adenosine by SAH hydrolase. This sequence of reactions is referred to as *transmethylation* and is ubiquitously present in cells. Homocysteine may be methylated back to methionine by the ubiquitously distributed methionine synthase (MS) and, also, in the liver as well as the kidney of some species, by betaine:homocysteine methyltransferase (BHMT). MS employs 5-methyl-THF as its methyl donor, whereas BHMT employs betaine, which is produced during choline oxidation as well as being provided by the diet (10). Both MS and BHMT effect *remethylation,* and the combination of *transmethylation* and *remethylation* comprise the *methionine cycle,* which occurs in most, if not all, cells.

[4] Abbreviations used: BHMT, betaine:homocysteine methyltransferase; CBS, cystathionine beta-synthase; CGL, cystathionine gamma-lyase; GNMT, glycine N-methyltransferase: MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

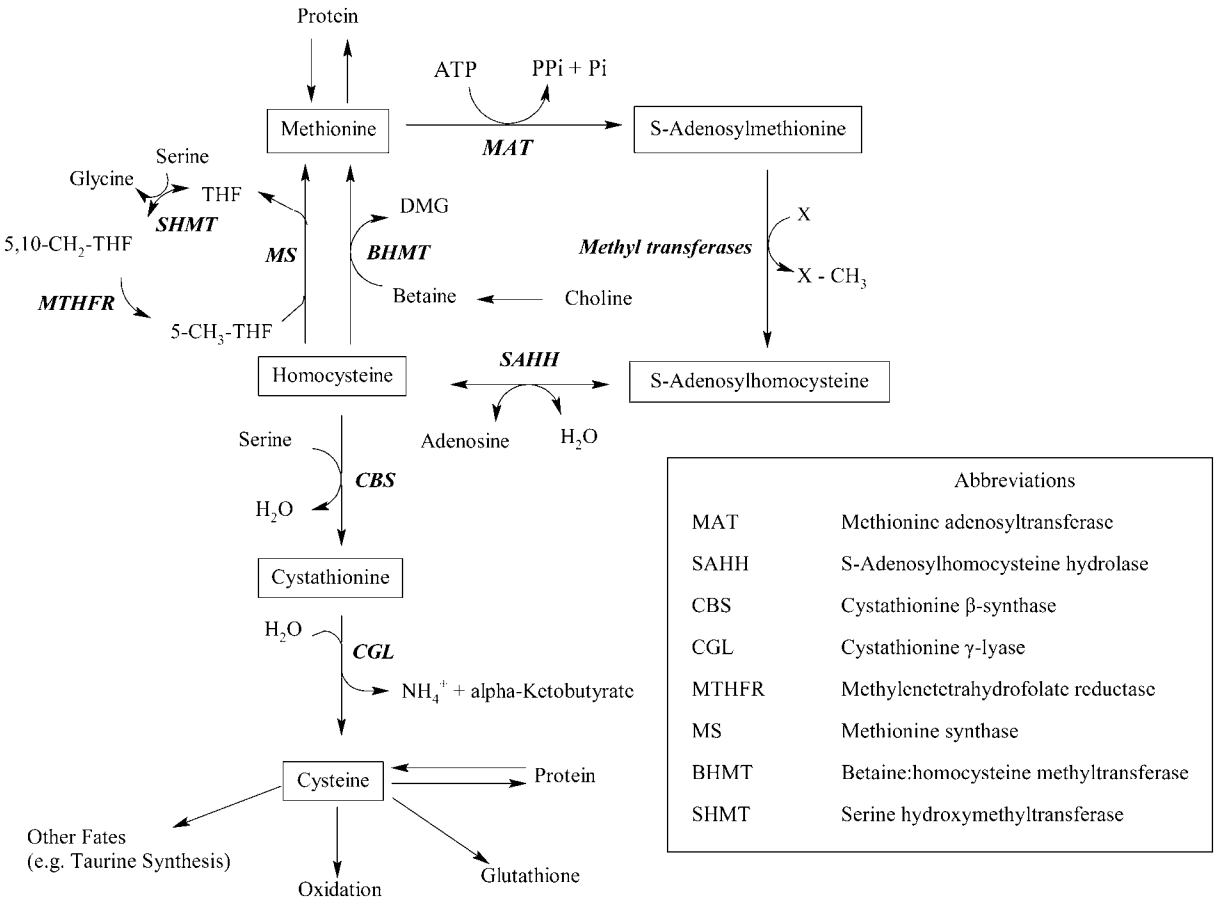

FIGURE 3 Major pathways of sulfur-containing amino acid metabolism.

The methionine cycle does not result in the catabolism of methionine. This is brought about by the *transsulfuration* pathway, which converts homocysteine to cysteine by the combined actions of cystathionine β-synthase (CBS) and cystathionine γ-lyase (CGL). The transsulfuration pathway has a very limited tissue distribution; it is restricted to the liver, kidney, intestine, and pancreas. The conversion of methionine to cysteine is an irreversible process, which accounts for the well-known nutritional principle that cysteine is not a dietary essential amino acid provided that adequate methionine is available, but methionine is a dietary essential amino acid, regardless of cysteine availability. This pathway for methionine catabolism suggests a paradox: is methionine catabolism constrained by the need for methylation reactions? If this were so, the methionine in a methionine-rich diet might exceed the methylation demand so that full catabolism could not occur via this pathway. GNMT methylates glycine to sarcosine, which may, in turn, be metabolized by sarcosine dehydrogenase to regenerate the glycine and oxidize the methyl group to 5,10-methylene-THF.

Application of sophisticated stable isotope tracer methodology to methionine metabolism in humans has yielded estimates of transmethylation, remethylation, and transsulfuration. Such studies reveal important points of regulation. For example, the sparing effect of cysteine on methionine requirements is evident as an increase in the fraction of the homocysteine pool that is remethylated and a decrease in the fraction that undergoes transsulfuration (12). In young adults ingesting a diet containing 1–1.5 g protein·kg^{-1}·d^{-1}, about 43% of the homocysteine pool was remethylated, and 57% was metabolized through the transsulfuration pathway (transmethylation = 9.7, transsulfuration = 5.4, remethylation = 4.4 μmol·kg^{-1}·h^{-1}) (13).

Methionine metabolism affords a remarkable example of the role of vitamins in cell chemistry. MS utilizes methylcobalamin as a prosthetic group, 1 of only 2 mammalian enzymes that are known to require Vitamin B-12. The methyl group utilized by MS is provided from the folic acid 1-carbon pool. Methylenetetrahydrofolate reductase (MTHFR), which reduces 5,10-methylene-THF to 5-methyl-THF, contains FAD as a prosthetic group. Both of the enzymes in the transsulfuration pathway (CBS and CGL) contain pyridoxal phosphate. It is hardly surprising, therefore, that deficiencies of each of these vitamins (Vitamin B-12, folic acid, riboflavin, and pyridoxine) are associated with elevated plasma homocysteine levels. The oxidative decarboxylation of the α-ketobutyrate produced by CGL is brought about by pyruvate dehydrogenase so that niacin (NAD), thiamine (thiamine pyrophosphate), and pantothenic acid (coenzyme A) may also be regarded as being required for methionine metabolism.

Not only are vitamins required for methionine metabolism, but methionine metabolism plays a crucial role in the cellular assimilation of folate. MS has 2 principal functions. In addition to its role in methionine conservation, MS converts 5-methyl-THF to THF, thereby making it available to support DNA synthesis and other functions. Because 5-methyl-THF is the dominant circulating form that is taken into cells, MS is essential for cellular folate assimilation. Impaired MS activity (e.g., brought about by cobalamin deficiency) results in the accumulation of the folate coenzymes as 5-methyl-THF, the so-called methyl trap (14). This hypothesis explains the fact that Vitamin B-12 deficiency causes a functional cellular folate deficiency.

The combined transmethylation and transsulfuration pathways are responsible for the catabolism of the great bulk of

methionine. However, there is also evidence for the occurrence of a SAM-independent catabolic pathway that begins with a transamination reaction (15). This is a very minor pathway under normal circumstances, but it becomes more significant at very high methionine concentrations. Because it produces powerful toxins such as methane thiol, it has been considered to be responsible for methionine toxicity. The identity of the initiating transaminase is uncertain; the glutamine transaminase can act on methionine, but it is thought to be unlikely to do so under physiological conditions (15). In view of the likelihood that this pathway plays a role in methionine toxicity, more work is warranted on its components, tissue distribution, and physiological function.

Regulation of methionine metabolism. The major means by which methionine metabolism is regulated are *1*) allosteric regulation by SAM and *2*) regulation of the expression of key enzymes. In the liver, SAM exerts powerful effects at a variety of loci. The liver-specific MAT has a high K_m for methionine and, therefore, is well fitted to remove excess dietary methionine. It exhibits the unusual property of feedback activation; it is activated by its product, SAM (16). This property has been incorporated into a computer model of hepatic methionine metabolism, and it is clear that it renders methionine disposal exquisitely sensitive to the methionine concentration (17). SAM is also an allosteric activator of CBS and an allosteric inhibitor of MTHFR (18). Therefore, elevated SAM promotes transsulfuration (methionine oxidation) and inhibits remethylation (methionine conservation). Many of the enzymes involved in methionine catabolism (MAT 1, GNMT, CBS) are increased in activity on ingestion of a high-protein diet (18).

In addition to its function in methionine catabolism, the transsulfuration pathway also provides cysteine for glutathione synthesis. Cysteine availability is often limiting for glutathione synthesis, and it appears that in a number of cells (e.g., hepatocytes), at least half of the cysteine required is provided by transsulfuration, even in the presence of physiological concentrations of cysteine (19). Transsulfuration is sensitive to the balance of prooxidants and antioxidants; peroxides increase the transsulfuration flux, whereas antioxidants decrease it (20). It is thought that redox regulation of the transsulfuration pathway occurs at the level of CBS, which contains a heme that may serve as a sensor of the oxidative environment (21).

Taurine. Taurine is remarkable, both for its high concentrations in animal tissues and because of the variety of functions that have been ascribed to it. Taurine is the most abundant free amino acid in animal tissues. **Table 1** shows that, although taurine accounts for only 3% of the free amino acid pool in plasma, it accounts for 25%, 50%, 53%, and 19%, respectively, of this pool in liver, kidney, muscle, and brain. The magnitude of the intracellular taurine pool deserves comment. For example, skeletal muscle contains 15.6 μmol of taurine per gram of tissue, which amounts to an intracellular concentration of about 25 mM. In addition to its role in the synthesis of the bile salt taurocholate, taurine has been proposed, inter alia, to act as an antioxidant, an intracellular osmolyte, a membrane stabilizer, and a neurotransmitter. It is an essential nutrient for cats; kittens born to mothers fed taurine-deficient diets exhibit retinal degeneration (24). Taurine is found in mother's milk, may be conditionally essential for human infants, and is routinely added to most infant formulas. Recent work has begun to reveal taurine's action in the retina. It appears that taurine, via an effect on a glycine receptor, promotes the generation of rod photoreceptor cells from retinal progenitor cells (25).

TABLE 1

Taurine concentrations in rat tissues (22,23)

Plasma	0.36 μmol/ml	(2.8%)
Liver	4.28 μmol/g	(24.6%)
Kidney	8.72 μmol/g	(50.1%)
Muscle	15.60 μmol/g	(52.7%)
Brain	5.09 μmol/g	(19.1%)

The numbers in parentheses give taurine as a percentage of the total free amino acid pool in each tissue.

Perspective. The sulfur-containing amino acids present a fascinating subject to the protein chemist, the nutritionist, and the metabolic scientist, alike. They play critical roles in protein synthesis, structure, and function. Their metabolism is vital for many critical functions. SAM, a remarkably versatile molecule, is said to be second, only to ATP, in the number of enzymes that require it. Vitamins play a crucial role in the metabolism of these amino acids, which, in turn, play a role in folic acid assimilation. Despite the great advances in our knowledge of the sulfur-containing amino acids, there are important areas where further work is required. These include methionine transamination and the molecular basis for the many functions of taurine.

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