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Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur

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Abstract

Synthesis of cysteine as a product of the transsulfuration pathway can be viewed as part of methionine or homocysteine degradation, with cysteine being the vehicle for sulfur conversion to end products (sulfate, taurine) that can be excreted in the urine. Transsulfuration is regulated by stimulation of cystathionine β-synthase and inhibition of methylene tetrahydrofolate reductase in response to changes in the level of S-adenosylmethionine, and this promotes homocysteine degradation when methionine availability is high. Cysteine is catabolized by several desulfuration reactions that release sulfur in a reduced oxidation state, generating sulfane sulfur or hydrogen sulfide (H_2S), which can be further oxidized to sulfate. Cysteine desulfuration is accomplished by alternate reactions catalyzed by cystathionine β-synthase and cystathionine γ-lyase. Cysteine is also catabolized by pathways that require the initial oxidation of the cysteine thiol by cysteine dioxygenase to form cysteinesulfinate. The oxidative pathway leads to production of taurine and sulfate in a ratio of approximately 2:1. Relative metabolism of cysteine by desulfuration versus oxidative pathways is influenced by cysteine dioxygenase activity, which is low in animals fed low-protein diets and high in animals fed excess sulfur amino acids. Thus, desulfuration reactions dominate when cysteine is deficient, whereas oxidative catabolism dominates when cysteine is in excess. In rats consuming a diet with an adequate level of sulfur amino acids, about two thirds of cysteine catabolism occurs by oxidative pathways and one third by desulfuration pathways. Cysteine dioxygenase is robustly regulated in response to cysteine availability and may function to provide a pathway to siphon cysteine to less toxic metabolites than those produced by cysteine desulfuration reactions.

The sulfur of methionine (Met) ends up forming the thiol group of homocysteine (Hcy) as a consequence of the transsulfuration pathway. In this review, the ultimate fate of this Met/Hcy sulfur atom is traced. Because transsulfuration moves the thiol group to a new carbon chain, forming cysteine (Cys), the discussion of Met/Hcy sulfur metabolism is, in actuality, largely a discussion of Cys catabolism.

Transsulfuration is necessary for homocysteine/methionine sulfur oxidation

Transsulfuration of Hcy to Cys is catalyzed by two pyridoxal 5′-phosphate (PLP)-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), as shown in Fig. 1. CBS catalyzes the condensation of Hcy and serine to form cystathionine. Cystathionine is

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then hydrolyzed by CSE to form Cys (now containing the sulfur atom from Hcy) and α-ketobutyrate (from the Hcy carbon chain) plus ammonia (from the amino group of Hcy). Alpha ketobutyrate can be further catabolized by oxidative decarboxylation to propionyl-coenzyme A (CoA), which enters the tricarboxylic acid cycle at the level of succinyl-CoA. Oxidative decarboxylation of α-ketobutyrate can be catalyzed by pyruvate and branched-chain keto acid dehydrogenase complexes. Thus, the transsulfuration pathway is responsible for catabolism of the carbon chain of Met, release of the amino nitrogen in a form that can be funneled into pathways of nitrogen excretion, and transfer of Met sulfur to serine to synthesize Cys.

In the steady-state metabolic condition in normal individuals, the intake of Met sulfur is balanced by metabolism of an almost equivalent amount of Hcy sulfur through the transsulfuration pathway (Mudd et al. 1980; Poole et al. 1975; Stipanuk 1986; Storch et al. 1988). This must be true regardless of the extent of remethylation. Little sulfur is oxidized or lost during Met metabolism, and essentially all Met sulfur is transferred to Cys prior to oxidation/excretion of the sulfur atom. CBS is a highly regulated enzyme, with S-adenosylmethionine acting as an allosteric activator that increases CBS activity as well as stabilizing the enzyme (and thus increasing its concentration) when Met is abundant (Janosik et al. 2001; Prudova et al. 2006), and with a heme-binding domain that possibly serves as a redox sensor to stimulate activity under oxidative conditions (Banerjee and Zou 2005).

Although the transsulfuration pathway converts Met/Hcy carbon and nitrogen into products that enter central pathways of fuel metabolism and nitrogen disposal, the sulfur atom is simply transferred to serine to form a new thiol-containing amino acid, Cys. Thus, further catabolism of Cys is essential for conversion of the sulfur to its normal end products (sulfate, taurine) that can be excreted in the urine. Although the transsulfuration pathway allows for biosynthesis of Cys from Met sulfur and serine, this reaction is less critical because Cys, as with other amino acids, can be obtained preformed in the diet. The conclusion that the major role of the transsulfuration pathway in animals is Met/Hcy degradation rather than cysteine biosynthesis seems consistent with its robust regulation by S-adenosylmethionine to minimize Met/Hcy degradation when Met availability is low and facilitate it when Met levels are high. Cys synthesis as a product of the transsulfuration pathway can be viewed as a part of Met/Hcy degradation, with Cys being the vehicle for Met/Hcy sulfur conversion to end products that can be excreted in the urine.

In mammals, Cys is catabolized by several nonoxidative cysteinesulfinate-independent desulfuration pathways as well as by oxidative cysteinesulfinate-dependent pathways (see Fig. 2). How much each of these routes contributes to Cys catabolism varies with sulfur-containing amino acid (SAA) intake or intracellular Cys and S-adenosylmethionine concentrations and with the particular species, tissue, or cell type being studied, as discussed below.

Cysteine undergoes desulfuration to hydrogen sulfide (H_2S) or sulfane sulfur

The nonoxidative pathways include desulfuration of cyst(e) ine by somewhat nonspecific reactions catalyzed by CSE (Cavallini et al. 1962a, b; Szczepkowski and Wood 1967), CBS (Lak et al. 1970; Braunstein et al. 1971; Porter et al. 1974), and possibly by aminotransferases in conjunction with 3-mercaptopyruvate sulfurtransferase (Meister et al. 1954; Ip et al. 1977; Ubuka et al. 1977a, b, [1978]; Taniguchi and Kimura 1974; Kun and Fanshier 1958, 1959a, b; Fiedler and Wood 1956). The reactions catalyzed by these enzymes are important in Cys catabolism and also in producing a pool of metabolically

reduced sulfur in the 0 or −2 oxidation state. Distribution of these enzymes varies among tissues.

Cystathionine β-synthase

CBS is well known for its role in the transsulfuration pathway, in which it catalyzes the β-replacement of the hydroxyl group of serine with Hcy, forming the thioether cystathionine with release of water (H_2O) (reaction 1). Under physiological conditions, Cys can substitute for serine, and the β-replacement of the sulfhydryl group of Cys with Hcy results in cystathionine formation with release of H_2S (reaction 2).

$$\text{Hcy} + \text{serine} \xrightarrow{\text{CBS}} \text{cystathionine} + H_2O \quad (1)$$

$$\text{Hcy} + \text{Cys} \xrightarrow{\text{CBS}} \text{cystathionine} + H_2S \quad (2)$$

This alternative reaction, using Cys instead of serine as the substrate that binds to enzyme site 1 (i.e., reaction 2), effectively removes Hcy and leads to Cys synthesis by transsulfuration. However, because Cys is both a substrate of reaction 2 and a product of the subsequent cleavage of cystathionine, no net Cys is produced by the transsulfuration pathway when CBS catalyzes Cys desulfuration. Other alternative reactions catalyzed by CBS appear to make a negligible contribution to Cys desulfuration (Braunstein et al 1971; Chen et al. 2004; Singh et al. 2009). Because the K_m values of mammalian CBS for serine and Cys (~1.9 mM and ~6.5 mM, respectively) are high relative to intracellular substrate concentrations, the extent to which serine versus Cys is used as substrate for cystathionine synthesis will largely be determined by the tissue concentrations of these substrates. Using concentrations of serine, Cys, and Hcy to mimic those measured in mouse liver (0.72, 0.47, and 0.58 mM, respectively) and human CBS, Chen et al. (2004) set up an in vitro reaction to assess the extent of cystathionine synthesis from serine versus Cys. They determined that about 5% of cystathionine formed in mouse liver was derived from Cys, indicating that the major reaction for the first step of the transsulfuration pathway involves serine as substrate but also that some H_2S production from Cys and Hcy is likely to occur in vivo by the alternative reaction. In addition, Chen et al. (2004) provided experimental data to show that the contribution of the alternative reaction for cystathionine synthesis increased as substrate concentrations increased: the alternative reaction with Cys accounted for 23% of total cystathionine synthesis when all substrates were present at 0.1 mM, 35% when substrates were present at 1 mM, and 44% when substrates were present at 10 mM. Singh et al. (2009) more recently evaluated the contribution of human CBS-catalyzed reactions to cystathionine and H_2S production by performing kinetic simulations at assumed physiological concentrations of serine, Cys, and Hcy (0.56, 0.10, and 0.01 mM, respectively). They predicted that the alternative reaction with Cys would contribute 56% of the cystathionine that is generated under normal conditions (see Table 2 or Fig. 1 of Singh et al. 2009), whereas that with serine would contribute only 44%. Based on this calculation, Cys desulfuration by CBS would be a major contributor to the transsulfuration pathway (Hcy disposal) and a significant producer of H_2S. It should be noted that this predicted contribution of the alternative reaction with Cys to cystathionine synthesis (56%) is much higher than the experimentally determined measure (5%) reported by Chen et al. (2004). Possible reasons for the different conclusions are that the assumed physiological substrate concentrations and the V_{max} or k_{cat} values used by the two groups differ markedly. Although Chen et al. (2004) used a high concentration of Hcy, this would not explain the

different results because the Singh et al. (2009) simulations predict that the Cys + Hcy reaction should contribute progressively less to total cystathionine synthesis as Hcy concentration is increased. The V_{max} determined by Chen et al. (2004) for the Cys + Hcy reaction was lower than that for the Ser + Hcy reaction (0.43 times the latter reaction); whereas the V_{max} determined by Singh et al. (2009) for the Cys + Hcy reaction was greater than that for the Ser + Hcy reaction (3.7 times the latter reaction). The direct competition experiments of Chen et al. (2004) yielded a 5–44% range for the proportion of cystathionine synthesized by the Cys + Hcy reaction, none of which was as high as the 56% predicted by Singh et al. (2009), clearly suggesting that the kinetic simulations of Singh et al. overestimated the contributions of the Cys + Hcy reaction in cystathionine synthesis. Although it is clear that Cys desulfuration by CBS occurs under physiological conditions, further work will be needed to define the quantitative contribution of Cys desulfuration by CBS to both cystathionine synthesis and Cys catabolism.

Cystathionine γ-lyase

The major function of CSE is cystathionine cleavage to yield Cys, α-ketobutyrate, and ammonia in the second step of the transsulfuration pathway (reaction 3). Side reactions with either Cys, Hcy, or both as substrate can lead to H_2S production. Chiku et al. (2009) ran simulations based on kinetic parameters of human CSE and predicted that the α,β elimination of Cys by CSE would be the major alternative reaction producing H_2S (reaction 4), accounting for about 70% of total H_2S production. The α, γ elimination of Hcy contributed about 29%. Catalysis of β- or γ-replacement reactions by CSE was negligible at physiological substrate concentrations. Thus, the α,β elimination of Cys is the major reaction catalyzed by human CSE that contributes to Cys desulfuration. The K_m of human CSE for H_2S generation from Cys is 1.7 mM (Chiku et al. 2009).

$$\text{Cystathionine} + H_2O \xrightarrow{\text{CSE}} \text{Cys} + \alpha - \text{ketobutyrate} + NH_3 \quad (3)$$

$$\text{Cys} + H_2O \xrightarrow{\text{CSE}} \text{serine} + H_2S \rightarrow \text{pyruvate} + NH_3(\text{human}) \quad (4)$$

In contrast to human CSE, rat CSE has been reported to catalyze desulfuration of the disulfide cystine, with a K_m of 0.07 mM for L-cystine (Cavallini et al. 1962a, b; Szczepkowski & Wood 1967; Uren et al. 1978; Yao et al. 1979). CSE is believed to catalyze a β-disulfide elimination reaction that results in production of pyruvate, ammonia, and thiocysteine (CySSH) (reaction 5). CySSH may then react with Cys or other thiols to form H_2S and cystine or the corresponding mixed disulfide. CySSH may also decompose to elemental sulfur and Cys, or the sulfur may be transferred from thiocysteine to acceptors such as sulfite or sulfinates. Although intracellular thiols in the cytosol are relatively reduced and cystine concentrations are low relative to Cys concentrations, measures of intracellular cystine concentrations suggest that these are high enough to compete favorably for binding to rat CSE. For example, Jones et al. (2004) reported intracellular cystine concentrations of 0.017–0.031 mM compared with 0.092–0.138 mM for Cys in cultured HT-29 cells.

$$\text{CySSCy} \rightarrow \text{Pyruvate} + NH_3 + \text{CySSH}[\rightarrow H_2S](\text{rat}) \quad (5)$$

Initial studies directed at elucidating contributions of various enzymes in Cys desulfuration suggest that both CSE and CBS play substantial roles in H_2S generation in rat liver and kidney homogenates (Stipanuk and Beck 1982). Propargylglycine, a suicide substrate of CSE, effectively inhibited CSE activity by >90% but had no effect on CBS activity in these tissue preparations. In support of CSE's contribution to cyst(e)ine desulfuration, propargylglycine inhibited H_2S production from Cys by >90% in liver and >74% in kidney preparations. However, these experiments with propargylglycine likely overestimated CSE contribution relative to CBS because the reaction mixtures did not contain S-adenosylmethionine or homocysteine, either of which would be needed for optimal levels of CBS-catalyzed Cys desulfuration. Addition of S-adenosylmethionine to enhance CBS activity resulted in increased H_2S production from Cys by about 50% in both liver and kidney, indicating a likely contribution of CBS to Cys desulfuration, especially under conditions in which the enzyme is allosterically activated by high S-adenosylmethionine levels (Stipanuk and Beck 1982). The influence of Hcy addition was not tested in these studies, but because we now know that condensation of Hcy with Cys is the major desulfuration reaction catalyzed by CBS (Chen et al. 2004), it seems clear that CBS contribution was underestimated in the Stipanuk and Beck (1982) work because no exogenous Hcy was added to the reaction mixtures.

Other studies in rat tissues support a substantial CSE contribution to Cys desulfuration reactions in rat tissues. Treatment of rat enterocytes, renal cortical tubules, or hepatocytes with propargylglycine to inhibit CSE resulted in inhibition of sulfur anion production or overall catabolism of Cys by about 50% (Drake et al. 1987; Stipanuk et al. 1990; Coloso and Stipanuk 1989). In addition, manipulation of the Cys to cystine ratio in the medium appeared to alter the relative catabolism of Cys by desulfuration versus oxidative, cysteinesulfinate-dependent pathways, with cystine formation favoring desulfuration (Coloso et al. 1990). Because rat liver CSE prefers cystine as substrate, this finding can be considered consistent with desulfuration of cyst(e)ine by CSE. Assuming intracellular Cys and cystine concentrations of 0.12 mM and 0.025 mM, respectively, human CSE has a reported K_m for Cys 14 times the intracellular Cys concentration, and rat CSE has a reported K_m for cystine three times the intracellular cystine concentration. These K_m values appear to be favorable for cyst(e)ine desulfuration by CSE when compared with the K_m of CBS for Cys, which is 54 times the intracellular Cys concentration.

More recently, Banerjee and colleagues (Singh et al. 2009) used kinetic simulations to assess relative contributions of human CBS and human CSE to H_2S production when the amounts of the two enzymes are equimolar, the substrate concentrations are near to assumed physiological levels (i.e., 0.56 mM serine, 0.10 mM Cys, 0.01 mM Hcy), and CBS is fully activated by its allosteric regulator S-adenosylmethionine. Because K_m values for substrates are high relative to their intracellular concentrations, the extent of various reactions will be largely determined by substrate availability. Assuming that Cys desulfuration only occurs due to reactions catalyzed by CBS and CSE, kinetic simulations reported by Singh et al. (2009) suggest that 75% of Cys desulfuration would be catalyzed by CBS and 25% by CSE. Their simulations also suggest that the relative contribution of CBS would decrease under conditions of severely elevated tissue Hcy levels. The possibility that these calculations are biased in favor of contributions from CBS should be considered based on the inclusion of a high concentration of S-adenosylmethionine to fully activate CBS and the possible bias of kinetic simulations toward the use of Cys versus serine as substrate for CBS, as discussed earlier.

Knockout murine models also provide insight into the roles of CBS and CSE in Cys desulfuration. Yang et al. (2008) found that endogenous H_2S levels in aorta and heart of homozygous $CSE^{-/-}$ mice were decreased by about 80%, whereas those in heterozygous

$CSE^{+/-}$ mice were decreased by about 50%. H_2S appears to be a physiological vasodilator and regulator of blood pressure. CSE-null mice developed age-dependent hypertension, with blood pressure of $CSE^{-/-}$ mice being more elevated than that of $CSE^{+/-}$ mice after 10 weeks of age. Consistent with other evidence that CSE is not the source of brain H_2S, endogenous H_2S levels in brains from $CSE^{-/-}$ mice were similar to those in wild-type mice. CBS knockout leads to severe accumulation of Hcy due to lack Hcy removal, and presumably, the β-replacement reactions of both serine and Cys would be blocked in these mice. In incubations of whole-brain homogenates with 10 mM L-Cys at pH 7.4, Shibuya et al. (2009) found that H_2S was produced at similar rates in brain homogenates from CBS knockout mice and wild-type mice. This production of H_2S by whole-brain homogenates was markedly increased by addition of 0.1 mM α-ketoglutarate. These findings, along with localization of aspartate aminotransferase and 3-mercaptopyruvate sulfur-transferase in brain, indicate that the transamination/sulfurtransferase pathway may also be active in rat tissues, although the relative contribution of this pathway at physiological Cys concentrations is not clear. Linden et al. (2008) reported the presence of both CBS and CSE messenger RNA (mRNA) in brain as well as liver, and H_2S production by tissue preparations incubated with 10 mM Cys and 2 mM PLP was blocked by inhibitors of PLP-dependent enzymes.

Cysteine transamination and 3-mercaptopyruvate sulfurtransferase

Although 3-mercaptopyruvate (the keto acid of Cys) is a good amino acceptor for transamination reactions, Cys is a poor amino donor for transamination reactions catalyzed by aspartate aminotransferase or other aminotransferases (reaction 6). For example, the K_m of aspartate aminotransferase for Cys is ~22 mM versus 0.06–0.5 mM for aspartate (Akagi 1982; Ubuka et al. 1978). On the other hand, 3-mercaptopyruvate is an excellent substrate for mercaptopyruvate sulfurtransferase (reaction 7). Although some transamination of cyst(e)ine to 3-mercaptopyruvate appears to occur in vivo based on the observed excretion of mercaptolactate in the urine of patients with 3-mercaptopyruvate sulfurtransferase deficiency (Crawhall et al. 1969; Niederwiesler et al. 1973; Hannestad et al. 1981; Sorbo 1987), transamination is not thought to be an important pathway for whole-body Cys catabolism or desulfuration under normal physiological conditions (Stipanuk and Beck 1982; Stipanuk 2004).

$$\text{Aminotransferases (Aspartate aminotransferase)}\ \text{Cys} + \text{keto acid} \rightarrow \text{mercaptopyruvate} + \text{amino acid} \quad (6)$$

$$\text{Mercaptopyruvate}\ (+\text{R}) \xrightarrow{\text{Mercaptopyruvate sulfurtransferase}} \text{pyruvate} + \text{HS}^- (\text{or RSH}) \quad (7)$$

At this point, there is insufficient evidence to definitively state the relative contributions of CBS, CSE, and transamination/mercaptopyruvate sulfurtransferase in Cys desulfhydration in any tissue, much less in the whole organism. Not only are the relative activities of the various enzymes unclear, expression and activity of these enzymes in various tissues and cell types are unclear. Furthermore, given the apparent different substrate specificities of rat and human orthologs of CSE, it is likely that the relative contributions will differ for different species. Nevertheless, existing data are sufficient to support important contributions of at least CBS and CSE in catalysis of H_2S production from cyst(e)ine.

Hydrogen sulfide (H_2S) as a signaling molecule and as a toxic compound

Hydrogen sulfide (H_2S) exists as a mixture of the gas and sulfide ions in aqueous solution, with about one third of H_2S remaining as the undissociated gas at pH 7.4. H_2S is known to be an extremely toxic substance for aerobic organisms, at least partially due to its ability to react with cytochrome c oxidase and other metal- or disulfide-containing proteins (Dorman et al. 2002). H_2S poisoning leads to inhibition of the mitochondrial electron transport chain and can lead to death through respiratory paralysis and pulmonary edema. Pathogenic roles of moderately elevated levels of metabolically produced H_2S in disease are yet uncertain but seem likely, given the recent identification of the ETHE1 gene, which is defective in individuals with the invariably fatal disorder known as ethylmalonic encephalopathy, as a sulfur dioxygenase. The ETHE1 gene encodes a mitochondrial sulfur dioxygenase that is a critical component of the first step of H_2S oxidation (Tiranti et al. 2009). Significant inhibition of cytochrome c oxidase has been shown to occur at 0.32 μM H_2S in studies with human colonocytes (Goubern et al. 2007).

More recently, H_2S has been shown to be a physiologically important signaling molecule in eukaryotes, being of particular importance in the brain, heart, and smooth muscle (Wang 2002; Lloyd 2006; Mancardi et al. 2009). In the cardiovascular system, H_2S relaxes vascular smooth muscle, inhibits platelet aggregation, and reduces the force output of the left ventricle of the heart (Wang et al. 2009; Wang 2002). In the gastrointestinal system, H_2S relaxes ileal smooth muscle, increases colonic secretion, and reduces gastric injury caused by nonsteroidal anti-inflammatory drugs (Fiorucci et al. 2006). In the central nervous system (CNS), H_2S increases the sensitivity of *N*-methyl-D-aspartate (NMDA) receptors to glutamate in hippocampal neurons to enhance synaptic transmission (Qu et al. 2008; Dominy and Stipanuk 2004). How H_2S signals is unclear, but it is known that H_2S sulfhydrates a wide range of proteins [i.e., the formation of Cys persulfenate residues (-CySSH) at targeted Cys sites] and that sulfhydration of target proteins can alter their biological activity (Jaffrey et al. 2001; Mustafa et al. 2009).

Oxidation of sulfur/sulfide to sulfite/sulfate

Sulfide (HS^- or H_2S) is oxidized to sulfate in mammalian mitochondria. Koj et al. (1967) and Szczepkowski et al. (1961) performed elegant experiments in the 1960s to demonstrate that thiosulfate plays a central role in sulfide oxidation by animal tissues. More recent work elucidated the details of thiosulfate formation from sulfide.

The first reaction (reaction 8) is carried out by a sequence of three mitochondrial enzymes: (1) sulfide: quinone oxidoreductase (SQR), which oxidizes sulfide to sulfane sulfur (bound to an SQR cysteinyl residue), transferring two electrons to the ubiquinone pool; (2) a sulfur dioxygenase, which oxidizes one SQR-bound per-sulfide sulfur to sulfite; and (3) a sulfur transferase (rhodanese) that transfers the second SQR-bound persulfide sulfur to sulfite to form thiosulfate (SSO_3^{2-} or $H_2S_2O_3$) (Hildebrandt and Grieshaber 2008). SQR is associated with the inner mitochondrial membrane, and both the sulfur dioxygenase and sulfur transferase are mitochondrial matrix enzymes. The K_m value of rat SQR for H_2S has been estimated at 2.9 uM (Hildebrandt and Grieshaber 2008).

$$\text{SQR/sulfur dioxygenase/sulfur transferase } 2\,H_2S + 1\,O_2 + 1\,H_2O \rightarrow H_2S_2O_3 + 4e^- + 4H^+ \quad (8)$$

The inner (oxidized) sulfur of thiosulfate is converted to sulfite (SO_3^{2-} or H_2SO_3), presumably by either a glutathione (GSH)-dependent thiosulfate reductase (reaction 9) or a thiosulfate sulfurtransferase (reaction 10). Involvement of the glutathione-dependent thiosulfate reductase in sulfite production is consistent with the glutathione dependence of

sulfate production by hepatocytes incubated with 10 mM Cys reported by Huang et al, (1998). Glutathione depletion markedly reduced sulfate production from Cys and resulted in accumulation of thiosulfate. When thiosulfate was used as a substrate, glutathione depletion similarly blocked its conversion to sulfate. On the other hand, thiosulfate sulfurtranserase (rhodanese) has a much higher K_m for thiosulfate than for sulfite, suggesting conversion of thiosulfate to sulfite by this enzyme would be limited. Use of thiosulfate to detoxify cyanide and similar transfers of sulfane sulfur from thiosulfate to other acceptors, however, would also release the oxidized sulfur of thiosulfate as sulfite.

$$\xrightarrow{\text{Thiosulfate reductase}}$$
$$H_2S_2O_3 + 2\,GSH \rightarrow H_2SO_3 + H_2S + GSSG \quad (9)$$

or

$$\xrightarrow{\text{Thiosulfate sulfurtransferase}}$$
$$H_2S_2O_3 + RSH(\text{or}\,CN^-) \rightarrow H_2SO_3 + RSSH(\text{or}\,SCN^-) \quad (10)$$

The final step of sulfide oxidation (reaction 11) is accomplished by the well-characterized mitochondrial enzyme sulfite oxidase, which converts sulfite to sulfate (SO_4^{2-}) (Feng et al. 2007). Sulfate can then either be excreted in the urine or activated to 3′-phosphoadenosine-5′-phosphosulfate, which, in turn, serves as a sulfate donor for sulfate ester formation. Sulfite oxidase is a heme-containing enzyme that is a member of the cytochrome b5 family and contains a molybdopterin cofactor. Along with oxidation of sulfite to sulfate, electrons are transferred, one at a time, from the molybdenum cofactor to the b5 heme of sulfite oxidase and then to the electron carrier cytochrome c. Inborn errors of the sulfite oxidase gene or errors affecting molybdenum cofactor synthesis have confirmed the critical role of this enzyme in the oxidation of sulfite to sulfate, and both thiosulfate and *S*-sulfocysteine ($CySSO_3^-$), which is formed nonenzymatically by reaction of Cys and sulfite, are elevated in the urine of patients with these inborn errors of metabolism (Crawhall 1985; Johnson and Rajagopalan 1995; Rupar et al. 1996; Touati et al. 2000).

$$\xrightarrow{\text{Sulfite oxidase}}$$
$$H_2SO_3 + H_2O \rightarrow H_2SO_4 + 2e^- + 2H^+ \quad (11)$$

The critical role of mitochondrial oxidation of sulfide is emphasized by recent identification of the ETHE1 gene as the mitochondrial sulfur dioxygenase (Tiranti et al. 2009). Ethylmalonic encephalopathy is an autosomal recessive and fatal disorder due to mutations in the ETHE1 gene. Ethylmalonic encephalopathy is characterized by encephalopathy, microangiopathy, chronic diarrhea, defective cytochrome c oxidase in muscle and brain, high concentrations of C4 and C5 acylcarnitines in blood (perhaps due to inhibition of short-chain acyl-CoA dehydrogenase by H_2S), and high excretion of ethylmalonic acid in urine (Burlina et al. 1991; Garcia-Silva et al. 1994). Patients with mutations in the ETHE1, or mitochondrial sulfur dioxygenase, gene also excrete large amounts of thiosulfate in the urine (possibly due to fixation of the accumulated H_2S to sulfite produced by the oxidative pathways of Cys catabolism, a reaction that may be catalyzed by thiosulfate sulfurtransferase). Similarly, ETHE1$^{-/-}$ mice had elevated tissue concentrations of sulfide and thiosulfate, low levels of cytochrome c oxidase in muscle and brain, elevated levels of lactate and C4 and C5 acylcarnitines in plasma, elevated ethylmalonic acid and thiosulfate in

urine, lower sulfate abundance in urine, and undetectable sulfite levels in urine (Tiranti et al. 2009).

Cys is oxidatively catabolized by cysteinesulfinate-dependent pathways, yielding either hypotaurine/taurine or sulfite/sulfate

In addition to the several possible routes of Cys desulfuration, mammals catabolize Cys by cysteinesulfinate-dependent pathways in which the sulfur is partially oxidized in the first step. Thus, in cysteinesulfinate-dependent pathways, sulfur is oxidized to a sulfinate prior to cleavage of the sulfur from the carbon chain or conversion to hypotaurine, as shown in Fig. 3. This initial oxidation of the Cys thiol group to a sulfinic acid group is carried out by cysteine dioxygenase (CDO), which adds molecular oxygen to the sulfur of Cys, forming cysteinesulfinate [also called cysteinesulfinic acid (CSA) or 3-sulfinoalanine]. The cysteinesulfinate formed by action of CDO on Cys may be decarboxylated to hypotaurine by cysteinesulfinate decarboxylase, and the hypotaurine may be subsequently oxidized to taurine. Alternatively, cysteinesulfinate may be transaminated (with α-ketoglutarate) in a unidirectional reaction to form the enzyme-bound keto acid β-sulfinylpyruvate, which spontaneously dissociates to gives rise to pyruvate and sulfur dioxide (SO_2,) which is hydrated to sulfite in vivo. The sulfite in turn is readily oxidized to sulfate by sulfite oxidase, as described earlier.

Overall flux of Cys through both of these cysteinesulfinate-dependent pathways is mainly regulated at the first step, the oxidation of Cys to cysteinesulfinate. High levels of CDO are expressed in liver, and substantial amounts are also present in kidney, lung, pancreas, and adipose tissue of mice and rats (see Fig. 4). Although CDO is widespread in liver and adipose tissue due to its expression by hepatocytes and adipocytes, it is found only in specialized cells in some other tissues, including goblet cells in the intestine, exocrine cells in the pancreas, and tubular epithelial cells in the kidney. As shown in Fig. 4, tissues that express high levels of CDO also have high levels of cysteinesulfinate decarboxylase (CSD) and thus have a complete pathway for synthesis of hypotaurine/taurine from Cys. Additionally, liver, kidney, and pancreas also have high levels of both CSE and CBS and thus a high capacity for Cys desulfuration.

CDO is one of the most highly regulated metabolic enzymes known to respond to diet: hepatic or adipocyte CDO concentration increases by up to 45-fold, and catalytic efficiency of the enzyme increases by up to 10-fold with increases in Cys availability (Fig. 5). For example, hepatic CDO activity is very low in animals fed a low-protein diet (e.g., 100 g casein per kg diet) but increases dramatically when the protein level in the diet is increased to 200 g/kg (near the requirement) and even more when it is increased to 400 g/kg (excess of the requirement), with the new steady-state levels of CDO activity being reached within hours of the diet change (Bella et al. 1999a,b;Stipanuk et al. 2002;Lee et al. 2004).

Regulation of CDO concentration and activity state

Regulation of CDO concentration, at least in hepatocytes and adipocytes, in response to Cys levels involves a block in the ubiquitination of CDO that in turn diminishes its rate of proteasomal degradation, allowing it to accumulate in the cell (Dominy et al. 2006a, b; Lee et al. 2004; Stipanuk et al. 2004a; Stipanuk and Ueki unpublished observations). Inhibitors of the 26S proteasome (e.g., proteasome inhibitor 1 and lactacystin) blocked CDO degradation in Cys-deficient cells but had little or no effect on CDO concentration in hepatocytes cultured with excess Cys in which CDO concentration was already elevated. Furthermore, high-molecular-mass CDO-ubiquitin conjugates that reacted with both anti-ubiquitin and anti-CDO were observed in cells cultured in Cys-deficient medium, whether or

not proteasome inhibitor was present; however, these CDO-ubiquitin conjugates were not observed or were much lower in cells cultured in Cys-supplemented medium with or without proteasome inhibitor.

To confirm that the diet-induced changes in CDO half-life observed in whole animals are due to regulation of CDO ubiquitination and degradation by the 26S proteasome, we explored CDO ubiquitination in response to diet in intact rats (Dominy et al. 2006a, b). In the rat, as anticipated from the studies with cultured cells, a Cys-supplemented diet (100 g casein +8.1 gL-Cys/kg) or a high-protein diet (400 g casein/kg) led to markedly higher hepatic CDO concentrations than were observed in rats fed a low-protein diet (100 g casein/kg). Inhibition of the proteasome in vivo with proteasome inhibitor 1 dramatically stabilized CDO in the liver under dietary conditions that normally favor its degradation (i.e., low-protein diet), and ubiquitinated CDO intermediates accumulated in the liver of these rats. Metabolic analyses showed that proteasome inhibitor 1 had a significant effect on sulfoxidation flux (accumulation of hypotaurine and, to a much lesser extent, taurine) secondary to CDO stabilization. Because proteasome inhibitor 1 had no significant effect on the intracellular Cys pool, the increased sulfoxidation flux could be attributed to increased enzyme (CDO) activity, demonstrating the physiological relevance of an increase in CDO activity, apart from increased Cys substrate concentration, in determining Cys flux to taurine.

The CDO activity state is regulated by Cys concentration via a substrate-turnover-dependent formation of a thioether cross-link between the sulfur of residue Cys93 and the aromatic side chain of residue Tyr157. The cross-linking of these two residues gives rise to an active-site protein-derived cofactor that enhances CDO's catalytic efficiency. Elucidation of this substrate-turnover-dependent regulation of CDO activity began with the discovery of this rare intramolecular cross-link between active-site residues Cys93 and Tyr157 in the X-ray crystal structure of CDO (Simmons et al. 2006b; McCoy et al. 2006; Ye et al. 2007). This discovery led us to postulate that cross-link formation was responsible for the commonly observed migration of CDO as two bands on sodium dodecyl sulfate polyacryl-amide gel electrophoresis (SDS-PAGE), and we verified that formation of this cross-link resulted in slightly faster migration of CDO on SDS-PAGE, with an apparent molecular mass of 22.5 kDa instead of the 23-kDa mass of immature CDO without the Cys-Tyr cross-link (Dominy et al. 2008). Confirmation of the correspondence of the presence of the intramolecular Cys-Tyr cross-link with a shift in CDO's electrophoretic migration provided an easy means to "assay" thioether cross-link formation.

Hence, we used this difference in electrophoretic migration to further evaluate the exact requirements for intramolecular thioether synthesis and contribution of thioether cofactor to the catalytic activity (Dominy et al. 2008). Some catalytic activity in the immature CDO was necessary for thioether cross-link formation; inactive mutant forms of CDO (e.g., His86Ala, leading to loss of the His that serves as a metal ligand in the active site) did not form any cross-link, and mutant forms of CDO with low activity (e.g., Arg60Ala, which has a markedly decreased affinity for Cys, probably due to the loss of the hydrogen bonding partner for the carboxylate of the substrate) formed the cross-link more slowly. Mutations of nonessential residues (e.g., Ser153Ala; Cys164Ala) had little effect. As with other amino-acid cofactor-containing enzymes, formation of the CDO Cys-Tyr cross-link required a transition metal cofactor [ferrous iron (Fe^{2+})] and oxygen (O_2)], but unlike other amino-acid cofactor-containing enzymes, biogenesis of the CDO cross-link did not occur immediately upon exposure to metal and O_2,, was not essential for basal catalytic activity, and was strictly dependent upon the presence of its specific substrate Cys. Substrate and substrate turnover were essential for CDO cofactor formation both in assays of purified CDO and of CDO in intact cells. Cofactor formation was much slower than the rates reported for other

amino acid cofactor-containing enzymes and, in fact, took hundreds of catalytic turnover cycles to occur. Nevertheless, the CDO Cys-Tyr moiety seems to serve a cofactor function in CDO. Although CDO possessed appreciable catalytic activity in the absence of the Cys-Tyr cofactor, cofactor formation increased CDO catalytic efficiency by ~10-fold (e.g., 3,200 $M^{-1}s^{-1}$ for wild-type CDO versus 220 $M^{-1}s^{-1}$ for Cys93Ser mutant CDO, which lacks the Cys residue needed for formation of the Cys-Tyr cofactor). Additionally, Cys-Tyr cofactor formation increased the catalytic lifetime of CDO, with the $t_{1/2}$ for catalytic activity measured in vitro being approximately double that of the immature enzyme. A transition of hepatic isoforms of CDO from mainly immature form in liver of rats fed a low-protein or cyst-(e)ine diet to mainly mature cross-link-containing form in liver of rats fed a diet containing a moderate to high level of cyst(e)ine or protein indicated that the presence of the two isoforms is physiologically relevant, and several studies from our laboratory have demonstrated the relationship of Cys concentration to mature CDO isoform formation in vivo (Stipanuk et al. 2004a, b; Dominy et al. 2006a, b; Ueki and Stipanuk 2009).

We also tested the possibility that Cys-Tyr thioether cofactor formation might alter the susceptibility of CDO to ubiquitination and proteasomal degradation by expressing wild-type and Cys93Ser mutant CDO in HepG2/C3A cells using a Tet-off system and Cys-supplemented medium. Following expression, both the wild-type CDO (converted to the mature form as a consequence of being cultured in Cys-supplemented medium) and the Cys93Ser mutant CDO (which could not be converted to the mature form due to loss of the Cys residue involved in thioether cross-link formation) appeared to be degraded at similar rates when cells were placed in Cys-deficient medium. Maintenance of a high rate of turnover of mature CDO is consistent with the cell retaining its ability to rapidly degrade CDO when Cys supply abruptly decreases. Nevertheless, synergistic coupling of the increase in CDO concentration that can be achieved by regulation of ubiquitination/degradation of CDO with the increase in catalytic efficiency that can be achieved by Cys-Tyr cofactor formation, plus the increased catalytic life time of the mature enzyme that results from Cys-Tyr cofactor formation, provides the cell with the capacity for a many-fold increase in CDO activity in response to a high intake of protein or sulfur amino acids.

Overall, our results indicate a remarkable responsiveness of mammals to changes in sulfur amino-acid availability—the ability to decrease CDO activity and conserve Cys when Cys is scarce and to rapidly increase CDO activity and catabolize Cys to prevent cytotoxicity when Cys supply is abundant. Changes in CDO activity state can be observed within minutes, and changes in CDO concentration can be observed within a few hours of changes in Cys availability, both in cells in culture and in liver of rats switched from a diet containing a low, inadequate level of sulfur-containing amino acids to one containing a high, excess level. Clearly, CDO is remarkable among metabolic enzymes in the degree to which its activity can be regulated in response to its substrate, suggesting that tight regulation of Cys levels is a critical physiological function.

CDO substrate specificity and the relation of cellular Cys levels to CDO activity

In contrast to the rather broad substrate specificity of the enzymes that catalyze Cys desulfuration, substrate specificity of CDO is very high (Yamaguchi et al. 1978; Dominy et al. 2006c). Observation of an Fe^{2+}-bound persulfenate intermediate in the active site of CDO that was fully buried, with no room to spare for additional atoms and with the full hydrogen-bonding potential of both the α-amino and α-carboxylate satisfied, provided a structural explanation for the high specificity of CDO for Cys as its only substrate (Simmons et al. 2008).

Cys concentration in most tissues is maintained between 0.03 and 0.2 μmol per gram, whereas kidney Cys levels are closer to 1.0 μmol per gram (Finkelstein et al. 1982; Lee et al.

2004). The K_m of CDO for Cys is on the order of 0.5–1.5 mM; the exact K_m of the enzyme is difficult to determine because of the marked inhibition of CDO in our in vitro assays by high concentrations of Cys (Dominy et al. 2006c; Simmons et al. 2006a). Regardless, CDO should respond to changes in tissue Cys concentrations by parallel changes in enzyme substrate saturation, resulting in changes in Cys catabolism (Lee et al. 2004; Stipanuk et al. 2002). In addition to a kinetic response to changes in Cys concentration, the catalytic turnover of Cys by CDO leads to conversion of CDO to its mature (Cys-Tyr cofactor-containing) form that has a markedly increased catalytic efficiency (k_{cat}/K_m) and longer catalytic half-life, as discussed earlier.

Cellular Cys concentration also appears to be the key metabolic regulator of CDO concentration via regulation of CDO degradation. Although dietary Met is as effective as dietary Cys in the upregulation of CDO, the effectiveness of Met depends upon a functioning transsulfuration pathway (Cresenzi et al. 2003; Kwon and Stipanuk 2001). Neither precursors nor products of Cys affect CDO concentration or activity, and no tested structural analogs of Cys or alternative thiols, with the exception of cysteamine in vitro, have any effect (Dominy et al. 2006a, b; Stipanuk et al. 2006, 2004a; Ueki and Stipanuk 2009; Kwon and Stipanuk 2001; Stipanuk et al. 2004a). Despite an effect of cysteamine on CDO stability in vitro, dramatic elevations of tissue cysteamine concentrations in intact rats fed a diet containing 7.2 g/kg L-cysteamine had no effect on CDO concentration, clearly demonstrating that physiological concentrations of cysteamine would be insufficient to regulate CDO turnover in vivo (Dominy et al. 2006a, b). CDO concentration in vivo appears to respond specifically to changes in Cys availability (Cresenzi et al. 2003).

The robust regulation of CDO in mammalian tissues by intracellular Cys levels and the direct correspondence of Cys levels with CDO levels in liver and adipose tissue suggest that CDO plays a role in controlling steady-state intracellular Cys levels. Direct experimental evidence also supports this claim. Expression of wild-type CDO, but not catalytically inactive His86Ala CDO, reduced intra-cellular Cys levels in HepG2/C3A cells, which do not express endogenous CDO protein, incubated in physiologically relevant concentrations of Cys (Dominy et al. 2007). Consistent with the dependence of cellular glutathione levels on cellular Cys levels, wild-type CDO expression also decreased the glutathione pool and potentiated cadmium chloride ($CdCl_2$) toxicity. In addition, in studies with 3T3-L1 cells, Cys supplementation of the culture medium led to a marked elevation of the intracellular Cys level in preadipocytes that express little or no CDO, whereas differentiating and differentiated adipocytes that do express CDO were able to maintain intracellular Cys levels that were not significantly greater ($P>0.05$) than those of cells cultured in basal medium (Ueki and Stipanuk 2009).

Further metabolism of cysteinesulfinate

The cysteinesulfinate formed by CDO action is further metabolized by CSD to hypotaurine or by aspartate (cysteinesulfinate) aminotransferase to pyruvate and sulfite. The product, hypotaurine (2-aminoethanesulfinate), is subsequently oxidized to taurine (2-aminoethanesulfonate), but whether this process is enzymatic or nonenzymatic remains to be determined.

Partitioning cysteinesulfinate between these two pathways is likely dependent upon the relative levels of CSD and aspartate aminotransferase in a given tissue in a given species as well as their affinities for the substrate. The K_m of CSD for cysteinesulfinate is ~0.04–0.17 mM (Guion-Rain et al. 1975; Oertel et al. 1981) versus ~3–25 mM for aspartate aminotransferase (Recasens et al. 1980; Yagi et al. 1979), suggesting that conversion of cysteinesulfinate to hypotaurine would be favored under physiological conditions if both enzymes are present. At the whole-body level, CDO partitioning is probably most dependent

on the levels of these enzymes within tissues that have high levels of CDO and hence can generate cysteinesulfinate, because most cysteinesulfinate is likely further metabolized within the tissue in which it is generated. As shown in Fig. 4, CSD expression is high in tissues that also express high levels of CDO.

When cysteinesulfinate itself was used as substrate, isolated hepatocytes, renal cortical tubules, enterocytes, perfused isolated hindquarter, and intact rats all exhibited a high capacity for cysteinesulfinate metabolism to carbon dioxide or sulfate, with rates of cysteinesulfinate oxidation far exceeding those for Cys catabolism to (Bagley and Stipanuk 1995; Stipanuk et al. 1990; Coloso and Stipanuk 1989; Ensunsa et al. 1993; Stipanuk and Rotter 1984). This large capacity of all tested tissues for cysteinesulfinate oxidation is largely due to the widespread distribution of aspartate aminotransferase, which can catalyze the transamination of cysteinesulfinate with α-ketoglutarate. Of the tissues tested, only hepatocytes had a high capacity for taurine synthesis from cysteinesulfinate, indicative of the presence of high levels of CSD.

In rodents, high levels of CDO are expressed in liver and adipose tissue, and both of these tissues also express high levels of CSD and have active taurine biosynthetic pathways (Stipanuk et al. 2002; Ueki and Stipanuk 2009). Arteriovenous difference measurements in rats indicated that net taurine synthesis is high in the liver but minimal in kidney (Garcia and Stipanuk 1992). Likewise, taurine synthesis from Cys has been demonstrated in isolated rat hepatocytes (Bagley and Stipanuk 1995; Bella et al. 1996; Bella and Stipanuk 1995), murine 3T3-L1 adipocytes (Ueki and Stipanuk 2009), and cultured rat astroglial cells (Brand et al. 1998), but not in rat enterocytes (Coloso and Stipanuk 1989) or rat renal cortical tubules (Stipanuk et al. 1990).

We made estimates of [^{35}S]cysteinesulfinate partitioning between taurine and sulfate in hepatocytes isolated from rats fed a semipurified diet with 100 g casein per kg without or with supplemental Cys or Met (Bagley and Stipanuk 1995). Hepatocytes isolated from these mice had CDO activities that ranged from 0.16 to 2.02 nmol/min^{-1}/mg protein^{-1} (a 13-fold range), whereas CSD activity in the hepatocytes remained similar. When these hepatocytes, which would presumably have different cysteinesulfinate pool sizes due to their different levels of CDO, were incubated with 0.2 mM [^{35}S]cysteinesulfinate, the fraction of [^{35}S]cysteinesulfinate converted to [^{35}S]taurine was 62–69% of the total [^{35}S]cysteinesulfinate catabolism to [^{35}S] taurine plus [^{35}S]sulfate. This predominant partitioning of cysteinesulfinate to taurine is similar to that observed for [^{35}S]Cys metabolism in hepatocytes (from rats fed a diet supplemented with 10 g Met/kg) that had the highest level of CDO and hence the most Cys catabolism by CDO-dependent pathways versus desulfuration pathways. This partitioning in hepatocytes is very close to the estimate made in intact animals using intraperitoneally administered cysteinesulfinate labeled with carbon 14 (^{14}C) in the 1 and 3 positions (Stipanuk and Rotter 1984). The estimated flux to taurine as a percentage of total cysteinesulfinate oxidation was 69–71%, regardless of whether the prior or test diet contained excess Cys. These results, together, suggest that partitioning of cysteinesulfinate to taurine versus sulfate at the whole-body level is largely determined by flux in tissues that contain high levels of CDO and is in the range of 66% to taurine and 34% to sulfate. Thus, a general estimate of total Cys flux through the oxidative cysteinesulfinate-dependent pathways may be obtained by dividing the taurine (or hypotaurine + taurine) production by 0.66.

Relative contributions of desulfuration vs. oxidative reactions in Cys catabolism depends on the dietary load of sulfur-containing amino acids (i.e., tissue Cys levels)

Using the estimate of 66% for the fraction of cysteinesulfinate-dependent Cys metabolism that yields taurine as the end product, we can further estimate the extent of Cys catabolism to taurine + sulfate by cysteinesulfinate-dependent pathways versus that of Cys catabolism to sulfate by cysteinesulfinate-independent (desulfuration) pathways. Figure 6 shows our estimates for cysteinesulfinate-dependent and cysteinesulfinate-independent catabolism of Cys by freshly isolated hepatocytes obtained from rats that had been fed diets with different levels of SAAs and then incubated in vitro with 0.2 mM Cys (Bagley and Stipanuk 1995). Rats used as a source of hepatocytes were adapted to diets that contained (per kg) 100 g casein (basal or low SAA diet), 100 g casein +3 g Met (adequate SAA diet), or 100 g casein +10 g Met (excess SAA diet). Hepatocytes isolated from the Met-supplemented rats had CDO activities 2.8-times (adequate SAA diet) or 12.6-times (excess SAA diet) those of rats fed the basal low-protein diet. Hepatocytes with low levels of CDO (basal group) had low levels of overall Cys catabolism, and only 33% of this catabolism occurred by the cysteinesulfinate-dependent pathways. Thus, when SAA intake is low, Cys metabolism by cysteinesulfinate-dependent pathways is also low, and catabolism occurs predominantly via the desulfuration pathways (67% of total). In hepatocytes from rats fed the SAA-adequate diet, the overall rate of Cys catabolism was 2.2-times that of the basal group, and flux through the cysteinesulfinate-dependent pathways increased markedly, whereas that through the desulfuration pathways remained about the same. Thus, in hepatocytes from rats fed an adequate diet, about 63% of Cys catabolism occurred via cysteinesulfinate-dependent pathways, with the desulfuration pathways contributing 37%. In hepatocytes from rats fed diets with excess SAA, both overall catabolic flux and the proportion of Cys catabolism via cysteinesulfinate-dependent pathways increased markedly. In these hepatocytes, total Cys catabolism was 4.1-times basal, and cysteinesulfinate-dependent catabolism accounted for 93% of total Cys catabolism. Under conditions of high levels of CDO, as present in liver of animals fed diets high in protein or SAAs, Cys catabolism occurs predominantly by the cysteinesulfinate-dependent pathways, and flux through the desulfuration pathways is held at the basal level.

Similarly, estimates for the whole animal based on excretion of sulfur metabolites (taurine and sulfate) in urine of rats fed either a basal low SAA diet or an adequate SAA diet are shown in Fig. 7 (Bella et al. 1999a). Hepatic CDO activity in rats fed the Met-supplemented adequate diet was 34 times that of the rats fed the low-protein diet. Thus, CDO level and Cys load were both increased in rats fed the SAA-adequate diet versus the SAA-deficient diet. Urinary taurine excretion increased from 3 mmol/24 h in rats on the basal diet to 279 mmol/24 h in rats fed the Met-supplemented diet, and sulfate excretion increased from 54 mmol/24 h to 320 mmol/24 h. Estimated percentage flux of Cys through cysteinesulfinate-dependent pathways is 8.0% [100(3/0.66)/57] for rats fed the basal SAA-deficient diet and 70.6% [100(279/0.66)/599] for rats fed the Met-supplemented SAA-adequate diet. This estimate of 71% for the proportion of Cys catabolized by cysteinesulfinate-dependent pathways in intact rats is similar to the estimate of 63% for hepatocytes isolated from rats fed an adequate diet and incubated with 0.2 mM Cys in vitro. Thus, it seems reasonable to assume that about two thirds of Cys catabolism occurs via cysteinesulfinate-dependent pathways in rats consuming standard diets.

Does the regulation of cellular Cys levels by CDO serve a fundamental biological role not met by the desulfuration pathways?

CDO is unique among enzymes of amino-acid metabolism in terms of its capacity to respond over an activity range of more than 100-fold to changes in substrate availability. CDO is synthesized whether it is needed or not but degraded when cysteine levels are low. This allows its concentration to change quickly in response to an increase in cysteine availability. It is highly specific for L-cysteine as substrate, and it is also highly regulated in response to changes in Cys concentration. Although both the desulfuration and the CDO-dependent oxidative pathways lead to production of inorganic sulfur that is further oxidized to sulfate, only the CDO-dependent pathway leads to taurine as an end product.

In all Cys catabolic pathways except that for taurine formation, the carbon chain of Cys is released as pyruvate, the sulfur is released as inorganic sulfur and ultimately converted to sulfate, and the amino group is released as ammonia or transferred to a keto-acid acceptor. When taurine is the end product, only the carboxyl carbon of Cys is released, and the other two carbons as well as the nitrogen and sulfur atoms remain in the end product. Thus, distribution of Cys among its catabolic pathways potentially affects utilization of amino-acid carbon chains for energy, net production of acid or fixed anions (sulfate), and synthesis of essential metabolites (inorganic sulfur and taurine). Although taurine and sulfate are end products of cellular Cys catabolism, both compounds participate in conjugation reactions and have a variety of essential physiological functions prior to their ultimate excretion. Production and excretion of taurine as an end product results in loss of the cysteine carbon chain (i.e., pyruvate) that could serve as a metabolic fuel, suggesting that taurine may have critical functions.

We can speculate about possible implications of this robust regulation of CDO. Tight regulation of CDO activity may facilitate maintenance of relatively low steady-state Cys concentrations so that Cys is available for essential functions (e.g., protein, CoA, glutathione, and H_2S synthesis) while also preventing high Cys concentrations, which could be toxic. There is some evidence for direct toxic effects of Cys leading to damage to cortical neurons (Sandberg et al. 1991), permanent retinal damage (Pedersen and Karlsen 1980), brain atrophy (Karlsen et al. 1981), and hyperactivity (Mathisen et al. 1996). It is possible that the desulfuration pathways alone do not provide sufficient capacity for catabolism of large amounts of Cys. A low capacity to oxidize Cys to sulfate has been observed in some individuals with liver diseases or rheumatoid arthritis (Bradley et al. 1994; Davies et al. 1995) and inconsistently in individuals with some chronic neurological diseases (Heafield et al. 1990; Perry et al. 1991). These individuals had elevated plasma ratios of Cys to sulfate due to lower sulfate and higher Cys concentrations, excreted a smaller percentage of a dose of acetaminophen as the sulfate versus the glucuronide conjugate, or had a lower sulfate concentration of synovial fluid, all of which are consistent with impaired Cys oxidation at the CDO level.

Another possibility is that CDO is induced when Cys levels increase to siphon Cys to less toxic metabolites than those produced by the Cys desulfuration pathways. It is known that H_2S or sulfane sulfur can be toxic, whereas taurine and sulfate are relatively innocuous end products (Dorman et al. 2002). It is also possible that high Cys concentrations would interfere with the regulated production of H_2S as a signaling molecule.

A third possibility is that hypotaurine/taurine synthesis has some type of protective effect in animals consuming diets high in SAAs. Although taurine is considered an essential metabolite or dietary component (Stipanuk 1986, 2004), Cys metabolism pathways generally do not seem to be regulated to conserve taurine synthesis when Cys availability is

low. We observed induction of CSD activity in tissues of animals depleted of taurine (Rentschler et al. 1986; Ueki and Stipanuk, unpublished observations), suggesting some attempt at regulation, which was, however, relatively unsuccessful because CDO is typically low under conditions in which severe taurine deficiency is observed.

Finally, it is possible that excess Cys catabolism by the desulfuration pathways would somehow interfere with normal Met/Hcy metabolism. The CDO-, or cysteinesulfinate-, dependent pathways provide routes for Cys catabolism that, unlike the desulfuration pathways, can be regulated separately from the transsulfuration pathway for Hcy/Met catabolism. Thus, Cys flux through the desulfuration pathways is constitutive and, perhaps, unregulated when Met levels are high due to increases in CBS activity/concentration when S-adenosylmethionine levels are elevated. Cys levels are also likely to increase when Met availability is high as a consequence of the transsulfuration pathway. Because the transsulfuration enzymes are involved in catabolism of Hcy/Met as well as in both producing and catabolizing Cys, the advantage of a pathway specifically for Cys catabolism that can be separately regulated is obvious in terms of control of tissue Cys concentrations.

Other ways to ask this question is: What would happen if CDO was absent? Would Cys accumulate and exert toxic effects? Would Cys flux through desulfuration pathways be increased with excess H_2S being produced? Would the lack of taurine biosynthesis have harmful effects? Would the lack of sulfate production by the cysteinesulfinate-dependent route result in a lack of sulfate, even though sulfide produced by the desulfuration pathways can be oxidized to sulfate? We are conducting studies to begin to answer these questions using a CDO knockout mouse model our laboratory has recently generated. Null mice have a high rate of neonatal mortality, but many null mice survive into adulthood. The possibilities for metabolic studies and dietary interventions with these mice are abundant, and we anticipate that the CDO knockout mouse model will provide answers to many of these questions.

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Abbreviations

Met	L-methionine
Cys	L-cysteine
Hcy	L-homocysteine
SAA	Sulfur-containing amino acids
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
CDO	Cysteine dioxygenase
CSD	Cysteinesulfinate decarboxylase

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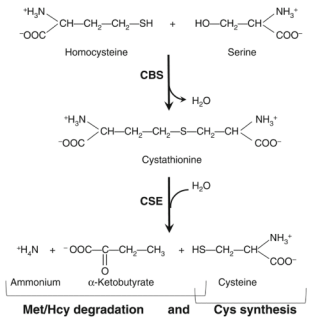

Fig. 1.
Transsulfuration pathway for homocysteine degradation and cysteine synthesis

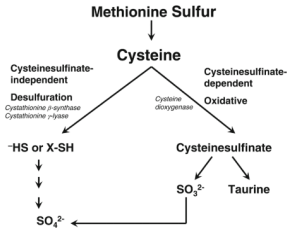

Fig. 2.
Overview of cysteine sulfur metabolism

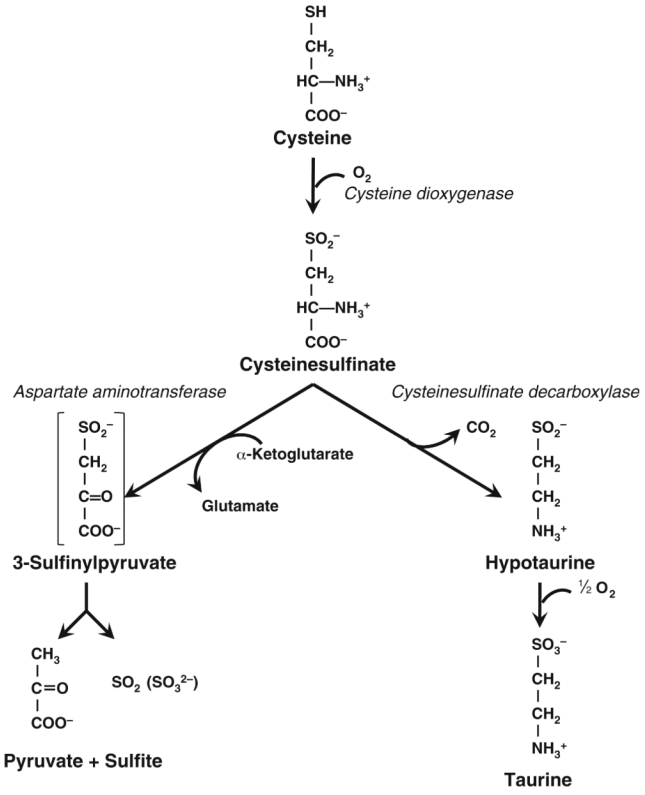

Fig. 3.
The cysteinesulfinate-dependent pathway of cysteine metabolism

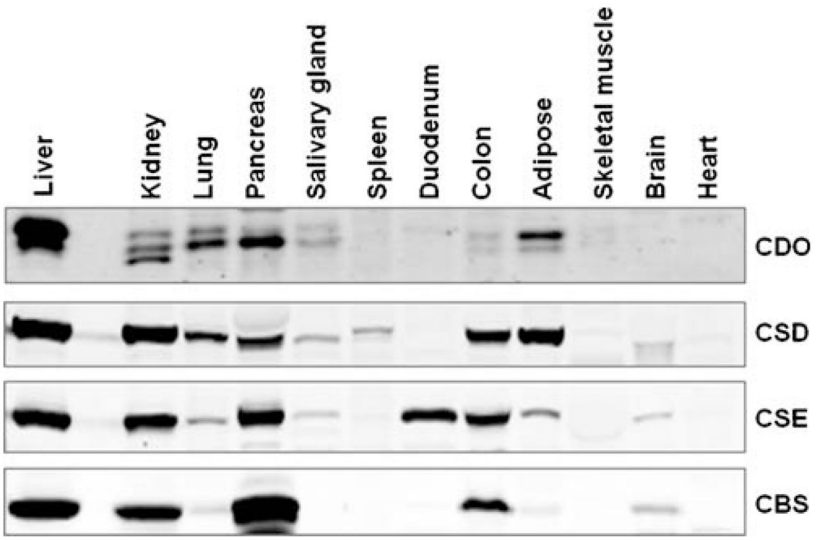

Fig. 4.

Distribution of cysteine dioxygenase (CDO), cysteinesulfinate decarboxylase (CSD), cystathionine γ-lyase (CSE), and cystathionine β-synthase (CBS) in murine tissues. Western blots of the soluble fraction of mouse tissue homogenates are shown. The CDO antibody was raised against rat CDO, and the CSD antibody was a gift from Dr. Marcel Tappaz (Institut National de la Santé et de la Recherche Médicale). Antibody for immunoblotting of CSE was obtained from Norvus Biologicals LLC, and antibody for CBS was obtained from Proteintech Group, Inc. Equal amounts of total protein (50 μg) were loaded per lane

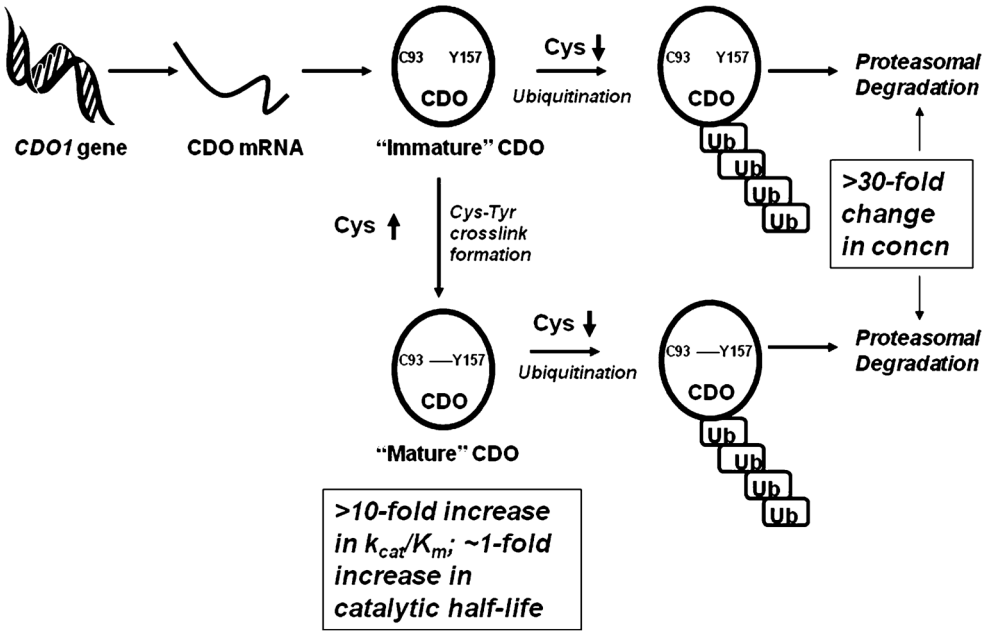

Fig. 5.

Overview of the regulation of cysteine dioxygenase (CDO) in response to changes in intracellular cysteine levels. Elevated cysteine levels promote protein cofactor formation, which increases catalytic efficiency (k_{cat}/K_m) and enzyme stability. Low cysteine levels promote ubiquitination and proteasomal degradation of CDO

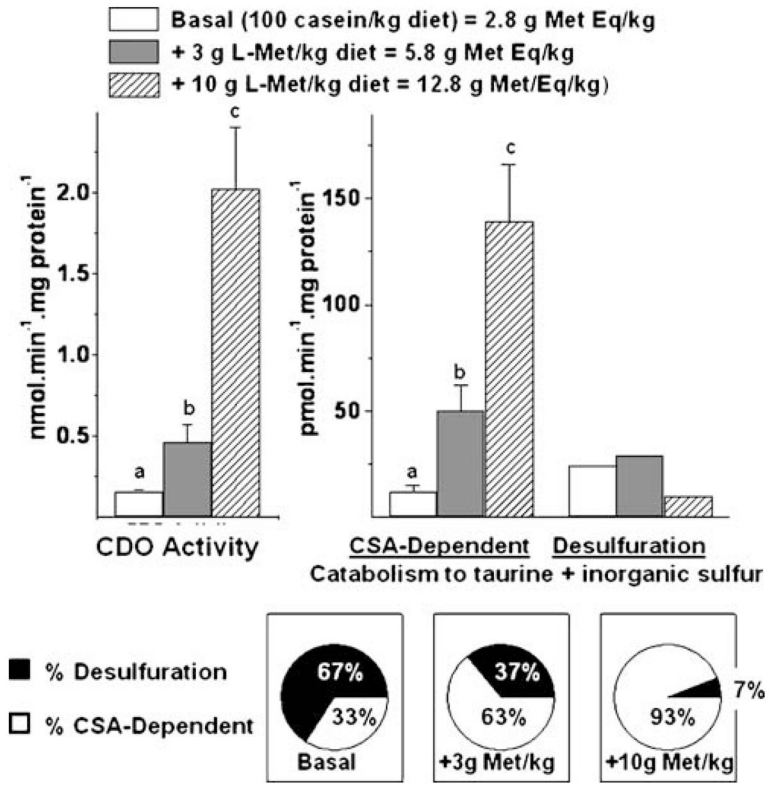

Fig. 6.

Estimates for cysteinesulfinate-dependent and cysteinesulfinate-independent catabolism of cysteine (Cys) by freshly isolated hepatocytes from rats that had been fed diets with different levels of sulfur-containing amino acids (SAAs). Diets and the total grams of methionine equivalents [1 g Cys=1.23 g methionine (Met)] per kilogram diet are shown in the l*egend*. Based on the data of Bagley and Stipanuk (1995) for hepatocytes incubated in vitro with 0.2 mM Cys or 0.2 mM cysteinesulfinate. Values are the mean ± standard error of mean (SEM) for hepatocyte preparations from six to seven rats. Values (*bars*) not designated by the same letter are significantly different at $P \leq 0.05$ by analysis of variance (ANOVA) and Dunnett's test. Estimates of Cys catabolism by Cys desulfuration were determined by difference. The *bar graph* shows values as actual flux through each route. The *pie chart* shows relative flux as a percentage of total Cys catabolism [*white* = cysteine sulfinic acid (CSA)- or cysteine dioxygenase (CDO)-dependent catabolism to taurine + sulfate; *black* = CSA-independent catabolism to sulfate via desulfuration]

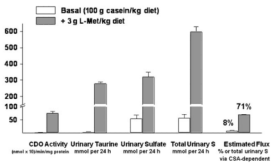

Fig. 7.

Estimates of cysteinesulfinate-dependent catabolism of cysteine (Cys) in intact rats fed either a basal low sulfur-containing amino acid (SAA) diet or an adequate SAA diet. Values for hepatic cysteine dioxygenase (CDO) activity are from Bella et al. (1999a). Estimates of cysteinesulfinate-dependent catabolism of Cys are based on the urinary excretion of sulfur metabolites (taurine and sulfate) in urine of rats, as reported by Bella et al. (1999a), and estimates of cysteinesulfinate partitioning are calculated from the data of Bagley and Stipanuk (1995) and Stipanuk and Rotter (1984). Values are mean ± standard error of mean (SEM) for six to seven rats