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Cysteine *S*-conjugate *β*-lyases

Review Article

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Summary. Cysteine *S*-conjugate β-lyases are pyridoxal 5′-phosphate-containing enzymes that catalyze β-elimination reactions with cysteine *S*-conjugates that possess an electron-withdrawing group attached at the sulfur. The end products of the β-lyase reaction are pyruvate, ammonium and a sulfur-containing fragment. If the sulfur-containing fragment is reactive, the parent cysteine *S*-conjugate may be toxic, particularly to kidney mitochondria. Halogenated alkenes are examples of electrophiles that are bioactivated (toxified) by conversion to cysteine *S*-conjugates. These conjugates are converted by cysteine *S*-conjugate β-lyases to thioacylating fragments. Several cysteine *S*-conjugates found in allium foods (garlic and onion) are β-lyase substrates. This finding may account in part for the chemopreventive activity of allium products. This review (1) identifies enzymes that catalyze cysteine *S*-conjugate β-lyase reactions, (2) suggests that toxicant channeling may contribute to halogenated cysteine *S*-conjugate-induced toxicity to mitochondria, and (3) proposes mechanisms that may contribute to the antiproliferative effects of sulfur-containing fragments eliminated from allium-derived cysteine *S*-conjugates.

Keywords: Cysteine *S*-conjugates – Cysteine *S*-conjugate β-lyases – *S*-(1,2-dichlorovinyl)-L-cysteine – Glutamine transaminase K – Mitochondrial aspartate aminotransferase – *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine – Allium-derived compounds

Abbreviations used: AlaAT, alanine aminotransferase; AGAT II, alanine-glyoxylate aminotransferase isoenzyme II; $BCAT_c$, cytosolic branched-chain aminotransferase; $BCAT_m$, mitochondrial branched-chain aminotransferase; BCDHC, branched-chain α-keto acid dehydrogenase complex; BTC, *S*-(2-benzothiazolyl)-L-cysteine; cytAspAT, cytosolic aspartate aminotransferase; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; GST, glutathione *S*-transferase; GTK, glutamine transaminase K; KAT I, kynurenine aminotransferase isoenzyme I; KGDHC, α-ketoglutarate dehydrogenase complex; mitAspAT, mitochondrial aspartate aminotransferase; PDHC, pyruvate dehydrogenase complex; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; SAC, *S*-allyl-L-cysteine; SAMC, *S*-allylmercapto-L-cysteine; TCA cycle, tricarboxylic acid cycle; TFEC, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine

Introduction

Many reactive endogenous and exogenous electrophiles are detoxified in mammalian tissues by the mercapturate pathway (Fig. 1), the outline of which was discovered over 120 years ago (e.g. Baumann, 1883). (For reviews of the mercapturate pathway, see, for example, Stevens et al. [1989], Cooper and Tate [1997].) The first step in the mercapturate pathway (formation of the corresponding glutathione *S*-conjugate by attack of glutathione thiolate [GS^-] on the electrophile) is catalyzed by a multigene superfamily of glutathione *S*-transferases (GSTs), many of which have overlapping specificities. GSTs are classified on the basis of their isoelectric point, substrate and inhibitor properties, antibody recognition, and N-terminal amino acid sequences. The main classes of GSTs are α, μ, π and θ. These enzymes may also be broadly classified into groups on the basis of their cellular location, namely, microsomal, mitochondrial and cytosolic (Hayes et al., 2005).

The glutathione *S*-conjugate (γ-GLU-CYS(X)-GLY) is converted sequentially to the L-cysteinylglycine *S*-conjugate (X-CYS-GLY), the L-cysteine *S*-conjugate (X-CYS) and finally to the *N*-acetyl-L-cysteinyl *S*-conjugate (*N*-ACETYL-CYS-X: mercapturate) by γ-glutamyltransferase, aminopeptidase M/cysteinylglycinase and an *N*-acetyltransferase, respectively (Fig. 1). Generally, the mercapturate is more polar and water-soluble than is the original electrophile, and is readily excreted in the urine

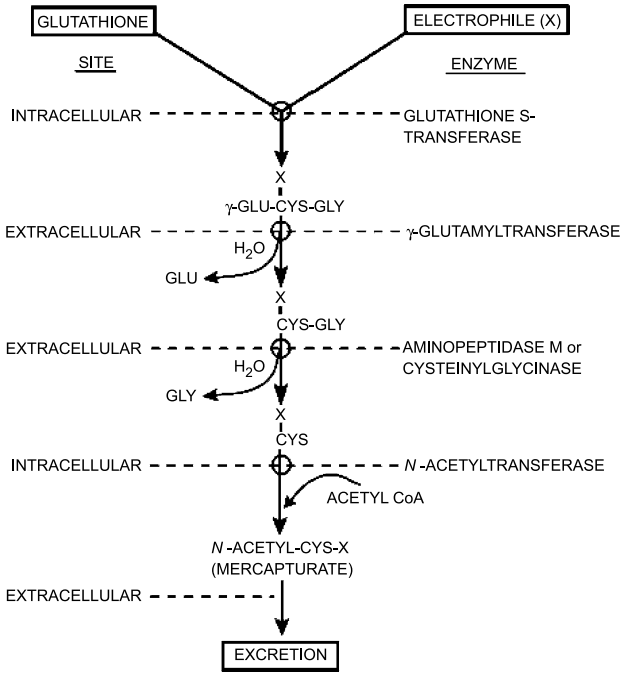


Fig. 1. Mercapturate pathway for the detoxification of electrophiles. The electrophile, denoted here by X, may be produced endogenously or ingested. Note that γ-glutamyltransferase, aminopeptidase M and cysteinylglycinase are ectoenzymes (occur on the cell surface), whereas glutathione *S*-transferases and *N*-acetyltransferases are intracellular. The acceptor substrate for the γ-glutamyltransferase-catalyzed reaction is shown as water, but can also be various amino acids, dipeptides or glutathione itself. Modified from Silbernagl and Heuner (1993)

and bile. GSTs catalyze three types of reaction. For example, haloalkenes and dichloroacetylene undergo GST-catalyzed vinylic substitution (S_NV) reactions, which may be either an addition reaction (e.g. with tetrafluoroethylene [Eq. (1)], dichloroacetylene [Eq. (2)]) or an addition elimination reaction (e.g. with trichloroethylene [Eq. (3)]). GSTs also catalyze reactions with epoxide moieties (e.g. in the conversion of leukotriene A_4 to leukotriene C_4) (reviewed by Cooper et al., 2002a). Glutathione *S*-conjugate formation with haloalkenes and dichloroacetylene is catalyzed by microsomal and cytosolic GSTs (Wolf et al., 1984; Dekant et al., 1988a, b; McLellan et al., 1989; Wallin et al., 1988; Cummings et al., 2000; Jolivette and Anders, 2002).

$$F_2C{=}CF_2 + GS^- + H^+ \rightarrow F_2C(H)CF_2SG \quad (1)$$

$$ClC{\equiv}CCl + GS^- + H^+ \rightarrow ClC(H){=}C(Cl)SG \quad (2)$$

$$Cl(H)C{=}CCl_2 + GS^- \rightarrow ClC(H){=}C(Cl)SG + Cl^- \quad (3)$$

Although the mercapturate pathway generally represents a mechanism by which potentially dangerous electrophiles are detoxified, the pathway can occasionally lead to bioactivation (toxification). Bioactivation occurs when (a) the cysteine *S*-conjugate is irreversibly diverted away from the mercapturate end product by the action of cysteine *S*-conjugate β-lyases, and (b) the sulfur-containing fragment eliminated by the cysteine *S*-conjugate β-lyase reaction is chemically reactive. Halogenated cysteine *S*-conjugates formed from trichloroethylene/dichloroacetylene and from tetrafluoroethylene (*S*-(1,2-dichlorovinyl)-L-cysteine [DCVC] and *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine [TFEC], respectively) are examples of cysteine *S*-conjugates that are toxic as a result of cysteine *S*-conjugate β-lyase activity. These cysteine *S*-conjugates and their elimination products are discussed below.

Discovery of cysteine *S*-conjugate β-lyases

In 1965, Colucci and Buyske identified a thiol metabolite of benzothiazolyl 2-sulfonamide in rabbits, rats, and dogs. This was probably the first example reported in the literature of a C-S lyase involved in the metabolism of a xenobiotic in mammals. Shortly thereafter, Bhattacharya and Schultze (1967) showed that DCVC is enzymatically cleaved by a C-S lyase. A sulfur-containing moiety was shown to attach covalently to macromolecules, but the nature of the reaction was not fully elucidated.

Tateishi et al. (1978) were the first authors to use the term "cysteine conjugate β-lyase". These authors noted that rat liver possesses an activity capable of cleaving the thioether linkage in cysteine *S*-conjugates of aromatic compounds such as 2,4-dinitrobenzene and *p*-bromobenzene. Equal quantities of pyruvate, ammonium and a sulfur-containing fragment (XSH) were generated via a β-elimination reaction (Eq. (4)). (Actually, the initial product of a β-elimination reaction with an α-amino acid is aminoacrylate [$CH_2{=}C(NH_3^+)CO_2^-$], which undergoes non-enzymatic rearrangement to the α-imino acid [$CH_3C(=NH_2^+)CO_2^-$] and subsequent hydrolysis to pyruvate and ammonium [Miles, 1986].) The β-lyase activity was purified about 500-fold, but the identity of the enzyme responsible was not determined (Tateishi et al., 1973).

$$XSCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + XSH \quad (4)$$

Identities and general properties of mammalian cysteine *S*-conjugate β-lyases

Stevens (1985) identified a cysteine *S*-conjugate β-lyase of rat liver cytosol as kynureninase. Shortly thereafter

Stevens and colleagues (1986) identified a cysteine *S*-conjugate β-lyase (DCVC as substrate) of rat kidney cytosol as glutamine transaminase K (GTK). GTK is a freely reversible glutamine (methionine) aromatic amino acid aminotransferase (Cooper and Meister, 1974, 1981). This enzyme is most likely involved in closure of the methionine salvage pathway, salvage of some α-keto acids and in the production of unusual sulfur-containing cyclic ketimines. GTK is identical to kynurenine aminotransferase isoenzyme I (KAT I) (reviewed by Cooper, 2004).

Mammalian tissues are now known to contain at least eleven enzymes capable of catalyzing cysteine *S*-conjugate β-lyase reactions in vitro (Table 1), and no doubt the list will continue to grow. The list of PLP-containing enzymes that can catalyze cysteine *S*-conjugate β-lyase reactions (Table 1) currently includes the cytosolic enzymes kynureninase, GTK, cytosolic aspartate aminotransferase (cytAspAT), alanine aminotransferase (AlaAT), and cytosolic branched-chain aminotransferase ($BCAT_c$) and the mitochondrial enzymes mitochondrial aspartate aminotransferase (mitAspAT), mitochondrial branched-chain aminotransferase ($BCAT_m$), alanine-glyoxylate aminotransferase isozyme II (AGAT II), and GABA aminotransferase. In addition, a high-M_r β-lyase occurs in both cytosolic and mitochondrial fractions. This high-M_r enzyme is discussed in more detail below.

All the cysteine *S*-conjugate β-lyases contain pyridoxal 5′-phosphate (PLP) as a coenzyme and, under normal

Table 1. Mammalian PLP-dependent enzymes with L-cysteine *S*-conjugate β-lyase activity[a,b]

Enzyme[c]	β-Lyase substrates			Syncatalytic inactivation	Competing transamination	Approximate specific activity (U/mg)[d]	Selected reference(s)
	DCVC	TFEC	BTC				
Cytosolic enzyme							
Kynureninase (R)	+	ND[e]	+	+	ND	0.25	Stevens (1985)
GTK/KAT I (R)[f]	+	+	−	−	+	0.6–6.4	Stevens et al. (1986), Yamauchi et al. (1993)
CytAspAT (R)	+	+	±	+	−	0.04–0.16	Gaskin et al. (1995), Adcock et al. (1996), Teesdale-Spittle et al. (1996), Kato et al. (1998), Buckberry et al. (1998)
AlaAT (P)	+	+	+	+	−	0.004–0.06	Gaskin et al. (1995), Adcock et al. (1996), Kato et al. (1998)
$BCAT_c$ (H)	+	+	+	+	−	0.3–0.5	Cooper et al. (2003a)
γ-Cystathionase (R)	−	+	−	−	−	0.05–0.1	Pinto et al. (2005)
Mitochondrial enzyme							
MitAspAT (R)	+	+	+	+	+	0.8–2.3	Cooper et al. (2002b)
$BCAT_m$ (H)	+	+	−	+	−	0.2–0.5	Cooper et al. (2003a)
AGAT II (R)	+	+	+	+	+	0.2	Cooper et al. (2003b)
GABA aminotransferase (P)[g]	ND	+	ND	ND	ND	0.016	Present work
High-M_r β-lyase (R)[h]	+	+	+	−	+	1.0–1.2	Abraham and Cooper (1991), Abraham et al. (1995), Cooper et al. (2001)

[a] This table is an update of that published in Cooper et al. (2002a). A unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of pyruvate per min (usually at 37°C, but temperature was not specified in all the references cited)

[b] AGAT II and γ-cystathionase are homotetramers with M_r values of ~210,000 and ~176,00, respectively. The high-M_r β-lyase of rat kidney and liver homogenates has a $M_r > 200{,}000$. All other enzymes listed are homodimers with M_r values for the intact holoenzymes of ~90,000–110,000

[c] Species abbreviations: *R*, rat; *P*, pig; *H*, human

[d] Activity with DCVC and/or TFEC

[e] *ND*, not determined

[f] Human liver contains a KAT with strong cysteine *S*-conjugate β-lyase activity (Buckberry et al., 1992). A cysteine *S*-conjugate β-lyase has been highly purified from human kidney (Lash, 1990a). The lyase activity co-purifies with GTK. The human GTK, unlike the rat enzyme, has activity with BTC (Lash et al., 1990a). In the rat, some GTK is also present in mitochondria (see the text)

[g] The assay mixture (0.02 ml) contained 20 mM TFEC, 50 mM Tris-HCl buffer (pH 8.0) and 14.4 μg GABA aminotransferase (17 U/mg). After incubation at 37°C for 30 min the amount of pyruvate formed was determined as the 2,4-dinitrophenylhydrazone according to the method of Cooper et al. (2002b)

[h] Also present in the cytosolic fraction of rat liver and kidneys (Abraham et al., 1995). The high-M_r β-lyase was shown to co-purify with HSP70 and protein disulfide isomerase (Cooper et al., 2001). The catalytic portion has been shown to be due, at least in part, to mitAspAT

conditions, are involved in amino acid metabolism. Most of the cysteine *S*-conjugate β-lyases discovered thus far are aminotransferases (Table 1), and an aminotransferase reaction may therefore compete with the β-elimination reaction. A half-transamination reaction will convert the PLP coenzyme to its pyridoxamine 5′-phosphate (PMP) form, which cannot catalyze a β-lyase reaction. When transamination of the cysteine *S*-conjugate competes with the β-lyase reaction, maximal β-lyase activity requires the presence of an α-keto acid substrate in the reaction mixture. The α-keto acid substrate forms a Schiff's base with PMP that is then converted to the corresponding amino acid and PLP. The PLP form of the enzyme can then support another round of β-lyase catalysis (Stevens et al., 1986; Cooper, 1998).

The cysteine *S*-conjugate β-lyases exhibit some degree of substrate specificity with the most commonly used substrates (DCVC, TFEC and *S*-(2-benzothiazolyl)-L-cysteine [BTC]). For example, DCVC and TFEC are β-lyase substrates of GTK, whereas BTC is not. On the other hand, all three are substrates of mitAspAT. TFEC is a β-lyase substrate of γ-cystathionase but DCVC is not (see Table 1).

Syncatalytic inactivation of cysteine *S*-conjugate β-lyases by end-product(s) may occur (Table 1). As noted above, one of the products eliminated from the β-lyase active site is aminoacrylate, which subsequently undergoes rearrangement and hydrolysis to pyruvate and ammonium. Aminoacrylate is known to inactivate pig heart cytAspAT by reacting with the PLP coenzyme to generate a PLP-pyruvate aldol condensation product (Ueno et al., 1982). Moreover, the sulfur-containing fragment released from the halogenated cysteine *S*-conjugate may react with a lysine residue leading to self-inactivation (see below). β-Lyase reactions with DCVC and TFEC catalyzed by GTK apparently do not lead to self-inactivation. However, mitAspAT-catalyzed β-elimination with TFEC leads to inactivation on average after several thousand turnover events (Cooper et al., 2002b). Inactivation of the cysteine *S*-conjugate β-lyase enzymes, $BCAT_m$ and $BCAT_c$, in the presence of TFEC occurs on average after about 200 and 45 turnover events, respectively (Cooper et al., 2003a). Thus, factors such as the presence or absence of "natural" α-keto acid substrate, length of incubation time, nature of cysteine *S*-conjugate used as substrate, and organ distribution of a given PLP-dependent enzyme, together with variable K_m values and pH optima will affect the apparent level of cysteine *S*-conjugate β-lyase activity measured in crude tissue homogenates.

β-Lyase activity toward analogues of cysteine S-conjugates containing a higher chalcogenide

S-Methyl-, *S*-ethyl-, *S*-allyl- and various *S*-aryl-L-cysteine *S*-conjugates are not measurable or are very limited β-lyase substrates of purified rat kidney GTK, but are moderately good aminotransferase substrates (Commandeur et al., 2000; see also Cooper and Pinto, 2005). By contrast, the selenocysteine *Se*-conjugates are 5- to 10-fold better aminotransferase substrates of GTK than their sulfur-containing counterparts. The selenocysteine *Se*-conjugates are also good β-lyase substrates of rat kidney GTK (Eq. (5)) (Commandeur et al., 2000). The greater reactivity of the selenocysteine *Se*-conjugates may be due to the weaker C–Se bond compared to the C–S bond and/or to the more facile abstraction of the β proton (Commandeur et al., 2002). Selenocysteine *Se*-conjugate β-lyase activity is present in human kidney cytosol, and it has been suggested that selenocysteine *Se*-conjugates may be useful as prodrugs to target pharmacologically active selenol compounds (XSeH; Eq. (5)) to the kidney (Rooseboom et al., 2000). *Se*-Benzyl-L-selenocysteine and *S*-benzyl-L-cysteine are oxidized to the corresponding sulfoxides by a flavin-containing monooxygenase in rat liver microsomes. *Se*-Benzyl-L-selenocysteine selenoxide, but not *S*-benzyl-L-cysteine sulfoxide, readily undergoes a non-enzymatic *syn* β-elimination reaction to yield aminoacrylate (and eventually pyruvate) and RSeOH (Rooseboom et al., 2001a). Selenocysteine *Se*-conjugates (but not cysteine *S*-conjugates) also undergo an L-amino acid oxidase-catalyzed β-elimination reaction (Rooseboom et al., 2001b).

Rooseboom et al. (2002) have shown that *Te*-phenyl-L-tellurocysteine is a good β-lyase substrate of purified rat kidney GTK (Eq. (6)). The authors suggested that tellurocysteine *Te*-conjugates may be an interesting novel class of prodrugs for the formation of biologically active tellurols. The lower pK_a and the much greater nucleophilicity of XSeH and XTeH compared to XSH should render the elimination fragments containing the higher chalcogenides catalytically much more reactive than the XSH elimination fragment.

$$XSeCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + XSeH \quad (5)$$

$$PheTeCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + PheTeH \quad (6)$$

Toxicity of haloalkenes and their corresponding cysteine *S*-conjugates

Haloalkenes enter the environment anthropogenically as degreasing solvents, organic solvents, organic reagents and inhalational anesthetic agents. The first evidence that haloalkenes are toxic was published early in the last century. Stockman (1916) reported that cattle fed soybean meal improperly treated with trichloroethylene developed aplastic anemia. Subsequently, compelling evidence was presented that a toxic factor derived from trichloroethylene is the corresponding cysteine *S*-conjugate, namely, DCVC (McKinney et al., 1959). Among the different species tested, cattle exclusively develop DCVC-induced aplastic anemia (Lock et al., 1996). However, DCVC is nephrotoxic/nephrocarcinogenic and hepatotoxic/hepatocarcinogenic in experimental animals (Dekant et al., 1990), and may also be neurotoxic under certain circumstances (reviewed in Cooper et al., 2002a; Anders, 2005). The glutathione-, cysteinylglycine-, cysteine- and *N*-acetylcysteine *S*-conjugates (mercapturates) of a number of haloalkenes, such as trichloroethylene, tetrachloroethylene, tetrafluoroethylene and hexachlorobutadiene, are all nephrotoxic/hepatotoxic, and in many cases nephrocarcinogenic/hepatocarcinogenic in experimental animals (e.g. Koob and Dekant, 1991; Dekant et al., 1990; Anders, 2004). Toxicity is due mostly to the action of cysteine *S*-conjugate β-lyases that irreversibly divert the cysteine *S*-conjugate away from mercapturate formation and simultaneously generate a reactive sulfur-containing fragment (see below). The mercapturates derived from the halogenated alkenes are toxic because they can be hydrolyzed back to the corresponding cysteine *S*-conjugate by aminoacylases (e.g. Uttamsingh et al., 2000; Pushkin et al., 2004). Thus, as noted above, the mercapturate pathway enzymes together with cysteine *S*-conjugate β-lyases can on occasion contribute to toxification (bioactivation) rather than to detoxification. (For reviews, see, for example, Cooper, 1994, 1998; Anders, 2004; Dekant et al., 1994.) The reactive sulfur-containing fragment generated from halogenated cysteine *S*-conjugates by the action of cysteine *S*-conjugate β-lyases are especially cytotoxic in experimental animals to the proximal renal tubules, in particular to the S3 regions and, to a lesser extent, the S2 regions (e.g. Nash et al., 1984; Jones et al., 1988). Toxicity of DCVC is associated in part with covalent modification of macromolecules, depletion of non-protein thiols (e.g. glutathione), and initiation of lipid peroxidation (Chen et al., 1990).

In rodents, major metabolites of trichloroethylene are dichloroacetate and trichloroacetate, which may contribute in part to the toxicity of this haloalkene (Bull et al., 2002). Only a small portion of trichloroethylene is metabolized through the glutathione *S*-transferase pathway (probably <1%) in the rat (Koob and Dekant, 1991). Nevertheless, there is compelling evidence that a major route for bioactivation of trichloroethylene involves the mercapturate pathway and cysteine *S*-conjugate formation (Anders, 2004). For other haloalkenes, the mercapturate pathway/cysteine *S*-conjugate formation is the major route for metabolism and consequently for bioactivation (e.g. Odum and Green, 1982; Lock and Ishmael, 1988; Jaffe et al., 1983; Dekant et al., 1988). DCVC induces expression of the proto-oncogenes c-*fos* and c-*myc* in LLC-PK1 cells (a pig kidney cell line) (Vamvakas and Köster, 1993; Vamvakas et al., 1993).

Halogenated alkenes (trichloroethylene, tetrachloroethylene, hexachlorobutadiene) are among the several hundred potentially dangerous toxicants listed as present in Superfund sites in the US (http://www.atsdr.cdc.gov/cxcx3.html). Some segments of the US population are exposed to haloalkenes from such sites and in the workplace (Wu and Schaum, 2000), and possibly through ''recreational'' abuse (Marjot and McLeod, 1989). Recent evidence suggests that heavy exposure to trichloroethylene is associated with an increased risk of kidney cancer in humans as a result of mutations in the von Hippel-Lindau (VHL) tumor suppressor gene (Brauch et al., 2004).

Another interesting example whereby cysteine *S*-conjugate β-lyases may contribute adversely to human health relates to exposure to breakdown products of inhalational anesthetic agents. Administration of various anesthetics can sometimes lead to damage to kidneys, liver and occasionally neural tissue (reviewed by Anders, 2005). When an anesthetic gas is used in association with a desiccated carbon dioxide absorbant, severe heat buildup in the anesthetic circuit, particularly in the CO_2 absorber canister, can lead to the conversion of trichloroethylene to dichloroacetylene, halothane to 2-bromo-2-chloro-1,1-difluoroethylene, sevoflurane to 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propylene (Compound A), and desflurane, isoflurane, and enflurane to CO. Dichloroacetylene, 2-bromo-2-chloro-1,1-difluoroethylene, and Compound A form glutathione *S*-conjugates that undergo hydrolysis to cysteine *S*-conjugates, and subsequent bioactivation to toxic sulfur-containing fragments by cysteine *S*-conjugate β-lyases (Anders, 2005). The cysteine *S*-conjugate derived from dichloroacetylene is DCVC. Glutathione *S*-conjugate formation with dichloroacetylene (Eq. (2)) is much more rapid than is glutathione *S*-conjugate formation from trichloroethylene (Eq. (3)). Thus, toxic DCVC forms

more readily from dichloroacetylene than from trichloroethylene (Kanhai et al., 1991). The above-mentioned findings have spurred the development of newer anesthetic gas ventilators that minimize the risk of conversion of anesthetics to toxic breakdown products (Anders, 2005).

Toxic fragments generated from halogenated cysteine *S*-conjugates by the action of cysteine *S*-conjugate *β*-lyases

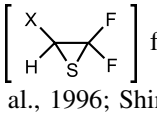
When the sulfur-containing fragment (XSH; Eq. (4)) eliminated in a cysteine *S*-conjugate β-lyase reaction is not reactive, the parent electrophile/cysteine *S*-conjugate is not particularly toxic. An example of a cysteine *S*-conjugate β-lyase substrate that is not toxic is BTC. The eliminated fragment (2-mercaptobenzothiazol) is *S*-glucuronidated by UDP-glucuronosyltransferase and excreted (Elfarra and Hwang, 1990). Conversely, toxicity may ensue when the eliminated sulfur-containing fragment is reactive. The toxicity of most halogenated cysteine *S*-conjugates is associated with the formation of a reactive thioacylating agents. For example, 1,2-dichloroethenethiol [ClC(H)=C(Cl)SH] formed from DCVC by the cysteine *S*-conjugate β-lyase reaction is highly reactive, most likely generating a thioketene (Eq. (7)), which thioacylates macromolecules, particularly at the ε-amino groups of lysyl residues in proteins (Eq. (8)) (Hayden et al., 1990; Dekant et al., 1994; Völkel and Dekant, 1998). The reactive metabolite eliminated from the toxic cysteine *S*-conjugate TFEC (Eq. (8)) yields difluorothionoacetyl fluoride (Eq. (9)) (Dekant et al., 1994), which also thioacylates lysyl moieties (Eq. (10)) (Oldum and Green, 1982; Lock and Ishmael, 1998).

$$[\mathrm{ClC(H)=C(Cl)SH}] \rightarrow \mathrm{ClC(H)=C=S} + \mathrm{HCl} \quad (7)$$

$$\mathrm{ClC(H)=C=S} + \mathrm{H_2NR} \rightarrow \mathrm{ClCH_2C(=S)NHR} \quad (8)$$

$$\mathrm{F_2C(H)CF_2SH} \rightarrow \mathrm{F_2C(H)C(=S)F} + \mathrm{HF} \quad (9)$$

$$\mathrm{F_2C(H)C(=S)F} + \mathrm{H_2NR} \rightarrow \mathrm{F_2C(H)C(=S)NHR} + \mathrm{HF} \quad (10)$$

Cysteine *S*-conjugates derived from bromine-containing fluoroalkenes are more mutagenic than those lacking bromine. The mutagenicity of these cysteine *S*-conjugates may be associated with the formation of a reactive 2,2-difluoro-3-halothiirane  following a β-lyase reaction (Finkelstein et al., 1996; Shim et al., 1997).

Targeting of mitochondria by toxic cysteine *S*-conjugates

Much evidence suggests that mitochondria are the principle targets of toxic, halogenated cysteine *S*-conjugates. Sulfoxidation (see Lash et al., 1994) may play a role in the mitochondrial toxicity of these compounds by a mechanism that is independent of a β-lyase reaction (Lash et al., 2003). However, there is compelling evidence that β-lyase reactions contribute importantly to the mitochondrial toxicity elicited by halogenated cysteine *S*-conjugates (see Cooper et al. [2002a] for a detailed discussion). Important findings are those of Bruschi et al. (1993), who showed that six mitochondrial proteins in kidney are thioacylated after rats were exposed to TFEC. No thioacylation of cytosolic proteins was detected. The six proteins have been identified as HSP60, mitochondrial HSP70, mitAspAT, the E2k subunit of the α-ketoglutarate dehydrogenase complex (KGDHC), the E3 subunit of KGDHC (and of the branched-chain keto acid dehydrogenase complex; BCDHC), and aconitase (Bruschi et al., 1993, 1994, 1998; James et al., 2002).

GTK rapidly catalyzes a β-lyase reaction with DCVC as substrate under optimal conditions in vitro (Stevens et al., 1986; Yamauchi et al., 1993). Thus, it seemed at first that GTK might be the major enzyme responsible for the β-lyase activity with halogenated cysteine *S*-conjugates in vivo. Indeed, GTK is often referred to as cysteine *S*-conjugate β-lyase, with the tacit assumption that it is the enzyme responsible for damage to the renal proximal tubules induced by DCVC and other halogenated cysteine *S*-conjugates. Certainly, GTK is well represented in the proximal tubules of the rat kidney (Jones et al., 1988; MacFarlane et al., 1989), but available data suggest that the contribution of this enzyme to halogenated cysteine *S*-conjugate-induced toxicity to the mitochondria in the proximal tubules may be less than originally proposed. GTK exists in cytosolic and mitochondrial forms due to the presence of two mRNAs derived from the same gene encoding proteins, respectively, with and without mitochondria-targeting leader sequences (Malherbe et al., 1995; Mosca et al., 2003). However, only about 10% of the total GTK activity in rat kidney fractions is due to the mitochondrial form (Cooper, 1988), and most of the cysteine *S*-conjugate β-lyase activity in rat kidney mitochondria cannot be assigned to GTK (Abraham et al., 1995a). A large fraction of the cysteine *S*-conjugate β-lyase activity in cytosolic and mitochondrial fractions obtained from rat kidney and liver is due to a high-M_r enzyme complex (Abraham et al., 1995b). Partial sequencing of proteins in the isolated rat kidney high-M_r complex

revealed the presence of HSP70 and protein disulfide isomerase (Cooper et al., 2001). This finding offers an explanation for the thioacylation of mitochondrial HSP70 in kidney mitochondria after rats were treated with TFEC. At first, the catalytic component of the high M_r-lyase was not recognized. However, recent work from our laboratory has shown that mitAspAT is present in the complex (A. C. L. Cooper, unpubl.). Other work from our laboratory has also shown that three aminotransferases present in kidney mitochondria (namely, mitAspAT, $BCAT_m$, AGAT II) possess cysteine *S*-conjugate β-lyase activity with TFEC as substrate (Cooper et al., 2002b, 2003a,b). It was estimated that 15–20% of the β-lyase activity toward TFEC in rat kidney mitochondria is due to mitAspAT (Cooper et al., 2002b). This finding is consistent with that of Bruschi et al. (1993). Those authors showed that mitAspAT is thioacylated in kidneys of rats treated with TFEC. Evidently the reactive sulfur-containing fragment released from TFEC at the active site of mitAspAT can cause auto-thioacylation.

Bruschi and colleagues have used a murine cell line (TAMH) to model TFEC-induced cell necrosis (Ho et al., 2005a, b). The TFEC-induced necrotic cell death in these cells is mediated by BAX and antagonized by the BCL-2 family member BCL-xL (Ho et al., 2005a). The TFEC-induced cytotoxicity in the TAMH cells was also shown to involve Nrf2 activation by an oxidative-stress-independent pathway (Ho et al., 2005b). Activation of Nrf2 involves redox mechanisms that mediate its release from Keap1, a cytosolic protein that sequesters and tethers the transcription factor in the cytoplasm. A widely accepted model for Nrf2 nuclear accumulation involves modification of Keap1 cysteine moieties by electrophilic compounds that lead directly to dissociation of the Keap1-Nrf2 complex and subsequent translocation of Nrf2 into the nucleus to drive gene transcription (e.g. Nguyen et al., 2005).

Toxicant channeling involving mitochondrial cysteine *S*-conjugate *β*-lyases

Although the E2k and E3 subunits of KGDHC are thioacylated in kidney mitochondria of rats administered TFEC, the E2p and E3 subunits of the pyruvate dehydrogenase complex (PDHC) are not (Bruschi et al., 1998; James et al., 2002). KGDHC activity, but not PDHC activity, is also diminished in the TFEC-treated rats. We have found that KGDHC, but not PDHC, is directly inhibited in PC12 cells exposed to 1 mM TFEC (Park et al., 1999). These findings are remarkable because E3 is a common component of KGDHC, PDHC and BCDHC (and the glycine cleavage system). PDHC is more resistant to in vitro inactivation than is KGDHC in the presence of TFEC and purified GTK (a source of thioacylating moieties) (Park et al., 1999), and there is some evidence that E3 subunits are packed differently in PDHC than in KGDHC (McCartney et al., 1998). Thus, part of the resistance of PDHC to thioacylation/inactivation in kidney mitochondria of TFEC-treated rats may be due to steric considerations. However, PDHC is not known to be associated with any aminotransferases/β-lyases in the mitochondria, whereas KGDHC is thought to be closely associated with mitAspAT (see below). Thus, the susceptibility of KGDHC to thioacylation may be due to close juxtapositioning of this enzyme complex to mitAspAT (James et al., 2002) and to the ready accessibility of the subunits to the thioacylating agent (Park et al., 1999).

Some evidence suggests that enzymes of the TCA cycle and ancillary enzymes are arranged in supramolecular complexes (metabolons) that facilitate substrate channeling. For example, mitAspAT is thought to be part of a metabolon that also contains KGDHC (Cooper et al., 2002a and references cited therein). On the basis of the findings from our laboratory and those of Bruschi and colleagues, we have proposed that it is not only possible for metabolites to be channeled through supramolecular

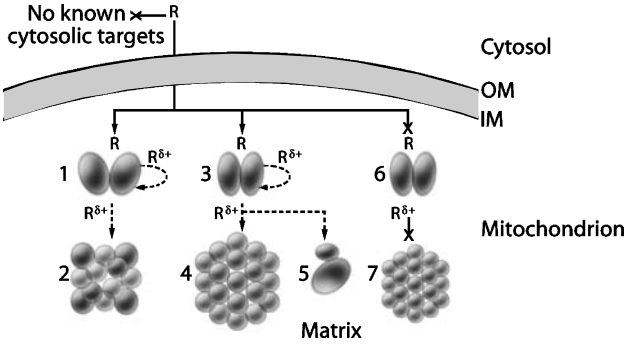


Fig. 2. Model for toxicant channeling in mitochondria in vivo. The protoxicant TFEC (R) is transported into the mitochondrion, where it is converted by cysteine *S*-conjugate β-lyases to a toxicant ($R^{\delta+}$, a thioacylating agent). The three α-keto acid dehydrogenase complexes – branched-chain α-keto acid dehydrogenase complex (BCDHC), α-ketoglutarate dehydrogenase complex (KGDHC) and pyruvate dehydrogenase complex (PDHC) – are represented as multimeric units labeled 2, 4 and 7, respectively. The mitochondrial cysteine *S*-conjugate β-lyases include the homodimeric $BCAT_m$ (1) and the homodimeric mitAspAT (3). The close juxtapositioning of $BCAT_m$ and mitAspAT to enzymes of energy metabolism results in channeling of the toxicant to BCDHC (2) and KGDHC (4)/aconitase (5), respectively, resulting in their inactivation. On the other hand, PDHC (7) is not known to be associated with any aminotransferase/cysteine *S*-conjugate β-lyase (6) and is not inactivated by direct thioacylation. The curved arrows represent "self-thioacylation" of $BCAT_m$ and mitAspAT, respectively. IM, inner membrane; OM, outer mitochondrial membrane. Modified from Cooper et al. (2002a)

complexes in the TCA cycle, but also for toxicants to be channeled through this complex (Cooper et al., 2002a) (Fig. 2). This concept may explain not only the susceptibility of KGDHC to TFEC-induced inactivation in rat kidney/cells in culture but also the susceptibility of aconitase and BCDHC to thioacylation/inactivation. Aconitase is thought to be part of the metabolon that includes KGDHC and mitAspAT (Ovadi and Srere, 2000), whereas BCDHC is thought to form a metabolon with $BCAT_m$ (Fig. 2) (Van Horn et al., 2004).

Prodrug/drug metabolism involving cysteine *S*-conjugate *β*-lyases

Elfarra and colleagues have synthesized prodrugs of the anti-cancer agent 6-mercaptopurine that were designed to release the active agent in the kidneys by the action of cysteine *S*-conjugate β-lyases (Hwang and Elfarra, 1991; Elfarra and Hwang, 1993; Elfarra et al., 1995; Lash et al., 1997). For example, it was shown that 30 min after administration of the prodrug *S*-(6-purinyl)-L-cysteine to rats the concentration of 6-mercaptopurine was nearly 2.4 times higher in the kidneys than in liver and 90 times higher than in plasma (Hwang and Elfarra, 1991). Other analogues, such as the mercapturate, glutathione *S*-conjugate and homocysteine *S*-conjugate also generated relatively high levels of 6-mercaptopurine in the kidney (Elfarra and Hwang, 1995). Evidently, the mercapturate and glutathione *S*-conjugates can be readily converted to the corresponding cysteine *S*-conjugate in the kidney. The cysteine *S*-conjugate, either taken up directly or formed from the mercapturate/glutathione *S*-conjugate, is a substrate of cysteine *S*-conjugate β-lyases thereby generating the active drug 6-mercaptopurine within the kidneys. Interestingly, *S*-(6-purinyl)-L-cysteine can undergo a novel rearrangement at neutral pH to yield the positional isomer *N*-(6-purinyl)-L-cysteine (Hwang and Elfarra, 1996). Rearrangement was much less favored with the homocysteine *S*-conjugate, and rearrangement was blocked in the mercapturate and glutathione *S*-conjugate (Hwang and Elfarra, 1996). In fact, *S*-(6-purinyl)glutathione may be an even better delivery agent for 6-mercaptopurine to the kidney than is *S*-(6-purinyl)-L-cysteine for treating renal tumors or for suppressing renal transplant rejection (Lash et al., 1997). Elfarra et al. (1995) also showed that *S*-(6-guaninyl)-L-cysteine is an effective prodrug of the anticancer agent 6-thioguanine and is bioactivated by cysteine *S*-conjugate β-lyase(s) in the kidney. Interestingly, the maximal specific activity of the β-lyase with *S*-(6-guaninyl)-L-cysteine as substrate was 45 times higher in a rat kidney mitochondrial fraction than in the cytosolic fraction. This finding shows that GTK cannot have been responsible for most of the β-lyase activity with *S*-(6-guaninyl)-L-cysteine. The possibility that mitAspAT may be the cysteine *S*-conjugate β-lyase responsible for the mitochondrial activity should be investigated. These studies with the various *S*-conjugate anti-cancer prodrugs have apparently not been followed up with clinical trials, but suggest that the strategy may generally be useful for targeting some drugs to cysteine *S*-conjugate β-lyases within human kidneys.

The carbonic anhydrase inhibitor methazolamide, which can cause ocular toxicity, is metabolized to glutathione *S*-conjugates and cysteine *S*-conjugates (Kishida et al., 1990). It was later shown that the cysteine *S*-conjugate is a substrate of β-lyases in bovine kidney and liver homogenates (Kishida et al., 2001). The eliminated SH-containing fragment was identified by elemental analysis, NMR and mass spectrometry to be *N*-(3-methyl)-5-mercapto-Δ^4-1,3,4-thiadiazol-2-yl)acetamide. This finding may account for the binding of a metabolite to macromolecules (Kishida et al., 2001).

Cisplatin has been used successfully to treat germ cell tumors, head and neck tumors and cervical cancer even when the cancer has metastasized. However, its effectiveness against other cancers is limited by its toxicity to renal proximal tubules. Studies from many laboratories have implicated DNA damage as the primary mechanism by which cisplatin kills tumor and other rapidly dividing cells. However, the renal proximal tubule cells are well-differentiated, non-dividing cells that are not killed by other DNA-damaging agents. Early work suggested that a metabolite of cisplatin is responsible for the nephrotoxicity and that mitochondria are the targets. (For original references, see Townsend et al. [2003] and Zhang and Hanigan [2003].) In part on the basis of the results of experiments with kidney tissue/cells and inhibitors of γ-glutamyltransferase and PLP-dependent enzymes, Hanigan and colleagues have concluded that damage to kidney cells is due to a bioactivation mechanism involving the cysteine *S*-conjugate (Townsend et al., 2003; Zhang and Hanigan, 2003). Cisplatin is converted to its glutathione *S*-conjugate and eventually to its cysteine *S*-conjugate. The cysteine *S*-conjugate is then acted upon by cysteine *S*-conjugate β-lyase(s) to generate a fragment containing a Pt-SH moiety (Eq. (11)). This Pt-SH fragment was proposed to react with macromolecules at thiophilic centers.

$$(H_2N)_2Pt(Cl)SCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + (NH_2)_2Pt(Cl)SH \quad (11)$$

If enzymes capable of catalyzing cysteine *S*-conjugate β-lyase reactions are widely distributed in mammalian tissues why are the kidney proximal tubules especially susceptible to halogenated cysteine *S*-conjugate-induced damage?

Many of the cysteine *S*-conjugate β-lyases are widespread in mammalian tissues. For example, in the rat, GTK is most active in kidney followed by the liver. However, this enzyme is present in almost every rat tissue analyzed (Cooper, 1988; Cooper and Meister, 1981). Similarly, mitAspAT is present in high concentrations in almost every tissue. Evidently, the tissue distribution of the cysteine *S*-conjugate β-lyases cannot explain the sensitivity of the kidney proximal tubules to the toxic, halogenated cysteine *S*-conjugates. Many factors are presumably involved, including relative distribution of *N*-acetyltransferase, acylases, cellular and mitochondrial uptake mechanisms, and concentrations of competing natural amino acid/α-keto acid substrates. However, a major contributing factor is likely to be the very large surface area of the renal proximal tubules coupled to the extraordinary high renal vascular perfusion. In humans, kidney receives 20% of the cardiac output; yet comprises <1% of the body weight (Pfaller and Gstraunthaler, 1998). The widespread distribution of cysteine *S*-conjugate β-lyases suggests that bioactivation may occur in many tissues, albeit at a lower level than in the kidneys. Indeed, as noted above, halogenated cysteine *S*-conjugates are toxic to liver and, on occasion, nervous tissue.

Cysteine *S*-conjugates in garlic extracts and their ability to undergo enzyme-catalyzed β-lyase reactions

Garlic and other members of the allium family contain alliin – a cysteine *S*-conjugate sulfoxide (Eq. (12)). On bruising or crushing of the bulb, alliin is converted to a number of odorous sulfur-containing compounds, which begins with the action of alliinase (a β-lyase) (Eq. (12)). The eliminated sulfur-containing compound (allylsulfenic acid) is converted non-enzymatically to allicin (Eq. (13)).

$$\underset{\text{Alliin}}{CH_2{=}CHCH_2S(O)CH_2CH(NH_3^+)CO_2^-} + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + \underset{\text{Allylsulfenic acid}}{CH_2{=}CHCH_2S(O)H} \quad (12)$$

$$2\,CH_2{=}CHCH_2S(O)H \rightarrow \underset{\text{Allicin}}{CH_2{=}CHCH_2S(O)SCH_2CH{=}CH_2} + H_2O \quad (13)$$

Through a series of disulfide/sulfide interchange reactions of allicin with cysteine, a number of cysteine *S*-conjugates are formed in fresh and aged garlic extracts including *S*-allyl-L-cysteine (SAC), *S*-allylmercapto-L-cysteine (SAMC), *S*-methylmercapto-L-cysteine, *S*-propylmercapto-L-cysteine, *S*-penta-1,3-dienylmercapto-L-cysteine, and L-ajocysteine (Cooper and Pinto, 2005). Each of these garlic-derived cysteine *S*-conjugates (except L-ajocysteine, which was not tested) was found to be an excellent to moderately good substrate of purified rat kidney GTK and snake venom L-amino acid oxidase (Cooper and Pinto, 2005). In addition, an activity in rat liver cytosol was able to catalyze a β-elimination reaction with SAC, SAMC, *S*-propylmercapto-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine (but not with *S*-methylmercapto-L-cysteine) (Table 2) (Cooper and Pinto, 2005). Tomisawa et al. (1988) previously showed that *S*-propyl-L-cysteine or cysteine *S*-conjugates containing larger alkyl groups, but not *S*-methyl- or *S*-ethyl-L-cysteine are

Table 2. Cysteine *S*-conjugates derived from L-alliin [$CH_2{=}CHCH_2S(O)CH_2CH(NH_3^+)CO_2^-$] in fresh and aged garlic extracts shown to be β-lyase substrates of γ-cystathionase, and predicted sulfur-containing β-elimination fragment[a]

Structure	Name	Predicted β-elimination fragment
$CH_2{=}CHCH_2SSCH_2CH(NH_3^+)CO_2^-$	*S*-allylmercapto-L-cysteine (SAMC)	$CH_2{=}CHCH_2SSH$
$CH_2{=}CHCH_2SCH_2CH(NH_3^+)CO_2^-$	*S*-allyl-L-cysteine (SAC)	$CH_2{=}CHCH_2SH$
$CH_3CH_2CH_2SSCH_2CH(NH_3^+)CO_2^-$	*S*-propylmercapto-L-cysteine	$CH_3CH_2CH_2SSH$
$CH_3CH{=}CHCH{=}CHSSCH_2CH(NH_3^+)CO_2^-$	*S*-penta-1,3-dienylmercapto-L-cysteine	$CH_3CH{=}CHCH{=}CHSSH$

[a] For the mechanism of formation of these cysteine *S*-conjugates from L-alliin, see Cooper and Pinto (2005). Each of the cysteine *S*-conjugates listed was shown to be converted to pyruvate by an enzyme present in rat liver homogenates (Cooper and Pinto, 2005), now identified as γ-cystathionase (Pinto et al., 2005). The structure of the sulfur-containing elimination fragment was not elucidated, so only the predicted structure is shown. With the notable exception of SAC, the predicted sulfur-containing elimination fragment is a persulfide (RSSH). L-Ajocysteine [$CH_2{=}CHCH_2S(O)CH_2CH{=}CHSSCH_2CH(NH_3^+)CO_2^-$], a cysteine *S*-conjugate that also occurs in garlic extracts, was not tested as a β-lyase substrate of γ-cystathionase but is expected to undergo a β-elimination reaction

β-lyase substrates of γ-cystathionase. On the basis of this finding and that from our laboratory of strong inhibition by propargylglycine, we suggested that the β-lyase activity in rat liver extracts toward the various garlic-derived cysteine *S*-conjugates is due to γ-cystathionase (Cooper and Pinto, 2005). This was subsequently confirmed when we showed that β-lyase activity toward SAMC co-purified with γ-cystathionase in rat liver preparations (Pinto et al., 2005). A mitochondrial enzyme may also be able to catalyze a β-elimination reaction with *S*-penta-1,3-dienylmercapto-L-cysteine but not with other garlic-derived cysteine *S*-conjugates tested (Cooper and Pinto, 2005). However, the identity of this enzyme is not known.

γ-Cystathionase can catalyze a β-elimination reaction with L-cystine (which may be regarded as a special case of a cysteine *S*-conjugate) generating pyruvate, ammonium and L-thiocysteine (L-cysteine persulfide) (Eq. (14)). Interaction of L-thiocysteine with unreacted L-cystine can generate L-cysteine and L-thiocystine (cystine persulfide) (Eq. (15)). At neutral pH, L-thiocystine slowly breaks down to L-cystine and elemental sulfur (S^0) (Eq. (16)). L-Thiocystine is a substrate of rhodanese, which can transfer S^0 (sulfane sulfur) to a suitable co-substrate such as cyanide, which generates the less toxic thiocyanate (Eq. (17)). (See Cooper and Pinto [2005] for original references.) Generally, interactions involving GSH and cysteine are slow at acid or neutral pH values. Therefore, it is probable that mercapto-L-cysteine *S*-conjugates can enter the bloodstream intact after ingestion. As discussed below, this raises some intriguing biochemical possibilities.

$$H_3{}^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2{}^-)NH_3{}^+ + H_2O \rightarrow CH_3C(O)CO_2{}^- + NH_4{}^+ + HSSCH_2CH(CO_2{}^-)NH_3{}^+ \quad (14)$$

$$H_3{}^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2{}^-)NH_3{}^+ + HSSCH_2CH(CO_2{}^-)NH_3{}^+ \rightarrow H_3{}^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2{}^-)NH_3{}^+ + HSCH_2CH(CO_2{}^-)NH_3{}^+ \quad (15)$$

$$H_3{}^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2{}^-)NH_3{}^+ \rightarrow H_3{}^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2{}^-)NH_3{}^+ + S^0 \quad (16)$$

$$H_3{}^+N(^-O_2C)CHCH_2SSSCH_2CH(CO_2{}^-)NH_3{}^+ + CN^- \rightarrow H_3{}^+N(^-O_2C)CHCH_2SSCH_2CH(CO_2{}^-)NH_3{}^+ + SCN^- \quad (17)$$

Biological implications of cysteine *S*-conjugate β-lyase catalyzed-elimination reactions with allium-derived cysteine *S*-conjugates

β-Lyase reactions involving the allium-derived mercapto-L-cysteine *S*-conjugates should theoretically generate reactive persulfide (hydrodisulfide) species (RSSH), in a fashion similar to the generation of persulfide from cystine (Table 2). The present finding that SAMC, *S*-propyl-L-cysteine and *S*-penta-1,3-dienylmercapto-L-cysteine are β-lyase substrates of γ-cystathionase in rat liver homogenates suggests that the eliminated persulfide fragment may be a source of S^0 through interactions with L-cystine or GSSG. This sulfane sulfur could exert biological effects through addition to cysteine domains on redox-sensitive proteins and through detoxification reactions involving rhodanese (Cooper and Pinto, 2005). Possible mechanisms whereby the eliminated sulfur-containing fragments interact with protein sulfhydryls are summarized in Fig. 3.

Meta-analysis of the literature revealed a diminished risk of stomach and colorectal cancer with increased garlic intake (Fleischauer et al., 2000). Additionally, numerous studies have shown that several garlic-derived cysteinyl *S*-conjugates have anti-proliferative and pro-apoptotic effects against a number of hormone-sensitive- and refractory human tumor cell lines, and inhibit growth of transplantable tumors in experimental animals. As reviewed by Toohey (1989, 2001) and by Iciek and Włodek (2001), these effects may be mediated by sulfane sulfur-containing compounds or sulfane progenitors. Toohey (1989, 2001) suggested that proliferation of malignant cells may be related to a paucity of sulfane sulfur in these cells and to uncontrolled activity of proliferative enzymes and/or signal factors normally inactivated by sulfane sulfur. If this hypothesis is correct, the anti-cancer effects of the allium-derived cysteine *S*-conjugates may be due at least in part to their ability to act as progenitors of sulfane sulfur via interactions involving γ-cystathionase.

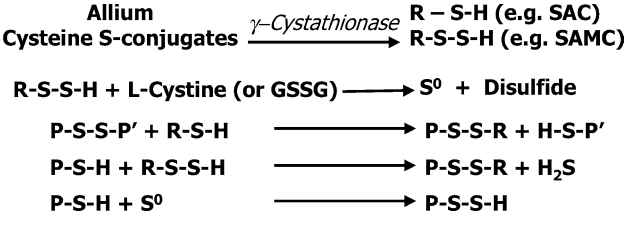


Fig. 3. Possible mechanisms whereby proteins are modified via products generated from the γ-cystathionase-catalyzed β-elimination reaction with various allium-derived cysteine *S*-conjugates. P and P′ represent protein backbones and R represents *S*-alkane and *S*-alkene moieties. From Cooper and Pinto, 2005

Flow cytometric analyses of DNA indicate that garlic-derived L-cysteine *S*-conjugates can prevent a variety of tumor cell lines from progressing through G_1/S phase while others are blocked in G_2/M. Studies on cell cycle progression suggest that allylsulfide derivatives can modify redox-sensitive signal transduction pathways that lead to expression of nuclear transcription factors. For example, a water-soluble allylsulfide derivative blocks activation of nuclear factor kappa B (NFκB) in Jurkat T cells, while one lipid-soluble allylpolysulfide compound induces apoptosis in human promyeloleukemic cells (reviewed by Pinto et al., 2001). Because of the interaction of garlic-derived cysteine *S*-conjugates with γ-cystathionase (and in one case a mitochondrial cysteine *S*-conjugate β-lyase) to produce reactive sulfhydryl and/or potential sulfane sulfur progenitors, the chemopreventive activity of these dietary factors may be due in part to their ability to modify intracellular redox potentials, to generate sulfane sulfur and/or to interact with thiols associated with cysteine moieties in regulatory or catalytic signal proteins.

γ-Elimination from homocysteine *S*-conjugates

Work from our laboratory using L-amino acid oxidase has shown that oxidation of the α-amino group to an α-imino group in amino acids containing a leaving group (denoted as R in Eq. (18)) in the γ-position leads to a non-enzymatic γ-elimination reaction with subsequent formation of ammonium and vinylglyoxylate (2-oxo-3-butenoic acid; $CH_2{=}CHC(O)CO_2^-$) (Eqs. (18–20)). This is due to the strong activation of the β C–H bond in the α-imino acid relative to that in the α-amino acid. Examples of amino acids that undergo this type of reaction with L-amino acid oxidase include L-methionine-*S,R*-sulfoximine (Cooper et al., 1976), L-canavanine (Hollander et al., 1989), and *S*-methyl-L-methionine (Rhodes et al., 1997), where the eliminated groups are methane sulfinamide, *N*-hydroxyguanine, and dimethylsulfide, respectively. γ-Elimination reactions also occur with α-keto acids generated from these amino acids via transamination reactions (Eqs. (21), (22)), although the reaction is less facile than that with α-imino acids. An interesting transamination/γ-elimination reaction occurs with *S*-adenosyl-L-methionine, which is the amino donor in the transamination of 7-oxo-8-aminopelargonic acid, an intermediate in the biotin biosynthetic pathway, to 7,8-diaminopelargonic acid in *Escherichia coli*. The α-keto acid analogue of *S*-adenosyl-L-methionine was shown to undergo a γ-elimination reaction in which 5′-methylthioadenosine is the leaving group (Stoner and Eisenberg, 1975).

$$RCH_2CH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow RCH_2CH_2C(=NH_2^+)CO_2^- + H_2O_2 \quad (18)$$

$$RCH_2CH_2C(=NH_2^+)CO_2^- \rightarrow RH + CH_2{=}CHC(=NH_2^+)CO_2^- \quad (19)$$

$$CH_2{=}CHC(=NH_2^+)CO_2^- + H_2O \rightarrow CH_2{=}CHC(O)CO_2^- + NH_4^+ \quad (20)$$

$$RCH_2CH_2CH(NH_3^+)CO_2^- + R'C(O)CO_2^- \rightarrow RCH_2CHC(O)CO_2^- + R'CH(NH_3^+)CO_2^- \quad (21)$$

$$RCH_2CHC(O)CO_2^- \rightarrow RH + CH_2{=}CHC(O)CO_2^- \quad (22)$$

The homocysteine *S*-conjugates *S*-(1,2,-dichlorovinyl)-L-homocysteine and *S*-(benzothiazolyl)-L-homocysteine) were observed to undergo an oxidation/γ-elimination reaction when incubated with L-amino acid oxidase (Lash et al., 1990b). *S*-(1,2,-Dichlorovinyl)-L-homocysteine is much more nephrotoxic than is DCVC. By contrast, the α-methyl homocysteine analogue, which cannot undergo a transamination reaction, is not toxic (Elfarra et al., 1986). *S*-(1,2,-Dichlorovinyl)-L-homocysteine is more toxic to isolated rat kidney cells and to mitochondria than is DCVC. *S*-(Benzothiazolyl)-L-homocysteine is toxic, whereas BTC is not. These data suggest that *S*-(1,2,-dichlorovinyl)-L-homocysteine and *S*-(benzothiazolyl)-L-homocysteine undergo a transamination reaction followed by a non-enzymatic γ-elimination reaction (shown in Eqs. (23), (24) for *S*-(1,2,-dichlorovinyl)-L-homocysteine) (Lash et al., 1990b). The increased toxicity of *S*-(1,2,-dichlorovinyl)-L-homocysteine and *S*-(benzothiazolyl)-L-homocysteine compared to the corresponding cysteine *S*-conjugates may be due to the formation of the toxic fragment, 2-oxo-3-butenoic acid, which is expected to readily participate in a Michael addition reaction (Elfarra et al., 1986; Lash et al., 1990b) forming a conjugate with a carbanion nucleophile, or possibly in a Robinson annulation reaction that leads to formation of a cyclic α,β-unsaturated ketone.

$$Cl(H)C{=}C(Cl)SCH_2CH_2CH(NH_3^+)CO_2^- + R'C(O)CO_2^- \rightarrow Cl(H)C{=}C(Cl)SCH_2CHC(O)CO_2^- + R'CH(NH_3^+)CO_2^- \quad (23)$$

$$Cl(H)C{=}C(Cl)SCH_2CHC(O)CO_2^- \rightarrow Cl(H)C{=}C(Cl)SH + CH_2{=}CHC(O)CO_2^- \quad (24)$$

As noted above, the homocysteine *S*-conjugate of 6-mercaptopurine acts as a prodrug directed toward the kidney. Presumably, the release of 6-mercaptopurine results from an enzymatic transamination followed by a non-enzymatic γ-elimination reaction (Elfarra and Hwang, 1995).

Conclusions

At least eleven PLP-dependent enzymes catalyze cysteine *S*-conjugate β-lyase side reactions. These reactions can have both negative and positive physiological consequences. Adverse effects may occur as a result of cysteine *S*-conjugate β-lyases catalyzing reactions that generate toxic sulfur-containing fragments. The toxicity of a number of halogenated cysteine *S*-conjugates may be attributable to at least two mechanisms (Cooper et al., 2002a). Syncatalytic inactivation may lead to inactivation of crucial PLP-dependent enzyme activity. For example, incubation of PC12 cells with 1 mM TFEC leads to a time-dependent loss of mitAspAT activity (Park et al., 1999). Similar results were obtained with rat astrocytes in culture (A. J. L. Cooper, unpubl. data). Inactivation of mitAspAT could lead to disruption of the malate:aspartate shuttle and thus to compromised energy metabolism. The sensitivity of both BCAT isozymes to syncatalytic inactivation in the presence of TFEC suggests that toxic halogenated cysteine *S*-conjugates may disrupt branched-chain amino acid metabolism. The brain is almost unique in its possession of both BCAT isozymes (Sweatt et al., 2004). This arrangement may allow crucial nitrogen cycling between astrocytes and neurons. Inhibition of the BCAT isozymes could lead to brain dysfunction and/or developmental alterations (Sweatt et al., 2004). Secondly, energy metabolism may be compromised by the selective loss of TCA cycle enzyme activities such as KGDHC and aconitase, in part resulting from close juxtapositioning to mitAspAT. Because cysteine *S*-conjugate β-lyase activity is widespread, the potential may exist for cysteine *S*-conjugate-induced toxicity in many tissues. Mitochondrial dysfunction and compromised energy metabolism occur in aging and in many neurodegenerative diseases (e.g. Beal, 2000). The possibility should be considered that exposure of mitochondria over many years to low levels of potentially toxic cysteine *S*-conjugates derived from endogenous and exogenous sources may contribute to mitochondrial dysfunction of aging and disease.

Possible beneficial or useful consequences of cysteine *S*-conjugate β-lyase activity include targeting of prodrugs. Although presently limited in scope, available studies show the potential for cysteine *S*-conjugate β-lyases to unmask suitably designed prodrugs in target tumors/tissues. Other favorable β-lyase reactions are associated with activation of *S*-cysteine conjugates derived from allium foods into reactive persulfide or sulfane sulfur progenitors. A large number of studies provides compelling evidence that allium-derived *S*-allylcysteinyl constituents are effective inhibitors of the cancer process. These studies reveal that the antiproliferative effects of cysteine *S*-conjugates reside in inhibited growth, cell cycle arrest in G_2/M, and induction of apoptotic signaling in several types of human cancers. For example, in colon cancer cells, immunofluorescence microscopy analyses reveal that treatment with SAMC causes rapid microtubule depolymerization, microtubule cytoskeleton disruption, centrosome fragmentation and Golgi dispersion in interphase cells, presumably manifested through direct interaction of eliminated sulfur-containing fragments from β-lyase reactions on sulfhydryl moieties in tubulin and other redox-sensitive signal proteins (Xiao et al., 2003). Thus, the eliminated products of garlic constituents by cysteine *S*-conjugate β-lyases may have anti-cancer properties. We conclude that the allium-derived compounds, such as SAMC, exert antiproliferative effects through a metabolite binding of sulfur-containing metabolites directly to redox-sensitive or thiophilic sites on signal proteins or transcription factor (Ganther, 1999), thus arresting cells in mitosis and triggering activation of other pro-apoptotic signaling pathways. Thus, the study of the biochemistry of allium-derived cysteine *S*-conjugates may lead to new avenues for cancer treatment.

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