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CHAPTER · DECEMBER 2007

DOI: 10.1007/978-0-387-77300-1_12

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Role of Cysteine S-Conjugate β-Lyases in the Bioactivation of Renal Toxicants

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Keywords: Cysteine S-conjugates · Cysteine S-conjugate β-lyases · Mercapturate Pathway · Beta-elimination · Pyridoxal 5′-phosphate Enzymes · Pyruvate · Sulfur-containing Elimination Fragment

12.1. Introduction

Halogenated alkenes (e.g., trichloroethylene, tetrachloroethylene, tetrafluoroethylene, and hexachloro-1:3-butadiene) are among several hundred potentially dangerous toxicants listed as present in Superfund sites in the US (http://www.atsdr.cdc.gov/cxcx3.html). Part of the US population is exposed to haloalkenes from such sites and in the workplace (Wu and Schaum 2000), and possibly through "recreational" abuse (Marjot and McLeod 1989). Haloalkenes are nephrotoxic in experimental animals (Dekant, Vamvakas and Anders 1994). In humans, heavy exposure to trichloroethylene is associated with an increased risk of kidney cancer (Brauch et al. 2004). The cysteine S-conjugate formed from trichloroethylene, namely *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC)[1], induces expression of the proto-oncogenes c-*fos* and c-*myc* in LLC-PK_1 cells (a pig kidney cell line) (Vamvakas and Köster 1993).

[1] Abbreviations used: AlaAT, alanine aminotransferase; AGAT II, alanine-glyoxylate aminotransferase isoenzyme II; AspAT, aspartate aminotransferase; AOA, aminooxyacetate; $BCAT_c$, cytosolic branched-chain aminotransferase; $BCAT_m$, mitochondrial branched-chain aminotransferase; BCDHC, branched-chain α-keto acid dehydrogenase complex; BTC, *S*-(2-benzothiazolyl)-L-cysteine; cyt, cytosolic; 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; mit, mitochondrial; PCBC, *S*-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine; PDHC, pyruvate dehydrogenase complex; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; TCA cycle, tricarboxylic acid cycle; TFEC, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine.

A.A. Elfarra (ed.), *Advances in Bioactivation Research*, DOI: 10.1007/978-0-387-77300-1_12,

The nephrotoxicity of halogenated alkenes is due at least in part to their conversion to the corresponding cysteine S-conjugate followed by the action of cysteine S-conjugate β-lyases. In this review, we describe the cysteine S-conjugate β-lyase bioactivation pathway and identify enzymes known to catalyze β-lyase reactions with nephrotoxic halogenated cysteine S-conjugates. We discuss mechanisms whereby reactive fragments generated from these conjugates by the action of cysteine S-conjugate β-lyases modify proteins, including enzymes of energy metabolism and heat shock proteins. We also provide examples of electrophilic xenobiotics other than haloalkenes that may also be bioactivated by mechanisms that involve cysteine S-conjugate β-lyases.

12.2. The Mercapturate Pathway

A large number of reactive electrophiles, whether generated endogenously or ingested, are converted to mercapturates. The mercapturate pathway may be depicted as X (electrophile) → GSX (glutathione S-conjugate) → L-cysteinylglycine S-conjugate → L-cysteine S-conjugate → *N*-acetyl-L-cysteine S-conjugate (mercapturate) (Figure 12.1). (For reviews see, for example, Chasseaud 1976; Stevens and Jones 1989; Cooper and Tate 1997) Mercapturates are often more polar and water soluble than the parent electrophile and are readily excreted. Excretion of the mercapturate represents a detoxification process. However, as discussed below, the mercapturate pathway may on occasion act as a bioactivation pathway if the cysteine S-conjugate is converted by cysteine S-conjugate β-lyases to a reactive sulfur-containing fragment.

The first step in the mercapturate pathway is attack of glutathione thiolate (GS^-) on the electrophile generating the corresponding glutathione S-conjugate. Glutathione S-conjugate formation may occur spontaneously, but in most cases it is catalyzed (or accelerated) by members of a superfamily of glutathione S-transferases (GSTs). Cytosolic GSTs are classified into several classes (A (alpha), M (mu), P (pi), T (theta), Z (zeta), O (omega), and S (sigma)) according to their isoelectric point, substrate and inhibitor properties, antibody recognition, and N-terminal amino acid sequences. Glutathione S-transferases may also be broadly classified on the basis of their cellular location, namely microsomal, mitochondrial, and cytosolic (Hayes, Flanagan and Jowsey 2005). Glutathione S-transferases catalyze three types of reactions (Anders 2004). 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. 12.1), dichloroacetylene (Eq. 12.2)), or an addition–elimination reaction (e.g., with trichloroethylene (Eq. 12.3)). Glutathione S-transferases also catalyze reactions with epoxide moieties (e.g., in the conversion of leukotriene A_4 to leukotriene C_4). Glutathione S-conjugate formation with haloalkenes is catalyzed by both microsomal (MGST1) and cytosolic GSTs in ratios that depend to some extent on the structure of the haloalkene (Anders 2004; Wolf et al. 1984; Wallin et al. 1988; McLellan, Wolf and Hayes 1989; Oesch and Wolf

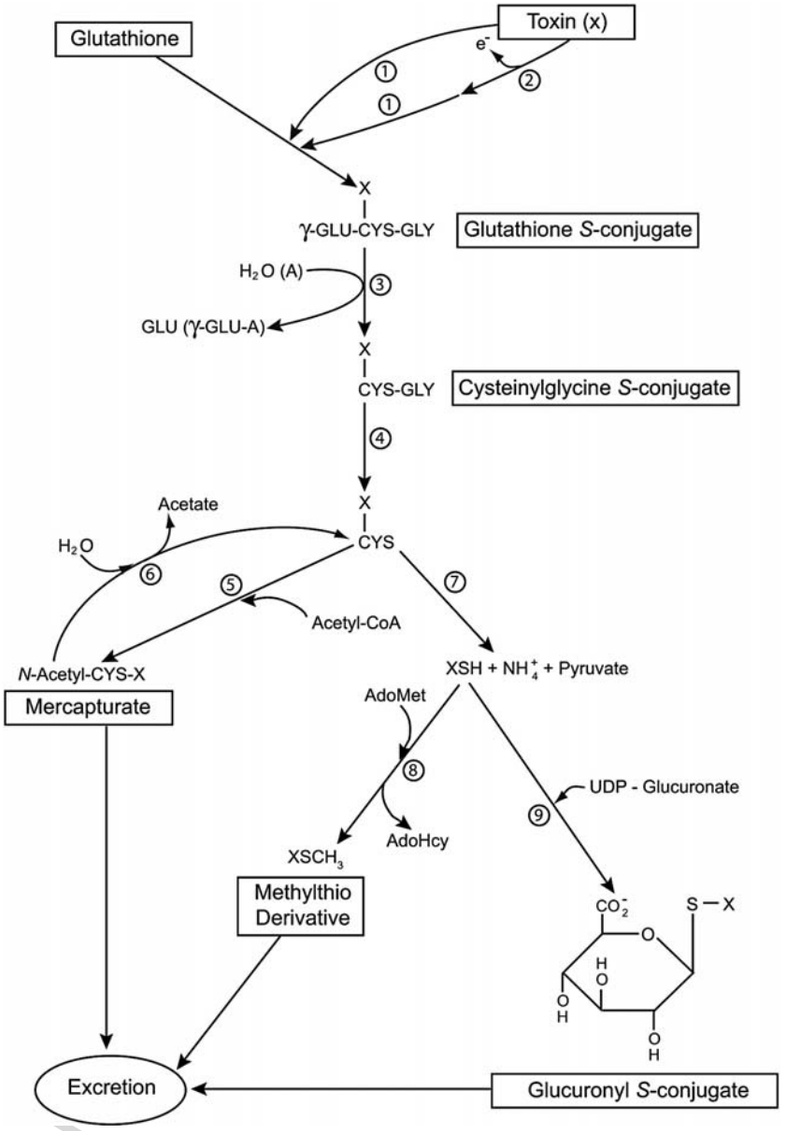

Figure 12.1 Detoxification via the mercapturate pathway and associated side reactions. If the potential toxin contains an electrophilic center it may react directly with GS^- (reaction 1). In that case the GSTs may be regarded as phase I detoxifying enzymes. Alternatively, the toxin may be oxidized via the P450 system prior to the GST-catalyzed reaction to generate a metabolite with an electrophilic center. In that case the glutathione S-transferases may be regarded as phase II detoxifying enzymes and glutathione S-conjugate formation will require two steps (reaction 2 followed by reaction 1). Enzymes: (1) glutathione S-transferases, (2) oxidases that generate an electrophilic center for attack by GS^-; (3) γ-glutamyltransferase (= γ-glutamyl transpeptidase); (4) aminopeptidase M/cysteinylglycinase; (5) *N*-acetyltransferases; (6) aminoacylases; (7) cysteine S-conjugate β-lyases; (8) thiomethyltransferase; (9) UDP-glucuronosyltransferases.
Abbreviations: AdoHcy, *S*-adenosyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine; A, amino acid or dipeptide acceptor for the γ-glutamyltransferase reaction; γ-GLU-A, γ-glutamyl amino acid (or γ-glutamyldipeptide).

1989; Hargus et al. 1991; Cummings, Parker and Lash 2000; Jolivette and Anders 2002, 2003).

$$F_2C = CF_2 + GS^- + H^+ \rightarrow F_2C(H)CF_2SG \quad (12.1)$$

$$ClC \equiv CCl + GS^- + H^+ \rightarrow ClC(H) = C(Cl)SG \quad (12.2)$$

$$Cl(H)C = CCl_2 + GS^- \rightarrow ClC(H) = C(Cl)SG + Cl^- \quad (12.3)$$

Conversion of the glutathione S-conjugate to the L-cysteinylglycine S-conjugate, L-cysteinyl S-conjugate, and mercapturate is catalyzed by the consecutive action of γ-glutamyltransferase, aminopeptidase M/cysteinylglycinase, and an N-acetyltransferase, respectively (Figure 12.1).

12.3. Nephrotoxic Haloalkene Glutathione- and Cysteine S-Conjugates

Trichloroethylene induces (1) aplastic anemia in cattle and (2) kidney damage in all animal species tested, including cattle (Lock et al. 1996). Depending on the structure, halogenated alkenes may be metabolized by cytochrome P450 isoenzymes (especially CYP2E1) and/or by glutathione S-conjugation. The ratio of the two activities varies markedly among the various halogenated alkenes. For example, hexachloro-1:3-butadiene (Wallin et al. 1988) and tetrafluoroethylene (Odum and Green 1984) are metabolized exclusively in rats via glutathione S-conjugation. However, trichloroethylene and tetrachloroethylene are metabolized mainly by cytochrome P450s. Only a small portion (<1%) is metabolized by glutathione S-conjugation (e.g., Koob and Dekant 1991). Nevertheless, although the cytochrome P450 pathway generates toxic species from trichloroethylene and tetrachloroethylene (Bull et al. 2002), a major contributor to the overall toxicity of trichloroethylene and tetrachloroethylene is the corresponding cysteine S-conjugate despite the fact that the glutathione S-conjugation pathway is quantitatively minor (Dekant 2003).

The glutathione S-conjugate, cysteinylglycine S-conjugate, cysteine S-conjugate, and *N*-acetylcysteine S-conjugate (mercapturate) derived from halogenated alkenes are all nephrotoxic/hepatotoxic, and in many cases nephrocarcinogenic/hepatocarcinogenic in experimental animals (e.g., Dekant 2003; Anders 2004; Koob and Dekant 1991; Anders and Dekant 1994, 1998). 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). As noted above, the nephrotoxicity of halogenated cysteine S-conjugates is due at least in part to the action of cysteine S-conjugate β-lyases. Thus, the mercapturate pathway enzymes together with cysteine S-conjugate β-lyases contribute to the bioactivation of halogenated alkenes. (For reviews see, for example, Cooper 1998; Dekant 2003; 2004; Dekant, Vamvakas and Anders 1994.) The reactive fragments generated from haloalkene 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 region and, to a lesser extent, the S2 region (e.g., Jones et al. 1988).

12.4. The Cysteine S-Conjugate β-Lyase Reaction

In 1965, Colucci and Buyske showed that benzothiazole-2-sulfonamide is converted in rats, rabbits, and dogs, not only to the corresponding mercapturate but also to 2-mercaptobenzothiazine in which the sulfur of the mercaptan moiety is derived from GSH. Work from Schultze and colleagues suggested that the toxicity of DCVC is associated with a "C-S" lyase reaction. Experiments with labeled DCVC showed the formation of a reactive sulfur-containing species that adds to macromolecules, including proteins and nucleic acids (e.g., Anderson and Schultze 1965; Bhattacharya and Schultze 1967, 1972). It was known for many years that several electrophilic xenobiotics (e.g., phenacetin, acetaminophen, *N*-hydroxy-2-acetylaminofluorene) are thiomethylated in vivo. It was initially assumed that thiomethylation involves formation of a sulfonium compound derived from methionine followed by decomposition of the sulfonium compound to homoserine lactone plus methylthio compound. However, Chatfield and Hunter (1973) showed that the conversion of 2-acetamido-4-chloromethylthiazole to 2-acetamido-4-methylthiomethylthiazole in rats involves the mercapturate pathway.

The mechanism for the thiomethylation reactions was elucidated by the work of Tateishi and colleagues (1978a,b). When bromazepam was administered to rats, the corresponding mercapturate and 6′-methylthiobromazepam were identified in the bile (Tateishi, Suzuki and Shimizu 1978a). The authors also showed that the thioether bond of the cysteine S-conjugates of 2,4-dinitrobenzene and bromobenzene was readily cleaved by an enzyme present in rat liver cytosol. Incubation of the purified enzyme with *S*-(2,4-dinitrophenyl)cysteine resulted in the formation of pyruvate, ammonium, and 2,4-dinitrobenzenethiol. Incubation of the thiol product with a microsomal thiomethyltransferase and *S*-adenosylmethionine resulted in formation of the corresponding methylthio compound (Tateishi, Suzuki and Shimizu 1978a). Tateishi, Suzuki and Shimizu (1978a,b) coined the term "cysteine conjugate β-lyase" to describe an enzyme that catalyzes β-elimination from a cysteine S-conjugate. The net cysteine S-conjugate β-lyase-catalyzed reaction is shown in Eq. 12.4.

$$XSCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + XSH \quad (12.4)$$

The actual products of the enzyme-catalyzed reaction are XSH and aminoacrylate ($CH_2=C(NH_3^+)CO_2^-$). The latter product undergoes nonenzymatic tautomerization to the α-imino acid ($CH_3C(=NH_2^+)CO_2^-$) and subsequent hydrolysis to pyruvate ($CH_3C(O)CO_2^-$) and ammonium. When the eliminated sulfur-containing fragment (XSH) is stable (i.e., does not contain electrophilic moieties that cause XSH to be converted to an

extremely reactive electrophile), the -SH group may be (1) methylated as in the case of the sulfur-containing fragment derived from *S*-(2,4-dinitrophenyl)cysteine (Tateishi, Suzuki and Shimizu 1978a; Figure 12.1, reaction 8), or (2) glucuronidated as in the case of 2-benzothiazole derived from benzothiazolyl-L-cysteine (BTC) (Elfarra and Hwang 1990; Figure 12.1, reaction 9). Mercapturates, methylthio derivatives, and S-glucuronates are readily excreted.

12.5. Identification of Cysteine S-Conjugate β-Lyases

Although Tateishi, Suzuki, and Shimizu (1978b) obtained a highly purified preparation of a cysteine S-conjugate β-lyase from rat liver they did not identify it. Subsequently, kynureninase (Stevens 1985) and glutamine transaminase K (GTK) (Stevens, Robbins and Byrd 1986) were identified as major cysteine S-conjugate β-lyases of rat liver and kidney cytosol, respectively. Over the last 20 years many more cysteine S-conjugate β-lyases have been identified (Table 12.1). All are pyridoxal 5′-phosphate

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Table 12.1 Mammalian PLP-dependent enzymes with L-cysteine S-conjugate β-lyase activity.[a,b]

	β-Lyase substrates			Syncatalytic inactivation	Competing transamination	Approximatespecific activity (U mg^{-1}[c])
	DCVC	TFEC	BTC			
Enzyme(cytosolic)						
Kynureninase (R)	+	ND	+	+	ND	0.25
GTK/KAT I (R)[d]	+	+	−	−	+	0.6–6.4
cytAspAT (R)	+	+	±	+	−	0.04–0.16
AlaAT (P)	+	+	+	+	−	0.004–0.06
$BCAT_c$ (H)	+	+	+	+	−	0.3–0.5
Cystathionine γ-lyase (R)	−	+	−	−	−	0.05–0.1
Enzyme (mitochondrial)						
mitAspAT (R)	+	+	+	+	+	0.8–2.3
$BCAT_m$ (H)	+	+	−	+	−	0.2–0.5
AGAT II (R)	+	+	+	+	+	0.2
GABA aminotransferase (P) ND	+	ND	ND	ND	0.016	
High-M_r β-lyase (R)	+	+	+	−	+	1.0–1.2

[a]This table is an update of that of Cooper and Pinto (2006). For original references see Cooper and Pinto (2006). 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 cases). ND, not determined. Species abbreviations: R, rat; P, pig; H, human.
[b]AGAT II and cystathionine γ-lyase are homotetramers with M_r values of ∼210 000 and ∼17 600, 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 holoenzyme of ∼90 000 – 110 000.
[c]Activity with DCVC and/or TFEC.
[d]Human liver contains a KAT with strong cysteine S-conjugate β-lyase activity. A cysteine S-conjugate β-lyase has been highly purified from human kidney. The lyase activity co-purifies with GTK. The human GTK, unlike the rat enzyme, has activity with BTC. In the rat, some GTK activity is also present in mitochondria.
Abbreviations: KAT I, kynurenine aminotransferase isoenzyme I.

(PLP)-containing enzymes and include (1) the cytosolic enzymes kynureninase, GTK, cytosolic aspartate aminotransferase (cytAspAT), alanine aminotransferase (AlaAT), and cytosolic branched-chain aminotransferase ($BCAT_c$), and (2) the mitochondrial enzymes mitochondrial aspartate aminotransferase (mitAspAT), mitochondrial branched-chain aminotransferase ($BCAT_m$), alanine-glyoxylate aminotransferase isozyme II (AGAT II), and GABA aminotransferase (Cooper and Pinto 2006). In addition, high-M_r β-lyases occur in both cytosolic and mitochondrial fractions of rat kidney and, to a lesser extent, rat liver. High-M_r forms in rat kidney cytosol and mitochondria contain GTK and mitAspAT, respectively (manuscript in preparation).

Most cysteine S-conjugate β-lyases identified thus far are aminotransferases (Table 12.1), and a transamination reaction may therefore compete with the β-elimination reaction. When transamination competes with the β-lyase reaction, an α-keto acid substrate (or PLP) must be present in the reaction mixture to maintain 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. The α-keto acid substrate forms a Schiffs base with PMP that is converted to the corresponding amino acid and PLP. The PLP form of the enzyme can then catalyze another round of the β-lyase reaction (Stevens, Robbins and Byrd 1986; Cooper 1998).

Table 12.1 indicates that cysteine S-conjugate β-lyases exhibit some degree of substrate specificity toward the commonly used β-lyase substrates (DCVC, TFEC, BTC). For example, DCVC and TFEC are β-lyase substrates of rat kidney GTK, whereas BTC is not. Curiously, however, BTC has been reported to be a substrate of human kidney GTK (Lash et al. 1990). On the other hand, all three cysteine S-conjugates are substrates of rat mitAspAT. *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC), but not DCVC, is a β-lyase substrate of γ-cystathionase. Table 12.1 also indicates that many of the cysteine S-conjugate β-lyases are prone to syncatalytic (i.e., self) inactivation. For example, rat liver mitAspAT-catalyzed β-elimination with TFEC leads to inactivation on average after about 2700 turnover events (Cooper, Bruschi and Anders 2002). Inactivation of human $BCAT_m$ and $BCAT_c$, in the presence of TFEC, occurs on average after about 200 and 45 turnover events, respectively (Cooper et al. 2003). Thus, factors such as the presence or absence of α-keto acids in the assay mixture, length of incubation time, the 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.

12.6. Nephrotoxicity of Haloalkene Cysteine S-Conjugates

Although GSTs are present in most tissues, the liver is the major organ contributing to the formation of glutathione S-conjugates (Anders 2004). As noted above, the conversion of the glutathione S-conjugate to the corresponding cysteine S-conjugate is catalyzed by the sequential

action of γ-glutamyltransferase and aminopeptidase M/cysteinylglycinase (Figure 12.1). These ectoenzymes are present in epithelial tissues such as bile duct, intestine, renal tubules, and choroid plexus. The cysteine S-conjugates are taken up in the kidneys by glomerular filtration or more importantly by active uptake (Anders 2004). Both Na^+-dependent and Na^+-independent transporters have been implicated in the renal uptake of DCVC (e.g., Lash and Anders 1989; Schaeffer and Stevens 1987a, 1987b; Anders 2004). Most of these studies have focused on uptake of DCVC across the basolateral (peritubular) membrane of the renal proximal cells (Wright et al. 1998). Wright et al. (1998) showed that DCVC is taken up by a Na^+-dependent uptake system in isolated renal brush-border membrane vesicles (Wright et al. 1998). Several neutral amino acids, but especially phenylalanine, cysteine, and leucine, compete with uptake of DCVC across the luminal membrane in this system (Wright et al. 1998).

Acivicin, a selective inhibitor of γ-glutamyltransferase, protects renal tubule cells against DCVC-induced toxicity (Elfarra, Jakobson and Anders 1986; Lash and Anders 1986). On the other hand, it has been reported that acivicin does not protect rats against the nephrotoxicity of hexachloro-1:3-butadiene (Davis 1988). However, others have shown that *S*-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine (PCBC; the cysteine S-conjugate of hexachloro-1:3-butadiene) is cytotoxic to LLC-PK_1 cells (Blackmore et al. 2002). Aminooxyacetate (AOA), a general inhibitor of PLP enzymes, protects against DCVC-induced toxicity (e.g., Lash et al. 1986; Beuter et al. 1989; Chen et al. 1990; Chen, Jones and Stevens 1994; Blackmore et al. 2002) and PCBC-induced toxicity (Blackmore et al. 2002) in LLC-PK_1 cells. AOA partially protects against DCVC-induced toxicity in isolated rat kidney proximal tubules (Lash and Anders 1986), but apparently not in isolated human renal proximal tubule cells (Cummings and Lash 2000). α-Keto acids stimulate the toxicity of DCVC toward rat renal cells (Elfarra, Lash and Anders 1987). Exacerbation of toxicity by α-keto acids suggests involvement of aminotransferases in the bioactivation process. Taken together, despite an occasional study to the contrary, the literature strongly supports important roles for γ-glutamyltransferase and cysteine S-conjugate β-lyases in promoting the nephrotoxicity of halogenated alkenes. However, it appears that human renal tissue is less susceptible than that of rat to damage from haloalkene cysteine S-conjugates, presumably as a result of lower specific activities of cysteine S-conjugate β-lyases in human renal tissue and allometric scaling (see discussion by Anders 2005).

Although the evidence is strong that cysteine S-conjugate β-lyases contribute to the nephrotoxicity of DCVC and other halogenated cysteine S-conjugates, other studies support additional mechanisms for bioactivation. DCVC can be oxidized by a flavin-containing monooxygenase to DCVC sulfoxide (Park et al. 1992), which may be even more nephrotoxic than DCVC itself (Lash et al. 1994, 2003). Moreover, the mercapturates derived from both trichloroethylene and tetrachloroethylene are both bioactivated to their sulfoxide forms by a cytochrome P450 (CYP3A) (Werner, Birner and Dekant 1996). One possibility for the toxicity of DCVC sulfoxide is that cysteine S-conjugate β-lyases catalyze

the elimination of a reactive sulfenic acid [RS(O)H], which forms a covalent adduct to macromolecules. However, AOA is not protective against the nephrotoxicity of DCVC sulfoxide in rats and only partially protects against DCVC (Lash et al. 1994). Thus, sulfoxidation of DCVC is a bioactivation event in addition to, and probably distinct from, the β-lyase pathway (Lash et al. 1994). DCVC sulfoxide was shown to react with GSH generating the glutathione S-conjugate, *S*-[1-chloro-2-(*S*-glutathionyl)vinyl]-L-cysteine sulfoxide, which was isolated from the bile of rats given DCVC sulfoxide (Sausen and Elfarra 1991). It has been suggested that the nephrotoxicity of DCVC sulfoxide is related in part to oxidative stress, possibly as a result of depleted mitochondrial GSH stores (Lash et al. 1994, 2003). The β-lyase pathway may result in greater bioactivation of DCVC than does the oxidation pathway in rat proximal tubules, whereas the converse may be true for human proximal tubules (Cummings and Lash 2000). Thus, although this review focuses on bioactivation of haloalkene cysteine S-conjugates via cysteine S-conjugate β-lyases, it is important to note that other bioactivation pathways exist. Moreover, species and sex differences in the mechanisms by which certain haloalkene cysteine S-conjugates are bioactivated must also be considered when evaluating the nephrotoxicity of halogenated cysteine S-conjugates.

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 (Anders 2004).

12.7. β-Lyase-Catalyzed Generation of Reactive Fragments from Nephrotoxic Halogenated Cysteine S-Conjugates

Active-site-generated aminoacrylate can cause syncatalytic inactivation of enzymes that catalyze β-elimination reactions with amino acids containing a good leaving group in the β-position. For example, pig heart cytAspAT is syncatalytically inactivated by β-chloro-L-alanine (Morino and Okamoto 1973) and L-serine O-sulfate (Ueno, Likos and Metzler 1982). Thiosulfate protects this enzyme against aminoacrylate generated from both L-serine O-sulfate and L-cysteine sulfinic acid (Cavallini et al. 1973). Michael addition of thiosulfate to the double bond yields cysteine sulfonic acid. Thus, thiosulfate competes with cytAspAT for interaction with aminoacrylate (Cavallini et al. 1973). Historically, it is interesting to note that the "C-S" lyase purified by Anderson and Schultze (1965) was rapidly inactivated by products of the reaction with DCVC. Moreover, protection was afforded in part by GSH (Anderson and Schultze 1965). Possibly, the "C-S" lyase was inactivated in part by aminoacrylate and in part by a reactive sulfur-containing fragment (see below). In that case, GSH may have protected against inactivation by competing with a susceptible enzyme moiety for Michael addition to aminoacrylate.

Initially, it was suggested that inactivation of pig heart cytAspAT by aminoacrylate, generated from β-lyase reactions, is due to modification of

an active-site lysine moiety (Morino and Okamoto 1973). Ueno and colleagues (1982), however, showed that aminoacrylate adds to the PLP coenzyme, resulting in the formation of a pyruvate-PLP aldol condensation product, which could also result in inactivation. Thus, the nephrotoxicity of halogenated cysteine S-conjugates could theoretically be due to the syncatalytic inactivation of key PLP enzymes by aminoacrylate either reacting with susceptible protein moieties or by direct attack on the aldehyde group of PLP. However, toxicity through these mechanisms may be limited to special cases because cysteine S-conjugates that undergo β-elimination are not generally toxic if the eliminated sulfur-containing fragment is unreactive (i.e., does not contain electronegative substituents), despite the fact that aminoacrylate will be produced. An example of a relatively nontoxic cysteine S-conjugate that undergoes enzyme-catalyzed β-elimination in vivo is BTC. As noted above, a sulfur-containing elimination fragment is glucuronidated and excreted in rats treated with BTC (Elfarra and Hwang 1990). Possibly, the aminoacrylate is scavenged by endogenous small-M_r nucleophiles (e.g., GSH), or syncatalytic loss of activity of PLP-containing enzymes is relatively slow.

On the other hand, if the eliminated sulfur-containing fragment is reactive (i.e., contains electronegative substituents, as is the case with cysteine S-conjugates derived from halogenated alkenes) the parent cysteine S-conjugate may be toxic, especially to the kidneys. The cysteine S-conjugate β-lyase reaction with DCVC (**1**) gives rise to pyruvate (**2**), ammonium and a sulfur-containing fragment that has the theoretical structure 1,2-dichloroethylenethiolate (**3**) (Figure 12.2). However, 1,2-dichloroethylenethiolate is extremely unstable and may tautomerize to chlorothioacetyl chloride ($ClC(=S)CH_2Cl$) (not shown) or spontaneously lose Cl^- to form the highly reactive chlorothioketene (**4**) (Anders 2004; and references cited therein). The chlorothioketene reacts with a variety of nucleophiles (Nu:) to generate a thioacylated adduct (**5**). In biological systems the nucleophile can be macromolecules, such as nucleic acids (Müller et al. 1998) and proteins (Eyre et al. 1995). This chemistry provides an explanation for the previous finding of Anderson and Schultze (1965), who as alluded to above, showed covalent incorporation of a sulfur-containing fragment from DCVC into macromolecules via the action of "C-S" lyases.

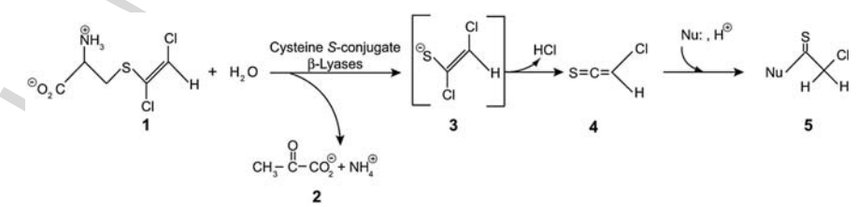

Figure 12.2 Bioactivation of DCVC by cysteine S-conjugate β-lyases. DCVC (**1**) is converted to pyruvate (**2**), ammonium, and a sulfur-containing fragment that has the theoretical structure 1,2-dichloroethylenethiolate (**3**). 1,2-Dichloroethylenethiolate is unstable and loses HCl to form the highly reactive chlorothioketene (**4**). The thioketene reacts with tissue nucleophiles (Nu:) to generate thioacylated products (**5**). Based in part on Anders (2004). AQ5

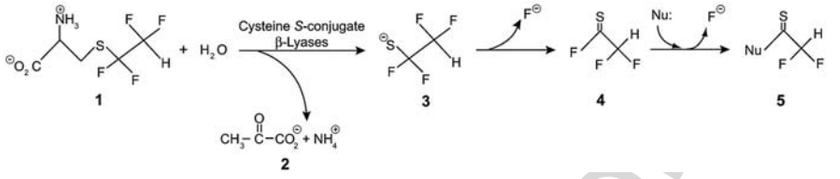

Figure 12.3 Bioactivation of TFEC by cysteine S-conjugate β-lyases. TFEC (**1**) is converted to pyruvate (**2**), ammonium, and a sulfur-containing fragment that has the structure 1,1,2,2-tetrafluoroethanethiolate (**3**). 1,1,2,2-Tetrafluoroethanethiolate (**3**) is unstable and loses F^- to form difluorothioacetyl fluoride (**4**). Difluorothioacetyl fluoride reacts with tissue nucleophiles (Nu:) to generate thioacylated products (**5**). Based in part on Anders (2004). Note that **3** may also react by a separate pathway. Thus, addition of **3** to a lysine residue in a protein may result in loss of H_2S and modification of the ε-amino group by replacement of an H with $-CF_2CF_2(H)$ (see Figure 12.4).

Cysteine S-conjugate β-lyase-catalyzed bioactivation of TFEC is shown in Figure 12.3. The β-lyase reaction with TFEC (**1**) results in the formation of pyruvate (**2**), ammonium and 1,1,2,2-tetrafluoroethanethiolate (**3**). 1,1,2,2-Tetrafluoroethanethiolate is very reactive, losing F^- to generate difluorothioacetyl fluoride (**4**), which thioacylates nucleophiles. ^{35}S-Labeling studies performed in vivo show that phosphatidylethanolamine is a major nucleophile thioacylated in mitochondria by the action of cysteine S-conjugate β-lyases on TFEC (Hayden et al. 1992). The ε-amino group of protein lysyl moieties are also especially vulnerable to thioacylation by **4** (Hayden and Stevens 1990; Harris, Dekant and Anders 1992; Fisher et al. 1993; Hayden et al. 1991).

Rat liver mitAspAT is syncatalytically inactivated by TFEC following fewer β-lyase turnover events than with β-chloroalanine (Cooper et al. 2002). This finding was ascribed to the fact that only one reactive fragment is generated from β-chloroalanine (i.e., aminoacrylate) whereas two reactive fragments are generated from TFEC, namely aminoacrylate and 1,1,2,2-tetrafluoroethanethiolate ($^-SC(F_2)C(F)_2H$). We have recently begun to investigate the mechanism by which rat liver mitAspAT is syncatalytically inactivated by TFEC. Mass spectrometric analysis showed that two active-site lysyl moieties are modified by addition of a fragment that results in a net gain of 100 amus for each lysyl moiety. The finding is consistent with a $-CF_2C(F)_2H$ linkage to the ε-amino group of a lysyl residue. This linkage apparently results from direct attack of the ε-amino group of a lysine residue on tetrafluoroethanethiolate with loss of hydrogen sulfide. This type of addition has not been described previously, and may have been obscured in studies with ^{35}S-labeled TFEC as a result of loss of radiolabel as H_2S. Interestingly, another lysyl moiety distal from the active site is modified by 96 amus, which is consistent with thioacylation. Finally, one cysteine residue was noted to be modified by 87 amus consistent with Michael addition of a cysteine thiolate to aminoacrylate generating a lanthionine moiety.[2] Mechanisms whereby fragments derived from a β-lyase reaction on TFEC form covalent adducts with proteins are shown in Figure 12.4.

[2] The mass spectral data have been presented at a national meeting (Villar et al. 2007).

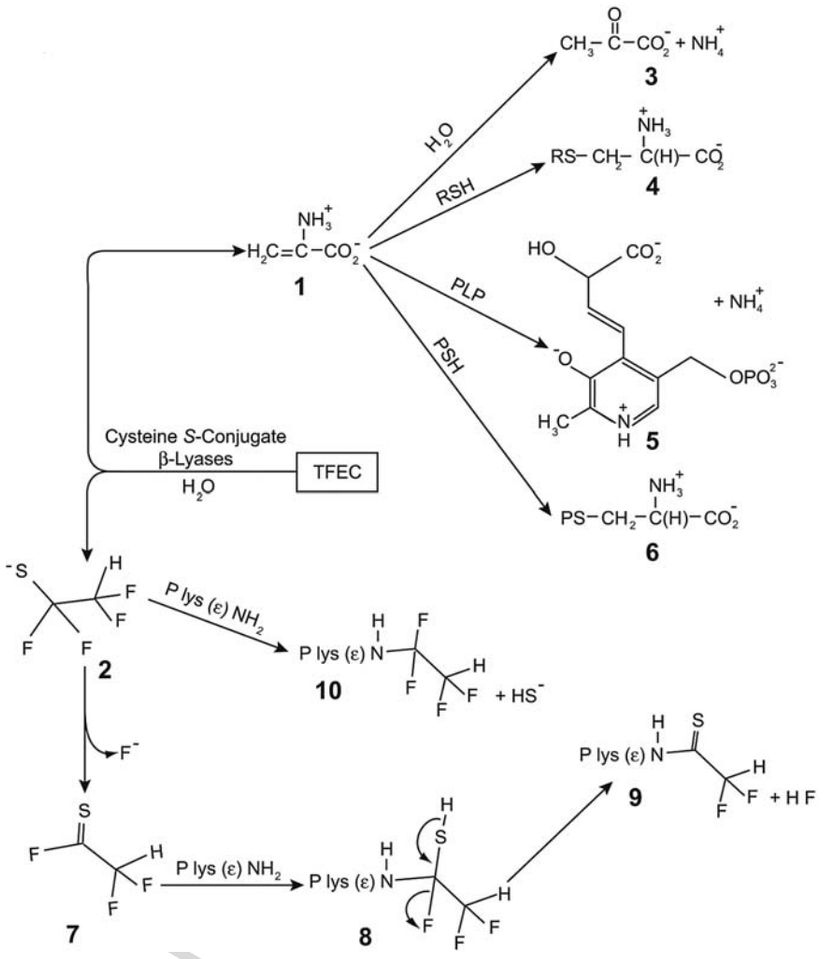

Figure 12.4 Protein modifications resulting from the β-lyase reaction on TFEC. The initial products of the β-lyase reaction are aminoacrylate (**1**) and 1,1,2,2-tetrafluoroethanethiolate (**2**). Aminoacrylate tautomerizes and hydrolyzes to pyruvate (**3**) and ammonium. However, in the presence of suitable nucleophiles such as RSH (e.g., GSH, thiosulfate), the aminoacrylate can be trapped as a "new" cysteine S-conjugate (**4**). It is also possible that aminoacrylate generated from TFEC will react with PLP at the active site of a susceptible enzyme to form the pyruvate-PLP aldol product (**5**). Michael addition of the sulfhydryl of a cysteinyl residue (PSH) to aminoacrylate will result in the formation of a protein-bound lanthionine residue [$PSCH_2CH(NH_3^+)CO_2^-$, **6**]. 1,1,2,2-Tetrafluoroethanethiolate (**2**) loses F^- to generate difluorothioacetyl fluoride (**7**). Reaction of the ε-amino group of a protein lysine residue (P-lys(ε)-NH_2) with **7** results in the formation of the tetrahedral intermediate (**8**). The tetrahedral intermediate (**8**) decomposes to thioacylated protein (**9**) plus HF. Interestingly, some of the 1,1,2,2-tetrafluoroethanethiolate (**2**) resulting from the β-elimination reaction with TFEC reacts directly with the ε-amino group of lysine residues generating a structure (**10**) that contains a $-C(F_2)C(F_2)H$ grouping attached at the N.

12.8. Mechanisms Contributing to the Nephrotoxicity of Haloalkene Cysteine S-Conjugates – Toxicant Channeling

Haloalkene cysteine S-conjugates are especially toxic to mitochondria (e.g., Groves et al. 1993; Chen et al. 2001). Thus, any mechanism that demonstrates nephrotoxicity of haloalkene cysteine S-conjugates must take into account the susceptibility of renal mitochondria and the bioactivation of these compounds via mitochondrial cysteine S-conjugate β-lyases.

Toxicity of DCVC to kidney cells has been associated with (1) covalent modification of macromolecules, (2) depletion of nonprotein thiols (presumably mostly GSH), and (3) lipid peroxidation (Beuter et al. 1989; Chen et al. 1990). These effects may be attributable to formation of metabolites derived from a β-lyase reaction since, as mentioned above, AOA can partially protect against DCVC toxicity in renal tissues and in renal cells in culture. Mechanisms by which metabolites of DCVC and other nephrotoxic haloalkene cysteine S-conjugates alter thiol status may involve direct inhibition of glutathione reductase (Lock and Schnellmann 1990) and generation of oxidative stress that leads indirectly to oxidation of thiol compounds (Chen et al. 1990). Oxidation of DCVC to DCVC sulfoxide followed by formation of a glutathione S-conjugate resulting from the reaction of DCVC sulfoxide with GSH may also lead to oxidative stress (Sausen and Elfarra 1991; Lash et al. 1994). Other studies lead us to suggest an additional possibility, namely that oxidative stress may result in part from Michael addition of thiols to aminoacrylate generated in the β-lyase reaction. In experiments in which LLC-PK_1 cells were exposed to DCVC, loss of nonprotein thiols was about 40%–60% (Chen et al. 1990). Interestingly, the authors suggested that depletion of nonprotein thiols to this extent was insufficient to kill the cells. As cited earlier, BTC is relatively nontoxic, despite the fact that it is metabolized in vivo via cysteine S-conjugate β-lyases (Elfarra and Hwang 1990). We are unaware of any studies on GSH status in renal cells/tissue resulting from exposure to BTC. Evidently, even if GSH is depleted in renal cells exposed to BTC, this insult may not be sufficient to induce overt toxicity. The implication of the work by Chen et al. (1990) is that in order for a cysteine S-conjugate to exhibit toxicity, it must generate reactive sulfur-containing fragments that covalently add to macromolecules. The toxicity may be enhanced, perhaps synergistically, by oxidative stress and by Michael adduction between aminoacrylate and susceptible nucleophiles such as GS^- and protein sulfhydryls and/or by modification of coenzyme in susceptible PLP enzymes.

Adduct formation with reactive fragments generated from a cysteine S-conjugate β-lyase reaction in vivo has been studied extensively using TFEC. For example, Bruschi et al. (1993) showed that six kidney mitochondrial proteins are thioacylated after rats are administered TFEC. No thioacylation of cytosolic proteins was detected. The six proteins were identified as HSP60, mitHSP70, mitAspAT, aconitase, the E_2k (dihydrolipoamide succinyl transferase) enzyme component of

the α-ketoglutarate dehydrogenase complex (KGDHC), the E_3 (dihydrolipoamide dehydrogenase) enzyme component of KGDHC, and the E_3 enzyme component of the branched-chain keto acid dehydrogenase complex (BCDHC) (Bruschi et al. 1993; Bruschi, Crabb and Stevens 1994; Bruschi, Lindsay and Crabb 1998; James et al. 2002). Interestingly, Lock and Schnellmann (1990) had previously reported that metabolites of haloalkene cysteine S-conjugates (DCVC, TFEC, PCBC) inhibited dihydrolipoyl dehydrogenase (E_3). Our findings that mitAspAT catalyzes a β-lyase reaction with TFEC in vitro and is syncatalytically inactivated in the process (Cooper et al. 2002) is consistent with the in vivo finding that this enzyme in kidney is thioacylated after administration of TFEC to rats. We also showed that a high-M_r β-lyase present in kidney co-purifies with HSP70 (Cooper et al. 2001). As cited earlier, the high-M_r β-lyase in rat kidney mitochondria contains mitAspAT. This finding provides a mechanism for the thioacylation of kidney mitHSP70 in rats administered TFEC. Conceivably, mitochondrial HSP60 may also associate with a PLP-containing enzyme that catalyzes a β-lyase reaction with TFEC.

Although the E_2k and E_3 enzyme components of KGDHC (and the E_3 enzyme component of BCDHC) are thioacylated in kidney mitochondria of rats administered TFEC, the E_2p (dihydrolipoamide acetyl transferase) and E_3 enzyme components of pyruvate dehydrogenase complex (PDHC) are not (Bruschi, Lindsay and Crabb 1998; James et al. 2002). Moreover, the specific activity of KGDHC, but not that of PDHC, is diminished in the kidneys of 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 E_3 is a common component of KGDHC, PDHC, and BCDHC (and the glycine cleavage system). In the presence of TFEC and purified GTK (a source of thioacylating moieties), purified PDHC is more resistant to in vitro inactivation than is purified KGDHC (Park et al. 1999). Moreover, there is some evidence that E_3 enzyme components are bound to the complex via E_2 enzyme components in PDHC, whereas E_3 enzyme components are bound to the complex via E_1 enzyme components 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 differences in the arrangement of its constituent enzymes. In addition, PDHC is not associated with any aminotransferases/β-lyases, whereas KGDHC is closely associated with mitAspAT. Thus, the susceptibility of KGDHC to thioacylation by a fragment derived from TFEC may be due to the proximity of mitAspAT or to actual co-localization with subunit enzymes (James et al. 2002; Park et al. 1999).

Several tricarboxylic acid cycle (TCA) and associated enzymes are arranged in supramolecular complexes (metabolons) that facilitate channeling of substrate from one enzyme to another. For example, mitAspAT is part of a metabolon affiliated or localized with KGDHC (see references cited in Cooper, Bruschi and Anders 2002). Based on our findings (Cooper et al. 2002, 2003) and those of Bruschi and colleagues (Bruschi, Lindsay and Crabb 1998; James et al. 2002), we have proposed that not only are metabolites channeled through supramolecular complexes in the TCA

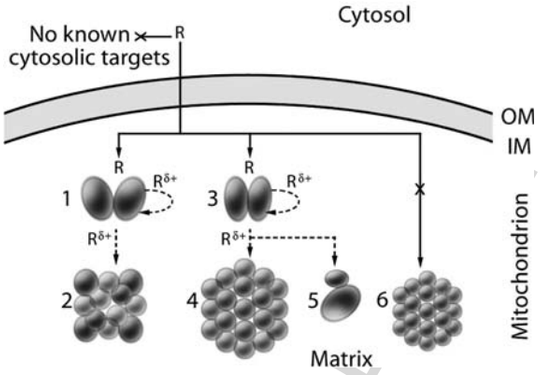

Figure 12.5 Model for toxicant channeling of β-lyase-derived TFEC products in mitochondria in vivo. TFEC (**R**) is transported into the mitochondrion where it is converted by cysteine S-conjugate β-lyases to toxicant(s) ($\mathbf{R}^{\delta+}$). The three mitochondrial α-keto acid dehydrogenase complexes (BCDHC, KGDHC, PDHC) are represented as multimeric units labeled **2**, **4**, and **7**, respectively. 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 toxicants to BCDHC (**2**) and KGDHC (**4**)/aconitase (**5**), respectively, resulting in their inactivation. PDHC (**7**) is not known to be associated with any aminotransferase/cysteine S-conjugate β-lyase (**6**) and is not directly inactivated. The curved arrows represent syncatalytic inactivation of $BCAT_m$ and mitAspAT, respectively.
Abbreviations: IM, inner membrane; OM outer mitochondrial membrane. From Cooper and Pinto (2006).

cycle, but toxicants as well (Cooper, Bruschi and Anders 2002; Figure 12.5). This concept explains not only the susceptibility of KGDHC to TFEC-induced inactivation in rat kidney and cells in culture, but also the susceptibility of aconitase and BCDHC to thioacylation/inactivation. Aconitase is part of a metabolon that includes KGDHC and mitAspAT (Ovádi and Srere 2000). BCDHC is part of a metabolon that includes $BCAT_m$ (Van Horn et al. 2004).

Cysteine S-conjugate β-lyases are ubiquitous in the body. For example, mitAspAT, a major mitochondrial cysteine S-conjugate β-lyase, is present in every cell that contains mitochondria. How then can one explain the unusual susceptibility of kidney to halogentaed cysteine S-conjugates? Many factors are presumably involved, including relative distribution of *N*-acetyltransferase, aminoacylases, cellular and mitochondrial uptake mechanisms, and concentrations of 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, the kidneys receive 20% of the cardiac output; yet comprise <1% of the body weight (Pfaller and Gstraunthaler 1998).

Despite these factors, haloalkene cysteine S-conjugate-induced toxicity is not necessarily confined to renal tubules. As we have noted above, toxicity may also occur in the liver and occasionally in neural tissue, presumably as a consequence of the widespread occurrence of cysteine S-conjugate β-lyases.

12.9. Metabolism of Electrophiles Other than Haloalkenes via the Mercapturate/Cysteine S-Conjugate β-Lyase Pathways

The carbonic anhydrase inhibitor methazolamide is metabolized to both a glutathione S- and a cysteine S-conjugate. The latter is a substrate of cysteine S-conjugate β-lyase(s) in bovine kidney and liver homogenates (Kishida et al. 2001). Since cysteine S-conjugate β-lyases are also presumably present in eye tissues, the β-elimination reaction may account for the binding of a metabolite of methazolamide to macromolecules and for the specific ocular toxicity (Kishida et al. 2001).

Cisplatin is used to treat germ cell tumors, head and neck tumors, and cervical cancer even when the cancer has metastasized. Unfortunately, its effectiveness against other cancers is limited because at the doses required to exhibit anticancer properties it is toxic to renal proximal tubule cells and especially to the mitochondria in these cells (reviewed in Zhang and Hanigan 2003). DNA damage is the primary mechanism by which cisplatin kills tumor and other rapidly dividing cells. However, the renal proximal tubule cells are well-differentiated, nondividing cells that are not killed by other DNA-damaging agents. Evidence has been presented that damage to kidney cells is due to conversion of cisplatin to its glutathione S-conjugate and subsequently to its cysteine S-conjugate. The cysteine S-conjugate is then bioactivated by cysteine S-conjugate β-lyase(s) to generate a fragment containing a Pt-SH moiety (Eq. 5). This Pt-SH fragment is proposed to react with macromolecules at thiophilic centers (Zhang and Hanigan 2003).

$$(H_2N)_2Pt(Cl)SCH_2CH(NH_3^+)CO_2^- + H_2O \rightarrow CH_3C(O)CO_2^- + NH_4^+ + (H_2N)_2Pt(Cl)SH \quad (12.5)$$

After mice were treated with cisplatin, proteins in kidney mitochondria were more platinated than proteins in the cytosolic fraction (Zhang et al. 2006). Moreover, the platination was decreased in the mitochondrial fraction, but not in the cytosolic fraction, in mice pretreated with AOA. The specific activities of mitAspAT, aconitase, and especially KGDHC were decreased in LLC-PK_1 cells treated with cisplatin (Zhang et al. 2006). The specific activity of KGDHC was decreased even further in cisplatin-treated LLC-PK_1 cells overexpressing mitAspAT. The data are consistent with the hypothesis that the cisplatin cysteine S-conjugate is a β-lyase substrate of mitAspAT and that the released Pt-SH fragment reacts with proteins in kidney mitochondria especially KGDHC.

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 kidney, liver, and occasionally neural tissue (reviewed by Anders 2005). When an anesthetic gas is used in association with a desiccated carbon dioxide absorbent, severe heat buildup in the anesthetic circuit, particularly in the CO_2 absorber canister, can lead to 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) (Kanhai et al. 1991). Thus, assuming that the activities of γ-glutamyltransferase and aminopeptidase M/cysteinylglycinase are not limiting, toxic DCVC should form more readily from dichloroacetylene than from trichloroethylene in vivo (Kanhai et al. 1991). The above-mentioned findings have spurred the development of newer anesthetic gas ventilators that minimize the risk of conversion of anesthetic gases to toxic breakdown products (Anders 2005). Toxic effects of trichloroethylene exposure to base have been well documented particularly to the facial nerves in humans (reviewed by Anders 2004). However, whereas compound A has been found to be nephrotoxic to rats and toxic to isolated human kidney tubules, injury to human kidneys in vivo appears to be minimal (Anders 2004; Kharasch et al. 2005). Most likely the difference between rats and humans resides in the lower overall renal cysteine S-conjugate β-lyase activity in humans than in rats.

2,4′,5-Trichlorobiphenyl (a polychlorinated biphenyl or PCB) is metabolized through the mercapturate pathway (Bakke, Bergman and Larsen 1982). This PCB is thought to be converted to an epoxide by the action of P450 enzymes. The epoxide then reacts with GS^- to form the corresponding glutathione S-conjugate, which is eventually converted to the mercapturate and excreted in the bile. However, several other metabolites were also detected in the feces of rats administered 2,4′,5-trichloro[^{14}C] biphenyl, namely 2,4′,5-trichlorobiphenyls ring-substituted with -SCH_3, -$S(O)CH_3$, or -$S(O_2)CH_3$ (Bakke, Bergman and Larsen 1982). Evidently, the glutathione S-conjugate of trichlorobiphenyl is converted to the corresponding cysteine S-conjugate, which then undergoes a β-lyase reaction with subsequent formation of a trichlorobiphenyl metabolite containing an -SH substituent that is methylated and partially oxidized. It is not clear whether the -SH-substituted PCB is more toxic (i.e., bioactivated) relative to the parent PCB. Metabolism of PCBs through the mercapturate/cysteine S-conjugate β-lyase pathways deserves more study.

4-Amino-2,6-dichlorophenol is nephrotoxic in Fischer 344 rats. Both AOA and the acivicin are protective (Hong et al. 1997; Song, Lang and Chen 1999), suggesting that nephrotoxicity involves conversion of a glutathione S-conjugate metabolite to a cysteine S-conjugate followed by a β-lyase reaction.

It is well known that high doses of acetaminophen are hepatotoxic especially to alcoholics and diabetics, but the exact mechanism is not known. The cytochrome P450 enzyme CYP2E1 appears to be a contributing factor in acetaminophen hepatotoxicity (e.g. Cheung et al. 2005). Acetaminophen is oxidized in vivo to a reactive quinoneimine by the action of CYP2E1. The quinoneimine can react with protein -SH groups and also with GSH to form a semistable *ipso* adduct or a relatively stable

adduct at the C3 position (reviewed in Monks and Jones 2002). If these glutathione S-conjugates are converted to the cysteine S-conjugates in vivo then they might be substrates of cysteine S-conjugate β-lyases. This possibility appears to have been largely overlooked. However, it is interesting that when [^{14}C]paracetamol (acetaminophen) was administered to hamsters, a small amount of labeled methyl 2-hydroxy-5-acetamidophenylsulfone was detected in the urine (Wong et al. 1976). Formation of this sulfone may be rationalized by invoking a β-lyase reaction on the cysteine S-conjugate of acetaminophen followed by methylation and oxidation of the sulfur.

12.10. Conclusion

Conversion of an electrophile to the corresponding mercapturate and excretion represents detoxification. However, the mercapturate pathway may sometimes bioactivate (toxify) an electrophile. If the cysteine S-conjugate formed in the mercapturate pathway contains a good electron-withdrawing group attached to the sulfur, it may undergo a β-elimination reaction. This reaction is catalyzed by several PLP-containing enzymes (cysteine S-conjugate β-lyases) that are normally involved in amino acid metabolism. If the eliminated sulfur-containing fragment is reactive (e.g., by adding to macromolecules) the parent cysteine S-conjugate may be toxic especially to the kidneys. Electrophiles that are bioactivated by this mechanism include halogenated alkenes and drugs such as methazolamide and cisplatin. Some PCBs and aminophenols may also be metabolized in part by pathways involving cysteine S-conjugate β-lyases. However, the contribution of the β-lyase reaction to the toxicity of these compounds is not clear. Mitochondrial enzymes of energy metabolism are especially vulnerable to reactive fragments generated from toxic cysteine S-conjugates, by a process that we have termed toxicant channeling. Humans are exposed to a large number of exogenously and endogenously produced electrophiles. It is, therefore, possible that cysteine S-conjugate β-lyases contribute to mitochondrial dysfunction of aging and disease.

In view of (1) the large number of mammalian cysteine S-conjugate β-lyases identified to date, (2) their overlapping specificities, (3) their widespread occurrence in tissues, and (4) their presence in different subcellular compartments (e.g., cytosol, mitochondria, and peroxisomes), the potential of these enzymes for generating toxic products in different subcellular compartments, not only in the kidneys but also in other tissues, is highly significant. We suggest that bioactivation of some natural products, certain drugs, and endogenously produced electrophiles via pathways that include cysteine S-conjugate β-lyases may be more common than is generally appreciated.

Acknowledgements. Part of the work cited from the authors' laboratory was supported by NIH grant RO1 ES8421. We thank Dr. Antonio Artigues, the University of Kansas Medical Center for Mass spectral analysis of TFEC-inactivated mitAspAT.

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Chapter 12

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