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# MECHANISM OF *S*-(1,2-DICHLOROVINYL)GLUTATHIONE-INDUCED NEPHROTOXICITY

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**Abstract**—*S*-(1,2-Dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-DL-cysteine are potent nephrotoxins. Agents that inhibit γ-glutamyl transpeptidase, cysteine conjugate β-lyase, and renal organic anion transport systems, namely L-(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125), aminooxyacetic acid, and probenecid, respectively, protected against *S*-conjugate-induced nephrotoxicity. Furthermore, *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine, which cannot be cleaved by cysteine conjugate β-lyase, was not nephrotoxic. These results strongly support a role for renal γ-glutamyl transpeptidase, cysteine conjugate β-lyase, and organic anion transport systems in *S*-(1,2-dichlorovinyl)glutathione- and *S*-(1,2,-dichlorovinyl)cysteine-induced nephrotoxicity.

An important mechanism for detoxification of many chemicals, including halogenated hydrocarbons, is the formation of glutathione conjugates [1]. Glutathione conjugates are eliminated in the bile or, after biotransformation to mercapturic acids, in the urine [1]. Glutathione conjugate formation, which is catalyzed by cytosolic or microsomal glutathione *S*-transferases, has been implicated in the activation of 1,2-dihaloethanes to potentially carcinogenic and nephrotoxic electrophiles [2–5]. Glutathione *S*-transferases are present in several tissues, but their activity in the liver is much higher than in the kidney [6]. In contrast, the activities of the enzymes that catalyze mercapturic acid formation (γ-glutamyl transpeptidase, cysteinylglycine dipeptidase, and cysteine conjugate *N*-acetyl transferase) are much higher in the kidney than in the liver [6, 7].

*S*-(1,2-Dichlorovinyl)-L-cysteine, the cysteine conjugate of trichloroethylene, is a potent nephrotoxin, which produces acute renal failure characterized by proximal tubular necrosis [8]. The metabolism of *S*-(1,2-dichlorovinyl)-L-cysteine by rat liver cytosol and mitochondria appears to be necessary for the *in vitro* inhibitory effect of *S*-(1,2-dichlorovinyl)-L-cysteine on mitochondrial lipoyl dehydrogenase, 2-oxoacid dehydrogenase, and cytosolic glutathione reductase activities [9–11]. *S*-(1,2-Dichlorovinyl)-L-cysteine is cleaved by cysteine conjugate β-lyase to yield pyruvic acid, ammonia, and unidentified sulfur-containing reactive metabolites. The reactive metabolites generated *in vitro* from *S*-(1,2-dichlorovinyl)-L-cysteine by cysteine conjugate β-lyase combine with proteins and nucleic acids and are presumed to be the ultimate nephrotoxins [12, 13], although direct evidence for a role of cysteine conjugate β-lyase in *S*-(1,2-dichlorovinyl)-L-cysteine-induced nephrotoxicity is lacking. Cysteine conjugate β-lyase has been isolated and purified from bovine kidney, bovine and rat liver, and intestinal microflora [14–17].

The experiments described here were designed to determine whether *S*-(1,2-dichlorovinyl)glutathione, the glutathione conjugate of trichloroethylene, is nephrotoxic and to examine the role of γ-glutamyl transpeptidase, cysteine conjugate β-lyase, and renal transport mechanisms in *S*-(1,2-dichlorovinyl)glutathione- and *S*-(1,2-dichlorovinyl)cysteine-induced nephrotoxicity.

## MATERIALS AND METHODS

*Chemicals*. Glutathione, L-cysteine, DL-cysteine, L-homocystine, *S*-benzyl-L-cysteine, *S*-benzyl-DL-homocysteine, *S*-ethyl-L-cysteine, L-methionine, L-serine-*O*-sulfate, aminooxyacetic acid (Fig. 1), and probenecid were purchased from the Sigma Chemical Co. (St. Louis, MO). Trichloroethylene, chloroacetone, benzyl mercaptan, sodium cyanide, sodium metal, and deuterium oxide were purchased from the Aldrich Chemical Co. (Milwaukee, WI). 3-(Trimethylsilyl)tetradeutero sodium propionate was purchased from the Wilmad Glass Co., Inc. (Buena, NJ). L-(α*S*,5*S*)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125, Fig. 1) (Acivicin, lot number 10985-DGM-153-15) was provided by Dr. Ruth D. Davis, National Cancer Institute, Bethesda, MD.

Melting points (uncorrected) were determined in open glass capillaries with a Mel-Temp apparatus (Laboratory Devices, Cambridge, MA). Proton-NMR spectra were recorded on a Bruker 270 MHz spectrometer. The elemental analyses (C, H, N, Cl, S) for *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine and *S*-(1,2-dicholorovinyl)-L-homocysteine were done by Midwest Microlab, Ltd. (Indianapolis, IN). Thin-layer chromatography was performed on Analtech silica gel HLF plates (Newark, DE), and the plates were visualized with a ninhydrin spray.

*Syntheses*. *S*-(1,2-Dichlorovinyl)glutathione, *S*-(1,2-dichlorovinyl)-L-cysteine, *S*-(1,2-dichlorovinyl)-DL-cysteine, *S*-(2-benzothiazolyl)-L-cysteine,

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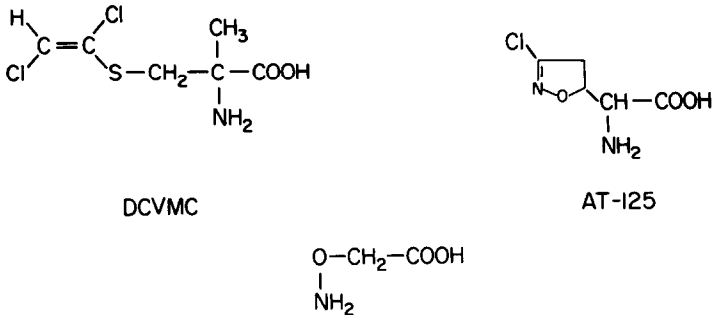


Fig. 1. Structures of *S*-(1,2-dicholorovinyl)-DL-$\alpha$-methylcysteine (DCVMC), L-($\alpha S$,5*S*)-$\alpha$-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (AT-125), and aminooxyacetic acid (AOA).

and *S*-benzyl-DL-$\alpha$-methylcysteine were synthesized by published procedures [18–20]. *S*-(1,2-Dichlorovinyl)-DL-$\alpha$-methylcysteine (Fig. 1) and *S*-(1,2-dichlorovinyl)-L-homocysteine were prepared by similar procedures by the reactions of $\alpha$-methyl-DL-cystine [20] or L-homocystine, respectively, with trichloroethylene in liquid ammonia after the complete addition of two equivalents of sodium metal. All products were pure by at least two TLC systems, and the structures were consistent with the respective $^1$H-NMR spectra. For the apparently new compounds: *S*-(1,2-dichlorovinyl)-DL-$\alpha$-methylcysteine (m.p. 188–190°), $\delta$ (ppm) 6.71 (1H, S), 3.60 (1H, d), 3.35 (1H, d), 1.57 (3H, S); Anal. Calc.: C, 31.32; H, 3.94; N, 6.09; S, 13.93; Cl, 30.81. Found: C, 31.46; H, 4.14; N, 6.20; S, 13.87; Cl, 30.63. *S*-(1,2-Dichlorovinyl)-L-homocysteine (m.p. 196–198°), $\delta$ (ppm) 6.77 (1H, S), 3.88 (1H, t), 3.09 (2H, t); 2.15 (2H, m); Anal. Calc.: C, 31.32; H, 3.94; N, 6.09; S, 13.93; Cl, 30.81. Found: C, 31.54; H, 4.19; N, 6.14; S, 14.17; Cl, 30.93.

*Cysteine conjugate $\beta$-lyase preparation.* Male Fischer 344 rats (175–225 g) were obtained from the Charles River Laboratories (Wilmington, MA). Rats were killed with carbon dioxide, and the livers and kidneys were removed. Subcellular fractionation by centrifugation was performed as described by Dohn and Anders [19]. These subcellular fractions were used as the enzyme source without further purification. The protein concentrations of the different fractions were determined according to Kalckar [21].

*Cysteine conjugate $\beta$-lyase activity.* Cysteine conjugate $\beta$-lyase activity was determined with *S*-(2-benzothiazolyl)-L-cysteine (0.4 mM) in 0.1 M sodium borate buffer (pH 8.6) as the substrate [19].

In vitro *inhibition of cysteine conjugate $\beta$-lyase.* The *in vitro* inhibition of cysteine conjugate $\beta$-lyase was measured with 1 mM inhibitor and 0.4 mM *S*-(2-benzothiazolyl)-L-cysteine. The velocity was measured in the presence and absence of inhibitor [19]. Inhibition constants, $K_i$, were determined from Dixon plots [22] with aminooxyacetic acid (0.2 to 2 mM, liver; 0.1 to 1 $\mu$M, kidney) as the inhibitor at various *S*-(2-benzothiazolyl)-L-cysteine concentrations (0.4 to 2 mM, liver; 0.4 to 1.2 mM, kidney) with liver cytosol and kidney mitochondrial fractions.

In vivo *inhibition of cysteine conjugate $\beta$-lyase.* Three rats were injected i.p. with 0.5 mmole/kg aminooxyacetic acid (in 4 ml/kg isotonic saline). After 1 hr the animals were killed, and the livers and kidneys were removed and fractionated as described above. The cysteine conjugate $\beta$-lyase activity in different fractions was compared, on a per gram (wet weight) tissue basis, with the activities obtained with untreated rats.

*Nephrotoxicity studies.* Male Fischer 344 rats were housed singly in plastic metabolic cages (Nalge Co., Rochester, NY) with a 9:00a.m. to 9:00p.m. light cycle. Feed (Rodent laboratory chow, Ralston Purina Co., St. Louis, MO) and water were provided *ad lib.* Rats were place in the metabolic cages for 2 days before treatment and were given *S*-(1,2-dichlorovinyl)glutathione, *S*-(1,2-dichlorovinyl)-DL-cysteine, or *S*-(1,2-dichlorovinyl)-DL-$\alpha$-methylcysteine in isotonic saline i.p. or saline alone. In some experiments, rats were given AT-125 (10 mg/kg), aminooxyacetic acid (0.5 mmole/kg), or probenecid (0.175 mmole/kg) i.p. 1 hr before treatment, and rats were killed by decapitation 24 or 48 hr after treatment. Blood samples were collected and were analyzed for blood urea nitrogen (BUN) and glucose concentrations with Beckman Kits 682233 and 682254 respectively (Beckman Instruments, Inc., Carlsbad, CA). Urine samples were collected and were analyzed for glucose as described above. Urine total glutathione concentrations after AT-125 treatment (10 mg/kg) were determined as described by Griffith [23].

*Statistics.* Statistical analyses were determined by analysis of variance with an MDCSTAT computer program (Micro Data Collection, Novato, CA). A level of $P \leq 0.05$ was chosen for acceptance or rejection of the null hypothesis.

## RESULTS

*Fractionation and subcellular distribution.* Typical results of the fractionation of the liver and kidneys, which show the cysteine conjugate $\beta$-lyase activity in different subcellular fractions, are presented in Table 1. Most of the activity in liver was found in the cytosol in contrast to kidneys, where most activity was detected in the mitochondrial fraction.

*Inhibitory effects.* The effects of inhibitors on liver cytosolic and renal mitochondrial cysteine conjugate $\beta$-lyase activities are presented in Table 2. With liver

Table 1. Cysteine conjugate β-lyase activity in various fractions of rat liver and kidney

| Fraction | Volume (ml) | Total activity (μmoles/min) | Specific activity (nmoles/min/mg) | Yield (%) |
|---|---|---|---|---|
| Liver | | | | |
| Whole homogenate | 49 | 94.4 | 22.7 | 100 |
| 750 *g* × 10 min pellet | 15 | 27.4 | 17.4 | 29 |
| 9800 *g* × 20 min pellet | 13 | 4.7 | 7.5 | 5 |
| 100,000 *g* × 60 min supernatant | 34 | 57.5 | 67.7 | 61 |
| 100,000 *g* × 60 min pellet | 10 | 5.4 | 6.4 | 6 |
| Kidney | | | | |
| Whole homogenate | 10 | 10.4 | 27.5 | 100 |
| 750 *g* × 10 min pellet | 8 | 4.3 | 24.1 | 41 |
| 9800 *g* × 20 min pellet | 10 | 5.0 | 32.4 | 48 |
| 100,000 *g* × 60 min supernatant | 8 | 2.5 | 20.5 | 24 |
| 100,000 *g* × 60 min pellet | 5 | 0 | 0 | 0 |

Cysteine conjugate β-lyase activity was determined by measuring the conversion of *S*-(2-benzothiazolyl)-L-cysteine (0.4 mM) to 2-mercaptobenzothiazole. Values represent the results obtained from a typical experiment.

cytosol, the most potent inhibitors were aminooxyacetic acid (84% inhibition) and *S*-(1,2-dichlorovinyl)-L-cysteine (55% inhibition). With kidney preparations, aminooxyacetic acid (97% inhibition) and *S*-(1,2-dichlorovinyl)-L-homocysteine (37% inhibition) were the most potent inhibitors. The type of inhibition by the various compounds in Table 2 was not investigated, except for aminooxyacetic acid where Dixon plots showed competitive inhibition of both liver cytosolic and kidney mitochondrial β-lyase activities. Inhibition constants, $K_i$, for aminooxyacetic acid were 0.2 mM (liver cytosol) and 0.1 μM (kidney mitochondria).

In vivo *inhibition*. Only about 10% of the total cysteine conjugate β-lyase activity in the homogenate as well as in the mitochondrial fraction in the kidneys was detected 1 hr after *in vivo* treatment with aminooxyacetic acid, compared to untreated rats. The corresponding values for liver homogenate and cytosol were 70 and 85% respectively.

*Effect of AT-125 on urine glutathione concentrations*. Rats given AT-125 showed a transient increase in urine glutathione concentrations. AT-125-treated rats excreted 13.6 ± 1.3 μmoles glutathione/12 hr (mean ± S.D.; N = 4). No glutathione was detected in the urine 12 hr after treatment with AT-125 or in the urine of rats given saline.

*S-Conjugate-induced nephrotoxicity*. Treatment of rats with *S*-(1,2-dichlorovinyl)glutathione or *S*-(1,2-dichlorovinyl)-DL-cysteine increased blood urea nitrogen concentrations and urine glucose excretion rates (Table 3). Equimolar doses of *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine failed to alter these renal function variables (Table 3).

*Effect of AT-125, aminooxyacetic acid, or probenecid on S-conjugate-induced nephrotoxicity*. Treatment of rats with AT-125 1 hr before giving *S*-(1,2-dichlorovinyl)glutathione resulted in reductions in *S*-(1,2-dichlorovinyl)glutathione-induced increases in urine glucose excretion rates (Table 3). Treatment of rats with aminooxyacetic acid or probenecid protected against *S*-(1,2-dichlorovinyl)-DL-cysteine-induced increases in urine glucose excretion rates (Table 3).

Table 2. Effects of various compounds on the cysteine conjugate β-lyase activity of rat liver cytosol and kidney mitochondrial fraction

| Inhibitor (1 mM) | Activity* (% of control) Liver cytosol | Kidney mitochondria |
|---|---|---|
| Aminooxyacetic acid | 16 ± 8 | 3 ± 3 |
| *S*-Benzyl-L-cysteine | 83 ± 13 | 93 ± 13 |
| *S*-Benzyl-DL-homocysteine | 82 ± 12 | 90 ± 9 |
| *S*-Benzyl-DL-α-methylcysteine | 95 ± 13 | 89 ± 6 |
| *S*-(1,2-Dichlorovinyl)-L-cysteine | 45 ± 5 | 92 ± 5 |
| *S*-(1,2-Dichlorovinyl)-L-homocysteine | 89 ± 12 | 63 ± 8 |
| *S*-(1,2-Dichlorovinyl)-DL-α-methylcysteine | 98 ± 8 | 88 ± 10 |
| *S*-Ethyl-L-cysteine | 89 ± 12 | 91 ± 10 |
| L-Methionine | 102 ± 4 | 83 ± 10 |
| L-Serine-*O*-sulfate | 96 ± 17 | 96 ± 10 |

* Cysteine conjugate β-lyase activity was determined by measuring the conversion of *S*-(2-benzothiazolyl)-L-cysteine (0.4 mM) to 2-mercaptobenzothiazole. The results are presented as mean ± S.D. from three experiments. Control values for liver cytosolic and kidney mitchondrial β-lyase activity were 68 and 32 nmoles 2-mercaptobenzothiazole/mg protein per min respectively.

Table 3. Effects of *S*-(1,2-dichlorovinyl)glutathione, *S*-(1,2-dichlorovinyl)-DL-cysteine, and *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine on blood urea nitrogen concentrations and urine glucose excretion rates

| Treatment* | Blood urea nitrogen (mg/100 ml) | | Urine glucose (mg/24 hr) | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 0–24 hr | 24–48 hr |
| Saline | 13 ± 2 | 16 ± 2 | 8 ± 3 | 11 ± 4 |
| *S*-(1,2-Dichlorovinyl)glutathione (0.23 mmole/kg) | 36 ± 3† | 59 ± 10† | 699 ± 146† | 423 ± 207† |
| *S*-(1,2-Dichlorovinyl)glutathione + AT-125 (10 mg/kg) | 35 ± 5 | 43 ± 10 | 225 ± 72‡ | 309 ± 38 |
| *S*-(1,2-Dichlorovinyl)-DL-cysteine (0.23 mmole/kg) | 110 ± 20† | 144 ± 31† | 177 ± 102† | 165 ± 97 |
| *S*-(1,2-Dichlorovinyl)-DL-cysteine (0.115 mmole/kg) | 23 ± 10 | 29 ± 16 | 609 ± 157† | 535 ± 145† |
| *S*-(1,2-Dichlorovinyl)-DL-cysteine (0.115 mmole/kg) + aminooxyacetic acid (0.5 mmole/kg) | 10 ± 2 | 19 ± 12 | 12 ± 17§ | 19 ± 17§ |
| *S*-(1,2-Dichlorovinyl)-DL-cysteine (0.115 mmole/kg) + probenecid (0.175 mmole/kg) | 8 ± 2 | 10 ± 3 | 267 ± 162§ | 541 ± 338 |
| *S*-(1,2-Dichlorovinyl)-DL-α-methylcysteine (0.115 mmole/kg) | 15 ± 1 | 17 ± 1 | 6 ± 1 | 7 ± 1 |
| *S*-(1,2-Dichlorovinyl)-DL-α-methylcysteine (0.23 mmole/kg) | 15 ± 2 | 19 ± 4 | 4 ± 2 | 7 ± 2 |
| AT-125 (10 mg/kg) | 17 ± 1 | 18 ± 1 | 6 ± 1 | 4 ± 1 |
| Aminooxyacetic acid (0.5 mmole/kg) | 22 ± 2 | 18 ± 2 | 6 ± 1 | 7 ± 1 |

Values are means ± S.D. of at least four animals per group.

* Male Fischer 344 rats (175–225 g) were given *S*-(1,2-dichlorovinyl)glutathione, *S*-(1,2-dichlorovinyl)-DL-cysteine, or *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine in isotonic saline i.p. or saline alone. Some rats were given AT-125 [Acivicin, L-(α*S*,5*S*)α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid], aminooxyacetic acid, or probenecid 1 hr before treatment.

† Significantly different ($P < 0.05$) from animals given saline alone at the same time.

‡ Significantly different ($P < 0.05$) from animals given 0.23 mmole/kg *S*-(1,2-dichlorovinyl)glutathione at the same time.

§ Significantly different ($P < 0.05$) from animals given 0.115 mmole/kg *S*-(1,2-dichlorovinyl)-DL-cysteine at the same time.

## DISCUSSION

The present study shows that *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-DL-cysteine are nephrotoxic. Nephrotoxicity was assessed by measuring urine glucose excretion rates and blood urea nitrogen concentrations. Renal glucose excretion rates are affected by changes in plasma glucose concentrations, glomerular filtration rates, and proximal tubular reabsorption [24]. In this study, *S*-(1,2-dichlorovinyl)glutathione or *S*-(1,2-dichlorovinyl)-DL-cysteine treatment did not alter blood glucose concentrations (data not shown), which suggests that the increases in urine glucose excretion rates seen after *S*-(1,2-dichlorovinyl)glutathione or *S*-(1,2-dichlorovinyl)-DL-cysteine treatment were the result of renal damage. These results are in agreement with histopathological studies with *S*-(1,2-dichlorovinyl)cysteine, which showed that the proximal tubules are the most affected after *S*-(1,2-dichlorivinylcysteine administration [8]. The finding that *S*-(1,2-dichlorovinyl)-DL-cysteine, at a dose of 0.115 mmole/kg, resulted in a larger increase in urine glucose excretion rates compared with a dose of 0.23 mmole/kg can be explained by decreases in glomerular filtration rates at the higher dose [24]. The expected dose response for *S*-(1,2-dichlorovinyl)-DL-cysteine-induced nephrotoxicity was seen with the BUN data.

The present experiments were designed to test the hypothesis that metabolism of *S*-(1,2-dichlorovinyl)-glutathione to *S*-(1,2-dichlorovinyl)-L-cysteine and subsequent bioactivation of *S*-(1,2-dichlorovinyl)-L-cysteine are involved in the nephrotoxic action of *S*-(1,2-dichlorovinyl)glutathione. A scheme for the metabolism of *S*-(1,2-dichlorovinyl)glutathione is shown in Fig. 2. Treatment of rats with AT-125, an irreversible inhibitor of γ-glutamyl transpeptidase [25, 26], resulted in a transient increase in urine glutathione concentrations, which is attributed to γ-glutamyl transpeptidase inhibition [25]. Although the effect of AT-125 on glomerular filtration rates was not studied, AT-125 treatment did not alter blood urea nitrogen concentrations or urine glucose excretion rates. The finding that AT-125 protected against *S*-(1,2-dichlorovinyl)glutathione-induced increases in urine glucose excretion rates suggests a role for γ-glutamyl transpeptidase in the activation of *S*-(1,2-dichlorovinyl)glutathione to the ultimate nephrotoxin. AT-125 treatment did not block completely the nephrotoxicity of *S*-(1,2-dichlorovinyl)glutathione, which suggests that the γ-glutamyl transpeptidase activity remaining after AT-125 treatment allows sufficient processing of *S*-(1,2-dichlorovinyl)glutathione to produce *S*-(1,2-dichlorovinyl)-L-cysteinylglycine in nephrotoxic amounts. Renal γ-glutamyl transpeptidase, which is present on the brush border of both convoluted and straight portions of the proximal tubule as well as on the anti-luminal region associated with the microvascular compartment, is present in the rat kidney in great excess [27, 28]. The enzyme on the vascular side is more susceptible to inhibition by AT-125 than the brush border enzyme [27, 28]. *S*-(1,2-Dichlorovinyl)-L-cysteinylglycine would then enter the cell and be hydrolyzed by brush border membrane peptidases [29] to yield *S*-(1,2-dichlorovinyl)-L-cysteine.

To ascertain the role of cysteine conjugate β-lyase in the nephrotoxicity of *S*-(1,2-dichlorovinyl)cysteine, the nephrotoxicity of *S*-(1,2-dichlorovinyl)-

DL-α-methylcysteine was studied. The catalytic mechanism of pyridoxal phosphate-dependent enzymes involves Schiff base formation with the α-amino group followed by abstraction of the proton from the α-carbon atom [30]; hence, compounds such as *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine, which lack a proton on the α-carbon atom, cannot be cleaved by the pyridoxal phosphate-dependent cysteine conjugate β-lyase. The failure of *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine to produce changes in blood urea nitrogen concentrations or in urine glucose excretion rates demonstrates a role for cysteine conjugate β-lyase in *S*-(1,2-dichlorovinyl)cysteine-induced nephrotoxicity.

To gain further evidence for the role of cysteine conjugate β-lyase in *S*-(1,2-dichlorovinyl)cysteine-induced nephrotoxicity, the inhibitory effects of various compounds on hepatic and renal cysteine conjugate β-lyase activity were studied. Aminooxyacetic acid, an inhibitor of pyridoxal phosphate-dependent enzymes [31, 32], was a more potent inhibitor of renal mitochondrial cysteine conjugate β-lyase activity than of hepatic cytosolic lyase activity and protected against *S*-(1,2-dichlorovinyl)-DL-cysteine-induced increases in urine glucose excretion rates. Although the effect of aminooxyacetic acid on the transport of *S*-(1,2-dichlorovinyl)-L-cysteine was not studied, the inhibitory effect of aminooxyacetic acid of renal mitochondrial β-lyase activity as well as the failure of *S*-(1,2-dichlorovinyl)-DL-α-methylcysteine to produce nephrotoxicity clearly implicate renal cysteine conjugate β-lyase in *S*-(1,2-dichlorovinyl)-DL-cysteine-induced nephrotoxicity. The difference between the susceptibility of hepatic cytosolic and renal mitochondrial β-lyase activities to inhibition by aminooxyacetic acid shows that the catalytic proteins in these tissues are different. Liver cytosolic β-lyase is identical to kyureninase [33], but the nature of the renal β-lyase activity has not been investigated in detail. It is possible that renal β-lyase activity is attributable to a single enzyme or to several enzymes, such as the L-amino acid transaminases or oxidases. Several of these enzymes catalyze the elimination of a good leaving group at the β-carbon atom of the amino acid [30, 34].

Finally, the role of transport in *S*-(1,2-dichlorovinyl)-DL-cysteine-induced nephrotoxicity was investigated with the organic anion transport inhibitor probenecid. Probenecid inhibits the active accumulation of *N*-acetyl *S*-benzyl-L-cysteine by isolated renal cortical tubules and causes a significant retardation of *N*-acetyl *S*-benzyl-L-[U-$^{14}$C]cysteine clearance from the plasma as well as inhibition of both renal accumulation and excretion into urine of *N*-acetyl *S*-benzyl-L-[U-$^{14}$C]cysteine [35, 36]. Thus, the protection against *S*-(1,2-dichlorovinyl)-DL-cysteine-induced increases in urine glucose excretion rates by probenecid suggests a role for the organic anion transport system in *S*-(1,2-dichlorovinyl)-DL-cysteine-induced nephrotoxicity.

The present study provides direct evidence for a role of γ-glutamyl transpeptidase and cysteine conjugate β-lyase in *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)cysteine-induced nephrotoxicity. Other nephrotoxic halogenated hydrocarbons that form glutathione conjugates, such as hexachloro-1,3-butadiene, chlorotrifluoroethylene, and tetrafluoroethylene, may produce renal damage by a similar mechanism [37–41]. This study also illustrates the usefulness of synthetic, nonmetabolizable analogues of toxic chemicals (α-methyl-substituted amino acids in the present study) as mechanistic probes. Finally, aminooxyacetic acid is a convenient tool for testing the role of pyridoxal phosphate-dependent enzymes, such as cysteine conjugate β-lyase, in the bioactivation of amino acids.

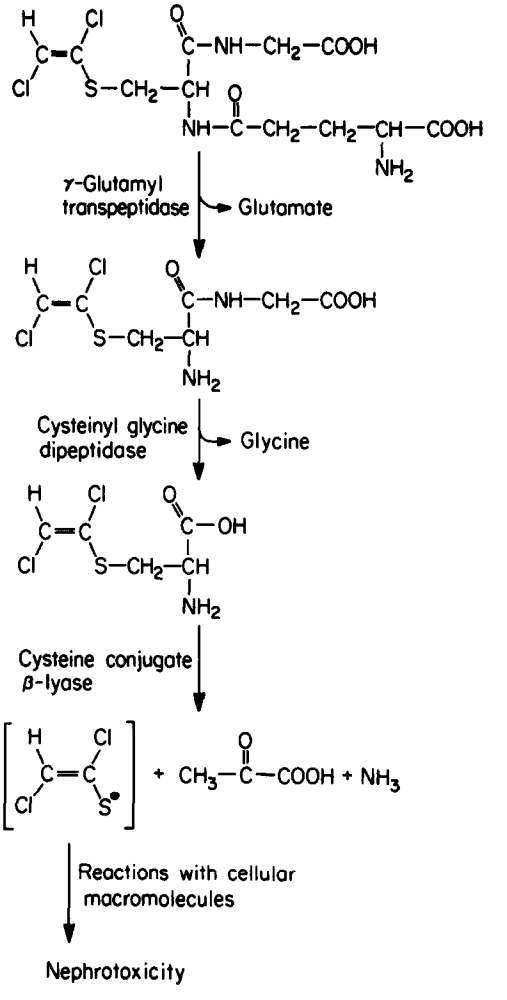


Fig. 2. Postulated mechanism of *S*-(1,2-dichlorovinyl)-glutathione-induced nephrotoxicity.

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