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S-(2-Chloroacetyl)glutathione, a Reactive Glutathione Thiol Ester and a Putative Metabolite of 1,1-Dichloroethylene[†]

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ABSTRACT: Conversion of the toxic vinyl halide 1,1-dichloroethylene (DCE) to *S*-(2-*S*-glutathionylacetyl)glutathione ($GSCH_2COSG$) involves sequential acylation and alkylation of two glutathione (GSH) molecules by the microsomal DCE metabolite $ClCH_2COCl$. To examine its possible role in DCE biotransformation, we synthesized the putative intermediate *S*-(2-chloroacetyl)glutathione ($ClCH_2COSG$). In aqueous buffer, $ClCH_2COSG$ did not hydrolyze to release GSH, but instead underwent a two-step rearrangement to yield a cyclic product. Product analyses by liquid secondary ion mass spectrometry and ^1H–^{13}C heteronuclear correlation nuclear magnetic resonance spectroscopy indicated that rearrangement involved initial transfer of the chloroacetyl moiety from the cysteinyl thiol to the γ-glutamyl α-amine. The cysteinyl thiol then displaced chloride from the 2-chloroacetyl methylene carbon to yield the cyclic product. Incubation of 2 mM $ClCH_2COSG$ with 20 mM GSH yielded approximately 4.5-fold more cyclic product than $GSCH_2COSG$. $ClCH_2COSG$ alkylated oxytocindithiol and *N*-acetyl-L-cysteine to yield *S*-[2-(alkylthio)acetyl]glutathione adducts analogous to $GSCH_2COSG$. S-2-Chloroacetylation products were absent. In reacting with thiols by alkylation and in decomposing by rearrangement, $ClCH_2COSG$ displayed properties strikingly different from those of $ClCH_2COCl$. Although much less reactive than its acyl halide precursor, $ClCH_2COSG$ may display greater selectivity in covalent modification of cellular targets in DCE intoxication.

By reacting with toxic chemicals and their metabolites, GSH[1] prevents modification of critical cellular targets and protects against tissue damage [reviewed in Larsson et al. (1983)]. Nonetheless, several recent reports implicate glutathione conjugates in chemical toxicity. GSH conjugates and their degradation products may exhibit diverse and toxicologically significant properties. *S*-(2-Haloethyl)glutathione conjugates, for example, are thought to mediate 1,2-dihaloethane covalent binding to cellular macromolecules (Koga et al., 1986; Foureman & Reed, 1987). Alternatively, some haloalkene conjugates undergo metabolic degradation to reactive intermediates (Elfarra et al., 1986; Elfarra & Anders, 1984). Glutathione conjugates derived from 2-bromohydroquinone may mediate the renal toxicity of bromobenzene (Monks et al., 1985).

Here we report studies of $ClCH_2COSG$, a putative intermediate in the biotransformation of the hepatotoxic vinyl halide DCE. Microsomal cytochromes P-450 convert DCE to Cl_2CHCHO and $ClCH_2COCl$ by a mechanism not involving an epoxide intermediate (Liebler & Guengerich, 1983). GSH-fortified microsomes and isolated rat hepatocytes convert DCE to $GSCH_2COSG$, a bis(glutathionyl) conjugate formally derived from reaction of $ClCH_2COCl$ with two GSH molecules

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[1] Abbreviations: $ClCH_2COSG$, *S*-(2-chloroacetyl)glutathione; DCE, 1,1-dichloroethylene; DTT, dithiothreitol; FAB, fast-atom bombardment; $GSCH_2COSG$, *S*-(2-*S*-glutathionylacetyl)glutathione; GSH, reduced glutathione; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; ^1H COSY, ^1H–^1H correlation spectroscopy; ^1H–^{13}C HETCOSY, ^1H–^{13}C heteronuclear correlation spectroscopy; LSIMS, liquid secondary ion mass spectrometry; TEAB, triethylammonium bicarbonate.

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(Liebler et al., 1984). $\mathrm{ClCH_2COCl}$ presumably acylates the GSH thiol. The resulting intermediate $\mathrm{ClCH_2COSG}$ then S-alkylates a second GSH to yield $\mathrm{GSCH_2COSG}$. Despite its enhanced stability to hydrolysis, $\mathrm{ClCH_2COSG}$ could be expected to display chemistry similar to its acyl chloride precursor.

To examine its putative role in DCE biotransformation, we synthesized $\mathrm{ClCH_2COSG}$ and studied its chemistry. Our results indicate that $\mathrm{ClCH_2COSG}$ indeed reacts with GSH to form $\mathrm{GSCH_2COSG}$. However, $\mathrm{ClCH_2COSG}$ displays properties that are quite different from those of $\mathrm{ClCH_2COCl}$. Reaction studies with model thiols suggest that $\mathrm{ClCH_2COSG}$ may covalently modify targets of toxicologic significance.

Experimental Procedures

Chemicals. GSH and *N*-acetyl-L-cysteine were purchased from Sigma (St. Louis, MO), $\mathrm{ClCH_2COCl}$ was from Aldrich (Milwaukee, WI), and oxytocin was from Peninsula Laboratories (Belmont, CA). ACS-grade dimethylformamide (Baker, Phillipsburg, NJ) was dried over 4-Å molecular sieves before use.

Synthesis, Purification, and Characterization of $\mathit{ClCH_2COSG}$. GSH (1.0 g; 3.26 mmol) was suspended in 200 mL of dry dimethylformamide containing 0.25 mL of glacial acetic acid. $\mathrm{ClCH_2COCl}$ (0.318 mL; 4.00 mmol) was slowly added over a 10-min period. The resulting clear solution was stirred for 60 min at 25 °C, the excess acyl chloride was quenched with 2 mL of ethanol, and the solvents were removed in vacuo. The oily, yellow residue was dissolved in 5 mL of 2-propanol, and a crude product containing $\mathrm{ClCH_2COSG}$ and GSH was then precipitated with 50 mL of diethyl ether. $\mathrm{ClCH_2COSG}$ was purified by HPLC on a 10 × 500 mm Partisil ODS-2 10-μm preparative column (Alltech, Deerfield, IL). Products were eluted isocratically with water/methanol/acetic acid (880:120:1) at a flow rate of 3 mL min^{-1} and detected by UV absorbance at 220 nm. $\mathrm{ClCH_2COSG}$ reacted with hydroxylamine in aqueous buffer at 45 °C to produce a hydroxamic acid derivative, which formed a colored ferric complex (λ_{max} = 460 nm). Negative ion LSIMS analysis of purified $\mathrm{ClCH_2COSG}$ produced signals at m/z 382 and 384 in a 3:1 ratio, which indicated that the purified product contained a single chlorine. Exact mass negative ion LSIMS measurement indicated an M − H mass of 382.0475 (calcd M − H mass 382.0475). On the basis of ^{1}H COSY analysis (Figure 1), the ^{1}H NMR signals were assigned as follows: δ 2.11 (m, 1 H) and 2.25 (m, 1 H), Glu-$\beta_{a,b}$; δ 2.51 (t, 2 H), Glu-γ; δ 2.96 (m, 2 H), Cys-β; δ 4.03 (s, 2 H), Gly-α; δ 4.21 (s, 2 H), Cl$\mathit{CH_2}$-; δ 4.44 (dd, 1 H), Glu-α; δ 4.60 (t, 1 H), Cys-α. Note that the Glu-β ^{1}H resonances are separated by approximately 0.15 ppm.

Rate Constants. Rate constants for $\mathrm{ClCH_2COSG}$ cyclization in 50 mM potassium phosphate were estimated from the loss of thiol ester absorbance at 232 nm versus time and were measured at 25 °C. The reaction of $\mathrm{ClCH_2COSG}$ with GSH was studied in incubations containing 2 mM $\mathrm{ClCH_2COSG}$, 20 mM GSH, and 100 mM potassium phosphate, pH 7.8. The pseudo-first-order rate constant for reaction of $\mathrm{ClCH_2COSG}$ with excess GSH (k_{bis}) was estimated by comparing yields of $\mathrm{GSCH_2COSG}$ and the cyclic $\mathrm{ClCH_2COSG}$ decomposition product according to

$$k_{bis} = k_{cyc}[\mathrm{GSCH_2COSG}]_{t=\infty}/[\text{cyclic conjugate}]_{t=\infty}$$

where k_{cyc} describes the first-order formation of the cyclic conjugate (0.027 min^{-1} at pH 7.8). The total yield of $\mathrm{GSCH_2COSG}$ was determined by HPLC analysis of its hydrolysis product $\mathrm{GSCH_2CO_2H}$ (Reed et al., 1980); that of the cyclic conjugate, by difference. Only $\mathrm{GSCH_2COSG}$, $\mathrm{GSCH_2CO_2H}$, and the cyclic conjugate were formed in the incubation.

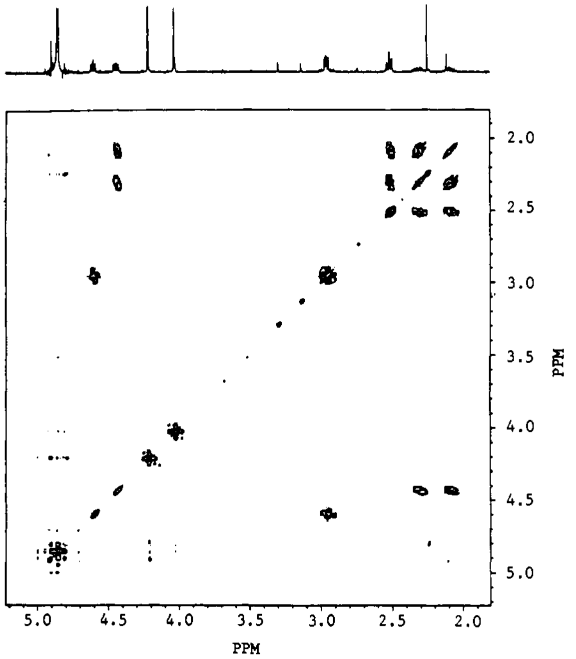

FIGURE 1: ^{1}H COSY spectrum of $\mathrm{ClCH_2COSG}$ in $\mathrm{D_2O}$. The singlet resonances at δ 2.11 and 2.25 are from unidentified contaminants.

Reaction of $\mathit{ClCH_2COSG}$ *with Oxytocindithiol.* Oxytocin (1 mg, 1 μmol) was treated with 5 mg of DTT in 0.5 mL of 20 mM TEAB, pH 7.7, at 25 °C for 60 min. DTT and its disulfide wre then removed by extraction with three 1-mL portions of ethyl acetate. $\mathrm{ClCH_2COSG}$ (1 mg, 2.5 μmol, dissolved in 20 mM TEAB, pH 7.7) was then added, and after being incubated for an additional 6 h, the solution was frozen and lyophilized for LSIMS analysis.

LSIMS Analysis of $\mathit{ClCH_2COSG}$ *and Reaction Products.* LSIMS was performed with a Varian CH-7 instrument equipped with a standard Ion-Tech FAB gun (Teddington, U.K.) that produced a 7-kV Xe beam. The accelerating voltage was 3 kV, and the source temperature was ambient. The system was equipped with a postacceleration switching system for negative ion analysis (AMD Intectra) and a System Industries 150 data system. $\mathrm{ClCH_2COSG}$ exact mass measurements and oxytocin–$\mathrm{ClCH_2COSG}$ adduct analyses were made with a Kratos MS-50 mass spectrometer (Manchester, U.K.) equipped with a standard Ion-Tech FAB gun and a Kratos DS-90 data system. Samples were dissolved in thioglycerol and applied to the probe tip. For exact mass measurements, samples were dissolved in glycerol.

NMR Spectroscopy. ^{1}H COSY and ^{1}H–^{13}C HETCOSY were performed with a Bruker AM-400 instrument equipped with an Aspect 3000 computer and operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ^{1}H COSY spectra were acquired with a 90°–τ–45° pulse sequence with 1024 data points in F_1 and 512 data points in F_2. Sine bell multiplication was applied before Fourier transformation. ^{1}H–^{13}C HETCOSY spectra were acquired with a 90°(^{1}H,x)–τ–90°(^{1}H,y)–90°(^{13}C,x) pulse sequence with a 1-s recycle delay. F_1 contained 256 data points, and F_2 contained 4000. Gaussian multiplication was applied before Fourier transformation.

Results

Decomposition of $\mathit{ClCH_2COSG}$. The rate of $\mathrm{ClCH_2COSG}$ degradation, monitored as loss of thiol ester absorbance at 232 nm, increased with increasing pH. At pH 7.5, $\mathrm{ClCH_2COSG}$

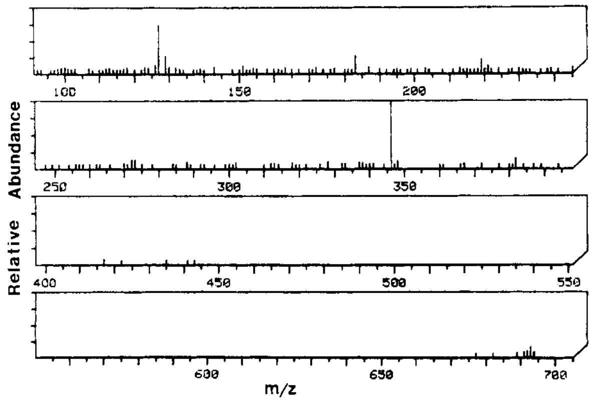

FIGURE 2: Negative ion LSIMS of $ClCH_2COSG$ cyclic degradation product. $ClCH_2COSG$ (5 mg; 13 mol) was dissolved in 1 mL of H_2O, and the pH was then adjusted to 9.0 with concentrated NH_4OH. After 30 min, the mixture was frozen and lyophilized for LSIMS analysis.

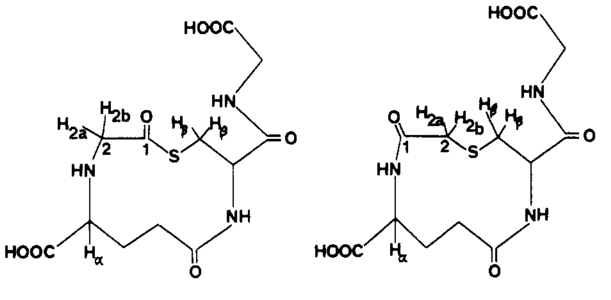

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FIGURE 3: Possible structures of $ClCH_2COSG$ cyclic degradation product.

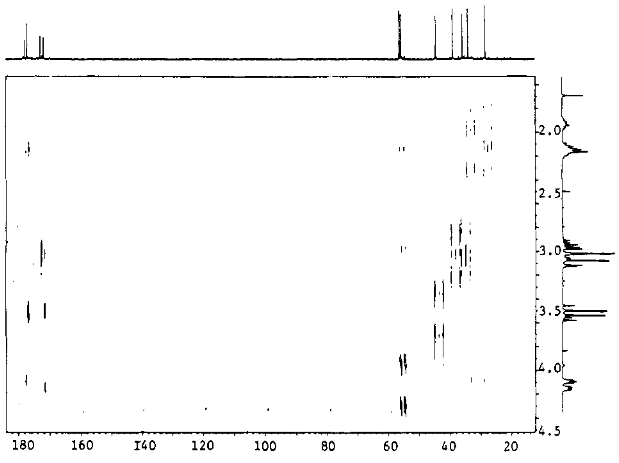

FIGURE 4: ^1H–^{13}C HETCOSY of $ClCH_2COSG$ cyclic degradation product. The cyclic product was prepared as described in the Figure 2 legend. Assignments for the ^1H and ^{13}C resonances are given in the text.

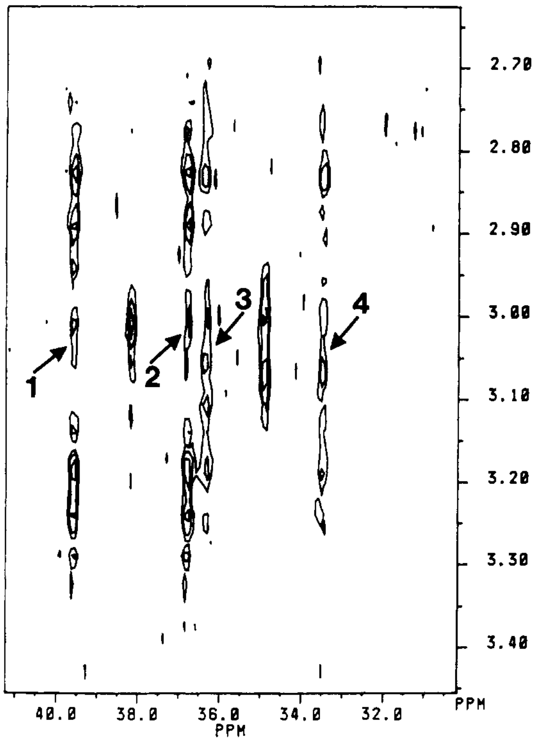

FIGURE 5: Expanded region of ^1H–^{13}C HETCOSY spectrum from Figure 4 containing the area between δ 30.5 and δ 41.2 (^{13}C axis) and between δ 2.62 and δ 3.45 (^1H axis). Spots 1 and 2 indicate three-bond coupling between C_2 and Cys β-protons; spots 3 and 4 indicate three-bond coupling between the cys β-carbon and $H_{2a,b}$ protons. The remaining spots along the δ36.9 and 39.6 projections indicate one-bond coupling between C_2 and its methylene protons $H_{2a,b}$. The remaining spots along the δ 33.5 and 36.4 projections indicate one-bond coupling between the Cys β-carbon and its methylene protons. Large spots on the δ 35.0 and 38.2 projections are artifacts occasionally produced by the pulse sequence used.

decomposed with a $t_{1/2}$ of 35 min; at pH 9.0, the $t_{1/2}$ was 6.4 min. $ClCH_2COSG$ did not decompose in aqueous buffer by hydrolysis. HPLC analyses of decomposition products (Reed et al., 1980) from incubation at pH values between 7.0 and 9.0 confirmed that no GSH was released. Negative ion LSIMS (Figure 2) revealed a single product ion of m/z 346, corresponding to a product molecular weight of 347 and consistent with a dehydrochlorination of $ClCH_2COSG$ to yield cyclic products **1** and/or **2** (Figure 3). Product **1** would arise directly from intramolecular displacement of the $ClCH_2COSG$ chloride by the glutamate α-amine:

$$H_2N\text{-Glu-Cys}(SCOCH_2Cl)\text{-Gly} \rightarrow \text{product } \mathbf{1}$$

Product **2** would be formed by a two-step mechanism involving chloroacetylation of the glutamate α-amine (1) followed by intramolecular displacement of chloride by the free thiol (2):

$$H_2N\text{-Glu-Cys}(SCOCH_2Cl)\text{-Gly} \rightarrow ClCH_2CONH\text{-Glu-Cys}(SH)\text{-Gly} \quad (1)$$

$$ClCH_2CONH\text{-Glu-Cys}(SH)\text{-Gly} \rightarrow \text{product } \mathbf{2} \quad (2)$$

We subjected the HPLC-purified cyclic product to ^1H–^{13}C HETCOSY (Figure 4) and ^1H COSY analysis (not shown). On the basis of these spectra, the ^1H and ^{13}C resonances were assigned. ^1H resonances: δ 1.93 (m, 1 H), Glu-β_a; δ 2.10–2.21 (m, 3 H), Glu-β_b and Glu-γ; δ 2.89–3.10 (m, 2 H), Cys-β; δ 2.96–3.12 (dd, 2 H), $H_{2a,b}$; δ 3.45–3.58 (dd, 2 H), Gly-α; δ 4.10 (m, 1 H), Glu-α; δ 4.15 (m, 1 H), Cys-α. ^{13}C resonances: δ 28.6, Glu-β; δ 33.6, Glu-γ; δ 34.9, Cys-β; δ 38.1 C_2; δ 44.1, Gly-α; δ 55.8, Cys-α; δ 56.3, Glu-α; δ 171.4, Cys-CO; δ 172.4, C_1; δ 177.1, Glu and Gly CO_2H; δ 178.2, γ-Glu CO.

^1H–^{13}C HETCOSY analysis was used to identify one-, two-, and three-bond couplings between ^1H and ^{13}C nuclei. Three-bond ^1H–^{13}C couplings were of interest because a product having structure **1** would display three-bond coupling between the methylene protons H_{2a}/H_{2b} and the glutamate α-carbon, coupling between the glutamate α-proton and methylene carbon 2, and coupling between the cysteine β-protons and carbonyl carbon 1. A product having structure **2** would display three-bond coupling between the cysteine β-protons and methylene carbon 2, between the methylene protons H_{2a} and H_{2b} and the cysteine β-carbon, and between the glutamate α-proton and carbonyl carbon 1.

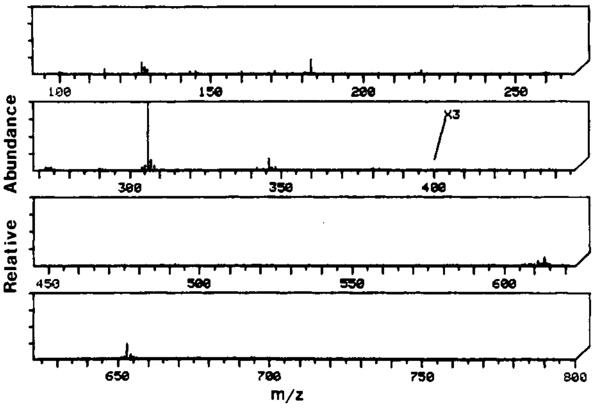

FIGURE 6: Negative ion LSIMS of reaction products of $ClCH_2COSG$ and GSH. $ClCH_2COSG$ (3 mg; 7.8 mol) and GSH (12 mg; 39 mol) were incubated together for 5 h at 25 °C in 20 mM TEAB, pH 7.7. The mixture was then frozen and lyophilized for LSIMS analysis.

Prominent coupling between the methylene protons H_{2a}/H_{2b} and the cysteine β-carbon was found (Figures 4 and 5). Coupling between the cysteine β-protons and methylene carbon 2 appeared in Figure 4 to be indistinct because of the extensive splitting of the cysteine β-proton signal. However, the expanded spectrum more clearly indicates both the one-bond coupling between the cysteine β-carbon and its methylene protons and the three-bond coupling between the cysteine β-carbon and the methylene protons H_{2a}/H_{2b}. The spectrum did not indicate coupling between the glutamate α-proton and carbonyl carbon 1. This coupling may be of very small magnitude. ^1H–^{13}C HETCOSY of the cyclic product did not show couplings consistent with structure **1**. These observations collectively favor structure **2** for the cyclic product.

Reaction of $ClCH_2COSG$ with GSH. $ClCH_2COSG$ incubated with GSH yielded $GSCH_2COSG$ and the cyclic product as indicated by ions at *m/z* 653 and 346, respectively, in negative ion LSIMS (Figure 6). (The signal at *m/z* 306 is from GSH.) $GSCH_2COSG$ was isolated by HPLC from a similar incubation mixture, and ^1H COSY was used to confirm the presence of a second GSH moiety in the conjugate (Figure 7). The ^1H resonance assignments are [a prime mark (′) denotes nuclei from the $-CH_2-$*GSH* moiety] as follows: δ 2.08–2.15 (m, 1 H), Glu-β_a; δ 2.21–2.26 (m, 2 H), Glu′-β; δ 2.28–δ 2.32 (m, 1 H), Glu-β_b; δ 2.52–2.56 (t, 2 H), Glu-γ; δ 2.58–2.63 (dd, 2 H), Glu′-γ; δ 2.97–3.01 (t, 2 H), Cys-β; δ 3.01–3.05 (dd, 1 H), Cys′-β_a; δ 3.14–3.19 (dd, 1 H), Cys′-β_b; δ 3.41 (s, 2 H) and 3.45 (s, 2 H), Gly-α, Gly′-α; δ 3.88–3.91 (t, 1 H), Glu′-α; δ 4.04 (s, 2 H), $-CH_2CO-$; δ 4.41–4.44 (dd, 1 H), Glu-α; δ 4.62–4.65 (dd, 1 H), Cys-α; δ 4.65–4.69 (dd, 1 H), Cys′-α. Pronounced splitting of the glutamate β-proton signals (presumably of the acyl-substituted GSH) was again observed. The resonances for the cysteine′ β-protons (adjacent to the thioether linkage) differed by about 0.15 ppm, a splitting close to the observed previously for these protons in GSSG (Koga et al., 1986). The resonances for the cysteine β-protons (adjacent to the thiol ester linkage) in $GSCH_2COSG$ are nearly coincident.

In 100 mM potassium phosphate, pH 7.8, the pseudo-first-order rate constant for reaction of 2 mM $ClCH_2COSG$ with 20 mM GSH was 0.0058 min^{-1}. The first-order rate constant for cyclization was 0.027 min^{-1}, approximately 4.5-fold greater than that for reaction with GSH.

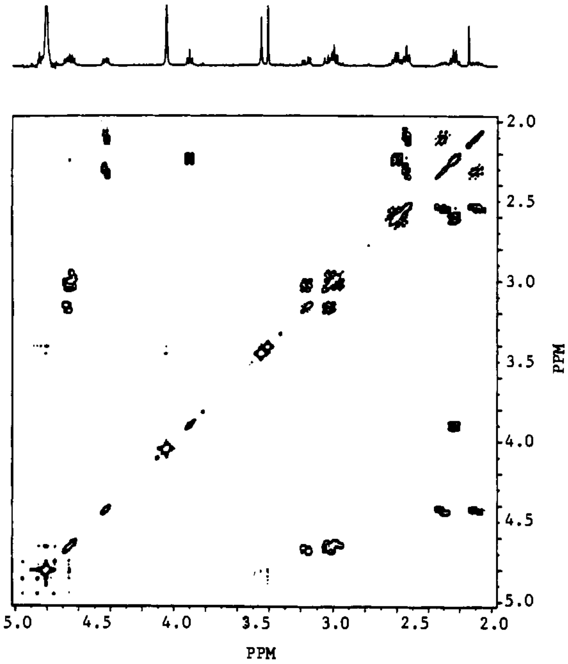

FIGURE 7: ^1H COSY spectrum of HPLC-purified $GSCH_2COSG$. $ClCH_2COSG$ (15 mg; 40 μmol) and GSH (61 mg; 200 μmol) were incubated together for 6 h at 25 °C in 100 mM potassium phosphate, pH 7.8. The mixture was then titrated with acetic acid to pH 4. $GSCH_2COSG$ was isolated by preparative HPLC as described under Experimental Procedures. The singlet resonance at δ 2.14 is from an unidentified contaminant.

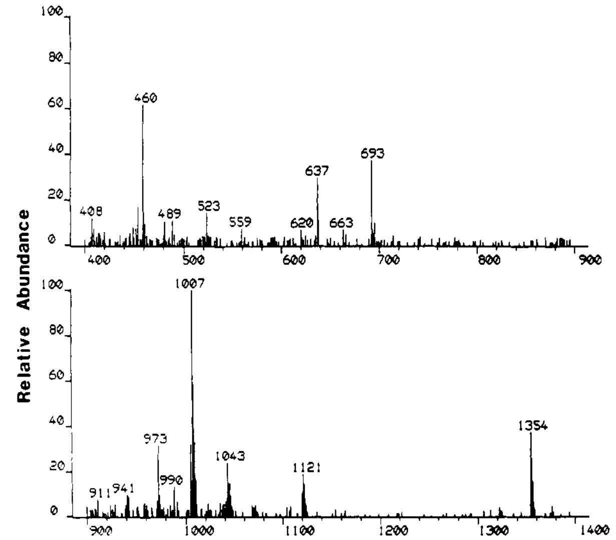

FIGURE 8: Negative ion LSIMS of $ClCH_2COSG$ and oxytocindithiol reaction products. Reaction conditions and sample preparation are described under Experimental Procedures.

Reaction of $ClCH_2COSG$ with Oxytocindithiol and N-Acetylcysteine. We employed oxytocindithiol as a model target to examine the selectivity of $ClCH_2COSG$ for reaction with different nucleophilic sites in the same peptide molecule. Oxytocin contains three primary amide nitrogens (Gln, Asn, Gly NH_2), one primary amine (Cys α-NH_2), and, upon disulfide reduction, two cysteinyl thiols. Treatment of oxytocin disulfide with DTT followed by incubation with $ClCH_2COSG$ produced an oxytocin–$ClCH_2COSG$ adduct. Negative ion LSIMS analysis indicated that, in addition to oxytocin (*m/z* 1007), oxytocin hydrochloride (*m/z* 1043), and an unidentified ion at *m/z* 1142 (present in controls), incubation mixtures yielded an oxytocin–$ClCH_2COSG$ adduct ion at *m/z* 1354 (Figure 8). This ion represents an oxytocindithiol modified by $GSCOCH_2-$ and is consistent with alkylation of either cysteinyl thiol by $ClCH_2COSG$. (Alkylation of a primary amine or amide would give rise to an adduct of mass 1353 with a negative ion at *m/z* 1352.) A negative ion signal for $ClCH_2CO$–S–oxytocin–SH, the product of chloroacetyl

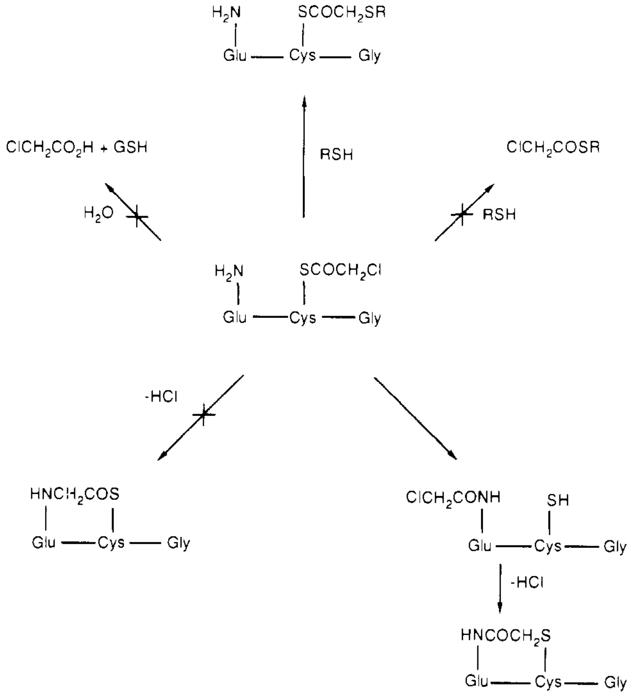

FIGURE 9: Summary of $ClCH_2COSG$ reactions. See text for discussion.

transfer from $ClCH_2COSG$, was not detected in LSIMS analyses.

Incubation of *N*-acetylcysteine with $ClCH_2COSG$ in 20 mM TEAB, pH 7.7, yielded a single adduct with a negative ion at *m/z* 509, which indicates addition of $-CH_2COSG$ to the *N*-acetylcysteine thiol (not shown). A negative ion signal for the expected S-chloroacetylation product (*m/z* 239) was absent.

DISCUSSION

Acyl halide intermediates occur frequently in halocarbon metabolism (Macdonald, 1982). Although these extremely reactive intermediates hydrolyze with rate constants exceeding $10^3\ s^{-1}$ at 22 °C (Palling & Jencks, 1984), they also react concomitantly with GSH to produce thiol esters (Pohl et al., 1981; Liebler et al., 1984). In DCE metabolism, $ClCH_2COCl$ reacts with two GSH molecules to yield $GSCH_2COSG$, a relatively stable thiol ester (Liebler et al., 1984). Our data indicate that $ClCH_2COSG$ displays properties that are consistent with its proposed role as a precursor to $GSCH_2COSG$.

$ClCH_2COSG$ displayed chemical properties that are distinctly different from those of $ClCH_2COCl$ (summarized in Figure 9). $ClCH_2COSG$ did not hydrolyze to yield $ClCH_2CO_2H$ and GSH even as the pH of the incubations was increased from 7.0 to 9.0. Moreover, we routinely prepared cyclic conjugate **2** by first dissolving $ClCH_2COSG$ in water and then adjusting the pH to 9.0–9.5 with ammonia. This procedure always yielded cyclic conjugate **2** as the sole product. $ClCH_2COSG$ did not undergo thiol exchange reactions with other thiols. *S*-Chloroacetyl adducts would be approximately as stable as $ClCH_2COSG$ and would have been detected in our experiments. Further, we did not find intra- or intermolecularly cross-linked oxytocin or *N*-acetylcysteine adducts. These could arise from secondary reactions of *S*-chloroacetyl adducts. Although $ClCH_2COSG$ did react with hydroxylamine, it failed to acylate the amine terminus of oxytocin. However, $ClCH_2COSG$ acylated the glutamate α-amine as the initial step in its degradation to a cyclic product. This preferential S–N acyl transfer may be dictated by the relatively slow rate of the competing hydrolysis reaction. Hydrolysis of $ClCH_2COSCoA$, for example, proceeds at approximately one-tenth the rate of S–N transfer in $ClCH_2COSG$ (Williams & Northrop, 1979). Although increasing pH might have been expected to cause $ClCH_2COSG$ hydrolysis, the competing S–N acyl transfer reaction was presumably accelerated by deprotonation of the γ-glutamyl α-amine ($pK = 9.12$), and no hydrolysis occurred. The cyclization step involves chloride displacement by free cysteinyl thiol and apparently occurs somewhat faster than S–N transfer. The exclusive formation of an 11-membered ring product is generally disfavored by thermodynamic considerations (Winnik, 1981). However, the presence of the chloroacetyl group on the cysteine sulfur and, subsequently, on the glutamate α-amine may cause $ClCH_2COSG$ to assume conformations that favor both S–N acyl transfer and the subsequent cyclization. In support of this suggestion, the glutamate β-protons, which have identical chemical shifts in GSH (Koga et al., 1986), have shifts that are separated by approximately 0.3 ppm in both $ClCH_2COSG$ and the cyclic product (see Figures 1 and 4). The similar shift separations suggest that molecular motion in the environment of both protons is constrained in a similar way and support the suggestion that $ClCH_2COSG$ and the cyclic product have some degree of conformational similarity. No accumulation of an N^{α}-(2-chloroacetyl)glutamyl intermediate was apparent in NMR studies of decomposing $ClCH_2COSG$. The rate of HCl release, measured with a pH-stat, was similar to that for loss of thiol ester absorbance.[2] However, small amounts of a dimeric product (*m/z* 693) were noted in LSIMS analyses of incubation mixtures (Figure 8 and unpublished observations). Such a dimeric product could be formed by reaction between two N^{α}-(2-chloroacetyl)glutamyl intermediates or possibly via a minor biomolecular pathway.

$ClCH_2COSG$ reacted with model thiols by alkylation to produce products in which the target thiol is cross-linked to GSH by a two-carbon bridge. Such adducts are structurally analogous to $GSCH_2COSG$, which can either exchange GSH for other thiols at the carbonyl carbon or undergo hydrolysis with a $t_{1/2}$ of about 4 h (Liebler et al., 1984). Although other thiols apparently do not displace GSH from $ClCH_2COSG$, GSH displacement from protein–S–CH_2COSG adducts may form protein thiol cross-links. Hydrolysis of the cross-links would yield carboxymethylated thiols. Protein thiols appear to be toxicologically significant cellular targets (DiMonte et al., 1984; Albano et al., 1985; Ku & Billings, 1986), and their modification by $ClCH_2COSG$ may contribute to DCE toxicity.

Although its properties make it a likely intermediate in DCE biotransformation, the role of $ClCH_2COSG$ in DCE toxicity is unclear. The extreme reactivity of acyl halides may limit their reaction with more distant targets inside or outside cells. Studies of protein covalent binding by reactive acyl halide metabolites of chloramphenicol and halothane indicate that cytochromes P-450, which produced the acyl halides, were modified almost exclusively (Halpert, 1981; Satoh et al., 1985). DCE metabolites from isolated rat hepatocytes reacted with extracellular GSH to form $GSCH_2COSG$ (Liebler et al., 1985). Although this result had been interpreted as evidence that $ClCH_2COCl$ crossed the hepatocyte plasma membrane, perhaps a more plausible interpretation is that $ClCH_2COSG$, rather than $ClCH_2COCl$, reacted with extracellular GSH. A contribution of $ClCH_2COSG$ to DCE toxicity may involve reactions with different targets than those modified by $ClCH_2COCl$. Enhanced stability relative to $ClCH_2COCl$ may

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[2] D. C. Liebler and D. S. Kling, unpublished observations.

allow $ClCH_2COSG$ to interact with targets in distant locations inside or outside the cell. In addition, reactive glutathione conjugates may interact specifically with proteins that contain specific GSH binding sites. The $ClCH_2COSG$ analogue $ClCH_2COSCoA$ irreversibly inhibited rabbit fatty acid synthetase by alkylating a thiol of the pantetheine prosthetic group (McCarthy & Hardie, 1982). Analogous reactions between $ClCH_2COSG$ and GSH-dependent detoxication enzymes and transport proteins could diminish cellular resistance to chemical injury. For example, selected hepatic glutathione-S-transferase activities are lost during DCE intoxication in vivo (Moslen & Reynolds, 1985). In addition, hepatocyte canilicular membranes, which contain transport proteins that extrude GSSG and glutathione conjugates from hepatocytes, are damaged early in DCE intoxication (Kanz & Reynolds, 1986). Further studies are necessary to determine whether reactive glutathione conjugates are involved in canilicular membrane injury, which coincides with the onset of DCE-induced cholestasis (Moslen et al., 1985).

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Registry No. **1**, 113668-39-6; **2**, 113668-40-9; DCE, 75-35-4; $ClCH_2COSG$, 113668-38-5; GSH, 70-18-8; $GSCH_2COSG$, 95102-11-7; 2-chloroacetyl chloride, 79-04-9; oxytocindithiol, 17912-60-6; *N*-acetyl-L-cysteine, 616-91-1; oxytocin–$ClCH_2COSG$, 113668-49-8; *N*-acetyl-L-cysteine–$ClCH_2COSG$, 113668-41-0.

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