# *S*-[2-($N^7$-Guanyl)ethyl]glutathione, the Major DNA Adduct Formed from 1,2-Dibromoethane†

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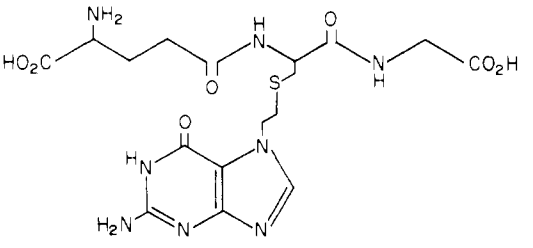
*Received July 18, 1985*

ABSTRACT: The reaction of 1,2-dibromoethane and glutathione with DNA in the presence of glutathione *S*-transferase results in the formation of a single major DNA adduct, which can be released by thermal hydrolysis at neutral pH and separated by octadecylsilyl and propylamino high-performance liquid chromatography. The same DNA adduct is the only major one formed in livers of rats treated with 1,2-dibromo[1,2-$^{14}$C]ethane. The DNA adduct was identified as *S*-[2-($N^7$-guanyl)ethyl]glutathione: (1) The chromatographic behavior was altered by treatment with γ-glutamyl transpeptidase or *Streptomyces griseus* protease. (2) The molecular ions observed in positive and negative mode fast atom bombardment mass spectrometry were those expected for the structure when either glycerol or a mixture of dithiothreitol and dithioerythritol was used as the bombardment matrix. (3) The two-dimensional $^1$H NMR correlated spectroscopy spectrum of the DNA adduct was compared to the spectra of glutathione, oxidized glutathione, and $N^7$-methylguanine and found to be consistent with the assigned structure. No evidence for in vitro or in vivo opening of the guanyl imidazole ring was observed under these conditions. The structure of the adduct supports a pathway involving enzyme-catalyzed conjugation of 1,2-dibromoethane with glutathione, nonenzymatic dehydrohalogenation of the resulting half-mustard to form a cyclic episulfonium ion, and attack of the $N^7$ nitrogen of DNA guanine on the episulfonium ion to generate this major DNA adduct, which may be related to the carcinogenicity of this chemical.

1,2-Dibromoethane (ethylene dibromide) has been used as a pesticide and lead scavenger in gasoline. Its commercial use has been restricted (*Chem. Eng. News*, 1983; Sun, 1984) because of its potential for producing liver, lung, stomach, mammary, adrenal, skin, and kidney tumors (Olson et al., 1972; Weisburger, 1977; National Toxicology Program, 1982; Wong et al., 1982; Huff, 1983). The deaths of two humans have been associated with acute exposure to 1,2-dibromoethane (Letz et al., 1984).

The manner in which 1,2-dibromoethane produces mutagenic and carcinogenic effects is of interest. 1,2-dibromoethane has some inherent chemical reactivity, but several lines of evidence indicate that its effects are due primarily to metabolites. The work of van Bladeren et al. (1981) suggests that the major portion of metabolism is oxidative, yielding 2-bromoacetaldehyde. 2-Bromoacetaldehyde is capable of reacting with DNA (Banerjee et al., 1979; Leonard, 1983), but the rate is very slow (Guengerich et al., 1981). 1,2-Dibromoethane has also been postulated to be conjugated with GSH[1] (Nachtomi, 1970). Experiments by Rannug et al. (1978) suggested that a mechanism for producing mutagens might involve conjugation of 1,2-dihaloethanes with GSH to form half-mustards and subsequent nucleophilic attack on these (via an episulfonium ion intermediate). Further evidence for a role of such a pathway with 1,2-dihaloethanes has been provided (Guengerich et al., 1980; Rannug, 1980; Sundheimer et al., 1982; White et al., 1983; MacFarland et al., 1984; Storer & Conolly, 1985).

Our own studies with 1,2-dichloroethane also support a primary role for such a GSH-dependent pathway in DNA damage (Guengerich et al., 1980). These studies suggested that GSH should become covalently attached to DNA after reaction with 1,2-dihaloethanes. We were able to demonstrate such attachment with 1,2-dibromoethane (Ozawa & Guengerich, 1983; Inskeep & Guengerich, 1984) using experiments with radioactively labeled GSH and 1,2-dibromoethane derivatives. Reductive desulfuration of the major DNA adduct (formed in vitro with purified GSH *S*-transferase) yielded $N^7$-ethylguanine, thus providing indirect evidence for the formation of *S*-[2-($N^7$-guanyl)ethyl]glutathione. In this report we provide chromatographic and spectral evidence that the major DNA adduct formed from 1,2-dibromoethane in vitro and in vivo in rat liver is *S*-[2-($N^7$-guanyl)ethyl]glutathione.



*S*-[2-($N^7$-guanyl)ethyl]glutathione

† This research was supported in part by USPHS Grants ES 01590 and ES 00267. The mass spectrometry work was supported in part by USPHS Grants RR 01688 and RR 05424 (Biomedical Research Support Grant). P.B.I. was supported by USPHS Training Grant ES 07028 and Postdoctoral Fellowship ES 05297. F.P.G. is a Burroughs Wellcome Scholar in Toxicology (1983–1988).

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[1] Abbreviations: GSH, glutathione; GSSG, oxidized glutathione; FAB-MS, fast atom bombardment mass spectrometry; HPLC, high-performance liquid chromatography; DSS, sodium 4,4-dimethyl-4-silapentane-1-sulfonate; COSY, correlated spectroscopy; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/86/0425-2192$01.50/0 

## Experimental Procedures

1,2-Dibromo[1,2-$^{14}$C]ethane was purchased from Amersham-Searle (Arlington Heights, IL). Male Sprague-Dawley rats (200 g) were obtained from Harlan Industries (Indianapolis, IN), and livers were used as a source of cytosolic enzymes in some experiments. $N^7$-Methylguanine, rat liver GSH *S*-transferase, *Streptomyces griseus* protease, herring sperm and calf thymus DNA, and γ-glutamyl transpeptidase were purchased from Sigma Chemical Co. (St. Louis, MO). The propylamine and octadecylsilyl HPLC columns were purchased from Altex Scientific, Berkeley, CA, unless indicated otherwise.

*Preparation of DNA Adducts.* Rats were administered a single ip injection of 37 mg of 1,2-dibromoethane (5 mCi/mmol)/kg body weight (dissolved in 0.4 mL of dimethyl sulfoxide for each rat) in in vivo labeling experiments. After 8 h, the livers from two rats were removed, suspended in 10 volumes of 6% (w/v) sodium aminosalicylate (pH 6.8), and minced with a scissors. The tissue was homogenized in a motor-driven Teflon glass device (0 °C) for 30 s at low speed. Sodium dodecyl sulfate was then added to final concentration of 1% (w/v) and homogenization continued for an additional 10 s. The homogenate was extracted 3 times with an equal volume of a $CHCl_3$–isoamyl alcohol–phenol mixture (24:1:25 v/v/v). The aqueous phase was sequentially washed with equal volumes of ethyl acetate (3 times) and ether (2 times). In each case the phases were separated by centrifugation ($10^4g$, 10 min). DNA was precipitated by the addition of 2.5 volumes of cold 100% ethanol. The precipitate obtained by centrifugation (as before) was dissolved by shaking overnight in 5 mL of 50 mM Tris-HCl buffer (pH 7.7). Five milliliters of 1 mM sodium phosphate buffer (pH 6.8) containing 2 M NaCl and 5 M urea was added, and the DNA was further purified by hydroxylapatite chromatography (5 g of dry gel used per column) as described in detail elsewhere (Inskeep & Guengerich, 1984); the material was divided among four individual columns.

Preparative in vitro DNA incubation involved a 1000-mL volume of rat liver cytosol (3.2 mg of protein $mL^{-1}$), herring sperm DNA (3 mg $mL^{-1}$), GSH (3 mM), and 1,2-dibromo-[1,2-$^{14}$C]ethane (10 mM, 75 mCi $mol^{-1}$) in 50 mM Tris-HCl buffer (pH 7.7) containing 15 mM sodium citrate. After the reaction proceeded for 120 min at 37 °C, DNA was recovered by ultracentrifugation in a sodium dodecyl sulfate solution (Inskeep & Guengerich, 1984). In some cases where the reaction was carried out on a smaller scale, calf thymus DNA was used instead of herring sperm.

In both the in vitro and in vivo (liver) labeling studies, approximately 1 nmol of adduct was formed per milligram of DNA (Ozawa & Guengerich, 1983; Inskeep & Guengerich, 1984). This level corresponds to about one alkylation in each 3000 bases, or one of every 700 guanines labeled. Adducts were released from DNA by heating for 30 min at 100 °C (pH 7.0). After the solution was cooled, DNA was precipitated by the addition of 2.5 volumes of cold ethanol and centrifuged for 30 min at $10^4g$. The supernatant was decanted and reduced to near dryness in vacuo (at 25 °C). When indicated, adducts were alternatively released enzymatically as described previously (Ozawa & Guengerich, 1983). Aliquots were chromatographed on HPLC columns: a propylamino ion-exchange system (Reed et al., 1980; Liebler et al., 1985) or a reverse-phase $C_{18}$ system described elsewhere (Ozawa & Guengerich, 1983). The NMR and mass spectra shown here were obtained with a sample that eluted in the chromatogram shown in Figure 1A between 14 and 17 min and subsequently in the chromatogram shown in Figure 2 between 23 and 25 min. This sample was rechromatographed in the reverse-phase HPLC system, passed through a Waters Sep-pak cartridge (washed with $CH_3OH$ prior to use; Waters Associates, Milford, MA), and lyophilized to dryness to give 0.7 mg of adduct.

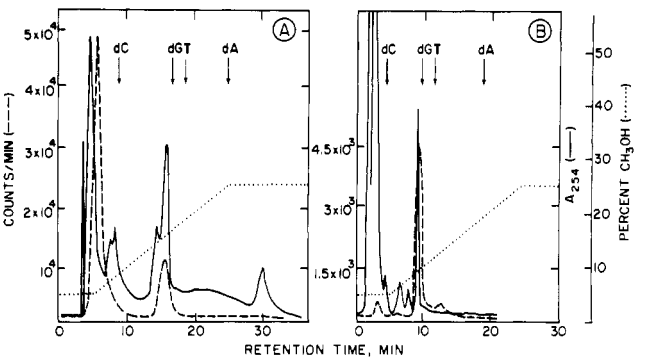


FIGURE 1: Reverse-phase HPLC chromatograms of DNA adducts formed from 1,2-dibromo[1,2-$^{14}$C]ethane and released by neutral thermal hydrolysis. (A) Herring sperm DNA was incubated in vitro with 1,2-dibromo[1,2-$^{14}$C]ethane and GSH as described and purified; an aliquot was hydrolyzed (neutral thermal) as described. A 20-min linear gradient of 5–25% $CH_3OH$ in 10 mM ammonium phosphate buffer (pH 4.0) was used to elute the 1 × 25 cm column. The flow rate was 3.0 mL $min^{-1}$. The positions of normal deoxyribonucleosides are indicated (dC, deoxycytidine; dT, thymidine; dG, deoxyguanosine; dA, deoxyadenosine). (B) Liver DNA recovered from a rat treated with 1,2-dibromo[1,2-$^{14}$C]ethane was hydrolyzed at neutral pH as described, and the resulting sample was chromatographed as described in (A), except that the size of the column was 0.4 × 25 cm and the flow rate was 1.0 mL $min^{-1}$; the positions of standard deoxyribonucleosides are indicated with arrows. [The order of migration of the deoxyribonucleosides has been observed to differ with the Altex (this figure) and Whatman (Figure 3) columns.]

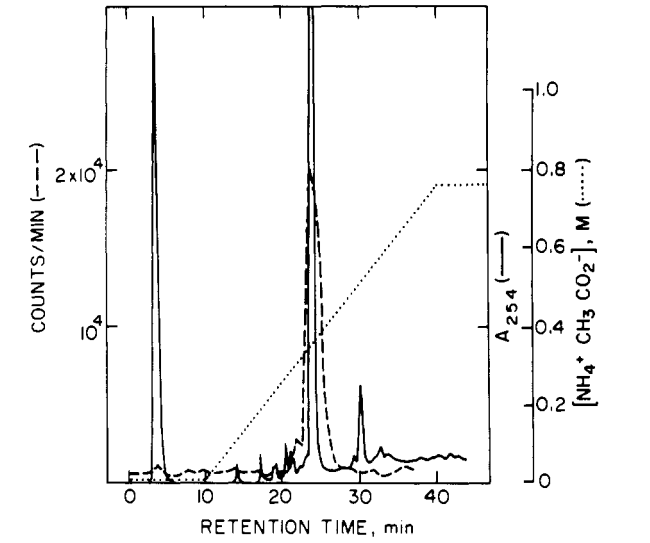


FIGURE 2: Ion-exchange HPLC of the GSH–1,2-dibromoethane–DNA adduct. The adduct recovered from the chromatography shown in Figure 1A (second radioactive peak) was chromatographed on a 0.4 × 25 cm propylamino column as described elsewhere (Reed et al., 1980). The flow rate was 1.0 mL $min^{-1}$, and the gradient was from 8 to 760 mM ammonium acetate (in 80% $CH_3OH$).

*FAB-MS.* Analysis was done at the Vanderbilt Mass Spectrometry Laboratory by Drs. B. Sweetman and I. A. Blair. The instrument used was a VG 70-250 system having extended geometry, a standard VG FAB ion source, a standard Ion-Tech saddle field FAB gun producing xenon atoms of 8 kV energy, and a VG 11/250 data system (VG, Manchester, U.K.). The temperature was ambient. Approximately 5 μg of the glutathione–1,2-dibromoethane–DNA adduct (formed in vitro) was mixed with the matrix (200 μg of glycerol or 500 μg of a 5:1

mixture of dithiothreitol and dithioerythritol) prior to placing the sample on the target. Spectra were recorded over the range $m/z$ 100–900, and matrix subtraction was not done in the data presented here.

*NMR Spectroscopy.* Spectra were obtained with a Bruker AM-400-NB spectrometer (Bruker, Billerica, MA) equipped with an Aspect 3000 computer and operating at 400.13 MHz for $^1H$ and with a probe temperature of 18 °C. Samples of the isolated 1,2-dibromoethane–DNA adduct (prepared in vitro), GSH, GSSG, and 7-methylguanine were prepared in $^2H_2O$ containing a small amount of DSS as internal standard; the DSS methyl singlet was defined as 0 ppm. Small signals for the DSS methylene signals are visible in several of the spectra. $C^2H_3CO_2{}^2H$ was added to the 7-methylguanine sample to achieve sufficient solubility. The residual $^1H^2HO$ signal at 4.7–4.9 was attenuated by continuous irradiation with the decoupler for the adduct and the GSH spectra; the $^1H^2HO$ signal was too close to the Cys-$\alpha$ signal for this procedure to be useful for GSSG. One-dimensional spectra were acquired by using 16 384 data points and spectral widths of approximately $4 \times 10^3$ Hz with a 30° pulse. Gaussian multiplication prior to Fourier transformation increased the spectral resolution. Two-dimensional COSY spectra were acquired by using a 90–$\tau$–45 pulse sequence with 1024 data points in $F_2$ and 256 points in $F_1$. In the two-dimensional spectra the intensity of the $^1H^2HO$ signal was reduced by gated irradiation with the decoupler turned off during data acquisition. The spectral window and transmitter frequence were chosen to observe the coupled signals with optimal data-point resolution. In the case of the adduct spectrum this led to folding of several extraneous signals that are visible in the upfield region of the contour plot; the one-dimensional spectrum was recorded with a wider spectral window to preclude spectral folding. Sine-bell multiplication was used prior to both transformations. The data were zero-filled once prior to the $F_1$ transformation and then symmetrized prior to plotting.

## Results and Discussion

Enzymatic digestion of DNA labeled in vitro with 1,2-dibromo[1,2-$^{14}$C]ethane yielded a single major product (Figure 3). This compound was more polar than that we had reported previously (Ozawa & Guengerich, 1983). This is tentatively identified as the nucleoside, which we were able to obtain by doing the DNA isolation and hydrolysis more rapidly. When the procedure is delayed or the sample is heated, hydrolysis of the glucosidic bond occurs.

Thus, we were able to utilize a relatively facile neutral thermal hydrolysis procedure to remove the adduct from DNA because of the $N^7$ substitution. We had previously suggested (Ozawa & Guengerich, 1983) the attachment of the GSH moiety to DNA; the presence of GSH allowed us to separate the adduct by anion-exchange chromatography. Radiolabeling studies indicated that >98% of the DNA-bound 1,2-dibromoethane (labeling done either in vitro or in vivo) was consistently released by neutral thermal hydrolysis and chromatographed as a single major peak on HPLC (Figure 1). In Figure 1A, a large peak was eluted near the void volume. This peak consisted of GSH conjugates, for in this particular case the DNA had not been purified by hydroxylapatite chromatography or phenol extraction. In other cases the size of this peak was much smaller [see Ozawa & Guengerich (1983)] and could be eliminated by purifying the DNA by hydroxylapatite chromatography. The DNA adducts formed in vitro and in vivo eluted at the same position in either HPLC system used, that is, with the same retention time as that of the deoxyguanosine standard. Reverse-phase HPLC

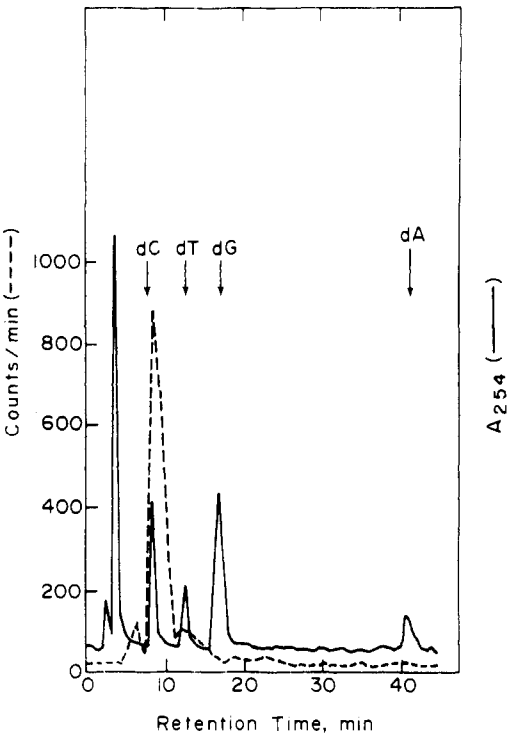


FIGURE 3: Reverse-phase HPLC chromatogram of DNA adducts formed from 1,2-dibromoethane and released by enzymatic hydrolysis. Calf thymus DNA was incubated in vitro with 1,2-dibromo[1,2-$^{14}$C]ethane and GSH as described, and the DNA (isolated by ultracentrifugation and hydroxylapatite chromatography) was enzymatically digested to nucleosides as described elsewhere (Ozawa & Guengerich, 1983); the elapsed time between the beginning of the incubation and analysis was 47 h. The extract was chromatographed on a 0.4 × 25 cm Partisil-ODS PXS 10/25 HPLC column (Whatman Separation Products, Clifton, NJ). After the sample was applied, the column was washed for 5 min with 10 mM ammonium phosphate buffer (pH 5.1) containing 8% (v/v) $CH_3OH$, and then the $CH_3OH$ concentration was increased to 20% (v/v) in a linear manner over a period of 40 min. The positions of normal deoxyribonucleosides are indicated (cf. Figure 1).

was used to purify the adduct formed in vitro. Further purification of the adduct was achieved by ion-exchange HPLC (Figure 2).

The retention time of the DNA adduct was decreased by about on-half in both HPLC systems after treatment with either *S. griseus* protease (Pronase) of $\gamma$-glutamyl transpeptidase (data not shown). These results suggested that the glutamate moiety of GSH was still present before treatment. Treatment of labeled DNA overnight with alkali (0.1 N NaOH, 23 °C) and subsequent acid hydrolysis (0.1 N HCl, 20 min, 70 °C) yielded the putative imidazole ring-opened guanyl adduct (formamidopyrimidine), which was eluted near the void volume in the reverse-phase HPLC system. (Ion-exchange HPLC did not separate the formamidopyrimidine and intact adducts well.) This adduct did not appear to be present in DNA (labeled in vitro or in vivo) that had not been treated with alkali, as judged by its absence from DNA which was acid-hydrolyzed with or without prior neutral thermal hydrolysis.

FAB-MS yielded a molecular ion at $m/z$ 483 in the negative mode and 485 in the positive mode (Figures 4 and 5). The data are consistent with the expected molecular weight of 484 for *S*-[2-($N^7$-guanyl)ethyl]glutathione. The negative ion spectrum was stronger than the positive ion spectrum, but an M + 1 ion (and sodium addition product) is clearly present in the glycerol spectrum (Figure 4). The signal-to-noise ratio was better in the positive ion spectrum obtained with the

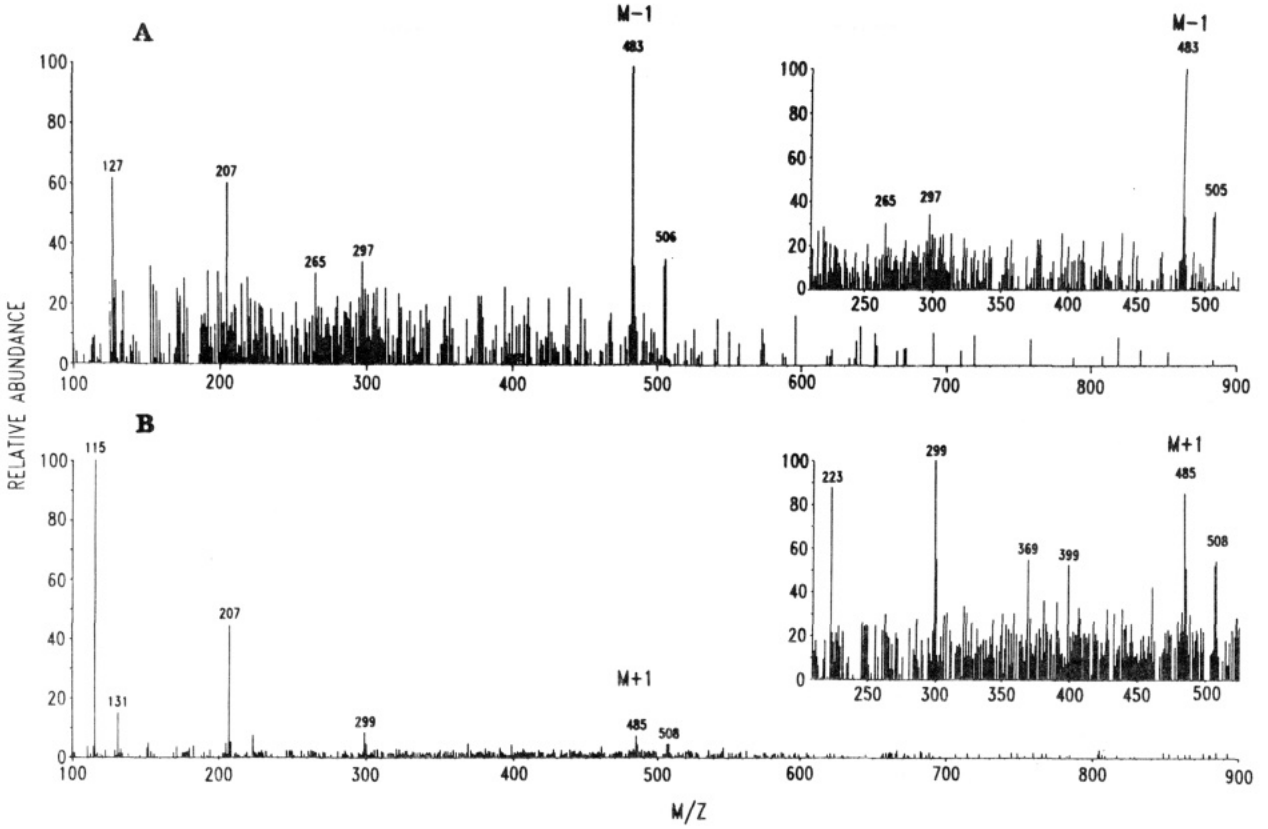


FIGURE 4: FAB-MS of the major 1,2-dibromoethane–DNA adduct formed in vitro by using a glycerol matrix. (A) Negative ion spectrum; (B) positive ion spectrum. The insets show the region near the molecular ion replotted for comparison.

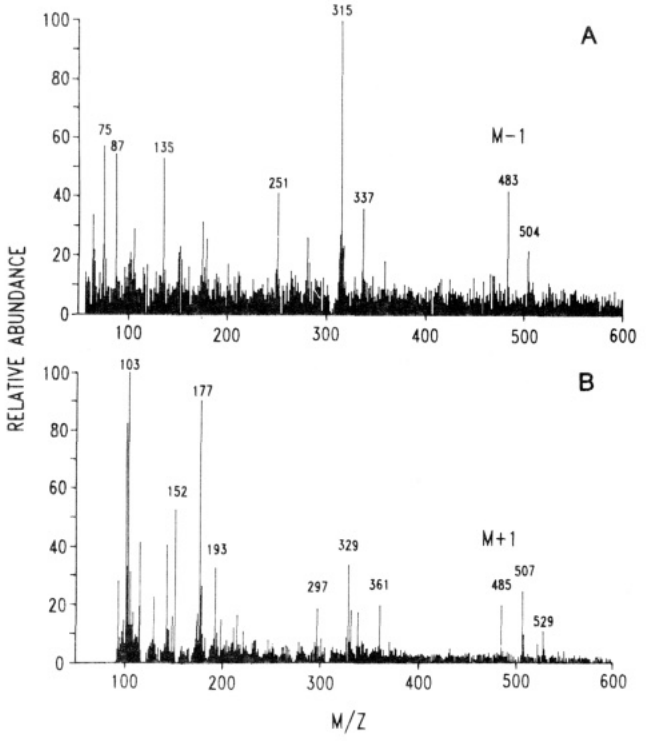


FIGURE 5: FAB-MS of the major 1,2-dibromoethane–DNA adduct formed in vitro by using a 5:1 mixture of dithiothreitol and dithioerythritol as the matrix. (A) Negative ion spectrum; (B) positive ion spectrum.

dithiothreitol–dithioerythritol matrix (Figure 5). [In other experiments, which are not presented here, we found that the addition of 1 μL of 10% (w/v) aqueous $CF_3CO_2H$ to the glycerol matrix also resulted in a similar improvement of the appearance of the parent ion in the positive-mode spectrum.]

Table I: NMR Chemical Shift Assignments for *S*-[2-($N^7$-Guanyl)ethyl]glutathione and Analogues

| | δ (relative to DSS) | | | |
|---|---|---|---|---|
| assignment | $N^7$-methyl-guanine | GSH | GSSG | *S*-[2-($N^7$-guanyl)ethyl]-glutathione |
| guanine H-8 | 8.59 | | | 7.97 br s |
| guanine $N^7$-$CH_3$ | 4.08 | | | |
| guanine $N^7$-$CH_2CH_2S$ | | | | |
| $H_a$ | | | | 4.28 m |
| $H_b$ | | | | 4.37 m |
| guanine $N^7$-$CH_2CH_2S$ | | | | |
| $H_a$ | | | | 2.91 m |
| $H_b$ | | | | 2.95 m |
| Cys β-$H_a$ | | 2.87 m | 2.95 dd | 2.51 dd |
| Cys β-$H_b$ | | 2.94 m | 3.30 dd | 2.77 dd |
| Cys α-H | | 4.56 t | 4.75 dd | 4.28[a] |
| Gly α-$H_a$ | | 3.96 m | 3.74 d | 3.62 d |
| Gly α-$H_b$ | | 3.97 m | 3.78 d | 3.67 d |
| Glu α-H | | 3.82 t | 3.70 t | 3.61 t |
| Glu β-methylene H | | 2.16 q | 2.12 q | 1.96 q |
| Glu γ-methylene H | | 2.56 m | 2.50 m | 2.31 t |

[a] Obscured by $CH_2N$ multiplet.

The molecular ions containing both one and two sodium atoms were present. The identities of major fragmentation peaks have not been determined. No higher molecular weight ions were found. The deoxyribose ring is clearly not attached, as expected from the release by neutral thermal hydrolysis. The absence of imidazole ring-opened guanine is clearly shown by the absence of any ions corresponding to a molecular weight of 502.

The one-dimensional NMR spectra of the DNA adduct, GSH, and GSSG are shown in Figures 6–8 along with the associated COSY-type two-dimensional spectra. The signal assignments for each of these are given in Table I, and a tabulation of readily measurable coupling constants in the

Table II: NMR Coupling Constants for *S*-[2-($N^7$-Guanyl)ethyl]glutathione and GSSG

| | *J* (Hz) | |
|---|---|---|
| | GSSG | *S*-[2-($N^7$-guanyl)ethyl]-glutathione |
| Gly–Gly′ | 17 | 17 |
| Cys α–Cys β-$H_a$ | 9.5 | 9 |
| Cys α–Cys β-$H_b$ | 4.5 | 5 |
| Cys β-$H_a$–Cys β-$H_b$ | 14 | 14 |
| Glu α–Glu β,β′ | 6.5 | 7.5 |
| Glu β,β′–Glu γ,γ′ | ~7 | ~8 |

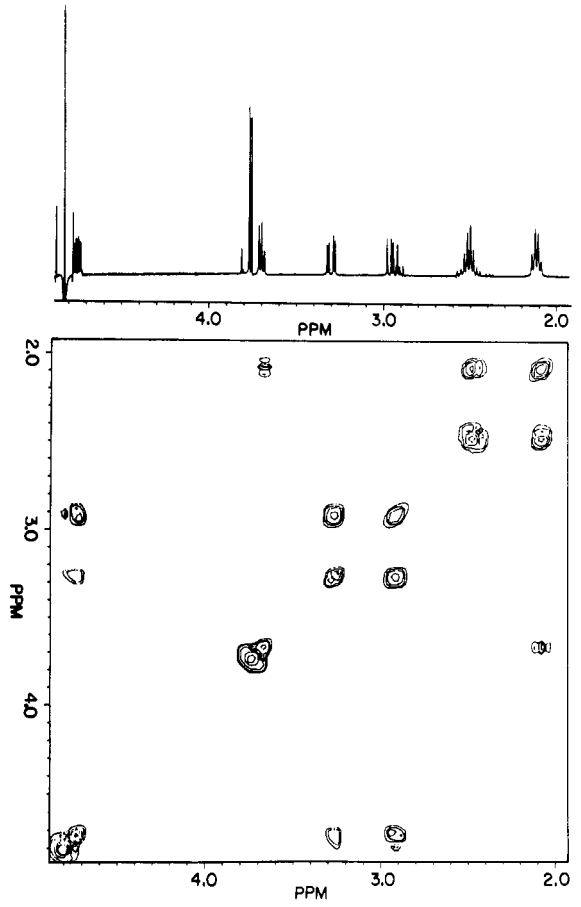


FIGURE 6: $^1$H NMR COSY spectrum of GSSG.

adduct and in GSSG appears in Table II. Substantial differences exist among the chemical shifts of individual peptide protons in going from GSSG to GSH and the GSH–1,2-dibromoethane–DNA adduct such that assignments cannot be made on the basis of simple chemical shift comparisons. More useful were the correlations obtained from the COSY spectra, the assignments derived from which were supported by coupling constant comparisons.

Looking first at the spectrum of GSSG (Figure 6), the Glu β-protons can immediately be assigned as the highest field signal, appearing as a quartet at δ 2.12. The correlation spectrum shows coupling to a multiplet at δ 2.50 and a triplet near δ 3.70, which can as a consequence be assigned as the Glu γ-protons and Glu α-protons, respectively. Three well-defined four-line patterns (doublets of doublets) appear at δ 2.95, 3.30, and 4.75; they show mutual coupling and can be assigned to the two Cys β-protons and to the Cys α-protons, respectively. The two Gly protons have slightly different chemical shifts and appear as a pair of doublets at δ 3.74 and 3.78. The weak outer line of the upfield doublet is obscured by the low-field line of the Glu α triplet. The compound has four pairs of methylene groups, and in each case the geminal

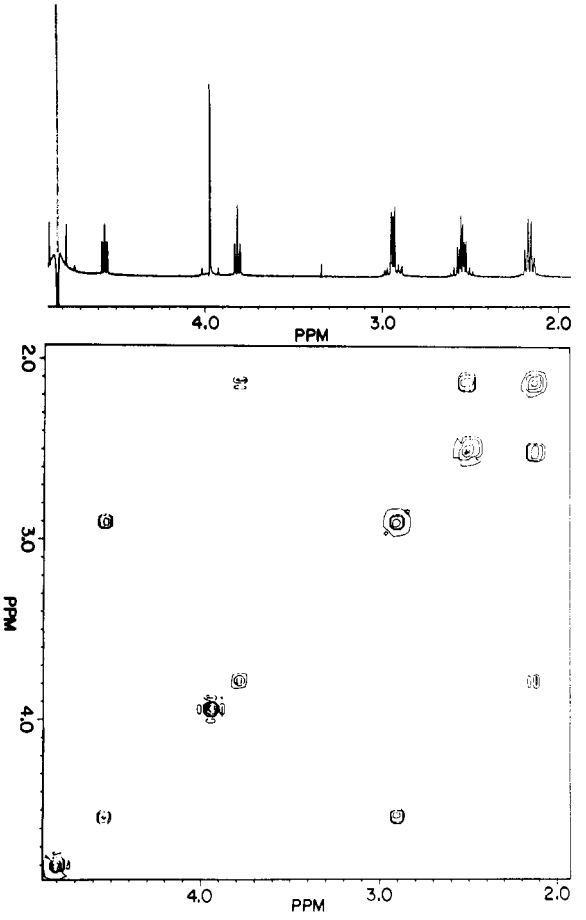


FIGURE 7: $^1$H NMR COSY spectrum of GSH.

protons are chemically nonequivalent because of the chirality of the molecule; however, the extent to which this nonequivalence leads to chemical shift differences is not uniform. The two Cys β-protons are displaced from each other by 0.35 ppm, whereas the Gly protons are separated by only 0.04 ppm. Close examination of the Glu γ-protons shows a small chemical shift difference, but the β-protons appear to be isochronous. The difference between the β-protons and γ-protons reflects the fact that the Glu residue is attached to the Cys via the γ-carboxyl group rather than the α one. The coupling constants support these assignments, particularly the Cys β assignments as a geminal pair because of the large (14-Hz) coupling constant.

The spectrum of GSH (Figure 7) is assigned similarly, although there are several noteworthy differences. One which is immediately obvious is that the Gly protons are almost indistinguishable and they are now separated sufficiently from the Glu α signal that the latter is clearly a triplet. The Cys β-protons are more nearly equivalent than in GSSG, appearing as a multiplet at δ 2.93, which is actually a closely spaced pair of four-line signals. The Cys α is a triplet. The differences in the Cys between GSSG and GSH can be accounted for by the fact that the $-CH_2SH$ group is freely rotating in GSH leading to an averaging of the two Cys α–Cys β coupling constants to give a triplet, whereas in GSSG the disulfide constrains the methylene group.

In the adduct spectrum (Figure 8) the three amino acid residues were assigned by using the same approach. The nonequivalence of the Cys β-protons was comparable to that in GSSG, as was that of the Gly protons. The accessible geminal and vicinal coupling constants are closely comparable to those of GSSG (Table II). The two methylene groups of

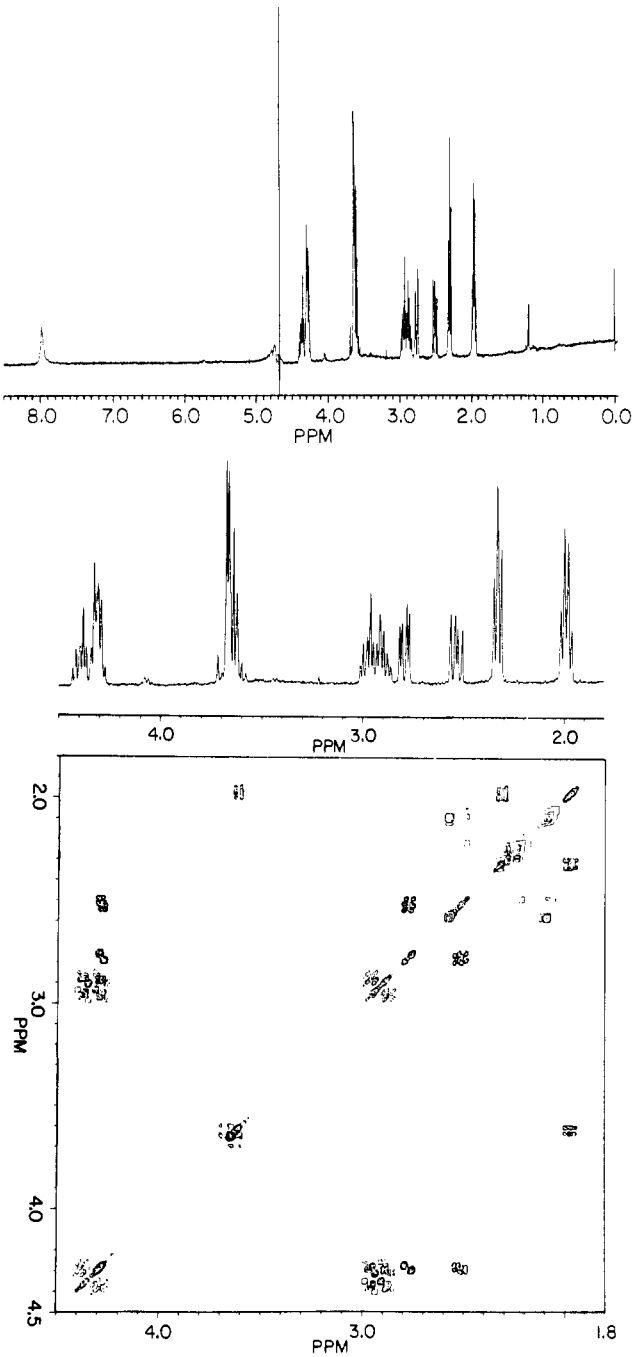


FIGURE 8: $^1$H NMR spectrum of the major 1,2-dibromoethane–DNA adduct formed in vitro: (A) $\delta$ 0–8.5 ppm; (B) $\delta$ 1.8–4.5 ppm; with COSY spectrum.

the ethylene bridge appear at approximately $\delta$ 2.93 and 4.33 and are assigned as the $S$-$CH_2$ and $N$-$CH_2$, respectively, on the basis of their shifts. Because of the chirality of the molecule, they are not an AA'XX' system but rather an ABXY system in which both sets of geminal protons show sufficient nonequivalence that they are clearly separated from each other and appear as five-line multiplets. One of the $N$-$CH_2$ protons is obscured by the Cys $\alpha$ signal, but the others are clearly visible. The coupling constants in the ethylene bridge have not been determined. The guanyl C-8 proton appears as a singlet at $\delta$ 7.97 and is significantly broadened by the adjacent quadrupolar nitrogen atoms. The spectrum of $N^7$-methylguanine was recorded for comparison; however, it was insufficiently soluble to obtain a spectrum unless sufficient $CD_3CO_2D$ was added to protonate it, under which conditions the C-8 proton appeared at $\delta$ 8.59.

## Conclusions

While we had previously suggested that $S$-[2-($N^7$-guanyl)ethyl]glutathione was a major DNA adduct formed from the carcinogen 1,2-dibromoethane, the approach of degradation of the adduct by reductive desulfuration left some questions open; e.g., was the entire GSH moiety attached to the DNA, did rearrangement occur during the reduction, and was the sugar moiety still attached to the guanine? We have now isolated the intact adduct by neutral thermal depurination of the DNA and chromatography. The same single major DNA adduct was found in vitro and in vivo. The nucleic acid base involved can only be guanine, the substitution is $N^7$, and the entire GSH moiety is present, as clearly indicated by the NMR and mass spectral evidence. Rearrangement of another adduct to yield $S$-[2-($N^7$-guanyl)ethyl]glutathione under the relatively mild isolation conditions used here cannot be unambiguously ruled out but seems unlikely in light of the available literature on DNA chemistry.

The finding that $S$-[2-($N^7$-guanyl)ethyl]glutathione is the major DNA adduct formed in vitro and in vivo is consistent with previous radiolabeling studies involving 1,2-dibromo-[1,2-$^{14}$C]ethane and [*Gly*-$^3$H]- and [$^{35}$S]GSH (Ozawa & Guengerich, 1983). Other pathways have been suggested for formation of DNA adducts involving 1,2-dibromoethane. They include oxidation to form 2-bromoacetaldehyde (Banerjee et al., 1979; Hill et al., 1978) and reductive metabolism (Tomasi et al., 1983), but these pathways are clearly not relevant. We had previously considered the potential of haloso oxidation products in our studies with 1,2-dichloroethane (Guengerich et al., 1980). However, since that time we have found that the apparent stimulation of microsomal DNA adduct formation by GSH was due to incomplete removal of GSH conjugates during analysis; moreover, $^2$H and $^{18}$O studies on the formation of 2-haloethanols and 2-haloacetaldehydes from 1,2-dihaloethanes are inconsistent with a major role for such a mechanism (Guengerich et al., 1986). Recently Foureman and Reed (1985) have reported that cleavage of the $\gamma$-glutamyl moiety from $S$-(2-chloroethyl)glutathione can increase rates of alkylation of model nucleophiles and have suggested a role for such proteolysis and a non-episulfonium mechanism. However, our results indicate that such a mechanism is not operative in alkylation of rat liver DNA by the analogous bromo compound.

The relevance of this unusual $S$-[2-($N^7$-guanyl)ethyl]glutathione adduct to the carcinogenicity of 1,2-dibromoethane has not yet been established. However, we have found that primary cultures of human hepatocytes can also carry out such DNA adduct formation, and further studies on the fate of this adduct in appropriate systems are in progress.

**Registry No.** GSH, 70-18-8; GSH $S$-transferase, 50812-37-8; $S$-[2-($N^7$-guanyl)ethyl]GSH, 100840-34-4; $Br(CH_2)_2Br$, 106-93-4.

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# Conformation of d(CpG) Modified by the Carcinogen 4-Aminobiphenyl: A Combined Experimental and Theoretical Analysis†

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ABSTRACT: The change in DNA conformation produced by the attachment of a reactive substance is likely to be a vital factor in determining the biological consequences of the reaction. We have prepared a deoxydinucleoside monophosphate containing the major adduct derived from the carcinogenic amine 4-aminobiphenyl and analyzed its conformation by theoretical and experimental methods. Reaction of d(CpG) with *N*-acetoxy-*N*-(trifluoroacetyl)-4-aminobiphenyl afforded the product modified at C-8 of guanine with 4-aminobiphenyl. After purification by reverse-phase high-performance liquid chromatography, milligram amounts of product were obtained. It was analyzed by circular dichroism, proton magnetic resonance, and minimized potential-energy calculations. A flexible molecule with a mixture of conformers is indicated. Both carcinogen–base-stacked states and base–base-stacked states, with guanine both syn and anti, contribute to the population mixture on the dimer level. The global minimum-energy conformation has *syn*-guanine and carcinogen–base stacking. Forms of this type are calculated to represent roughly 58% of the conformer population. Because of the twisted nature of the biphenyl moiety, carcinogen–base stacking inherently involves less overlap than that in the planar and rigid three-ringed aminofluorene analogue. This difference might relate to the diminished effectiveness of the aminobiphenyl vs. the aminofluorene adduct as a frameshift mutagen in *Salmonella typhimurium* 1538.

Only a slight structural modification often distinguishes potent carcinogens and mutagens from related compounds that are innocuous. This difference may involve the presence or location of a methyl group, for example, or the location of a ring junction (Dipple et al., 1984; Garner et al., 1984). In the case of the *anti*-benzo[*a*]pyrenediol epoxides (Buening et al., 1978), two enantiomers showed vastly different biological effects. It would be valuable if some simple principle could explain these differences. Carcinogenesis and mutagenesis by chemicals is the result of a series of processes, however, all of which must be considered if the relation of structure to biological effect is to be understood. These steps include entry of a chemical into the cell, metabolic activation, reaction with the critical target (presumably DNA), and response of the modified DNA to the repair and replication systems that subsequently interact with it.

† This work was supported jointly by DOE Contract DE-ACO2-81 ER60015, by USPHS Grant 1R01 CA 28038-05, awarded by the National Cancer Institute, DHHS, by BRSG Grant RR07062, awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health, by National Science Foundation Grant DMB8416009, and by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract DE-AC05-84OR21400 with Martin-Marietta Energy Systems, Inc. (B.E.H.).
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0006-2960/86/0425-2198$01.50/0 