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Activation of Dihaloalkanes by Glutathione Conjugation and Formation of DNA Adducts

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Ethylene dibromide (1,2-dibromoethane, EDB) can be activated to electrophilic species by either oxidative metabolism or conjugation with glutathione. Although conjugation is generally a route of detoxication, in this case it leads to genetic damage. The major DNA adduct has been identified as S-[2-(N^7-guanyl)ethyl]glutathione, which is believed to arise via half-mustard and episulfonium ion intermediates. The adduct has a half-life of about 70 to 100 hr and does not appear to migrate to other DNA sites. Glutathione-dependent DNA damage by EDB was also demonstrated in human hepatocyte preparations. The possible relevance of this DNA adduct to genetic damage is discussed.

Introduction

Many halogenated hydrocarbons are of concern because of industrial and environmental exposure. With the apparent exception of halogenated dioxins, biphenyls, and related compounds, most of these chemicals require metabolic bioactivation to exert their biological effects, at least genetic damage. Several enzymatic pathways can be involved in such bioactivation, including mixed-function oxidation and reduction by cytochrome P-450 (P-450) enzymes. In recent years another mechanism for activation has been identified, that of conjugation with the physiological nucleophile glutathione (GSH). This reaction appears to be largely restricted to 1,2-dihaloalkanes. Several of these are of particular concern. 1,2-Dibromoethane (ethylene dibromide, EDB) has been used as a pesticide and gasoline additive; it produces tumors at a number of sites in rodents (*1–3*) and is acutely toxic to rodents (*1*) and man (*4*). 1,2-Dichloroethane (ethylene dichloride, EDC) is used in the production of vinyl chloride; it can produce tumors in mice when administered via specific routes (*5*). Evidence for the existence of this reaction in the production of DNA adducts from *vic*-dihaloalkanes is reviewed and the relevance of these adducts is discussed.

Chemistry of Formation of DNA Adducts

The work in this laboratory was based upon the reports of Rannug et al. (*6*) that bacterial mutagenesis induced by EDC was dependent upon the action of GSH-dependent cytosolic enzymes and not microsomal mixed-function oxidases. We repeated these experiments and also found that the covalent binding of radioactivity from [1,2-^{14}C]-EDC followed a similar pattern (*7*). The pathway postulated by Rannug (*8*) would be expected to yield covalent attachment of GSH to DNA, and subsequent experiments with purified GSH S-transferases and isolated rat hepatocytes showed that radiolabels from both EDB and GSH became covalently bound to DNA in equimolar ratios (*9*).

The DNA adduct formed from EDB *in vitro* was cleaved enzymatically and partially purified. Reduction of the resulting sample with a modified Raney nickel procedure yielded N^7-ethylguanine, consistent with the view that the original adduct was S-[2-(N^7-guanyl) ethyl]-GSH (*9*). Subsequently, the adduct could be released from DNA by neutral thermal hydrolysis and isolated using a combination of reversed phase and anion exchange high performance liquid chromatography (HPLC). The structure S-[2-(N^7-guanyl)ethyl]GSH (Fig.1) was unambiguously established by fast atom bombardment mass spectrometry and two-dimensional homonuclear NMR correlated spectroscopy (*10*). No evidence for opening of the imidazole ring was observed in these experiments. In the case of [1,2-^{14}C]-EDB adducts formed *in vitro* or *in vivo* (rat liver or kidney), more than 97% of the radioactivity bound to DNA could be released by neutral thermal hydrolysis and, of this, >90% migrated as S-[2-(N^7-guanyl)ethyl]GSH upon HPLC.

Several mechanisms are possible for the formation of the adduct; we prefer a pathway involving an episul-

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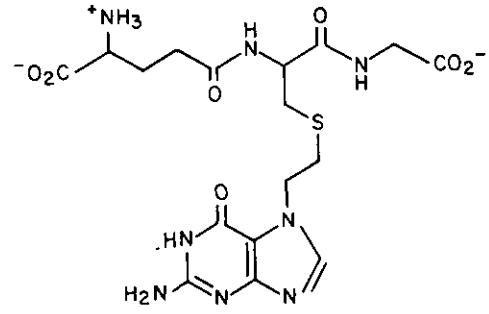

FIGURE 1. Structure of S-[2-(N^7-guanyl)ethyl]GSH.

fonium (thiiranium) ion intermediate (Fig. 2). Studies on the alkylation of model nucleophiles by S-(2-chloroethyl)cysteine derivatives indicate that the thioether moiety is essential for alkylation. A protonated amine retards alkylation; alkylation is facilitated by raising the pH to deprotonate the amine, acetylation of the amine, or the addition of a methylene group (homocysteine analog) to introduce an inductive effect and move the positively charged amine away from the incipient episulfonium ion. These results are consistent with a mechanism which involves an episulfonium ion; in addition, these model studies argue that if the γ-glutamyl bond were cleaved to generate a free amine at neutral pH, the alkylation of DNA should be retarded and not enhanced.

The above model studies argue against a role for γ-glutamyl transpeptidase in modulating the alkylation of DNA. In other studies we found that inhibition of γ-glutamyl transpeptidase activity *in vitro* or *in vivo* did not affect the level of DNA adducts formed in liver or kidney (*11*). These findings are consistent with the high level of DNA adducts formed in rat liver, a tissue devoid of γ-glutamyl transpeptidase.

Fate of S-[2-(N^7-guanyl)ethyl]GSH

One question concerning S-[2-(N^7-guanyl)ethyl]GSH adducts in DNA is whether they can break down to regenerate the episulfonium ion, i.e., whether guanine can act as a leaving group. This question is relevant in considering the potential of EDB adducts in meat, milk, and other foods to alkylate host DNA after ingestion. We incubated S-[2-(N^7-guanyl)(1,2-^{14}C)ethyl]GSH with calf thymus DNA for 3 hr at neutral pH; under these conditions no labeling of the DNA could be detected (*11*). Thus, such a transalkylation reaction does not appear to be a matter of concern.

We established a half-life of S-[2-(N^7-guanyl)ethyl]-GSH in calf thymus DNA of about 150 hr at neutral pH and 37°C. In rat liver, kidney, lung, and stomach the *in vivo* half-life was 70 to 100 hr (*11*). In the case of liver, comparison of data based on total DNA-bound radioactivity and on HPLC separation of S-[2-(N^7-guanyl)ethyl]GSH as a function of time yielded similar half-life values (*11*).

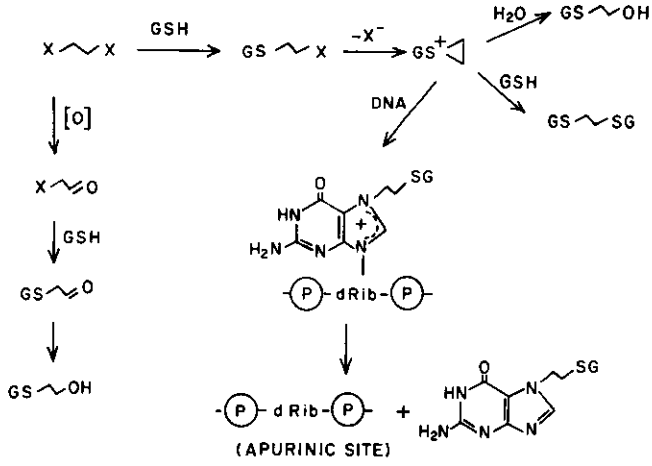

FIGURE 2. Reactions related to metabolism of EDB. See text.

Consideration of Other Dihalides

EDC appears to be activated by the same mechanism as EDB (*6,7*). We also detected S-[2-(N^7-guanyl)ethyl]-GSH in the liver and kidney DNA of rats treated with EDC, although the levels of binding were much lower and, in the case of kidney, other adducts appeared to be present as well (*11*). The mixed halide 1-bromo-2-chloroethane is activated via the same mechanism, and the expected halide order is observed (*9*).

Insertion of more methylene groups into these α,ω-substituted haloalkanes abolished the reaction with DNA (although these are actually better substrates for GSH S-transferase). *In vitro* evidence for some DNA adduct formation via the GSH-conjugation pathway could be obtained for 1,2-dibromo-3-chloropropane and *tris*(2,3-dibromopropyl)phosphate, although the contribution of oxidative pathways seems to be more important (*12*). The question of dihalomethanes is quite relevant: The GSH-dependent formation of HCHO from these compounds has been reported (*13*), but only very limited data implicating GSH/GSH S-transferase-mediated bacterial mutagenesis are available (*14–16*).

Experiments Involving Humans

Hepatocytes were isolated from five individual human liver samples using the technique of collagenase incubation of liver slices. The level of DNA adduct formation in the human samples was about 40% of that in rat hepatocytes prepared in the same manner (0.16 vs. 0.45 nmole adducts formed/mg DNA/2 hr).

In other experiments (with M. J. Meredith), human hepatocytes were isolated and allowed to form monolayers. EDB dose-dependent incorporation of ^{3}H-thymidine into DNA (unscheduled DNA synthesis) could be demonstrated over the range of 10^{-7} to 10^{-3} M EDB. The unscheduled DNA synthesis could be nearly abolished if the cells were treated with 0.5 mM diethylmaleate (prior to EDB incubation) to deplete GSH. However, treatment with diethylmaleate after EDB incubation had only a small effect on the EDB dose-

dependent unscheduled DNA synthesis, consistent with the view that both EDB and GSH are required for DNA alkylation. These studies also suggest that some enzymatic DNA repair can occur.

Recently, Letz et al. (*4*) reported the death of two workers due to EDB intoxication. We have isolated DNA samples from one of these individuals and are presently attempting to estimate the levels of S-[2-(N^7-guanyl)ethyl]GSH using a postlabeling method.

Relevance to Risk Assessment and Future Considerations

This article has reviewed evidence that the major DNA adduct formed from EDB and EDC is S-[2-(N^7-guanyl)ethyl]GSH, which is formed via reaction of S-(2-haloethyl)GSH and episulfonium intermediates with the N^7 position of guanine to yield a bulky adduct (Fig. 3). The physiological half-life of the adduct is 70 to 100 hr and transalkylation does not apparently occur. The basic reaction appears to occur in human samples and with some related *vic*-dihaloalkanes.

The question arises as to whether or not this particular DNA adduct is responsible for the biological properties of *vic*-dihaloalkanes, particularly tumor initiation. Several lines of evidence support the view that GSH-dependent reactions are related to genetic damage (Table 1). However, a problem exists in that guanyl N^7 adducts are not in the DNA base-pairing region, and among the N^7 purine lesions, only aflatoxin adducts are thought to be associated with mutation. However, aflatoxin B_1 apparently forms only N^7 guanyl adducts and the resulting imidazole ring-opened derivatives as DNA adducts, but these are highly effective in causing mutations; further, aflatoxin B_1 also gives rise to a mutation at the guanyl residue in codon 12 of the c-ras^K gene to yield an active oncogene capable of transforming NIH 3T3 cells in culture (*21*). Nevertheless, we cannot at this time discount the possibility that other DNA adducts are formed in small amounts and are responsible for the biological effects of EDB and other *vic*-dihaloalkanes. Further experiments are in progress in this laboratory to test the hypothesis that S-[2-(N^7-guanyl)ethyl]GSH is the major mutagenic lesion formed.

The point should be made that GSH conjugation is generally a detoxicating process, but here the reaction can place the organism at risk. Attempts to intervene in metabolic processes and increase GSH or GSH S-transferase levels could render the host at greater risk.

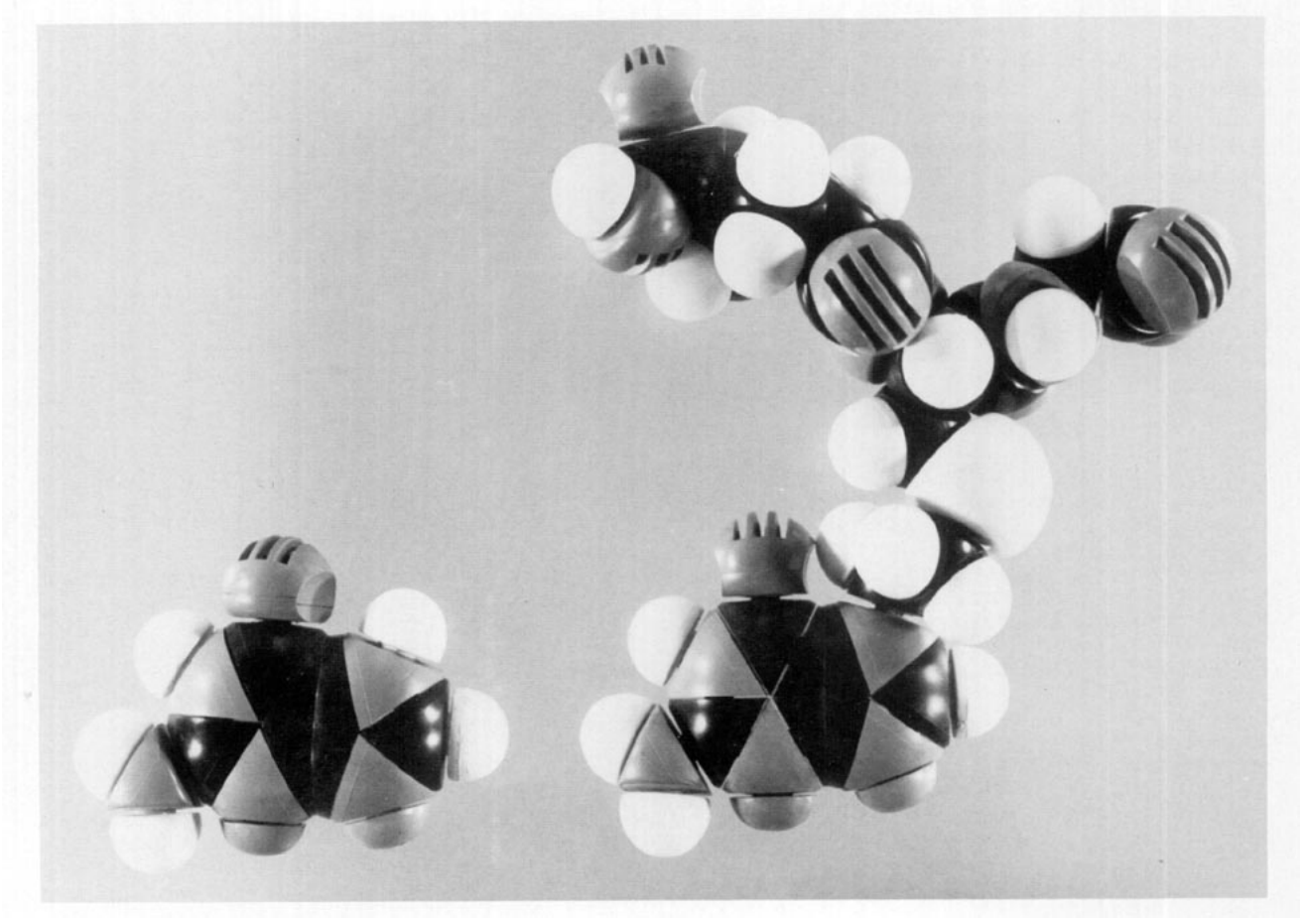

FIGURE 3. Space-filling models of (left) guanine and (right) S-[2-(N^7-guanyl)ethyl]GSH.

Table 1. Evidence for the involvement of GSH conjugation in genetic damage of *vic*-dihaloalkanes.

	Reference
Bacterial mutagenicity depends upon cytosolic enzymes and GSH	(*8*)
In vitro DNA binding depends upon cytosolic enzymes and GSH	(*7*)
DNA strand breaks enhanced by deuterium substitution	(*17*)
DNA strand breaks enhanced by cytochrome P-450 inhibitors	(*18*)
S-(2-Haloethyl)cysteine derivatives are mutagenic	(*19*)
Major DNA adduct contains GSH	(*9,10*)
In vitro unscheduled DNA synthesis is GSH-dependent	This report, (*20*)

Further, this case points out the inherent dangers in assuming that all electrophilic and reactive chemical species are identical. Oxidation of EDB and EDC produces the 2-haloacetaldehydes, which react rapidly with thiols but not with DNA (*22*). Further, the level of 2-haloacetaldehydes produced *in vivo* appears to be considerably higher than S-(2-haloethyl)GSH (*23*). An additional complication in all of these considerations is that GSH still has at least two detoxicating roles (Fig. 2), and estimating rate constants for all of the individual reactions and modeling the overall scheme is difficult.

Several lines of evidence indicate that these reactions probably do occur in humans. However, the data to date are limited and meaningful rodent/human extrapolations are difficult. The possibility exists that depurination of S-[2-(N^7-guanyl)ethyl]GSH may be followed by processing to yield a urinary mercapturic acid to expedite dosimetry.

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