Stereoselective Formation of in Vitro Nucleic Acid Adducts by 2,3-Epoxy-4-hydroxynonanal

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ABSTRACT

This paper describes the reactions of purine nucleosides and nucleic acids with 2,3-epoxy-4-hydroxynonanal. 2,3-Epoxy-4-hydroxynonanal was produced with tert-butyl hydroperoxide by epoxidation of trans-4-hydroxy-2-nonenal, a lipid peroxidation product. The epoxy aldehyde exists as a pair of diastereomers, I and II. Because these isomers could not be completely separated under the chromatographic conditions used, reactions were carried out with a mixture of known proportions of isomers I and II. Reaction of adenine nucleosides with the epoxy aldehyde yielded diastereomers A1 and A2, which structures were assigned on the basis of their spectroscopic data and by chemical synthesis as 1,N2-etheno adducts possessing a heptyl group at C8. These adducts were formed from isomers I and II in a stereoselective manner. Isomer I appeared to be responsible for the formation of A2, whereas isomer II favored the production of A1. Stereoselectivity of isomers I and II was also observed upon reaction with guanine nucleosides in the formation of adducts G1, G2, G3, G4, G5, and G6. G2, G3, G5, and G6 were unstable in base and could be converted quantitatively to G1. The structures of these adducts were reported (Sodum, R. S., and Chung, F. L. Chem. Res. Toxicol., 2: 23-28, 1989). G5 and G6 were the products formed predominantly from reactions in which isomer I was in excess, whereas G1 and G4 were the major products in reactions enriched with isomer II. Incubation of DNA with the epoxy aldehyde at 37°C and pH 7.0 yielded a modified DNA containing 1,N2-etheno-oxyguanosine (G1) at levels of 10 pmol/mg DNA. Although G2, G3, G5, and G6 were not readily detected in this DNA hydrolysate, base conversion of fractions corresponding to these adducts to G1 indicated that the total yield of these adducts was equivalent to approximately 20% of that of G1. A1 and A2 were not found in this DNA. Contrary to the reactions with native DNA, reactions of single-stranded DNA resulted in the formation of primarily A1 and A2, with a total adduct level of 30 nmol/mg DNA. In this DNA, the yield of guanine adducts was relatively small, estimated at 0.73 nmol/mg DNA based on conversion to G1. RNA was extensively modified by the epoxy aldehyde, yielding both adenine and guanine nucleosides. The levels of adenine nucleoside adducts formed in RNA were greater than 50 nmol/mg RNA. The yields of guanine nucleoside adducts were 7.0 and 50 nmol/mg RNA depending on the proportion of isomers I and II in the reaction. Although isomer II showed a comparable reactivity for adenine and guanine nucleosides in RNA, isomer I seemed to be more reactive toward adenine than toward guanine, yielding levels of adenine adducts at least 7-fold greater than those of guanine adducts. Stereoselectivity of isomers I and II similar to that observed with monomers was also demonstrated.

INTRODUCTION

EH2 is a potential activated metabolite of trans-4-hydroxy-2-nonenal, a biologically active aldehyde generated by lipid peroxidation (1). Because it possesses two chemically reactive functional groups, an epoxide and an aldehyde, EH readily reacts with deoxyguanosine, producing cyclic adducts (2, 3). The structures of deoxyguanosine adducts have been fully characterized (3). Two types of structural modifications were identified. One consists of a novel tetracyclic ring structure resulting from ring-extension from the 1 and N2 positions of guanine, and the other involves the formation of a new 1,N2-etheno ring moiety. The former modification resulted in adducts that can be quantitatively converted to 1,N2-etheno-oxyguanosine under mildly basic conditions. These results suggest that 1,N2-etheno-oxyguanosine may be used as a general marker for this damage in nucleic acids. Because of its reactivity, it is speculated that EH could produce adducts upon reaction with nucleic acids and thus is a likely mutagen and carcinogen. Glycidaldehyde, an epoxide of acrolein and a lower homologue of EH, is tumorigenic (4). Related cyclic adducts were also reported upon reaction of glycidaldehyde with guanine nucleoside (5). These results prompted us to investigate the reaction of EH with nucleic acids. In this paper we describe the structural characterizations of adenine nucleoside adducts of EH and its reaction in vitro with DNA and RNA. The stereoselective formation of adducts with diastereomers of EH was also demonstrated.

MATERIALS AND METHODS

Chemicals. trans-4-Hydroxy-2-nonenal was synthesized by a published method (6) and further purified on a silica gel column, as previously described (3). [2,3-3H]trans-4-Hydroxy-2-nonenal (438 mCi/mmol) was obtained from Chemsyn Science Laboratories (Lexena, KS). Tritium was incorporated by reduction with LiAlH4 of the corresponding 1,1-diethoxy-4-hydroxy-2-ynone, followed by quenching with triethylamine in THF. EH was prepared from [2,3-3H]-trans-4-hydroxy-2-nonenal in THF with tert-butyl hydroperoxide as described (3). This reaction yielded a pair of diastereomers (Fig. 1) composed of 75% isomer I and 25% isomer II as determined by integrations of aldehydic protons in the proton NMR spectrum (Fig. 2). To obtain fractions enriched with isomer I or II (75%-95%), the crude product was applied to a silica gel column using CH2Cl2 to remove nonpolar material and then partially separated by elution with CH2Cl2/methanol (97/3). Tritium-labeled epoxide was prepared by the following procedure. tert-Butyl hydroperoxide (10 µl) in 0.2 ml of THF containing 20 µl of 1 N NaOH was added with stirring to [2,3-3H]trans-4-hydroxy-2-nonenal (100 µCi) in 0.25 ml THF at room temperature. After 10 min of stirring, this solution was neutralized with 1 N HCl and evaporated to dryness in a vacuum. The residue was extracted with CHCl3 (4 ml). The extract was evaporated to afford [2,3,3H]EH in 90% yield. The identity of this product was confirmed by comparing its mobility on thin layer chromatography with that of the unlabeled standard. Deoxyadenosine and deoxyguanosine were purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ), and adenosine and guanosine were obtained from Aldrich Chemical Co. (Milwaukee, WI). Calf thymus DNA and calf liver RNA were obtained from Sigma Chemical Co. (St. Louis, MO). Denatured DNA was produced by heating a DNA solution in a phosphate buffer for 20 min followed immediately by cooling in an ice bath. Enzymes for nucleic acid hydrolysis were obtained from Sigma. 1,N2-Ethenoadenosine was synthesized from a reaction of adenine with
choloracetalddehyde by a published method (7). Conversion of deoxyadenosine adducts A1 or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7). Conversion of deoxyadenosine adducts Al or A2 to Structure 3 (Fig. 5) with sodium chloroacetaldehyde by a published method (7).
Stereoselective formation of in vitro nucleic acid adducts

eomeric epoxy aldehydes designated isomers I and II. Each isomer could consist of a pair of enantiomers. The structures are shown in Fig. 1. The chemical-ionization mass spectrum of the epoxy aldehyde showed an increment of 16 (m/e 173, M^+ + 1) in comparison with that of the parent aldehyde, indicating the incorporation of an oxygen atom via epoxidation. Its proton NMR spectrum, given in Fig. 2, shows that the aldehydic protons appeared as two doublets (J = 6.0 Hz) at 9.10 and 9.15 ppm, suggesting that two isomers are formed. Decoupling experiments showed that these aldehydic protons are independent of each other. These results confirmed that these signals are derived from two diastereomeric epoxy aldehydes. The ratio of these isomers (isomer I : isomer II) was 3, based on the integration of the aldehydic protons. Decoupling experiments showed that these aldehydic protons are independent of each other. These results confirmed that these signals are derived from two diastereomeric epoxy aldehydes. The ratio of these isomers (isomer I : isomer II) was 3, based on the integration of the aldehydic protons. A complete separation of isomers I and II was not possible under the chromatographic conditions used. However, a fraction enriched with isomer II (75 to 95%) was obtained from a silica gel column (see "Materials and Methods"). Fractions enriched in either isomer I or isomer II were used to demonstrate their stereoselectivity in reactions with nucleosides and nucleic acids. The absolute configurations of isomers I and II were not assigned in this study.

Reaction with Nucleosides. Reverse-phase HPLC analysis of the reaction mixture of adenine nucleoside with EH showed two major peaks, A1 and A2, in addition to the unreacted adenine nucleoside. A typical HPLC chromatogram is shown in Fig. 3a. A2 accounted for more than 70% of the yield. The UV spectra of A1 and A2 were identical. The fluorescence spectra of A1 at pH 7.0 showed an emission maximum at 420 nm (excitation at 278 nm) and excitation maxima at 238, 278 (sh) and 310 nm (emission at 420 nm). Similar spectra were observed with A2, which showed an emission maximum at 420 nm (excitation at 279 nm) and excitation maxima at 238, 278 (sh) and 308 nm (emission at 420 nm). The fluorescent intensity at neutrality was 10-fold greater than that in acidic solution and 7-8-fold greater than that in basic solution. These spectra are characteristic of 1,N^6-ethenoadenine nucleoside derivatives (7, 8). Fig. 4 shows a proton NMR spectrum of A2. It has two singlets at 8.45 and 9.23 ppm due to aromatic protons at C2 and C5, respectively. A signal at 7.55 ppm was assigned to an etheno proton. The fact that it appeared as a singlet indicates that one of the etheno carbons is substituted. The multiplets between 1.2 and 1.5 ppm were derived from methylene protons.
of the aliphatic chain. A triplet at 0.80 ppm was assigned to the CH₃. The two carboxil protons appeared as a doublet and a multiplet at 5.10 and 4.05 ppm. The remaining signals were from sugar protons. All of these assignments were verified by decoupling experiments. The proton NMR spectra of A1 and A2 are almost superimposable, with only a small difference in chemical shifts ranging from 0.05 to 0.10 ppm. These data support the structures of A1 and A2 as a pair of diastereomers possessing a 1,N⁴-etheno moiety substituted with a heptyl group at one of the etheno carbons. Further evidence for the structures was provided by chemical conversion of A1 or A2 of deoxyadenosine to a known Structure 3 with periodide oxidation followed by sodium borohydride reduction (Fig. 5). The UV and proton NMR spectra of this product agreed entirely with the published data for structure 3 (9). The site of substitution on the etheno carbons was established by comparing the chemical shift (7.55 ppm) of the etheno proton in A1 or A2 with those (7.45 and 7.85 ppm) of 1,N⁴-etheno-adenosine synthesized from the reaction with chloroacetaldehyde by a published procedure (7). The proton with higher chemical shift in 1,N⁴-etheno-adenosine was previously shown to be bound to the carbon adjacent to N1 of adenine (8). Thus, the absence of a signal around 7.85 ppm in A1 or A2 indicated that the alkyl group is attached to the etheno carbon next to N1. The structure of A1 or A2 is given in Fig. 5; its full name is 7-(1,2-dihydroxy-heptyl)-3,2-D-ribofuranosylimidazo[2,1-α]purine or the corresponding deoxyribonanosyl derivative. The absolute configurations of A1 and A2 were not determined.

As expected, the reaction of EH with guanosine produced an adduct pattern similar to that formed in the deoxyguanosine reaction (3). Fig. 3b is a typical HPLC chromatogram obtained from the reaction of guanosine. These peaks are assigned as G1, G2, G3, G4, G5, and G6. The identities of these adducts were established by comparing their UV spectra with those of the corresponding deoxyguanosine adducts as well as by conversion of G2, G3, G5, and G6 to 1,N²-ethenoguanosine (G1) in base. Similar conversion was observed with the corresponding deoxyguanosine adducts. Peaks G7 and G8, however, were not identified and appeared to be secondary products due to the excess epoxy aldehyde in the reaction. Structures of guanine nucleoside adducts are shown in Fig. 6.

Stereoselective Formation of Guanine and Adenine Nucleoside Adducts. Reactions of adenosine and guanosine with epoxy aldehyde containing different proportions of isomers I and II were carried out at 37° and pH 7 under identical conditions. Fig. 3 compares the HPLC chromatograms obtained from analysis of these reactions. Although pure isomers I and II were not available for these reactions, the results clearly showed that adducts were formed in a stereoselective manner. Decreasing the proportion of isomer I and increasing the proportion of isomer II resulted in a lower yield of A2 and a greater yield of A1, indicating that A2 was primarily derived from reaction with isomer I whereas A1 was derived from isomer II (Fig. 3, a and c). The preferential formation of G5 and G6 was observed with an increased amount of isomer I (Fig. 3b). Reaction with the greater proportion of isomer II resulted in greater yields of G1 and G4. Furthermore, two isomers of G4 were formed in reaction with isomer II. This was evidenced by their characteristic UV spectra of the 1,N²-ethenoguanine nucleosides and further confirmed by conversion of each isomer with sodium periodate followed by sodium borohydride reduction to 7-hydroxymethyl-substituted 1,N²-etheno derivative (3). Table 1 shows the amounts and percentages of yield of these adducts.

Reaction with Double-stranded DNA. Initially we investigated the reaction of DNA with unlabeled EH. However, the amount of adduct formed in native DNA was too low to be detected by UV. To enhance the sensitivity of detection, radiolabeled epoxy aldehyde was synthesized. Fig. 7 shows the HPLC chromatograms obtained from analysis of the enzymatic hydrolysates of DNA modified with tritium-labeled EH. A peak at 28 min coeluted with standard 1,N²-ethenodeoxyguanine (G1). To confirm its identity, it was collected from the HPLC and depurinated in 0.1 N HCl at 37° for 16 h. The acid-hydrolyzed product again coeluted with 1,N²-ethenodeoxyguanine in two HPLC systems. In System 2, using the reverse-phase column, the depurinated product eluted at 25.5 min; in System 6, using two strong cation exchange columns, it eluted at 13.5 min. In addition, HPLC analysis of the acid hydrolysates of DNA modified with [2,3-³H]EH also gave a peak that co-eluted with 1,N²-ethenoguanine. A few minor peaks were observed after 28 min. However, only a small amount of radioactivity was detected between 40 and 50 min, which corresponds to the retention times of other deoxyguanosine adducts and A1 and A2. It is difficult to differentiate them as distinct peaks. The majority of the radioactivity in the DNA hydrolysate was detected in fractions between 10 and 20 min. These peaks could be derived from a number of possible sources: hydrolyzed polar products of the epoxy aldehyde, phosphodiesters, and/or other deoxyribonucleoside ad-
of the fractions of DNA hydrolysate corresponding to the adenine nucleoside. Guanine nucleoside adducts were not enriched with isomer I, both A1 and A2 were formed in relative high amounts. The levels of these adducts were 6 and 21 pmol/mg DNA or 14 ¿¿mol/mol guanine. This was calculated based on one-half of the specific activity of [2,3--'H]2,3-epoxy-hydrolysates without base treatment was estimated to be 10 nmol/mg DNA. These results demonstrated that deoxyadenosine is more reactive than deoxyguanosine toward isomer I and, thus, is selectively modified in single-stranded DNA.

Reaction with RNA. Reaction with RNA yielded both adenine and guanosine adducts. Although the total levels of adenine adducts were 8-fold greater than those of guanosine adducts in the reaction with isomer I, this selectivity was not observed with isomer II. Consistent with the stereoselectivity observed with monomers, A2, G5, and G6 were favored adducts in RNA reacted with isomer I, whereas A1, G1, and G4 were predominant with isomer II. Levels of these adducts are summarized in Table 2. Interestingly, little product other than the purine nucleoside adducts was found in the hydrolysate of RNA or single-stranded DNA modified by the epoxy aldehyde.

DISCUSSION

The reaction of adenine nucleosides with EH yielded two diastereomeric adenine derivatives possessing an extended 1,N^6-etheno ring with a heptyl substituent at C8. Formation of the cyclic 1,N^6-etheno adduct requires nucleophilic addition by the N1 and N6 in adenine. A possible mechanism for their formation is depicted in Fig. 5. It involves an initial addition to the aldehydic carbon by the exocyclic amino group to give a carbinolamine (1), followed by ring closure via nucleophilic attack by N1 at the epoxide carbon to yield Intermediate 2. This is followed by dehydration of 2, forming 1,N^6-etheno adducts A1 and A2. A similar mechanism has been postulated for the formation of 1,N^6-ethenoadenosine by chloroacetaldelyde (10). Nair and Offerman reported the formation of a substituted 1,N^6-etheno adduct in the reaction of adenine with 2,3-epoxybutanol (9). However, the stereochemistry was not mentioned in that study. Four diastereomeric adducts would be expected to be formed because of two chiral carbinol carbons in the alkyl side chain and the β configuration of the sugar.

Table 2 Levels of adenosine and guanosine adducts in RNA after reaction with 2,3-epoxy-4-hydroxynonanal

<table>
<thead>
<tr>
<th>Epoxy aldehyde</th>
<th>A1</th>
<th>A2</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
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<tr>
<td>Isomer I*</td>
<td>3</td>
<td>50</td>
<td>0.6</td>
<td>ND</td>
<td>ND</td>
<td>2.0</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Isomer II*</td>
<td>46</td>
<td>8</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>28</td>
<td>0.1</td>
<td>0.1</td>
</tr>
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* Epoxy aldehyde containing predominantly isomer I (75% isomer I and 25% isomer II).  
* ND, not detected.  
* Combined yield of G4 isomers.
moiety. However, only two peaks were observed upon HPLC analysis of the reaction mixture. This result suggests that either ring-opening of the epoxide intermediate 1 was stereospecific or the isomers were not separable under the conditions used.

Nucleic acids were modified by EH in a stereoselective manner analogous to that observed with monomers. Depending on the relative amounts of isomers I and II, reaction of RNA yielded different proportions of diastereomeric adenosine or guanosine adducts. In addition, comparable yields of adducts were obtained from reactions with adenine nucleosides using different proportions of isomers I and II, indicating that isomers I and II have similar degrees of reactivity and stability toward adenine. However, isomer II appears to be more reactive than isomer I in its reaction with guanine nucleosides because the yields of guanine adducts increased with increasing proportions of isomer II. Similar results were observed with RNA; yields of adenosine adducts from reaction with isomer I were considerably greater than those of guanosine adducts. Furthermore, the epoxy aldehyde enriched with isomer I reacted preferentially in single-stranded DNA, yielding A1 and A2 as predominant products. Thus, it appeared that isomer I reacted selectively with adenine in single-stranded DNA as well as in RNA. However, this greater reactivity with adenine than with guanine was not observed in the reactions with isomer II, indicating the importance of stereochemical factors in influencing the base modifications in nucleic acids. The steric effects on the production of adduct are similar in reactions with nucleosides and nucleic acids, suggesting that a common steric factor(s) governed the selectivity of these reactions. In contrast to the single-stranded nucleic acids, reaction of native DNA produced only deoxyguanosine adducts. In double-stranded DNA, yields of adduct were considerably lower than in single-stranded DNA or RNA. Adenine and guanine in double-stranded DNA are known to be resistant to the attack by chloroacetaldehyde (10, 11). Binding of EH to nucleic acids involves N-1 and N² of guanine as well as N-1 and N⁸ of adenine. These sites are hydrogen-bonded in double-stranded DNA and, therefore, are much less accessible in native DNA than in RNA and single-stranded DNA. Interestingly, little pyrimidine adduct was found in RNA and DNA hydrolysates; all of the major peaks in HPLC analysis were accounted for as adducts of guanine and adenine. The stereoselective bindings of epoxy aldehyde isomers toward nucleic acids described in the present study are reminiscent of those observed with (±)benzo(a)pyrene-7,8-di-hydrodiol-9,10-oxide, which was extensively studied both in vitro and in vivo (12, 13). It is possible that the stereochemistry of the epoxy aldehyde is an important determinant of binding to nucleic acids in vivo.

At least two possible mechanisms could lead to DNA damage induced by trans-4-hydroxy-2-nonenal. These mechanisms entail either direct Michael addition, forming an adduct such as 1,N²-propanoguanine (14), or damage via its epoxide that reacts with nucleic acids, forming adducts described in this paper. A number of α,β-unsaturated carbonyls can form cyclic adducts upon reaction with nucleosides (15–17). The in vivo formation of cyclic adducts via the former pathway has recently been demonstrated. Crotonaldehyde released during the metabolism of the hepatic carcinogen, N-nitrosopyrrolidine, or applied topically on mouse skin reacts with rat liver DNA or mouse skin DNA, yielding low levels of 1,N²-propanoguanine adducts (18). It is expected that binding of enals to nucleic acids would be low because enals conjugate with cellular sulfhydryls readily (19). Acrylonitrile, an α,β-unsaturated nitrile, reacted rapidly with proteins in vitro but showed little binding to calf thymus DNA (20). In contrast, its epoxide, 2-cyanoethylene oxide, was more reactive with DNA than with proteins (20, 21). From the DNA binding and metabolism studies, it is thought that 2-cyanoethylene oxide could be important for the carcinogenesis of its parent compound. We have found that EH is more reactive toward nucleic acids than is its parent aldehyde, suggesting the importance of epoxidation in enal tumorigenesis. The relative rates of enals and their epoxides in reacting with nucleic acids and proteins would be important factors in assessing their potential role in tumorigenesis.

Although the epoxidation of endogenous trans-4-hydroxy-2-nonenal has not yet been shown to occur in vivo, several pathways for its formation can be envisaged. For example, epoxidation could occur with fatty acid hydroperoxides generated by lipid peroxidation, could occur through prostaglandin biosynthesis, and/or could be mediated by microsomal enzymes. Peroxy radical-dependent epoxidation is involved in the in vitro as well as in vivo activation of polyaromatic hydrocarbons such as benzo(a)pyrene (22–24). The results of this study show that, if the epoxy aldehyde is indeed formed in vivo, it could be one of the potential sources for endogenous adduct formation that contributes to tumorigenesis. The formation of EH from its parent compound in these systems is currently being investigated.

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