1,2-Deoxyguanosines Adducts of Acrolein, Crotonaldehyde, and trans-4-Hydroxynonenal Cross-link to Peptides via Schiff Base Linkage

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DNA-protein cross-links (DPCs) are formed upon exposure to a variety of chemical and physical agents and pose a threat to genomic integrity. In particular, acrolein and related aldehydes produce DPCs, although the chemical linkages for such cross-links have not been identified. Here, we report that oligodeoxynucleotides containing 1,2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal can form cross-links with the tetrapeptide Lys-Trp-Lys-Lys. We concluded that complex formation does not occur by a Schiff base linkage because DNA-peptide complexes were covalently trapped following reduction with sodium cyanoborohydride and pre-reduction of added DNAs inhibited complex formation. A previous NMR study demonstrated that duplex DNA catalyzes ring opening for the acrolein-derived 2-propa- acetyldenuosine adduct to yield an aldehydic function (de los Santos, C., Zaliznyak, T., and Johnson, F. (2001) J. Biol. Chem. 276, 9077–9082). Consistent with this earlier observation, the adducts under investigation were more reactive in duplex DNA than in single-stranded DNA, and we concluded that the ring-open aldehydic moiety is the induced tautomer in duplex DNA for adducts exhibiting high relative reactivity. Ad- ducted DNA cross-linked to Arg-Trp-Arg and Lys-Trp-Lys with comparable efficiency, and N-acetylation of peptides dramatically inhibited trapping; thus, the reactive nucleophile is located at the N-terminal α-amino of the peptide. These data suggest that Schiff base chemistry can mediate DPC formation in vivo following the formation of stable aldehyde-derived DNA adducts.

DNA-protein cross-links (DPCs) are formed upon exposure to several exogenous and endogenous agents, including ionizing radiation, metal compounds, oxygen radicals, X-rays, and reactive aldehydes (1–6). The histones and nuclear matrix proteins are the predominant substrates involved in DPC formation (7–9), and chromatin structure significantly affects cross-linking efficiency (10–12). Not surprisingly, aldehydes with established DPC-forming ability disrupt DNA replication for the SV40 minichromosome following exogenous exposure (13), suggesting that DPC damage presents a major obstacle to the mammalian DNA replication (and transcription) machinery. We envisage that a DNA repair and/or damage avoidance pathway exists to prevent interruptions to these normal cellular events, although a unified repair scheme has not been elucidated for all DPC lesions. In particular, studies conducted in xeroderma pigmentosum cells have implicated nucleotide excision repair (NER) in the removal of DPCs induced by trans-1,2-diamino dichloride (1); however, studies on formaldehyde-induced DPCs indicate that NER is a dispensable pathway in the active repair of these lesions (14–16).

Among the agents that induce DPCs, acrolein and crotonaldehyde are bifunctional electrophiles belonging to a group of highly reactive aldehydes termed 2-alkenals. These compounds retain two electrophilic reaction centers and are capable of forming various DNA and protein adducts as well as DPCs (2–19). It has been postulated that the 2-alkenals and also the structurally related 4-hydroxy-2-alkenals (e.g. trans-4-hydroxynonenal (HNE)) represent significant sources of endoge- nous DNA damage because of their presence as metabolites of lipid peroxidation (19, 20). Acrolein and crotonaldehyde are known carcinogens and pose an environmental health risk as constituents of automotive exhaust and tobacco smoke (21, 22); however, because these 2-alkenals cause damage to a multi- tude of cellular macromolecules, what role DPC formation plays in their observed mutagenic and carcinogenic effects is as yet unclear. Likewise, although the formation of 4-hydroxynon- enal-derived protein adducts has been correlated with degenerative conditions such as cardiovascular and Parkinson’s diseases (23, 24), demonstration that HNE can induce DPCs may suggest alternative mechanisms to explain the observed cytotoxicity of this compound.

In the case of formaldehyde- and malondialdehyde-induced DPCs, the sequence of reactivity in cross-link formation appears to involve a rapid primary reaction to form a protein adduct, followed by a slower secondary reaction with DNA amines to form a DPC (25, 26). However, the detection of stable acrolein-, crotonaldehyde-, and 4-hydroxynonenal-derived DNA adducts in vivo (27–29) suggests that bifunctional electrophiles can react to form primary DNA adducts capable of participating in secondary reactions with proteins. Acrolein reacts with DNA to form a major exocyclic adduct, γ-hydroxy-1,2-deoxy-2-propa-2-acetyldenuosine (γ-HOPdG); and recently, this adduct was shown to undergo ring opening in duplex DNA to...
yield an aldehydic moiety (30). Consistent with the predicted reactivity of the ring-open aldehydic tautomer, the \( \gamma \)-HOPdG adduct was shown to form an interstrand DNA-DNA cross-link to the \( N^2 \)-position of an opposing guanine base in a 5'-CpG sequence context, mediated by a Schiff base (or carbinolamine) linkage (31). To test the possibility that peptide amines might also provide suitable substrates for cross-link formation, we evaluated the propensity for the major 1,\( N^2 \)-deoxyguanosine adducts of acrolein, crotonaldehyde, and HNE (Fig. 1) to form cross-links with the tetrapeptide Lys-Trp-Lys-Lys, and HNE (d–g).

For standard trapping reactions, adducted DNA (75 nM) was incubated with peptide in 50 mM HEPES (pH 7.0) and 100 mM NaCl at 4 °C. An aqueous solution of NaCNBH\(_3\) was prepared on the day of use and added to each reaction (final concentration of 50 mM) immediately preceding the addition of peptide. Unless otherwise stated, the concentration of peptide in the trapping reactions was 1.0 mM, and reactions were quenched by the addition of a freshly prepared aqueous solution of NaCNBH\(_3\) (final concentration of 100 mM). Each reaction mixture was subsequently diluted 5-fold by the addition of 1.25 \( \times \) loading buffer (59% [v/v] formamide, 12.5 mM EDTA, 0.0125% [w/v] bromphenol blue, and 0.02% [w/v] xylene cyanol) and heated at 90 °C for 2 min. An aliquot of each reaction was loaded onto a 15% denaturing polyacrylamide gel (8.3 M urea) in sequencing buffer (134 mM Tris base, 44 mM boric acid, and 10 mM EDTA), and products were separated by electrophoresis for 5 hr at 1500 V. The complement to the sequenced adducted base. Oligodeoxynucleotides containing the acrolein and crotonaldehyde adducts were a generous gift of Drs. Constance Harris and Thomas Harris (Vanderbilt University). The structure-specific synthesis of the HNE-adducted deoxynucleosides has been described previously (34). The synthesis of oligodeoxynucleotides containing HNE-derived adducts was carried out by Hao Wang (Vanderbilt University); these DNAs were a generous gift of Dr. Carmelo Rizzo (Vanderbilt University).

**Aldehyde-derived DNA Adducts Cross-link to Peptides**

**EXPERIMENTAL PROCEDURES**

**Materials**—All peptides were prepared by the NIEHS Center in Environmental Toxicology–Protein Chemistry Laboratory (University of Texas Medical Branch). Sodium borohydride was obtained from Sigma, and sodium cyanoborohydride was obtained from Aldrich. \( \gamma^{32} \text{P} \)-ATP was obtained from PerkinElmer Life Sciences.

**Peptide Quantitation**—Following initial syntheses, peptides were analyzed by mass spectrometry, and the composition of each was confirmed by observing a major peak corresponding to the predicted molecular mass. Peptides were subsequently purified by preparative HPLC and resuspended in a solution of 20:80 acetonitrile/water. Because all peptides used in this study contained a single Trp residue, the concentration of each peptide solution was determined by monitoring Trp absorbance at 280 nm using a Shimadzu BioSpec-1601 spectrophotometer. Concentrations were calculated using 5500 m\(^{-1} \cdot \) cm\(^{-1} \) as the Trp molar extinction coefficient (36).

**Adducted Oligodeoxynucleotides**—The synthesis of adducted deoxynucleosides was carried out by Nechev et al. as described previously for the proximal deoxyguanosine adduct of acrolein (32) and the \( 6R,6R \)- and \( 6S,6S \)-crotonaldehyde adducts (33). These adducted deoxynucleosides were constructed into 12-mer oligodeoxynucleotides with the sequence 5'-GCTAGCG*A/AGTCC-3', where G* denotes the adducted base. Oligodeoxynucleotides containing the acrolein and crotonaldehyde adducts were a generous gift of Drs. Constance Harris and Thomas Harris (Vanderbilt University). The structure-specific synthesis of the HNE-adducted deoxynucleosides has been described previously (34). The synthesis of oligodeoxynucleotides containing HNE-derived adducts was carried out by Hao Wang (Vanderbilt University); these DNAs were a generous gift of Dr. Carmelo Rizzo (Vanderbilt University).

**Determination of Duplex DNA Integrity**—The complement to the adducted 12-mers, with sequence 3'-CGATCCGCTCAGG-5', was synthesized by Midland Certified Reagent Co. and was gel-purified by standard procedures prior to use. For the preparation of duplex DNAs, adducted 12-mers were annealed to the complementary strand (20-fold molar excess) in 1x NaCl by heating at 90 °C for 3 min and cooling slowly to 4 °C. For experiments requiring single-stranded DNA in trapping reactions, the annealing step was omitted. To verify the integrity of the double-stranded substrate for trapping reactions, single- and double-stranded DNAs were incubated under standard reaction conditions (50 mM HEPES (pH 7.0) and 100 mM NaCl) for 30 min at 4 °C. To each reaction, 0.2 volumes of loading buffer (0.25% [w/v] bromphenol blue, 0.25% xylene cyanol, and 40% [w/v] sucrose in H\(_2\)O), and DNAs were analyzed on a 12.5% native polyacrylamide gel in 0.5X TBE buffer containing 45 mM Tris borate and 1.0 mM EDTA. Duplex DNAs were visualized as bands migrating with slower mobility relative to the labeled single-stranded oligodeoxynucleotides, and quantitative annealing was observed for all duplexes (data not shown).

**Trapping of Covalent DNA-Peptide Complexes Using NaCNBH\(_3\)**—For standard trapping reactions, adducted DNA (75 nM) was incubated with peptide in 50 mM HEPES (pH 7.0) and 100 mM NaCl at 4 °C. An aqueous solution of NaCNBH\(_3\) was prepared on the day of use and added to each reaction (final concentration of 50 mM) immediately preceding the addition of peptide. Unless otherwise stated, the concentration of peptide in the trapping reactions was 1.0 mM, and reactions were quenched by the addition of a freshly prepared aqueous solution of NaCNBH\(_3\) (final concentration of 100 mM). Each reaction mixture was subsequently diluted 5-fold by the addition of 1.25 \( \times \) loading buffer (59% [v/v] formamide, 12.5 mM EDTA, 0.0125% [w/v] bromphenol blue, and 0.02% [w/v] xylene cyanol) and heated at 90 °C for 2 min. An aliquot of each reaction was loaded onto a 15% denaturing polyacrylamide gel (8.3 M urea) in sequencing buffer (134 mM Tris base, 44 mM boric acid, and 10 mM EDTA), and products were separated by electrophoresis for 5 hr at 1500 V.

**Illustrations—Results**—Results were visualized from wet gels by PhosphorImager analysis, and product bands were quantitated using ImageQuant software. In all cases, the trapped Schiff base complex was calculated as the amount of major shifted complex on the gel as a percentage of the substrate DNA + major shifted complex. Electronic gel files were processed with Adobe Photoshop Version 5.5 and Illustrator Version 9.0.
DNAs and the major reduced Schiff base complexes (12-mer/H11001 Experimental Procedures, DNAs were separated through a denaturing gel as described under an asterisk this study. The position of the adducted deoxyguanosine is denoted by are indicated.

![Diagram A](image)

![Diagram B](image)

![Diagram C](image)

**Fig. 2.** A, shown is the sequence of the 12-bp oligonucleotide used in this study. The position of the adducted deoxyguanosine is denoted by an asterisk on the upper stand, and the position of the 32P label is shown. B, shown are the structures of the ring-open (species 1) and ring-closed (species 2) forms of the acrolein-derived γ-HOPdG adduct. A peptide amine reacts with the aldehydic DNA adduct to form a protoxynucleotide substrate containing a centrally located γ-HOPdG adduct (Fig. 2A) was obtained, and this DNA was reacted with the Lys-Trp-Lys-Lys peptide in the presence of NaCNBH3. In this experiment, a complex was observed that migrated slower on a denaturing polyacrylamide gel compared with the adducted 12-mer, and the amount of gel-shifted complex that was observed was dependent on the concentration of peptide in the reaction (Fig. 2C). A shifted DNA band that migrated only slightly slower than the substrate DNA was also observed when reducing agent was present without peptide; this band was competed away by the addition of increasing peptide concentrations. One possibility may be that this species represents a subpopulation of substrate DNA (with altered gel mobility) in which the ring-open γ-HOPdG aldehyde has reacted to form a Schiff base complex with Tris base in the reaction mixture. Such a complex should also be reducible to form a stable covalent species, and experiments are currently underway to test this hypothesis utilizing mass spectrometry to identify the chemical composition of this species. Very low levels of complex were observed in the absence of reducing agent for the γ-HOPdG-adducted DNA; and when non-adducted DNA was examined as a negative control, a shifted complex was not observed in the presence of absence of NaCNBH3.

**RESULTS**

Covalent Trapping of Lys-Trp-Lys-Lys at the γ-HOPdG Ad-duct—A prior NMR study on the γ-HOPdG adduct showed that this exocyclic ring-closed adduct is able to tautomerase to a ring-open aldehydic moiety in duplex DNA (Fig. 2B, species 1 and 2), a result that may be rationalized, at least in part, because of stabilization of the ring-open structure by pairing of a cytosine base opposite the lesion (30). This structural observation prompted our investigation into the reactivity of the γ-HOPdG adduct with peptides and, in particular, whether this adduct could form a Schiff base-mediated cross-link between a peptide amine and the aldehydic function at the adducted deoxyguanosine (Fig. 2B, species 3). It is the presence of a reducing agent, such a complex can be trapped as a reduced Schiff base (Fig. 2B, species 4); thus, formation of this stable covalent species was monitored as the experimental end point in cross-link formation. For the trapping assay, a 12-mer oligodeoxyxynucleotide substrate containing a centrally located γ-HOPdG adduct (Fig. 2A) was obtained, and this DNA was reacted with the Lys-Trp-Lys-Lys peptide in the presence of NaCNBH3. In this experiment, a complex was observed that migrated slower on a denaturing polyacrylamide gel compared with the adducted 12-mer, and the amount of gel-shifted complex that was observed was dependent on the concentration of peptide in the reaction (Fig. 2C). A shifted DNA band that migrated only slightly slower than the substrate DNA was also observed when reducing agent was present without peptide; this band was competed away by the addition of increasing peptide concentrations. One possibility may be that this species represents a subpopulation of substrate DNA (with altered gel mobility) in which the ring-open γ-HOPdG aldehyde has reacted to form a Schiff base complex with Tris base in the reaction mixture. Such a complex should also be reducible to form a stable covalent species, and experiments are currently underway to test this hypothesis utilizing mass spectrometry to identify the chemical composition of this species. Very low levels of complex were observed in the absence of reducing agent for the γ-HOPdG-adducted DNA; and when non-adducted DNA was examined as a negative control, a shifted complex was not observed in the presence of absence of NaCNBH3.

**Peptide Trapping Kinetics for Aldehyde-derived 1,N2-Deoxy- guanosine Adducts—**We next investigated the possibility that other 1,N2-deoxyguanosine adducts, i.e. crotonaldehyde- and HNE-derived adducts (Fig. 1), could undergo ring opening in duplex DNA in a fashion analogous to the γ-HOPdG adduct by evaluating their propensity to form covalent complexes with Lys-Trp-Lys-Lys in the trapping assay. To directly compare reaction efficiencies, the crotonaldehyde and HNE adducts were constructed into the same 12-mer sequence as shown for the γ-HOPdG adduct (Fig. 2A), where G denotes the position of the adducted deoxyguanosine base. Each of the singly adducted oligodeoxyxynucleotides was individually reacted with Lys-Trp-Lys-Lys in the presence of NaCNBH3, and the kinetics of complex formation were evaluated by monitoring the accumulation of a major DNA-peptide product on a denaturing gel. The designated time points, NaBH4 was added to quench the reaction mixture to prevent further complex accumulation by facilitating the rapid reduction of the aldehyde substrate. During a 2-h time course, similar trapping kinetics were observed for the γ-HOPdG adduct and the crotonaldehyde adducts, and the total amount of trapped complex observed at the 2-h time point for the (6R,6R)- and (6S,6S)-crotonaldehyde adducts was virtually identical at 73 and 71%, respectively (Fig. 3). In contrast, oligodeoxyxynucleotides containing the four stereoisomers of the HNE adduct showed significant differences in reactivity (Fig. 4). Appreciable complex formation was observed for the (6S,8R,11S)-HNE adduct by the end of the 2-h time course (59%) and an intermediate reactivity for the (6R,8S,11R)-HNE adduct (17%) under identical reaction conditions. Each of the other two HNE adducts reacted with poor relative efficiency and showed only 8% complex formation for the (6R,8S,11S)-HNE adduct and 7% complex formation for the (6S,8R,11R)-HNE adduct at the 2-h time point.

**Inhibition of Cross-link Formation by Pre-reduction of Alde- hyde-adducted DNAs—**To provide further evidence that the observed complexes were mediated by Schiff base chemistry, adducted oligodeoxyxynucleotides were pretreated with the strong reducing agent NaBH4 to inhibit DNA-peptide cross-link formation. As stated above, NaBH4 reduces an aldehydic

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*A. M. Sanchez, personal communication.*
function to a primary alcohol, thereby rendering this group nonreactive with amines; thus, if the observed DNA-peptide complex in the trapping assay was the result of a Schiff base linkage, we reasoned that pre-reduction of the adducts should prevent cross-link formation. A previous investigation demonstrated that the half-life of a nonreduced abasic site aldehyde is 12 s at pH 6.8 in the presence of 100 mM NaBH₄ (37); however, we could not confidently predict that the reduction kinetics of the adducts under study here would be equally fast. Specifically, to allow the opportunity for the subpopulation of the ring-closed conformer of each adduct to shift toward a readily reducible ring-open form, substrates were preincubated with 75 mM NaBH₄ for 1 h to achieve quantitative reduction of the ring-open aldehyde adducts. For NaBH₄ trapping inhibition analysis, each of the 1,N²-deoxyguanosine-adducted oligodeoxyribonucleotides that reacted with high relative efficiency in our trapping assay was selected, viz. the acrolein-derived γ-HOPdG adduct, the (6R,8R)- and (6S,8S)-crotonaldehyde adducts, and the (6S,8R,11S)-HNE adduct. In each case, preincubation of substrate DNA with NaBH₄ dramatically inhibited the subsequent formation of covalent DNA-peptide cross-links in a standard trapping assay with Lys-Trp-Lys-Lys and NaCNBH₃ (Fig. 5). In addition, virtually no shifted complex was observed.
in the absence of any reducing agent for each of the aldehyde-
ducted DNAs, consistent with the result obtained for the
γ-HOPdG adduct. From these combined results, we concluded
that the formation of a Schiff base mediates covalent attach-
ment of the Lys-Trp-Lys peptide to the acrolein-derived
γ-HOPdG adduct and the crotonaldehyde- and HNE-derived
deoxyguanosine adducts.

Reactivity of Single- Versus Double-stranded Aldehyde-ad-
ducted DNAs—As stated above, a prior NMR study of the
γ-HOPdG adduct showed that this adduct assumes a ring-open
structure in duplex DNA (30); however, it was also reported in
that study that the γ-HOPdG deoxynucleoside exists primarily
in the ring-closed conformation. This difference in tautomer
formation prompted an investigation into the relative reactiv-
ity of single- versus double-stranded adducted oligodeoxynucle-
otides because the rate of formation of DNA-peptide cross-links
should be dependent on the concentration of the ring-open
aldehyde adduct under our reaction conditions. In particular, if
the ring-closed tautomer predominates in single-stranded DNA
for the γ-HOPdG adduct (Fig. 2B, species 2), and the ring-open
aldehyde predominates in duplex DNA (species 1), a slower rate
of DNA-peptide complex formation should be observed in our
trapping assay for the single-stranded γ-HOPdG-adducted sub-
strate versus the duplex substrate. Using the same 12-mer se-
quency context as shown in Fig. 2A, we observed that the
double-stranded γ-HOPdG-adducted DNA formed 88% complex
by the end of a 2-h time course, whereas the single-stranded
DNA formed only 29% complex (Fig. 6). We next conducted a
similar comparison for the (6R,8R)- and (6S,8S)-crotonaldehyde
adducts and the (6S,8R,11S)-HNE adduct; and in each
case, the single-stranded adducted DNAs reacted with much
slower reaction kinetics compared with duplex DNAs (Fig. 6).

From these combined results, we concluded that the ring-open
aldehyde tautomer is stabilized upon formation of duplex DNA
and that the concentration of ring-open aldehyde is greater in
duplex DNA than in single-stranded DNA for each of the ad-
ducts tested.

Investigation of the Reactive Nucleophile—Peptides capable
catalyzing β-elimination at abasic sites in DNA, such as Lys-Trp-
Lys, initiate this chemistry via the formation of a Schiff base
between a peptide amine and C-1

\( \text{amine} \) on the deoxyribose sugar (38, 39). In a recent study, we demonstrated that lysine-containing
peptides utilize the N-terminal α-amine as the nucleophile in this
reaction (35). Because the formation of a Schiff base requires a
neutral (deprotonated) amine, we concluded that this result is
consistent with the lower intrinsic \( pK_a \) of an α-amine versus an
ε-amine. Specifically, the concentration of neutral α-amine
should be far greater than the relative concentration of ε-amine
at a given reaction pH for a lysine-containing peptide. This line of
reasoning also predicts that an ε-amine should mediate Schiff
base formation in the case of the adducts under study in this
work; thus, the location of the reactive nucleophile for Lys-Trp-
Lys was interrogated by examining trapping at the
(6S,8R,11S)-HNE adduct as a representative case. To first test
whether a lysine residue is absolutely required for Schiff base
formation, Arg-Arg-Arg-Arg was substituted for Lys-Trp-Lys
in the standard trapping assay with the (6S,8R,11S)-HNE
adducted duplex DNA. This peptide was chosen because arginine
residues should retain positively charged side chain moieties
under reaction conditions at pH 7.0, mimicking the electrostatic
contribution of lysine in DNA binding. In this experiment, a
similar kinetics profile for the accumulation of the DNA-peptide
complex was observed for each peptide, and the total amounts of
trapped complex observed at the 2-h time point for Lys-Trp-Lys-
acetyl moiety. If the reactive nucleophile was located at the
mM NaBH₄. The kinetics of trapped complex formation are plotted over
0, 15, 30, 60, 90, and 120 min and were quenched by the addition of 100
\( \text{mM} \) HEPES (pH 7.0), 100 mM NaCl, and 50 mM NaCNBH₃ for
0, 15, 30, 60, 90, and 120 min and were quenched by the addition of 100
mM NaHCO₃. The kinetics of trapped complex formation are plotted over a
2-h time course for Lys-Trp-Lys-Lys (free N terminus (A) and N⁺-
acetyl terminus (B)) and Arg-Trp-Arg-Arg (free N terminus (C) and
N⁺-acetyl terminus (D)).

Lys and Arg-Trp-Arg-Arg were 68 and 81%, respectively (Fig. 7).
To directly evaluate the role of the N-terminal ω-amino in cross-
link formation for each peptide, the Lys-Trp-Lys-Lys and Arg-
Trp-Arg-Arg peptides were modified by the addition of an N⁺-
acetyl moiety. If the reactive nucleophile was located at the
α-amino, N⁺-acytelyation should prevent Schiff base formation by
generating an amide at the N terminus. For both peptides, N⁺-
acytelyation dramatically inhibited the formation of a DNA-
peptide complex at the (65,8R,11S)-HNE-adducted DNA, con-
fiming the participation of the amino terminus as the reactive
nucleophile (Fig. 7).

**DISCUSSION**

It has been well documented that aldehydes react with biomol-
ecules to form a variety of adducts, many of which have been
related to mutagenic, carcinogenic, and cytotoxic conse-
quences. In particular, acrolein and crotonaldehyde were shown
previously to form protein adducts at lysine residues by Schiff
base and Michael addition pathways (17, 18); however, although
these compounds can also produce DPCs, the chemistry under-
lying the formation of these adducts has not been elucidated. We
are unaware of any prior study demonstrating that HNE can
produce DPCs, although Uchida and Studtman (40) proposed that
HNE-derived Michael addition products may participate in
secondary reactions to form lysine-mediated inter- and intrasub-
unit protein cross-links. This observation suggests that an ana-
logous reaction of HNE with a protein lysine and a nucleic acid
amine might represent a plausible pathway in the formation of
DPCs. In addition, the detection of 1,N²-deoxyguanosine adducts
of acrolein, crotonaldehyde, and 4-hydroxynonenal in human and
rodent tissues, both as endogenous adducts and following chem-
ical treatment (27–29, 41–43), may indicate that these stable
DNA adducts exist as intermediates along a pathway of forma-
tion for DPCs. Consistent with such a mechanism, the results
presented here demonstrate that peptide amines can react with
these DNA adducts to form DNA-peptide cross-links via Schiff
base linkage. We propose that this pathway may account for a
subset of DPCs formed within the cell whereby nucleophilic func-
tions on proteins react with primary aldehyde-derived DNA ad-
ducts. For example, the case in which a lysine-rich histone pro-
tein is juxtaposed with a 1,N²-deoxyguanosine adduct, as might
be found in the nucleosome, may provide the proper scenario for
such a reaction. As an alternative to this mechanism, Voitkun
and Zhitkovich (26) demonstrated previously that malondialde-
hyde (a bifunctional electrophile and known DPC inducer) reacts
preferentially with the histone H1 protein compared with DNA
in vitro, leading to an adducted protein intermediate that pre-
cedes the formation of a DPC. However, a number of investiga-
tions have established that the malondialdehyde-derived
3-(2'-deoxy-β-D-erythro-pentofuranosyl)-pyrimido[1,2-a]purin-
10(3H)-one (M₁G) DNA adduct is a prominent endogenous lesion
(44–46), indicating that malondialdehyde reacts to form a signif-
ificant number of DNA adducts in vivo. It is likely that the com-
plexity of reactions for aldehydes with biomolecules within the
cell is not accurately mimicked by an in vitro examination, par-
ticularly with respect to a multistep reaction leading to the for-
mation of a DPC. Rather, the spectrum of products observed in
vitro provides insight into the possible reaction pathways for
DPC formation, which may be kinetically favored or disfavored
within the cell, depending on a variety of factors. These combined
observations indicate a multiplicity of pathways that lead to the
formation of DPCs in vivo, one of which may include the forma-
tion of stable aldehyde-derived DNA adducts as reaction
intermediates.

The borohydride trapping methodology employed in this study
was utilized previously to isolate Schiff base intermediates in
peptide-catalyzed β-elimination at abasic sites (35). In particular,
detection of DNA-peptide complexes by denaturing PAGE neces-
sitated the stabilization of the cross-links by reduction, and the
continuous presence of NaCNBH₃ in our reactions irreversibly
shifted the equilibrium toward the accumulation of a reduced
Schiff base (Fig. 2B, species 4). As a result, we could not evaluate
the stability of a nonreduced Schiff base complex from the exper-
iments presented here. However, because we observed a small
detectable level of DNA-peptide complex when reacting Lys-TRp-
Lys-Lys with γ-HOPOG in the absence of reducing agent (Fig. 2),
we speculate that the lifetime of a nonreduced Schiff base-
mediated DPC is likely sufficient to interfere with replication or
transcription in vivo.

A major factor affecting the formation and stability of a
Schiff base complex should be the pKₐ of the reactive amine
because nucleophilic attack at the ring-open aldehyde adducts
requires a neutral deprotonated amine. Here, we found that
the preferred reactive nucleophile for peptides is located at the
α-amino on the peptide (Fig. 7), consistent with the lower
intrinsic pKₐ of an α-amino (pKₐ = 7.6) compared with an
ω-amino (pKₐ = 10.3) in a random coil peptide (47). Alterna-
tively, high local concentrations of positively charged surface
residues on a protein may facilitate reaction of an ω-amino on
a neighboring lysine because such an environment will serve to
depress the pKₐ of the amine. In a previous study, lysine
residues were implicated in the formation of acetaldehyde-
deinduced DPCs (48); and based on the above reasoning, we
suggest that lysines may also react to form Schiff base medi-
ated cross-links at the 1,N²-deoxyguanosine adducts under in-
vestigation here. Importantly, such a linkage may represent
one of several possibilities for DPC formation at these adducts
because acrolein, crotonaldehyde, and HNE may also react at
histidine and cysteine residues on proteins (19, 23, 49).

As depicted in Fig. 2B (species 1), a requisite step in the
reaction of peptides to form DNA-peptide cross-links with the
DNA adducts under study here is the ring opening of the adduct
to form a reactive aldehyde. Recently, an NMR study confirmed
that duplex DNA catalyzes rearrangement of the ring-closed
γ-HOPOG adduct to the ring-open tautomer (30), and a similar
result has also been observed for the structurally related malon-
dialdehyde-derived M₁G adduct (50). Consistent with these ear-
lier observations, the γ-HOPdG adduct exhibited faster reaction kinetics to form DNA-peptide cross-links in duplex DNA versus single-stranded DNA in our analyses (Fig. 6). Structural studies have not yet been conducted for the crotonaldehyde and HNE adducts, although each of the adducts tested gave a result similar to that obtained with the γ-HOPdG adduct in such an experiment. Thus, we concluded that the formation of duplex DNA most probably catalyzes ring opening for the (6R,8R)- and (6S,8S)-crotonaldehyde adducts and the (6S,8R,11S)-HNE adduct in a fashion analogous to the γ-HOPdG adduct. For duplex oligonucleotides containing the (6R,8S,11R), (6R,8S,11S), and (6S,8R,11S) adducts, the observation that these substrates do not readily form cross-links may indicate that these adducts behave in a chemically distinct fashion from the (6S,8R,11S)-HNE adduct. It is as yet unclear how differences in stereochemistry might affect the ring-open/ring-closed conformational equilibria for these non-reactive diastereomers. One possibility might be that such adducts exist as ring-closed tautomers in the syn-conformation about the glycosidic bond, as shown previously for the 1,N2-propanodeoxyguanosine adduct (51). Interestingly, the potential for the γ-HOPdG adduct to assume a ring-open structure (capable of forming a canonical Watson-Crick base pair) has been demonstrated in human polymerase η preferentially incorporates a cytosine base opposite a γ-HOPdG lesion in which the adduct is trapped as a ring-open structure by reduction, whereas the nonreduced adduct gives rise to incorporation of adenine and guanine in addition to cytosine (52). Because the experiments presented here are able to discriminate between an apparent ring-open versus ring-closed structure (dependent upon the use of duplex or single-stranded DNA as a starting substrate) we suggest that peptide trapping methodology may provide a means to probe the ring structure at a primer-template junction.

Although a significant amount of literature has enumerated the various agents inducing DPC damage, the potential for repair has not been clearly elucidated for these DNA lesions. When the 16-kDa DNA repair enzyme T4 pyrimidine glycosylase is covalently attached to DNA as an artificial DPC lesion, the bacterial UvrABC NER proteins are able to recognize and to initiate repair in vitro at a rate comparable to that observed for other well characterized NER substrates (53). In mammalian cells, NER has been implicated in the repair of DPC lesions for only a select number of DPC-substrates (53). In mammalian cells, NER has been implicated in the repair of DPC lesions in which the adduct is trapped as a ring-open structure (51). Interestingly, the potential for the γ-HOPdG adduct to assume a ring-open structure (capable of forming a canonical Watson-Crick base pair) has been demonstrated in human polymerase η preferentially incorporates a cytosine base opposite a γ-HOPdG lesion in which the adduct is trapped as a ring-open structure by reduction, whereas the nonreduced adduct gives rise to incorporation of adenine and guanine in addition to cytosine (52). Because the experiments presented here are able to discriminate between an apparent ring-open versus ring-closed structure (dependent upon the use of duplex or single-stranded DNA as a starting substrate) we suggest that peptide trapping methodology may provide a means to probe the ring structure at a primer-template junction.

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1,N\textsuperscript{2}-Deoxyguanosine Adducts of Acrolein, Crotonaldehyde, and trans-4-Hydroxynonenal Cross-link to Peptides via Schiff Base Linkage
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