Comparative Mutagenesis of the C8-Guanine Adducts of 1-Nitropyrene and 1,6- and 1,8-Dinitropyrene in a CpG Repeat Sequence

A SLIPPED FRAMESHIFT INTERMEDIATE MODEL FOR DINUCLEOTIDE DELETION*[S]

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In the Ames Salmonella typhimurium reversion assay, 1,6- and 1,8-dinitropyrenes (1,6- and 1,8-DNPs) are much more potent mutagens than 1-nitropyrene (1-NP). Genetic experiments established that certain differences in the metabolism of the DNPs, which in turn result in increased DNA adduction, play a role. It remained unclear, however, if the DNP adducts, N-(guanin-8-yl)-1-amino-6 (8)-nitropyrene (Gua-C8–1,6-ANP and Gua-C8–1,8-ANP), which contain a nitro group on the pyrene ring covalently linked to the guanine C8, are more mutagenic than the major 1-NP adduct, N-(guanin-8-yl)-1-amino-6-nitropyrene (Gua-C8-AP). In order to address this, we have compared the mutation frequency of the three guanine C8 adducts, Gua-C8-AP, Gua-C8–1,6-ANP, and Gua-C8–1,8-ANP in a CGCGCG hotspot sequence upstream of the hisD3052 mutation. A remarkable difference in the induced CpG deletion frequency between these adducts was noted. In repair-competent cells the 1-NP adduct induced 1.7% CpG deletions without SOS, whereas the 1,6- and 1,8-DNP adducts induced 6.8 and 10.0% two-base deletions, respectively. With SOS, CpG deletions increased up to 1.9, 11.1, and 15.1% by 1-NP, 1,6-, and 1,8-DNP adducts, respectively. This result unequivocally established that DNP adducts are more mutagenic than the 1-NP adduct in the repetitive CpG sequence. In each case the mutation frequency was significantly increased in a mutS strain, which is impaired in methyl-directed mismatch repair, and a dnaQ strain, which carries a defect in proofreading activity of the DNA polymerase III. Modeling studies showed that the nitro group on the pyrene ring at the 8-position can provide additional stabilization to the two-nucleotide extraheiral loop in the promutagenic slipped frameshift intermediate through its added hydrogen-bonding capability. This could account for the increase in CpG deletions in the M13 vector with the nitro-containing adducts compared with the Gua-C8-AP adduct itself.

1-Nitropyrene (1-NP) and the dinitropyrenes are common environmental pollutants (1–3). Most of the nitropyrenes are mutagenic (for a review see Ref. 4) and tumorigenic (5–8), but their potency sometimes differs by more than an order of magnitude. Nitroreduction is a major pathway of bioactivation of all nitropyrenes, whereas O-estherification enzymes, in addition, play a crucial role in the mutagenicity of DNPs. 1-NP and the DNP s revert Salmonella typhimurium frameshift tester strains TA98 and TA1538 more efficiently than the strains TA100 and TA1535 that detect base pair substitutions (9). The most frequent mutation among the revertants in TA98 is a two-base deletion of a GpC or CpG pair within a CGCGCGCG hotspot sequence upstream of the hisD3052 mutation (10). The frequency of reversion induced by 1-NP in TA98 drops sharply in TA98NR that lacks the classical nitroreductase (11). By contrast, the frequency of reversion by 1,6- and 1,8-DNP is only slightly lower in TA98NR but is significantly reduced in TA98/1,8-DNP₆, which is deficient in a specific aryldihydroxylamine esterification enzyme (11, 12). It appears that this enzyme is necessary for the expression of mutagenicity of the DNPs but not for that of 1-NP. The C8 guanine adducts of 1-NP and DNPs (Fig. 1) have been thought to be responsible for a major fraction of their mutagenicity. Several site-specific studies from our laboratory showed that the 1-NP adduct N-(guanin-8-yl)-1-amino-8-nitropyrene (Gua-C8-AP) is mutagenic in Escherichia coli (13–15). However, the type and frequency of mutations are dependent on DNA sequence context. Despite the acknowledged role of metabolism and the ability of each nitropyrene to induce frameshifts, neither the frequency nor the genetic requirements for mutagenesis of the adducts formed by these carcinogens have ever been compared in the same organism. In order to address such structure-activity relationships, in the current work we have examined mutagenicity of the C8 guanine adducts formed by 1-NP and 1,6- and 1,8-DNP in a repetitive CpG sequence. We have constructed single-stranded M13 bacteriophage genomes in which an adduct was placed at the underscored G of an inserted CGCGCG sequence in the lacZ α fragment. Mutagenicity of the three adducts was examined in strains that are either repair-

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[S] The on-line version of this article (available at http://www.jbc.org) contains Fig. S1 and Tables S1–S3.

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† The abbreviations used are: 1-NP, 1-nitropyrene; AP, 1-aminopyrene; Gua-C8-AP, the corresponding base, N-(guanin-8-yl)-1-aminopyrene; DNPs, dinitropyrene; 1,6-ANP, 1-amino-6-nitropyrene; Gua-C8–1,6-ANP, N-(guanin-8-yl)-1-amino-6-nitropyrene; 1,8-ANP, 1-amino-8-nitropyrene; Gua-C8–1,8-ANP, N-(guanin-8-yl)-1-amino-8-nitropyrene; AAF, N-acetyl-2-aminofluorene; IPTG, isopropyl β-D-thigalactosidase; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; Gua, guanine; MF, mutation frequency; HPLC, high pressure liquid chromatography.
ambient temperature with 15/H9262

M13mp7L2 DNA was prepared as described (18).

E. coli

nitrosonitropyrenes were from Aldrich. Ethidium bromide and polyeth-

,F

::Tn

::Tn
dnaQ49

atives NR9294 (mutS101

ara, thi,

purchased from Amersham Biosciences. Isopropyl

by the nitro group as likely sources of added stabilization.

Our findings suggest added possibilities for hydrogen bonding

hypothesized intermediate and to examine plausible reasons

bility of which may be linked to the mutation frequency. A

cative mismatch repair. Our results suggest formation of an

the effects of this adduct in a strain with impaired postrepli-

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/H11032

uvr

purchased from New England Biolabs (Beverly, MA). [Bss

otide kinase and DNA ligase were obtained from Invitrogen.

X=Y=H, dG-C8-AP

X=H, Y=NO2, dG-C8-1,6-ANP

X=NO2, Y=H, dG-C8-1,8-ANP

FIG. 1. Structures of the major 2'-deoxyguanosine adducts formed by reductively activated 1-AP and the DNPs. Torsion angles \( \chi, \alpha \), and \( \beta \) are defined as follows: \( \chi, O^\prime-C1-N9-C4; \)

\( \alpha, N9-C8-N(AP)\cdot C1(AP); \) and \( \beta, C8-N(AP)\cdot C1(AP)\cdot C10A(AP).\)

COMPETENT or contained a defect in the dnaQ gene that encodes the

e-subunit of DNA polymerase III holoenzyme. Pol III from

these strains has been shown to be defective in 3'-5'-proofread-

ing exonuclease activity (16). In addition, we have determined

the effects of this adduct in a strain with impaired postrepli-

cation at 37

°C, which provide charges that are compatible with the rest of the DUPLEX

charges for these adducts in modified nucleosides. CNDO calculations,

package Gaussian 98 (24). These provided the bond lengths, bond

angles, and dihedral angles needed for the coordinate generator in

SOS Induction and Transformation in E. coli—E. coli

cells were grown in 100-mL cultures to 1 \times 10^8 cells/mL and then harvested by centrifugation at 5,000 \times g for 15 min at 0

°C. All E. coli cells were grown in Luria broth, except the dnaQ49 strain NR11446, which was grown in minimal medium. The cells were resuspended in an equal

volume of ice-cold deionized water and recentrifuged at 5,000 \times g for 30

min. This procedure was repeated except the cells were resuspended in 50

mL of water. The bacterial pellet was resuspended in 1 mL of glycerol/

water (10% v/v) and kept on ice until further use. To induce SOS, the

following additional steps were introduced after the first centrifugation.

The cells were resuspended in 50 mL of 10 mM MgSO4 and treated with

UV light (354 nm) (either 20 or 50 J/m^2) in 25-mL aliquots in 150

× 50-mm plastic Petri dishes. The cultures were incubated in Luria broth

at 37

°C for 40 min in order to express SOS functions maximally. Following SOS induction, these cells were centrifuged, deionized, and resuspended in glycerol/water in a similar manner as described earlier (13) except all manipulations were carried out in subdued light.

Before transformation, the constructed genome was subjected to

another round of EcoRI digestion to digest any uncutf or religated

M13mp7L2 DNA. A 10-fold molar excess of a 46-mer that contained the

daDNA sequence complementary to the scaffold oligomer was added to each DNA solution, and the mixture was heated at 100

°C for 2 min to remove the scaffold and rapidly cooled to 0

°C. To monitor the extent of removal of the scaffold, a gapped genome was taken through the same

protocol. An aliquot of each of these DNA solutions was subjected to

electrophoresis to ensure that the scaffold was quantita-

tively denatured. For each transformation, 40 \( \mu L \) of the cell suspension was mixed with 4 \( \mu L \) (500 ng) of DNA solution and transferred to the bottom of an ice-cold Bio-Rad Gene-Pulser cuvette (0.1-cm electrode
gap). Electroporation of cells was carried out in a Bio-Rad Gene-Pulser

apparatus at 25 microfarads and 1.8 kV with the pulse controller set at

200 ohms. Immediately after electroporation, 1 mL of SOC medium was added, and the mixture was transferred to a 1.5-mL microcentrifuge
tube. Part of the cells was plated following a 15-min recovery at 37

°C in the presence of the plating bacteria E. coli

GW5100, IPTG, and X-gal to determine the number of independent transformants. The remainder of cells was centrifuged at 15,000

\( \times g \) for 5 min to isolate the phage-

containing supernatant. Minus-two and plus-one mutant phages were detected directly from the progeny as blue plaques after 18 h of incubation at 37

°C. All other colorless mutants were detected and isolated by oligonucleotide hybridization as described (14).

Modeling Studies—The molecular mechanics program DUPLEX (21, 22) was employed to obtain energy minimized structures for the AP and 1,8-ANP adducts in slipped mutagenic intermediate structures (23). Using this method, a two-base bulge in the DNA was assumed. Optimized geometries for the AP and 1,8-ANP adducts were computed in modified nucleosides, using Hartree-Fock calculations with 6-31+G* basis set, employing the program
gaussian 98 (24). These provided the bond lengths, bond

angles, and dihedral angles needed for the coordinate generator in

DUPLEX. Gaussian 98 (24) was also employed to compute the partial charges for these adducts in modified nucleosides. ONDO calculations,

which provide charges that are compatible with the rest of the DUPLEX

model were used for this purpose. These are given in Table S1 of the Supplemental Material. Other DUPLEX force field parameters for the AP and 1,8-ANP adducts are the same as those employed previously (25).
RESULTS

Construction and Characterization of M13 Genomes Containing a Single Adduct—Deoxyhexanucleotides, d(CGCG*CG) containing the C8 guanine adduct of 1-NP, 1,6-DNP, or 1,8-DNP, were synthesized by reacting N-hydroxy derivative of 1-aminopyrene or 1-amino-6(8)-nitropyrene in N,N-dimethylformamide/water (1:9), pH 5.0, 0.4 mm containing the C8 guanine adduct of 1-NP, 1,6-DNP, or 1,8-DNP, were synthesized by reacting N-hydroxy derivative of 1-aminopyrene or 1-amino-6(8)-nitropyrene in N,N-dimethylformamide/water (1:9), pH 5.0, for 16–20 h at ambient temperature and purified by HPLC followed by PAGE (19, 20). The added oligonucleotides were characterized by enzymatic digestion to nucleosides followed by HPLC analysis and by electrophoretic migration pattern of the fragments after piperidine cleavage (at 90 °C for 1 h) in comparison with Maxam-Gilbert G reaction products of the unmodified hexamer (see Refs. 13 and 20 for details). They were also examined by electrospray ionization-mass spectrometry analysis. The negative ion electrospray ionization-mass spectra of the hexamer containing Gua-C8-AP, Gua-C8-ANP, and Gua-C8–1,8-ANP contained an intense M – H peak at 2007.45 Da (theoretical 2052.34), respectively. It is noteworthy that in each synthesis the major product contained the C8 guanine adduct at the second G, which eluted 7–8 min after the unmodified peak and 6–12 min before the other modified hexamers by reverse-phase HPLC (see Fig. S1 of the Supplemental Material for a typical chromatogram). Reionization of the purified d(CGCG*CG) on a reverse-phase column showed no detectable level of either the unmodified or the other modified hexamers, and based on the chromatographic analysis, we estimated that the modified hexamers were in excess of 98% pure. Since further PAGE purification of each oligonucleotide was carried out, and the migration pattern of each adducted hexamer was different (data not shown), the purity of the hexamers used in this work was expected to be significantly higher than 98%. Fig. 2 shows a typical autoradiogram of the unmodified and the three adducted hexamers by denaturing PAGE.

The hairpin region of a +2 frameshift mutant clone of M13mp7 was digested with EcoRI (26). This was recircularized noncovalently by annealing a scaffold 46-mer, the two ends of which were complementary to the terminal 20 nucleotides of the linearized vector. The central segment of the oligomer was complementary to 5′-CGCGCG, which allowed the ligation of 5′-CGCG*CG to the ends of M13 vector by T4 DNA ligase. A control (i.e. unmodified) genome was constructed in a similar manner. A mock ligation was also carried out, in which no oligonucleotide was added.

In order to visualize the unmodified and modified DNA constructs, a portion of each of these genomes was run on a 1% agarose gel in the presence of ethidium bromide (1 μg/ml). Lanes 1 and 9 show the single-stranded M13mp7L2 DNA. Lanes 2 and 3 show EcoRI-digested M13mp7L2 before and after annealing the 46-mer scaffold, respectively. The M13 genomes after ligation of unmodified and 1,6-ANP-dG-, 1,8-ANP-dG-, and AP-dG-modified hexamers followed by removal of the scaffold 46-mer are shown in lanes 4–7, respectively. Lane 8 is the same as lane 4 except no hexamer was included in the ligation mixture (i.e. mock).

![Fig. 2. Denaturing PAGE of 32P-end-labeled d(CGCGCG) (lane 1), and d(CGCG(1,8-ANP)CG) (lane 2), d(CGCG(1,6-ANP)CG) (lane 3), and d(CGCG(1,8-ANP)CG) (lane 4). Electrophoresis was carried out at room temperature at 1800 V for 3.5 h on a 16% polyacrylamide gel (30 × 40 cm × 0.4 mm) containing 8 M urea.](http://www.jbc.org/)

**Fig. 3. Characterization of the M13 constructs.** The DNA samples were electrophoresed on 1% agarose gel in the presence of ethidium bromide (1 μg/ml). Lanes 2 and 3 show the single-stranded M13mp7L2 DNA. Lanes 2 and 3 show EcoRI-digested M13mp7L2 before and after annealing the 46-mer scaffold, respectively. The M13 genomes after ligation of unmodified and 1,6-ANP-dG-, 1,8-ANP-dG-, and AP-dG-modified hexamers followed by removal of the scaffold 46-mer are shown in lanes 4-7, respectively. Lane 8 is the same as in lanes 4-7 except no hexamer was included in the ligation mixture (i.e. mock).

To remove the 46-mer scaffold from the M13 DNA, each DNA solution was heated at 100 °C for 2 min and rapidly cooled to 0 °C. Prior to heating, a 10-fold molar excess of a 46-mer that contained the DNA sequence complementary to the scaffold oligomer was added to the DNA solution to ensure that the scaffold, once denatured, did not reanneal on the M13 DNA. As shown in Fig. 3, subsequent to removal of the scaffold 46-mer, a band for the circular DNA construct was observed on an agarose gel when either a control or adducted hexamer was included in the ligation mix, but no circular DNA was detectable (<2%) in the mock construct.

**Mutagenesis of the Three Adducts in Repair-proficient and-deficient Background—**The constructed M13 vector used in this study is a +2 derivative, which should generate colorless plaques in the presence of IPTG and X-gal. Either a −2 or a +1 frameshift can restore the reading frame to a Lac phenotype generating blue plaques. Spontaneous mutagenesis of the unmodified vector as determined phenotypically by the reversion to blue plaques was 2 × 10⁻⁴ or less (Table I). In the repair-competent strain DL7 the presence of Gua-C8-AP increased the MF to 1.7% as determined by an increased proportion of blue plaques (Table I and Fig. 4A). With SOS (50 J/m²), the MF of Gua-C8-AP increased to 1.9%. The MF of the two DNP adducts, Gua-C8–1,6-ANP and Gua-C8–1,8-ANP, were 6.8 and 10.1% without SOS, which increased to 8.2 and 12.8%, respectively, with SOS (20 J/m²). SOS induction with a higher dosage of UV (50 J/m²) resulted in further increase in MF of the two DNP adducts, which was found to be 11.2 and 15.0%, respectively, for Gua-C8–1,6-ANP and Gua-C8–1,8-ANP (Table I and Fig. 4A). In another repair-competent strain, NR9295, the numbers were somewhat lower, although the trend was similar (Table I and Fig. 4B).

In a nucleotide excision repair-impaired strain (uvrA) the MF did not increase without SOS, but with SOS (20 J/m²) there was a notable increase in MF, much more so than what we observed for the repair-proficient strains (Table I and Fig. 4A).
with SOS (Table I and Fig. 4B). The extent of increase in MF for the two DNP adducts was much less pronounced in the dnaQ strain. Both with and without SOS, MF was ~3-fold relative to the repair-competent strain. A population of blue plaques from each transformation was subjected to DNA sequencing. In each case more than 90% of the blue plaques contained CpG deletions, although a small population of one-base additions was also detected.

To investigate phenotypically undetectable base substitutions and other types of frameshifts, we used oligonucleotide hybridization with a 17-mer probe complementary to the region of M13 where the 6-mer was inserted. The probe was designed to bind only to the non-mutant plaques. Therefore, all non-hybridized or weakly hybridized plaques were considered putative mutants and subjected to DNA sequencing. In the progeny from the control construct with SOS ~1% progeny (13/1357) were mutants, which showed one-base deletions in various sites in the CGCGCG insert. The MF of clear plaques did not increase in the addeduct vectors, although both base substitutions and one-base deletions were detected. We conclude that in this repetitive CpG sequence, the predominant mutation induced by the three adducts is ~2 frameshifts.

Modeling Studies—To investigate possible structural reasons for the enhancement in two-base deletions when the adducts contained a nitro group on the pyrene ring, we have carried out a computer modeling study. Specifically, we wished to obtain structures of the slipped mutagenic intermediate, which has been proposed to cause two-base frameshifts in NarI-type sequences (23). We investigated the following Sequence 1,

$$5'$$-C1-G2-C3-G4*-C5-G6-A7-3'

$$3'$$-C11-G10——-C9-T8-5'

**Sequence 1**

Even though these bulky adducts are likely to be repaired by the nucleotide excision repair system, in single-stranded DNA it is unlikely to have a significant effect. We believe that the higher MF with SOS was due to a more pronounced SOS response, which in turn was a result of the persistence of the UV lesions in the *E. coli* DNA. For the mismatch repair-deficient strain (*mutS*), there was ~2-fold increase in MF of each of the three adducts compared with the repair-proficient strain. In the *dnaQ* strain, the increase in MF was most pronounced, and for Gua-C8-AP in the absence of SOS, MF was ~8-fold of the same in repair-competent strain, which increased to 11-fold with SOS (Table I and Fig. 4B).

### Table I

| Strain (genotype) | SOS  | Control* | AP* | 1,6-ANP* | 1,8-ANP* |
|------------------|------|----------|-----|----------|----------|
| DL7 (wild type)  |      | 0.04     | 1.7 | 6.8      | 10.1     |
| DL6 (uvrA)      | +20 J/m² | 0.18    | 1.7 | 8.2      | 12.8     |
|                  | +50 J/m² | < 0.01  | 1.9 | 11.2     | 15.0     |
| NR9295 (wild type) |      | < 0.01  | 3.1 | 16.2     | 17.2     |
| NR9294 (mutS)   |      | 0.12     | 1.9 | 5.2      | 6.2      |
| NR11446 ( dnaQ) |      | 0.11     | 2.1 | 9.0      | 12.4     |

* Mutation frequency (%).
ANP. These revealed that the oxygens of the NO$_2$ are out of plane with the AP ring system, with a dihedral angle O1–N–C9–C10 (see Fig. 1) of $-31.6$ degrees. An in-plane position of these oxygens produces steric crowding with hydrogens in the adjacent ring system. The DUPLEX molecular mechanics calculations employed this quantum mechanically derived orientation of the NO$_2$.

Stereo views of the modeled structures are shown in Fig. 5. In both cases, a base-displaced intercalated conformation is adopted with the aromatic ring system stacked on the G6-C9 base pair. The modified, unpartnered G4* has a syn-glycosidic torsion angle and is displaced into the major groove, with the covalently linked pyrene ring system directed toward the minor groove; the bulged out, unpartnered C5 is positioned on the minor groove side and interacts on one face with the edge of the pyrene ring system. These structures share features with the NMR solution structure of the AP adduct in a DNA duplex with normal partner C in adopting a syn-guanine base-displaced intercalated conformation, with the modified G displaced into the major groove (28). In the duplex structure the looped out

**Fig. 5. Stereo views of DUPLEX generated −2 frameshift structures of the AP adduct and 1,8-ANP adduct.** A, the AP adduct. B, the 1,8-ANP adduct and the proposed water-mediated hydrogen bond network. The AP and 1,8-ANP are in green. The adducted G4 is in magenta, and the bulged out C5 is in cyan. The atoms involved in the hydrogen bond network are colored by atom types. The N of the nitro group is blue and the two oxygens are red. Also designated in red are two other key oxygens: O2 of C5 and O4 of the G6 sugar. The water oxygens are red, and hydrogens are white. Other residues and atoms are in gray.
partner C was displaced into the major groove, and the NMR data indicated conformational heterogeneity for this residue. Key torsion angles \( \chi, \alpha' \) and \( \beta' \) defining the orientation of the carcinogenic moiety (\( \chi, O4'\)-C1'-N9-C4; \( \alpha', N9-C8-N(AP)-C1(AP); \beta', C8-N(AP)-C11(AP)-C10A(AP) \)) (Fig. 1) are very similar in our modeled slippage structures and in the NMR solution structure of the duplex (see Table S2 of the Supplemental Material).

Even though the slippage structures for the AP and 1,8-ANP are very similar, a key feature distinguishes them that can account for greater stability in the case of the 8-nitro derivative. Specifically, the nitro group oxygens are placed in a position to permit water-mediated hydrogen bonds between the carbonyl oxygen of the looped out C5 and one of the nitro oxygens; furthermore, this same nitro-oxygen can also form a second water-mediated hydrogen bond to the sugar O4 of G6. Table S3 of the Supplemental Material gives geometric features of these hydrogen bonds. In Fig. 5C we have modeled in these waters explicitly. This network of water-mediated hydrogen bonds is a feature only of the nitro-containing Gua-C8–1,8-ANP and would serve to stabilize its slippage structure, including especially the looped out C; the latter would be more mobile in the AP slippage structure, as is the looped out partner C to the AP-modified G in the NMR solution structure of the duplex.

**DISCUSSION**

This study unequivocally demonstrated that the guanine C8 adducts of 1,6- and 1,8-DNP are significantly more mutagenic in *E. coli* cells than the 1-NP adduct. With SOS, MF of each adduct increases. The trend is similar in all repair-competent and repair-impaired cells, even though the magnitude of frameshift is highly dependent on the type of repair defect.

We hypothesize that the mechanism of two-base deletion involves generation of a promutagenic slipped frameshift intermediate whose structure is similar in the different adducts, but whose stability is greater in the more mutagenic adduct. Whereas these intermediates may form spontaneously in repetitive sequences (29), certain lesions such as the guanine C8 adducts of nitropyrenes and AAF are particularly efficient in generating such intermediates in high frequency (13, 30).

Scheme I shows our working model and also suggests how the slippage and elongation might be linked to proofreading and mismatch repair activity. To investigate if this may be the case, we have performed a computer modeling study comparing Gua-C8-AP with Gua-C8–1,8-ANP. This study supports our hypothesis and shows how the 8-nitro substituent can stabilize the looped structure in solution.

High fidelity of DNA replication is maintained by at least three major steps (16). A high level of efficiency of the DNA polymerase in incorporating correct nucleotides is certainly very important. Nevertheless errors do occur, and in a large fraction of such cases the 3'-5'-exonuclease activity associated with the DNA polymerase can remove the incorrectly incorporated nucleotides. In addition, a DNA mismatch repair system detects and corrects mismatched nucleotides shortly after replication. It has been estimated that nucleotide selection discriminates against errors by 200,000–2,000,000-fold, proof-reading by 40–200-fold, and mismatch repair by 20–400-fold, each depending on the type of error (31). However, the ability of the DNA polymerase to incorporate the correct nucleotide opposite many carcinogen-DNA adducts is impaired, and the types and frequencies of misincorporation are often dependent on the DNA sequence surrounding the lesion. It has been suggested that when the replicative polymerase, pol III, encounters a replication-blocking lesion, it detaches from the replication terminus and translesional polymerases are recruited (32). Other polymerases such as pol II, pol IV, and pol
V have been reported to be involved at this stage for translesion synthesis (32–35). Some of these bypass polymerases are error-prone. The current work indicates the roles of two complementary repair systems in correcting the promutagenic slipped frameshift intermediate. As we have suggested in Scheme I, when either repair is impaired, there would be an increase in CpG deletion events. The guanine C8 adduct formed by AAF, a potent frameshift mutagenic lesion, which also intercalates with base displacement (36) and promotes slipped frameshift intermediates, shares some of the properties of the nitropyrene-DNA adducts (30, 34, 35, 37, 38). Our modeling studies of this slipped frameshift intermediate suggest that the bulged structure is more stable in the case of the 1,8-ANP compared with the AP adduct by virtue of its added hydrogen-bonding potential.

Current investigations in E. coli indicate that mutagenic bypass of bulky lesions can be carried out by pol II, pol III, pol IV, or pol V depending on the specific lesion and the base sequence context (35, 39). Relaxed steric constraints in the active site appear to be a feature common to Y family bypass DNA polymerases, such as pol IV (40, 41). Our modeling efforts suggest that specific hydrogen bonding capabilities of the nitro, through water-mediated hydrogen bonds within the bulge, are plausible structural features that would stabilize the slipped mutagenic intermediate more in the 1,8-ANP adduct compared with the AP adduct itself. It is also conceivable that there may exist stabilizing water-mediated or direct hydrogen-bonding interactions of the nitro group with specific amino acid residues within the polymerase. Since the 1,6-ANP adduct compared with the AP adduct itself. It is also conceivable that there may exist stabilizing water-mediated or direct hydrogen-bonding interactions of the nitro group with specific amino acid residues within the polymerase. Since the 1,6-ANP adduct would also allow for such unique hydrogen-bonding interactions, stabilization of a slipped intermediate through an analogous structural mechanism could be envisioned.

Recent studies (32–35), including the current work, suggest three major issues relating to translesion synthesis. First, it is important to analyze the structure, conformation, and stability of the promutagenic intermediate induced by the lesion, such as the slipped frameshift intermediate described here. Second, the DNA polymerases that can bypass the damage either with or without help from accessory proteins play crucial roles in both error-free and error-prone bypass events. This area of research has lately generated some intriguing data (32, 42). Finally, the roles of DNA repair proteins that are involved either before or after the replication process must also be taken into account. It is fascinating how the different branches of chemistry and genetics are merging to decipher these puzzles of replication and mutagenesis.

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