Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 6615–6620, June 1999
Biochemistry, Chemistry

Duplex DNA catalyzes the chemical rearrangement of a malondialdehyde deoxyguanosine adduct

HUI MAO*†, NATHALIE C. SCHNETZ-BOUTAUD‡, JASON P. WEISENSEEL*, LAWRENCE J. MARNETT‡, AND MICHAEL P. STONE*§

Departments of *Chemistry and ‡Biochemistry, Center in Molecular Toxicology and the E. Bronson Ingram Cancer Center, Vanderbilt University, Nashville, TN 37235

Communicated by Gerald N. Wogan, Massachusetts Institute of Technology, Cambridge, MA, March 26, 1999 (received for review December 10, 1998)

ABSTRACT **The primary DNA lesion induced by malondialdehyde, a byproduct of lipid peroxidation and prostaglandin synthesis, is 3-(2′-deoxy-β-D-erythro-pentofuranosyl)-pyrimido[1,2-a]purin-10(3H)-one (M_1G). When placed opposite cytosine (underlined) at neutral pH in either the d(GGTMTCCG)·d(CGGACACC) or d(ATCGCMCGGCATG)· d(CATGCCGCGCGAT) duplexes, M_1G spontaneously and quantitatively converts to the ring-opened derivative N^2-(3-oxo-1-propenyl)-dG. Ring-opening is reversible on thermal denaturation. Ring-opening does not occur at neutral pH in single-stranded oligodeoxynucleotides or when T is placed opposite to M_1G in a duplex. The presence of a complementary cytosine is not required to stabilize N^2-(3-oxo-1-propenyl)-dG in duplex DNA at neutral pH. When N^2-(3-oxo-1-propenyl)-dG is placed opposite to thymine in a duplex, it does not revert to M_1G. A mechanism for the conversion of M_1G to N^2-(3-oxo-1-propenyl)-dG is proposed in which the exocyclic amino group of the complementary cytosine attacks the C8 position of the M_1G exocyclic ring and facilitates ring opening via formation of a transient Schiff base. Addition of water to the Schiff base regenerates the catalytic cytosine and generates N^2-(3-oxo-1-propenyl)-dG. These results document the ability of duplex DNA to catalyze the transformation of one adduct into another, which may have important consequences for mutagenesis and repair.**

Malondialdehyde is a toxic and mutagenic metabolite produced by lipid peroxidation (1) and prostaglandin biosynthesis (2, 3). It is of interest in the etiology of human cancer because it is produced endogenously, and it is mutagenic in bacterial (4, 5) and mammalian cells (6) and is carcinogenic in rats (7). Malondialdehyde reacts with DNA (8–11), and, at physiological pH, the major adduct is an exocyclic pyrimidopurinone termed M_1G (8, 9, 12–14). This 1,N^2 exocyclic guanine adduct is unstable under basic conditions, rearranging to the ring-opened derivative N^2-(3-oxo-1-propenyl)-dG (15) (Fig. 1). M_1G has been identified in DNA from rodent (16) and human (17, 18) tissue samples, as have other exocyclic purine lesions (19–21), suggesting that these exocyclic lesions are formed *in vivo*. M_1G represents the most abundant exocyclic DNA adduct present endogenously in human DNA, as quantitated by mass spectroscopic (11, 17, 22), postlabeling (23, 24), and immunochemical (25) techniques. Site-specific mutagenesis experiments indicate M_1G is an efficient premutagenic lesion (26), suggesting that it is a mediator of human genetic disease.

The development of methodology to allow site-specific preparation of M_1G-adducted oligodeoxynucleotides (15) allowed M_1G to be introduced into the oligodeoxynucleotides d(GGTMTCCG) and d(ATCGCMCGGCATG). These oligodeoxynucleotides were derived from the *hisD3052* mutation within the histidinol dehydrogenase gene in the corresponding frameshift-sensitive tester strain of *Salmonella typhimurium* (27, 28). In d(GGTMTCCG), M_1G was centered in a palindromic sequence; in d(ATCGCMCGGCATG), M_1G was inserted into an iterated CG repeat sequence. Both of these sequence contexts were of interest for site-specific mutagenesis studies and also for solution structural studies because both were suspected to be loci for frameshift mutations that caused reversion of the *hisD3052* mutation. Studies using the structural analog 1,N^2-propanodeoxyguanosine (PdG) revealed that PdG equilibrated between the anti- and syn-conformations of the glycosyl bond within each of these sequences (29, 30).

The present results reveal that, when combined with the appropriate complementary strands to form the d(GGTXTCCG)·d(CGGACACC) or d(ATCGCXCGGCATG)· d(CATGCCG CGCGAT) duplexes, such that M_1G is placed opposite cytosine, M_1G undergoes quantitative transformation into the ring-opened N^2-(3-oxo-1-propenyl)-dG derivative (Fig. 2). Ring-opening is reversible on thermal dissociation of the duplexes, such that M_1G is regenerated. Ring-opening is not observed at pH 6.8 in the single-stranded oligodeoxynucleotides d(GGTMTCCG) and d(ATCGCMCGGCATG). When the N^2-(3-oxo-1-propenyl)-dG derivative is placed complementary to thymine in duplex DNA, it does not revert to M_1G, indicating that the presence of cytosine is not necessary to stabilize the ring-opened derivative. The conversion of M_1G to N^2-(3-oxo-1-propenyl)-dG is proposed to occur via formation of a transient Schiff base when the exocyclic amino group of the complementary cytosine attacks the C8 position of the M_1G exocyclic ring. These data provide the first evidence for the catalyzed chemical transformation of one adduct into another by duplex DNA, which may have important consequences for mutagenesis and repair.

MATERIALS AND METHODS

Materials. The oligodeoxynucleotides d(GGTMTCCG) and d(ATCGCMCGGCATG) were synthesized by using the 2-(acetoxymethyl)-benzoyl protecting group, which was removed with anhydrous potassium carbonate/methanol (15). The M_1G-modified oligodeoxynucleotides (15) were purified by reverse-phase and anion-exchange HPLC. The samples were maintained between pH 6 and pH 7 during purification. The adducted oligodeoxynucleotides were desalted by using gel filtration (BioGel P-2, Bio-Rad). Their purities were verified by ^{32}P-end labeling followed by denaturing polyacrylamide gel electrophoresis. Unmodified oligode-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

Abbreviation: PdG, propanodeoxyguanosine.

†Present address: Department of Radiology, Frederik Philips Magnetic Resonance Research Center, Emory University School of Medicine, Atlanta, GA 30322.

§To whom reprint requests should be addressed. e-mail: stone@toxicology.mc.vanderbilt.edu.

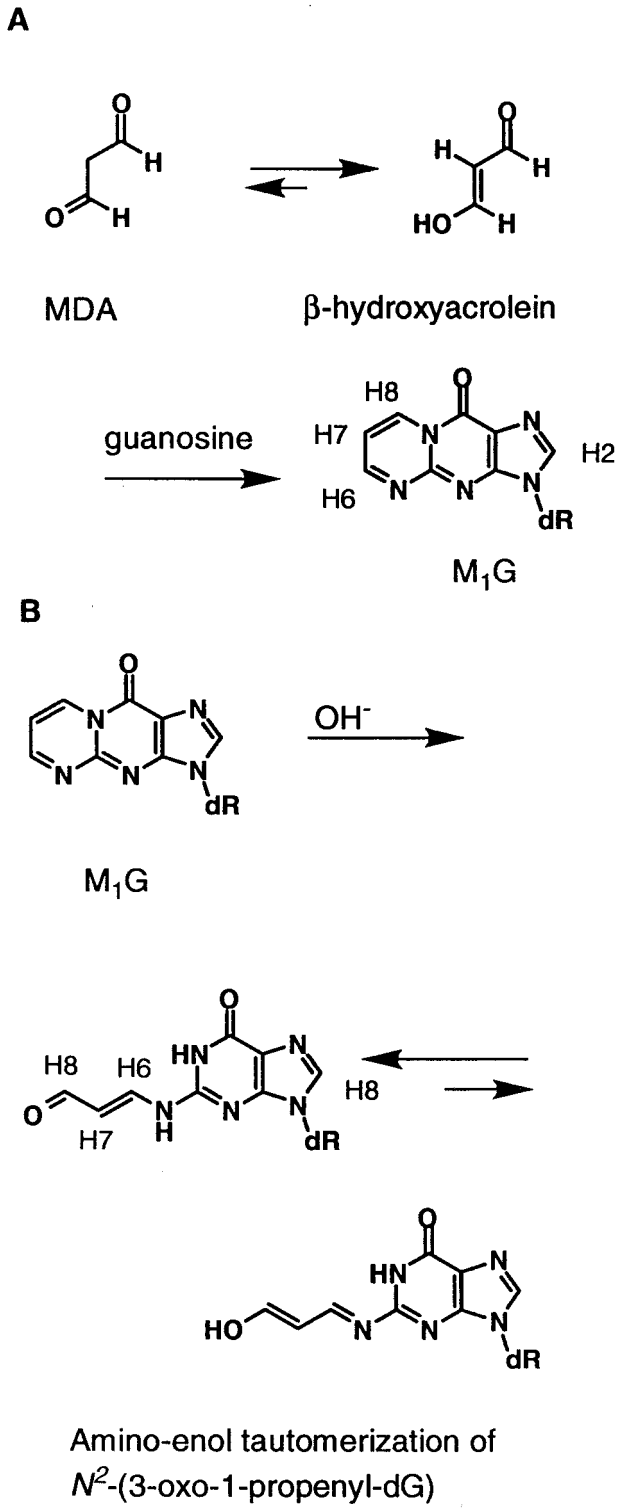

FIG. 1. (*A*) Formation of M_1G from [1,2a] cyclization of deoxyguanosine by β-hydroxyacrolein. (*B*) Ring-opening to the N^2-(3-oxo-1-propenyl)-dG derivative occurs at basic pH; the ring-opened species equilibrate between the favored amino and the enol tautomers. Note the numbering system for the M_1G protons. In oligodeoxynucleotides, M_1G is designated as M, and N^2-(3-oxo-1-propenyl)-dG is designated as X.

oxynucleotides were purchased from Midland Certified Reagent (Midland, TX). They were purified by reverse-phase and anion-exchange HPLC. The sample purities were checked by using SureCheck oligodeoxynucleotide analysis kits (Amersham Pharmacia).

NMR Sample Preparation. The NMR samples (0.5 ml) of d(GGTMTCCG) and d(ATCGCMCGGCATG) were prepared in 0.1 M NaCl, 10 mM NaH_2PO_4, and 50 μM Na_2EDTA (pH 6.8). The complementary strands were prepared in 99.96% D_2O. The solutions were lyophilized and exchanged with 99.96% D_2O. Duplex samples were made by titrating the appropriate complementary strands into the NMR tube containing the modified strand of interest at 60°C until the integration of the M^4 H2 resonance and the H8 resonance in the complementary strand reached a 1:1 ratio. Duplex samples were reconcentrated to 0.5 ml (0.7 mM strand concentration) in buffer of 0.1 M NaCl, 10 mM NaH_2PO_4, and 50 μM Na_2EDTA (pH 6.8). Samples used for examining exchangeable protons were prepared in the above buffer containing 9:1 H_2O:D_2O. For monitoring pH, a microprobe (Ingold, Wilmington, MA) was inserted into NMR sample tube before and after experiments.

NMR. Experiments were performed at the ^1H frequency of 500.13 MHz at 10°C. 2,2-dimethyl-2-silapentane-5-sulfonate was used to reference the spectra. Phase-sensitive two-dimensional nuclear Overhauser effect spectra for resonance assignment were recorded by using time-proportional phase increment quadrature detection with a mixing time of 300 ms. For examination of exchangeable protons, experiments were carried out in 9:1 H_2O/D_2O at 5°C by using a field-gradient watergate pulse sequence (31) for water suppression. Phase-sensitive total correlation spectra were obtained by using a MLEV17 (32) spin lock pulse at 2 G for a mixing time of 100 ms with time-proportional phase increment quadrature detection. Typically, 1,024 real data in the t1 dimension and 2,048 real data in the t2 dimension were collected. The data were processed by using FELIX (Molecular Simulations, Inc. San Diego) on an Indigo^2 workstation (Silicon Graphics, Mountain View, CA). The data in the t1 dimension were zero-filled to give a matrix of 2,048 × 2,048 real points. A skewed sinebell square apodization function with a 90° phase-shift was used in both dimensions.

To monitor the formation of N^2-(3-oxo-1-propenyl)-dG in duplex DNA when cytosine was placed opposite M_1G at neutral pH, an equimolar mixture of d(GGTMTCCG) and d(CGGACACC) was equilibrated at 60°C and pH 6.8. The sample was rapidly cooled to 5°C and then was maintained at that temperature. To monitor the formation of M_1G in heat-denatured DNA at neutral pH, a duplex sample of d(GGTXTCCG)·d(CGGACACC) was equilibrated at 5°C and pH 6.8. The sample was rapidly heated to 60°C and then was maintained at that temperature. To monitor the formation of M_1G when N^2-(3-oxo-1-propenyl)-dG was placed opposite thymine in duplex DNA at neutral pH, an equimolar mixture of d(GGTMTCCG) and d(CGGATACC) was equilibrated at 60°C, pH 10. The sample was rapidly cooled to 5°C. Immediately thereafter, the pH was rapidly adjusted to pH 6.8. The sample was maintained at that temperature and pH. The presence of M_1G or N^2-(3-oxo-1-propenyl)-dG was monitored by integration of the H6 and H8 ^1H NMR resonances of M_1G or N^2-(3-oxo-1-propenyl)-dG. Alternatively, UV absorbance was monitored at 320 nm. For NMR experiments, the strand concentration of oligodeoxynucleotide was 0.7 mM, in buffer of 0.1 M NaCl, 10 mM NaH_2PO_4, and 50 μM Na_2EDTA. For UV detection, the strand concentration was ≈10 μM in 1.0 M NaCl, 10 mM NaH_2PO_4, and 50 μM Na_2EDTA.

Molecular Modeling. Potential energy minimization calculations were performed by using XPLOR 2.4 (33) implemented with the CHARMM (34) force field. The empirical energy function (35) consisted of energy terms for bonds, bond angles, torsion angles, tetrahedral and planar geometry, hydrogen bonding, and nonbonded interactions, including van der Waals and electrostatic forces. The van der Waals energy term was approximated by using the Lennard-Jones potential energy function. The electrostatic term used the Coulomb function,

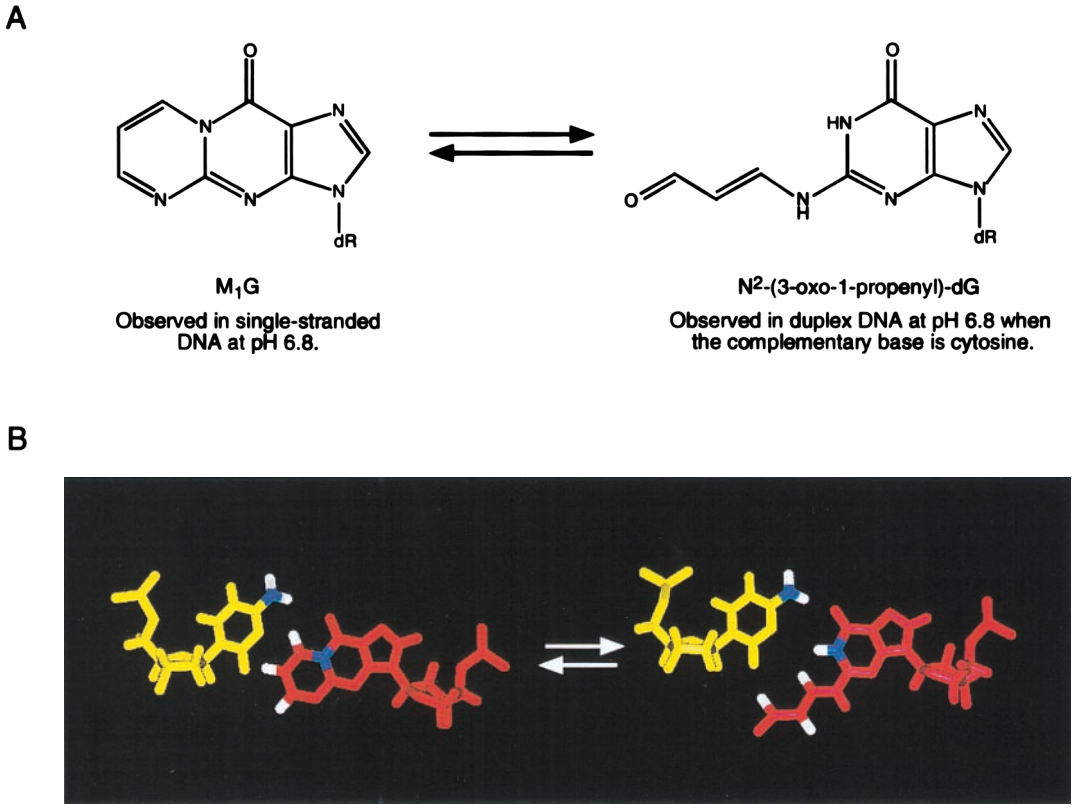

FIG. 2. (*A*) Analysis of d(GGTXTCCG)·d(CGGACACC) and d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT) revealed that, at pH 6.8, M_1G had undergone a quantitative chemical transformation into N^2-(3-oxo-1-propenyl)-dG. On strand dissociation at neutral pH, M_1G was regenerated. (*B*) Molecular modeling revealed that the exocyclic N^4 amino group of the complementary cytosine was positioned in duplex DNA to catalyze opening of M_1G, probably via attack at the C8 position of M_1G.

based on a full set of partial charges (−1/residue). A distance dependent dielectric constant of 4 was applied for all calculations.

RESULTS

M_1G Spontaneously Converts to N^2-(3-oxo-1-propenyl)-dG at Neutral pH When Placed Opposite Cytosine in Duplex Oligodeoxynucleotides. On addition of the complementary strand to the M_1G-modified oligodeoxynucleotides at neutral pH, thus placing M_1G opposite cytosine, spontaneous and quantitative conversion to N^2-(3-oxo-1-propenyl)-dG occurred. This was observed both for the 5′-TMT-3′·5′-ACA-3′ and the 5′-CMC-3′·5′-GCG-3′ sequence contexts in d(GGTXTCCG)·d(CGGACACC) and d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT). Below the melting temperature, the H6, H7, and H8 resonances derived from the malondialdehyde moiety were observed at ≈8.9, 5.8, and 7.8 ppm, respectively, consistent with conversion to N^2-(3-oxo-1-propenyl)-dG. The imidazole proton H2 (corresponding to H8 in deoxyguanosine) shifted to 7.9 ppm. Small sequence-specific differences in the chemical shifts were observed in the two oligodeoxynucleotides. A coupling constant of $J_{H7,H8}$ = 12 Hz indicated coupling from an aldehyde proton into a trans vinylic system. Likewise, $J_{H6,H7}$ = 18 Hz was consistent with trans orientation about the vinylic bond. The long range coupling $J_{H6,H8}$ was concluded to be <5Hz because it was not resolved (Fig. 3). The trans configuration also was suggested by the strong nuclear Overhauser effect between the propenyl H6 and H8 protons. The linewidths of the H6, H7, and H8 protons increased as compared with M_1G, which was attributed to amino-enol tautomerism (Fig. 1). The NMR evidence argued that the amino tautomer predominated in both duplexes. This was indicated by observation of the aldehyde H8 resonance in the 9-ppm range (Fig. 3) and the characteristic aldehyde carbon resonance, which was observed in the ^{13}C spectrum at 190 ppm.

For both d(GGTXTCCG)·d(CGGACACC) and d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT), the opening of the exocyclic ring of M_1G allowed observation of a resonance for the imino proton N1H of N^2-(3-oxo-1-propenyl)-dG in double-stranded DNA (Fig. 4). This was not present in M_1G because of the exocyclic 1,N^2 ring of the modified guanine. This resonance was in both instances observed at ≈11.4 ppm, ≈1 ppm upfield of the chemical shift range normally observed for Watson-Crick hydrogen bonded G·C base pairs. The upfield shift of X^4 N1H in both instances suggested that ring-opening of M_1G to the N^2-(3-oxo-1-propenyl)-dG derivative was not accompanied by base pair formation with the cytosine in the complementary strand.

The modified oligodeoxynucleotides remained in right-handed duplexes. Sequential ^1H resonance assignments for the unmodified and modified strands were obtained from nuclear Overhauser effect spectroscopy, double quantum-filtered correlated spectroscopy, and total correlation spectra by using established protocols (36, 37). Complete sets of aromatic-to-anomeric proton sequential connectivities were observed for both modified and complementary strands. There were no interruptions to the sequential NOEs at or adjacent to the adducted site. The NMR data revealed that the expected cytosine H5 → H6 cross-peaks, thymine CH_3 → H6 cross-peaks, and all expected H1′/H2′ and H1′/H2′′ cross-peaks were observed in their characteristic spectral regions. The magnitudes of the nuclear Overhauser effects between the H8 (imidazole) protons and the H1′ (anomeric) protons of the N^2-(3-oxo-1-propenyl)-dG derivatives were consistent with the N^2-(3-oxo-1-propenyl)-dG derivatives existing in the anti-conformation about the glycosyl bond χ.

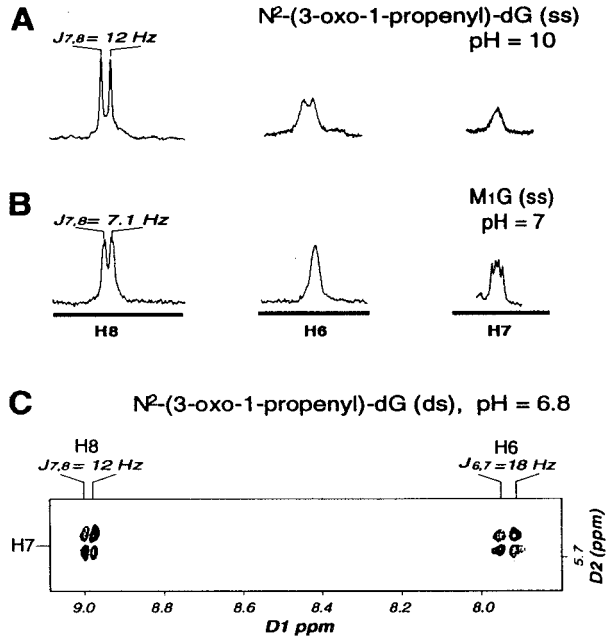

FIG. 3. Cytosine-mediated opening of M_1G to N^2-(3-oxo-1-propenyl)-dG is reflected by changes in scalar coupling constants. (*A*) ^1H spectrum of N^2-(3-oxo-1-propenyl)-dG resonances H6, H7, and H8 at pH 10. (*B*) ^1H spectrum of M_1G resonances H6, H7, and H8 at pH 7. (*C*) Expanded region of a ^1H double quantum-filtered correlated spectroscopy spectrum of N^2-(3-oxo-1-propenyl)-dG crosspeaks at pH 7.

Cytosine Accelerates the Rate of M_1G Ring-Opening at Neutral pH. At neutral pH, opening of M_1G to N^2-(3-oxo-1-propenyl)-dG occurred at an observable rate only when cytosine was placed opposite M_1G in duplex DNA. For the 0.7 mM d(GGTMTCCG)·d(CGGACACC) sample containing 0.1 M NaCl, 10 mM Na_2HPO_4, and 50 μM Na_2EDTA, ring-opening at 5°C was 90% complete in <5 min. For both d(GGTMTCCG)·d(CGGACACC) and d(ATCGCMCGGCATG)·d(CATGCCGCGCGAT), cytosine-promoted ring-opening was reversible when the duplexes were thermally denatured. On heating the duplexes above their melting temperatures, M_1G was regenerated. For the 0.7 mM d(GGTXTCCG)·d(CGGACACC) sample containing 0.1 M NaCl, 10 mM Na_2HPO_4, and 50 μM Na_2EDTA at pH 7, at 60°C N^2-(3-oxo-1-propenyl)-dG was 90% converted to M_1G in <5 min.

M_1G in the single-stranded oligodeoxynucleotides, as well as M_1G nucleotide, was stable indefinitely at neutral pH (15). The extent to which M_1G and N^2-(3-oxo-1-propenyl)-dG might be in rapid equilibrium, favoring M_1G at neutral pH, was tested in D_2O buffer by monitoring for deuterium exchange at M_1G H8. No exchange was observed when the sample was monitored for a period of several weeks. To examine the role of the complementary cytosine in promoting ring opening of M_1G, d(GGTMTCCG)·d(CGGATACC) was synthesized in which M_1G was placed opposite T. M_1G was stable at neutral pH in the M_1G·T duplex and did not undergo ring-opening, evidenced by the observation of resonances for H6, H7, and H8, which were in agreement with the M_1G in the single strand at pH 7 (Fig. 5). In that instance, the M_1G H6, H7, and H8 resonances were assigned at ≈7.4, 9.0, and 9.3 ppm from total correlation spectroscopy experiments; the imidazole H2 (H8 in deoxyguanosine) resonances were at ≈8.4 ppm. Double quantum-filtered correlated spectra revealed $J_{H6,H7}$ of ≈4 Hz and $J_{H7,H8}$ of 7.1 Hz. Inspection of the imino region of the d(GGTMTCCG)·d(CGGATACC) mismatched duplex confirmed that M_1G remained intact (Fig. 4). No resonance for a non-hydrogen-bonded guanine N1H proton was observed at or near 11.4 ppm. Integration of the imino region of the spectrum yielded a total of seven Watson-Crick base pairs.

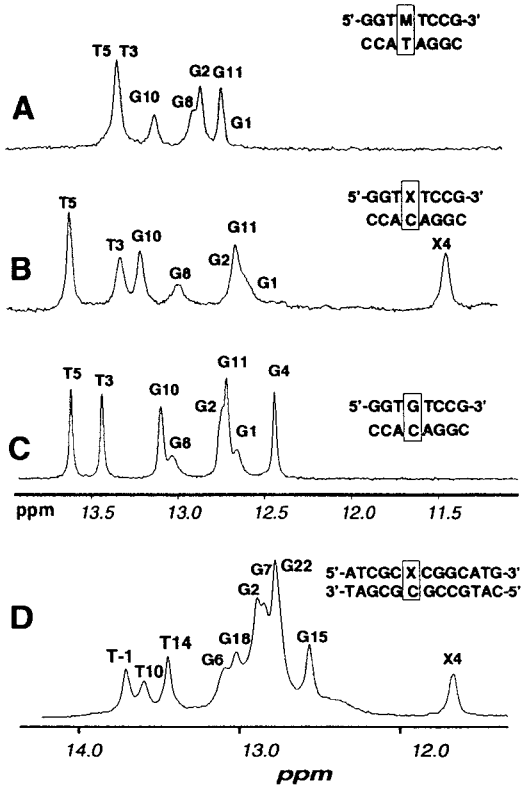

FIG. 4. ^1H NMR spectra of the far downfield imino proton region at 5°C and pH 6.8. (*A*) The unadducted duplex d(GGTGTCCG)·d(CGGACACC). (*B*) The duplex d(GGTXTCCG)·d(CGGACACC). (*C*) The duplex d(GGTMTCCG)·d(CGGATACC). (*D*) The duplex d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT).

The imino resonance of the non-hydrogen-bonded thymine at the mismatch site was not observed, which was attributed to rapid exchange with solvent. This was consistent with the broadening of the M_1G resonances and lower thermal stability of this duplex.

In the absence of cytosine, M_1G underwent measureable ring opening to N^2-(3-oxo-1-propenyl)-dG only under basic conditions. Moreover, the rate of ring-opening at pH 10 was slower than in the presence of cytosine at neutral pH. For both d(GGTMTCCG) and d(ATCGCMCGGCATG) at pH 10, M_1G underwent slow ring-opening. As investigated for d(GGTMTCCG) at 25°C, after 30 min, and at pH 10, the conversion of M_1G to N^2-(3-oxo-1-propenyl)-dG was 50% complete. About 90% of the M_1G converted after 5 hr. A small quantity of M_1G remained detectable in the spectrum after 24 hr at pH 10. Under these conditions, the H6, H7, and H8 resonances shifted to ≈8.0, 5.6, and 9.0 ppm. The imidazole H8 resonances were at ≈8 ppm. The scalar couplings $J_{H6,H7}$ of 18 Hz and $J_{H7,H8}$ of 12 Hz were observed. Ring opening was reversible in the single-stranded oligodeoxynucleotides on lowering pH. The reverse rate of conversion from N^2-(3-oxo-1-propenyl)-dG to M_1G was investigated in the single-stranded oligodeoxynucleotides at pH 6.5. In the case of d(GGTXTCCG), ≈50% of N^2-(3-oxo-1-propenyl)-dG converted to M_1G after 30 min. Almost all of the N^2-(3-oxo-1-propenyl)-dG converted to M_1G after 12 hr at pH 6.5, although a detectable amount of N^2-(3-oxo-1-propenyl)-dG remained.

The Presence of Cytosine Opposite the Lesion Is Not Required to Stabilize N^2-(3-oxo-1-propenyl)-dG in Duplex DNA. N^2-(3-oxo-1-propenyl)-dG was intentionally placed opposite thymine in duplex DNA at neutral pH to determine whether the ring-opened derivative would spontaneously recyclize to M_1G in the absence of cytosine complementary to the lesion. For the 0.7 mM d(GGTXTCCG)·d(CGGATACC)

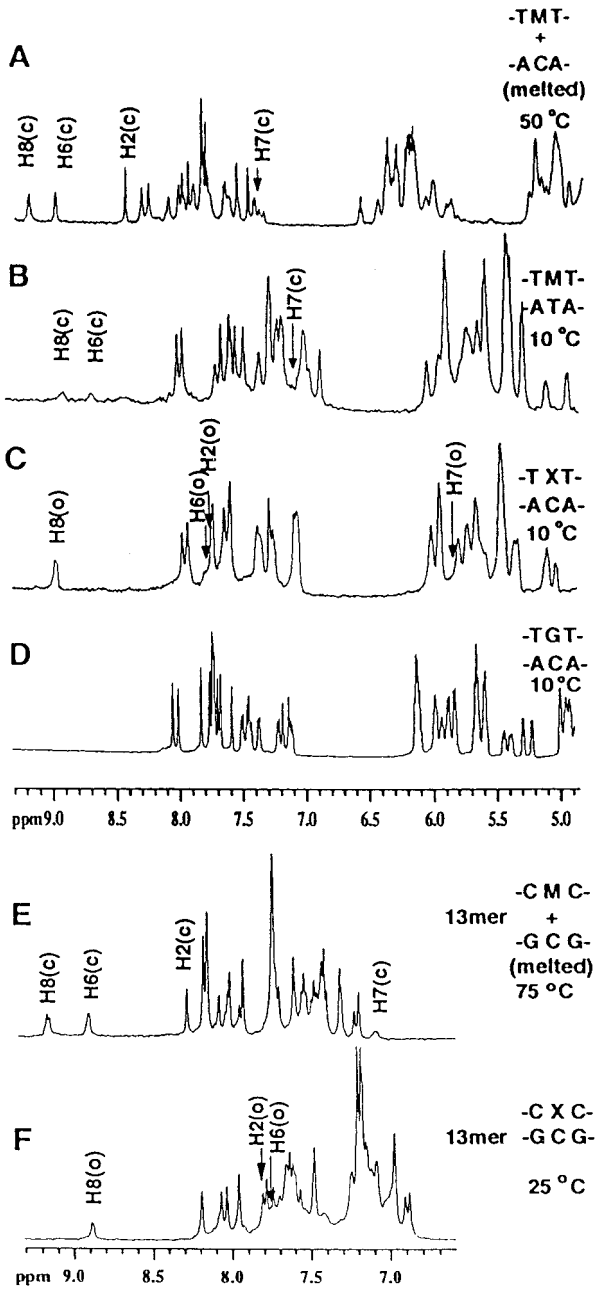

FIG. 5. Aromatic and anomeric regions of ^{1}H NMR spectra at pH 6.8. (*A*) The unmodified duplex d(GGTGTCCG)·d(CGGACACC) at 10°C. (*B*) The duplex d(GGTXTCCG)·d(CGGACACC) at 10°C. (*C*) The duplex d(GGTMTCCG)·d(CGGATACC) at 10°C. The broadening of the M_1G H6, H7, and H8 resonances was attributed to the low thermal stability of the M_1G·T mismatch. An additional source could be conformational exchange of M_1G about the glycosyl bond, as observed for its saturated analog PdG (38). (*D*) The duplex d(GGTXTCCG)·d(CGGACACC) at 50°C after thermal denaturation. (*E*) The duplex d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT) at 10°C. (*F*) The duplex d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT) after thermal denaturation.

sample containing 0.1 M NaCl, 10 mM Na_2HPO_4, and 50 μM Na_2EDTA, no measureable recyclization occurred after 60 min at 5°C. Under these conditions, the N^2-(3-oxo-1-propenyl)-dG derivative remained stable in duplex DNA. The stability of the N^2-(3-oxo-1-propenyl)-dG lesion depended on maintenance of the DNA duplex. The T_m of the X·T mismatched duplex was below 20°C at neutral pH, and, as the temperature was raised further, significant denaturation caused the N^2-(3-oxo-1-propenyl)-dG lesion to be rapidly recyclized to M_1G.

DISCUSSION

Detection of the ring-opened N^2-(3-oxo-1-propenyl)-dG derivative of M_1G in d(GGTXTCCG)·d(CGGATACC) and d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT) reveals the ability of duplex DNA to catalyze the chemical transformation of one adduct into another. The observation that this occurs when M_1G is placed in either the 5′-TMT-3′ or 5′-CMC-3′ sequences opposite cytosine in the complementary strand, but not when M_1G is positioned opposite thymine in the complementary strand, suggests that it represents a general phenomenon associated with the positioning of M_1G opposite cytosine in the complementary strand of duplex DNA.

Cytosine-Catalyzed Ring-Opening of M_1G. The observation that, in duplex DNA at neutral pH, M_1G ring-opening occurred only when cytosine was placed opposite the adduct suggested the role of cytosine in catalyzing the ring-opening of M_1G to the N^2-(3-oxo-1-propenyl)-dG derivative. An alternative hypothesis, that the complementary cytosine acted by stabilizing N^2-(3-oxo-1-propenyl)-dG by lowering its free energy, was considered. However, this was not consistent with the observation that N^2-(3-oxo-1-propenyl)-dG was not formed in the absence of cytosine. Also, ^{1}H NMR data in the 5′-CXC-3′·5′-GCG-3′ sequence showed that N^2-(3-oxo-1-propenyl)-dG did not interact with cytosine but, rather, was oriented into the minor groove (Fig. 6). Watson–Crick base pairing was not observed between the N^2-(3-oxo-1-propenyl)-dG derivative and the complementary cytosine. Finally, the observation that N^2-(3-oxo-1-propenyl)-dG was stable when intentionally placed opposite thymine in duplex DNA suggested that cytosine was not necessary for stabilization of the ring-opened species.

^{1}H NMR data for the N^2-(3-oxo-1-propenyl)-dG derivative contained in the 5′-CXC-3′·5′-GCG-3′ sequence provided a plausible explanation as to why, at neutral pH in duplex DNA, cytosine-promoted formation of N^2-(3-oxo-1-propenyl)-dG was facile (Fig. 6). The ring-opened derivative exhibited the trans configuration of the propenal, as demonstrated by ^{1}H J-coupling values. Consequently, both the amino proton and the 3-oxo-1-propenyl derivative were oriented into the minor groove, and the ring-opened species was easily accommodated by the DNA duplex with minimal structural perturbation. In contrast, modeling predicted that accommodation of the exocyclic ring of M_1G would require substantial perturbation to

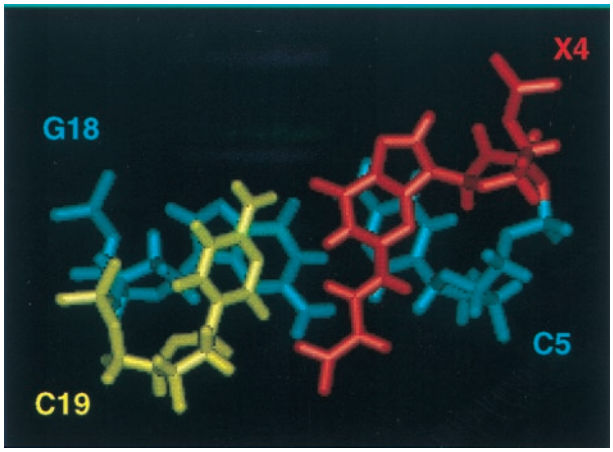

FIG. 6. Structure obtained from molecular dynamics calculations by using NMR distance restraints for the (3-oxo-1-propenyl)-dG derivative of M_1G in d(ATCGCXCGGCATG)·d(CATGCCGCGCGAT).

duplex DNA, as observed for its fully saturated analog, 1,N^2-propanodeoxyguanosine (PdG) (38).

Proposed Mechanism of M_1G Ring-Opening. The spatial relationship between the exocyclic amino group of the cytosine complementary to M_1G and the C8 position of M_1G was examined by constructing a model duplex in which M_1G in the anticonformation about the glycosyl bond χ was positioned opposite cytosine in the complementary strand, followed by potential energy minimization. The energy minimized structure suggested that, in the anti-conformation, the cytosine amino group was positioned to attack M_1G at the major groove face of the exocyclic ring, i.e., from the N1 point of closure as opposed to the N^2 side of the pyrimidopurinone moiety (Fig. 2*B*). This would facilitate ring opening at neutral pH, probably via formation of a transient Schiff base (Fig. 7). Addition of water to the Schiff base would regenerate the catalytic cytosine and would result in the formation of the N^2-(3-oxo-1-propenyl)-dG derivative. This hypothesis derived support from UV and NMR spectroscopic experiments that revealed the existence of Schiff bases when M_1G-dR was reacted with tris-hydroxymethylaminomethane (39) and with glycine (N.C.S.-B., H.M., G. Ramachandra Reddy, and L.J.M., unpublished work).

Biological Implications. Structurally, M_1G and its N^2-(3-oxo-1-propenyl)-dG derivative are sufficiently different that their independent presence in DNA may result in significant differences in mutagenic potential and susceptibility to repair. The reversible transformation of M_1G to the N^2-(3-oxo-1-propenyl)-dG derivative may explain the observation that M_1G located on viral genomes is more mutagenic when the base opposite the adduct is T than when it is C (40). M_1G positioned opposite T in the M13 phage genomes was ≈5-fold more mutagenic than M_1G positioned opposite C, but the mutation spectra were identical in the two vectors. When similar experiments were conducted with the stable M_1G analog PdG, no difference in the frequency of mutations induced was detected between vectors containing C or T opposite the lesion. One interpretation is that M_1G is considerably more mutagenic than N^2-(3-oxo-1-propenyl)-dG, so that the frequency of mutations induced at the adduct site depends on the amount of M_1G present at the replication fork. In vectors with M_1G positioned opposite C, the adduct is predicted to be present as N^2-(3-oxo-1-propenyl)-dG, and the mutation frequency would depend on the rate of its recyclization to M_1G after helicase-catalyzed strand separation.

Summary. The idea that DNA contains complementary binding pockets analogous to those of enzymes for their substrates has been advanced to explain the exquisite sequence specificity controlling the binding of many electrophiles to DNA (41). Our findings extend the enzyme–substrate analogy by showing that the DNA duplex actively catalyzes a chemical transformation in a site-specific manner. In this instance, the DNA duplex plays an active role in directing the metabolism of a genotoxic chemical, catalyzing the chemical transformation of one adduct into another.

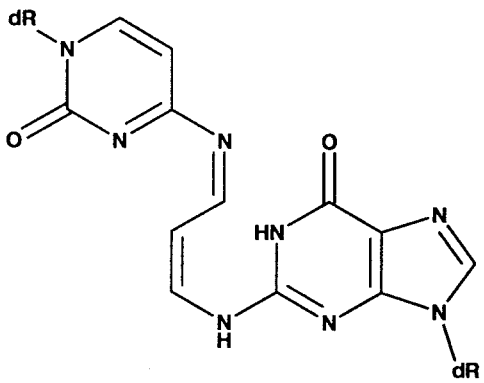

FIG. 7. Proposed Schiff base intermediate after attack by the exocyclic amino group of cytosine at C8 of M_1G.

This work was supported by National Institutes of Health Grants CA-55678 (to M.P.S.) and CA-47479 (to L.J.M.). J.P.W. received support from a training grant in Molecular Biophysics, Grant GM-08320, from the National Institutes of Health. Funding for the NMR spectrometer was supplied by National Institutes of Health Grant RR-05805 and Vanderbilt Center in Molecular Toxicology Grant ES-00267. This project was supported by Center Grant CA-68485, to the E. Bronson Ingram Cancer Center at Vanderbilt University.

1. Pryor, W. A. & Stanley, J. P. (1975) *J. Org. Chem.* **40,** 3615–3617.
2. Hamberg, M. & Samuelsson, B. (1967) *J. Biol. Chem.* **242,** 5336–5343.
3. Diczfalusy, U., Falardeau, P. & Hammarstrom, S. (1977) *FEBS Lett.* **84,** 271–274.
4. Mukai, F. H. & Goldstein, B. D. (1976) *Science* **191,** 868–869.
5. Benamira, M., Johnson, K., Chaudhary, A., Bruner, K., Tibbetts, C. & Marnett, L. J. (1995) *Carcinogenesis* **16,** 93–99.
6. Yau, T. M. (1979) *Mech. Ageing Dev.* **11,** 137–144.
7. Spalding, J. W. (1988) *Natl. Toxicol. Progr. Tech. Rep. Ser.* **331,** 5–13.
8. Marnett, L. J., Basu, A. K., O'Hara, S. M., Weller, P. E., Rahman, A. F. M. M. & Oliver, J. P. (1986) *J. Am. Chem. Soc.* **108,** 1348–1350.
9. Basu, A. K., O'Hara, S. M., Valladier, P., Stone, K., Mols, O. & Marnett, L. J. (1988) *Chem. Res. Toxicol.* **1,** 53–59.
10. Chaudhary, A. K., Nokubo, M., Oglesby, T. D., Marnett, L. J. & Blair, I. A. (1995) *J. Mass Spectrom.* **30,** 1157–1166.
11. Chaudhary, A. K., Reddy, R. G., Blair, I. A. & Marnett, L. J. (1996) *Carcinogenesis* **17,** 1167–1170.
12. Seto, H., Okuda, T., Takesue, T. & Ikemura, T. (1983) *Bull. Chem. Soc. Jpn.* **56,** 1799–1802.
13. Seto, H., Seto, T., Takesue, T. & Ikemura, T. (1986) *Chem. Pharm. Bull.* **34,** 5079–5085.
14. Reddy, G. R. & Marnett, L. J. (1996) *Chem. Res. Toxicol.* **9,** 12–15.
15. Reddy, G. R. & Marnett, L. J. (1995) *J. Am. Chem. Soc.* **117,** 5007–5008.
16. Wang, M. Y. & Liehr, J. G. (1995) *Carcinogenesis* **16,** 1941–1945.
17. Chaudhary, A. K., Nokubo, M., Reddy, G. R., Yeola, S. N., Morrow, J. D., Blair, I. A. & Marnett, L. J. (1994) *Science* **265,** 1580–1582.
18. Wang, M., Dhingra, K., Hittleman, W. N., Liehr, J. G., de Andrade, M. & Li, D. (1996) *Cancer Epidemiol.* **5,** 705–710.
19. Nath, R. G. & Chung, F.-L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7491–7495.
20. Nath, R. G. & Chung, F.-L. (1993) *Proc. Am. Assoc. Cancer Res.* **34,** 137.
21. O'Nair, J., Barbin, A., Guichard, Y. & Bartsch, H. (1995) *Carcinogenesis* **16,** 613–617.
22. Rouzer, C. A., Chaudhary, A. K., Nokubo, M., Ferguson, D. M., Reddy, G. R., Blair, I. A. & Marnett, L. J. (1997) *Chem. Res. Toxicol.* **10,** 181–188.
23. Vaca, C. E., Fang, J. L., Mutanen, M. & Valsta, L. (1995) *Carcinogenesis* **16,** 1847–1851.
24. Fang, J. L., Vaca, C. E., Valsta, L. M. & Mutanen, M. (1996) *Carcinogenesis* **17,** 1035–1040.
25. Sevilla, C. L., Mahle, N. H., Eliezer, N., Uzieblo, A., O'Hara, S. M., Nokubo, M., Miller, R., Rouzer, C. A. & Marnett, L. J. (1997) *Chem. Res. Toxicol.* **2,** 172–180.
26. Fink, S. P., Reddy, G. R. & Marnett, L. J. (1997) *Proc. Natl. Acad. Sci. USA* **16,** 8652–8657.
27. O'Hara, S. M. & Marnett, L. J. (1991) *Mutat. Res.* **247,** 45–56.
28. Benamira, M. & Marnett, L. J. (1993) *Chem. Res. Toxicol.* **6,** 317–327.
29. Moe, J. G., Reddy, G. R., Marnett, L. J. & Stone, M. P. (1994) *Chem. Res. Toxicol.* **7,** 319–328.
30. Weisenseel, J. P., Moe, J. G., Reddy, G. R., Marnett, L. J. & Stone, M. P. (1995) *Biochemistry* **34,** 50–64.
31. Piotto, M., Saudek, V. & Sklenar, V. (1992) *J. Mol. Biol.* **6,** 661–665.
32. Bax, A. & Davis, D. G. (1985) *J. Magn. Reson.* **65,** 355–360.
33. Brunger, A. T. (1992) XPLOR 3.1*: A System for X-Ray Crystallography and NMR* (Yale Univ. Press, New Haven, CT).
34. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S. & Karplus, M. (1983) *J. Comput. Chem.* **4,** 187–217.
35. Nilsson, L., Clore, G. M., Gronenborn, A. M., Brunger, A. T. & Karplus, M. (1986) *J. Mol. Biol.* **188,** 455–475.
36. Reid, B. R. (1987) *Q. Rev. Biophys.* **20,** 2–28.
37. Patel, D. J., Shapiro, L. & Hare, D. (1987) *Q. Rev. Biophys.* **20,** 35–112.
38. Singh, U. S., Moe, J. G., Reddy, G. R., Weisenseel, J. P., Marnett, L. J. & Stone, M. P. (1993) *Chem. Res. Toxicol.* **6,** 825–836.
39. Niedernhofer, L. J., Riley, M., Schnetz-Boutand, N., Sanduwaran, G., Chaudhary, A. K., Reddy, G. R. & Marnett, L. J. (1997) *Chem. Res. Toxicol.* **10,** 556–561.
40. Niedernhofer, L. J. (1996) Ph.D. dissertation (Vanderbilt Univ., Nashville, TN).
41. Warpehoski, M. A. & Hurley, L. H. (1988) *Chem. Res. Toxicol.* **1,** 315–333.