The ability of the methyl-directed mismatch repair system to recognize and repair the exocyclic adducts propanodeoxyguanosine (PdG) and pyrimido[1,2-a]purin-10(3H)-one (M1G), the major adduct derived from the endogenous mutagen malondialdehyde, has been assessed both in vivo and in vitro. Both adducts were site-specifically incorporated into M13MB102 DNA, and the adducted genomes were electroporated into wild-type or mutS-deficient Escherichia coli strains. A decrease in mutation frequency observed when hemiexcision repair. This hypothesis was supported by the differences in mutation frequency observed when hemimethylated genomes containing PdG on the (−) strand were electroporated into a uvrA-deficient strain. The ability of purified MutS to bind to PdG- or M1G-containing 31-mer duplexes in vitro was assessed using both surface plasmon resonance and gel shift assays. MutS bound to M1G; T-containing duplexes with similar affinity to a G:T mismatch but less strongly to M1G:C- and PdG-containing duplexes. Dissociation from each of the adduct-containing duplexes occurred at a faster rate than from a G:T mismatch. The present results indicate that MutS can bind to exocyclic adducts resulting from endogenous DNA damage and trigger their removal by mismatch repair or protect them from removal by nucleotide excision repair.

Malondialdehyde (MDA) is produced endogenously through the processes of lipid peroxidation and eicosanoid biosynthesis (1, 2). MDA is mutagenic in bacterial and mammalian cell assays and carcinogenic in rodents (3–7). It also induces p53-independent cell cycle arrest at the G1/S and G2/M checkpoints (8). MDA reacts with DNA, forming a pyrimidopurinone adduct to deoxyguanosine (M1G) and an oxopropenyl adduct to deoxyadenosine (N6-[3-(1-oxopropenyl)](deoxyadenosine) (M1A) (Fig. 1) (9–12). M1G also can be formed by the reaction of the oxidative DNA damage product, base propenal, with deoxyguanosine (13). M1G is an abundant constituent of DNA from healthy human beings. It has been detected in several human tissues at levels from 2–150 per 10^8 bases (14–17). M1G may account for a significant portion of the genotoxic and cell cycle regulatory activity of MDA. Site-specific mutagenesis experiments indicate that M1G induces mutations to T and A on replication in Escherichia coli and is a block to replication (18).

Given the high biological activity of M1G, it is important to identify the pathways by which it may be removed from DNA. We have shown that M1G and a structural analog, propanodeoxyguanosine (PdG), are removed in E. coli by nucleotide excision repair and that PdG is excised by both E. coli and mammalian nucleotide excision repair complexes in vitro (18, 19). The experiments described in the present report were designed to test the hypothesis that M1G is recognized by the mismatch repair system. Mismatch repair exists to correct errors that arise during DNA replication, but recent studies indicate that it recognizes and acts on damaged DNA, including duplexes containing alkylated or platinated bases (20–26). In both cases, the mismatch repair system attempts to remove the normal base opposite the adduct, which sets up a futile cycle of repair and replication that leads to cell toxicity. M1G and other exocyclic adducts are relatively small adducts that resemble normal DNA bases, so they may be substrates for removal by mismatch repair. Transfection of M13 genomes containing single M1G or PdG adducts into E. coli strains deficient in mismatch repair suggested that both adducts are recognized and repaired by MutS-dependent mismatch repair. This conclusion was supported by in vitro studies of purified MutS protein binding to M1G- and PdG-containing duplexes.

**Experimental Procedures**

Kap1 was purchased from Roche Molecular Biochemicals, and BstHII and T4 DNA ligase were from New England Biolabs (Beverly, MA). Formamide was from Aldrich Chemical. GELase was purchased from Epicentre Technologies(Madison, WI). T4 polynucleotide kinase and purified MutS protein were purchased from Amersham Pharmacia Biotech. The MutS protein ran as a single band at 95 kDa on SDS-polyacrylamide gel electrophoresis. Tris-HCl, EDTA, MOPS, calf thymus DNA, lauryl sulfate, ATP, ADP, and nonhydrolyzable analogs were purchased from Sigma. X-gal and isopropyl-β-D-thiogalactoside were from Gold Biotechnology (St. Louis, MO). Nitrocellulose transfer membranes (BA85, 82.5-mm diameter) were purchased from Schleicher & Schuell. Ultrafree probrin filters (0.45 μm) were from Millipore (Bedford, MA). [γ-32P]ATP (10 μCi/ml) was from NEN Life Science Products. Unadducted and PdG-adducted 8-mer oligonucleotides used in mutagenesis experiments, 5′-GGTCTCGG-3′ (X = G, PdG), and 31-mer oligonucleotides used for gel shift and surface plasmon resonance assays 5′-GCCAGATTCCCGATCCGTGACTCGAGTCGACGAGTACG-3′ were synthesized by Midland Certified Reagent Company (Midland, TX) and ran as single bands on a 20% polyacrylamide gel. The M1G-adducted oligo...
nucleotides were synthesized, purified, and characterized as described. Following purification, the M5G oligonucleotides were determined to be 99.7% pure by 20% polyacrylamide gel electrophoresis. For the surface plasmon resonance assays, duplexes were 5'-biotinylated by phosphoramidite chemistry on the nonadducted strand. Oligonucleotides used as hybridization probes were prepared using an Applied Biosystems (Foster City, CA) automated DNA synthesizer in the Vanderbilt University Center in Molecular Toxicology Molecular Genetics Core and purified using a SurePure™ oligonucleotide purification kit from Amersham Pharmacia Biotech.

**Bacterial Strain and DNA Isolation**—Single-stranded M13MB102 for the construction of M5G-C, M5G-T, or G-GC genomes was isolated as described (27). Replicase form M13MB102 DNA was harvested using Qiagen columns (Chatsworth, CA). Briefly, bacteria in logarithmic phase growth were SOS-induced with UV light before making them competent for transformation. The UV dose was determined by irradiating cells at increasing times from 0 to 3 min and then plating dilutions of the irradiated cells on LB plates. The optimal UV dose corresponded to roughly a 10% survival of the cells as compared with no exposure. For transformation, 3 μl of DNA sample (~25 ng/μl) was added to 20 μl of cells. The cell/DNA mixture was placed into a chilled microelectroporation cuvette (Life Technologies, Inc.), and the electroporations were performed at 1.5 kV/cm using a Life Technologies, Inc. Cell-Porator E. coli electroporation system. After electroporation, 1 ml of SOC medium was added, and the bacteria were plated on LB plates in the presence of competent bacteria and isopropyl-β-D-thiogalactoside and allowed to grow overnight.

To determine mutation frequencies, phage were eluted from the primary transformation plates, diluted, and then replated with JM105 on X-gal/isonicotinamide-β-D-thiogalactoside indicator plates to give roughly 300 plaques/plate. The plaques on the secondary plates were then lifted using nitrocellulose membranes and probed for base pair substitution mutations at position 6256 by differential hybridization with 13-mer probes. Membranes from 12 modified phage plates and 12 unmodified phage plates were split evenly into four dishes. Each dish contained one of the four probes. Because there was only one lift/plate and not four identical lifts with one membrane being placed into each dish, the summation of mutations detected along with G hybridizations sometimes did not add up to 100%. The specificity of the probes for a 1-base change at position 6256 has been shown previously (28, 30).

**In Vivo Gel Mobility Shift Assays**—A 31-mer strand oligonucleotides were phosphorylated with 10 μCi of [γ-32P]ATP using 10 units of T4 polynucleotide kinase and 5 μl of kinase dilution buffer. The reaction was incubated at 37 °C for 30 min and then heated to 70 °C for 10 min to inactivate the enzyme. The labeled oligonucleotide was purified by elution from a G50 microspin (Amersham Pharmacia Biotech) column and annealed to a 5-fold excess of its unlabeled complementary strand. All binding reactions were carried out in 20-μl incubations on ice. 10 μl of 0–400 nm purified MutS protein was added to 5 μl (0.8 pmol/μl) of labeled oligonucleotide and 5 μl of reaction buffer (0.1 M MOPS-KOH, pH 7.4, 0.15 M NaCl, 8.4 mM MgCl2, 3.4 mM EDTA, 10 μg/ml poly[dI-dC]), 15% glycerol). Reactions were incubated on ice for 15 min, before loading onto a 4% polyacrylamide, 4% glycerol gel with a 40 mM MOPS, 1 mM MgCl2 buffer run at constant voltage of 200 V for 2.5 h. Gels were then dried and analyzed using a 400E PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Surface Plasmon Resonance Measurements**—Surface plasmon resonance measurements were performed using a streptavidin chip (Amersham Pharmacia Biotech) and duplex DNA containing a 5'-biotin on the nonadducted strand. Homo- or heteroduplex 51-mers were prepared by digesting J53 at position 6256 by differential hybridization with 13-mer probes. Membranes from 12 modified phage plates and 12 unmodified phage plates were split evenly into four dishes. Each dish contained one of the four probes. Because there was only one lift/plate and not four identical lifts with one membrane being placed into each dish, the summation of mutations detected along with G hybridizations sometimes did not add up to 100%. The specificity of the probes for a 1-base change at position 6256 has been shown previously (28, 30).

**Briefly, double-stranded M13MB102 DNA was linearized with**

**Construction of Unadducted or Adducted M13MB102 Viral Genomes**—Construction of gapped duplex M13MB102 DNA and ligation of adducted or unadducted 8-mers were as described previously (28). Briefly, double-stranded M13MB102 DNA was linearized with KspI and BstHI and then dialyzed with a 10-fold excess of single-stranded M13MB102 DNA in decreasing concentrations of formamide. In some experiments, the single strand contained uracil in place of thymine to minimize replication of the nonadducted strand. The result-
Effect of mismatch repair deficiency on the induction of mutations by PdG or M1G

| Strain          | M1G → G | M1G → A | M1G → T | M1G → C | Mutations |
|-----------------|---------|---------|---------|---------|-----------|
| LM102 (wt)      | 96.9 ± 0.8 | 1.5 ± 0.4 | 1.5 ± 0.3 | 0.1 ± 0.1 | 3.1       |
| PdG:C           | 100     | <0.09   | <0.08   | <0.09   |           |
| dG:C            | 99.2 ± 0.4 | 0.35 ± 0.09 | 0.4 ± 0.2 | 0.12 ± 0.06 | 0.9       |
| M1G:C           |         |         |         |         |           |
| LM107 (mutS−)   | 99.2 ± 0.4 | 0.2 ± 0.1 | 0.5 ± 0.2 | 0.2 ± 0.1 | 0.9       |
| PdG:C           | 100     | <0.13   | <0.17   | <0.23   |           |
| dG:C            | 100     | <0.13   | <0.14   | <0.12   |           |
| M1G:C−         |         |         |         |         |           |

*Results are expressed as percentages ± S.D. Each value is the average of six independent DNA constructions, transformations, and hybridizations.

*Values are from a single transformation experiment.

FIG. 2. Competitive binding of PdG by MutS and UvrA,B. In the wild-type strain, both MutS and UvrA,B bind PdG, but only the UvrAB/C complex repairs the adduct. In the uvrA− strain, only MutS is present so the repair of PdG decreases and the mutation frequency increases. In the mutS− strain, there is no competition between binding of UvrA,B and binding of MutS. Repair of PdG by UvrAB/C increases, thus decreasing the mutation frequency.

RESULTS

Detection of M1G Mutagenicity in Wild-type and Mismatch Repair Deficient Backgrounds—Duplex M13MB102 genomes containing G, PdG, or M1G at position 6256 were constructed by ligation of 8-mer oligonucleotides into gapped duplexes as described previously. The resulting vectors were electroporated into SOS-induced E. coli strains that were wild type or deficient in mismatch repair, and progeny phage were probed for mutations. Single base pair substitutions were detected by differential hybridization of plaque DNA with radiolabeled 13-mer probes specific for each type of base pair substitution at position 6526. It was anticipated that the involvement of mismatch repair in the removal of M1G or PdG would result in an increased mutation frequency in cells that were deficient in mismatch repair. However, in the mutS− strain, uvrA2B is able to repair the adduct, and a decreased mutation frequency is observed. These observations and the hypothesis to explain them raise the question of whether the mismatch repair system is capable of removing PdG or M1G.

Mismatch Repair in Differentially Methylated DNA—To answer this question, we constructed hemimethylated plasmids containing PdG on the (+)-strand and methyl groups on either the (+)- or (−)-strand. Methylated single-stranded or double-stranded M13MB102 was isolated from JM105 (dam+), and unmethylated single- or double-stranded DNA was isolated from JM110 (dam−). Hemimethylated DNA was prepared by formamide dialysis, and gapped duplex DNA was prepared with methyl groups placed selectively on the (+)- or (−)-strand. Hemimethylated, adducted plasmids were transformed into E. coli, and the percentage of base pair substitutions at position 6526 was determined. The frequency of PdG → A transitions and PdG → T transversions were equivalent in all strains tested, so the total percentage of mutations is reported for simplicity. The percentage of base pair substitutions observed in JM105 (wt) was 1.5% when PdG was on the methylated strand and 1.0% and 1.1% when the strand opposite PdG was methylated or both strands were methylated (Table II). A similar percentage of mutations was observed in LM102. The lower
mutation frequencies recorded in these experiments relative to those summarized in Table I resulted from the absence of uracil residues in the (+)-strand in the experiments summarized in Table II. The presence of uracil residues in the (+)-strand lowers the replication of the (+)-strand and increases the detection of mutations induced by adducts on the (-)-strand.

To probe for PdG removal by mismatch repair, differentially methylated genomes was transformed into uvrA-deficient cells. This was necessary to eliminate competitive nucleotide excision repair. As expected from previous results, the percentage of mutations observed in the uvrA− background was 3–4-fold higher when methyl groups were present on the PdG-containing strand or on both strands. In contrast, the percentage of mutations was much lower when methyl groups were present on the strand opposite PdG. This is consistent with removal of PdG by the methyl-directed mismatch repair system.

When differentially methylated, PdG-containing genomes were transformed into mutS− cells, a decrease in mutations comparable with that reported in Table I was observed regardless of the methylation status of the genome. This suggests that removal of PdG by nucleotide excision repair is not sensitive to the presence of methyl groups on either the adducted or non-adducted strand.

**MutS Binding Detection by Surface Plasmon Resonance Assay**—The results of the in vivo experiments suggested that MutS binds to PdG and M1G, triggering their removal by mismatch repair if the adduct-containing strand is not methylated. Therefore, we sought to confirm the ability of MutS to bind M1G or PdG in duplex DNA using an in vitro assay for protein-DNA interaction. Duplex 31-mers containing PdG or M1G at position 15 and C or T opposite the adduct were synthesized and bound to a streptavidin chip by the addition of a biotin residue to the 5′ terminus of the unadducted oligonucleotide. A duplex oligonucleotide of identical sequence but containing a G:T mismatch at position 15 was used as a control.

**MutS Binding to a G:T Mismatch**—Two separate flow cells were derivatized individually with approximately 60 RU of either 5′-biotinylated G:T duplex or G:C duplex. The binding of solutions containing various concentrations of MutS were assessed as the MutS-containing solutions were passed across the derivatized chips (Fig. 3). After the binding of a 400 nM MutS solution had reached equilibrium, the binding to the G:T duplex produced an absolute change of 616 RU (100%) compared with a change of 61 RU (9.9%) in the G:C duplex. The latter indicated a weak nonspecific interaction between MutS and the G:C duplex. This nonspecific binding was subtracted out of all binding sensograms produced from G:T- or adduct-containing duplexes.

Binding to the G:T mismatch was assessed at various concentrations of MutS to produce binding isotherms that were subsequently analyzed for kinetic and thermodynamic parameters. At MutS concentrations of 50–400 nM, isotherms were globally fitted to a simple 1:1 Langmuir binding model. The BIAEVAL 3.0 program simultaneously determines kinetic association and dissociation constants from the rate of change of response with respect to time (slope of association and dissociation curves). The G:T mismatch had an apparent thermodynamic dissociation constant (K_D) of 18 nM. Kinetic measurements indicated a k_a of 2.44 × 10^3 s^{-1} and k_d of 3.36 × 10^2 s^{-1} with a chi square value of 1.10, falling well within accepted statistical values for Biaeval 3.0 analysis. These thermodynamic constants compare favorably with a K_D of 25 ± 5 nM determined by DNA footprinting and gel shift analysis (32).

**MutS Binding to PdG and M1G Adducts**—The relative binding of MutS to PdG and M1G adducts was assessed in a manner similar to the G:T mismatch. Each adduct was probed at several different concentrations of MutS, and nonspecific binding was subtracted out, and the sensograms were subjected to global kinetic analysis. As illustrated in Fig. 4, when all adduct-containing duplexes are probed with the same concentration of 200 nM MutS, the equilibrium level of binding of MutS to the M1G-T duplex is very close to that of a G:T mismatch (240 RU versus 219 RU). Binding to an M1G-G duplex gave a lower absolute response of 138 RU, and binding to both PdG-C and PdG:T duplexes was even less significant. PdG:C showed an

| Strain        | PdG Me | PdG | PdG Me |
|---------------|--------|-----|--------|
| LM102 (wt)    | 1.2 ± 0.4 | ND  | ND     |
| LM105 (uvrA−) | 5.0 ± 2.0 | 1.2 ± 0.2 | 4.3 ± 0.5 |
| LM107 (mutS−) | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| JM105 (wt)    | 1.5 ± 0.2 | 1.0 ± 0.2 | 1.1 ± 0.1 |

**FIG. 3. Specificity of binding of MutS to G:T mismatch.** Homoduplexes containing a G:T mismatch were created by annealing a 5-fold excess of the nonbiotinylated strand to the 5′-biotinylated strand. DNA (60 RU) was immobilized on a streptavidin surface in separate flow cells of a Biacore chip. A, a saturating amount of MutS protein (50 μl, 400 nM) was washed over each flow cell at a flow rate of 20 μl/min before the protein was washed off with 20 μl of 0.5% SDS (not shown). B, the bulk refractive index contribution from buffer solution was subtracted from sensograms to display the low level of nonspecific binding to the G:C homoduplex.

TABLE II

| Strain | PdG Me | PdG | PdG Me |
|--------|--------|-----|--------|
| LM102 (wt)    | 1.2 ± 0.4 | ND  | ND     |
| LM105 (uvrA−) | 5.0 ± 2.0 | 1.2 ± 0.2 | 4.3 ± 0.5 |
| LM107 (mutS−) | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| JM105 (wt)    | 1.5 ± 0.2 | 1.0 ± 0.2 | 1.1 ± 0.1 |

**Effect of methylation status on mutation frequency**

Base pair substitution mutation frequencies (%) induced by PdG on hemimethylated DNA transformed into repair-deficient strains. The (+)-strand in these constructions did not contain uracil. ND, not determined.
equilibrium response of 85 RU, and PdG:T showed only 41 RU. Although the thermodynamic interactions between MutS and M1G:T or G:T duplexes are similar, Fig. 4 illustrates that there are differences in the rates of association and dissociation. Whereas MutS binds to PdG and M1G duplexes with a $k_a$ comparable with that of a G:T duplex, dissociation from PdG or M1G duplexes occurs significantly faster than dissociation from the G:T duplex. This observation is confirmed by the kinetic and thermodynamic constants that were extrapolated from sensogram data (Table III). The dissociation of MutS from M1G and PdG duplexes occurs approximately 5-fold faster than dissociation from the G:T duplex. Because the association rates are comparable, the lower $K_d$ for the MutS-G:T duplex reflects the lower rate of dissociation.

**MutS Binding to M1G:T in the Presence of ATP or ADP—**

Adenine nucleotide-binding sites have been found to be highly conserved in both eukaryotic and prokaryotic MutS homologs (33). Several of the known MutS homologs fail to form complexes with mismatches in the presence of ATP by gel shift analysis. Therefore, we attempted to use adenine nucleotide modulation as a probe for MutS-adduct binding. The oligonucleotide derivatized chips were probed with different concentrations of MutS as above, but the buffer contained a 2-fold excess of either ADP, ATP, or a nonhydrolyzable analog of ATP (AMP-PNP or ATP-$\gamma$-S) with respect to MutS. As demonstrated in Fig. 5, the extent of binding of MutS to the M1G:T duplex is significantly reduced in the presence of ATP. In contrast, addition of ADP appeared to stabilize the interaction between MutS and the M1G:T duplex, denoted on the sensogram by a slower rate of dissociation of the MutS from the oligonucleotide surface.

**MutS Binding to M1G and PdG Adducts by Gel Shift Analysis—**

We attempted to detect MutS binding to M1G and PdG duplexes by gel shift analysis. Radiolabeled 31-mer oligonucleotides containing mismatches or adducts were incubated with increasing amounts of purified MutS protein. MutS binding to the mismatch or adduct should retard the migration of the duplex through the gel. Fig. 6 depicts an average band shift experiment. Although MutS was incubated with duplexes in the presence of excess poly(dI-dC), a small amount of non-specific binding was detected and was used as background for comparison with mismatch and adduct binding. Incubation of a G:T duplex with increasing amounts of MutS protein resulted in a strong gel shift, consistent with previous reports. The presence of MutS was required for detection of this shift. MutS incubation with a duplex containing M1G:T produced a shift above background levels. Significant binding was detected at concentrations as low as 200 nM. Incubation of MutS with M1G:C showed only a small amount of binding that was not far above background levels, and incubation with a PdG:C or PdG:T containing duplex did not yield any band shift. The results of these gel shift experiments are consistent with the results of the surface plasmon resonance experiments.

**DISCUSSION**

The present study provides *in vivo* and *in vitro* evidence that M1G and PdG are recognized by MutS and that they can be...
MutS Binding to Exocyclic DNA Adducts

repaired by methyl-directed mismatch repair when the genome is not methylated on the adduct-containing strand. The interaction with MutS appears to be competitive with binding and repair of the adducts by nucleotide excision repair. Thus, when M1G and PdG are introduced on vectors methylated on both strands, they are not repaired by mismatch repair but are protected against nucleotide excision repair. Conversely, when mutS is deleted, the adducts are efficiently repaired by nucleotide excision repair resulting in a significant reduction in mutations relative to wild type. To probe for the binding of MutS to PdG and M1G in vitro, we employed a surface plasmon resonance assay and a gel mobility shift assay. The surface plasmon resonance assay provided kinetic and thermodynamic data for the protein-nucleic acid interaction. The dissociation constant for MutS binding to the G:T duplex was very similar to the value determined previously by gel shift analysis (32). Binding isotherms illustrated that the affinity for M1G:T was similar to that for G:T, but its affinity for M1G:C, PdG:C, and PdG:T was lower. Furthermore, the dissociation rates for MutS release from each of these adducts were approximately 10-fold faster than the dissociation rate from G:T.

MutS binding to G:T and M1G:T duplexes also was detected by gel mobility shift analysis, but binding to M1G:C, PdG:T or PdG:C was not. We attribute this to the lower affinity of MutS for these duplexes and their higher dissociation rates as measured by surface plasmon resonance. These experiments illustrate the utility of surface plasmon resonance for detection of rapidly reversible protein-nucleic acid interactions. Although the kinetics of dissociation of MutS from M1G- and PdG-containing duplexes are rapid, the extent of binding is sufficient to protect M1G- or PdG-adducted genomes from nucleotide excision repair or to initiate methyl-directed mismatch repair as judged by the in vivo data. MutS binding to these genomes in vivo may be stabilized by the larger size of the genome relative to the 31-mer oligonucleotides used for the in vitro experiments or by binding to additional protein factors.

Although mismatch repair exists to remove errors made during DNA replication, the system does recognize and attempt to repair other DNA adducts. For example, MutS binds to O6-methylguanine and attempts to repair the strand opposite it (23). This leads to a futile cycle of removal and resynthesis that eventually causes cell death. A similar effect is observed with DNA containing G-G intrastrand cross-links induced by cis-platinum (35, 36). The attempted repair of these lesions or protection from nucleotide excision repair provided by MutS binding is important to the therapeutic activity of methylating agents and cis-platinum. Tumor cells that have lost mismatch repair capability are more resistant to the cytotoxic action of both alkylating agents and cis-platinum (37, 38). In fact, high levels of expression of the mismatch repair system appear to be an important determinant of the efficacy of cis-platinum against testicular tumors (39).

The affinity of MutS for M1G and PdG duplexes is comparable with that of MutS for the cis-platinum intrasstrand G-G cross-link (23-107 nM versus 67 nM, respectively) (35). The latter value was measured by gel mobility shift analysis, which suggests that the dissociation rate of MutS from the cis-platinum adduct is lower than that from M1G or PdG. However, the affinity is strongly dependent on the base opposite the modified guanines. For example, no binding of human MutS to a cis-platinum adduct is observed when the base opposite each of the modified Gs is C or when the base opposite the 5’ G is T (40). However, binding is optimal when the base opposite the 3’ G is T. In our hands, MutS binds twice as strongly to M1G:T relative to M1G:C. This is further support for the observation that the base opposite the damaged DNA base is an important determinant of MutS binding.

An added feature of the binding of MutS to M1G is the chemical structure of the M1G as a function of the base opposite M1G. For example, no binding of human MutS to a cis-platinum adduct is observed when the base opposite each of the modified Gs is C or when the base opposite the 5’ G is T (40). However, binding is optimal when the base opposite the 3’ G is T. In our hands, MutS binds twice as strongly to M1G:T relative to M1G:C. This is further support for the observation that the base opposite the damaged DNA base is an important determinant of MutS binding.

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1) (41). Ring opening is reversed when the duplex is heat-denatured. No ring opening is detected when M1G is placed in duplexes opposite T. Thus, the reduced affinity of MutS for M1G:T duplexes may be a result of the presence of the ring-opened form of M1G in this duplex.

This and previous studies establish that some overlap exists between the repair of small DNA adducts by global repair systems such as nucleotide excision repair and mismatch repair. Furthermore, binding of MutS to small lesions, even in the absence of subsequent repair, can protect these lesions from removal by nucleotide excision repair. M1G is among the most abundant exocyclic adducts found in the DNA of healthy humans (17). Its formation is linked to polyunsaturated fatty acid metabolism through lipid peroxidation or prostaglandin biosynthesis. Indeed, women consuming diets rich in polyunsaturated fatty acids exhibit a 10–20-fold increase in the level of M1G:C duplexes opposite T. Thus, the reduced affinity of MutS for denatured. No ring opening is detected when M1G is placed in heat-denatured. No ring opening is detected when M1G is placed in duplexes opposite T. Thus, the reduced affinity of MutS for M1G:T duplexes may be a result of the presence of the ring-opened form of M1G in this duplex.

Acknowledgment—We are grateful to R. Mernaugh for assistance with surface plasmon resonance measurements and for helpful discussions.

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