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MutS Preferentially Recognizes Cisplatin- over Oxaliplatin-modified DNA*

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Zoran Z. Zdraveski‡§, Jill A. Mello‡§, Christine K. Farinelli‡§, John M. Essigmann‡§¶‖, and Martin G. Marinus¶‡‡**

*From the ‡Department of Chemistry and §Division of Bioengineering and Environmental Health, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 and the **Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605*

Loss of mismatch repair leads to tumor resistance by desensitizing cells to specific DNA-damaging agents, including the anticancer drug cisplatin. Cisplatin analogs with a diamminocyclohexane (DACH) carrier ligand, such as oxaliplatin and Pt(DACH)Cl_2, do not elicit resistance in mismatch repair-deficient cells and therefore present promising therapeutic agents. This study compared the interactions of the purified *Escherichia coli* mismatch repair protein MutS with DNA modified to contain cisplatin and DACH adducts. MutS recognized the cisplatin-modified DNA with 2-fold higher affinity in comparison to the DACH-modified DNA. ADP stimulated the binding of MutS to cisplatin-modified DNA, whereas it had no effect on the MutS interaction with DNA modified by DACH or EN adducts. In parallel cytotoxicity experiments, methylation-deficient *E. coli dam* mutants were 2-fold more sensitive to cisplatin than DACH compounds. A panel of recombination-deficient mutants showed striking sensitivity to both compounds, indicating that both types of adducts are strong replication blocks. The differential affinity of MutS for DNA modified with the different platinum analogs could provide the molecular basis for the distinctive cellular responses to cisplatin and oxaliplatin.

Cisplatin (*cis*-diamminedichloroplatinum(II), Fig. 1) is a DNA-damaging drug that has shown spectacular success in the treatment of testicular, ovarian, and other tumors (1). The detailed biochemical mechanism underlying the clinical effectiveness of cisplatin is incompletely understood, but most likely it results from the formation of DNA adducts that block replication and elicit a variety of cellular responses including nucleotide excision repair (2, 3), recombinational repair (4), and the triggering of apoptosis (5). Cisplatin forms predominantly (>90%) 1,2-d(GpG), 1,2-d(ApG), and 1,3-d(GpNpG, where N is any nucleotide) intrastrand adducts, and a small number of monofunctional adducts and interstrand cross-links (6, 7). The 1,2-intrastrand cisplatin-DNA adducts induce significant distortions of the double helix and provide a structural signal for specific recognition by a variety of cellular proteins, including those involved in mismatch repair (2, 8–10).

Mismatch repair maintains genomic integrity by correcting polymerase replication errors and by ensuring the fidelity and frequency of recombination events (11–13). In eukaryotes, mismatch repair also plays a role in apoptotic signaling and cell cycle regulation (5, 14). It has been established that mismatch repair proteins mediate the cellular responses to cisplatin damage, but paradoxically they seem to sensitize rather than protect the cell. In both *Escherichia coli* and eukaryotes, loss of mismatch repair confers cellular resistance to cisplatin cytotoxicity (2, 15–18). Cisplatin resistance by tumors (mismatch repair-deficient) presents a serious clinical problem (19), and it has stimulated a great deal of interest in the design of novel platinum compounds that would overcome this drawback. One of the earliest leads involved complexes with diamminocyclohexane (DACH)[1] carrier ligand (20), such as oxaliplatin ((*trans-R,R*)-(DACH)oxalatoplatinum(II), Fig. 1) and Pt(DACH)Cl_2 ((1,2-DACH)dichloroplatinum(II), Fig. 1). Loss of mismatch repair does not seem to confer resistance to oxaliplatin (16), and, in recent years, oxaliplatin has shown great potential for clinical use (20, 21). Oxaliplatin and Pt(DACH)Cl_2 form a similar DNA adduct profile to cisplatin (22, 23), and modeling studies have suggested that the adducts of both cisplatin and DACH could induce similar distortions of secondary DNA structure (24). Adducts of the DACH compounds differ from cisplatin by their bulky, nonpolar ligand that probably protrudes in the major groove. The presence of the nonpolar cyclohexane ligand in the mostly polar major groove would certainly present a distinct recognition environment for the mismatch repair proteins or any other cellular proteins that interact with platinum adducts. For example, high mobility group 1 (HMG-1) box proteins, which recognize cisplatin-DNA adducts with great affinity (3), poorly recognize DACH-DNA adducts (25).

We set out to examine if the differential mismatch repair-mediated cellular responses to the cisplatin and DACH compounds result from differential recognition of their platinum-DNA adducts by mismatch repair proteins. The eukaryotic mismatch repair proteins hMSH2 (26) and MutSα (27) bind to oligonucleotides modified to contain the major cisplatin-DNA adduct, a 1,2-d(GpG) intrastrand cross-link. To date, however, there have been no studies of the interaction of their bacterial homologue, MutS, with DNA modified with cisplatin or DACH compounds. To address this gap in knowledge, we examined

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¶ Each laboratory contributed equally to this work.
‖ To whom correspondence may be addressed. Tel.: 617-253-6224; Fax: 617-253-5445; E-mail: jessig@mit.edu.
‡‡ To whom correspondence may be addressed. Tel.: 508-856-3330; Fax: 508-856-3036; E-mail: martin.marinus@umassmed.edu.

[1] The abbreviations used are: DACH, diamminocyclohexane; HMG-1, high mobility group 1; *trans*-DDP, *trans*-diamminedichloroplatinum-(II); DIEN, diethylenetriamine; EN, ethylenediammine.

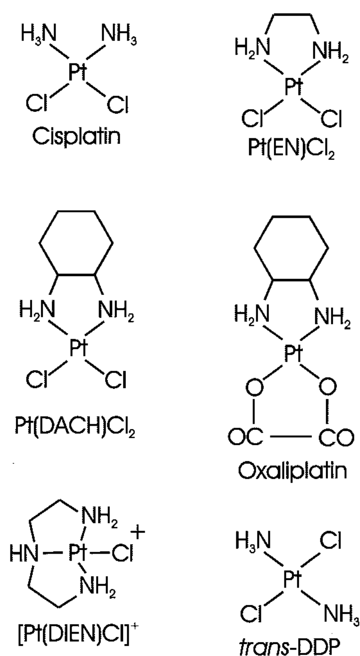

FIG. 1. **The structures of cisplatin and cisplatin analogs used in this study.** Cisplatin, $Pt(EN)Cl_2$, oxaliplatin, and $Pt(DACH)Cl_2$ are therapeutically active platinum complexes and they all have chloride ligands in *cis* geometry. Oxaliplatin and $Pt(DACH)Cl_2$ have different leaving groups, but they form identical DNA adducts. The *trans* isomer of cisplatin, *trans*-DDP and $Pt(DIEN)Cl^+$ are clinically ineffective cisplatin analogs.

the interactions of MutS with oligonucleotides differentially modified with platinum compounds. In addition, we assembled a panel of *E. coli* mutants deficient in the major mismatch repair and recombination pathways and we compared their sensitivity to treatment with the cisplatin and DACH compounds.

MATERIALS AND METHODS

Preparation of Platinum-modified DNA Probes—Platinum compounds were purchased from Sigma-Aldrich, except for $Pt(EN)Cl_2$ (*cis*-ethylenediammine dichloroplatinum(II)) and $[Pt(DIEN)Cl]^+$ (diethylenetriamine platinum(II)chloride), which were synthesized as previously described (28, 29). Oxaliplatin was a generous gift from Dr. S. B. Howell (University of California, San Diego, CA). Platinum-modified DNA probes were prepared as described previously (26). In brief, restriction enzyme digests of pSTR3 with *Cla*I and *Eco*RV yielded 162- and 4205-bp restriction fragments. DNA probes of 162 bp were purified from the 4205-bp restriction fragment on native 5% polyacrylamide gels. Platination reactions of the restriction fragment were carried out in 3 mM NaCl, 1 mM Na_2HPO_4 (pH 7.4) with 100 μg/ml DNA and appropriate platinum compound:DNA molar ratios by incubating at 37 °C for 16 h. Unreacted platinum compounds were removed by dialysis (24 h) against 10 mM Tris-HCl buffer (pH 8.0), 1 mM EDTA (TE). Levels of platinum modification were determined by flameless atomic absorption spectroscopy on a Varian AA1475 instrument equipped with a GTA95 graphite furnace. DNA probes of 162 bp were radiolabeled with [γ-^{32}P]dATP (6000 Ci $mmol^{-1}$, PerkinElmer Life Sciences) and resuspended in TE to 5000–10000 cpm/μl. It should be noted that for oligonucleotide modifications we used (*trans-R,R*)-$Pt(DACH)Cl_2$ whose chloride leaving groups differ from the oxalato group of oxaliplatin. However, following biotransformation both compounds should form identical adducts with DNA. ^{1}H NMR spectroscopic analysis of the $Pt(DACH)Cl_2$ compound was used to demonstrate that the compound was in the *trans-R,R* conformation.

Protein Purification—MutS was purified as previously described (30). The host strain was BL21 (λDE3) (pLysS), and the plasmid used was pMQ372 (31). In brief, the strain was transformed with pMQ372 and grown at 37 °C to an A_{600} of 0.8, shifted to room temperature, and isopropyl-1-thio-β-D-galactopyranoside was added to 50 μM final concentration. Incubation was continued for 2 h at room temperature, and the cells were harvested and lysed in a French pressure cell (Aminco). The lysate was treated with streptomycin sulfate and ammonium sulfate as described (30). We used a heparin-agarose (Sigma) column instead of heparin-Sepharose. Two fractions (IVa and IVb) from the hydroxylapatite chromatography were saved and stored at −70 °C. The IVb fraction was used in the binding assays. Protein concentration was assayed using the Bradford reagent (Bio-Rad).

Binding Assays—Binding assays contained radiolabeled 162-bp DNA probes (present at 100–200 pM, 5000–10,000 cpm) either unmodified or modified with platinum compounds, and purified MutS present at 0–300 nM concentration. Binding reactions were carried out in a 15-μl volume containing 20 mM Tris base, 5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1 mM DTT, 0.01 mM EDTA, and 50 ng of nonspecific competitor chicken erythrocyte DNA. The binding reactions were incubated for 30 min on ice. Samples were then loaded onto 4% (29:1 acrylamide:bis) native gels containing TAE buffer (90 mM Tris base (pH 8.0), 2.0 mM EDTA, 90 mM boric acid) and 5% sucrose, and separated by electrophoresis at room temperature in TAE buffer at ~25 mA (140 V) for 2 h. Amounts of bound and unbound radiolabeled probe were determined by quantitative analysis of gels using a Molecular Dynamics Storm system and ImageQuant software. The $K_{d(app)}$ was determined by a nonlinear least squares fitting of the binding data to the standard Hill equation. In the reactions that contained nucleotides, ADP or ATP (Roche) was added to a 100 μM reaction concentration. Titration of increasing amounts of ADP (up to 300 μM) did not further increase the percentage of MutS bound to the modified probes.

Bacterial Strains—The strains used in this study are listed in Table I. The strains are derivatives of GM112, used in the toxicity experiments with the mismatch repair- and methylation-deficient mutants, or AB1157, used in the experiments with the recombination-deficient mutants. The auxotrophic phenotype of each mutant was conformed by growth on the appropriate supplemented minimal medium.

Cytotoxicity Analysis—Overnight cultures were diluted 1000-fold and grown in Luria-Bertani medium until the density of the population reached 2×10^8 cells/ml as determined by A_{600}. The exponentially growing cells were resuspended in M9 minimal medium (32) and treated with drug dissolved in H_2O for 2 h at 37 °C. Appropriate dilutions in M9 medium were plated on Luria-Bertani plates and incubated at 37 °C until colonies could be counted. Results from three to six independent experiments plated in duplicate were averaged and plotted against drug concentration, ± S.E. (standard error of the mean). IC_{37} (inhibitory concentration of 37%) was determined as the drug concentration where there was 37% of survival in comparison to the untreated control.

RESULTS

MutS Preferentially Binds to DNA Globally Modified with Cisplatin—To examine if the bacterial MutS binds to DNA modified to contain cisplatin adducts, purified *E. coli* MutS was used in an electrophoretic mobility shift assay with DNA globally modified by cisplatin. Three types of globally modified cisplatin duplexes were constructed that differed in the level of modification. (i) Cisplatin-3 had, on the average, 3 cisplatin adducts/oligonucleotide molecule (drug-to-nucleotide ratio (r_b) = 0.0009); (ii) cisplatin-7 had 7 adducts/oligonucleotide (r_b = 0.0021); and (iii) cisplatin-11 had 11 adducts/oligonucleotide (r_b = 0.0033). Binding of MutS to these radiolabeled 162-bp probes was readily observed by the retarded band migration that represented the bound probe (Fig. 2). The fraction of bound probe increased proportionately as the cisplatin modification level increased, 4.9% for cisplatin-3, 12% for cisplatin-7, and 30% for cisplatin-11. The increased fraction of shifted material was probably caused by an increasing population of modified DNA, reinforcing the specific nature of the interaction.

In the same assay, we examined the ability of MutS to recognize DNA modified with DACH adducts. The probe DACH-9 had on the average nine DACH adducts per DNA molecule (r_b = 0.0027). MutS showed affinity for the DNA modified with DACH adducts, but the fraction of the shifted material was lower in comparison to the cisplatin-modified probes, showing only 2.9% bound probe for DACH-9 (Fig. 2). Under identical conditions the MutS protein did not cause a shift of the corresponding control unmodified homoduplex. To assess if the recognition of the globally modified cisplatin duplex were a consequence of nonspecific MutS interactions, we

TABLE I
Genotypes of E. coli K-12 strains used in this study

All strains are F^-. Abbreviations used: Am, *amber* mutation; AS, *amber* suppressor; Δ, deletion; Oc, ochre mutation; Str, streptomycin; Kan, kanamycin; Tn*5* and Tn*10* encode kanamycin and tetracycline resistance, respectively.

Strain	Genotype	Source
AB1157	*thr-1 ara-14 leuB6 − (gpt-proA)*62 *lacY1 tsx-33 glnV44*(AS) *galK2*(Oc) *hisG4*(Oc) *rfbD1 mgl-51 rpoS396*(Am) *rpsL31*(Str^R) *kdgK51 xylA5 mtl-1 argE3*(Oc) *thi-1*	E. A. Adelberg
GM112	F^- thr-1 ara-14 leuB6 DE(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 metB1 rfbD1 mgl-51 rpsL31 kdgK51 mtl-1 thi-1 thyA12 deoB16	Laboratory stock
GM113	As GM112 but dam-3	Laboratory stock
GM150	As GM112 but mutL451 dam-3	Laboratory stock
GM169	As AB1157 but mutS453 dam-3	Laboratory stock
AM547	As AB1157 but Δ*ruvABC65*	R. G. Lloyd
JC9239	As AB1157 but *recF143*	A. J. Clark
KM21	As AB1157 but Δ*recBCD*::Kan	K. M. Murphy

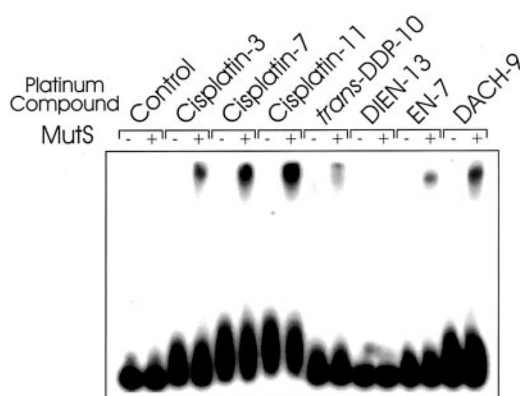

FIG. 2. **Selectivity of MutS for DNA modified with therapeutically active platinum compounds.** A radiolabeled 162-bp probe was modified to contain 3, 7, and 11 cisplatin adducts; 10 *trans*-DDP adducts; 13 DIEN adducts; 7 EN adducts; and 9 DACH adducts. Unmodified 162-bp probe was used as a control. DNA probes were incubated in the absence (−) or presence (+) of MutS (40 nM). Discrete, shifted bands were observed only when MutS was incubated with DNA modified by the therapeutically active complexes cisplatin, $Pt(EN)Cl_2$, and $Pt(DACH)Cl_2$. The binding of MutS to the cisplatin-modified probes increased as the degree of cisplatin modification increased.

examined the interaction of MutS with the identical DNA modified by the panel of platinum compounds shown in Fig. 1. It is of interest to note that the electrophoretic mobility of the modified probes in the absence of MutS reflects the differential structural distortions the respective adducts induce to the double helix (3, 33). As mentioned, cisplatin induces strong directional bend and distortion of the double helix, and higher levels of modification with cisplatin result in significantly altered electrophoretic mobility of the oligonucleotide. Other platinum compounds do not induce strong bending and unwinding of the double helix and, as a result, even high levels of modification do not alter the mobility of the oligonucleotide; *trans*-DDP (*trans*-diamminedichloroplatinum(II)) adducts induce a hinge-like bend in the DNA, whereas $[Pt(DIEN)Cl]^+$ produces only minimally disruptive monofunctional adducts. MutS showed affinity for the DNA modified with adducts of the cisplatin analog $Pt(EN)Cl_2$, an analog with an ethylenediammine (EN) ligand. The $Pt(EN)Cl_2$-modified 162-bp probe had, on the average, seven EN adducts ($r_b = 0.0021$), and the fraction of the MutS bound probe was 3.4%. This result is in line with previously published data that have shown that $Pt(EN)Cl_2$-modified DNA is recognized by the MutS homologue hMSH2 (26). In contrast, MutS had low affinity for DNA that contained adducts of the clinically inactive platinum complexes *trans*-DDP and the monofunctional $[Pt(DIEN)Cl]^+$ (1.1 and 0.4% bound probe, respectively) even though, on the average, the *trans*-DDP-modified oligonucleotide contained 10 ($r_b = 0.0030$) and the DIEN-modified oligonucleotide contained 13 platinum adducts ($r_b = 0.0039$). The specificity of the interactions of MutS with the cisplatin-, EN-, and DACH-modified oligonucleotides was confirmed by competition band-shift experiments (described in detail in Ref. 26, and data not shown).

Specificity of MutS Binding to Cisplatin- and DACH-DNA Adducts—To characterize the nature of the interaction between MutS and cisplatin- or DACH-modified DNA further, MutS protein was titrated into binding reactions containing a constant concentration of duplex DNA modified by adducts of the two drugs (Fig. 3*A*). The 162-bp oligonucleotide used in this experiment contained, on the average, seven cisplatin adducts (cisplatin-7) or nine DACH adducts (DACH-9) per duplex DNA molecule (one cisplatin adduct per 23 bp, and one DACH adduct per 18 bp). The addition of increasing amounts of protein caused the complex to be proportionally shifted through the gel, presumably because multiple protein complexes bound to the multiplatinated probes. The binding isotherm (Fig. 3*B*) reveals that the fraction of bound platinated DNA increases to saturation over a narrow range of MutS concentrations, consistent with positive cooperative binding (Hill coefficient, $n_H = 2.9$ for cisplatin-7; $n_H = 2.7$ for DACH-9). The observed apparent cooperative binding behavior may be a consequence of multiple platinum sites situated in close proximity in the duplex DNA. MutS produces a 20-bp DNase I footprint at a mismatch site (30), and the crystal structure reveals protein DNA contacts that extend to 13 nucleotides proximal to the mismatch (34, 35). The binding of a MutS dimer to an adduct may render the subsequent binding of a second MutS dimer to a nearby platinum adduct more favorable, or it may facilitate the formation of higher ordered complexes (tetramers and higher oligomers) that have been observed in experiments with high MutS (or MutSα) concentrations (data not shown and Ref. 36). Generation of the binding isotherm yielded a $K_{d(\text{app})} = 57$ nM for the cisplatin-modified probe and $K_{d(\text{app})} = 120$ nM for the DACH-modified probe. Neither the active fraction of our MutS preparation nor the aggregation state of the protein were established; thus, our estimation of the dissociation constant assumes that MutS binds as a dimer and that 100% of the protein is active in binding. These considerations, taken together with the observed complex nature of the MutS-DNA interactions, dictate that the dissociation constant should be considered an approximation of the affinity of MutS for the platinum-modified DNA. However, the value obtained for the interaction of MutS cisplatin-modified DNA is in accordance with the previously reported value for the interaction of hMSH2 with a cisplatin-modified probe of similar size and level of modification (26). No previous reports of MutS binding to DNA modified with DACH adducts exist for comparisons.

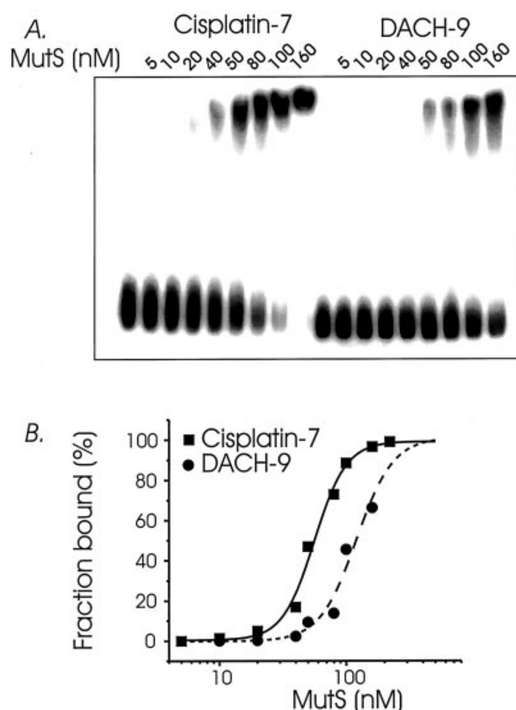

FIG. 3. **Binding isotherm describing the interaction between MutS and cisplatin- and DACH-modified DNA.** *A*, MutS protein was titrated into binding reactions containing radiolabeled 162-bp probe that contained an average of seven cisplatin adducts or nine DACH adducts. *B*, the fraction of bound probe in each lane was quantitated by Storm PhosphorImager analysis and is presented as a function of the concentration of MutS present in the binding reactions. Fitting these binding data to the Hill equation generated the binding curve.

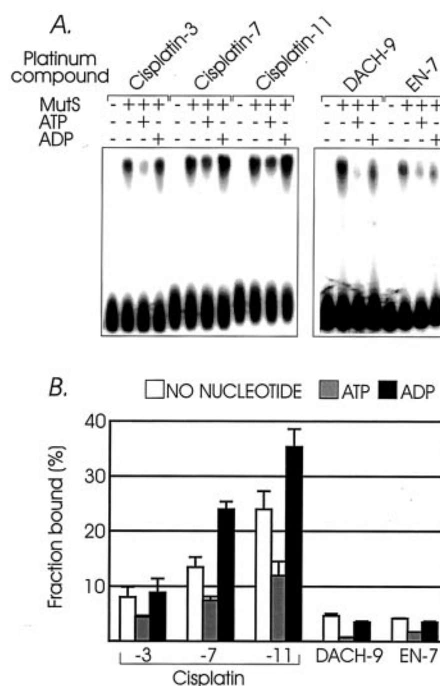

FIG. 4. **Effects of nucleotides on binding of MutS to platinated DNA.** Radiolabeled 162-bp probe modified to contain 3, 7, and 11 cisplatin adducts; 9 DACH adducts; and 7 EN adducts was incubated with MutS (40 nM). ATP or ADP was added to the binding reaction to a final concentration of 100 μM. *A*, retarded bands were observed similar to the ones in Fig. 1. *B*, specific binding diminished with the addition of ATP, whereas it increased with the addition of ADP to significant levels only when MutS was incubated with cisplatin-modified probes. Mean ± standard deviation (n = 3).

Nucleotide Effects on MutS Binding to DNA Modified to Contain Platinum Adducts—Nucleotide (ATP or ADP) binding to MutS mediates the conformation of the protein dimer and its binding affinity for DNA and mismatches. Addition of ATP to mismatch-bound MutS can cause the protein to dissociate or translocate from the mismatch, whereas addition of ADP stimulates MutS binding (36–38). To investigate the effects of nucleotides on the interaction of MutS with platinum-modified DNA, ATP or ADP was added to a binding reaction containing MutS and the previously described platinum-modified probes (Fig. 4*A*). In the binding reactions that contained DNA probes modified with cisplatin adducts, the addition of ADP increased the proportion of the shifted probe, whereas the addition of ATP caused a decrease in the portion of the shifted probe (Fig. 4*B*). For the cisplatin-7 probe, the addition of ADP increased the amount of the shifted probe 1.8-fold, from 13 ± 2.1% to 23.9 ± 1.5%, whereas addition of ATP decreased the amount of shifted probe by a factor of 2, from 13.5 ± 2.1% to 7.4 ± 1.3%. Similar nucleotide effects were observed with the cisplatin-3 and the cisplatin-11 probe (Fig. 4*B*). In contrast to the results with cisplatin-modified probes, addition of ADP to the binding reactions that contained DNA modified with DACH or EN adducts did not increase the percentage of binding observed; actually, it slightly decreased it from 4.6 ± 0.65% to 3.4 ± 0.54% and from 4.2 ± 0.30% to 3.5 ± 0.95%, respectively. The addition of ATP to the binding reaction containing the DACH- and EN-modified probes also resulted in a decrease of the fraction of the bound probe.

Sensitivity of Methylation- and Mismatch Repair-deficient Mutants to Cisplatin Analogs—Methylation-deficient (*dam*) mutants in *E. coli* show high sensitivity to cisplatin, and this sensitivity is abrogated by additional mutations in either of the mismatch repair genes MutS or MutL (repeated in Fig. 5; Ref. 39). The biochemical basis for this observation is not known, but it has been proposed that it involves mismatch repair-initiated cycles of futile repair of cisplatin adducts (because of the absence of a strand discrimination signal in the *dam* mutants). We examined the survival of *dam*, *dam mutS*, and *dam mutL* mutants following treatment with increasing concentrations of $Pt(DACH)Cl_2$ (Fig. 5). The wild type showed higher sensitivity to equimolar $Pt(DACH)Cl_2$ than cisplatin, which was expected because higher toxicity for DACH compounds has been reported previously in other systems (20). The methylation-deficient *dam* mutants demonstrated high sensitivity to both drugs in comparison to the wild type. When compared, the wild type/*dam* IC_{37} ratios for both compounds revealed that the *dam* mutants were ~2-fold more resistant to $Pt(DACH)Cl_2$ than cisplatin. The IC_{37} ratio was 1.4 for $Pt(DACH)Cl_2$ ($IC_{37(\text{wild type})}$ = 21 μM, $IC_{37(dam)}$ = 15 μM) and 2.7 for cisplatin ($IC_{37(\text{wild type})}$ = 73 μM, $IC_{37(dam)}$ = 27 μM). This difference could reflect the degree of sensitivity added by the *dam* mutation, presumably because of the previously discussed abortive repair model. Introduction of an additional mutation in the mismatch repair gene MutS or MutL (*dam mutS*, *dam mutL*) abrogated the *dam* sensitivity to $Pt(DACH)Cl_2$ and cisplatin to similar levels. Similar results were observed in experiments where oxaliplatin was used in place of $Pt(DACH)Cl_2$ (data not shown).

Recombination-deficient Mutants Are Equally Sensitive to Cisplatin and DACH Compounds—Cisplatin-DNA adducts present strong blocks to replication *in vitro* and *in vivo* (2, 40), and these frequent replication blocks require various recombination pathways for their repair or tolerance (4). DACH adducts also present replication blocks, and it has been shown *in vitro* and *in vivo* that the DACH-DNA adducts are bypassed more efficiently than cisplatin adducts by various polymerases

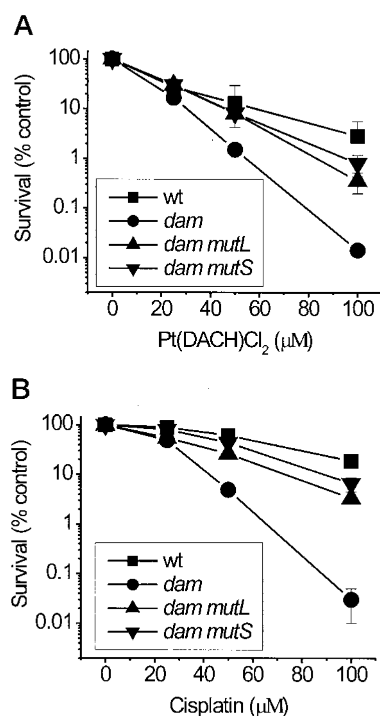

FIG. 5. **Survival of mismatch repair-deficient *E. coli* strains treated with Pt(DACH)Cl$_2$ and cisplatin.** For each data point, results shown are the mean of at least three independent experiments plated in duplicate, ± S.E.

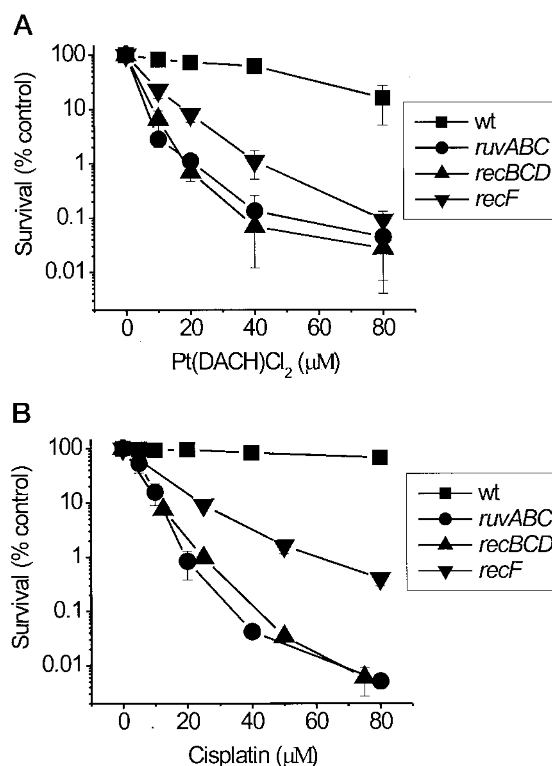

FIG. 6. **Survival of recombination-deficient *E. coli* strains treated with Pt(DACH)Cl$_2$ and cisplatin.** For each data point, results shown are the mean of at least three independent experiments plated in duplicate, ± S.E.

(41–43). We examined whether there would be a difference in *E. coli* in the capacity of the two drugs to induce replication-blocking lesions that would require recombination for their repair. We determined the sensitivity of a panel of mutants deficient in the major pathways of recombination to increasing concentrations of DACH compounds. The *recF* mutant is deficient in repair of daughter strand gaps that follow replication blocks and it is sensitive to UV treatment (44). The *ruvABC* mutant is deficient in branch migration and resolution of various recombination intermediates such as Holliday junctions, and these mutants are sensitive to certain types of DNA damage, including UV treatment, cisplatin, and γ irradiation (45). The *recBCD* mutants are deficient in the repair of double strand breaks and are sensitive to γ irradiation (46). As shown on Fig. 6, all of the mutants tested showed sensitivity to treatment with Pt(DACH)Cl$_2$. These results are comparable with the cisplatin sensitivity reported previously for this panel of mutants, shown here in Fig. 6 for better comparison (4). Taken together, these data suggested that cisplatin and DACH compounds require recombinational repair for cellular survival, presumably because in *E. coli* both types of adducts present replication blocks. A similar pattern of sensitivity was observed when the strains were treated with oxaliplatin (data not shown).

DISCUSSION

Mismatch repair-deficient cells have shown differential sensitivity to the two platinum analogs, cisplatin and oxaliplatin, and several mutually nonexclusive mechanisms have been proposed that account for this phenomenon. Mismatch repair could initiate abortive repair of cisplatin-DNA adducts, selectively inhibit their replicative or recombinational bypass, or directly trigger apoptotic signaling. A key common upstream event in these proposed mechanisms is the recognition of platinum-DNA adducts by mismatch repair proteins. Our working hypothesis was that mismatch repair proteins preferentially recognize cisplatin over oxaliplatin DNA adducts and that this preferential recognition could lead to the observed differential cellular responses to the two drugs.

Our results showed that MutS recognized both types of adducts, but it recognized DNA modified with cisplatin adducts with a 2-fold higher affinity than DNA modified with DACH adducts. This could represent a biologically significant difference, especially when it is considered that mismatch repair proteins have only 10–20-fold higher affinity for mismatches than they have for homoduplex DNA (47). The possible reasons for weaker MutS interactions with DACH-modified DNA can be extrapolated from the recently reported crystal structure of MutS bound to a mismatch (34, 35). While the bending and unwinding caused by a cisplatin adduct would favor MutS recognition and possibly intercalation of MutS residues in the double helix, the nonpolar DACH ligand is likely to protrude into the major groove where it could disrupt the nonspecific, polar major groove interactions between the positively charged surface of the clamp portion of MutS and the phosphate backbone.

Another difference specific for the MutS interaction with cisplatin-modified DNA was observed when nucleotides were added to the binding reaction. Addition of ADP increased the MutS affinity for the cisplatin-modified DNA, but it did not have an observable effect on the affinity of MutS for DNA modified with DACH or EN adducts. The function of nucleotide hydrolysis in the function of MutS, or its mammalian homologues, is currently unclear. It could provide the energy for bidirectional DNA scanning (48) or, it could form a molecular switch, signaling between ADP bound/on and ATP bound/off states to downstream components (36, 49). These downstream components include the reminder of the mismatch repair machinery and, in eukaryotes, apoptotic pathways as well. It has been shown that cisplatin-DNA damage can trigger c-Abl/p73 mismatch repair-mediated apoptosis (5). This response is ab-

sent in mismatch repair-deficient cells, and oxaliplatin failed to show detectable activation of c-Jun N-terminal kinase or c-Abl kinases regardless of the mismatch repair status of the cells (50). It is attractive to speculate that the selective ADP modulation of MutS binding to cisplatin adducts underlines a potential mechanism of damage recognition-specific signaling.

In parallel to the MutS binding assays, survival experiments showed that methylation-deficient mutants *dam* were more sensitive to cisplatin than DACH compounds when compared at a dose equitoxic to the wild type. In *dam* mutants, where the strand discrimination signal is absent, mismatch repair could initiate futile cycles of abortive repair opposite platinum adducts (39). In support of this model, an additional mutation in mismatch repair genes abrogates the cisplatin sensitivity. This model has been extrapolated to account for the cisplatin resistance of eukaryotic mismatch repair-deficient cells as well (26). If abortive repair were operational, preferential recognition of cisplatin in comparison to DACH adducts should lead to higher level of abortive repair in *dam* mutants and thus, enhanced toxicity. Our results support this model to the extent that the 2-fold higher sensitivity of the *dam* mutants to cisplatin in comparison to $Pt(DACH)Cl_2$, reflects the 2-fold higher affinity of MutS for cisplatin over DACH-modified DNA. These results are also in line with studies done in mismatch repair-deficient cell lines, where it has been observed that defects in mismatch repair result in increased cisplatin, but not oxaliplatin, resistance (18).

It is also possible that mismatch repair proteins could mediate cisplatin toxicity by inhibiting replication or recombination dependent bypass of platinum DNA adducts. Studies have shown that DACH compounds are more efficiently bypassed by eukaryotic polymerases in comparison to cisplatin adducts (42, 43). Our survival experiments showed that recombination-deficient mutants were strikingly, but equally sensitive to both cisplatin and DACH compounds. This results suggests that the primary mechanism of cytotoxicity for both types of compounds, at least in *E. coli*, involves the formation of DNA adducts that form replication blocks that require recombination for their repair. The replicative bypass of cisplatin adducts is enhanced when a mismatch repair inactivating mutation is introduced (51), an observation that has led to the speculation that direct interactions of cellular proteins and cisplatin adducts could result in enhanced replication blocks. The HMG-1 box protein can selectively inhibit translesion synthesis of cisplatin over oxaliplatin damaged templates, presumably because of a stronger affinity of HMG-1 box protein for cisplatin over oxaliplatin DNA adducts (25). It is possible that in our study the replication blocks, in at least in part, were also a consequence of preferential interactions between cellular proteins such as MutS and cisplatin adducts.

In addition to the models for mismatch repair-mediated responses to cisplatin and oxaliplatin discussed above, other, yet undiscovered mechanisms by which these compounds contribute to cellular toxicity could exist. Because oxaliplatin and $Pt(DACH)Cl_2$ are more toxic than cisplatin for equimolar doses, yet they have a substantially lower rate of formation of DNA adducts in comparison to cisplatin (22), it is possible that the cellular responses to the DACH compounds are significantly modulated by their interactions with proteins or other cellular components. Our results add information to the biochemical framework within which the differential cellular responses to the two platinum analogs can be viewed. Further biochemical elucidations within this framework could have clinical importance in that they may lead to the development of novel successful antitumor drugs based on the parental structure of cisplatin.

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