Binding discrimination of MutS to a set of lesions and compound lesions (base damage and mismatch) reveals its potential role as a cisplatin-damaged DNA sensing protein

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Running Title : MutS binding and cisplatin-damaged DNA substrates

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#Supported by a grant from the Ministère de l’Enseignement Supérieur et de la Recherche.
Abbreviations: cisplatin, cis-diaminedichloroplatinum(II); MMR, DNA mismatch repair; $K_d$, dissociation constant; $k_{on}$, association rate; $k_{off}$, dissociation rate; PAGE, polyacrylamide gel electrophoresis.
The DNA mismatch repair (MMR) system plays a critical role in sensitizing both prokaryotic and eukaryotic cells to the clinically potent anticancer drug cisplatin. It is thought to mediate cytotoxicity through recognition of cisplatin DNA lesions. This drug generates a range of lesions which may also give rise to compound lesions resulting from the misincorporation of a base during translesion synthesis. Using gel mobility shift competition assays and surface plasmon resonance, we have analyzed the interaction of *Escherichia coli* MutS protein with site-specifically modified DNA oligonucleotides containing each of the four cisplatin cross-links or a set of compound lesions. The major 1,2-d(GpG) cisplatin intrastrand cross-link was recognized with only a 1.5-fold specificity whereas a 47-fold specificity was found with a natural G/T containing DNA substrate. The rate of association, $k_{on}$, for binding to 1,2-d(GpG) adduct was $3.1 \times 10^4$ M$^{-1}$ s$^{-1}$ and the specificity of binding was essentially dependent on $k_{off}$. DNA duplexes containing a single 1,2-d(ApG), 1,3-d(GpCpG) adduct and an interstrand cross-link of cisplatin were not preferentially recognized. Among twelve DNA substrates each containing a different cisplatin compound lesion derived from replicative misincorporation of one base opposite either of the 1,2-intrastrand adducts, ten were specifically recognized including those which are more likely formed *in vivo* based on cisplatin mutation spectra. Moreover, among these lesions, two compound lesions formed when an adenine was misincorporated opposite a 1,2-d(GpG) adduct were not substrates for the MutY-dependent mismatch repair pathway. The ability of MutS to sense differentially various platinated DNA substrates suggests that cisplatin compound lesions formed during misincorporation of a base opposite either adducted base of both 1,2-intrastrand cross-links are more plausible critical lesions for MMR-mediated cisplatin cytotoxicity.
Cisplatin reacts preferentially with the N7 atoms of purine residues in DNA. The major adducts (90%) are 1,2-intrastrand cross-links at d(GpG) and d(ApG) sites and the minor adducts correspond to 1,3-intrastrand cross-links at d(GpNpG) (N being a nucleotide residue) and interstrand cross-links formed at d(GpC/GpC) sites (7-10). Once formed, cisplatin lesions such as the major 1,2-intrastrand cross-links can undergo replication bypass in cells treated with cisplatin (11, 12). Because these lesions are often miscoding, they give rise to another form of lesion that is called a compound lesion (13), namely a lesion in one strand and a mismatch in the other. In both prokaryotes and eukaryotes, the pattern of cisplatin mutagenesis suggests that the major 1,2-intrastrand cross-links give rise to an array of compound lesions of which the majority are those produced when an adenine is misincorporated opposite the adducts (14-17). In vitro, DNA polymerases have been shown to bypass the major cisplatin adduct in an error-prone manner. For instance, misincorporation of a guanine by yeast polymerase ζ and misinsertion of a thymine by human polymerases η and β can occur opposite the 3’ adducted guanine (18, 19); human polymerase η can also misincorporate an adenine opposite the 5’ platinated guanine (20).

Recently, studies showed that the DNA mismatch repair (MMR) pathway contributes to the cytotoxicity elicited by cisplatin. In eukaryotes, MMR has been involved as an upstream component of a cisplatin-induced apoptotic pathway mediated by the tumor suppressor p73 or by the retinoblastoma tumor suppressor (21, 22). Importantly, a relationship between the loss of a functional MMR pathway and cellular resistance to cisplatin cytotoxicity has been established in vitro and in vivo (23-26). As cisplatin resistance is a major reason for treatment failure, it is therefore of great interest to understand how MMR-dependent molecular mechanisms...
participate in the modulation of cellular sensitivity to the drug. Several models have been proposed to explain the possible biochemical link between the MMR pathway and cisplatin cytotoxicity (reviewed in 26-29) but no precise mechanism has yet been defined. An important molecular determinant of such mechanisms was the identification of MMR proteins implicated in cisplatin cytotoxicity. Early experiments in *Escherichia coli* have shown that loss of MMR activity due to mutations in *mutS* or *mutL* was correlated with cisplatin resistance (30). In eukaryotes, mismatch repair defects due to loss of either human MSH2 or MSH6 subunits of hMutS\(\alpha\) or the human MLH1 or PMS2 subunits of the hMutL\(\alpha\) complex result in cisplatin resistance (23, 24, 26). Thus MutS or MutL and their respective eukaryotic counterparts hMutS\(\alpha\) or hMutL\(\alpha\) are MMR proteins implicated in cisplatin cytotoxicity.

Because the primary role of MutS and hMutS\(\alpha\) appears to be recognition of mispaired bases and small base insertion mismatches (31-33) and as recognition of cisplatin-damage DNA is a necessary step in all proposed models, the first mechanistically important question concerns both the capability of MMR proteins to sense cisplatin damaged DNA and the identification of the cisplatin lesions specifically recognized. A quantitative study reported that hMutS\(\alpha\) displayed weak affinity for 1,2-d(GpG) cisplatin intrastrand cross-links (34) but in contrast a qualitative study reported that this protein exhibits a strong preference for cisplatin compound lesions formed when a thymine is misincorporated opposite the major cisplatin adduct (35). This protein was also found to have a reduced affinity for a G/T mismatch in the context of a 1,2-d(GpG) adduct (13) suggesting that compound lesions were not involved in MMR activity (36). hMSH2 alone also displayed weak affinity for the major intrastrand cross-link (37). *E. coli* MutS was shown to recognize DNA globally modified by cisplatin (38) but the precise nature of the bound adducts is not yet known. Therefore, a sensitive assay is needed in order to gain insight into the nature of the suspected cytotoxic lesions recognized by MMR binding activities. In particular, no extensive interaction study has been carried out with cisplatin compound lesions, defined here as a cisplatin lesion in one strand and a mismatch in the other.
In the present study, we investigated the binding properties of *E. coli* MutS protein with various platinated DNA substrates containing a single centrally located cisplatin adduct in order to define the precise nature of the bound adducts. Experimental conditions were used in which sensitivity was high due to the absence of carrier DNA and to low nonspecific background binding. Within the sequence context studied here, the 1,2-d(GpG) adduct is the only bifunctional cisplatin lesion that is recognized albeit with a very low relative affinity. In contrast, MutS recognized with higher specificity an extended array of cisplatin compound lesions which are likely to be formed *in vivo* based on cisplatin mutation spectra. Surface plasmon resonance was employed for the first time to study MutS binding kinetics to several platinated DNA substrates; the weak binding of MutS to the 1,2-d(GpG) intrastrand cross-link is essentially dependent on a fast rate of dissociation. Taken together, our interaction data with MutS and different platinated DNA substrates suggest that cisplatin compound lesions formed during misincorporation of a base opposite either adducted base of both 1,2-intrastrand cross-links are the most probable critical lesions for MMR-mediated cisplatin cytotoxicity.

**EXPERIMENTAL PROCEDURES**

*Materials* — Single-stranded 24-mer oligonucleotides, biotinylated oligonucleotides and [γ-^32^P]ATP were purchased from Amersham Pharmacia Biotech. T4 polynucleotide kinase was from New England Biolabs. Untagged MutS was obtained from IntegraGen (Evry, France). Solutions of 40% acrylamide and 2% bisacrylamide were from Biorad. Cisplatin was from Sigma. Surfactant P20 and streptavidin-coated SA sensor chips were purchased from BIAcore AB (Uppsala, Sweden).

*Protein Purification* - Hexameric histidine-tagged MutS protein was prepared as previously described (39). The host strain was BL21 (lambda DE3) (Novagen) and the expression vector pMQ382 was kindly provided by Dr. M. G. Marinus. The strain transformed with pMQ382 was grown at 37°C with shaking to an OD 600 of O.6. The culture was then shifted to 25°C and induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG) at a final concentration of 50 µM for 3 hours. After centrifugation, the cells were resuspended in a sonication buffer (20 mM Tris-HCl pH 7.6, 500 mM NaCl, 20 mM imidazole, 5 mM β-mercaptoethanol, 10% glycerol)
supplemented with protease inhibitor cocktail (Sigma), frozen at –20°C and then sonicated. All
purification steps were performed at 4°C. The lysate obtained after centrifugation of the extract
was mixed with a nickel affinity resin (Qiagen) and MutS was batch-eluted with elution buffer
containing 100 mM imidazole. After overnight dialysis MutS was stored at –80°C in a buffer
containing 350 mM NaCl, 20 mM Tris-HCl pH 7.6, 0.1 mM EDTA, 1 mM dithiothreitol and 50
% glycerol. The concentration of MutS protein was determined using the Bradford reagent
(Pierce) with BSA as standard and the purity was estimated as > 95 % using coomassie staining
of a sodium dodecyl sulphate acrylamide gel. The concentration was 1.5-2 mg/ml.
Preparation of the glutathione-S-transferase-MutY fusion protein was carried out as previously
described (41). The host strain was BL21 (lambda DE3) and the expression vector pGEX-MutY
was kindly provided by Dr. S. Yonei. The strain transformed with pGEX-MutY was grown at
25°C with shaking to an OD 600 of 0.6. Expression of the GST-MutY fusion protein was
induced by the addition of 50 µM IPTG, and growth was continued overnight at 25°C. After
centrifugation, the cells were resuspended in phosphate-buffered saline (PBS) supplemented
with protease inhibitor cocktail, frozen at –20°C and then sonicated. After centrifugation the
supernatant was applied to a glutathione-Sepharose 4B column (Amersham Pharmacia Biotech)
at 4°C. The bound protein was eluted with 15 mM glutathione in 50 mM Tris-HCl pH 8. After
dialysis, the GST-MutY fusion protein was cleaved in PBS by thrombin protease by overnight
incubation at 4°C and the final concentration was 0.5 mg/ml.

Platination and DNA purification - The sequences of the oligonucleotides are shown in Fig. 1.
Single-stranded oligonucleotides including those with a 3’-biotin label, were purified on a 24 %
denaturating polyacrylamide gel. The preparation and purification of oligonucleotides
containing single 1,2-d(GpG), d(ApG) or d(GpCpG) cisplatin intrastrand cross-links and the
synthesis of the cisplatin interstrand cross-link was as described (42). The single-stranded
purine rich oligonucleotide 5’-AGGAGTAGAGATCGAGAGAGTAAG-3’ was 5’-end labeled
with T4 polynucleotide kinase and γ-32P-ATP. Radiolabeled heteroduplexes GG/CT and
G*G*/CT were prepared by mixing the 32P-labeled oligonucleotide with a 1.5 molar excess of
complementary purine-rich oligonucleotide containing a GG platination site without or with a
cisplatin residue in annealing buffer (60 mM KCl, 5 mM Tris-HCl pH 7.5, 0.1 mM EDTA), heating at 75°C for 5 min and slowly cooling at 4°C. Radiolabeled duplexes GG/CT and G*G*/CT were then purified on non-denaturating 10% polyacrylamide gel. Unlabeled duplexes for competition experiments were annealed by combining equimolar amounts of the two complementary strands at concentrations of 1-3.5 µM in annealing buffer at 75°C followed by cooling to 4°C.

**Electrophoretic Mobility Shift Competition Assays** - MutS (22 nM) was incubated in binding buffer (100 mM NaCl, 5 mM MgCl₂, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 1 mM dithiothreitol, 80 µg/ml acetylated bovine serum albumin and 2% glycerol) with mixtures of ³²P-labeled duplex GG/CT (2 nM) and particular unlabeled duplex competitors at varying concentrations as indicated in 20 µl. Reactions were allowed to reach equilibrium by incubation at 4°C for 20 min. Gel loading buffer was added, and the reaction mixture was loaded on a 6% native polyacrylamide gel (37.5:1 acrylamide:bisacrylamide (w/w); 45 mM Tris-borate, 0.5 mM EDTA, pH 8.3). Gels were electrophoresed for 1 h at 4°C at 13 V/cm, dried and exposed to a Molecular Dynamics phosphorimager screen overnight. The fraction bound (radioactivity of the bound complex/total radioactivity) was determined by ImageQuant 5.1 software (Molecular Dynamics, Sunnyvale, CA). Data analysis was carried out as described (43). We determined the concentration of various competitors necessary to decrease by 2-fold the amount of DNA shifted in the absence of competitor by linear regression of plots of the fraction of DNA bound in the absence of competitor divided by the fraction of DNA bound in the presence of competitor as a function of the competitor concentration.

**Direct binding experiments** - Varying concentrations of MutS (0 to 100 nM) were used to titrate ³²P-labeled duplex GG/CT or G*G*/CT (2 nM) in binding conditions as described above with the exception that no competitor DNA was present. The subsequent steps were the same as described under “Electrophoretic Mobility Shift Competition Assay”. The fraction bound was calculated and plotted as a function of the protein concentration. Data were least-squares fitted to an equation for a simple two-state binding process (44) using Origin 6.0 software (Microcal, Northampton, MA).
Surface Plasmon Resonance (SPR) measurements - SPR measurements were performed on a BIAcore 2000 at 15°C using a streptavidin coated chip and duplex DNA containing a 3’-biotin on the nonadducted purine-rich strand. 24-mer oligonucleotides used for SPR were the same as those presented in Fig. 1 with the exception that the purine-rich strand oligonucleotides have a 3’-biotin. Various biotinylated duplexes were prepared by mixing biotinylated purine-rich strand with a 3-fold molar excess of pyrimidine-rich strand in annealing buffer at 75°C followed by cooling to 4°C. Platinated and unplatinated biotinylated DNA duplexes were bound to the streptavidin-coated SA sensor chip by injecting 10 µl of 750 mM NaCl, 10 mM HEPES-KOH pH 7.4, 3 mM EDTA, 0.005% surfactant P20 and 1.7 µM biotinylated duplexes GG/CC, G*G*/CC, GG/CT, G*G*/CT, GG/TC or G*G*/TC. MutS protein was injected at a flow rate of 20 µl/min in running buffer (100 mM NaCl, 5 mM MgCl₂, 10 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 1 mM dithiothreitol, 0.0005% Tween 20 and 2% glycerol). Two washes of 10 µl of running buffer containing 0.04% SDS were used to regenerate the surface after each injection of MutS. Binding to each duplex was assayed at five different concentrations of MutS (0-1000 µM). To obtain kinetic parameters, late association phases and early dissociation phases of the resulting sensorgrams were fit to the Langmuir binding model using BIAevaluation software (version 2.0). The ratio of bound MutS per duplex DNA substrate was deduced from the stoichiometry formula (MutS response . DNA MW)/ (DNA response . MutS MW) where MutS response is the maximal response (in RU) obtained at the plateau level, DNA response (in RU) corresponds to the plateau value for a DNA substrate and DNA and MutS MW are molecular weights of the DNA substrate and MutS, respectively.

To check the chemical stability of bound platinum residue within biotinylated duplexes, the platinated pyrimidine-rich strand was 5’-32P-end labeled followed by hybridization with matched or mismatched complementary biotinylated strand. Labeled duplexes were then incubated in 100 mM NaCl, 5 mM MgCl₂, 5 mM Tris-HCl pH 7.5, 0.5 mM EDTA at 25°C for 24 h and loaded on a 24% denaturating polyacrylamide gel. After migration no product corresponding to unplatinated pyrimidine-rich strand was detected indicating that the platinum residue was not displaced from the strand.
MutY Adenine Glycosylase assay - Radiolabeled double-stranded 24-mer oligonucleotides GG/CC, GG/AC and G*G*/AC were prepared and purified as described above (with the purine-rich strand 5'-32P-end-labeled). Reactions were as previously described with minor modifications (41). Briefly, duplexes (40 fmol) were incubated with MutY (130 ng) at room temperature in 10 µl of reaction buffer containing 20 mM Tris-HCl pH 7.5, 50 µg/ml BSA and 10 mM EDTA followed by the addition of 2 µl of 2.5 M NaOH and heating at 95°C for 5 min at the times indicated. The denaturated samples were electrophoresed on a 24% denaturating polyacrylamide gel followed by quantification by phosphorimaging.

RESULTS
Relative binding affinities of MutS for cisplatin bifunctional adducts within the context of homoduplex DNA. We were first interested in determining whether bifunctional adducts of cisplatin could be recognized by the bacterial MutS within the context of homoduplex DNA. For this purpose we designed 24-mer oligonucleotide duplexes (Fig. 1) containing each of the four cisplatin bifunctional adducts considered as critical lesions for drug cytotoxicity: the 1,2-intrastrand d(GpG) and d(ApG) cross-links, the 1,3-d(GpCpG) intrastrand cross-link and the interstrand cross-link formed at a d(GC/GC) site. To determine the relative affinities of purified *E. coli* MutS for the different platinated duplexes, we performed competition experiments using an electrophoretic mobility shift assay. We compared the abilities of the various platinated unlabeled 24-mer duplexes to compete with 32P-labeled G/T containing duplex GG/CT. In agreement with previous data (45), the dissociation constant (Kd) for MutS with the duplex GG/CT was found to be 19 ± 3 nM as determined by direct titration experiments (data not shown). We confirmed that tagged MutS protein yielded the same results as untagged protein for all the binding experiments presented in this study. Fig. 2A shows typical competition experiments for which the 32P-labeled duplex GG/CT was mixed with different amounts of the unlabeled duplex competitors GG/CT, GG/CC and G*G*/CC which contained the major 1,2-intrastrand cisplatin cross-link. As expected, addition of unlabeled duplex GG/CT competes away the binding of MutS to the probe as judged by the decrease of the bound DNA (migrating with reduced electrophoretic mobility). Duplexes GG/CC and G*G*/CC inhibit weakly the
binding of MutS to the probe, with the platinated duplex showing slightly greater inhibition. Data from competition experiments were plotted as the fractional binding of labeled duplex GG/CT in the absence of competitor relative to binding at various competitor concentrations as a function of competitor DNA concentrations as previously reported (43) (Fig. 2B). The concentration of competitor duplexes necessary to reduce GG/CT binding by 50% was used to determine the specificity for various competitors relative to homoduplex DNA substrates. MutS was found to recognize specifically the homoduplex G*G*/CC containing a cisplatin 1,2-d(GpG) adduct. However, the 1,2-d(GpG) intrastrand cross-link is recognized with only a 1.5-fold specificity (Table I) whereas the duplex GG/CT containing a natural G/T mismatch is bound by MutS with a 47-fold higher preference (Table II). The adduct recognition appears to be sequence-independent as MutS binding was the same when the nature of the base pairs flanking the platination site was changed (CGGC or TGGT as central sequence for the purine-rich strand) or when the entire sequence was changed by using oligonucleotides previously studied in binding experiments with hMutSα (34, 35) (data not shown). These data for MutS and those of Duckett et al. (34) for hMutSα show weak binding to the 1,2-d(GpG) intrastrand cross-link with 30- and 10-fold respectively weaker efficiencies as compared to a single G/T mispair.

Following the same procedure, recognition of DNA substrates containing other cisplatin bifunctional adducts were tested. Competitors containing either a 1,2-d(ApG) intrastrand cross-link, a 1,3-d(GpCpG) intrastrand cross-link or an interstrand cross-link formed at a d(GC/GC) site compete less efficiently with the labeled G/T heteroduplex than the corresponding unplatinated competitors as deduced from the values of specificity reported in Table I. This implies that the 1,2-d(GpG) intrastrand adduct is the unique cisplatin bifunctional adduct recognized specifically by MutS. This suggests that 1,2-d(GpG) intrastrand cross-link is the lesion recognized in vitro by MutS in globally cisplatin-modified DNA (38).

*MutS recognition of cisplatin intrastrand cross-links when a mismatched thymine is opposite one platinated guanine.* hMutSα was found to recognize more efficiently a d(GpG) adduct
when it is part of two compound lesions formed after misincorporation of a thymine opposite either 3' or 5' platinated guanine (35). Because these substrates have not been studied for binding with MutS, we first investigated the recognition by MutS of the duplex G*G*/CT containing a 1,2-d(GpG) cisplatin intrastrand cross-link with a thymine opposite the 3' modified guanine. As shown in Fig. 3, MutS binds strongly to the mismatched G*G*/CT substrate. The determined Kd value of 7 ± 1 nM shows that the presence of the adduct stimulates the binding activity of the protein by about 3 times as compared to the unplatinated G/T substrate (Kd=19 ± 3 nM, above). As shown in Fig. 4 and Table II, this difference in binding was also confirmed in competition experiments. Next, we tested recognition of another compound lesion in which a thymine residue is now located opposite the 5' platinated guanine of the 1,2-d(GpG) adduct. The duplex G*G*/TC competes less efficiently than GG/TC (Fig. 4); the presence of the adduct decreases the affinity of the protein with a factor of 4-fold (Table II). These data indicate that according to the position of the mismatched thymine, the cisplatin adduct can stimulate or can impair MutS recognition. Interestingly, this effect has also been observed with hMutSα (35) suggesting that both bacterial and human proteins exhibit similar binding properties with cisplatin compound lesions.

In contrast to the stimulation by the adduct to binding of MutS with the duplex G*G*/CT, a DNA substrate containing a cisplatin 1,2-d(ApG) intrastrand cross-link with a T opposite the platinated guanine competes with the same order of magnitude as the corresponding unplatinated heteroduplex (Fig. 4, Table II). Thus, the 1,2-d(ApG) intrastrand cross-link can be recognized due to the presence of the misincorporated thymine opposite the adducted guanine.

MutS recognizes an extensive set of cisplatin 1,2-intrastrand cross-link/base mismatches. Results on cisplatin mutagenicity in both prokaryotic and eukaryotic cells indicate that several different compound lesions can be produced and that adenine or guanine rather than thymine are preferentially mispaired opposite 1,2-intrastrand adducts (14-17). In order to identify the compound lesions specifically bound by a MMR binding activity, we analyzed interaction of MutS with platinated duplexes containing all other possible mispaired bases opposite the 5' or the 3' platinated base of the 1,2-d(GpG) or -d(ApG) cross-links. Again, the abilities of various
mismatched DNA substrates with or without a 1,2-intrastrand cross-link to compete with radiolabeled duplex GG/CT were compared by competition experiments; the relative binding affinities for MutS are reported in Table II. The trend in affinity for mismatches is $G/T > G/G > G/A$ for unplatinated duplexes in the GG series and $G/T > G/G > A/C > A/A > G/A$ for unplatinated duplexes in the AG series. This order of affinity qualitatively agrees with that reported previously and the specific recognition of these mismatches by MutS is consistent with their repair both \textit{in vitro} and \textit{in vivo} \cite{46, 47}. Among nine platinated duplexes of the GG and AG series with G/G, G/A, A/A and A/C mismatches, seven are specifically bound by MutS (Table II). This result illustrates the capacity of MutS to bind DNA containing a large variety of cisplatin compound lesions. Mutagenesis studies have shown that d(GpG) and d(ApG) adducts primarily induced respectively G to T and A to T transversions and less frequently G to C and A to C transversions, with a preponderance located at the 5’-modified base in prokaryotes \cite{14}. Our finding that MutS recognizes specifically the 1,2-d(GpG) adduct when the complementary strand contains an adenine or a guanine opposite the 5' platinated guanine and the d(ApG) adduct with a mispaired adenine opposite the 5' platinated adenine strengthens the likelihood of a role of such compound lesions in mechanisms linking cisplatin cytotoxicity and MMR.

Among the seven platinated duplexes with mispaired bases opposite either modified base that we have found to act as specific competitors, five are more effective than the corresponding unadducted heteroduplexes. The increase in affinity occurs with the major cisplatin adduct for all G/A and G/G mispairs tested and is independent of the mispair location (opposite 3’ or 5’ platinated guanine). As compared to the mismatched duplexes, the presence of 1,2-d(GpG) adduct increased the specificity of MutS by 5-, 3- and 2-fold for the duplexes G*G*/CG, G*G*/AC and G*G*/GC, respectively. This effect also occurs with 1,2-d(ApG) adduct in the case of the duplex A*G*/TA. Inversely, the presence of this adduct can decrease the relative ability of a mismatched substrate to compete, as observed with the competitor A*G*/TG. An even more dramatic effect was observed with the duplexes A*G*/CC and A*G*/GC as the presence of the adduct abolishes MutS recognition of A/C and A/G mismatches, respectively.
These results indicate that the nature of the 1,2-intrastrand adduct can play a differential role in mismatch recognition of cisplatin compound lesions.

Surface plasmon resonance (SPR) analysis of MutS binding to platinated DNA substrates. We used SPR to examine the kinetics of binding of MutS to oligonucleotide duplexes containing either a 1,2-d(GpG) intrastrand cross-link or a 1,2-d(GpG) adduct with a mismatched thymine opposite either the 5’ or the 3’ platinated guanine.

For binding of platinated and unplatinated 24 base pairs duplexes to the Biacore streptavidin chip, a biotin residue was added to the 3’ end of the unadducted purine-rich strand. Because platinum has affinity for sulfur donors (48), we showed that the stability of the 1,2-d(GpG) adduct within a duplex was unaffected by the presence of a sulfur-containing biotin residue (see Experimental Procedures). After immobilizing the appropriate biotinylated duplexes to separate flow cells, MutS was injected over the surfaces. As shown in Fig. 5A, the maximum level of binding of MutS to the heteroduplex GG/CT is much higher as compared to that of the perfectly paired duplex, as expected for a mismatch binding protein. As deduced at saturation binding obtained at a higher protein concentration (RU_max=650, data not shown), MutS as a dimer was found to bind to the heteroduplex in a 1:1 manner, in agreement with previous studies (40, 49).

Adding a cisplatin 1,2-intrastrand adduct to the GG/CC homoduplex results in an increase of the plateau level. A similar increase occurred when the mismatched thymine was opposite the 3’ platinated guanine. Examination of the association and dissociation phases shows that the kinetic parameters responsible for the affinity increase are different for homoduplex and heteroduplex: with the homoduplex, this increase relates to variations of the dissociation rate whereas for the heteroduplex it is the association step that is changed. In contrast, when the mismatched thymine is opposite the 5’ modified guanine, the plateau level of the platinated heteroduplex is lower (Fig. 5B). In this case, MutS dissociation is more rapid as compared with the corresponding unplatinated mismatched duplex GG/TC. The SPR data are in qualitative agreement with results obtained from competition experiments.

The association and dissociation rate constants and the relative affinities of MutS for the different DNA substrates were determined by injecting increasing concentrations of MutS
through the cells (data not shown). Most of the kinetograms were fitted with a 1:1 Langmuir model. The association rate for MutS-G*G*/CT complex was not determined due to a complex biphasic type of interaction; a fit of the dissociation phase using a single exponential decay model provided the dissociation rate constant for this platinated substrate. Summary of data are reported in Fig. 5C. Our thermodynamic parameters compare well with those previously obtained with G/T-containing DNA substrates (50). The dissociation rate of MutS from the platinated homoduplex is 2-fold lower as compared to the corresponding unplatinated duplex and the affinity decrease for duplex G*G*/TC is related to a 4.8-fold increase in MutS dissociation as compared to duplex GG/TC. Thus, these data confirmed that the variations in the dissociation phase can explain the differences in MutS affinity for duplexes G*G*/CC and G*G*/TC as compared to their corresponding unplatinated duplexes. Compound lesions with adenine opposite either platinated guanine of the major cisplatin intrastrand cross-link are not substrates for the bacterial DNA glycosylase MutY. Although MutS recognizes specifically a 1,2-d(GpG) cisplatin cross-link when an adenine is opposite either platinated guanine, it is possible that in vivo, these lesions are primarily substrates for MutY, a DNA glycosylase responsible for the first step of base excision repair of adenine misincorporated opposite guanine or opposite the damaged guanine, 8-oxoguanine (51). Due its glycosylase activity, it is difficult to study the binding of MutY with DNA substrates containing a G/A mismatch. Therefore, we compared the glycosylase activity of MutY with the same 24-mer duplexes as above containing a mispaired adenine opposite unplatinated or platinated guanine in a 1,2-d(GpG) cross-link. In the experimental conditions used here, apurinic sites generated by the removal of mispaired adenines by MutY were chemically converted to strand breaks. The resulting fragmented products were then analyzed on a denaturating gel. As shown in Fig. 6A, the 24-mer double-stranded oligonucleotide GG/AC bearing a mismatched adenine yields a product comigrating with a 12-mer oligonucleotide thus showing the glycosylase activity of MutY. No cleavage was detected with the corresponding matched duplex GG/CC. Incubation of MutY with a DNA substrate containing a mispaired adenine opposite the 5’ platinated guanine of 1,2-d(GpG) cross-link did not result in cleavage. The same result was
obtained with duplex G*G*/CA (data not shown). This loss of activity could result from either a lack of duplex recognition by MutY or an inhibition of the glycosylase activity by cisplatin. To test the former hypothesis, we compared the efficiency of competitor duplexes GG/CC or G*G*/AC in inhibiting the cleavage reaction of G/A mispair-containing duplex (Fig. 6B). We found that duplexes G*G*/AC inhibited the cleavage reaction but with the same efficiency as the matched duplex GG/CC. The same result was obtained with duplexes G*G*/CA (data not shown). Thus the major cisplatin intrastrand cross-link abolishes the specific recognition of a G/A mispair by MutY. Although competition between MutS and MutY does occur for the repair of G/A mispairs, our data show that it no longer occurs in the presence of a 1,2-d(GpG) cisplatin cross-link.

**DISCUSSION**

Recognition of cisplatin-DNA adducts by MutS or its human homolog MutSα is thought to be a necessary event in the biochemical processes linking the MMR system with drug cytotoxicity. In this study, we have examined the ability of MutS to specifically recognize cisplatin-modified matched or mismatched DNA substrates.

Cisplatin generates a range of bifunctional DNA lesions but our study showed that MutS specific recognition is limited to the most abundant 1,2-d(GpG) adduct (Table I). The fact that MutS does not recognize specifically a d(ApG) adduct is unexpected because the DNA structure induced by both adducts is considered to be very similar. Indeed these adducts induced a bending and an unwinding of the DNA helix to the same extent (2), but distortions are more pronounced for the 5’ adducted base pair of the 1,2-d(ApG) adduct as compared with the 1,2-d(GpG) cross-link indicating structural differences at the nucleotide level (52). On the other hand, because mispair recognition by MutS was previously shown to be affected by the nature of the flanking base pairs (46, 53), it is possible that the different chemical nature of the 5’ base pair of the cross-linked d(GpG) and d(ApG) sites may also play a role. In contrast to the 1,2-d(ApG) adduct, the structures of 1,3-intrastrand and interstrand cross-links differ significantly from that of the major cisplatin lesion (2, 10) and these adducts were not preferentially recognized by MutS (Table I). These data demonstrate the ability of MutS to distinguish
between 1,2-d(GpG) intrastrand cross-links and other bifunctional cisplatin lesions and hence illustrate its potential role as a cisplatin DNA damage sensor.

In our assay conditions enabling high affinity and specificity, competition binding analysis revealed that the 1,2-d(GpG) intrastrand cross-link is poorly recognized by MutS with a 1.5-fold specificity as compared to an equivalent unadducted DNA substrate (Fig. 2B). Despite this relatively weak affinity, this binding might be sufficient for MMR-dependent processing of the 1,2-d(GpG) cross-link as a G/A mismatch which shows similar binding is known to be repaired by MMR in vitro and in vivo (46, 53). On the other hand, this adduct can also interact with a variety of cellular proteins as demonstrated in eukaryotes (for reviews see 54 and 55), and as a consequence MMR binding activity to this adduct might be less favored. In this case, SPR kinetic data can be helpful to predict the result of the competition between different cisplatin DNA damage binding proteins. Indeed, the competition of two proteins for a platinated DNA substrate has recently been correlated to their rates of association. Box B of the high-mobility-group box protein 1 (HMGB1) whose $k_{on}$ is close to the diffusion limit ($\approx 10^9 \text{ M}^{-1}\text{s}^{-1}$) (56) selectively binds to a 1,2-d(GpG) intrastrand cross-link in the presence of human replication protein A (57), a DNA damage recognition protein in the nucleotide excision repair pathway that associates with this lesion at a rate that is about two orders of magnitude lower (58). In the present study our SPR assay demonstrated a $k_{on}$ of $3.1 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ for MutS binding to the 1,2-d(GpG) intrastrand cross-link in agreement with values published for MutS and eukaryotic MutSα interacting with various DNA substrates (50, 59, 60). Thus the kinetic data obtained with MutS reveal a significant slower rate of association as compared to box B of HMGB1 and replication protein A, and so suggest that these proteins occupy the major cisplatin intrastrand cross-link before the MMR system can trigger downstream events. In support for this view, the nucleotide excision repair activity of human cellular extracts with a DNA substrate containing the major 1,2-intrastrand cross-link was shown to be unaffected by the presence of hMutSα (13) and a number of cellular proteins including HMGB, replication protein A, TATA-binding protein, human upstream binding factor and Ku autoantigen have been identified in human cell extracts as cisplatin DNA damage binding proteins but not mismatch binding proteins (35, 55).
Several observations both in vitro and in vivo are consistent with the existence of a variety of cisplatin compound lesions which are formed during replicative bypass of 1,2-intrastrand cross-links with misincorporation of a base opposite one of the adducted purines. In the course of this work, all three possible mispaired bases opposite the adducted purines (T, G, A and G, A, C opposite the adducted guanines and adenine, respectively) were tested for binding with MutS. We found that all the compound lesions with 1,2-d(GpG) cross-link and four out of six with the 1,2-d(ApG) intrastrand adduct were specifically recognized by MutS (Table II). Duplexes containing the 1,2-d(GpG) intrastrand cross-link were recognized more efficiently when a mispaired base was opposite either platinated guanine; the ability of the protein to bind DNA substrates with the 1,2-d(ApG) intrastrand adduct was dependent on a mismatched base opposite the adduct except in two cases. These results are similar to other reports that have shown that a substrate with O(6)-methylguanine opposite a thymine is preferentially bound by MutS (61) and hMutSα (62); ultraviolet light photoproducts when opposite mismatched bases are also preferentially bound by hMutSα (43). However, an unexpected effect reported here is that the 1,2-d(GpG) intrastrand cross-link results in increase of MutS mismatch binding activity. Such an effect occurs when all possible mispaired bases are placed opposite either platinated guanine except when a mispaired thymine is opposite the 5’ platinated guanine (Fig. 4, Table II). Compound lesions with a 1,2-d(ApG) adduct are less well recognized and the stimulation of binding they cause is quantitatively less important than that observed with the 1,2-d(GpG) cross-link. On the other hand, it is noteworthy that the nature of the mispaired base is also an important parameter for binding to compound lesions. Indeed, with respect to MutS binding efficiency, compound lesions follow the same order as the corresponding unplatinated mismatches. These results are consistent with a mutual influence of both the cisplatin adduct and the mispair on the recognition of cisplatin compound lesions by MutS. Taken together, our data clearly show that a large spectrum of cisplatin compound lesions with both 1,2-intrastrand cross-links are good substrates for MutS recognition including those that are principally formed in vivo, namely, compound lesions with an adenine opposite the 5’ purine of either 1,2-intrastrand cross-link. We have yet to determine if a cisplatin lesion could activate MutS to
engage MMR activity or other downstream events following the recognition step of a compound lesion. However, it is conceivable that the significant binding activity of MutS for a majority of compound lesions and even more the preference shown for these compound lesions over mismatched bases could have a bearing on MMR activity, especially as MutS binding affinity does correspond to the efficiency of mismatch correction (46).

We cannot exclude the in vivo recognition of compound lesions by binding proteins other than MutS. In particular, a subset of compound lesions could be substrates for other mismatch processing pathways. To address this question, we showed that significant cisplatin compound lesions containing G/A mispairs are not recognized by the MutY-dependent mismatch repair pathway indicating that recognition of such lesions could be restricted to MutS. Similarly, in eukaryotes, binding activity of human cell extracts with two cisplatin compound lesions (a mismatched thymine opposite either adducted guanine in a 1,2-d(GpG) adduct) seemed strictly dependent on hMutSα (35). However, repair of these lesions in human cell extracts seems to argue that proteins of the nucleotide excision repair (NER) pathway may interact with compound lesions (63). Further studies will be needed to determine whether proteins from NER and MMR compete for binding to cisplatin compound lesions.

Models have been proposed to explain the connection between the MMR pathway and cytotoxicity of cisplatin (26-29). Some of them include molecular mechanisms initially proposed for recognition of cisplatin DNA damage by protein such as HMG-box proteins. In these models, a first obligatory step is a relatively tight binding of a protein to a cisplatin lesion but not to compound lesions. As a consequence, the resulting nucleoprotein complex could block replication past an adduct leading to either a cytotoxic response and/or inhibition of the nucleotide excision repair by shielding the lesion, therefore allowing its persistence in the genome. As shown in the present study, the fact that MutS recognized poorly the major cisplatin 1,2-d(GpG) intrastrand cross-link reduces considerably the likelihood of such models. Our interaction study is more consistent with other models whereby compound lesions are the critical lesions. A well known model already proposed for cisplatin and other DNA damaging agents is called the futile repair model (29). It was proposed that MMR could be capable of
repeatedly initiating repair of the mispaired base opposite a cisplatin adduct within the newly replicated strand leading to the accumulation of secondary cytotoxic lesions (DNA strand breaks) (24, 26). Another attractive model involves an antirecombinogenic activity of MMR. Such a possibility has been recently proposed in *Saccharomyces cerevisiae* and is thought to involve a RAD52-dependent recombinational bypass during replication (27). In bacterial cells, recombination has recently been implicated in the replicative bypass of cisplatin lesions (64). One possibility is that MutS binds to cisplatin compound lesions that could be formed during recombination bypass, leading to inhibition of survival mediated by recombination-dependent bypass. In this context, the fact that MutS binds less efficiently to the 1,2-d(ApG) intrastrand adduct than to the 1,2-d(GpG) intrastrand cross-link when they are part of a compound lesion could contribute to a higher proportion of error-prone bypass of 1,2-d(ApG) adducts which could explain in part the higher mutagenic effect of this adduct in MMR proficient bacterial cells (14, 15). It would be of interest to investigate cisplatin mutagenesis in mismatch repair deficient cells to ascertain such a relationship. As an alternative to the models presented above, a more direct signaling pathway has been proposed (28); in such a model, the assembly of the eukaryotic hMutSα and hMutLα proteins at a specific lesion would then directly trigger apoptotic processes by yet unknown biochemical events that depend on the presence of a lesion (62). It would be of interest to test such a model with various cisplatin compound lesions.

The results presented here have identified critical primary DNA lesions of cisplatin which recognition is a prerequisite step in the chain of events leading to cisplatin cytotoxicity mediated by MMR. Correlation have been established between loss of MMR in cancer cells and resistance or tolerance to cisplatin. Continued investigations into the molecular mechanisms by which MMR pathways can modulate the cellular response to cisplatin may be helpful to improve the therapeutic use of this drug and in the design of new platinum-based drugs.

ACKNOWLEDGMENTS

We thank F. Culard and M.-J. Giraud-Panis for critical reading of the manuscript, M. Marinus and S. Yonei for respectively the MutS and MutY expression vectors, C. Marcaillou for expert technical assistance and J. Weissenbach for helpful encouragement. This work was
partially supported by the Ligue contre le Cancer, the Association pour la Recherche sur le Cancer and COST Chemistry Actions D20. P.B. was on leave at Genoscope from the Centre National de la Recherche Scientifique.
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FIGURE LEGENDS

TABLE I: Relative affinities of MutS binding to cisplatin-modified matched DNA substrates with respect to homoduplex DNA

Specificity of MutS for platinated homoduplex DNA substrates containing a site-specifically placed cisplatin bifunctional adduct was deduced from experiments carried out by competition binding assays shown in Fig. 2. Unlabeled competitor concentrations corresponding to 50% reduction in binding (ordinate of 2.0 in Fig. 2B) were used to determine the affinities relative to homoduplex DNA substrates (reciprocals of ratios of concentrations showing 50% reduction relative to the concentrations of homoduplex DNA substrates for each series GG, AG, GCG and GC).

TABLE II: Relative affinities of MutS binding to cisplatin-modified mismatched DNA substrates with respect to homoduplex DNA

Specificity of MutS for various platinated and unplatinated heteroduplex DNAs was determined from titration experiments carried out by competition binding assays shown in Fig. 2 and Fig. 4. The affinities relative to homoduplex DNA substrates were determined as described in Table I. The DNA substrates belonging to both GG and AG series are ordered according to the specificity of MutS for platinated DNA substrates.

FIGURE 1: (A) *cis*-Diamminedichloroplatinum(II) (Cisplatin); (B) sequences of oligodeoxyribonucleotides used in this paper. The pyrimidine-rich strands (left) each contain a platination site indicated in bold and each series refers to the sequence of a platination site. Within the complementary purine-rich strands (right), the nucleotide shown in bold indicates the location of a non-complementary base after hybridization with the complementary pyrimidine-rich strand. In the text, duplexes formed by hybridization of pyrimidine- and purine-rich strands...
are abbreviated as exemplified at the bottom of the figure. G*G*, A*G*, G*CG* and G*C/G*C denotes the presence of 1,2-d(GpG), 1,2-d(ApG), 1,3-d(GpCpG) intrastrand cross-links and interstrand cross-link at d(GC/GC) sites, respectively.

FIGURE 2: Specificity of MutS binding to the 1,2-d(GpG) cisplatin intrastrand cross-link. (A) A typical titration experiment with incubation of MutS (21 nM) with mixtures of 32P-labeled heteroduplex GG/CT (2 nM) and varying concentrations of unlabeled competitor DNAs as indicated. (B) Data from (A) are plotted as the fraction of bound radiolabeled G/T probe in the absence of unlabeled competitor divided by the fraction bound at indicated concentrations of unlabeled competitor as a function of the concentration of unlabeled competitor DNAs: heteroduplex GG/CT (solid squares), homoduplex GG/CC (solid diamonds), homoduplex G*G*/CC containing a 1,2-d(GpG) intrastrand cross-link (open diamonds). Data points are means of triplicate measurements with error bars showing standard deviations.

FIGURE 3: Interaction of MutS with DNA substrates with cisplatin compound lesions, containing a 1,2-d(GpG) intrastrand cross-link in one strand and a mispaired thymine opposite the 3’ platinated guanine in the other. (A) MutS was incubated with 32P-labeled duplex G*G*/CT (2 nM) and binding was assayed as described under “direct binding experiments”. The concentration of MutS was varied as indicated. (B) Plot of the data from panel (A); data were fitted to an equation for a simple two-state binding process.

FIGURE 4: Competition for MutS binding by DNA substrates containing cisplatin compound lesions. Data from competition binding experiments are plotted as described in Fig. 2B. Competition binding experiments were similar to those shown in Fig. 2A with labeled duplex GG/CT (2 nM), MutS (21 nM) and unlabeled DNA duplex competitors (20 to 620 nM). Data points are means of triplicate measurements with error bars showing standard deviations.
FIGURE 5: BIAcore analysis of MutS binding to matched and mismatched DNA substrates containing a 1,2-d(GpG) cisplatin intrastrand cross-link. (A) Kinetograms of binding and dissociation phases of MutS (100 nM) interacting with the following immobilized duplexes: GG/CC (30 RU), G*G*/CC (26 RU), GG/CT (55 RU) G*G*/CT (50 RU). (B), as for (A) but with 120 nM MutS and with GG/TC (29 RU) and G*G*/TC (33 RU). (C) Kinetic and thermodynamic parameters were obtained by monitoring kinetograms at different MutS concentrations for each DNA substrate used in (A) and (B). The resulting kinetograms were fit to a 1:1 Langmuir binding model using BIAevaluation software. K_d values are “apparent K_d” and are determined only to enable comparison between the different substrates. ND, not determined.

FIGURE 6: Interaction of the base excision repair protein MutY with a cisplatin compound lesion formed when a misincorporated adenine is opposite the 5’ guanine of 1,2-d(GpG) intrastrand cross-link. (A) A cleavage assay with mismatched adenine-containing unplatinated and platinated duplexes was carried out with MutY. The strand of duplexes containing the mispaired adenine on the corresponding strand for the homoduplex GG/CC were 5’-end radiolabeled. Each radiolabeled duplex was incubated with MutY, aliquots were withdrawn at the times indicated and the reaction was quenched by heating in NaOH, which also served to induce strand cleavage at the abasic site. The amount of cleavage at the apurinic site was monitored by denaturing PAGE. The removal of adenine by MutY activity is shown by the appearance of a reaction product comigrating with a 12-mer oligonucleotide (lane M). (B) Comparative inhibition of MutY glycosylase activity with duplexes GG/CC and G*G*/AC. Radioactive mispaired adenine-containing duplex GG/AC (4 nM) and MutY (130 ng) were incubated with various concentrations of unlabeled competitor. Lanes 1 and 6, no competitor DNA; lanes 2-5, homoduplex GG/CC; lanes 7-10, heteroduplex G*G*/AC containing a misincorporated adenine opposite the 5’ adducted guanine of a 1,2-d(GpG) intrastrand cross-link; lane M, standard 12-mer oligonucleotide. ★, 32P-labeled strand at the 5’ terminus.
| Platinated Homoduplex DNAs | -GG- | -AG- | -GCG- | -GC- | -CG- |
|---------------------------|------|------|-------|------|------|
| Relative Affinity         | 1.5  | 0.9  | 0.5   | 0.3  |      |
|        | GG series | AG series |
|--------|-----------|-----------|
| -GG-CT- | 47.4      | -AG-TT-   |
|        | -GG-CT-  | 129       | -AG-TT-   |
|        |          | 129       | -AG-TT-   |
| -GG-CG- | 2.6       | -AG-TG-   |
|        | -GG-CG-  | 10.3      | -AG-TG-   |
|        |          | 10.3      | -AG-TG-   |
| -GG-TC- | 24.1      | -AG-TA-   |
|        | -GG-TC-  | 5.9       | -AG-TA-   |
|        |          | 5.9       | -AG-TA-   |
| -GG-GC- | 2.6       | -AG-AC-   |
|        | -GG-GC-  | 5.5       | -AG-AC-   |
|        |          | 5.5       | -AG-AC-   |
| -GG-AC- | 1.8       | -AG-CC-   |
|        | -GG-AC-  | 5.5       | -AG-CC-   |
|        |          | 5.5       | -AG-CC-   |
| -GG-CA- | 1.6       | -AG-GC-   |
|        | -GG-CA-  | 2.0       | -AG-GC-   |
|        |          | 2.0       | -AG-GC-   |

Table II

Relative Affinity

GG series  AG series

-GG-CT-  47.4  -AG-TT-  24.3  -AG-TT-  27.1
-GG-CG-  2.6   -GG-CG-  10.3 -AG-TG-  6.6   -AG-TG-  5.8
-GG-TC-  24.1  -AG-TA-  2.5   -AG-TA-  2.9
-AG-TT-  129   -AG-TT-  27.1 -AG-TT-  27.1
-AG-TG-  6.6   -AG-TG-  5.8  -AG-TG-  5.8
-AG-TA-  2.5   -AG-TA-  2.9  -AG-TA-  2.9
-AG-AC-  2.7   -AG-AC-  1.8  -AG-AC-  1.8
-AG-CC-  4.6   -AG-CC-  0.8  -AG-CC-  0.8
-AG-GC-  1.5   -AG-GC-  0.3  -AG-GC-  0.3
Figure 1

A. Cisplatin

B. Pyrimidine-rich strands with the platination site in bold (5'-3')

| Strand  | Complementary purine-rich strands with the non-complementary base in bold (5'-3') |
|---------|-----------------------------------------------------------------------------------|
| CTTACTCTCTCGGCTCTACTCCT | AGGAGTAGAGACCGAGAGATTAAG |
| CTTACTCTCTCAGCTCTACTCCT | AGGAGTAGAGACCGAGAGATTAAG |
| CTTACTCTCTCGGTCTCTACTCCT | GGAGTAGAGACCGAGAGATTAAG |
| CTTACTCTCTCGGCTCTACTCCT | AGGAGTAGAGACCGAGAGATTAAG |

Example of unplatinated and platinated DNA duplexes used in this study with their abbreviations:

5'CTTACTCTCGGCTCTACTCCT 3' 5'CTTACTCTCGGCTCTACTCCT 3' 5'GG/CT 5'GG/CT
3'GAATGAGAGAGCATAGATGAGA 5'GAATGAGAGAGCATAGATGAGA 5'G*G*/CT
Figure 2

A.

Unlabeled competitor: $[^{32}P]$-GG/CT ratios

| competitors | 0  | 4  | 8  | 16 | 32 | 64 | 64 | 400 | 800 | 1200 |
|-------------|----|----|----|----|----|----|----|-----|-----|------|
| -GG-CT-     |    |    |    |    |    |    |    |     |     |      |
| -GG-CC-     |    |    |    |    |    |    |    |     |     |      |
| -GG-CC-     |    |    |    |    |    |    |    |     |     | 1200 |

bound →

free →

B.

Fraction of DNA bound (no competitor) / Fraction of DNA bound (competitor)

[Competitor DNA] (nM)
Figure 3

A.

![Image of gel with bands labeled bound and free]

B.

![Graph showing fraction bound vs. [MutS] (M)]

- Fraction bound
- [MutS] (M)
Figure 4

![Graph showing fraction of DNA bound (no competitor) / fraction of DNA bound (competitor) vs. [Competitor DNA] (nM). The graph includes various DNA base pair combinations: G*G*CT, GG/CT, G*G*/TC, GG/TC, A*G*/TT, AG/TT, GG/CC, AG/TC. Each data point is accompanied by error bars.](http://www.jbc.org)
Figure 5

A.

B.

C.

|                  | $K_d$ (M) | $k_{on}$ (M$^{-1}$s$^{-1}$) | $k_{off}$ (s$^{-1}$) |
|------------------|-----------|----------------------------|----------------------|
| -GG- CT-         | N.D.      | N.D.                       | $6.8 \times 10^{-4}$ |
| -GG- CT-         | $2.5 \times 10^{-8}$ | $3.3 \times 10^{4}$     | $8.2 \times 10^{-4}$ |
| -GG- TC-         | $31.5 \times 10^{-8}$ | $2.5 \times 10^{4}$     | $7.8 \times 10^{-3}$ |
| -GG- TC-         | $5.5 \times 10^{-8}$ | $3.0 \times 10^{4}$     | $1.6 \times 10^{-3}$ |
| -GG- CC-         | $36.3 \times 10^{-8}$ | $3.1 \times 10^{4}$     | $1.1 \times 10^{-2}$ |
| -GG- CC-         | $72.5 \times 10^{-8}$ | $3.2 \times 10^{4}$     | $2.3 \times 10^{-2}$ |
Figure 6

A.

- GG-CC-
- GG-AC-
- G-AC-

| 0 | 0.5 | 1 | 3 | 6 |
|---|-----|---|---|---|
| 0 | 0.5 | 1 | 3 | 6 |
| 0 | 0.5 | 1 | 3 | 6 |
| 0 | 0.5 | 1 | 3 | 6 |

M

time (h)

24-mer

12-mer

B.

Unlabeled competitor: $[^{32}P]$-labeled GG/AC ratios

- GG-CC-
- G-AC-

| 0 | 5 | 10 | 20 | 25 |
|---|---|----|----|----|
| 0 | 5 | 10 | 20 | 25 |
| 0 | 5 | 10 | 20 | 25 |

competitors

24-mer

12-mer
Binding discrimination of MutS to a set of lesions and compound lesions (base damage and mismatch) reveals its potential role as a cisplatin-damaged DNA sensing protein
Laurence Fourrier, Peter Brooks and Jean-Marc Malinge

*J. Biol. Chem.* published online March 24, 2003

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