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DNA Lesion Recognition by the Bacterial Repair Enzyme MutM*

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MutM is a bacterial DNA glycosylase that removes the mutagenic lesion 8-oxoguanine (oxoG) from duplex DNA. The means of oxoG recognition by MutM (also known as Fpg) is of fundamental interest, in light of the vast excess of normal guanine bases present in genomic DNA. The crystal structure of a recognition-competent but catalytically inactive version of MutM in complex with oxoG-containing DNA reveals the structural basis for recognition. MutM binds the oxoG nucleoside in the *syn* glycosidic configuration and distinguishes oxoG from guanine by reading out the protonation state of the N7 atom. The segment of MutM principally responsible for oxoG recognition is a flexible loop, suggesting that conformational mobility influences lesion recognition and catalysis. Furthermore, the structure of MutM in complex with DNA containing an alternative substrate, dihydrouracil, demonstrates how MutM is able to recognize lesions other than oxoG.

The reactive byproducts of aerobic respiration oxidize DNA to generate a number of deleterious adducts, among which the most widely studied is 8-oxoguanine (oxoG).¹ Because oxoG mispairs with adenine during replication, this oxidative lesion is a source of G-C to T-A transversion mutations. Nearly all organisms possess enzymes that safeguard against the genotoxic effects of oxoG. The oxoG resistance pathway in bacteria, known as the “GO” system, comprises three components: MutT, MutY, and MutM (1–4). MutT hydrolyzes oxo-dGTP to oxo-dGMP and inorganic pyrophosphate so as to prevent *de novo* incorporation of oxoG into the genome during DNA replication. MutY initiates the repair of misreplicated oxoG-A pairs in DNA by catalyzing hydrolytic excision of the adenine base. MutM (also known as Fpg) is a bifunctional DNA glycosylase/lyase that catalyzes complete excision of oxoG lesion nucleosides when paired opposite C in DNA via a complex multistep reaction cascade. Most eukaryotes possess a GO system related to

that in bacteria, with orthologous versions of MutT and MutY; however, in higher organisms, MutM is replaced by the functionally analogous but structurally unrelated enzyme Ogg1 (5, 6). Orthologs of MutM have been discovered in mammals, but these do not appear to be primarily involved in oxoG repair (7–10).

Recent structural studies of MutM-DNA complexes have revealed the overall architecture of the protein-DNA complex. These structures have also provided insights into the general features of lesion presentation to the MutM active site, suggesting that the oxoG nucleoside is swiveled out of the DNA helix during repair, a theme common throughout the structural biology of base excision DNA repair. A segment of the protein near the active site is disordered in the DNA-bound MutM structures lacking an oxoG nucleobase, yet is ordered in the absence of DNA, thus leading to the tantalizing suggestion that induced fit might contribute to lesion base recognition. Understanding the structural determinants of lesion recognition by MutM has been hampered by the fact that none of the available high resolution structures contain a damaged base (11–13).

In general, obtaining crystals of complexes comprising a DNA glycosylase bound to lesion-containing DNA is always complicated by the fact that the wild-type enzyme processes its substrate much faster than the time scale of crystallization and data collection. In several cases, this problem has been solved through introduction of amino acid changes that abrogate catalysis while preserving lesion recognition, thus enabling the preparation of homogeneous stalled glycosylase/DNA complexes (14–16). Studies with *Escherichia coli* MutM have demonstrated that conversion of a conserved active site glutamate at position 3 to glutamine provides a mutant protein lacking base excision (glycosylase) activity but retaining the ability to catalyze DNA strand scission (lyase activity) (17). We reasoned that an E3Q mutant version of MutM might allow the enzyme to bind stably to oxoG-containing DNA, thus enabling us to obtain a stalled oxoG recognition complex. Here we report the crystallization of this complex and the determination of its structure to 2.34-Å resolution. Furthermore, we have determined the 1.63-Å structure of a complex with DNA containing an alternative substrate, dihydrouracil (DHU). These structures reveal the mode of lesion recognition and hint at a substrate discrimination mechanism that couples substrate recognition with catalysis.

EXPERIMENTAL PROCEDURES

DNA Synthesis—The DNA oligonucleotides 5'-AGGTAGACCTG-GACGC-3' and 5'-TGCCTCCAXGTCTACC-3' (X = oxoG or DHU) were synthesized on an ABI 392 DNA synthesizer utilizing standard phosphoramidite chemistry, using the four normal phosphoramidites plus the 8-oxo-2'-deoxyguanosine or 5,6-dihydro-2'-deoxyuridine phosphoramidites (Glen Research). Deprotection of the oxoG-containing oligonucleotide was carried out in concentrated NH₄OH supplemented with 250 mM 2-mercaptoethanol for 18 h at 55 °C. Deprotection of the DHU-containing oligonucleotide was carried out in concentrated

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The atomic coordinates and structure factors (code 1R2Y and 1R2Z) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (<http://www.rcsb.org/>).

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¹ The abbreviations used are: oxoG, 8-oxoguanine; oxo-dGTP, 8-oxo-2'-deoxyguanosine-5'-triphosphate; oxo-dG, 8-oxo-2'-deoxyguanosine; oxo-dGTP, 8-oxo-deoxyguanosine triphosphate; oxo-dGMP, 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-monophosphate; DHU, dihydrouracil; hogg1, human 8-oxoguanine glycosylase-1.

TABLE I
Data collection and refinement statistics

	OxoG-DNA complex	Dihydrouracil-DNA complex
Data collection statistics		
Radiation Source	Rigaku laboratory source	NLSL X25
Resolution (Å)	50–2.34	50–1.63
Unique reflections	18601	53944
Completeness (%) ^a	100.0 (100.0)	98.9 (93.9)
Redundancy ^a	7.5 (7.4)	5.3 (4.8)
$R_{\text{merge}}^{a,b}$	0.090 (0.395)	0.048 (0.357)
$\langle I/\sigma \rangle^a$	23.0 (4.8)	31.0 (4.0)
Refinement and model statistics		
Protein Data Bank accession code	1R2Y	1R2Z
Resolution (Å)	50–2.34	50–1.63
$R_{\text{work}}^{a,c}$ (%)	21.1 (21.7)	20.8 (24.7)
$R_{\text{free}}^{a,c}$ (%)	25.7 (26.5)	23.3 (24.9)
Mean B-value, all atoms (Å ²)	33.2	29.5
Root mean square deviation from ideality		
Bond lengths (Å)	0.006	0.005
Bond angles (°)	1.2	1.2
Dihedral angles (°)	22.5	22.5
Ramachandran plot ^d (%)		
Most favored	89.7	90.9
Additionally allowed	9.9	8.7
Generously allowed	0.4	0.4
Non-water atoms	2596	2590
Water atoms	135	318

^a Values in parenthesis refer to the highest of ten resolution bins.

^b $R_{\text{merge}} = \sum I - \langle I \rangle / \sum I$, where I is the observed intensity.

^c $R_{\text{work}} = \sum F_o - F_c / \sum F_o$; where F_o and F_c are the observed and calculated structure factor amplitudes, respectively. R_{free} was calculated based on data randomly selected and omitted throughout structure refinement (21).

^d Values were calculated using PROCHECK (43).

NH₄OH at room temperature for 2 h. After lyophilization, all oligonucleotides were purified by urea-PAGE.

Mutagenesis and Protein Purification—An expression construct of the E3Q mutant version of *Bacillus stearothermophilus* MutM was created by subcloning (via introduction of a GAA to CAA mutation with the oligonucleotide 5'-ggctaacatcccCAAttgcggaggtggaacg-3' used as an N-terminal primer) starting with the previously described expression construct for the wild-type protein (13). The *B. stearothermophilus* E3Q MutM protein was overexpressed and purified as described (13), except that an additional purification step employing a MonoS cation exchange column (Amersham Biosciences) was included (gradient from 100 to 500 mM NaCl). Activity assays were performed essentially as described for hOgg1 (18) to confirm that *B. stearothermophilus* MutM cleaves DHU-containing DNA and that *B. stearothermophilus* E3Q MutM does not cleave oxoG- or DHU-containing DNA. A native electrophoretic mobility shift assay using a 7% native-PAGE gel run in 1× Tris-borate-EDTA and stained with SYBR-GOLD (Molecular Probes) was performed using 1 μM DNA and 1 μM protein to verify that *B. stearothermophilus* MutM E3Q forms stable complexes with both oxoG- and DHU-containing DNA.

Crystallization, Data Collection, and Structure Determination—Crystallization and cryo-protection for data collection were carried out as described (13). Data were collected at 100 K on a Rigaku R-axis IV²⁺ detector using a copper rotating anode x-ray source (oxoG-containing DNA complex) or on an ADSC Q315 detector at the X25 beamline of the National Synchrotron Light Source (DHU-containing DNA complex). All data were processed with HKL2000 (19). The structure of the oxoG-containing DNA complex was determined first, as follows: a modified model of the structure of *B. stearothermophilus* MutM bound to DNA containing a reduced abasic site (Protein Data Bank accession code 1L1T) in which the lesion nucleoside and all waters were completely omitted was used as a starting point for rigid body refinement followed by simulated annealing torsion angle dynamics refinement in CNS (20). Electron density maps calculated at this stage revealed clear density for the oxoG nucleoside and the amino acid residues (221–234) that were formerly disordered in the structure of the reduced abasic site-containing DNA complex. A revised model was built to account for all difference density, and several rounds of simulated annealing, energy minimization, and individual B-factor refinement, together with model-corrections and the addition of water molecules, were carried out while monitoring R_{free} (21). The structure of the DHU-containing DNA complex was determined and refined in a similar manner using the oxoG-containing DNA complex as a starting point. Data collection, refinement, and model statistics are presented in Table I.

RESULTS AND DISCUSSION

In the initial biochemical phase of this investigation, we verified that a mutant version of *B. stearothermophilus* MutM containing the E3Q modification (E3Q MutM) bound specifically to oxoG-containing DNA but failed to perform chemistry on it (data not shown). Crystals of E3Q MutM in complex with an oxoG-containing duplex 15-mer oligonucleotide were grown under the previously reported conditions (13). X-ray diffraction data were collected, and the structure of the lesion recognition complex was determined by difference-Fourier analysis (Table I). Not surprisingly, the overall structure of the oxoG recognition complex is very similar to those of previously reported MutM co-complex structures lacking a cognate lesion, with a protein backbone root mean square deviation of 0.37 Å for the present structure *versus* that of MutM bound to DNA containing a reduced abasic site (13). The most significant difference is the appearance of an ordered active site loop that was previously absent from the electron density maps of MutM/DNA structures lacking a lesion nucleobase (Fig. 1, purple). We refer to this segment, which resembles a “figure 8,” as the βF-α10 loop, owing to its location between β-sheet F and α-helix 10 (22).

Features of 8-Oxoguanine Recognition—The electron density maps in the active site region of the lesion recognition complex clearly reveal the presence of the entire oxo-dG lesion nucleoside (Fig. 2). The oxo-dG moiety is extruded from the DNA duplex and inserted into the extrahelical MutM active site, as anticipated from the oxoG-less structures (11–13) and in common with human Ogg1 (hOgg1) and other structurally characterized DNA glycosylases that act on single-base lesions. Apart from this point of similarity, the oxoG recognition mode of MutM is quite distinct from that of hOgg1. Whereas hOgg1 bears a rigid, preformed pocket into which the oxoG base fits snugly (15, 23–25), MutM possesses a relatively open, plastic oxoG recognition motif that is fully assembled only upon closure of the βF-α10 loop over the oxoG nucleobase. In the MutM active site, the oxo-dG nucleoside adopts a *syn* glycosidic tor-

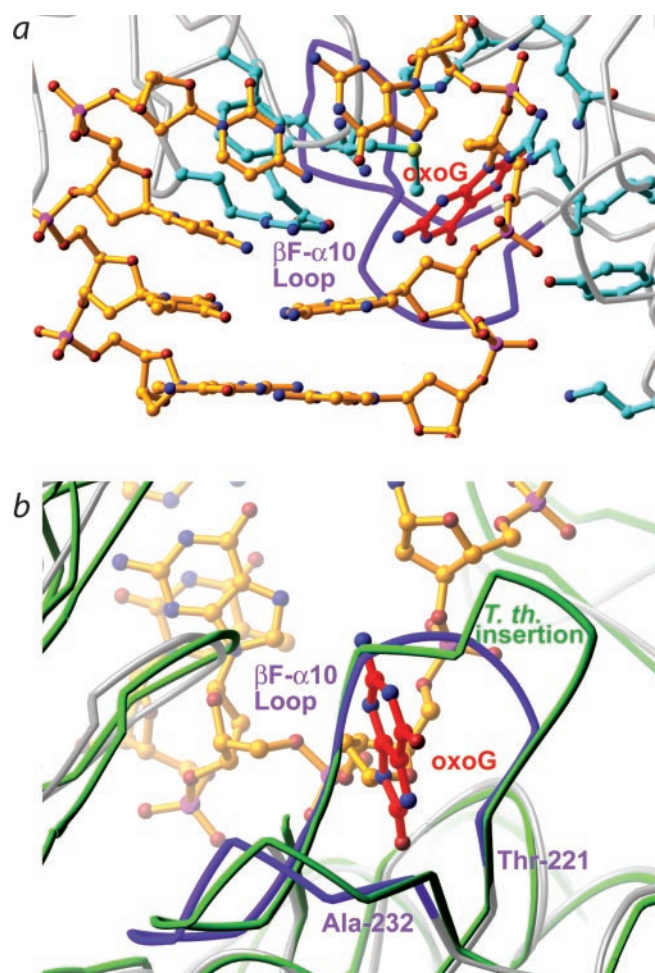


FIG. 1. **The E3Q MutM-DNA interface.** *a*, view of the protein-DNA interface, with the DNA colored gold, the oxoG lesion base colored red, and protein side chains colored in teal. The backbone trace of MutM is shown as a gray ribbon, except for the β F- α 10 loop, which is colored purple. *b*, superposition of the unliganded *T. thermophilus* (*T. Th.*) MutM structure (22), colored green, onto the structure of the E3Q *B. stearothermophilus* MutM-DNA complex (present work), colored as in *a*. The ribbon is drawn so as to trace through the positions of the α -carbons; approximate locations of specific amino acids residues are indicated. The location of the two-amino acid insertion in *T. thermophilus* MutM is denoted. Note in *a* and *b* that the loop is roughly shaped like a figure 8.

sion angle (Fig. 2, *a* and *b*); conversely, the same lesion is recognized by hOgg1 in the *anti* glycosidic configuration (15). The *syn* configuration is thermodynamically favored over *anti* for an isolated oxoG nucleoside (26), but both rotamers are found in oxoG-containing duplex DNA, with *syn* being preferred for oxoG paired opposite A (27, 28) and *anti* being preferred for oxoG opposite C (29, 30).

The β F- α 10 loop plays a key role in recognition of the oxoG base. A discrete projection from the loop wraps itself around the exocyclic O⁶ of oxoG (Fig. 2*a*), with four consecutive main-chain amide protons (residues 222–225) making hydrogen bonds to this one single atom of the nucleobase. None of these four residues that contact O⁶ is strictly conserved, perhaps unsurprisingly since lesion recognition involves only main-chain amides, and only sparse interactions are observed between side chains of the β F- α 10 loop and the main body of MutM. The structure of the O⁶-binding projection appears to be stabilized and anchored by a bridging hydrogen bond between the side-chain hydroxyl group of Thr-221, which is conserved (Thr or Ser), and the main-chain carbonyl oxygen of Tyr-225 (Fig. 2*c*). On the other hand, hOgg1 makes no direct contact to

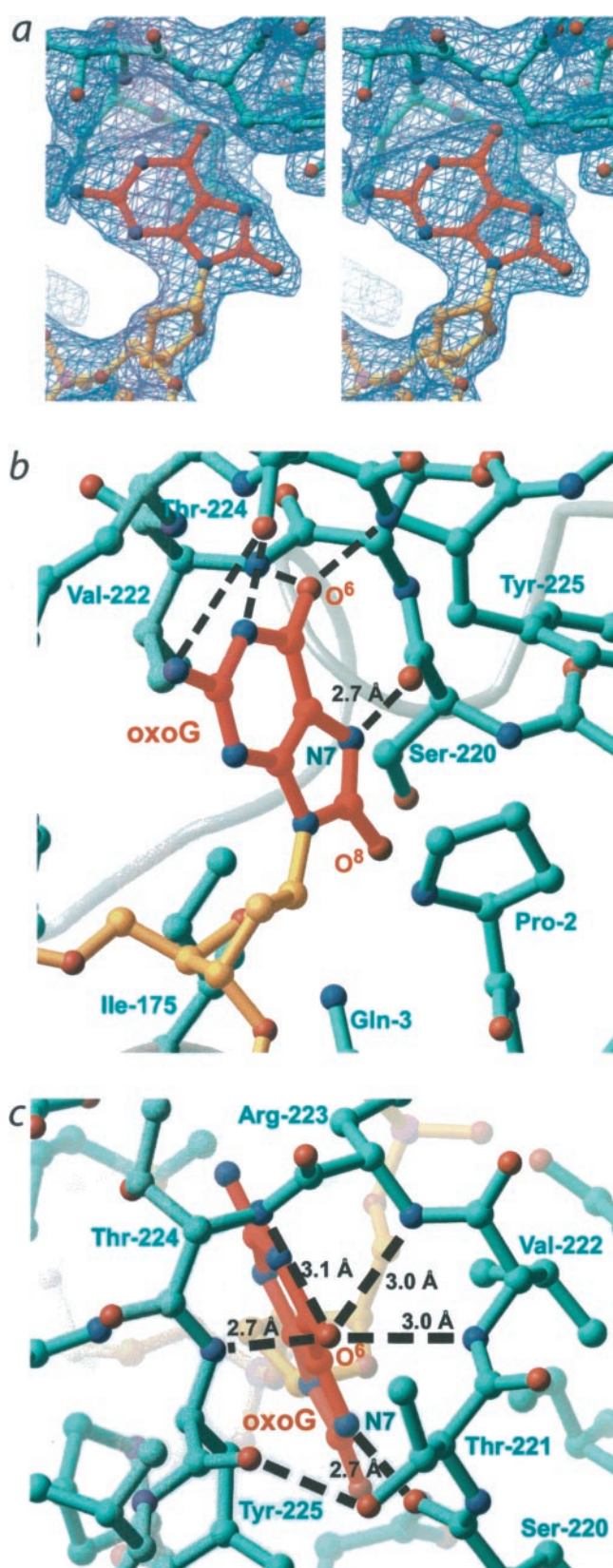


FIG. 2. **Close-up views of the 8-oxoguanine recognition complex active site.** Inferred hydrogen bonds are shown as dashed lines, and coloring is the same as in Fig. 1. *a*, stereo view of the active site region, with the final model superimposed on a σ_A -weighted $F_o - F_c$ (44) electron density map calculated to 2.34 Å using simulated annealing omit phases and contoured at 2.8 σ . *b*, view from similar perspective as *a*. *c*, view from above the active site, looking down through the β F- α 10 loop.

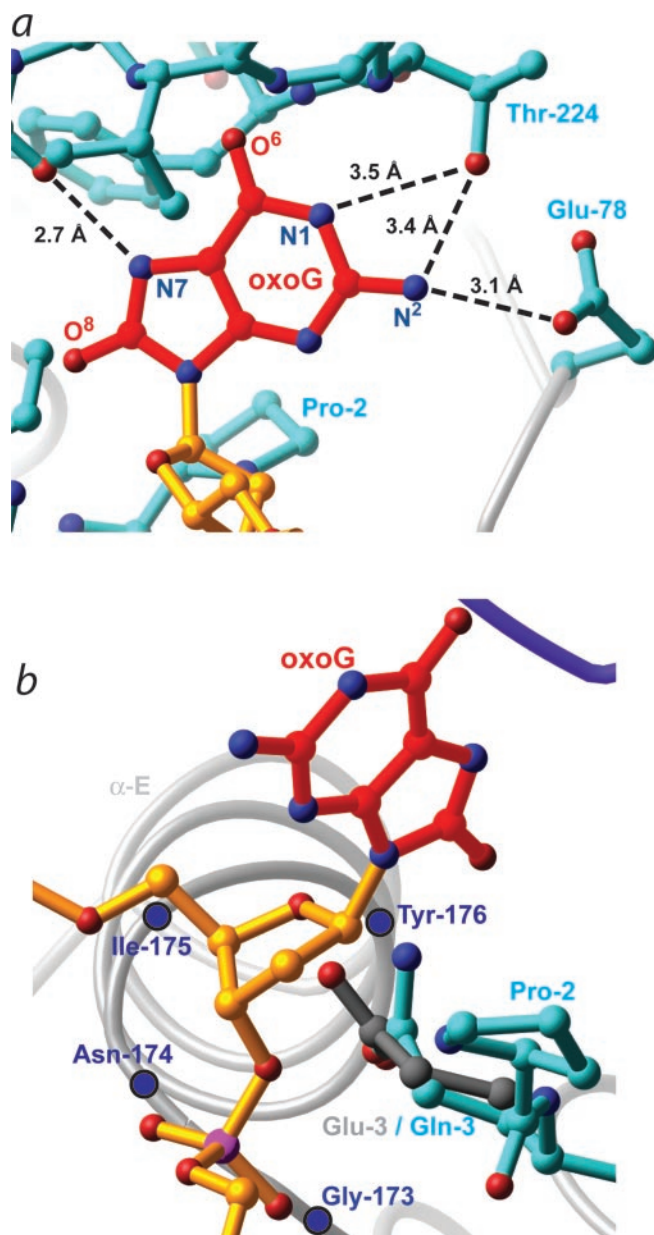


FIG. 3. *a*, interaction of the Watson-Crick face of oxoG with MutM. *b*, close-up view of the oxoG recognition complex active site with Glu-3, as determined in a previous study (13) shown in dark gray, superimposed for purposes of comparison with Gln-3 (this study). The approximate locations of main-chain amides involved in hydrogen bonds with Glu-3 are shown as labeled blue circles. Coloring is as in Fig. 1.

O⁶ of oxoG, but instead has bridging contacts through the intermediacy of two ordered water molecules (15). No water-mediated contacts to oxoG are evident in the present structure.

MutM also recognizes the Watson-Crick face of oxoG through hydrogen-bonding contacts to two residues, Thr-224 and Glu-78. Specifically, the side-chain hydroxyl of Thr-224 is positioned suitably to interact simultaneously with both N1 and N² of oxoG, whereas the side-chain carboxylate of Glu-78 is separated by a somewhat shorter distance from N² (Fig. 3*a*). In the case of hOgg1, a single residue, Gln-315, makes a bifurcated hydrogen-bonding interaction with both N1 and N² (15), similar to Thr-224 of MutM.

MutM makes no significant interactions with the π -face of oxoG, which results in the lesion recognition pocket having a rather spacious interior. Again, this differs from hOgg1, in which the oxoG is sandwiched between two residues (Cys-253

and Phe-319), each of which contacts one of the two π -faces of the lesion nucleobase.

A central question of this study is the means by which MutM distinguishes oxoG from undamaged DNA bases. The aforementioned interactions involving O⁶, N1, N², and the π -face of oxoG provide an obvious basis for distinguishing oxoG from adenine (A), cytosine (C), and thymine (T) because these normal bases bear distinct functionalities at the corresponding positions. These contacts, however, do not provide a clear basis for distinguishing oxoG from G because the two bear identical functionalities at the indicated positions. The structural differences between G and oxoG are localized to their five-membered heterocyclic rings, with C–H *versus* C=O at the 8-position and N: *versus* N–H at the 7-position, respectively. MutM exploits the difference in protonation state at N7 to distinguish oxoG from G. Specifically, the main-chain carbonyl of Ser-220 lies in close hydrogen-bonding contact with the N7 amide proton of oxoG, an interaction that would be repulsed by the N7 lone electron pair on G. Although the C=O group in oxoG does appear to be hydrogen-bonded to the side-chain amide of Gln-3, this contact may be an artifact of the E3Q mutation; the side-chain carboxylate of Glu-3 would instead experience a repulsive interaction with the 8-carbonyl oxygen (see below). Taking this into account, we conclude that the enzyme indirectly detects the occurrence of oxidation at C8 by sensing the attendant addition of a proton at N7. This strategy for oxoG/G discrimination is remarkably similar to that used by hOgg1, namely, the eukaryotic protein does not contact the C8 of oxoG but rather uses the main-chain carbonyl of Gly-42 to recognize the protonated N7 position (15).

In an effort to model the active form of the enzyme, we used least-squares superposition to transplant Glu-3 into the E3Q structure (Fig. 3*b*). Confidence in this model is strengthened by the fact that the active site structure, including the position and conformation of Glu-3, is nearly identical in several independently determined x-ray co-crystal structures of MutM (11–13) and EndoVIII (31) representing different stages of the repair reaction. The only significant point of divergence among all the DNA-bound structures, apart from the differences in covalent structure of the bound lesion, is the degree of disorder in the β F- α 10 loop. In all these structures, Glu-3 serves as an *N*-cap for helix α -E, with one carbonyl oxygen directed toward the amide NHs of Gly-173 and Tyr-176 (or the corresponding residue) and the other pointed toward the amide NHs of Asn-174 and Ile-175 (Fig. 3*b*). In the present structure of the E3Q mutant protein, the side-chain amide carbonyl of Gln-3 maintains a helix-capping interaction with Gly-173 and Tyr-176, but the side chain is swiveled to avoid a repulsive interaction between its amide –NH₂ group and the backbone amides of Asn-174 and Ile-175. This swiveling enables the Gly-3 amide to hydrogen bond to O⁸ of oxoG. It is at least a formal possibility that the carboxylate side chain of Glu-3 is actually protonated when the wild-type enzyme binds oxoG and that the acidic –OH group of the protonated carboxyl hydrogen bonds to O⁸ of oxoG in a manner much like the amide –NH₂ of Gln-3. An attraction of this scenario is that it would obviously facilitate the glycosyl transfer step by activating the oxoG leaving group; a difficulty is that carboxylates involved in helix-capping interactions have unusually high acidity and are therefore unlikely to be protonated (32).

The glycosylase reaction proceeds via nucleophilic substitution at C1' by the α -amino group of Pro-2 (33, 34). The α -nitrogen atom of Pro-2 lies close to C1' but is not well aligned for an in-line S_N2-type displacement of oxoG. Alternatively, the reaction could proceed by an S_N1-type mechanism, in which the base is excised first to yield a reactive oxocarbenium ion inter-

mediate, which is subsequently attacked by the α -N of Pro-2 (Figs. 2B and 3B). Kinetic isotope effect studies (35) and computational evidence (36) indicate a substrate-assisted S_N1 mechanism for uracil DNA glycosylase, and it is not unreasonable to expect that MutM performs catalysis by a similar mechanism. Additional support for a MutM-catalyzed S_N1 mechanism is found in the extensive array of hydrogen-bonding interactions to O⁶ of oxoG, reminiscent of the oxyanion hole of serine proteases, which would activate the oxoG base as a leaving group; additional activation could possibly be gained via protonation at C8 by Glu-3.

Recognition of an Alternative Substrate—In addition to oxoG, MutM repairs a number of alternative substrates, most notably formamidopyrimidine (37–39), 5-hydroxycytosine (40), and the hydantoin lesions (41). We tested the activity of MutM on DNA containing DHU, the product resulting from reduction of the C5=C6 double bond in uridine to furnish the corresponding singly bonded CH5–CH6 system. We determined that MutM catalyzes excision and strand-nicking of DHU-containing DNA and that E3Q MutM binds stably to this lesion without catalysis (data not shown). We grew crystals of the E3Q MutM-DHU-containing DNA complex and determined the structure by difference-Fourier analysis (Table I). Electron density for the DHU moiety is clearly visible in the active site, and the density is distinct enough to observe the puckering of the non-aromatic reduced pyrimidine ring (Fig. 4a).

The two carbonyl groups of DHU (O² and O⁴) reside in locations virtually identical to those of oxoG (O⁸ and O⁶, respectively). Additionally, the protonated N3 atom of DHU is in roughly the same location as the protonated N7 atom of oxoG (Fig. 4b). Most active site residues are in essentially the same conformations in both recognition complexes, preserving the β F- α 10 loop structure through interaction with O⁴ of DHU or O⁶ of oxoG. One exception is the side chain of Thr-224, which is disordered in the DHU recognition complex. Thus, the interactions with N1 and N² of oxoG described above are necessary for ordering of this side chain. As a consequence of this disorder, the loop is more flexible in the vicinity of Thr-224 in the DHU recognition complex, judged by weaker electron density and nearly 2-fold higher B-factors when compared with nearby residues. Another difference between the two different recognition complex structures is the positioning of the Glu-78 side chain. This side chain is retracted from the DHU lesion base when compared with the position it occupies when interacting with N² of oxoG.

Based on these two structures of recognition complexes with different lesions, the primary structural requirements for competent MutM substrates appear to be a hydrogen bond donor and acceptor positioned approximately where N7 and O⁶, respectively, are located in the oxoG recognition complex. Formamidopyrimidine and the hydantoin lesions also meet these requirements. The determinants for specific recognition of 5-hydroxycytosine are less obvious; perhaps the N⁴-H₂ and 5-OH groups are recognized in the same ways as the N7-H and O⁶ of oxoG, respectively.

Consequences of β F- α 10 Loop Flexibility—Despite being disordered in DNA-bound structures without lesion base (11–13), the β F- α 10 loop is ordered in the crystal structure of *Thermus thermophilus* MutM determined in the absence of DNA (22). Superimposing the *T. thermophilus* MutM structure on the present structure indicates that the β F- α 10 loop retains approximately the same folded structure in both (Fig. 1b). This comparison is complicated by the presence of a two-residue insertion in the *T. thermophilus* MutM sequence; however, this insertion is articulated merely as an extension of the tip of the loop in the *T. thermophilus* MutM structure.

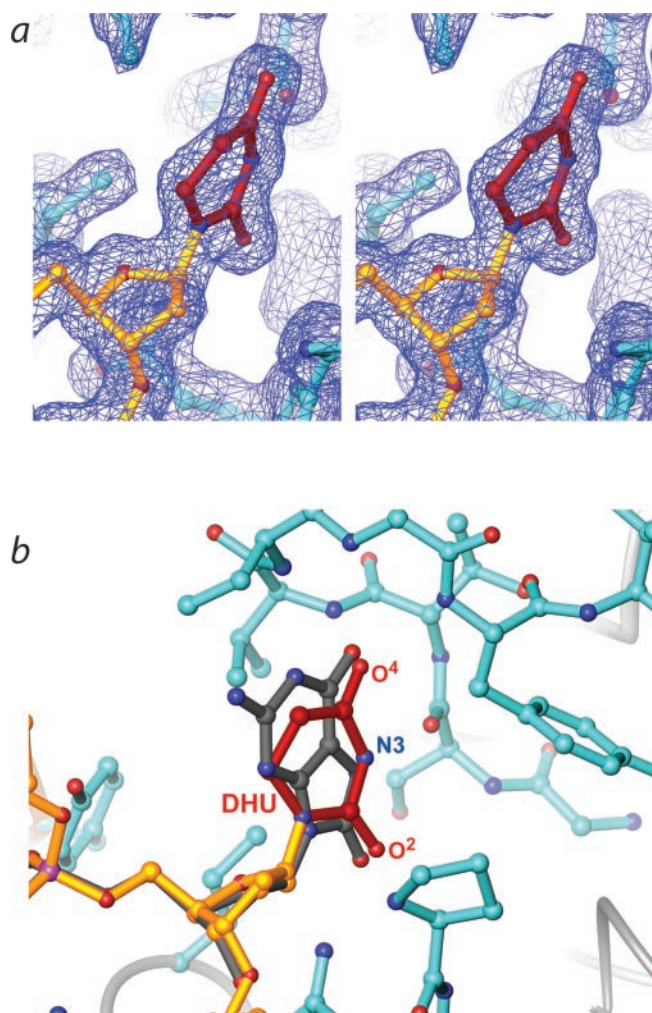


FIG. 4. **The dihydrouracil recognition complex.** *a*, stereo view of the active site region, with the final model superimposed on a $2F_o - F_c$ electron density map calculated to 1.63 Å using simulated annealing omit phases and contoured at 1.0 σ . *b*, superposition of the oxoG and DHU recognition complexes. Coloring as in Fig. 1, except that the dihydrouracil moiety is colored red, and the oxoG nucleoside is dark gray.

The large body of structural data now available for MutM (Refs. 11–13 and this work) suggest that the β F- α 10 loop is ordered prior to DNA binding and following oxoG insertion into the active site but becomes disordered following base excision. One way to rationalize this behavior is to hypothesize that the loop is in a relaxed state in the unliganded protein. DNA binding induces strain in the protein, perhaps by slight flexion at the domain interface, thereby causing the loop to become disordered. When oxoG is covalently linked to the DNA backbone and inserted into the lesion recognition pocket, the favorable enthalpy of loop/oxoG interactions provides conformational stability to the loop. An ordered β F- α 10 loop may be necessary for catalysis of base excision chemistry, bearing in mind the wealth of main-chain amide protons employed by the enzyme to recognize O⁶. These amide protons may act as Lewis acids, similar to the oxyanion hole found in proteases, to accelerate base excision. The N7-discriminating Ser-220 residue (see above) lies at the N terminus of the mobile loop, and stabilization of Ser-220 via interaction with N7-H may be a prerequisite for loop ordering. If the interactions of the β F- α 10 loop with O⁶ are accordingly contingent upon hydrogen bonding to the N7-H of oxoG, MutM would thereby couple oxoG recognition to catalysis using the β F- α 10 loop.

Once oxoG has been cleaved, it becomes entropically favored

for the loop to become disordered, and the loop probably remains disordered during 3'- and 5'-strand-nicking that occurs via β - and δ -elimination reactions, respectively (42), because it is disordered in a borohydride-trapped reaction intermediate and in a DNA-bound end product complex (13). The significance of β F- α 10 loop flexibility upon base release is not clear. It is possible that the entropic advantage gained by disordering drives lesion base excision in the forward direction. In this scenario, the cost of ordering the loop is paid by the enthalpy of interactions with oxo-dG, but the interactions between the loop and the free oxoG base are not strong enough to balance the entropic cost of holding onto the base and keeping the loop ordered. Finally, disordering of the β F- α 10 loop following base excision provides a means for water to enter the active site; water is thought to be the most likely candidate for a general acid/base to catalyze the β - and δ -elimination reactions (13).

Conclusions—We have determined structures of the catalytically inactive E3Q mutant MutM bound to an oxoG-containing DNA duplex and to a DHU-containing duplex. These structures reveal that the oxoG nucleoside is bound in the *syn* configuration about the glycosidic bond. The β F- α 10 loop, known to be disordered in DNA-bound structures of MutM lacking a lesion base, is primarily responsible for recognizing the oxoG base through hydrogen bonds to main-chain atoms. Notably, the prime determinant of oxoG *versus* G selectivity is the protonation state of N7 on the nucleobase. MutM binds to the distinct DHU lesion in a manner similar to oxoG, utilizing a few identical functionalities on the smaller base. The flexibility of the β F- α 10 loop suggests a possible functional role of loop ordering and disordering in oxoG recognition and catalysis by MutM.

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