The trajectory of intrahelical lesion recognition and extrusion by the human 8-oxoguanine DNA glycosylase

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Efficient search for DNA damage embedded in vast expanses of the DNA genome presents one of the greatest challenges to DNA repair enzymes. We report here crystal structures of human 8-oxoguanine (oxoG) DNA glycosylase, hOGG1, that interact with the DNA containing the damaged base oxoG and the normal base G while they are nested in the DNA helical stack. The structures reveal that hOGG1 engages the DNA using different protein-DNA contacts from those observed in the previously determined lesion recognition complex and other hOGG1-DNA complexes. By applying molecular dynamics simulations, we have determined the pathways taken by the lesion and normal bases when extruded from the DNA helix and their associated free energy profiles. These results reveal how the human oxoG DNA glycosylase hOGG1 locates the lesions inside the DNA helix and facilitates their extrusion for repair.
The chemical instability of the genome toward attack by a variety of reactive species with which it comes into routine contact has given rise to dedicated surveillance and maintenance systems that seek out DNA damage and repair it. Most forms of damage to the heterocyclic nucleobases of DNA are repaired by mechanistically related pathways collectively referred to as the base-excision repair (BER) mechanism. Both the recognition of damaged sites and initiation of repair by BER are performed by DNA glycosylases, lesion-specific enzymes that recognize specific nucleobase damages in the genome and catalyze their excision through cleavage of the glycosidic bond.

DNA glycosylases carry out the formidable task of locating, on average, one aberrant base embedded among $10^6$–$10^7$ normal bases. Many of these lesions differ from their undamaged counterparts by at most a few atoms and cause no frank distortion or little energetic destabilization of the DNA duplex. Among them, 8-oxoguanine (oxoG, $\text{G}^\text{Ox}$) presents the greatest challenge to the DNA glycosylases responsible for its repair, due to its unique combination of structural innocuousness, extraordinary mutagenicity, and chronic low-level production. Aminoguanidine mutagenesis, and chronic low-level production. Arising from its unique combination of structural innocuousness, extrordinary mutagenicity, and chronic low-level production. Aminoguanidine mutagenesis, and chronic low-level production.

Results and discussion

Entrainment of hOGG1 complex with intrahelical DNA.

Numerous structures of hOGG1 complexed with DNA have been reported, but all represent states with the target nucleotide in the course of being extruded from the DNA helix. To entrap the complex of hOGG1 with intrahelical G or oxoG, we identified several potential sites for DXL in the protein–DNA interface based on the native lesion-recognition complex (LRG) of hOGG1 and prepared the corresponding mutant proteins, containing a single cysteine at the relevant positions. Given that, a
Unique Interactions of hOGG1 with DNA. Several features of the IC and EC structures support the conclusion that the two structures represent the state of the enzyme at its initial encounter with the DNA. They are characterized below in terms of three key elements, which are different from the native LRC: (1) unique conformation of the DNA backbone, (2) rearrangement of the active site, and (3) different interaction with the C opposite oxoG (hereafter referred to as the estranged C).

First, the DNA conformation of the IC and EC structures differs substantially from that of LRC. The least-squares superposition of the IC and EC structures with LRC, using only the protein component in the superposition, clearly shows a well-defined anchor point on the 3′ side of the target strand (Fig. 3). The backbone of the target strand is held in place by the main-chain hydrogen bonds with G245, Q/K249 and V250 of the signature helix-hairpin-helix motif, which are found in all hOGG1–DNA structures solved to date.16,23,31–33. On the other hand, the 5′ side of the target strand and the non-target strand of the IC and EC structures are noticeably different from those of LRC (Fig. 3).

Secondly, in the LRC, F319 and C253 interact with both π-faces of the extruded oxoG, which sandwich the base in the active site of hOGG1. The extruded oxoG is further stabilized by hydrogen bonding with the sidechain carbonyl of Q315 and also with the backbone carbonyl of G42 (Supplementary Fig. 3a). The interaction with G42 is specific to oxoG and contributes to the discrimination of oxoG in the active site.16,23 These interactions are not possible in the IC and EC structures as there is no base extrusion. In addition, the α-helix, which includes F319 and Q315 in the IC and EC structures, retracts away from that of LRC (Supplementary Fig. 3b, c), leaving the active site open for lesion binding. This active site rearrangement was observed previously in the exo-site structure of hOGG1 (Supplementary Fig. 3d), suggesting that the rearrangement of the α-helix relative to LRC takes place after or in concert with the insertion of the base into the active site.16,23.

Finally, hOGG1 in the IC and EC structures interacts differently with the estranged C (Fig. 4). In LRC16, Y203 wedges into the DNA helical stack on the 5′-side of the estranged C stabilizing a helical bend. N149 enters the space left vacant by the oxoG extrusion and hydrogen bonds with the Watson-Crick face of the estranged C. In addition, the estranged C is stabilized by R154 and R204 that form bidentate hydrogen bonds together with N149. On the target strand, the 3′ and 5′ phosphotyrosyl groups of oxoG are anchored to hOGG1 by main chain hydrogen bonds with N150 of the conserved NNN motif (Fig. 4a). This mode of interaction was observed in all the hOGG1–DNA complex structures published to date, including the exo-site structure.23.
In the IC and EC structures, due to the presence of oxoG/G in the helical stack, the residues mentioned above engage in a different mode of interactions (Fig. 4b, c). For example, R154 and R204 contact the non-target strand backbone, instead of interacting with the C opposite to oxoG (i.e., the extranged C in LRC). In addition, Y203 does not invade the helical stack and remains at the periphery of the minor-groove face of DNA. Similarly, N149 rests on the minor-groove face of the target G/C (in IC)/oxoG/G base-pair (in EC) and hydrogen bonds with both bases (Fig. 4b, c and Supplementary Fig. 4a–c). In these hydrogen bonds, the interaction with N2 of G/oxoG is specific to G and oxoG. Adenine (A) cannot interact with N149 in this orientation (Supplementary Fig. 5). For C and T, although their C2=O carbonyl can form a hydrogen bond with N149, its sidechain is too short to reach C and T on the target strand. OxoG can also adopt a syn conformation and mis-pair with adenine (Supplementary Fig. 5d). Although N149 could in principle interact with the C8=O in the syn conformation, similar to C and T, the side chain of N149 is too short to reach the C8=O group of oxoG (syn), so it is unlikely that N149 will make a productive engagement with the oxoG (syn):A pair. Biochemical data also support that hOGG1 is specific to oxoG(anti):C and does not catalyze oxoG (syn):A pair efficiently35.

**Intrahelical lesion recognition by hOGG1.** Between IC and EC, while the target strand follows a similar backbone trajectory, the non-target strand of IC has translocated by half a nucleotide step toward its 3′ end relative to EC (Fig. 3b). Despite the conformational differences of DNA between the IC and EC structures, their DNA backbone structures around the target nucleotide are similar to each other (Supplementary Fig. 6a). The only notable difference is in the longer distance between C8 and C5′ of oxoG compared to the corresponding distance of IC (5.4 Å in EC vs 4.4 Å in IC).

The X-ray structures also reveal that, in EC, a water molecule bridges the oxoG to its 5′-backbone phosphate (Supplementary Fig. 6b) so as to attenuate the repulsion between them. Previously, it had been shown that the repulsion between C8=O of oxoG and its backbone phosphate plays a key role in the oxoG-specific intrahelical recognition by MutM22. The repulsion forces the oxoG ribose to adopt an alternative sugar pucker and/or a rotation of phosphodiester groups around oxoG. In the EC structure of hOGG1, the longer distance and the bridging water molecule between C8=O of oxoG and the C5′ and 5′′-backbone phosphate, respectively, could help bend the DNA at the target site, bringing these repulsive functional groups (i.e., oxoG and backbone phosphate) close to each other, thereby initiating base extrusion. Through this repulsion the enzyme discriminates oxoG and G, even at the initial encounter of the (intrahelical) lesion, prior to base extrusion and the conformational change to LRC.

MD simulations reveal an important consequence of the structural differences at the target site between EC and IC. As presented in Supplementary Fig. 7, in the presence of oxoG, hOGG1 establishes a stable contact with the target strand, while in the case of G it fluctuates back-and-forth. Consistent with this, the target strand with oxoG shows smaller root-mean-square fluctuation (RMSF) of atomic position than that with G (Supplementary Fig. 8). This suggests that the enzyme can quickly translocate to the next base pair in IC, while in the case of oxoG, it is locked at the target site.

**Lesion discrimination and base extrusion mechanism.** In the IC and EC structures, hOGG1 does not form any direct interaction with the discriminatory major groove face of oxoG and G (Fig. 1a). This raises the question: by what mechanism does hOGG1 discriminate G and oxoG in the early phase of extrusion? To answer this question, we simulated the extrusion of G and oxoG out of the DNA helix into an extrahelical state and determined the associated free energy change by use of the string method36. The free energy profiles determined for the oxoG and G extrusions in this event are presented in Fig. 5a, in which the progression of the base extrusion is described by a normalized reaction coordinate α. The simulations show that the extrusion proceeds in three steps: (1) the target base is extruded out of a helical stack through a major groove of DNA, (2) the extruded base binds transiently at the exo-site, and (3) it then enters the active site of the enzyme with concomitant closure of the active site (see Supplementary Movie 1).

The MD simulation shows that G has a higher barrier than oxoG (13.3 kcal/mol for G versus 8.1 kcal/mol for oxoG; Fig. 5a). Moreover, the free energy of G continues to increase up to 21.2 kcal/mol near the exo-site (Supplementary Fig. 9). Two protein residues, H270 and K249, stabilize the extruded oxoG through C8=O but not G (Fig. 5b); both residues are indispensable for the oxoG cleavage37. In particular, H270 forms a hydrogen bond with C8=O of the extruded oxoG between a = 0.5 and 0.9, through its backbone amine (Fig. 5b); Supplementary Fig. 9 shows the change of the distance between H270 and oxoG C8=O group along the entire base extrusion process. As the oxoG extrusion continues,
the C8=O group begins to interact with the K249 sidechain. These interactions lead to a relatively flat free energy profile between $\alpha = 0.5$ and 1.0 (Fig. 5a). Since these interactions are not possible for G, its free energy remains high, thus G return quickly to the intrahelical position. This difference suggests that hOGG1 kinetically discriminates the DNA lesion during its extrusion as shown in Fig. 6.

Despite the significant difference of the free energy barrier between oxoG and G, the free energy of the two systems increases very similarly at the beginning of the base extrusion between $0 < \alpha < 0.4$...
between the major30,42 and minor groove base extrusions22,29. The base extrusion process are shown in Supplementary Fig. 9. The extrusion was also proposed from the stopped-base extrusion. Poor discrimination in the early phase of base active site of the enzyme to initiate its catalytic repair. The free energy pro presented in Fig.5a, and the free energy pro

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DNA contacts to induce DNA bending at the target site. This orientation. In silico molecular dynamics simulations and free energy calculations corroborate the structural results and help to elucidate the role of the human enzyme in discriminating oxoG from G prior to a complete extrusion from the DNA stack. The results presented here broaden our understanding of one of the earliest events that occur as this extraordinary enzyme patrols genome in its surveillance of DNA damage.

Methods

Cross-linked complex formation and crystallization. A fragment of hOGG1 (amino acids 12–327, UniProtKB-015527) bearing the Y207C, Y207C/C253W, and Y207C/K249Q mutation was expressed in Escherichia coli BL21(DE3)LyS3 cells. The cells were lysed by sonication in solution of 50 mM sodium phosphate pH 8.0, 10 mM imidazole, 500 mM NaCl, 5 mM BME, and 10% glycerol. The protein was immobilized by Ni-NTA resin (Qiagen) and eluted with 50 mM sodium phosphate pH 8.0, 250 mM imidazole, 500 mM NaCl, 5 mM BME, and 10% glycerol. Protein was concentrated, centrifuged, and diluted with 10 mM Tris pH 7.4 to 50 mM NaCl, loaded to Hi-Trap SP column (GE Healthcare) and eluted with increasing NaCl concentration. The N-terminal histidine tag was cleaved by enterokinase digestion (New England Biolabs) using a 1:1 solution of 1 M CaCl2 for 36 h at 4 °C. Protein was further purified by Superdex-200 gel filtration chromatography (GE Healthcare) equilibrated with 10 mM Tris 7.4, 100 mM NaCl, 1 mM EDTA, and 10% glycerol41. Each mutant was prepared using QuickChange mutagenesis kit (Stratagene) and confirmed by sequencing (see Supplementary Table 2 for primer sequences used in mutagenesis). Phosphoramidite derivatives of 8-oxoG and 2-F-dI were purchased from Glen research. DNA oligomers 5’-AGGTCAGXG*TCTACG-3’ where X denotes 8-oxoG or G and G* refers the site of modification with the thiol-bearing tether, were synthesized using ABI Expedite 8909 DNA synthesizer and functionalized with X2 (NH2.CH2.CH2.OCH2.CH2.OCH2.CH2.S-), using post synthetic modification44. DNA oligonucleotides were deprotected with ammonium hydroxide and purified in 20% denaturing urea polyacrylamide gel electrophoresis (PAGE). For DNA containing tether and oxoG on the same strand, 50 mM β- me was added to prevent oxidative degradation of 8-oxoG. DNA was purified by 20% urea-PAGE and dissolved in 10 mM Tris, pH 8.0, 1 mM EDTA, and annealed with complimentary strand 5’-TGTTAGAACCCTGGACCC-3’.

Cross-linked complexes were formed by mixing duplex DNA with 2-fold molar excess protein and incubating at 4 °C for several days. Unreacted DNA and protein were removed by Mono Q chromatography (GE Healthcare). The purified complexes were buffer-exchanged to 10 mM Tris 7.4 and 100 mM NaCl, concentrated and crystallized by hanging droplet vapor diffusion at 20 °C. For each complex, crystals were allowed to grow for several days, transferred to a cryoprotectant solution containing mother liquor supplemented with 25% glycerol, and frozen in liquid nitrogen for data collection. For LRC (Y207C/K249Q), diffraction quality crystals appeared with 16.4 mg/mL complex concentration (protein concentration measured using Bradford assay) within a few days in well solution containing 100 mM sodium cacodylate, pH 6.5, 200 mM MgCl2, and 18% polyethylene glycol 8000. For IC (Y207C), diffraction quality crystals appeared with 12 mg/mL complex concentration within a few days in well solution containing 200 mM NH4NO3, and 20% polyethylene glycol 3350. For EC (Y207C/C253W), diffraction quality crystals appeared with ~17 mg/mL complex concentration within a few days in well solution containing 100 mM sodium cacodylate, pH 6.1, 200 mM MgOAc, and 17% polyethylene glycol 8000. Structure determination: Diffraction datasets were collected at ~170 °C at the 24-ID-C and 24-ID-E beamlines (NE-CAT) of the Advanced Photon Source and processed using the HKL program suite45. Initial molecular replacement solutions were obtained by Phaser in the CCP4 suite46,47, using the coordinates of previously determined hOGG1 structure (PDB ID:1EBM16) but omitting DNA as search models. Each hOGG1–DNA model was built through iterative cycles of manual model building in COOT48 and structure refinement using REFMAC549,50.
and PHENIX. The Ramachandran plots, calculated by MolProbity (http://molprobity.biochem.duke.edu), confirmed no residues in disallowed regions for all structures. Additional details on the data collection and structure refinement are provided in Supplementary Table 1. PyMol (The PyMOL Molecular Graphics System, Version 2.0, Schrödinger, LLC) was used to prepare all structure model figures presented in the paper.

System preparation for molecular dynamics (MD) simulations. Two systems were prepared based on the intrahelical IC (for G) and EC (for oxoG) structures, respectively. In the preparation of the IC system, we further refined the IC X-ray structure to build more base pairs. Although this resulted in slightly lower quality of DNA structure, the core DNA base pairs were essentially unchanged. We used this refined structure in the IC system building. In each system, protonation states of all ionizable residues were determined based on their hydrogen bonding interactions deduced from the X-ray structures as well as on their pKa values in water. All crystal waters were included. For DNA, the central 14 base pairs of the sequence presented in Fig. 1d were used in the simulations, in which any missing nucleotide coordinates from the crystal structures were model built as the standard B-form DNA. Then, the HBUILD facility of the CHARMM program was used to assign atomic coordinates of hydrogen atoms. The resulting systems were solvated with a rhombic dodecahedron (RHDO) box of 11,712 TIP3P water molecules and any water molecules within 2.5 Å from any heavy atom of protein, DNA, and crystal water were removed, leaving, for example, 9554 TIP3P waters for the IC system. Finally, each system was neutralized by adding 50 Na+ and 26 Cl− ions at random positions, making its ionic concentration equal to 150 mM. In addition, two additional systems were prepared based on the LRC X-ray structure (PDB-ID: 1YQR) to be used as the reference state of the targeted molecular dynamics (TMD) simulations (see the Supplementary Methods for details).

MD simulations. Each system was first energy minimized for 5000 steps and equilibrated for 500 ps at 300 K. The energy minimization and equilibration procedures were very similar to those employed in our previous studies of MutM/DNA complexes. Then, the production MD was carried out for 500 ns for the IC and EC systems, during which the atomic coordinates of the entire system were saved at every 2 ps for later analysis. The all-atom CHARMM and 27 force fields were used to represent the protein, DNA, and ions, the CMAP correction for protein backbone dihedrals, and the TIP3P water model for water molecules, respectively. For oxoG, we used the force field parameters developed in our previous study. The RHDO periodic boundary conditions were imposed with the lattice length parameter of 7.85 Å. Electrostatic interactions were evaluated using the smooth particle mesh Ewald (PME) sum method and van der Waals interactions were evaluated using a switching function between 9.0 Å and 11.0 Å. All MD simulations were performed with a 2 fs integration time step and SHAKApplied to all bonds involving hydrogen atoms. The Langevin thermostat was used to maintain the system temperature at 300 K. In all simulations, we also applied harmonic restraints to the terminal base pairs to avoid them fraying away from each other.

System preparations, trajectory analysis, the TMD simulations, and the string method simulations (see below) were carried out using the CHARMM program (version c37a2) and the 500 ns production MD simulations were performed using the NAMD program.

String method (SM) simulations. The base extrusion pathways for G and oxoG were determined by applying the string method in collective variables (SMCV). In SMCV, a path connecting two end states (i.e., the intrahelical IC/EC and LRC conformations) is represented by N discretized images (called MD replica) which are evenly distributed along the path. In the present work, we used N = 46 discretized images to represent the entire base extrusion pathway described by a total of 45 CVs defined in Supplementary Fig. 10a. Starting from the initial path generated by the TMD simulation (Supplementary Methods), each path was optimized for 25 ns in an iterative manner. A total of 2.8 μs path optimization MD was performed. Then, the Markovian milestoning simulations with Voronoi tessellations were performed for 10 ns for each MD replica (thus, 0.64 μs MD simulations collectively) to determine the free energy change along the optimized base extrusion paths. The details of the SMCV path optimization and Voronoi tessellations simulations are provided in the Supplementary Methods.

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