Coupling of Substrate Recognition and Catalysis by a Human Base-Excision DNA Repair Protein

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Most damaged bases in DNA are removed by the base-excision DNA repair pathway.[1] The key components of this pathway are DNA glycosylases, enzymes that recognize damaged bases and catalyze scission of their glycosidic bond.[2] The human 8-oxoguanine DNA glycosylase (hOGG1) is responsible for repair of the mutagenic lesion 7,8-dihydro-8-oxoguanine (oxoG).[3] The X-ray structure of a catalytically inactive mutant of hOGG1 bound to oxoG-containing DNA revealed the molecular basis for lesion recognition[4] but left unanswered many questions concerning the catalytic mechanism. Here we report the X-ray structure of wild-type hOGG1 bound to a substrate-based inhibitor. The structure lends insight into the coupling of base recognition and catalysis, and suggests a previously unanticipated role for a key aspartic acid residue in the enzyme.

hOGG1 employs a covalent catalysis mechanism in which the ϵ-amino group of Lys 249 attacks C-1′ of oxoG, thereby expelling the oxoG base[5] (Figure 1). A key question concerns the mechanism by which the enzyme activates the nucleophilicity of Lys 249. This issue is obscured in the X-ray structure of the mutant hOGG1-DNA complex[4] by the presence of glutamine (Q) in place of lysine (K) at position 249 (K249Q hOGG1), which was introduced to enable the formation of a stable recognition complex. To illuminate details of the active-site structure and function, we decided to cocrystallize the wild-type hOGG1 with a "mutant" substrate to which the enzyme can bind but not perform catalysis.[6] DNA containing the abasic tetrahydrofuranyl (THF) moiety[7] in DNA is one such substrate-based inhibitor of hOGG1, binding the enzyme with an equilibrium dissociation constant of 0.01 nM (S.D.B. and G.L.V., unpublished results). We therefore attempted to cocrystallize a ternary complex containing wild-type hOGG1, oxoG base, and a 16-mer duplex containing a single THF residue.[8] Diffraction data from the resulting crystals were used to solve the structure and refine it to a resolution of 2.6 Å.[9]

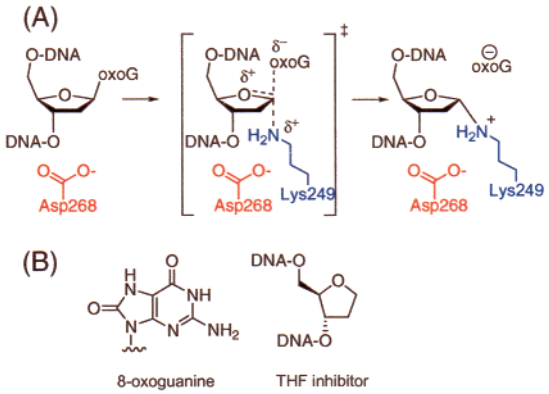

Figure 1. Putative mechanism of base excision catalyzed by human oxoguanine DNA glycosylase (hOGG1) and structures of the oxoG substrate and tetrahydrofuranyl (THF) abasic inhibitor.

The global structure of the THF−hOGG1 complex is similar to that of K249Q hOGG1, with a root-mean-square deviation of 0.59 Å for the protein backbone (Figure 2). The most significant differences between the two structures lie in the region of the base-recognition pocket. Specifically, electron density corresponding to the oxoG base is absent in the THF−hOGG1 structure,[10] despite the presence of oxoG in the crystallization medium. Vacancy of the oxoG recognition pocket is accompanied by local adjustments of the protein backbone and of several amino acid side-chains (Figure 2C). In the K249Q structure, Phe 319 π-stacks with oxoG, and His 270 hydrogen bonds to the phosphate on the 5′-side of oxoG. In the absence of oxoG, the O-helix is shifted away from the active site (see Supporting Information), resulting in retraction of Phe 319. Furthermore, His 270 is no longer hydrogen-bonded to its phosphate but instead is swiveled to π-stack with the Phe 319 aryl ring. The hydrocarbon side-chain of Gln 315, the residue that interacts with the Watson−Crick face of oxoG, is relaxed from a *gauche* to *trans* conformation in the absence of the base, with its amide headgroup extended slightly into the base recognition pocket (Figures 2C and 3). A significant backbone rearrangement involving Pro 40 and Ser 41 of the α-A/β-B loop is also evident, but neither the driving force nor the consequences of this change are immediately apparent from the structure. The absence of oxoG from the base-recognition pocket does not lead to collapse of the pocket but instead results in its expansion (Figure 3).

The overall conformation of the DNA in the THF−hOGG1 complex, including the helical bend of ∼70°, is nearly identical to that in the oxoG−K249Q complex.[4] The only significant difference is a crankshaft motion involving the C4′−C5′−O5′−P bonds on the 5′-side of the lesion, which results in a ∼30° change in the tilt angle of the plane of the sugar ring (Figure 2C). The conformation about these bonds in the THF structure, although typical of B-form DNA,[11] would engender a steric clash between the 8-oxo carbonyl of oxoG and O5′, whereas the atypical conformation seen in the K249Q structure avoids such a clash. Such

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(8) The protein was prepared as described in ref 4. Full experimental details are given in the Supporting Information

(9) Diffraction data collected at the 19-ID beamline at APS on a 3 × 3 Mosaic CCD detector were indexed and scaled using DENZO/SCALEPACK (Otwinowski, Z. M. W. *Methods Enzymol.* **1996**, *276*, 307−326). The structure was solved using CNS (Brunger, A. T. *Acta Crystallogr.* **1998**, *D 54*, 905−921.) by molecular replacement using the K249Q hOGG1 complex as a model. The structure was refined to $R = 0.22$ and $R_{free} = 0.27$. The coordinates for this structure are deposited in the RCSB Protein Data Bank, accession number 1FN7. See the Supporting Information for additional information and statistics.

(10) Additional details and graphics are available in the Supporting Information.

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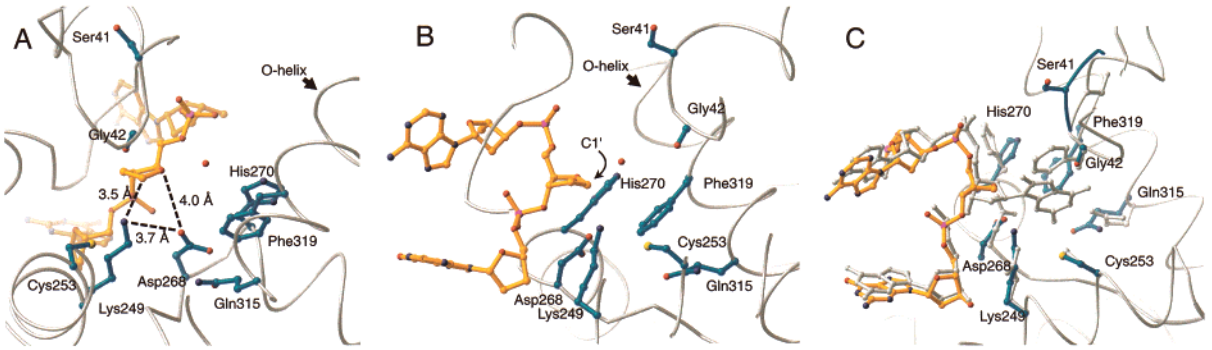

Figure 2. Active-site structure of hOGG1 bound to DNA. (a and b) Orthogonal views of the THF−hOGG1 structure, related by 90° rotation about the vertical axis. The protein backbone is presented as an α-carbon trace in gray; side-chains of key residues are shown in blue. The DNA is shown in gold. An ordered water molecule in the active site is depicted as a red sphere. (c) Least-squares superposition of the K249Q X-ray structure (ref 4, gray) with relevant parts of the THF−hOGG1 X-ray structure (this work; DNA, gold; protein side chains, blue). A short loop containing Ser 41 is also shown in blue, as the conformation of this loop differs significantly in the two structures. The retraction of the O-helix, not shown in (c), can be seen in the Supporting Information.

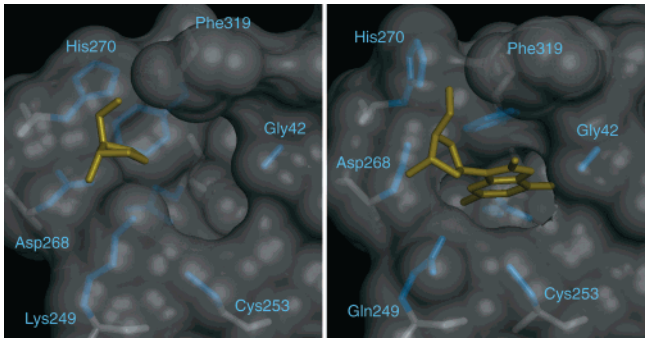

Figure 3. View of the solvent accessible surface looking into the base-recognition pocket of the K249Q structure (left, ref 4) and the THF−hOGG1 structure (right, this work). Note the expansion of the base-recognition pocket in the absence of oxoG.

conformational changes involving the sugar may contribute thermodynamically to specific recognition of the correct base.

The structure of wild-type hOGG1 in complex with the THF inhibitor clearly shows the active-site nucleophile, Lys 249, extending toward C1′, with the ϵ-amino group being well-positioned for in-line attack, both in terms of distance (3.5 Å) and trajectory (Figure 2A). Asp 268, the only active-site residue that is absolutely conserved in all members of the protein superfamily to which hOGG1 belongs,[12−14] has been suggested to activate Lys 249 by deprotonating it to the free amine. Unexpectedly, however, Asp 268 is far removed from Lys 249, with the closest N−O distance being 3.7 Å. Furthermore, Asp 268 appears to have lost the hydrogen-bonding interaction with His 270 observed in the K249Q structure (Figure 2A). In an attempt to simulate the actual Michaelis complex, we modeled Lys 249 from the THF−hOGG1 structure into the K249Q structure. This model[10] has two noteworthy features: (i) Asp 268 lies well outside hydrogen-bonding distance from Lys 249 (4.9 Å) and in fact is closer to the endocyclic sugar oxygen (O1′, O−O distance 3.2 Å), and (ii) Lys 249 makes a very close approach to C1′ (2.5 Å).

The present analysis has several significant implications for understanding the mechanism of base excision by hOGG1. First, it appears that the catalytic apparatus of hOGG1 is assembled properly only upon insertion of a cognate base into the oxoG recognition pocket. This draws the O-helix and Phe 319 inward, freeing His 270 to hydrogen bond with the 5′-phosphate and Asp 268, thereby positioning this critical amino acid side-chain for catalysis. Insertion of a noncognate base into the active site would be unlikely to trigger the same sequence of events, owing to poor chemical complementarity with the residues lining the base-recognition pocket. Second, the structural evidence neither supports nor refutes the notion that Asp 268 acts as a general base to deprotonate Lys 249. The data do suggest that another plausible role for Asp 268 should be considered, namely electrostatic stabilization of the developing positive charge developing in the transition state on the deoxyribose moiety,[15] especially at O1′ and C1′ (Figure 1A). Finally, proper insertion of oxoG into its recognition pocket on hOGG1 delivers C1′ precisely to Lys 249 and appears to compress these reactive centers together, thus providing both enthalpic and entropic driving force for the reaction to ensue.

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Supporting Information Available: Backbone least-squares superpositions, difference electron density maps, modeled active site of the complex formed between wild-type hOGG1 and oxoG-containing DNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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