Disulfide Trapped Structure of a Repair Enzyme Interrogating Undamaged DNA Sheds Light on Damaged DNA Recognition

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The integrity of the genome is constantly threatened in living cells by spontaneous alterations in the chemical structure of DNA that are caused by endogenous and exogenous agents. DNA repair pathways counteract these modifications and restore DNA to its undamaged state. Enzymes known as DNA glycosylases initiate the process, called base excision repair (BER), by locating a damaged nucleobase in the genome and then excising it from double-stranded (duplex) DNA. But how DNA glycosylases discriminate between very few damage sites amongst a huge amount of normal DNA is not well understood. We have used an efficient trapping strategy to capture a human repair protein, called 8-oxoguanine DNA glycosylase I (hOGG1), in the act of interrogating normal DNA for damage. By combining the structures of the protein-DNA complexes with free-energy calculations, we have gained novel insights into the mechanism by which hOGG1 is able to discriminate between normal and damaged DNA, and is able to prevent aberrant cleavage of normal DNA.

In humans, the DNA glycosylase hOGG1 is responsible for recognizing and removing 8-oxoguanine (oxoG), which is an oxidized form of the DNA base guanine (G) (Figure 1c). In general, DNA glycosylases bind at the site of the damaged base (lesion base), bend the duplex DNA considerably, and flip the lesion out in an active site pocket so that excision may occur (Figure 2, left). To understand how hOGG1 discriminates between the substrate oxoG and its normal counterpart G, which differ by only two atoms, it is imperative to obtain a three-dimensional structure of hOGG1 bound to undamaged DNA and compare it with existing oxoG-bound structures. The challenge lies in obtaining a homogeneous complex of hOGG1 with undamaged DNA in solution in the absence of a damaged base in the DNA to fix the “binding register.” This problem was solved using the “disulfide crosslinking” technique (Figure 3a) that we have developed, which stabilizes protein-DNA complexes that would otherwise be transient. We use existing structural and/or biochemical data to decipher sites in which the protein and the DNA are in close proximity. The residue on the protein is then mutated to the amino acid cysteine and a disulfide-bearing tether is introduced on the DNA.

In the case of hOGG1, we reasoned that if the normal base G is extruded from the DNA helix — even transiently — we should be able to implant a disulfide bond between a tether emanating from G’s partner base, cytosine (also called estranged C), and Asn149 (the amino acid asparagine, which is mutated to cysteine). Asn149 is one of the residues involved in recognizing the estranged C when the duplex DNA is invaded by hOGG1 in the complex with oxoG-containing DNA (Figure 3b,c).

The structure of the crosslinked complex of hOGG1 with undamaged DNA containing a G (G-complex) (Figure 2, right) resembled the structure of hOGG1 with oxoG-containing DNA (oxoG-complex) (Figure 2, left) that was solved earlier in our lab. However, the G residue, in spite of being extruded from duplex DNA, is rejected from entering the active site and lies at an alternative extrahelical site (exo-site). This explains how hOGG1 is able to scan long lengths of DNA without
accidentally excising normal bases. A superposition of the DNA molecules in the two structures reveals that large rotations about three backbone bonds in the DNA are enough to shift the G from the exo-site into the active site, leading us to propose that the G-complex is probably analogous to a late-stage intermediate in the base-extrusion pathway of oxoG. Contacts between the phosphate backbone of the DNA and the protein are almost identical on the 3’ side of the oxoG/G. On the 5’ side, however, the sole DNA contact (mediated by histidine, His270) is dislodged in the G-complex, leading to an over-twisted and considerably different conformation of the DNA on that side (Figure 1a).

Free-energy calculations using the G-complex and the oxoG-complex of hOGG1 help reveal how hOGG1 discriminates between oxoG and G. Computational work indicates that Lys249 (lysine), which participates in the excision reaction, forms a salt bridge with a conserved cysteine residue at the active site. Further analysis shows, quite unexpectedly, that the favorable electrostatic interaction of the salt-bridge dipole with oxoG and the unfavorable interaction with G (Figure 1b) is a major factor contributing to hOGG1’s discrimination between G and oxoG. Although G is denied entry into the active site, computational data suggested that analogs of G (with a C-H replacing a nitrogen atom, N7) should be stabilized in the active site. We solved the structures of hOGG1 crosslinked to DNA containing analogs of guanine (Figure 1c). Indeed, the analogs were inserted into the active site at exactly the same place as oxoG.

Figure 2. A comparison of the overall structures of the trapped complexes obtained with oxoG-containing (top) or G-containing (bottom) DNA. Both protein and DNA are represented as backbone ribbon traces, with the protein in cyan and the DNA in gold. The estranged C (magenta) and oxoG or G (red) are rendered in ball-and-stick representations. Note that oxoG is bound in the lesion-recognition pocket, while the G is bound at the alternative extrahelical site.

Figure 3. An overview of the strategy for obtaining a complex with G-containing DNA. (a) Based on the structure of the recognition complex, crosslinking sites are chosen first. The crosslinking strategy is validated by inspecting the structure of the crosslinked complex with oxoG-containing DNA and ensuring the absence of crosslinking-induced structural perturbations. Finally, oxoG in the DNA is replaced by G to obtain a crosslinked complex. (b) Details of the crosslinking strategy used in this case. The Asn149 contact with the estranged C was replaced with a disulfide crosslink by introducing a Cys149 point mutation and synthetically modifying DNA by introducing a tether with a disulfide linkage on the exocyclic amine. Note that introducing the tether does not perturb the Watson-Crick edge of the C as evident from existing structural information on similarly tethered DNA duplex. (c) The sequence of the DNA duplex. The crosslinking site is on the complementary strand of the oxoG/G-containing strand.

Figure 1. (a) Superposition of the oxoG-complex with the G-complex in the region around the protein/DNA interface. The overlay uses the protein backbone only (gray) for superposition, with the DNA backbone of the oxoG-complex in green and the G-complex in gold. Spheres are Ca$^{2+}$ ions. The residues that interact with DNA through the backbone amid nitrogen atoms are denoted in magenta, and those that interact through side-chains are shown in black. Dotted lines are hydrogen bonds. (b) The electrostatic potential difference (from computational data) between oxoG and G. Regions of positive charge are in blue and negative regions are in red. Dipoles are in cyan, with Mulliken charges indicated. (c) A structural representation of the expected interactions between the main-chain carbonyl Gly42 and nucleobases examined in this study. Whereas oxoG is known to hydrogen-bond with Gly42, the lone pair of electrons on G produces a repulsive interaction with the lone pairs on Gly42. 7-deazaG and 7-deaza-8-aza-G are analogs of G.