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Two sequential phosphates 3′ adjacent to the 8-oxoguanosine are crucial for lesion excision by *E. coli* Fpg protein and human 8-oxoguanine-DNA glycosylase

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Abstract

Escherichia coli formamidopyrimidine-DNA glycosylase (Fpg) and human 8-oxoguanine-DNA glycosylase (hOGG1) are base excision repair enzymes involved in the 8-oxoguanine (oxoG) repair pathway. Specific contacts between these enzymes and DNA phosphate groups play a significant role in DNA–protein interactions. To reveal the phosphates crucial for lesion excision by Fpg and hOGG1, modified DNA duplexes containing pyrophosphate and *OEt*-substituted pyrophosphate internucleotide (SPI) groups near the oxoG were tested as substrate analogues for both proteins. We have shown that Fpg and hOGG1 recognize and specifically bind the DNA duplexes tested. We have found that both enzymes were not able to excise the oxoG residue from DNA containing modified phosphates immediately 3′ to the 8-oxoguanosine (oxodG) and one nucleotide 3′ away from it. In contrast, they efficiently incised DNA duplexes bearing the same phosphate modifications 5′ to the oxodG and two nucleotides 3′ away from the lesion. The effect of these phosphate modifications on the substrate properties of oxoG-containing DNA duplexes is discussed. Non-cleavable oxoG-containing DNA duplexes bearing pyrophosphate or SPI groups immediately 3′ to the oxodG or one nucleotide 3′ away from it are specific inhibitors for both 8-oxoguanine-DNA glycosylases and can be used for structural studies of complexes comprising a wild-type enzymes bound to oxoG-containing DNA.

Keywords: Fpg; hOGG1; 8-Oxoguanine; Modified DNA duplexes; Pyrophosphate and substituted pyrophosphate internucleotide groups

1. Introduction

Escherichia coli formamidopyrimidine-DNA glycosylase (Fpg, also known as MutM) and human 8-oxoguanine-DNA glycosylase (hOGG1) are DNA repair enzymes that catalyze the removal of 8-oxoguanine (oxoG) residues and cleave the DNA strand [1–3]. OxoG is a major mutagenic base lesion produced in DNA by reactive oxygen species that are generated by cellular metabolism, cell injury and exposure to physical and chemical oxygen radical-forming agents [4,5]. It is a miscoding lesion since it pairs preferentially with adenine rather when cytosine and induces GC→TA transversions in vivo and in vitro [6,7]. The physiological function of these repair enzymes is to prevent the mutagenic effects of oxoG residues in DNA and to maintain the genome integrity.

Fpg protein and hOGG1 have identical substrate specificity, but various cleavage mechanisms and molecular architecture [8]. Recently, several co-crystal structures of hOGG1 and Fpg proteins from different species associated with abasic analogues of damaged DNA ligands have been solved [9–11]. The high-resolution structures containing mutant recognition-competent but catalytically inactive ΔP1 and E3Q forms of Fpg proteins from *Lactococcus lactis* and *Bacillus stearothermophilus* as well as K249Q, D268 E, D268 N, and D268Q mutant forms of hOGG1 in complex with lesion-containing DNA have been elucidated [12–15]. It has been proved that

Abbreviations: AP sites, abasic sites; Fpg, *Escherichia coli* formamidopyrimidine-DNA glycosylase; hOGG1, human 8-oxoguanine-DNA glycosylase; oxoG, 8-oxoguanine; oxodG, 8-oxo-2′-deoxyguanosine; SPI groups, substituted pyrophosphate internucleotide groups; UDG, uracil-DNA glycosylase.

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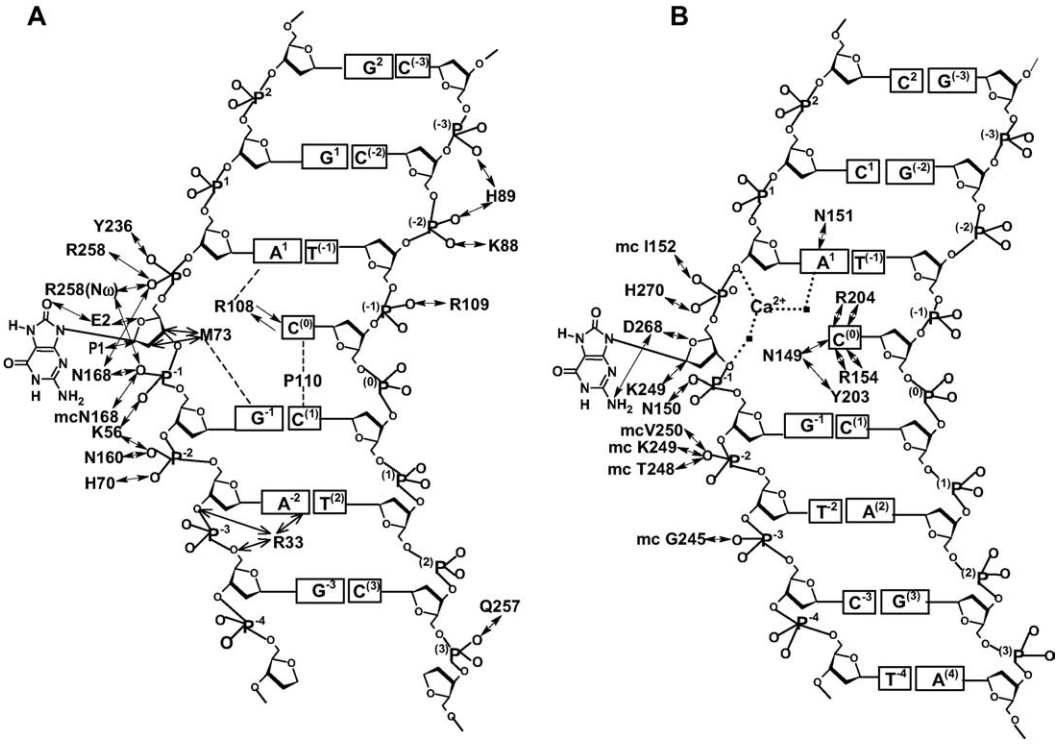

Fig. 1. Schematic representation of interactions between the DNA phosphate groups and Fpg (**A**) or hOGG1 (**B**). The central nucleotides of the modified and complementary strands are 8-oxoG^0 and C$^{(0)}$, respectively. Nucleotides are numbered as shown with those in the complementary strand in parentheses. The interactions are deduced from the crystal structures of wild-type Fpg and hOGG1 bound to abasic DNAs (1K82 and 1FN7 in the Protein Data Bank) and mutant forms of Fpg and hOgg1 bound to oxoG-containing DNA (1R2Y, 1N3C and 1EBM). •, Water molecule. mcK249 refers to main chain of Lys249. Y236 refers to side chain of Tyr236, and R258(Nω) refers to the terminal nitrogen atom of Arg258. The arrow ⟷ illustrates the hydrogen bonds, which are ≤3.5 Å in length; ↔, van der Waals contacts; ----, π-interactions; ·····, electrostatic interactions.

binding to DNA involves extensive interactions between the enzymes and DNA. Specific contacts with DNA phosphate groups play a significant role in DNA–Fpg and DNA–hOGG1 interactions [8,14]. The relevant enzymes-phosphate contacts seen in crystal structures are illustrated in Fig. 1. As follows from Fig. 1, both enzymes mainly contact the damaged strand of DNA. Several amino acid residues in hOGG1 form specific contacts with p^{-1}, p^{-2} and p^{-3} phosphates of the damaged DNA. On the 5′ side of the oxodG residue, two contacts are formed with p^0, but hOGG1 makes no further backbone contacts. Fpg also mainly contacts the damaged strand 3′ to the abasic residue via side chains and backbone amides of highly conserved basic amino acid residues [8] (Fig. 1). Recently, using chemical crosslinking, we have identified specific contacts between nucleophilic amino acids in Fpg and DNA phosphate groups 5′ and 3′ adjacent to the oxodG moiety [16].

Although the folds of Fpg and hOGG1 are completely unrelated, their mechanisms of cleavage are different and different residues are used as the catalytic nucleophile, the similarity in the interaction of these proteins with DNA can be revealed [8,14]. In particular, both enzymes have similar values of the contact area between protein and DNA (2512 Å for Fpg protein and 2268 Å for hOGG1) and possess some local structural similarity in the core of their active sites. They partially unwind the DNA, creating a sharp kink in the substrate. The enzymes extrude oxoG out of the DNA helix and insert the extrahelical base into the active site pocket. In addition, both enzymes primarily utilize the phosphate groups 3′ to the lesion site to form specific contacts with the DNA backbone. Based on these data, we have suggested that modifications of the contacting phosphates, e.g. their replacement with related internucleotide groups can greatly affect the activity of both repair enzymes and might be crucial for lesion excision. Previously, we have demonstrated that Fpg was not capable of cleaving DNA duplexes that contained pyrophosphate or substituted pyrophosphate internucleotide (SPI) groups 3′ adjacent to the oxodG [16]. In the present work, modified DNA duplexes carrying these alterations 3′ and 5′ to the oxodG and one and two nucleotides 3′ away from it have been tested as substrate analogues for both Fpg protein and hOGG1. The mechanism of their interaction with enzymes has been investigated. As a result, we have identified the phosphate groups that are crucial for lesion excision by Fpg and hOGG1.

2. Materials and methods

2.1. Oligonucleotides

Oligonucleotides and DNA duplexes used in this study are shown in Fig. 2. The oxoG residue was introduced into the oligonucleotides forming DNA duplexes I–V and IIpp by a standard phosphoramidite procedure using an Applied Bio-