Depurination of N7-Methylguanine by DNA Glycosylase AlkD Is Dependent on the DNA Backbone

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S *Supporting Information*

ABSTRACT: DNA glycosylase AlkD excises N7-methyl-guanine (7mG) by a unique but unknown mechanism, in which the damaged nucleotide is positioned away from the protein and the phosphate backbone is distorted. Here, we show by methylphosphonate substitution that a phosphate proximal to the lesion has a significant effect on the rate enhancement of 7mG depurination by the enzyme. Thus, instead of a conventional mechanism whereby protein side chains participate in N-glycosidic bond cleavage, AlkD remodels the DNA into an active site composed exclusively of DNA functional groups that provide the necessary chemistry to catalyze depurination.

DNA glycosylases liberate aberrant nucleobases from the genome by catalyzing the hydrolysis of the N-glycosidic bond. Most glycosylases flip the target nucleobase into an active site that contains conserved side chains necessary for general acid–base catalysis (Figure 1A).[1] Kinetic isotope effects and quantum
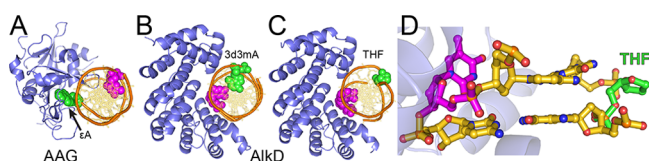

Figure 1. AlkD traps the lesion away from the protein. (A) Crystal structure of human alkyladenine DNA glycosylase (AAG) in complex with 1,N^6-ethenoadenine (εA)-DNA. (B and C) Crystal structures of AlkD in complex with (B) 3-deaza-3-methyladenine (3d3mA)-DNA and (C) tetrahydrofuran (THF)-DNA. The modified base pairs are rendered as CPK spheres, with εA, 3d3mA, and THF colored green and the opposing thymidine colored magenta. (D) Side view of the extrahelical THF-T bulge trapped by AlkD (blue).

mechanical calculations for several monofunctional DNA and RNA glycosylases are consistent with a dissociative (D_N*A_N) mechanism involving a cationic oxocarbenium intermediate that is converted to an abasic site by a water nucleophile.[2−7] Catalysis by a variety of glycosylases is driven largely by a conserved carboxylate side chain, which can electrostatically stabilize the oxocarbenium intermediate and/or activate the water nucleophile.[8−11] In the case of purine excision, a general acid protonates N7 to activate the nucleobase leaving group.[4−7,12−15] In addition to protein functional groups, the DNA backbone has been shown to play a role in base excision by several enzymes.[4,7,16−18] The

best studied example is human uracil DNA glycosylase, in which DNA phosphates promote glycosidic bond cleavage by stabilizing the charge or conformation of the oxocarbenium intermediate.[4,7,16,17,19−21]

N3- and N7-alkylated purine nucleobases are highly detrimental to the cell.[22,23] 3mA and a ring-opened formamido-pyrimidyl derivative of 7mG, 2,6-diamino-4-hydroxy-N^5-methyl-formamidopyrimidine, are cytotoxic by virtue of their ability to inhibit DNA synthesis.[24−26] As a consequence of their positively charged purine rings, 3mA and 7mG are highly susceptible to spontaneous depurination, leading to formation of abasic sites that are both cytotoxic and mutagenic.[27−29] Thus, glycosylase excision of cationic 3mA and 7mG does not require activation by a general acid or a substantial amount of catalytic power.[6] Interestingly, most 3mA-specific glycosylases retain excision activity in the absence of specific catalytic residues, suggesting that direct side chain chemistry does not fully account for the observed rate enhancements by these enzymes.[30−34] Because spontaneous depurination rates of N7-alkylguanines depend on the DNA secondary structural context,[14,35−37] it is reasonable to postulate that the specific DNA conformation in the vicinity of the lesion contributes to excision of these adducts, although this idea has not been explored in any detail.

We recently determined several crystal structures of a unique 3mA/7mG DNA glycosylase, AlkD, in complex with alkylpurine, mismatched, and abasic DNA, all of which exhibit the same general protein–DNA binding regime.[34,37−40] AlkD does not flip the lesion into a binding pocket but instead binds the undamaged DNA strand and positions the lesion on the opposite face of the DNA helix from the protein binding surface (Figure 1B,C). Most strikingly, there are no contacts between the protein and the lesion. The alkylpurine and mismatched base pairs are highly sheared but remain stacked in the duplex, whereas the abasic site and its opposite nucleotide are rotated out of the helix to create a one-nucleotide bubble with the flanking base pairs stacked (Figure 1D). This distortion to the DNA backbone positions the flipped ribose ring closer to the phosphate of the nucleotide immediately 5′ to the lesion [position M1 (Figure 2)]. The distance between this M1 phosphate and C1′ of the flipped nucleotide is 20% shorter than in the unflipped AlkD alkylpurine/mismatch complexes and in normal B-DNA (Figure S3 of the Supporting Information).
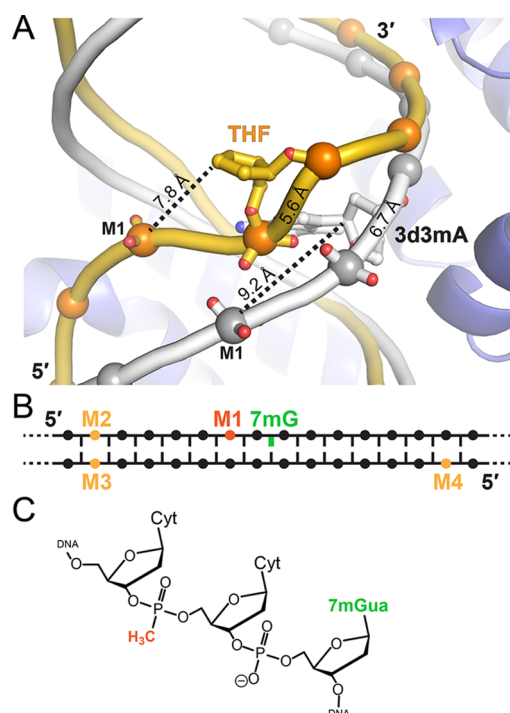
Figure 2. (A) Overlay of AlkD THF-DNA (gold) and 3d3mA-DNA (gray) complexes. The phosphate 5′ to the lesion is designated M1. Distances between M1 and C1′ of the lesion are marked with dashed lines, and distances between the phosphates covalently attached to the lesion are shown on the backbone. (B) Schematic showing the relative positions of 7mG and MeP substitutions used in this study. (C) Chemical structure of MeP in the M1 sequence context.

Table 1. Rates of AlkD-Catalyzed and Spontaneous Depuration of 7mG from MeP-DNA

	k_{st} $(\times 10^{-3}\ s^{-1})^a$	k_{non} $(\times 10^{-6}\ s^{-1})^a$	k_{st}/k_{non}
M0	23.6 ± 1.1	1.0 ± 0.1	2.4×10^4
M1	1.6 ± 0.4	1.8 ± 0.2	8.9×10^2
M2	23.6 ± 1.1	1.0 ± 0.1	2.4×10^4
M3	23.2 ± 1.2	1.1 ± 0.03	2.1×10^4
M4	7.7 ± 0.5	1.1 ± 0.1	7.0×10^3

^{a}Single-turnover (k_{st}) and nonenzymatic (k_{non}) rate constants for depuration of 7mG from 25-mer oligonucleotides at 37 °C, pH 7.5, and an ionic strength of 150 mM. M1−M4 each contained one MeP at the positions shown in Figure 2B. Data for M0 (no MeP) were taken from ref 37. DNA sequences and kinetic data can be found in the Supporting Information.

The skewed DNA conformation and the absence of protein contacts to the lesion in the AlkD-DNA complex led us to hypothesize that the phosphate backbone plays a substantial role in 7mG depuration. To test this, we measured the rates of AlkD-catalyzed and spontaneous release of 7mG from oligonucleotides containing nonbridging methylphosphonate (MeP) substitutions at various phosphate positions (Figure 2B). MeP eliminates the negative charge at that position (Figure 2C) and was a key strategy in determining the importance of phosphates to UDG catalysis.[16,17] We introduced a MeP at the proximal nucleotide 5′ to the 7mG (M1), and as controls for general effects of MeP substitution, at three sites located six nucleotides from the lesion and outside of the protein binding region on both damaged (M2) and undamaged (M3 and M4) strands (Figure 2B).

Compared to the substrate containing no MeP (M0), control sequences M2 and M3 had no effect on either the AlkD single-turnover (k_{st}) or nonenzymatic (k_{non}) rates of 7mG depuration, and M4 had an only modest (3-fold) effect on k_{st} (Table 1 and Figure S4 of the Supporting Information). In contrast, the M1 substitution resulted in a 15-fold reduction in AlkD activity and a 30-fold (96%) reduction in rate enhancement (k_{st}/k_{non}) compared to those of M0 (Table 1). Thus, excision of 7mG by AlkD is largely dependent on the conformation of the phosphate backbone in the vicinity of the lesion. Interestingly, the modest increase in k_{non} for M1-MeP is similar to that of 7mG depuration from single-stranded DNA $(2.8 \times 10^{-6}\ s^{-1})$,[37] suggesting that the MeP substitution perturbs the secondary structure of the unbound DNA. Regardless of this effect, the larger effect of MeP substitution on the AlkD-catalyzed rate versus the spontaneous rate of 7mG depuration indicates that the 96% reduction in M1/M0 rate enhancement is attributed to the specific enzyme-DNA complex and not to an effect on the DNA alone.

The residual 7mG depuration activity in the M1 substrate may stem from the phosphates immediately 5′ and 3′ to the lesion, which reside 5 Å from C1′ of the lesion in the flipped THF complex (Figure S3 of the Supporting Information). However, we were unable to substitute MeP at these positions because of the manner in which the 7mG substrate was prepared (see the Supporting Information). Although these flanking phosphates are closer to the lesion than M1, only M1 moves closer to the lesion upon base flipping (Figure S3 of the Supporting Information). The modest reduction in the level of excision of 7mG from the M4 substrate is consistent with preferential binding of the enzyme to the unmodified strand and may reflect a preference of the enzyme to initially bind the 5′ ends of an oligonucleotide.

Given the absence of protein functional groups in the vicinity of the lesion, we conclude that catalysis of 7mG excision by AlkD is driven by the DNA and speculate that the proximal phosphates facilitate depuration by either stabilizing the oxocarbenium intermediate or positioning the attacking water. Introduction of MeP, which eliminates the negative charge and replaces a polar oxygen with an aliphatic methyl group, could have effected either the electrostatic environment or the specific conformation of the DNA. The decrease in the distance from the M1 phosphate to the lesion upon base flipping suggests that the observed effect of MeP substitution may have disrupted a stabilizing electrostatic interaction between the anionic phosphate backbone and the oxocarbenium (Figure 3). For this to be true, however, the M1 phosphate would need to be closer to the lesion than in the THF structure (observed distance of 7.8 Å), and it is not unreasonable to postulate that a cationic 3mA or 7mG substrate would have such an additional distortive effect. Alternatively, the phosphate group may stabilize a particular DNA conformation necessary for catalysis. Indeed, the nucleic acid secondary structure is the basis for designed DNAs (DNAzymes) that catalyze various metal-dependent RNA cleavage and DNA ligation reactions.[41,42] In addition to a direct interaction, the phosphate could deprotonate or stabilize any developing positive charge on the attacking water. Regardless of the specific mechanism employed, this work, together with the crystal structures, establishes that the specific conformation of the DNA backbone captured by the enzyme positions the phosphate for catalysis. To the best of our knowledge, this is the first example of an active site composed exclusively of DNA atoms.
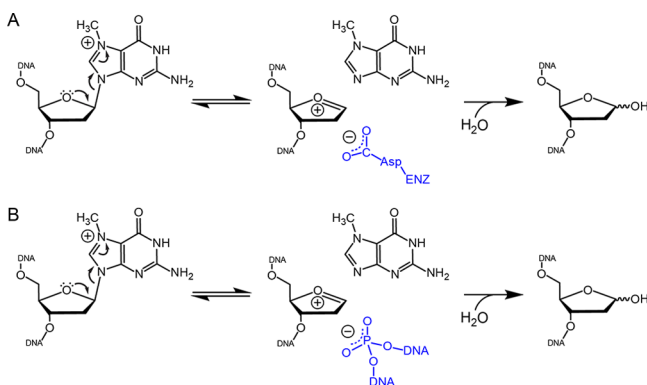
Figure 3. Protein and/or DNA functional groups provide an anionic environment to drive base excision. The chemical mechanism of 7mG depurination is shown, with stabilization of the oxocarbenium reaction intermediate by (A) a conserved carboxylate side chain and (B) a DNA phosphate moiety.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and representative kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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