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Rapid Report

N-Glycosyl Bond Formation Catalyzed by Human Alkyladenine DNA Glycosylase[†]

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ABSTRACT: The removal of damaged bases by DNA glycosylases is thought to be effectively irreversible, because of an overall equilibrium that favors hydrolysis over synthesis of the *N*-glycosyl bond. Surprisingly, human alkyladenine DNA glycosylase (AAG) can make damaged DNA by catalyzing formation of an *N*-glycosyl bond between 1,N^6-ethenoadenine (εA) and abasic DNA. We attribute the ready reversibility of this glycosylase reaction to the exceptionally tight binding and slow subsequent hydrolysis of DNA containing an εA lesion. In principle, reversibility could provide a mechanism for direct reversal of base damage by a DNA glycosylase, allowing the glycosylase to bypass the rest of the base excision repair pathway.

The equilibria for hydrolysis of the *N*-glycosyl bonds of nucleotides and nucleosides favor their nucleobase and ribose products (*1*, *2*). Indeed, de novo and salvage pathways for nucleotide biosynthesis typically use 5-phosphoribosyl-α-pyrophosphate (PRPP) as a substrate for *N*-glycosyl bond formation, as its utilization is driven by the subsequent hydrolysis of released PP_i. Base excision repair DNA glycosylases hydrolyze the analogous *N*-glycosyl bond between a damaged base and C1 of a deoxyribosyl moiety, giving rise to a free base and an abasic site within DNA (*3*). Although the overall equilibrium for this reaction favors hydrolysis, human alkyladenine DNA glycosylase (AAG) unexpectedly synthesizes 1,N^6-ethenodeoxyadenosine (εdA) within DNA when supplied with 1,N^6-ethenoadenine (εA) and abasic DNA. The internal equilibrium constant (K_{int}) for this enzymatic transformation is near unity.

Mutagenic εdA lesions form when adenine bases in DNA react with certain lipid peroxidation products or pollutants (*4*, *5*). AAG is the only human glycosylase known to excise εA from εdA-containing DNA, and its mechanism has been proposed to include fast two-step DNA binding, rate-limiting *N*-glycosyl bond hydrolysis, and rapid dissociation of εA (*6*). We explored possible inhibition of the AAG reaction by its εA product by adding the free base to AAG·εdA-DNA, where εdA-DNA is a 25 bp oligonucleotide that contains a centrally located εdA (Scheme 1). The single-turnover rate constant of 0.04 min^{-1} obtained for the AAG-catalyzed hydrolysis of εdA-DNA to ab-DNA in the absence of added εA matched the value of k_{hyd} measured previously [Figure 1A (■)] (*6*). Addition of 0.6 mM εA to an otherwise identical reaction resulted in faster, but only partial

Scheme 1: Duplex DNA Oligonucleotides Used in This Study

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5'-CGATAGCATCCTxCCTTCTCTCCAT
3'-GCTATCGTAGGATGGAAGAGAGGTA
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εdA-DNA: x = εdA
ab-DNA: x = deoxyribose

(~70%), conversion of εdA-DNA to ab-DNA [Figure 1A (□)], whereas a slower reaction that proceeded to completion was expected for simple product inhibition of AAG by εA.

We tested the possibility that εA reacts reversibly with ab-DNA to regenerate εdA-DNA by adding free εA to AAG·ab-DNA. Reactions containing 0.6 mM εA that were initiated with either AAG·ab-DNA or AAG·εdA-DNA proceeded to the same end point of ~0.3 [Figure 1A (open symbols)], which shows the same equilibrium between AAG-bound species being approached from opposite directions. To verify that the plateau reached in the AAG·ab-DNA reaction represented the equilibrium between AAG·ab-DNA and newly synthesized AAG·εdA-DNA, we tried to perturb it predictably. We first increased the AAG·ab-DNA concentration while maintaining the εA concentration at 0.6 mM and followed the conversion of ab-DNA to εdA-DNA for 100 min, until the expected end point of ~0.3 had been attained. The reaction mixture was then diluted 25-fold, resulting in a mixture containing only 24 μM εA, and the decay of the putative εdA-DNA was followed to its new equilibrium position (Figure 1B). The rates of decay of the putative εdA-DNA and authentic εdA-DNA were the same within error (Figure 1B, inset), consistent with the model in which AAG converts ab-DNA and εA into εdA-DNA via direct reversal of the glycosylase hydrolysis step (Figure 2, k_{syn}).

We next investigated the dependence of the synthesis reaction on the concentration of εA. As shown in Figure 3A, reactions of AAG·ab-DNA with εA proceeded with faster initial rates to higher end points as the εA concentration increased. The reaction end points are plotted as a function of εA concentration in Figure 3B, and saturation with εA is observed with a $K_{1/2}$ of 0.9 mM. At saturating concentrations of εA, the fractional concentration of AAG·εdA-DNA is 0.7 (the maximum end point), and the fractional concentration of AAG·ab-DNA·εA is correspondingly 0.3, indicating that εdA-DNA is more stable than its hydrolysis products when bound to AAG. The internal equilibrium constant for these AAG-bound species is the ratio of their fractional concentrations (K_{int} = 0.3/0.7 = 0.4). K_{int} is also equivalent to k_{hyd}/k_{syn}, the ratio of the respective rate constants for formation of AAG·ab-DNA·εA and AAG·εdA-DNA from one another, so a value of 0.1 min^{-1} for k_{syn} can be calculated from K_{int} and the reported value of 0.04 min^{-1} for

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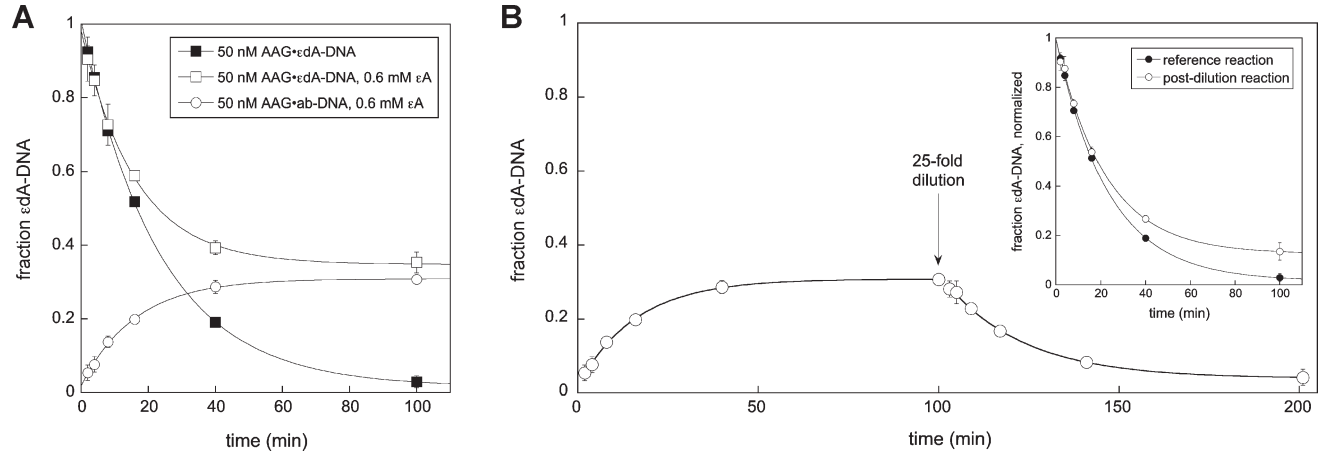
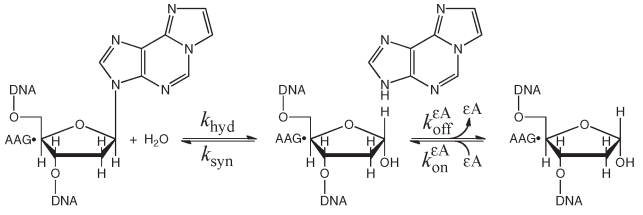


FIGURE 1: AAG synthesizes εdA-DNA from εA and ab-DNA. (A) The fraction of total DNA present as εdA-DNA in quenched samples from single-turnover reactions of AAG with εdA-DNA or ab-DNA was determined using a gel-based glycosylase assay (see the Supporting Information). Error bars indicate the standard deviation from the mean for at least two independent determinations, and lines are the best fits to single-exponential equations. (B) A single-turnover reaction containing 1.25 μM AAG·ab-DNA and 0.6 mM εA was followed to completion and then diluted 25-fold with buffer, resulting in a postdilution reaction of 50 nM AAG·DNA and 24 μM εA that was followed to its new equilibrium position. The inset shows the normalized postdilution reaction alongside a single-turnover reference reaction of 50 nM AAG·εdA-DNA. Although best fits of both inset reactions to single-exponential equations give k_{obs} values of 0.04 min^{-1}, representing the rate constant for single-turnover excision of εA from εdA-DNA (k_{hyd}), the postdilution reaction does not reach an end point of zero because of the residual εA that it contains. All reactions were performed at 25 °C and pH 6.5, and εdA-DNA reactions were corrected to account for the small percentage of ring-opened substrate that is refractory to AAG reaction (see the Supporting Information).



K_{int} = [AAG•ab-DNA•εA]/[AAG•εdA-DNA]
= k_{hyd}/k_{syn}

FIGURE 2: Model for reversible excision of εA from εdA-DNA by AAG under single-turnover conditions, where all the DNA present is bound to AAG.

k_{hyd} [Figure 1A (*6*)]. The best global fit of the data in Figure 3A to the model in Figure 2 gives slightly different values of 0.2 for K_{int} and 0.2 min^{-1} for k_{syn} (see the Supporting Information), so we report an average value of 0.3 ± 0.1 for K_{int}.

To the best of our knowledge, the conversion of ab-DNA and εA to εdA-DNA by AAG is the first example of *N*-glycosyl bond synthesis by a DNA glycosylase. We assessed the generality of this reverse reaction by investigating several related reactions. AAG did not insert either adenine or hypoxanthine into ab-DNA, despite extended incubation times with millimolar concentrations of each free base, nor was any reaction observed when a different DNA glycosylase, 3-methyladenine DNA glycosylase II (AlkA), was incubated with ab-DNA and εA (data not shown). Thus, the ability of AAG to stably synthesize εdA-DNA appears to be unusual. A related enzyme activity that inserts purines into depurinated DNA has been reported previously (*7*, *8*), and kinetic isotope effects for the DNA glycosylase MutY are consistent with a dissociative mechanism in which reversible C−N bond cleavage precedes irreversible nucleophilic attack by water (*9*).

Interestingly, the structure of AAG crystallized in the presence of an εdA-containing DNA substrate revealed an intact *N*-glycosyl bond (*10*). Although the authors proposed that the high concentration of $MgCl_2$ present during crystallization may have inhibited the glycosylase activity of AAG, our results raise the possibility that the substrate may have instead persisted because of the internal equilibrium that favors synthesis of εdA-containing DNA over its hydrolysis by AAG. The crystal structure shows εdA flipped into the AAG active site, securely held by aromatic stacking interactions and a hydrogen bond between the backbone amide of His-136 and N^6 of the nucleobase. This snug fit helps to account for the observed tight binding of εdA-containing DNA by AAG (*6*, *11*, *12*) and the highly favorable equilibrium for flipping εdA into the active site [$K_{flip} \sim 1300$ (*6*)]. The unusual stability of εdA-DNA when bound to AAG, which enabled us to detect its synthesis and to measure the internal equilibrium constant for its hydrolysis, presumably arises from its tight binding to the active site coupled with slow subsequent hydrolysis of its *N*-glycosyl bond. In contrast, human uracil DNA glycosylase (UDG) has been crystallized bound to its uracil and abasic DNA products (*13*, *14*), consistent with an internal equilibrium for UDG that favors hydrolysis of uracil-containing DNA (*15*).

As described above, results from reactions at saturating εA concentrations allowed determination of a value of 0.3 for K_{int}, the equilibrium constant for hydrolysis of the *N*-glycosyl bond of AAG-bound εdA-DNA. K_{int} values near unity are considered to be optimal for enzymes that operate reversibly (*16*). While it stands to reason that certain metabolic enzymes that act at the *N*-glycosyl bond may benefit from reversibility (e.g., nucleosidases and nucleoside phosphorylases), synthesis of εdA-containing DNA by AAG is expected to be negligible under typical conditions, because of low cellular concentrations of abasic DNA and εA. In principle, however, ready reversibility provides a potential mechanism for direct repair by DNA glycosylases, in which excision and dissociation of an aberrant base from DNA are

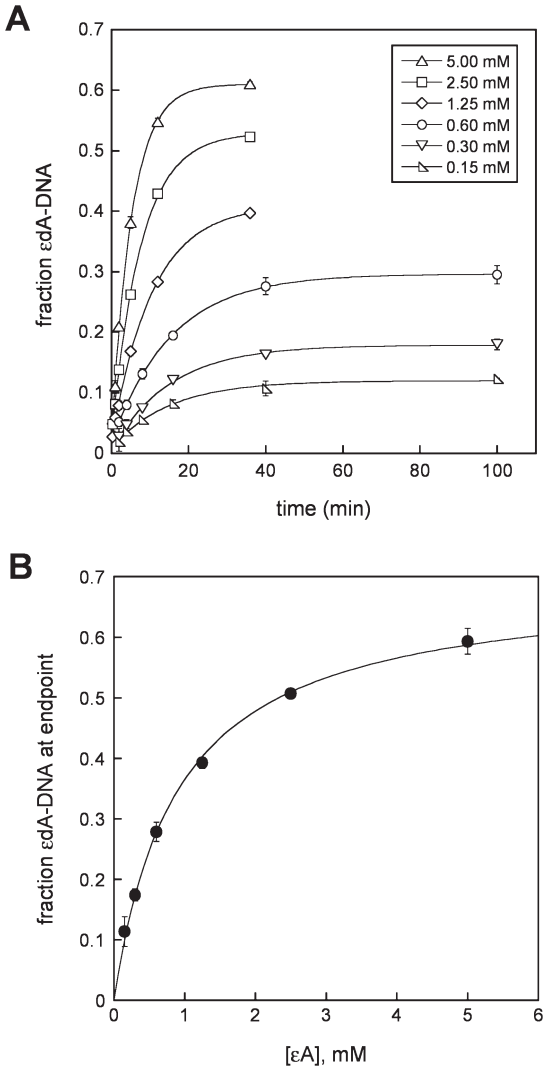


FIGURE 3: Dependence of AAG-catalyzed synthesis of εdA-DNA on the concentration of εA. (A) Reactions of 50 nM AAG·ab-DNA at varying εA concentrations, as indicated in the legend. Error bars indicate the standard deviation from the mean for at least two independent determinations, and lines are the best fits to single-exponential equations. All reactions were performed at 25 °C and pH 6.5. (B) Reaction end points from the curves in panel A plotted as a function of εA concentration. A maximum end point value of 0.7 and a $K_{1/2}$ of 0.9 mM were obtained from the best fit to a saturation equation: end point = [(end point$_{max}$)[εA]]/($K_{1/2}$ + [εA]).

followed by binding and stable incorporation of the appropriate base. Such a repair mechanism, if present in nature, would be reminiscent of the overall base exchange processes catalyzed by tRNA guanine transglycosylases, nucleotide deoxyribosyltransferases, and nucleoside ribosyltransferases (*17*, *18*).

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SUPPORTING INFORMATION AVAILABLE

Detailed experimental procedures and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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