Supplementary Information

Direct Repair of the Exocyclic DNA Adduct 1,N^6-Ethenoadenine by the DNA Repair AlkB Proteins

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Protein Purification and Oligonucleotide Synthesis

Wild type *E. coli* AlkB and human ABH3 were overexpressed in *E. coli* BL21(DE3) and purified as described previously.[1,2] Etheno-dA-CE phosphoramidite was purchased from Glen Research Inc. This base was incorporated into T(εA)T using a solid state DNA synthesizer (ABI Expedite 8909). TAT was also prepared as the standard for the repaired product. Both oligonucleotides were purified by denaturing polyacrylamide gel electrophoresis. The identities of the oligonucleotides were confirmed by mass spectrometry, and the concentrations were determined by UV-vis.

Assays for the Repair Reaction

For the reactions with T(εA)T, purified proteins were dialyzed into a buffer containing 50 mM MES (for pH 4.0 - 6.5) or 50 mM HEPES (for pH 7.0 – 8.5). These buffers were used for the repair reaction. *E. coli* AlkB (0.005 or 0.01mM) and T(εA)T (0.1 mM) were incubated together with 0.2 mM $Fe(NH_4)_2(SO_4)_2$ (Acros), 2.6 mM α-ketoglutarate (Sigma), 5.2 mM ascorbate (Aldrich) at 37 °C for varying amounts of time in the reaction buffer (100 μL). For human ABH3, 0.01 mM, 0.05 mM or 0.1 mM of protein and 0.1 mM of T(εA)T were used in the reaction at room temperature for 16 h at pH 6.0. After the reaction the product mixture was quenched with EDTA (Sigma) and analyzed directly by HPLC (Varian) or MALDI-TOF Mass Spectrometry (MALDI-MS, ABI Voyager System 6187). HPLC was carried out with a Varian Microsorb C-18 column (150 x 4.6 mm) and the elutant was analyzed using a Photodiode Array Detector. The column was run at 0.8 ml/min in 50 mM ammonium acetate (pH 6.5) for 5 min. A linear gradient from 0 to 45% MeOH over 45 min was applied to separate TAT and T(εA)T.

For the reactions with TTTTT(εA)TTTTT, purified proteins were dialyzed into a buffer containing 50 mM MES (pH 6.0) or 50 mM HEPES (pH 7.0). 0.1 mM TTTTT(εA)TTTTT was incubated with 0.005 mM AlkB for 30 min at 37 ^{o}C. The reaction was quenched with EDTA, and a published procedure was followed to digest the DNA to nucleosides.[3] Reaction buffers with 0.1 mM DNA standards (TTTTTT(εA)TTTTT and TTTTTATTTTT, with respective masses confirmed by MALDI) with no protein were used as controls to test the nucleoside formation. The product formation was analyzed by HPLC [4] using a Phenomenex C-12 column (250 x 4.6 mm) and the elutant was analyzed using a Photodiode Array Detector at 254 nm. The column was run at 1.0 ml/min in 250 mM ammonium acetate (pH 6.0) for 5 min. A linear gradient from 5 to 25% of 40% acetonitrile over 15 min was used to separate the nucleosides.

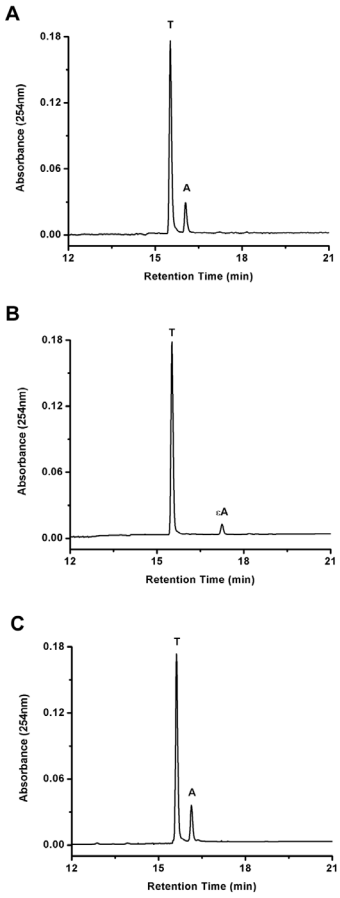

Figure S1. Reaction of AlkB with 11-mer DNA TTTTT(εA)TTTTT, observed with HPLC after enzymatic digestion. (A) 0.1 mM TTTTT(εA)TTTTT DNA standard after digestion into nucleosides. (B) 0.1 mM TTTTTATTTTT DNA standard after digestion into nucleosides. (C) 0.1 mM TTTTT(εA)TTTTT after reaction with 0.005 mM AlkB at 37^{o}C for 30 min at pH 7.0. The HPLC analysis (after digestion) indicates the complete conversion of εA to A by AlkB (20 turnovers).

Detection of Glyoxal

A published procedure was followed to derivatize glyoxal (Fluka) with pentafluorobenzylhydroxylamine (PFBHA, Aldrich) to form a PFBHA-dioxime product [5] which can be detected and quantified by HPLC analysis. A portion of PFBHA (3 mg) was added to the repair solution. The mixture was adjusted to pH 3 and shaken at room temperature for 1 h. PFBHA-dioxime was extracted twice with 1:1/diethyl ether:hexane (1 mL). The combined organic solution was concentrated to 100 μL and analyzed by HPLC with a Varian Microsorb-mv column (250 x 4.6 mm). The PFBHA-dioxime was separated by eluting with hexane at 2 mL/min. In repeated runs 67-85% of glyoxal (based on 1 eauiv of the DNA substrate) was detected with this method. With the use of a reverse phase column we can also separate the dioxime by using 50 mM ammonium acetate buffer (pH 6.5) for 5 min, followed by a linear gradient of MeOH (0-90%) applied over 20 min, and 90% MeOH for 5 min at the end to give the similar result. In both methods a small background peak is observed which seems to come from the buffer even in the absence of protein and DNA. This small background only accounts to less than 5% of the peak observed with the use of the Varian Microsorb-mv column.

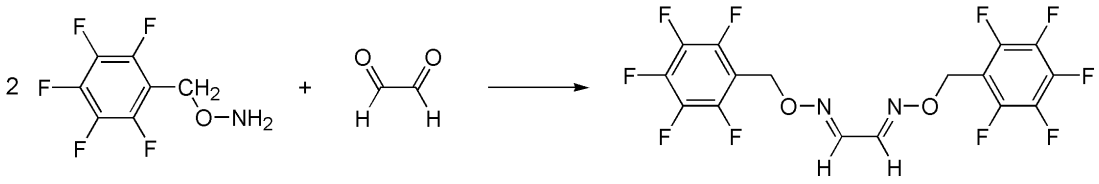

Figure S2. Trapping of glyoxal by pentafluorobenzylhydroxylamine (PFBHA) to form PFBHA-dioxime.

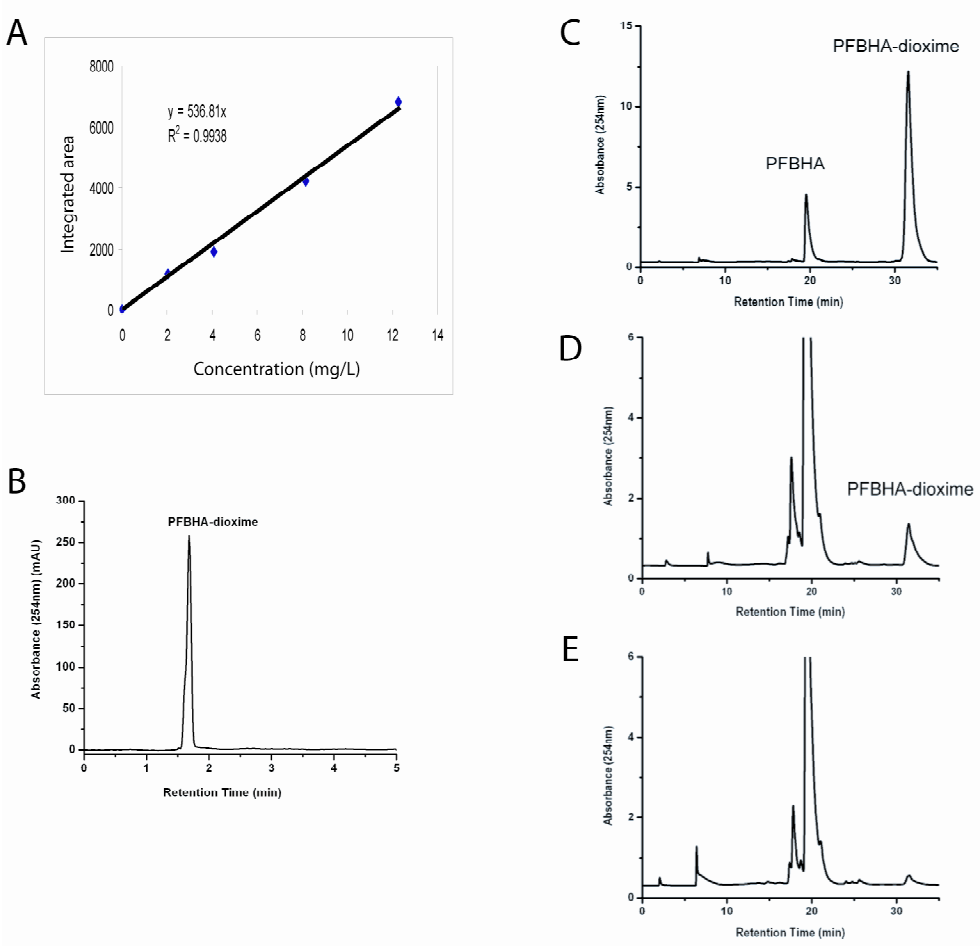

Figure S3. Identification of glyoxal produced from the AlkB-mediated repair of T(εA)T. (A) The calibration curve of the PFBHA-dioxime peak correlates with the concentrations of glyoxal presented in reaction buffer solution (with the use of a Varian Microsorb-mv column). (B) The PFBHA-dioxime peak was observed by treating the repair reaction product (reacting with 0.1 mM of T(εA)T in 250 μL solution) with PFBHA (PFBHA is polar and comes out much later). About 0.7 equivalent of glyoxal (several runs gave 67-85% of glyoxal based on T(εA)T) was produced based on the calibration curve (a background < 5% of the peak intensity was observed in the absence of the protein and DNA). (C) The PFBHA-dioxime peak can also be detected with a reverse phase column. (D) The PFBHA-dioxime peak was observed by treating the repair reaction product with PFBHA as well. (E) The control experiment excluding only AlkB (a small background peak was observed).

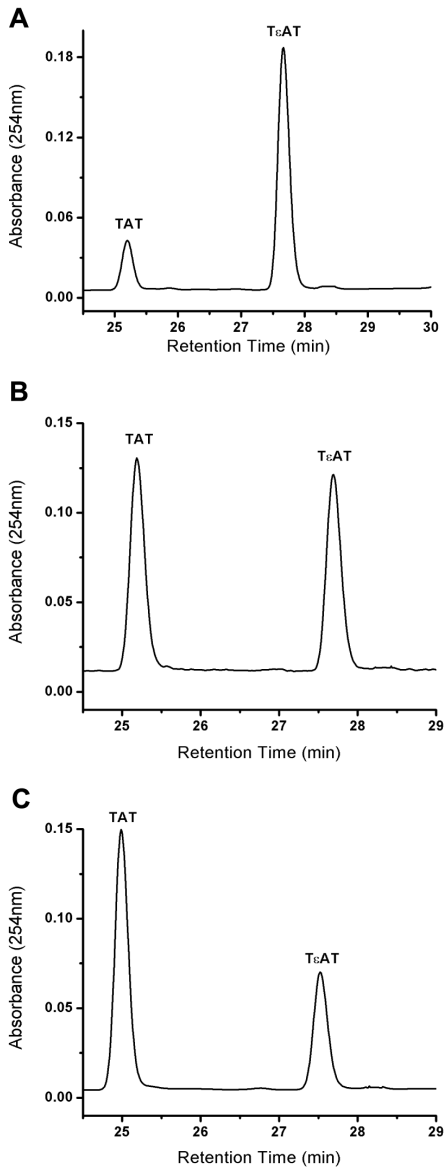

Figure S4. Repair of T(εA)T by human ABH3. T(εA)T (0.1 mM) was treated with 0.01 mM (A), 0.05 mM (B), and 0.1 mM (C) human ABH3 at pH 6.0 (100 μL) for 16 h at room temperature. Production of 16% (A), 51% (B), and 67% (C) of TAT could be observed by integration of the area under the peaks at 254 nm by HPLC analysis.

REFERENCES

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