A Designed Inhibitor of Base-Excision DNA Repair

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DNA repair enzymes are expressed in all known organisms to counter the deleterious effects of endogenous and exogenous DNA damaging agents.[1] The important function served by these enzymes has recently been underscored by the finding that certain hereditary cancers arise from defects in DNA repair genes.[2] Although some insights have been gained into the recognition and processing of damaged bases by DNA repair enzymes,[3] a more complete understanding of these systems will require high-resolution structural analysis of enzyme−DNA complexes. Efforts to obtain such information have been frustrated by the fleeting nature of enzyme−substrate complexes formed along the catalytic pathway. As a first step toward solving this problem, we recently reported the use of transition state mimicry to dissect binding from catalysis by a base-excision DNA repair protein.[4] Here we report an alternative and complementary approach, involving transition state destabilization, which now affords a potent inhibitor of DNA repair that differs from the native substrate only in the replacement of one hydrogen atom for fluorine.

The subject of the present study is a human repair enzyme, the so-called alkyl-*N*-purine DNA glycosylase (ANPG), which catalyzes the removal of several modified bases, including 3-methyladenine, 7-methylguanine, 1,N^6-ethenoadenine (εA), and hypoxanthine, through glycosidic bond hydrolysis (Figure 1).[5] Because ANPG directly counters the action of chemotherapeutic alkylating agents,[6] this enzyme represents a potential target for drug discovery.

The current mechanistic model for ANPG, based primarily on studies of enzymes that catalyze other glycosyl transfer reactions, invokes one or more transition states in which substantial positive charge is accumulated in the 2′-deoxyribose ring, notably at O-1′ and C-1′.[7] We reasoned that placement of an electron-withdrawing fluorine substituent at C-2′ should destabilize the transition state by increasing its charge density, thereby decreasing the reaction rate.[8] It remained to be seen whether the elevation in transition state energy would be sufficient to preclude the glycosyl transfer reaction altogether. For reasons of synthetic expedience, we chose to introduce the fluoro substituent in the arabino configuration and to use 1,N^6-ethenoadenine as the target base (Figure 1, FεdA).

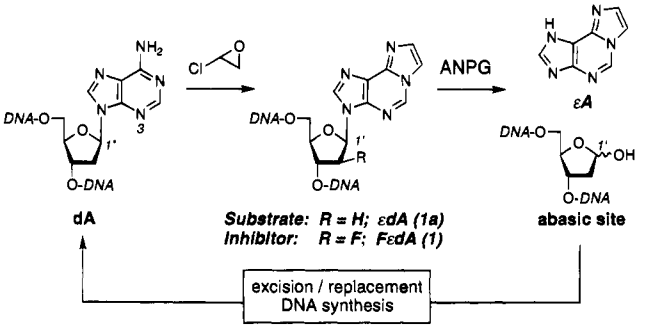
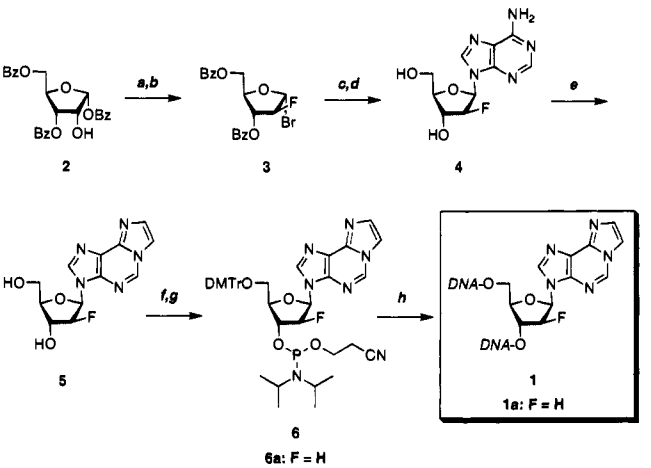


Figure 1. Production and repair of 1,N^6-ethenoadenine residues in DNA. Alkylation of an adenine residue by chloroethylene oxide (a metabolite of vinylchloride) generates 2′-deoxy-1,N^6-ethenoadenosine (εdA). ANPG catalyzes the excision of the aberrant base through glycosidic bond hydrolysis. The abasic site thus generated is processed by additional enzymes in the overall repair pathway, ultimately regenerating the original dA residue. The inhibitor, FεdA, differs from the substrate only in having the 2′β-H replaced by F.

Scheme 1[a]



[a] Reagents and conditions: (a) DAST, CH_2Cl_2, 40 °C, 75%; (b) 30% HBr/HOAc, CH_2Cl_2, 96%; (c) 6-chloropurine, NaH, CH_3CN, 50 °C, 51%; (d) NH_3, MeOH, heat, 70%; (e) $ClCH_2CHO$, H_2O, pH 4.5, 90%; (f) DMTrCl, DMAP, Et_3N, pyridine, 71%; (g) $iPr_2NP(Cl)OC_2H_4CN$, iPr_2NEt, THF, 86%; (h) solid phase DNA synthesis.

The commercially available ribose derivative **2** was fluorinated in good yield by treatment with (diethylamino)sulfur trifluoride (DAST) and then treated with HBr to furnish the known glycosyl bromide **3** (Scheme 1).[9,10] Glycosidation of **3** with the sodium salt of 6-chloropurine[11] yielded principally the desired β-anomer, which was treated with ammonia-saturated methanol to provide the known 9-(2′-deoxy-2′-fluoro-arabinofuranosyl)adenine (**4**).[12] Condensation with chloroacetaldehyde in buffered aqueous solution provided the fluoro-1,N^6-ethenodA nucleoside **5** (FεdA).[13] Standard tritylation and phosphitylation yielded phosphoramidite **6**.[14] Using analogous procedures, the phosphoramidite of the parent 2′-deoxy-1,N^6-ethenoadenosine (**6a**)[15] was synthesized. εdA is labile toward alkaline hydrolysis, which requires the use of protecting groups that can

(1) Lindahl, T. *Nature* **1993**, *362*, 709−715.
(2) Papadopoulos, N.; Nicolaides, N. C.; Liu, B.; Parsons, R.; Lengauer, C.; Palombo, F.; D'Arrigo, A.; Markovitz, S.; Willson, J. K. V.; Kinzler, K. W.; Jiricny, J.; Vogelstein, B. *Science* **1995**, *268*, 1915−1917. Modrich, P. *Science* **1994**, *266*, 1959−1960.
(3) Savva, R.; McAuley-Hecht, K.; Brown, T.; Pearl, L. *Nature* **1995**, *373*, 487−493. Mol, C. D.; Arvai, A. S.; Slupphaug, G.; Kavil, B.; Alseth, I.; Krokan, H. E.; Tainer, J. A. *Cell* **1995**, *80*, 869−878. See also: Myers, L. C.; Verdine, G. L. *Curr. Opin. Struct. Biol.* **1994**, *4*, 51−59. Tainer, J. A.; Thayer, M. M.; Cunningham, R. P. *Curr. Opin. Struct. Biol.* **1995**, *5*, 20−26. Park, H.-W.; Kim, S.-T.; Sancar, A.; Deisenhofer, J. *Science* **1995**, *268*, 1866−1872.
(4) Schärer, O. D.; Ortholand, J.-Y.; Ganesan, A.; Ezaz-Nikpay, K.; Verdine, G. L. *J. Am. Chem. Soc.* **1995**, *117*, 6623−6624.
(5) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*; ASM Press: Washington, DC, 1995; and references therein.
(6) Matijasevic, Z.; Boosalis, M.; Mackay, W.; Samson, L.; Ludlum, D. B. *Proc. Natl. Acad. Sci U.S.A.* **1993**, *90*, 11855−11859.
(7) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202. Schramm, V. L.; Horenstein, B. A.; Kline, P. C. *J. Biol. Chem.* **1994**, *269*, 18259−18262.
(8) Marquez, V. E.; Tseng, C. K.-H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. *J. Med. Chem.* **1990**, *33*, 978−985. York, J. L. *J. Org. Chem.* **1981**, *46*, 2171−2173.
(9) Additional details are provided in the supporting information, which can also be obtained directly from the authors by FAX: (617) 495−8755.
(10) Howell, H. G.; Brodfuehrer, P. R.; Brundidge, S. P.; Benigni, D. A.; Sapino, C., Jr. *J. Org. Chem.* **1988**, *53*, 85−88.
(11) Kazimierczuk, Z.; Cottam, H. B.; Revnakar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379−6382.
(12) Chu, C. K.; Matulic-Admic, J.; Huang, J.-T.; Chou, T.-C.; Burchenal, J. H.; Fox, J. J.; Watanabe, K. A. *Chem. Pharm. Bull.* **1989**, *37*, 336−339.
(13) Secrist, J. A., III; Barrio, J. R.; Leonard, N. J.; Weber, G. *Biochemistry* **1972**, *11*, 3499−3506.
(14) Gait, M. J. *Oligonucleotide Synthesis, A Practical Approach*; IRL Press Ltd.: Oxford, England, 1984.

0002-7863/95/1517-10781$09.00/0

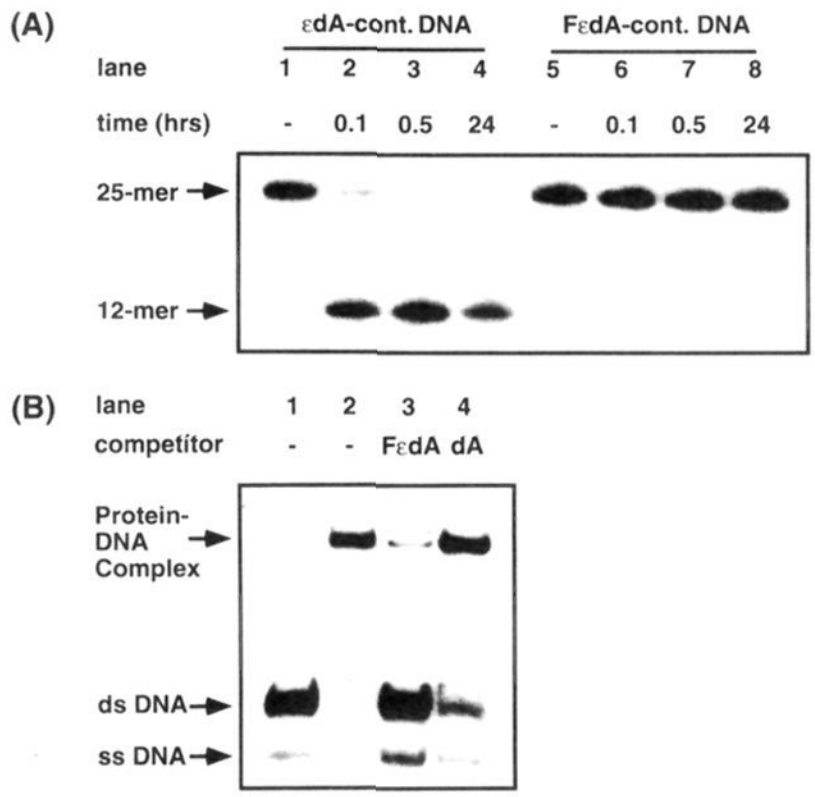


Figure 2. (A) Cleavage assay to detect the processing of ϵdA and FϵdA containing oligos by ANPG. Substrate concentration, 2 nM; enzyme concentration, 5 nM. The 12-mer product that results from processing of the substrate by ANPG followed by piperidine-mediated DNA strand scission was identified by comparison with an authentic standard. (B) EMSA assay to detect specific binding of FϵdA-containing DNA to ANPG. Oligonucletide concentration, 0.5 nM; ANPG concentrations, lane 1, no protein, lane 2−4, 2.5 nM; concentration of unlabeled competitor oligonucleotide in lanes 3 and 4, 50 nM (100-fold molar excess over labeled substrate).

be removed under mildly basic, nonaqueous conditions.[15b] Therefore, the phenoxyacetyl (PAC) protecting group was used for the unmodified bases in the solid phase synthesis of two 25-mer oligonucleotides having a central modified base (5′-GGA TAG TGT CCA **N** GTT ACT CGA AGC-3′, in which **N** = **FϵdA** or **ϵdA**). These oligonucleotides were deprotected using ammonia-saturated methanol at room temperature,[15b] concentrated by lyophilization, and purified by denaturing polyacrylamide gel electrophoresis.[9] The oligonucleotides were 5′-^{32}P-end labeled and annealed to a complementary 25-mer (5′-GCT TCG AGT AAC **T** TGG ACA CTA TCC-3′) containing a thymine residue opposite the FϵdA or ϵdA residue.

The reaction catalyzed by ANPG generates an abasic site in DNA, which degrades upon treatment with aqueous piperidine, resulting in specific strand scission; the cleavage product can be detected on the basis of its increased mobility in a polyacrylamide gel. For example, when the ϵdA-containing 25-mer duplex was incubated with ANPG for varying amounts of time and treated with piperidine, time-dependent appearance of the expected cleavage product was observed (Figure 2A, lanes 1−4).[16,17] By contrast, when FϵdA was used as a prospective ANPG substrate in this assay, no cleavage was observed, even after 24 h of incubation with a 2.5-fold molar excess of enzyme (Figure 2A, lanes 5−8).

The inability of the FϵdA to undergo ANPG-catalyzed glycosidic bond hydrolysis could result from a failure of the enzyme to bind this fluorinated substrate. To test this possibility, we carried out electrophoretic mobility shift assays (EMSA),[18] which detect the difference in electrophoretic mobility between protein-bound and free DNA. As shown in Figure 2B, ANPG readily binds the FϵdA-containing duplex (lane 2). This binding is specific for the presence of the FϵdA moiety, because it is susceptible to competition by a 100-fold excess of unlabeled FϵdA-containing duplex (lane 3), yet almost completely resistant to competition by a 100-fold excess of an unlabeled duplex containing dA in place of FϵdA (lane 4).

To determine quantitatively the effect of replacing the 2′-H in ϵdA with 2′-F in FϵdA, we carried out EMSA titration experiments under K_d conditions.[9] The dissociation constants thus determined were 30 pM for ϵdA-containing oligonucleotides versus 80 pM for FϵdA-containing oligonucleotides. Thus, the presence of the 2′-fluoro substituent in FϵdA decreases the binding strength of the oligonucleotide to ANPG by only 0.4 kcal/mol (25 °C). This modest difference suggests that the ANPG complexes with the natural and the fluorinated substrates are likely to posses similar structures.

In other cases of catalytic DNA binding proteins, such frozen enzyme−pseudosubstrate complexes have yielded fascinating insights into the architectural features governing recognition and catalysis in macromolecular complexes.[19] The present results should pave the way for cocrystal structural studies of ANPG bound to an FϵdA-containing DNA duplex. Furthermore, the transition state destabilization approach demonstrated here may be more generally applicable to other DNA glycosylases that recognize diverse lesions. In a separate series of experiments (data not shown), we used the piperidine cleavage assay to demonstrate that FϵdA-containing oligonucleotides inhibit the catalytic activity of ANPG on an ϵdA-containing substrate. These findings raise the prospect that FϵdA-containing oligonucleotides or their derivatives might be used to increase the potency of chemotherapeutic alkylating agents by inhibiting the action of ANPG.

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Supporting Information Available: Synthetic procedures and ^{1}H-NMR and HRMS data for all new compounds, HPLC traces of nucleoside composition analysis, and procedures for cleavage and EMSA assays (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA952502W

(15) (a) Basu, A. K.; Niedernhofer, L. J.; Essigmann, J. M. *Biochemistry* **1987**, *26*, 5626−5635. (b) Srivastava, S. C.; Raza, S. K.; Misra, R. *Nucleic Acids Res.* **1994**, *22*, 1296−1304.

(16) For these experiments, we have used a truncated revsion of ANPG that is more stable than the native protein while retaining full DNA glycosylase activity *in vitro*. O'Connor, T. R. *Nucleic Acids Res.* **1993**, *21*, 5561−5569.

(17) Iwai et al. have shown that a ribo-configured fluoronucleoside substrate undergoes base excision by T4 endonuclease V but fails to undergo the subsequent β-elimination. These studies demonstrate that the abasic site of a fluoro sugar can be degraded by piperidine, resulting in strand scission. Iwai, S.; Maeda, M.; Shirai, M.; Shimada, Y.; Osafune, T.; Murata, T.; Ohtsuka, E. *Biochemistry* **1995**, *34*, 4601−4609.

(18) Klimasauskas, S.; Kumar, S.; Roberts, R. J.; Cheng, X. *Cell* **1994**, *76*, 357−369. Reinisch, K. M.; Chen, L.; Verdine, G. L.; Lipscomb, W. N. *Cell* **1995**, *82*, 143−153.

(19) Fried, M. G.; Crothers, D. M. *J. Mol. Biol.* **1984**, *172*, 263−282.