EFFECT OF HUMAN APE1 ON hNTH1 ACTIVITY*

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Base excision repair of oxidized pyrimidines in human DNA is initiated by the DNA N-glycosylase/apurinic/apyrimidinic (AP) lyase, human NTH1 (hNTH1), the homolog of *Escherichia coli* endonuclease III (Nth). In contrast to Nth, the DNA N-glycosylase activity of hNTH1 is 7-fold greater than its AP lyase activity when the DNA substrate contains a thymine glycol (Tg) opposite adenine (TgA) (Marenstein, D. R., Ocampo, M. T. A., Chan, M. K., Altamirano, A., Basu, A. K., Boorstein, R. J., Cunningham, R. P., and Teebor, G. W. (2001) *J. Biol. Chem.* 276, 21242–21249). When Tg is opposite guanine (TgG), the two activities are of the same specific activity as the AP lyase activity of hNTH1 against TgA (Ocampo, M. T. A., Chaung, W., Marenstein, D. R., Chan, M. K., Altamirano, A., Basu, A. K., Boorstein, R. J., Cunningham, R. P., and Teebor, G. W. (2002) *Mol. Cell. Biol.* 22, 6111–6121). We demonstrate here that hNTH1 was inhibited by the product of its DNA N-glycosylase activity directed against TgG, the AP:G site. In contrast, hNTH1 was not inhibited by the AP:A site arising from release of Tg from TgA. Addition of human APE1 (AP endonuclease-1) increased dissociation of hNTH1 from the DNA N-glycosylase-generated AP:A site, resulting in abrogation of AP lyase activity and an increase in turnover of the DNA N-glycosylase activity of hNTH1. Addition of APE1 did not abrogate hNTH1 AP lyase activity against TgG. The stimulatory protein YB-1 (Marenstein et al.), added to APE1, resulted in an additive increase in both activities of hNTH1 regardless of base pairing. TgA is formed by oxidative attack on thymine opposite adenine. TgG is formed by oxidative attack on 5-methylcytosine opposite guanine (Zuo, S., Boorstein, R. J., and Teebor, G. W. (1995) *Nucleic Acids Res.* 23, 3239–3243). It is possible that the in vitro substrate selectivity of mammalian NTH1 and the concomitant selective stimulation of activity by APE1 are indicative of selective repair of oxidative damage in different regions of the genome.

Like its *Escherichia coli* homolog, endonuclease III (Nth), hNTH1 is a bifunctional DNA N-glycosylase/apurinic/apyrimidinic (AP) lyase that removes ring-saturated pyrimidines, be they hydrated, reduced, or oxidized, from the DNA backbone as the initial step of base excision repair (BER) of such modified residues (1). The oxidation product of thymine, 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol (Tg)) is a widely studied substrate for BER. This enzyme activity is mediated via formation of a transient Schiff base imino enzyme-DNA intermediate, an example of covalent catalysis and a characteristic of all known bifunctional DNA N-glycosylases/AP lyases (4, 5). The Schiff base moiety has been hypothesized to be required for the enzymatic catalysis of the β-elimination reaction, which effects DNA strand cleavage 3′ to the abasic (AP) site formed as a product of the release of the base from the 2′-deoxyribose moiety to which it was linked. The hydrolysis of the Schiff base intermediate can occur in the absence of or following enzyme-catalyzed β-elimination (AP lyase activity), resulting in DNA strand cleavage. The enzymatic catalysis of β-elimination by DNA N-glycosylases/AP lyases has been shown to be initiated via abstraction of the deoxyribose pre-S-2′-hydrogen by a basic amino acid in the enzyme active site. Several factors, including pH, can affect the efficiency of the AP lyase step by affecting substrate binding and proton abstraction (5).

Based on the results of studies with Nth, the two activities of hNTH1 (DNA N-glycosylase and β-elimination catalysis) require the formation of the Schiff base intermediate and were thought to occur concomitantly. However, data from our laboratory (1) and from other laboratories (6–8) indicate that DNA N-glycosylase and β-elimination catalysis by mammalian members of the endonuclease III enzyme superfamily are not concurrent under the assay conditions employed. We were the first to report that the DNA N-glycosylase and AP lyase activities of hNTH1 are not concurrent, i.e. that the rate of AP lyase-mediated strand cleavage is much slower than the rate of DNA N-glycosylase-mediated base release. These results are similar to the non-concurrency of base release and strand cleavage reported for the mammalian 8-oxoguanine-DNA N-glycosylase homolog OGG1 (8). In this report, we present data demonstrating that the dissociation of the two activities of hNTH1 is dependent on the nature of the orphan base opposite the Tg residue in DNA.

In our studies of the properties of hNTH1, we have focused on

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3 The abbreviations used are: hNTH1, human NTH1; AP, apurinic/apyrimidinic; BER, base excision repair; Tg, thymine glycol; hOGG1, human OGG1.

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our attention on protein modulators of hNTH1 activities. This approach resulted from our original observation that the enzyme properties of mammalian endonuclease III homologs are strikingly different from those of bacterial endonuclease III. Our laboratory was the first to identify mammalian endonuclease III homologs and to describe their distinct kinetic properties (9, 10). These observations spurred us to isolate novel proteins that might modulate the enzyme activities of mammalian NTH1. A yeast two-hybrid search resulted in identification of the pluripotent transcription factor YB-1 (Y-box-binding protein-1; also known as DNA-binding protein B) as a stimulator of hNTH1 activity (1).

We now report additional aspects of the proteomics of hNTH1-initiated BER based on the effects of human APE1 (AP endonuclease-1; HAPR/REF1/APE1) on hNTH1 activity. APE1, a human homolog of *E. coli* exonuclease III (Xth), catalyzes the hydrolysis of the phosphodiester bond 5′ to AP sites, generating a free 3′-hydroxyl end for the initiation of DNA polymerase repair synthesis. APE1 has recently been demonstrated to play a significant role in the coordination of BER. APE1 has been shown to modulate the activities of several major DNA N-glycosylases, although physical interaction between APE1 and most of these DNA N-glycosylases has not been demonstrated (6, 7, 11, 12). The functional interaction between APE1 and DNA N-glycosylases is consistent with the model of the coordinated activities of BER enzymes at sites of DNA damage (13–16). Two groups independently demonstrated the stimulation of human OGG1 (hOGG1) by APE1 (6, 7). Both groups demonstrated that APE1 replaces hOGG1 bound to the DNA site following base release and prior to hOGG1-catalyzed β-elimination. This resulted in bypass of the AP lyase step and an increase in hOGG1 turnover. In this study, we report similar findings with hNTH1 and APE1. However, stimulation by APE1 proved to be dependent on the nature of the orphan base opposite the Tg residue in DNA.

**EXPERIMENTAL PROCEDURES**

**Proteins—**Expression and purification of recombinant hNTH1 were induced as described previously (10). hNTH1 concentration was quantified using an extinction coefficient of 1 absorbance unit at λ494 μM for the C-terminal cubic {Fe4-S4} cluster (17). YB-1 was expressed with a His tag in *Trichosporon* *ni* cells and purified as described (1). DNA polymerase β was a gift from Dr. Samuel H. Wilson (NIEHS), and APE1 was a gift from Dr. David M. Wilson III (University of California). Formamidopyrimidine-DNA N-glycosylase was purchased from New England Biolabs Inc., and uracil-DNA N-glycosylase was purchased from Invitrogen.

2′-Deoxyribose Oligonucleotides—The 30-mer 2′-deoxyribose oligonucleotide substrate containing a Tg residue at position 13 (d[GATCCTCTAGATCGACCTGAGGCGATGCA]) was prepared as described (1). The 30-mer 2′-deoxyribose oligonucleotide substrate containing a uracil residue at position 13 of the identical sequence and the complementary 2′-deoxyribose oligonucleotides were synthesized by the Department of Cell Biology of the New York University School of Medicine. 2′-Deoxyribose oligonucleotides were dephosphorylated, deprotected, and purified by 20% denaturing PAGE. The 2′-deoxyribose oligonucleotides were gel-purified and labeled at the 5′-end using [γ-32P]ATP (PerkinElmer Life Sciences) and T4 polynucleotide kinase (Invitrogen) or at the 3′-end using [α-32P]dATP (Amersham Biosciences) and terminal transferase (New England Biolabs Inc.). All labeled nucleotides were purified by Sephadex G-25 spin column filtration (Amersham Biosciences) and annealed to the complementary strand, which contained an adenine or guanine base opposite the Tg or uracil residue.

**Enzyme Assays—**Unless otherwise indicated, assays were performed at 37 °C in buffer containing 50 mM HEPES (pH 7.6), 100 mM KCl, 0.1 mg/ml bovine serum albumin, 5 mM MgCl2, and 1 mM dithiothreitol. Enzyme reactions and substrate were directed to working conditions in assay buffer and equilibrated at 37 °C. MgCl2-independent assays were performed with the above reaction buffer minus MgCl2 in the presence of 5 mM EDTA, which permits binding of APE1 to DNA, but inhibits its endonuclease activity (18). Reactions contained 40 nM [32P]-5′-end-labeled 2′-deoxyribose oligonucleotide duplex substrate and 5 nM hNTH1 with or without 20 nM APE1. Two sets of 10-μl aliquots were taken at the indicated time periods and snap-frozen in ethanol and dry ice, after which one set was treated with 5 μl of 0.5 M putrescine (pH 8.0) to measure base release. The treated assay mixtures were then heated at 95 °C for 5 min, followed by addition of 15 μl of loading dye (95% denatured formamide, 10 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol). To measure strand cleavage, samples were treated with an equal volume of loading dye. All samples were then heated at 55 °C for 5 min, and products were separated by 20% PAGE in 7 M urea and 1× Tris borate/EDTA. Low substrate concentration assays were performed with 20 nM hNTH1 and 20 nM [32P]-5′-end-labeled 2′-deoxyribose oligonucleotide duplex substrate. At the indicated times, aliquots were taken and treated as described above. Multiple-turnover reactions were performed individually in 10-μl volumes containing reaction buffer, the indicated concentration of [32P]-5′-end-labeled 2′-deoxyribose oligonucleotide duplex substrate, and 20 nM hNTH1 with or without 100 nM YB-1 and/or 100 nM APE1. The reactions were split into two 5-μl aliquots, snap-frozen in ethanol and dry ice, and treated as described above.

To discriminate between endonucleolytic and AP lyase products, reactions were performed using 2′-deoxyribose oligonucleotide duplex substrate labeled at the 3′-end with [α-32P]dAMP and 100 nM hNTH1 with or without 100 nM APE1 in a volume of 220 nM of 5′-phosphate moiety (19). The products were purified by Sephadex G-25 spin column filtration prior to separation and analysis.

Single-turnover assays contained 10 nM 2′-deoxyribose oligonucleotide duplex labeled at the 3′-end with [α-32P]dAMP and 100 nM hNTH1 with or without 100 nM APE1 in a volume of 220 μl. Enzyme reaction mixtures were incubated together for 2 min prior to initiation of the assay upon addition of substrate. To measure base release, 5-μl aliquots were removed at the indicated time periods, snap-frozen, and treated with 5 μl of 0.5 M putrescine (pH 8.0). The treated assay mixtures were then heated at 95 °C for 5 min, followed by addition of 10 μl of loading dye. To measure strand cleavage and to determine incision products, the 5-μl aliquots were removed at the indicated time periods, snap-frozen, and treated with an equal volume of 0.5 M NaBH4 at 37 °C for 20 min for reduction of the 2′-deoxyribose 5′-phosphate moiety. Samples were then filtered through a Sephadex G-25 spin column, treated with an equal volume of loading dye, and heated at 55 °C for 5 min, and products were separated by 20% PAGE in 7 M urea and 1× Tris borate/EDTA. All products were analyzed quantitatively via phosphorimaging using a Molecular Imager FX system with Quantity One software (Bio-Rad).

**Cross-linking of Enzyme to 2′-Deoxyribose Oligonucleotide Substrate (Enzyme Trapping)—**For NaBH4 reduction, assays contained the indicated [32P]-5′-end-labeled 2′-deoxyribose oligonucleotide duplex (400 nM) and 20 nM hNTH1 with or without 100 nM APE1 and/or 100 nM YB-1 in a volume of 10 μl. After a 15-min incubation, reaction mixtures were treated with 5 μl of 0.5 M NaBH4 and incubated at 37 °C for 5 min. After addition of an equal volume of Laemmli SDS loading buffer, samples were boiled in preparation for separation and analysis. All samples were separated by 12% SDS-PAGE and analyzed quantitatively via phosphorimaging as described above.

**RESULTS**

**Differential Processing of Tg Opposite Adenine and Tg Opposite Guanine by hNTH1—**We previously demonstrated, using a Tg:A-containing substrate, that the initial rate of DNA N-glycosylase-mediated release of Tg by hNTH1 is much greater than the rate of AP lyase-mediated DNA strand cleavage (1). Because all previous assays with Tg have been done with adenine as the opposite base and because this may not be the only context in which Tg occurs in *vivo*, we decided to look at hNTH1 activity against substrates containing Tg paired opposite guanine (2). Fig. 1 is confirmation of our previous data. The assay was carried out in a shorter reaction period than we previously used and revealed that the difference between the rate of base release and strand cleavage of a Tg:A substrate is even greater than we had reported, differing by an order of magnitude. In sharp contrast, the AP lyase activity of hNTH1 was the same as its DNA N-glycosylase activity against a Tg:G substrate. The DNA N-glycosylase activity of the en-
zyme against Tg:A appeared to reach $V_{\text{max}}$ at substrate concentrations >400 nM. However, as we previously reported, hNTH1 does not follow Michaelis-Menten pseudo zero-order kinetics (1). Instead, the apparent $k_{\text{cat}}$ (which includes the product release rates) increased with increasing hNTH1 concentrations, suggesting positive cooperativity in hNTH1 substrate processing. This observation and interpretation have been corroborated and expanded upon by Liu and Roy (20). Because of this phenomenon, $k_{\text{cat}}$ and $K_m$ values for the 2'-deoxyribose oligonucleotide duplex substrates vary with enzyme concentration. As we previously suggested, the $V$ versus $S$ data of Fig. 1 reflect the fact that the enzyme was dissociating from the DNA N-glycosylase-generated AP:A site and initiating base release on new Tg:A substrates without catalyzing β-elimination (1). However, when the substrate was Tg:G, there was no evidence of enzyme dissociation from AP:G (2).

APE1 Increases hNTH1 Processing of Tg:A-containing 2'-Deoxyribose Oligonucleotide Substrates—We looked at the effect of APE1 on the activities of hNTH1. There is evidence that APE1 interacts with DNA N-glycosylases in BER as part of a “single-nucleotide BER relay” involved in the coordination of processing of BER pathway intermediates (13, 21, 22).

Fig. 2 shows that addition of APE1 increased hNTH1 activity against a Tg:A substrate. The stimulation of hNTH1 activity was independent of the enzyme activity of APE1. The assay mixture lacked MgCl$_2$ and contained sufficient EDTA to abrogate APE1-mediated endonucleolytic cleavage, but not the binding of APE1 to DNA (18). Identical experiments with Tg:G substrates showed no increase in hNTH1 substrate processing in the presence of APE1 (data not shown). We found that the optimum ratio for stimulation of hNTH1 by APE1 varied with both hNTH1 and substrate concentrations, ranging from 1:1 to 1:10. We suggest that this variability was due, at least in part, to the positive cooperativity exhibited by hNTH1 (1, 20).

APE1 Abrogates hNTH1 AP Lyase Activity on Tg:A-containing Substrates, but Not on Tg:G-containing Substrates—Having determined that the effect of APE1 on hNTH1 activity differed with substrate, we characterized the incision products generated by APE1 and hNTH1 with the two substrates. The incision products generated by hNTH1 in the presence of APE1 differed with each substrate. Fig. 3 is the gel analysis of the incision products of 32P-5'-end-labeled Tg:A- and Tg:G-containing 2'-deoxyribonucleotide duplex substrates generated by hNTH1 in the presence or absence of APE1. In contrast to the experiments shown in Fig. 2, MgCl$_2$ was added to the reaction to activate the endonucleolytic activity of APE1. The 2'-deoxyribose 5'-phosphate residue generated by APE1-catalyzed endonucleolytic cleavage of an AP site is extremely labile and not retained during electrophoresis. Reduction by NaBH$_4$ prior to electrophoresis stabilizes the sugar moiety and permits size discrimination between 5'-endonucleolytic and 3'-β-elimination AP lyase products (19). The AP lyase product (p-17-p*ddA) can be seen in lanes containing hNTH1 with either the Tg:A or Tg:G substrate (lanes 2 and 4, respectively). Addition of APE1 to hNTH1 using Tg:A produced a higher molecular weight incision product, which was the endonucleolytic product (p-rAP-p-17-p*ddA), where rAP is reduced apurinic/apyrimidinic generated by APE1 (lane 3). In the case of the Tg:G substrate, no change in the size of the incision product was detected upon APE1 addition, indicating that the AP lyase activity of hNTH1 was unaffected by APE1 with this substrate (lane 5).
γ-elimination product, one with the TgG-containing substrate and one with the U:A-containing substrate (lanes 6 and 8, respectively). Formamidopyrimidine-DNA N-glycosylase has γ/β-elimination activity. Because the substrate was 3′-labeled, the only product observed with formamidopyrimidine-DNA N-glycosylase was the β-elimination product (lane 6). A second marker lane for the β-elimination product is the AP site-containing substrate generated by incubation of the U:A-containing substrate with uracil-DNA N-glycosylase and hNTH1 to generate the AP lyase product (p-17-p*ddA) (lane 8). The U:A-containing substrate incubated with uracil-DNA N-glycosylase and APE1 serves as the marker for the endonucleolytic product (p-rAP-p-17-p*ddA) (lane 7).

Incision Products Generated by hNTH1 in the Presence or Absence of APE1, YB-1, and DNA Polymerase β Differ in Vitro—Because our previous work identified YB-1 as a modifier of hNTH1 activity (1), we next investigated whether addition of YB-1 together with APE1 had any effect on the nature of the incision products of hNTH1 with TgA-containing 2′-deoxyribonucleotide oligonucleotide substrates. Reactions contained 20 nm dNTPs, 16.6 nm TgA-containing 2′-deoxyribonucleotide duplex substrate labeled at the 3′-end with [32P]ddAMP, 16.6 nm hNTH1, 8.3 nm APE1, 8.3 nm DNA polymerase β (β-pol), and 83 nm YB-1 as indicated. Lane 9 contained 1 unit of formamidopyrimidine-DNA N-glycosylase (Fpg) as a marker for the β-elimination product (p-17-p*ddA, where the asterisk denotes the labeled phosphate). Reactions were incubated at 37 °C for 30 min prior to addition of NaBH₄, followed by analysis as described under “Experimental Procedures.”

Because hNTH1 did not demonstrate AP lyase activity against Tg:A sites in the presence of APE1, we investigated whether the removal of the 2′-deoxyribose 5′-phosphate residue could be effected by DNA polymerase β. Fig. 4 demonstrates the reappearance of the β-elimination product upon addition of DNA polymerase β to hNTH1 and APE1 with a TgA substrate (lane 7). This observation is consistent with the known 2′-deoxyribose 5′-phosphatase activity of DNA polymerase β (22). The marker lane for the β-elimination product (p-17-p*ddA) contains formamidopyrimidine-DNA N-glycosylase (lane 9). Similar experiments with an N-terminal deletion mutant of hNTH1 lacking amino acids 1–57 produced the same results in the presence of APE1 and YB-1 (data not shown), suggesting that the effects of YB-1 and APE1 are not mediated by the N terminus of hNTH1.

APE1 Does Not Increase the DNA N-Glycosylase Activity of hNTH1 under Conditions of Single Turnover—To elucidate the mechanism by which APE1 affects hNTH1 activity, we investigated whether APE1 changed the catalytic properties of hNTH1 or whether it affected product inhibition. To address this, we conducted the reaction under single-turnover conditions in which [E] ≫ [S] using substrates labeled at the 3′-end with [32P]ddAMP to determine the nature of the incision product. Because all substrate molecules are bound by enzyme under single-turnover conditions, the rates that determine product formation are dependent only on the rate of base release (Schiff base formation) and β-elimination (24). If APE1 did not affect either of these catalytic properties, then there should be no stimulation under conditions of single turnover.

Fig. 5 illustrates that no stimulatory effect of DNA N-glycosylase activity was observed under conditions in which neither enzyme turnover nor substrate binding contributed significantly to the rate of the reaction. A complete abrogation of the AP lyase-mediated AP site cleavage was seen with the Tg:A substrate (Fig. 5A). This is consistent with the results of Figs. 3 and 4 (lanes 3).

Interestingly, in the case of the TgG substrate, addition of APE1 increased the rate of hNTH1 AP lyase activity (Fig. 5B). We previously mentioned that addition of APE1 did not result in increased product formation by hNTH1 under turnover con-
substrates as a function of time under multiple-turnover conditions. Reactions contained 20 nM $^{32}$P-5′-end-labeled Tg:A-containing (circles) or Tg:G-containing (squares) 2′-deoxyribose oligonucleotide duplex substrate as indicated with 20 nM hNTH1. Open symbols denote DNA N-glycosylase activity determined by putrescine treatment of reaction products. Closed symbols denote AP lyase activity.

APE1 Stimulates hNTH1 Activity under Conditions of $V_{\text{max}}$—To determine whether APE1 increased the maximal rate of turnover for the enzyme, we tested stimulation by APE1 under conditions of $V_{\text{max}}$ for both DNA N-glycosylase and AP lyase activities using 20 nM hNTH1 and 400 nM Tg:A substrate, identical to the conditions of Fig. 1. However, in experiments of Fig. 7, MgCl$_2$ was added to activate APE1 endonucleolytic activity. In reactions containing hNTH1 alone or with YB-1, DNA cleavage was due solely to hNTH1-catalyzed $\beta$-elimination. For all four sets of experiments, DNA N-glycosylase activity was quantified by measuring the cleavage of hNTH1-generated AP sites by treatment with organic base (putrescine), which effects cleavage via $\beta$-elimination (25). In reactions containing APE1, all cleavages were the result of APE1 activity. Addition of APE1 resulted in a 2-fold increase in the number of hNTH1-generated AP sites compared with hNTH1 alone. Addition of YB-1 also caused a 2–3-fold increase in DNA N-glycosylase activity, as we previously described (1). Like YB-1, APE1 increased the $k_{\text{cat}}$ of hNTH1 (as a DNA N-glycosylase) under conditions of substrate excess. The last experiment of this group indicates that the effects of APE1 and YB-1 were additive, resulting in a close to 4-fold increase in product. This combined stimulation could actually be much greater because, under our experimental conditions, most of the substrate had been processed within 15 min.

Corroborating our results of experiments performed under low Tg:G substrate concentrations, addition of APE1 to hNTH1 using substrate under conditions of $V_{\text{max}}$ (400 nM Tg:G) did not result in increased product formation (data not shown). Interestingly, hNTH1 activity against Tg:G was stimulated by addition of YB-1, but there was no additive effect with APE1 (data not shown).

APE1 Addition Decreases the Half-life of Covalent Enzyme-Substrate Complexes Formed by hNTH1 with Its Substrate—To investigate the effect of APE1 on the reaction intermediates of hNTH1 with its substrate, we used the reducing agent NaBH$_4$ to trap the covalent enzyme-substrate intermediate (Fig. 8). NaBH$_4$ is a strong reducing agent, capable of reducing the Schiff base as well as existing AP sites (26). Therefore, the trapping of the covalent reaction intermediate with NaBH$_4$ (Fig. 8) revealed the number of covalent complexes present in the reaction mixture at a given time point, in this case, 15 min. For the Tg:A substrate under steady-state conditions, addition of APE1 resulted in a decrease in the steady-state number of complexes initiated by hNTH1 15 min after the start of the reaction when the rate of product formation was still linear with time. There was no significant change in the number of steady-state complexes with the Tg:G substrate upon APE1 addition. Addition of YB-1 resulted in a greater number of complexes with both substrates, corroborating our previous data (Ref. 1 and data not shown). The marked reduction in the number of enzyme-substrate complexes (Tg:A) in the presence of both YB-1 and APE1 is a reflection of the increased stimulation of hNTH1 turnover, resulting in almost total substrate processing within 15 min. These data suggest that APE1 decreased the half-life of the enzyme-substrate covalent intermediate, thereby stimulating dissociation of hNTH1 from the AP-A site without catalyzing $\beta$-elimination, consistent with results of other laboratories studying the effect of APE1 on hOGG1 (6, 7).

**DISCUSSION**

The generation of Tg in DNA occurs through oxidative attack on the pyrimidine bases. Tg opposite adenine can be formed by direct hydroxyl radical attack under aerobic conditions on thymine in situ opposite its correctly paired base. As a blocker of both DNA and RNA polymerases, Tg opposite adenine is a cytotoxic lesion (27). When Tg is bypassed by a DNA polymer-
substrate with 20 nM hNTH1, 100 nM YB-1, and/or 100 nM APE1 as indicated. Reactions were incubated at 37 °C for 15 min; NaBH₄ was then added to a concentration of 100 nM, and the reactions were incubated for another 2 min at 37 °C prior to addition of Laemmli SDS sample buffer.

The different activities that we observed with hNTH1 in...
vitro may be indicative of the involvement of different substrate-dependent interacting and modifying proteins in vivo. Certain lesions, by virtue of their mutagenic or toxic characteristics, may be preferentially channeled down a particular BER pathway, i.e. long- or short-patch BER. It has been suggested that the intrinsic properties of the DNA N-glycosylase involved in the initial recognition and removal of the damaged base may be responsible for BER pathway selection (30). In this case, the intrinsic properties may include the pairing base discrimination exhibited by hNTH1.

The in vivo significance of AP lyase activity is poorly understood. The removal of the damaged base by the DNA N-glycosylase generates an AP site, which is a labile and therefore dangerous intermediate for the cell. AP sites are the universal byproduct of the repair of damaged bases and are substrates for several AP lyases, which remove the AP site and restore DNA to its original conformation. The fact that DNA polymerases and endonucleases, in addition to BER factors, can process AP sites (22, 33) suggests the probability of multiprotein complex formation in the initial events of BER.

Currently, the removal of the damaged base by the DNA N-glycosylase/AP lyase activity by bifunctional DNA N-glycosylases/AP lyases would result in the simultaneous formation of a large number of damaged bases throughout cellular DNA (1). In theory, the spontaneous removal of the damage via concomitant DNA N-glycosylase/AP lyase activity by bifunctional DNA N-glycosylases/AP lyases would result in the simultaneous formation of a large number of DNA strand breaks, a cytotoxic intermediate. In support of this hypothesis, recent data on H₂O₂ sensitivity in E. coli identified the intermediates of Tg repair, rather than the persistence of Tg, as significant contributors to cell death (32). The delayed AP lyase activity and product inhibition inherent in hNTH1 activity may allow for the regulation of AP site processing. The substrate-specific product inhibition exhibited by hNTH1 may also represent the channeling of AP site processing into specific BER pathways in different regions of the genome. The rate of removal of Tg from cellular DNA has been shown to be very slow compared with the removal of uracil or the repair of AP sites (22, 33).

BER factors to the DNA repair synthesis enzymes, such as DNA polymerase ɛ, FEN1 (Flap endonuclease-1), and DNA ligase (13, 21, 31). Data from our laboratory (1) and others (6, 7, 36–39) have shown that interactions between BER enzymes and other proteins can stimulate damage recognition or excision, suggesting the probability of multiprotein complex formation in the initial events of BER.

APE1 is not limited to its role in BER, but is a composite of the different activities of the enzyme as a redox factor (40). Notably, APE1 has been shown to control the activity of p53 through redox alteration; and in turn, p53 has been shown to play a role in BER (40–44). Like YB-1, APE1 has been shown to be a pluripotent factor and may be a key effector of the relationships between mammalian BER, oxidative signaling, transcription regulation, and cell cycle control (40, 45).

We previously demonstrated that YB-1 affects hNTH1 activity via the steady-state equilibrium between the covalent (E – S₃p) and noncovalent (Eₜ) enzyme-DNA intermediate (1). We proposed that this equilibrium may be a checkpoint for modulation of hNTH1 activity (1). In this study, we have shown that APE1 modulated hNTH1 activity at the same equilibrium point, albeit to a different effect. Thus, although YB-1 and APE1 seem to modulate hNTH1 activity in opposite ways, they have a synergistic effect in vitro. The overall effect of these two factors on hNTH1 activity is to increase hNTH1 turnover and substrate processing. Whether this has significance in vivo remains to be determined.

In conclusion, we have performed further analyses into the mechanism of hNTH1 activity and identified substrate-specific enzyme properties. We have also demonstrated a functional interaction between hNTH1 and the major mammalian AP endonuclease, APE1. This interaction is dependent on the nature of the orphan base opposite Tg.

We recently reported (2), as has another laboratory (46), that hNTH1 may play a role in BER (40–44). Notably, APE1 has been shown to control the activity of p53 through redox alteration; and in turn, p53 has been shown to play a role in BER (40). The different activities of the enzyme as a redox factor (40, 45) have shown that interactions between BER enzymes and other proteins can stimulate damage recognition or excision, suggesting the probability of multiprotein complex formation in the initial events of BER.
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