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Mouse NEIL1 Protein Is Specific for Excision of 2,6-Diamino-4-hydroxy-5-formamidopyrimidine and 4,6-Diamino-5-formamidopyrimidine from Oxidatively Damaged DNA

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ABSTRACT: A functional homologue of human DNA glycosylase NEIL1 (hNEIL1) in mouse has recently been cloned, isolated, characterized, and named mouse NEIL1 (mNEIL1). This enzyme exhibited specificity for excision of oxidatively modified pyrimidine bases such as thymine glycol, 5,6-dihydrouracil, and 5-hydroxypyrimidines, using oligonucleotides with a single base lesion incorporated at a specific site. It also acted upon AP sites; however, no significant excision of 8-hydroxyguanine was observed [Rosenquist, T. A., Zaika, E., Fernandes, A. S., Zharkov, D. O., Miller, H., and Grollman, A. P. (2003) *DNA Repair 2,* 581−591]. We investigated the substrate specificity and excision kinetics of mNEIL1 for excision of oxidatively modified bases from high-molecular weight DNA with multiple lesions, which were generated by exposure of DNA in aqueous solution to ionizing radiation. Among a large number of pyrimidine- and purine-derived lesions detected and quantified in DNA, only purine-derived lesions 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine were significantly excised. This finding establishes that mNEIL1 and its functional homologue hNEIL1 possess common substrates, namely, 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 4,6-diamino-5-formamidopyrimidine. Measurement of excision kinetics showed that mNEIL1 possesses equal specificity for these two formamidopyrimidines. This enzyme also excised thymine-derived lesions thymine glycol and 5-hydroxy-5-methylhydantoin, albeit at a much lower rate. A comparison of the specificity and excision kinetics of mNEIL1 with other DNA glycosylases shows that this enzyme is as efficient as those DNA glycosylases, which specifically remove the formamidopyrimidines from DNA.

Oxidative damage to DNA occurs when oxygen-derived species such as free radicals and other oxidizing agents react with the constituents of DNA, causing the formation of modified bases and sugars, DNA−protein cross links, strand breaks, and base-free sites (reviewed in refs *1* and *2*). Oxidative DNA damage is repaired in cells by a variety of repair pathways including base-excision repair (BER)[1] and nucleotide-excision repair (reviewed in ref *3*). There is evidence that modified bases in DNA are repaired mainly by BER, which is conserved from bacteria to mammals (reviewed in ref *4*). BER involves the removal of modified bases from DNA in the first step by DNA glycosylases, some of which also possess lyase activities (reviewed in ref *5*). The presence and mechanisms of action of DNA glycosylases in bacteria have widely been elucidated. In *Escherichia coli*, three DNA glycosylases, formamidopyrimidine DNA glycosylase (Fpg), endonuclease III (Nth), and endonuclease VIII (Nei) act on a variety of purine- and pyrimidine-derived lesions in oxidatively damaged DNA (reviewed in refs *6*−*9*). When DNA containing a multiplicity of modified bases is used as a substrate, Fpg is specific for purine-derived lesions and excises 8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), and 4,6-diamino-5-formamidopyrimidine (FapyAde) with similar excision kinetics (*10*, *11*). Nth and Nei primarily remove pyrimidine-derived lesions, although they also possess specificity for the purine-derived lesion FapyAde (*8*, *12*, *13*). However, these enzymes also exhibit cross reactivity in the case of oligonucleotides containing a single lesion incorporated at a specific position. Thus, Fpg efficiently removes pyrimidine-derived lesions from oligonucleotides (reviewed in ref *6*).

In eukaryotic cells, the counterparts of Fpg, Nth, and Nei were discovered (*7*). Nth-type glycosylases in human and yeast cells, human NTH1 (hNTH1), *Schizosaccharomyces*

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[1] Abbreviations: AP sites, abasic sites; BER, base-excision repair; Fpg, *E. coli* formamidopyrimidine DNA glycosylase; Nei, *E. coli* endonuclease VIII; GC/MS, gas chromatography/mass spectrometry; 8-OH-Gua, 7,8-dihydro-8-oxoguanine, 8-hydroxyguanine (also known as 8-oxoguanine); FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyAde, 4,6-diamino-5-formamidopyrimidine; Me-FapyGua, 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine; 5-OH-5-Me-Hyd, 5-hydroxy-5-methylhydantoin; ThyGly, thymine glycol; 5-OH-Cyt, 5-hydroxycytosine; 5-OH-Ura, 5-hydroxyuracil; 5,6-diHThy, 5,6-dihydrothymine; 5,6-diHUra, 5,6-dihydrouracil.

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pombe Nth, and *Saccharomyces cerevisiae* Ntg1 and Ntg2 (*y*Ntg1 and *y*Ntg2) act on pyrimidine-derived lesions, although the latter two enzymes also excise formamidopyrimidines from DNA (*7*, *8*). Other Nth-family enzymes, human, *S. cerevisiae*, *Drosophila melanogaster*, and *Arabidopsis thaliana* Ogg1 proteins (hOgg1, *y*Ogg1, *d*Ogg1, and *At*Ogg1, respectively) and *D. melanogaster* ribosomal protein S3 possess functional but no structural similarity to Fpg and remove 8-OH-Gua and FapyGua but not FapyAde from DNA with multiple lesions (*8*, *14*−*16*). Recently, novel Fpg/Nei family enzymes that exhibit sequence similarity to Nei were discovered in human and mouse cells (*17*−*22*) and named human NEIL1 and NEIL2 (hNEIL1 and hNEIL2) and mouse NEIL1 (mNEIL1). The term human FPG1 was also used for hNEIL1 (*20*) (It should be pointed out that the human gene map nomenclature committee's accepted name is NEIL1). Their substrate specificities were determined using a variety of oligonucleotides with a single lesion or DNA with multiple lesions. Oligonucleotides contained thymine glycol (ThyGly), 5-hydroxycytosine (5-OH-Cyt), 5-hydroxyuracil (5-OH-Ura), 5,6-dihydrothymine (5,6-diHThy), 5,6-dihydrouracil (5,6-diHUra), 2,6-diamino-4-hydroxy-5-*N*-methylformamidopyrimidine (Me-FapyGua), or 8-OH-Gua incorporated at a specific position. hNEIL1 and mNEIL1 exhibited substantial activity toward pyrimidine-derived lesions and the methylation product of guanine, Me-FapyGua; however, 8-OH-Gua paired with Cyt was not a significant substrate (*17*−*19*, *21*−*23*). In contrast, another study reported an efficient excision of 8-OH-Gua by human FPG1 from a duplex oligonucleotide, albeit a 10-fold greater activity by hOgg1 was observed (*20*). When high-molecular weight DNA with multiple lesions was used as a substrate, hNEIL1 excised only FapyGua and FapyAde among other purine- and pyrimidine-derived lesions (*18*). hNEIL2 exhibited activity for 8-OH-Gua in the DNA bubble structure (*23*).

In the present work, we investigated the substrate specificity and excision kinetics of mNEIL1 using high-molecular weight DNA with multiple lesions. The technique of gas chromatography/mass spectrometry (GC/MS) with isotope dilution was used for this purpose.

MATERIALS AND METHODS

Materials.[2] Modified DNA bases, their stable isotope-labeled analogues, and other materials for GC/MS were obtained as described (*24*, *25*). Calf thymus DNA was purchased from Sigma. The preparation of N_2O-saturated aqueous solutions of DNA and their exposure to ionizing radiation in a ^{60}Co γ source were performed as described (*26*). Aliquots of nonirradiated and irradiated DNA samples (100 μg) were dried in a SpeedVac under vacuum.

Expression and Purification of mNEIL1. C-Terminal hexahistidine-tagged mNEIL1 was expressed and purified as described previously (*22*).

Preparation of DNA Samples, Enzymic Assays, and GC/MS. Enzymic assays were performed as described (*27*). For the measurement of excision kinetics, DNA solutions were γ-irradiated at 5, 10, 20, 40, 60, and 80 Gy. Aliquots (100 μg) of irradiated DNA samples were dried in a SpeedVac under vacuum. Two sets of these samples with three replicates were prepared. One set of the samples was incubated with 1 μg of mNEIL1 at 37 °C for 30 min. The amount of the enzyme corresponded to a concentration of 242 nM. After incubation, 250 μL of cold ethanol (−20 °C) was added to the samples to stop the reaction and precipitate DNA. The samples were kept at −20 °C for 2 h. Aliquots of stable isotope-labeled analogues of modified DNA bases as internal standards and an additional 180 μL of cold ethanol (−20 °C) were added. After storage at −20 °C for 2 h, the samples were centrifuged at 15000 *g* for 30 min at 4 °C. DNA pellets and supernatant fractions were separated. Ethanol was removed from supernatant fractions under vacuum in a SpeedVac. Aqueous supernatant fractions were lyophilized to dryness for 18 h. The other set of irradiated samples was used to determine the levels of modified DNA bases in each sample. After addition of aliquots of stable isotope-labeled analogues of modified bases as internal standards, the samples were dried in a SpeedVac under vacuum and hydrolyzed with 0.5 mL of 60% formic acid in evacuated and sealed tubes for 30 min at 140 °C. The hydrolysates were frozen in liquid nitrogen and lyophilized for 18 h.

GC/MS measurements were performed using a gas chromatograph (Model 6890 Series) mass selective detector (Model 5973N) system (Agilent Technologies, Rockville, MD) according to the published procedures for trimethylsilylation of dried supernatant fractions and DNA hydrolysates and for the use of the GC column and other experimental conditions (*28*).

RESULTS

Substrate Specificity of mNEIL1. Excision of modified bases from DNA by mNEIL1 was investigated using DNA samples exposed to ionizing radiation in N_2O-saturated buffered aqueous solution. A total of 17 modified bases were identified and quantified in DNA samples using GC/MS. To find out as to which modified bases might be excised from DNA containing multiple lesions, we first incubated the DNA samples irradiated at a dose of 60 Gy with either the active or heat-inactivated enzyme or no enzyme. Supernatant and pellet fractions of DNA samples were separated and then analyzed by GC/MS. The selected-ion monitoring (SIM) mode was used, and the characteristic ions of the trimethylsilyl derivatives of modified DNA bases and those of their stable isotope-labeled analogues were monitored (*29*). An efficient excision of FapyGua, FapyAde, ThyGly, and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd) by mNEIL1 was observed. Other modified bases including 8-OH-Gua were not significantly excised. Figure 1 illustrates the levels of FapyGua and FapyAde in supernatant and pellet fractions of DNA samples in the case of mNEIL1. The evidence for the excision of these compounds was their appearance in supernatant fractions combined with the reduction of their levels in pellet fractions of DNA samples incubated with active enzyme when compared with DNA samples incubated with inactivated enzyme or no enzyme. The fact that the amount of each compound removed from the pellet fraction by the active enzyme was similar to its amount found in the supernatant fraction unequivocally proved FapyGua and

[2] Certain commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

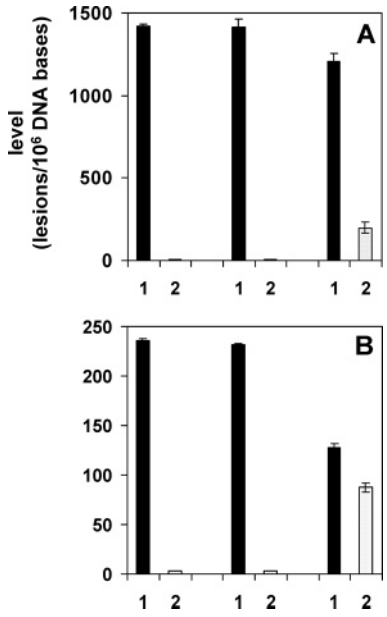

FIGURE 1: Excision of FapyGua (A) and FapyAde (B) by mNEIL1 from DNA γ-irradiated under N_2O. Dark columns (1), pellets; light columns (2), supernatant fractions. Left, DNA incubated with no enzyme; middle, DNA incubated with heat-inactivated enzyme; and right, DNA incubated with active enzyme. The enzyme amount was 2 μg/100 μg of DNA γ-irradiated at 60 Gy. The incubation time was 30 min at 37 °C. Each column represents the mean ± standard deviation of the values obtained by GC/MS analysis of three independently prepared samples.

FapyAde as substrates of mNEIL1. The heat-inactivated enzyme had no activity. Figure 2 illustrates the dependence of excision of FapyGua, FapyAde, ThyGly, and 5-OH-5-MeHyd on the enzyme amount. Excision steadily increased and reached a plateau above 2 μg of mNEIL1/100 μg of DNA. Figure 2A also shows that no significant excision of 8-OH-Gua from DNA was observed. A time course of excision was measured using incubation times of 5, 10, 20, and 30 min. Excision increased with time and reached a plateau after 30 min (Figure 3).

Excision Kinetics. DNA samples were irradiated at six radiation doses, i.e., 5, 10, 20, 40, 60, and 80 Gy, to obtain different levels of modified bases to measure the dependence of excision on the substrate concentration. Excised levels of modified bases found in supernatant fractions were used for the determination of the kinetic parameters. Concentration ranges of FapyGua and FapyAde in DNA samples incubated with the enzyme were 1.15−6.10 and 0.20−1.12 μM, respectively. Excision followed Michaelis−Menten kinetics (*30*). Lineweaver−Burk plots for excision of FapyGua and FapyAde are illustrated in Figure 4. The calculation of the kinetic constants and standard deviations ($n = 6$) was achieved using a computer program with the linear least-squares analysis of the data. Kinetic constants of the excision of FapyGua and FapyAde are given in Table 1. Relatively low excision of ThyGly and 5-OH-5-MeHyd (Figure 2) from DNA did not permit an accurate determination of their excision kinetics. There was no statistically significant difference between the maximum velocities (V_{max}), Michaelis constants (K_m), or the specificity constants (k_{cat}/K_m) for excision of FapyGua and FapyAde, indicating a similar preference of mNEIL1 for both compounds. There are numerous prokaryotic and eukaryotic DNA glycosylases that

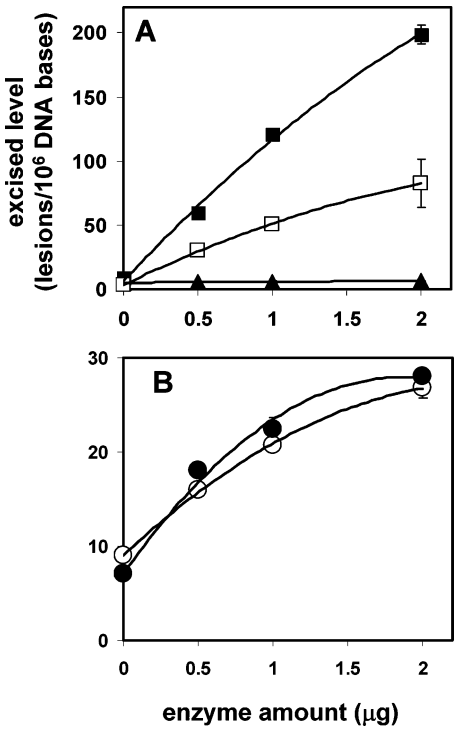

FIGURE 2: Dependence of excision on the enzyme amount. The incubation time was 30 min at 37 °C. The levels given on the *y* axis represent those found in the supernatant fractions. The calculation of the level of each lesion per 10^6 DNA bases was based on the known total level of each lesion in DNA samples prior to enzymic hydrolysis. Each data point represents the mean ± standard deviation of the values obtained by GC/MS analysis of three independently prepared DNA samples, which were γ-irradiated under N_2O at 60 Gy. (A) ■, FapyGua; □, FapyAde; ▲, 8-OH-Gua. (B) ○, ThyGly; ●, 5-OH-5-MeHyd. Some standard deviations are not seen in these graphs because they were less than 5% of the corresponding data points.

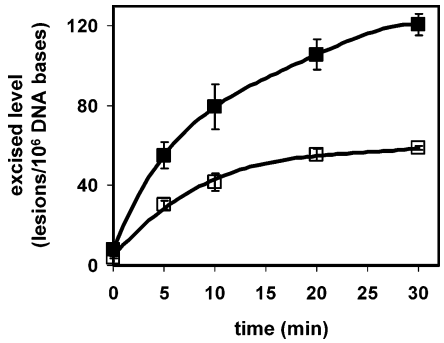

FIGURE 3: Dependence of excision on the incubation time. The enzyme amount was 1 μg/100 μg of DNA. Other details are the same as in Figure 2. ■, FapyGua; □, FapyAde. In some cases, the standard deviations are not seen in this graph because they were less than 5% of the corresponding data points.

are specific for excision of FapyGua and FapyAde from DNA. Table 2 shows a comparison of the specificity constants of these enzymes with that of mNEIL1. It should be pointed out that Fpg and *Dr*Fpg remove 8-OH-Gua from DNA in addition to FapyGua and FapyAde and that Nei, Nth, *y*Ntg1, and *y*Ntg2 are also specific for pyrimidine-derived lesions (reviewed in ref *8*). Furthermore, eukaryotic Ogg1 proteins act on 8-OH-Gua in addition to FapyGua but not on FapyAde (*8*). Kinetics of hNEIL1 was not reported (*18*). The comparison shows that Fpg is the most efficient enzyme to remove both FapyGua and FapyAde from DNA.

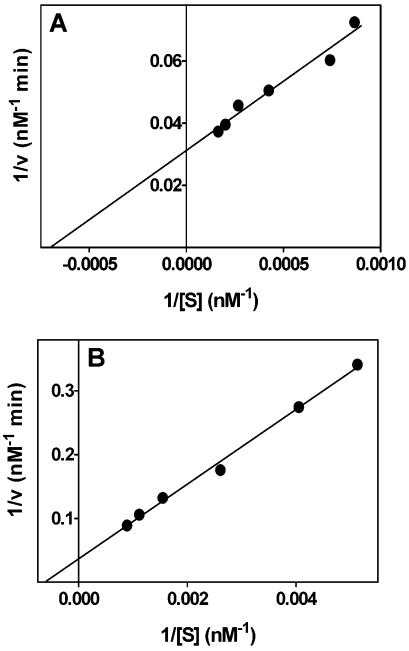

FIGURE 4: Lineweaver−Burk plots for excision of FapyGua (A) and FapyAde (B) by mNEIL1 from DNA γ-irradiated under N_2O at doses 5, 10, 20, 40, 60, and 80 Gy. The incubation time was 30 min at 37 °C. The enzyme amount was 1 μg/100 μg of DNA. Concentration ranges of FapyGua and FapyAde in DNA samples treated with the active enzyme were 1.15−6.10 and 0.20−1.12 μM, respectively. Amounts of products found in the supernatant fractions were used for the initial velocity. *S*, concentration of FapyGua or FapyAde; v, initial velocity of excision of FapyGua or FapyAde.

Table 1: Kinetic Constants for Excision of FapyGua and FapyAde by mNEIL1 from DNA γ-Irradiated under N_2O

substrate	V_{max} (nM min^{-1})	k_{cat} (min^{-1})	K_m (nM)	$k_{cat}/K_m \times 10^5$ (min^{-1} nM^{-1})
FapyGua	32.1 ± 2.2[a]	0.132 ± 0.009	1422 ± 165	9.3 ± 0.6
FapyAde	27.4 ± 4.6	0.113 ± 0.019	1605 ± 274	7.1 ± 0.1

[a] Values represent the mean ± standard deviation ($n = 6$). ($k_{cat} = V_{max}$/[enzyme]). The enzyme amount was 1 μg/100 μg of DNA, corresponding to an enzyme concentration of 242 nM. Concentration ranges of FapyGua and FapyAde were 1.15−6.10 and 0.20−1.12 μM, respectively. For comparison, the yields of other purine- and pyrimidine-derived lesions in DNA irradiated under N_2O, which were measured under identical experimental conditions, can be found elsewhere (e.g., see refs *49* and *50*).

Otherwise, the efficiency of mNEIL1 is similar to or greater than those of the enzymes listed in Table 2.

DISCUSSION

Mouse NEIL1 possesses both DNA glycosylase and β-δ lyase activities similar to other members of the Fpg/Nei family (*9*). Essentially, it is a functional hybrid of Fpg and Nei. Mouse embryonic stem cells deficient in mNEIL1 exhibit a hypersensitivity to the effects of ionizing radiation, indicating the role of this protein in the repair of oxidative DNA damage (*22*). It was shown earlier that mNEIL1 readily excises 5,6-diHUra, Me-FapyGua, a methylation product of guanine, and two stereoisomers of ThyGly from double-stranded oligonucleotides with a single lesion and also acts on AP sites (*22*). Cytosine-derived lesions such as 5-OH-Cyt and 5-OH-Ura were also removed, albeit at a lower rate. Accumulation of these lesions in mouse cells because of the lack of mNEIL1 may account for observed hypersensitivity to ionizing radiation (*22*). On the other hand, mNEIL1 did not significantly act on 8-OH-Gua paired with Cyt. However, recent evidence indicated that mNEIL1 removes the further oxidation products of 8-OH-Gua, i.e., spiroiminodihydantoin and guanidinohydantoin, from oligonucleotides (*31*). Our current study does not include these compounds.

The present study shows for the first time that mNEIL1 efficiently excises FapyGua and FapyAde from high-molecular weight DNA containing multiple lesions formed by ionizing radiation-generated hydroxyl radicals and H atoms. Albeit to a lesser extent, mNEIL1 also acted on ThyGly and 5-OH-5-Me-Hyd in DNA. FapyGua and FapyAde are major hydroxyl radical-induced products in DNA, generated by addition of the hydroxyl radical at the C8 position of guanine and adenine, respectively, followed by one-electron reduction of thus-formed C8-OH-adduct radicals (reviewed in ref *32*). These compounds are also formed in DNA by UV radiation by a different mechanism, which involves the formation of a purine radical cation by photoionization, followed by hydration (addition of $^-$OH) of the imidazole ring and subsequent reduction (*33*, *34*). Excision of FapyGua and FapyAde from oligonucleotides by mNEIL1 was not investigated because of the lack of oligodeoxynucleotides containing these compounds. Such studies might be possible in future using recently synthesized oligodeoxynucleotides that contain FapyGua or FapyAde incorporated at a specific position (*35*, *36*). Recent studies unequivocally provided the evidence that both FapyGua and FapyAde are premutagenic lesions (*37*, *38*). This indicates the importance of these lesions in biological effects of oxidative DNA base damage.

The excision of FapyGua, FapyAde, and 5-OH-5-Me-Hyd extends the substrate specificity of mNEIL1 previously determined using oligonucleotides with a single lesion. Past work recognized mNEIL1 as a DNA glycosylase specific for pyrimidine-derived lesions. The present work shows that this enzyme is also specific for purine-derived lesions, even more so when high-molecular weight DNA with multiple

Table 2: Comparison of the Specificity Constants [$k_{cat}/K_m \times 10^5$ (min^{-1} nM^{-1})][a] for Excision of FapyGua and FapyAde from DNA by mNEIL1, Fpg (*11*), *Dr*Fpg (*50*), Nei (*13*), Nth (*26*), *y*Ntg1 and *y*Ntg2 (*51*), hOgg1 (hOgg1-Ser^{326}) (*52*), *y*Ogg1 (*53*), *At*Ogg1 (*16*), and *d*Ogg1 (*54*)[b]

substrate	mNEIL1	Fpg	*Dr*Fpg	Nei	Nth	*y*Ntg1	*y*Ntg2	hOgg1	*y*Ogg1	*At*Ogg1	*d*Ogg1
FapyGua	9.3 ± 0.6[c]	25.7 ± 2.9[d,e]	8.6 ± 0.9[f]	*g*	*g*	3.6 ± 0.5[h]	2.4 ± 0.6[e,h]	7.4 ± 0.2[i]	1.5 ± 0.1[j]	6.6 ± 0.2[k]	11.2 ± 0.2
FapyAde	7.1 ± 0.1[l]	17.6 ± 1.2[m]	6.5 ± 0.6[n]	5.0 ± 0.3[n]	3.1 ± 0.3[o]	4.9 ± 0.7	4.7 ± 0.6	*g*	*g*	*g*	*g*

[a] Values represent the mean ± standard deviation ($n = 6$). [b] DNA irradiated under N_2O was used. [c] Statistically different from the values in columns 2 and 6−10 ($p < 0.05$). [d] Statistically different from the values in columns 3 and 6−11 ($p < 0.05$). [e] Statistically different from the value in line 2 ($p < 0.05$). [f] Statistically different from the values in columns 6, 7, 9, and 10 ($p < 0.05$). [g] No excision observed. [h] Statistically different from the values in columns 8−11 ($p < 0.05$). [i] Statistically different from the value in columns 9 and 11 ($p < 0.05$). [j] Statistically different from the values in columns 10 and 11 ($p < 0.05$). [k] Statistically different from the values in column 11 ($p < 0.05$). [l] Statistically different from the values in columns 2, 4, and 5−7 ($p < 0.05$). [m] Statistically different from the values in columns 3−7 ($p < 0.05$). [n] Statistically different from the value in column 5 ($p < 0.05$). [o] Statistically different from the value in columns 6 and 7 ($p < 0.05$).

lesions is used rather than oligonucleotides. The excision of a methylated analogue of FapyGua, i.e., Me-FapyGua by mNEIL1 or by human FPG1 was also reported (*20*, *22*). It should be noted, however, that the mechanisms of formation of Me-FapyGua and FapyGua are entirely different. The former is a methylation product of guanine, whereas the latter results from guanine by hydroxyl radical attack (*32*) or by UV radiation (*33*, *34*). Thus, these compounds should not be confused with each other. Furthermore, a recent study unequivocally showed that FapyGua is a strongly mutagenic lesion (*37*), whereas Me-FapyGua has been recognized for a long time as a block to DNA polymerases, thus a lethal lesion (*39*, *40*).

The present study unequivocally establishes that mNEIL1 possesses common substrates, FapyGua and FapyAde with its functional homologue hNEIL1 (*18*). No significant excision of other lesions was observed, including 8-OH-Gua. Excision of 8-OH-Gua by the bacterial Fpg/Nei super family of DNA glycosylases has been controversial (*9*, *18*−*22*). Our results are in agreement with the lack of activity on 8-OH-Gua of mNEIL1 and hNEIL1 enzymes from several different laboratories that used oligonucleotides containing a single 8-OH-Gua (*18*, *19*, *21*, *22*) or DNA with multiple lesions (*18*).

K_m values of excision by mNEIL1 as obtained in the present work using high-molecular weight DNA with multiple lesions are substantially different from those recently reported using oligonucleotides with a single ThyGly (*22*). Previously, this phenomenon was also observed with other DNA glycosylases, although k_{cat} values were somewhat similar (*8*, *41*). High K_m values may be due to the fact that the enzyme encounters only one substrate in an oligonucleotide, whereas it is presented with several substrates simultaneously when high-molecular weight DNA is used. Thus, each lesion may compete with other lesions for excision by a given enzyme and act as an inhibitor with respect to others. This may result in kinetic parameters of excision from DNA being dramatically different from those obtained with oligonucleotides with a single lesion. An additional reason may be a very high effective concentration of a lesion in an oligonucleotide, such as 1 lesion in 20−40 base pairs, in contrast to DNA with multiple lesions at concentration levels at least 2 orders of magnitude smaller than those in oligonucleotides. The phenomenon of high K_m values obtained with the use of high-molecular weight DNA is consistent with the one-dimensional model for proteins scanning DNA (*41*−*44*). Essentially, this model suggests that the translocation time of an enzyme to search for a DNA lesion without dissociation of the enzyme−DNA complex will be increased by the size of DNA molecule, causing an increase in the K_m value. An excellent discussion of this phenomenon regarding the action of DNA glycosylases can be found elsewhere (*41*).

This phenomenon also explains why lesions removed by a DNA glycosylase from oligonucleotides are not recognized by the same enzyme in high-molecular weight DNA (reviewed in ref *8*). Thus, as the present work shows, mNEIL1 excises only FapyGua and FapyAde as the primary substrates from high-molecular weight DNA and ThyGly and 5-OH-5-MeHyd to a lesser degree, while it efficiently excises pyrimidine-derived lesions ThyGly, 5,6-diHUra, 5-OH-Cyt, and 5-OH-Ura in oligonucleotides (*22*). hNEIL1 also exhibits the same type of activity (*18*). Another excellent example is that Fpg excises 8-OH-Gua and pyrimidine-derived lesions such as ThyGly, 5-OH-Cyt, 5,6-dihydrothymine, and others from oligonucleotides with a single lesion (*22*, *45*−*48*). In contrast, this enzyme removes only 8-OH-Gua, FapyGua, and FapyAde from high-molecular weight DNA (*10*, *11*).

In conclusion, we show that mNEIL1 possesses specificity, with similar efficiency of excision, for two purine-derived formamidopyrimidines that are formed in DNA by hydroxyl radical attack or by UV radiation. This specificity reveals that mNEIL1 has common substrates with its functional homologue hNEIL1. A comparison of kinetics of mNEIL1 with other enzymes shows that this enzyme is as efficient as other DNA glycosylases, which specifically remove the formamidopyrimidines from DNA.

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