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Deoxyribophosphate lyase activity of mammalian endonuclease VIII-like proteins

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Abstract Base excision repair (BER) protects cells from nucleobase DNA damage. In eukaryotic BER, DNA glycosylases generate abasic sites, which are then converted to deoxyribo-5′-phosphate (dRP) and excised by a dRP lyase (dRPase) activity of DNA polymerase β (Polβ). Here, we demonstrate that NEIL1 and NEIL2, mammalian homologs of bacterial endonuclease VIII, excise dRP by β-elimination with the efficiency similar to Polβ. DNA duplexes imitating BER intermediates after insertion of a single nucleotide were better substrates. NEIL1 and NEIL2 supplied dRPase activity in BER reconstituted with dRPase-null Polβ. Our results suggest a role for NEILs as backup dRPases in mammalian cells.

Keywords: DNA repair; Base excision; Deoxyribophosphate lyase; NEIL; DNA polymerase β

1. Introduction

Base excision repair (BER) is responsible for cleansing DNA of non-bulky, frequently occurring base lesions [1]. During BER, the lesion is first located by one of DNA glycosylases, enzymes that excise damaged bases. This results in an abasic (AP) site, which is hydrolyzed at its 5′-side by an AP endonuclease, leaving a nick flanked by a 3′-hydroxyl of an undamaged deoxynucleotide and a deoxyribo-5′-phosphate (dRP) to which the damaged base was formerly connected. DNA polymerase then inserts a normal deoxynucleotide; however, ligation to restore intact DNA is impossible because of the dangling dRP moiety. The situation is resolved by a special enzymatic activity, deoxyribophosphatase (dRPase), excising dRP (short-patch BER, Fig. 1), or by continuing DNA synthesis with strand displacement, followed by degradation of the displaced strand (long-patch BER). The whole process currently draws much attention due to its antimutagenic and tumor suppression role [2].

The dRPase activity, a rate-limiting step in BER [3], plays a central role in switching between its short- and long-patch branches. In *Escherichia coli*, two main dRPase activities have been observed. A Mg^{2+}-dependent RecJ deoxyribophosphodiesterase hydrolyzes the 3′-phosphodiester bond in dRP releasing deoxyribo-5′-phosphate [4]. Formamidopyrimidine-DNA glycosylase (Fpg) does not depend on Mg^{2+} and catalyzes β-elimination of dRP rather than its hydrolysis (deoxyribophosphate lyase), the product being 2-hydroxy-5-oxopent-3-enyl phosphate [5]. Both RecJ and Fpg leave a 5′-terminal phosphate in DNA, creating a substrate for DNA ligase. Little Mg^{2+}-independent activity was observed in *fpg* null cells [5], although dRPase activity *in vitro* has also been reported for *E. coli* endonuclease VIII (Nei), a homolog of Fpg [6]. Nei dRPase activity may explain the lack of phenotype in *fpg recJ* double mutants [4].

The major dRPase in mammalian cells is DNA polymerase β (Polβ) [7,8], which has a dRP lyase domain [9,10]. Polβ-deficient cells show low dRPase activity [7], but some residual dRP removal by extracts from these cells is still present [11]. A dRPase activity in vertebrates was also shown for the mitochondrial DNA polymerase γ [12–14], translesion DNA polymerases ι [15] and λ [16], and a Mg^{2+}-dependent activity was purified from human cells and calf thymus [17]. It is possible that while Polβ carries out the bulk of dRP removal, other activities could be more specifically employed for some lesions, cell or tissue types, or at certain cell cycle points.

Recently, three mammalian homologs of Fpg and Nei have been identified and termed NEIL (Nei-like, or endonuclease VIII-like)-1, -2, and -3 [18–23]. Based on the similarity of their active sites to those of Fpg and Nei (Fig. 2), one could expect that they also display dRPase activity. In this report we show that two of these proteins, NEIL1 and NEIL2, are capable of removing dRP from DNA with the efficiency comparable to that of Polβ, and that they can substitute for Polβ dRPase activity in a reconstituted BER system.

2. Materials and methods

2.1. Oligonucleotides and enzymes

ODNs were synthesized from phosphoramidite precursors (Glen Research) using established protocols. The modified 23-mer strand, 5′-CTCTCCCTTCXCTCCTTTCCTCT-3′, where X is uracil (U) or 8-oxoguanine (8-oxoG), was 5′-labeled using γ[^{32}P]-ATP and polynucleotide kinase, purified by PAGE, precipitated and annealed to a complementary 23-mer strand 5′-AGAGGAAAGGAGNGAAGGGAGAG-3′ (N = A, C, G, or T). To label the modified ODNs at the 3′-terminus, they were annealed to a 25-mer complementary strand, 5′-GTAGAGGAAAGGAGNGAAGGGAGAG-3′, and the overhang

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Abbreviations: AP, apurinic/apyrimidinic; BER, base excision repair; dRP, deoxyribo-5′-phosphate; dRPase, deoxyribo-5′-phosphate lyase; ODN, oligodeoxynucleotide; PAGE, polyacrylamide gel electrophoresis; Polβ, DNA polymerase β; mPolβ, Polβ K35A/K68A/K72A mutant; SDS, sodium dodecyl sulfate

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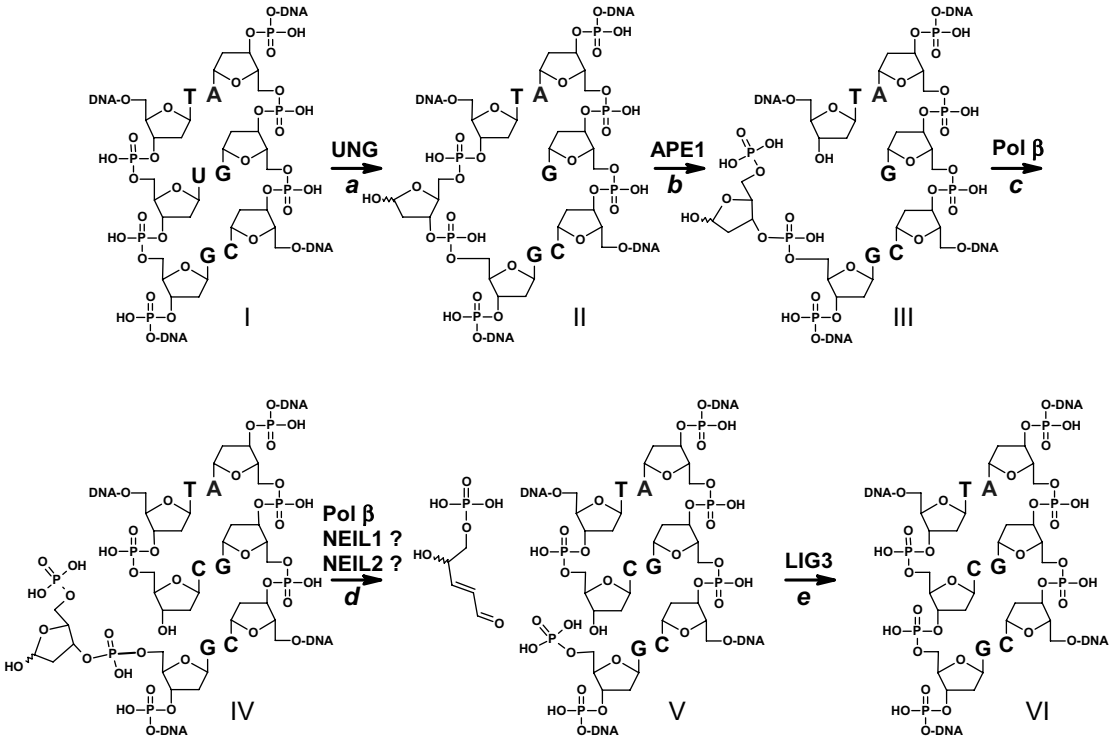

Fig. 1. General scheme of base excision repair. Main stages of the short-patch BER sub-pathway and the relevant enzymes are shown schematically for a U:G mispair (I) formed by spontaneous cytosine deamination: (a), excision of the damaged base by a DNA glycosylase (UNG) with formation of an AP site (II); (b), 5′-incision of the AP site by AP endonuclease (APE1) with formation of a dRP site (III); (c), insertion of a correct nucleotide by a DNA polymerase (Polβ for mammalian short-patch BER) with formation of a ''hanging'' dRP site (IV); (d), elimination of the dRP site by a dRP lyase (Polβ, or, possibly, NEIL1/NEIL2) with formation of a nick in DNA (V); (e), ligation of the nick by a DNA ligase (DNA ligase III for mammalian short-patch BER) and restoration of undamaged DNA (VI).

was partially filled by Klenow fragment using α[^{32}P]-dATP. T4 polynucleotide kinase, T4 DNA ligase and *E. coli* uracil-DNA glycosylase (Eco-Ung) were purchased from New England Biolabs. Exonuclease-deficient Klenow fragment was a gift from Dr. Holly Miller (SUNY Stony Brook); human uracil-DNA glycosylase (UNG) was kindly provided by Dr. Alexander Ischenko (Institut Gustave Roussy, France). NEIL1 and NEIL2 proteins, wild-type and dRPase-deficient Polβ were expressed in *E. coli* and purified as described [24–26]. 8-Oxoguanine-DNA glycosylase (OGG1) and AP endonuclease (APE1) were expressed as His_6-tagged proteins and purified using Ni^{2+}-chelate chromatography [27]. Concentrations of active forms of NEIL1 and NEIL2 were determined by $NaBH_4$-stabilized crosslinking of the enzyme (10 nM) to saturating amounts (5 μM) of a dRP substrate as described in the following section.

2.2. dRPase and crosslinking assays

To prepare a dRP substrate, the 3′-labeled U-containing duplex (20 nM unless indicated otherwise) was treated with 1 U Eco-Ung and 1 μM APE1 in 25 mM K–phosphate (pH 7.4), 5 mM $MgCl_2$ and 1 mM dithiothreitol for 10 min at 25 °C. To obtain a substrate with an inserted nucleotide, this reaction mixture was supplemented with 40 nM mPolβ and 1 mM dGTP. To analyze dRPase activity, NEIL1, NEIL2, or Polβ was added (20 μl final reaction volume) and incubated for 10 min at 25 °C. The reaction products were stabilized by 50 mM $NaBH_4$ for 30 min on ice. The reaction products were resolved by 20% denaturing PAGE and quantified using Molecular Imager FX (Bio-Rad). To analyze crosslinking, 50 mM $NaBH_4$ was added together with dRP lyases and incubated for 30 min on ice. The products were resolved by 12% SDS–PAGE and imaged as above.

2.3. Base excision repair reconstitution assay

The reaction mixtures (20 μl) included 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 25 μg/ml bovine serum albumin, 10 nM substrate duplex, 1 mM dGTP, 600 nM APE1, 500 nM Polβ or mPolβ, T4 DNA ligase (1 Weiss unit), and, if needed, 75 nM NEIL1 or 150 nM NEIL2. When the repair of an AP site was reconstituted, the U-containing substrate was pre-treated with 1 U Eco-Ung as above. When the repair of U or 8-oxoG was studied, the reaction mixture was supplemented with 400 nM UNG or OGG1, respectively. The reaction mixture was incubated for 20 min at 25 °C and analyzed by 20% denaturing PAGE.

3. Results

dRPase activity can be revealed with 3′-labeled nicked abasic ODN substrates, which were prepared by end-filling of 5′-overhanging ODN duplexes with ^{32}P-labeled dATP and the treatment of the duplex with Ung and APE1. As the resulting dRP site is unstable in nucleophilic buffers and is degraded during migration through Tris-containing gels, the products were stabilized by $NaBH_4$ reduction immediately after the dRPase reaction. Under these conditions, β-elimination of dRP leads to a product with a slightly higher mobility (the bottom arrow in Fig. 3A) compared to the dRP-containing substrate (the middle arrow in Fig. 3A). Fig. 3A illustrates that both NEIL1 and NEIL2 possess a dRP-removing activity. This activity was similar in potassium phosphate and Tris–HCl buffers and was not affected by the presence or absence of Mg^{2+} ions in the reaction mixture (data not shown). The dRPase activities of NEIL1 and NEIL2 demonstrated the enzyme concentration and time dependence expected of an enzyme-catalyzed reaction (Fig. 3B and data not shown). The activity of NEIL1 in these experiments appeared higher than that of NEIL2 (Fig. 3B). Both NEIL1 and NEIL2 excised