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AP-site cleavage activity of tyrosyl-DNA phosphodiesterase 1

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ABSTRACT

APE-independent base excision repair (BER) pathway plays an important role in the regulation of DNA repair mechanisms. In this study it has been found that recently discovered tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the AP site cleavage reaction to generate breaks with the 3′- and 5′-phosphate termini. The removal of the 3′-phosphate is performed by polynucleotide kinase phosphatase (PNKP). Tdp1 is known to interact stably with BER proteins: DNA polymerase beta (Pol β), XRCC1, PARP1 and DNA ligase III. The data suggest a role of Tdp1 in the new APE-independent BER pathway in mammals.

1. Introduction

Tyrosyl-DNA phosphodiesterase (Tdp1) was discovered as an enzymatic activity from *Saccharomyces cerevisiae* that specifically hydrolyzes the phosphodiester linkage between the O-4 atom of a tyrosine and a DNA 3′ phosphate [1]. This type of linkage is typical for the covalent reaction intermediate produced upon Topoisomerase 1 cleavage of one DNA strand. Human Tdp1 can also hydrolyze other 3′-end DNA alterations that are covalently linked to the DNA, indicating that it may function as a general 3′-DNA phosphodiesterase and repair enzyme [2]. Oxidative damage at DNA ends (i.e. the termini of DNA single- or double-strand breaks) or intermediates in the base excision repair (BER) process may represent substrates for Tdp1 in vivo. For example, it is conceivable that Tdp1 acts on the 3′ phospho α, β unsaturated aldehyde (3′ dRP) that results from β-elimination by the base-specific mammalian DNA glycosylases/AP lyases (for example, OGG1 or NTH1) [3,4]. Also Tdp1 can remove the tetrahydrofuran moiety from the 3′-end of DNA [5].

Human Tdp1 always cleaves at the most 3′ backbone phosphate to remove one nucleoside from either DNA or RNA and leave a 3′ phosphate terminus. The yeast enzyme was reported to be unable to cleave the DNA phosphate backbone, but it is possible that the assay used was not sensitive enough to detect this activity [6]. The 3′-phosphate-end generated by Tdp1 has to be hydrolyzed to a 3′-hydroxyl in order to enable the following DNA repair. Polynucleotide kinase phosphatase (PNKP), a bifunctional enzyme with 5′-kinase and 3′-phosphatase activities, has been suggested as a reasonable candidate in human cells for the repair of these 3′-phosphate lesions. Tdp1 has been shown to exist in complex with PNKP in human cells [7,8].

Since several thousand apurinic/apyrimidinic (abasic or AP) sites are generated spontaneously every day in a living cell [3,9] and this structure is the key intermediate of BER pathway, it was of particular interest to find out whether Tdp1 can hydrolyze of AP site. It is known that the first step in BER, excision of damaged base, is catalyzed by DNA glycosylases, many of which are monofunctional and hydrolyze *N*-glycosidic bonds to generate abasic sites. The major enzyme of eukaryotic cells catalyzing the endonuclease cleavage of AP sites is apurinic/apyrimidinic endonuclease 1 (APE1) that cleaves the phosphodiester bond at the 5′ end of abasic site. Bifunctional DNA glycosylases that excise oxidized bases also possess an intrinsic lyase activity, cleaving the DNA at the resultant AP sites [10]. These DNA glycosylase/AP lyases belong to two broad classes of enzymes, based on their reaction mechanism. *Escherichia coli* Nth is the representative of one class that utilizes an internal lysine as the active site nucleophile and cleaves the DNA strand at the AP site by β elimination, generating a 3′ phospho α,β-unsaturated aldehyde (3′-dRP) at the strand break [11]. In contrast, another class of mammalian DNA glycosylases, belonging to the family of *E. coli* Nei and Fpg (named Nei-like (NEIL)) catalyzes βδ elimination at the AP site and

Abbreviations: BER, base excision repair; Tdp1, tyrosyl-DNA phosphodiesterase 1; PNKP, polynucleotide kinase phosphatase; Pol β, DNA polymerase beta

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removes the deoxyribose residue to produce a 3′ phosphate terminus at the DNA strand break [12].

In this report we show that Tdp1 generates DNA strand break with the 3′ phosphate termini from the abasic (AP) site therefore it can function in a fashion similar to NEIL1 in the APE-independent BER pathway.

2. Materials and methods

2.1. Materials

[γ-^{32}P]ATP (5000 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci//mmol) were produced in the Laboratory of Radiochemistry (ICBFM, Novosibirsk); phage T4 polynucleotide kinase was purchased from Biosan (Russia); stained molecular mass markers were from Fermentas (Lithuania), reagents for electrophoresis and buffer components from Sigma (USA). Ultrapure dNTPs were from SibEnzyme (Russia).

The recombinant Tdp1 was purified to homogeneity as described [13] with the additional purification steps using plasmid pET 16B-Tdp1 kindly provided by Dr. K.W. Caldecott (University of Sussex, United Kingdom). Purification protocol and coomassie stained protein gel are shown in Supplementary data. The recombinant purified UDG, DNA polymerase β, APE1 and XRCC1 were a generous gift from Dr. S.N. Khodyreva (ICBFM, Novosibirsk). Phage T4 endonuclease III, NEIL1, PNKP and DNA ligase III were kindly donated by Dr. D.O. Zharkov (ICBFM, Novosibirsk).

2.2. Radioactive labeling of oligonucleotides

Oligodeoxynucleotides were 5′-[^{32}P]-labeled with T4 polynucleotide kinase and [γ^{32}P] ATP as described [14]. Unreacted [γ^{32}P] ATP was removed by passing the mixture over a MicroSpin™ G-25 column (Amersham, USA) using the manufacturer's suggested protocol. Complementary oligodeoxynucleotides were annealed in equimolar amounts by heating a solution to 95 °C for 3 min, followed by slow cooling to room temperature. The sequences of the oligonucleotides used in experiments are shown:

AP-DNA: 5′-GGCGATTAAGTTGGG**U**AACGTCAGGGTCTTCC-3′
3′-CCGCTAATTCAACCC GTTGCAGTCCCAGAAGG-5′
THF-DNA: 5′-GGCGATTAAGTTGGG**THF**AACGTCAGGGTCTTCC-3′
3′-CCGCTAATTCAACCC G TTGCAGTCCCAGAAGG-5′

2.3. Endonuclease assays

Standard reaction mixtures (10 μl) contained 50 mM Tris–HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 10 nM 5′-[^{32}P]-labeled DNA substrate and necessary enzymes (Tdp1, NEIL1, APE1 or EndoIII). For the preparation of natural AP site, a AP-DNA duplex was first incubated in reaction buffer with UDG (0.5 U/μl) for 15 min at 37 °C. The reaction mixtures were incubated at 37 °C for 30 min. Then reactions were terminated by adding of the formamide dye and the mixtures were heated for 3 min at 90 °C. The products were analyzed by electrophoresis in 20% polyacrylamide gel with 8 M urea followed by autoradiography [14].

2.4. DNA repair reconstitution assay

The reaction mixture (10 μl) contained 10 nM of the labeled substrate in a buffer containing 50 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.5 mM dCTP, 1 mM ATP. Different combinations of Tdp1 (100 nM), DNA polymerase beta (Pol β) (50 nM), PNKP (300 nM), and DNA ligase III (10 nM) were added as required. The mixtures were incubated at 37 °C for 30 min and analyzed as above.

3. Results and discussion

To identify a new activity of Tdp1, we analyzed the capability of enzyme to cleave DNA structure containing a natural abasic site. The 32 mer DNA duplex bearing a single uridine moiety in the 5′ end-labeled strand was treated with UDG to produce AP site that was efficiently cleaved by Tdp1 (Fig. 1A, lane 1). Without addition of UDG, DNA duplex with uridine residue was not cleaved by Tdp1. This activity was compared with the action of bifunctional DNA glycosylase NEIL1 (lane 2) as well as with activity of two endonucleases, APE1 and T4 endonuclease III (EndoIII) (lanes 3 and 4, respectively). AP site cleavage mechanisms catalyzed by Endo III, APE1, and NEIL1 are shown schematically in Fig. 1B. One can see from the experimental data (Fig. 1A, lanes 1 and 2) that the mobility of the product of cleavage produced by Tdp1 was the same as generated by AP lyase activity of NEIL1, which creates the 3′ terminal phosphate (3′-P) in the AP site cleavage reaction. Both APE1 and EndoIII cleavage products bearing the 3′-OH and the 3′-dRP groups, respectively, demonstrate the lower mobility in gel electrophoresis (lanes 3 and 4). This data point out that, Tdp1 cleaves AP-DNA generating the 3′-P termini.

We also tested Tdp1 activity towards AP sites located in ssDNA or dsDNA in comparison with the same DNA structures containing an inside strand tetrahydrofuran (THF) moiety as a synthetic analogue of abasic site (Fig. 2). Tdp1 is more active in the cleavage of ssDNA containing THF moiety (Fig. 2, lane 2) or natural AP site (lane 9) in comparison with dsDNA containing THF or natural AP site in one of the strands (lanes 5 and 12, respectively). APE1 shows preferentially hydrolysis of the AP site or its synthetic analogue located in one strand of dsDNA (compare lanes 4 and 11 with lanes 1 and 8). Unlike Tdp1 NEIL1 was unable to cleave DNA with THF moiety (lanes 3 and 6), but catalyzed the hydrolysis of both ss and dsDNA containing natural AP site (lanes 10 and 13). It should be noted that the experiments with Tdp1 also showed the product P1. This indicates that Tdp1 was able to remove a 3′ terminal mononucleoside moiety in both ssDNA and DNA duplex to produce 3′-phosphate whereas resulting DNA containing 3′-phosphate

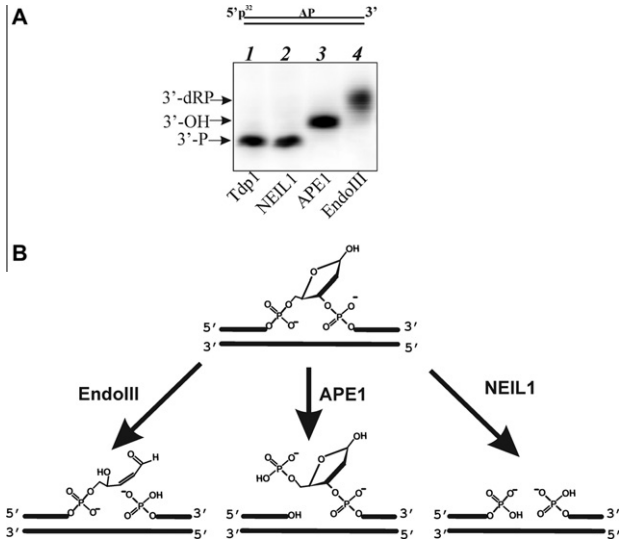

Fig. 1. Tdp1 is able to cleave 5′-end labeled AP-DNA structure. (A) The 15mer product with 3′-phospate (3′-P) was generated by incubation of the 5′-end labeled AP-DNA with Tdp1 (lane 1). The same product with the 3′-P was observed after incubation with NEIL1 (lane 2). Lane 3 shows the 15mer product with the 3′-OH after incubating AP-DNA with APE1 and lane 4 shows the product with 3′-dRP after hydrolysis of AP-DNA with EndoIII. (B) Scheme of hydrolysis of the AP site in one strand of DNA duplex by different enzymes.