Biochimie 94 (2012) 1749–1753

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Tyrosyl-DNA phosphodiesterase 1 initiates repair of apurinic/apyrimidinic sites

Natalia A. Lebedeva [a], Nadejda I. Rechkunova [a], Sherif F. El-Khamisy [b,c,2], Olga I. Lavrik [a,*,1]

[a] Institute of Chemical Biology and Fundamental Medicine, Lavrentiev av. 8, 630090 Novosibirsk, Russia
[b] MRC Genome Damage and Stability Centre, University of Sussex, Brighton, UK
[c] Department of Biochemistry, Faculty of Pharmacy, Ain Shams University, Egypt

ARTICLE INFO

Article history:
Received 29 February 2012
Accepted 4 April 2012
Available online 12 April 2012

Keywords:
Base excision repair
AP site
Tyrosyl-DNA phosphodiesterase 1

ABSTRACT

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the hydrolysis of the phosphodiester linkage between the DNA 3′ phosphate and a tyrosine residue as well as a variety of other DNA 3′ damaged termini. Recently we have shown that Tdp1 can liberate the 3′ DNA phosphate termini from apurinic/apyrimidinic (AP) sites. Here, we found that Tdp1 is more active in the cleavage of the AP sites inside bubble-DNA structure in comparison to ssDNA containing AP site. Furthermore, Tdp1 hydrolyzes AP sites opposite to bulky fluorescein adduct faster than AP sites located in dsDNA. Whilst the Tdp1 H493R (SCAN1) and H263A mutants retain the ability to bind an AP site-containing DNA, both mutants do not reveal endonuclease activity, further suggesting the specificity of the AP cleavage activity. We suggest that this Tdp1 activity can contribute to the repair of AP sites particularly in DNA structures containing ssDNA region or AP sites in the context of clustered DNA lesions.

1. Introduction

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is an enzyme that catalyzes the hydrolysis of 3′-phosphotyrosyl bonds. It was shown earlier that Tdp1 shows preference to remove a tyrosine residue from the ends of single- or double-stranded DNA substrates in vitro rather than tyrosine located at a nick of duplex DNA [1–4]. It suggests involvement of the enzyme in the removal of the adducts from DNA ends rather than located at the internal sites of DNA. Human Tdp1 can also hydrolyze other 3′-end DNA alterations including phosphoglycolates and 3′-abasic sites indicating that it may function as a general 3′-DNA phosphodiesterase and repair enzyme [5]. In addition, Tdp1 possesses a 3′-nucleosidase activity in which a single nucleoside is removed from 3′-hydroxy terminated ribo- and deoxyribonucleosides [6].

Recently we have shown that Tdp1 can cleave an AP site with the formation of 3′-phosphate termini [7]. Apurinic/apyrimidinic (AP) sites arise as a result of excision of the oxidatively damaged bases by DNA glycosylases that initiate base excision repair (BER), or by spontaneous hydrolysis that generates several thousand AP sites per day in a living cell [8,9]. The major enzyme in eukaryotic cells that catalyzes the cleavage of AP sites is apurinic/apyrimidinic endonuclease 1 (APE1). APE1 hydrolyzes the phosphodiester bond on the 5′-side of the abasic sites. We found that human Tdp1 also can initiate repair of AP sites. The 3′-phosphates generated by Tdp1 are efficiently removed by polynucleotide kinase 3′-phosphatase (PNKP) to produce a 3′-hydroxyl, which can be processed further and repaired by DNA polymerases and ligases [10–12]. Tdp1 is known to interact with base excision repair proteins: DNA polymerase beta (Pol β), XRCC1, poly(ADPribose)polymerase 1 (PARP1) and DNA ligase III [5,13–15]. A role for Tdp1 to maintain mitochondrial genetic integrity has recently been proposed [16] and a role in clearing alkylation DNA damage *in vivo* has also been reported for yeast *Schizosaccharomyces pombe* [17].

The reaction catalyzed by Tdp1 involves a covalent intermediate in which an active site histidine (His263 in human Tdp1) is linked by a phosphamide bond to the DNA 3′-phosphate of the substrate [18,19]. The importance of Tdp1 in humans is highlighted by the observation that a recessive mutation in the *TDP1* is responsible for the inherited disorder, spinocerebellar ataxia with axonal neuropathy (SCAN1) [15,20] in which a H493R mutation in the Tdp1 causes the accumulation of both Top1-DNA and Tdp1-DNA covalent intermediates *in vivo* [13,21–23].

In the present study we analyzed the AP site cleavage activity of Tdp1 using different DNA substrates and examined the possibility to repair AP sites located opposite to bulky DNA lesions. Our data suggest a novel APE-independent pathway for processing AP sites.

* Corresponding author. Tel.: +7 383 363 5195; fax: +7 383 363 5153.
E-mail addresses: smfame20@sussex.ac.uk (S.F. El-Khamisy), lavrik@niboch.nsc.ru (O.I. Lavrik).
[1] Fax: +7 383 333 3677.
[2] Fax: +44 (0) 1273 678 121.

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2012.04.004

2. Materials and methods

2.1. Materials

[γ^{32}P]ATP (5000 Ci/mmol) was 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 wild-type Tdp1 and mutant human Tdp1 proteins (SCAN1 and H263A) were purified to homogeneity by the chromatography on Ni-chelating resin and phosphocellulose P11 as described [18]. Wild-type Tdp1 was additionally purified by gel filtration through S-200 Sephacryl column using buffer containing Triton X-100 (0.01%) and 0.15 M NaCl followed by chromatography on heparin sepharose. The recombinant plasmid coding mutant Tdp1 (H263A) was a generous gift from Dr. James Champoux (University of Washington, Seattle). The recombinant purified UDG and DNA polymerase β were a generous gift from Dr. S.N. Khodyreva (ICBFM, Novosibirsk). The recombinant purified XRCC1 was a generous gift from Dr. I.A. Vasil'eva (ICBFM, Novosibirsk). 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 [24]. 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 were as follows:

ssAP-DNA
5′-ctat ggcg aggc gatt aagt tggg **U**ac gtca gggt cttc cgaa cgac
dsAP-DNA
5′-ctat ggcg aggc gatt aagt tggg **U**ac gtca gggt cttc cgaa cgac
3′-gata ccgc tccg ctaa ttca accc gttg cagt ccca gaag gctt gctg
AP-DNA/Flu
5′-ctat ggcg aggc gatt aagt tggg **U**ac gtca gggt cttc cgaa cgac
3′-gata ccgc tccg ctaa ttca accc g**F**tg cagt ccca gaag gctt gctg
AP-DNA/bubble
5′-ctat ggcg aggc gatt aagt tggg **U**ac gtca gggt cttc cgaa cgac
3′-gata ccgc tccg ctaa tagt tggg taac gtca ccca gaag gctt gctg,

where U designates dUMP residue, which is converted to AP site by the following UDG treatment; F designates 5-{3-[6-(carboxyamidofluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate residue (Flu-dUMP, see Fig. 1 for structure).

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, SCAN1 or H263A). For the preparation of natural AP site, an AP-DNA duplex was first incubated in reaction buffer with UDG (0.5 U/μl) for 15 min at 37 °C. After adding Tdp1 (100 nM) 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 [24].

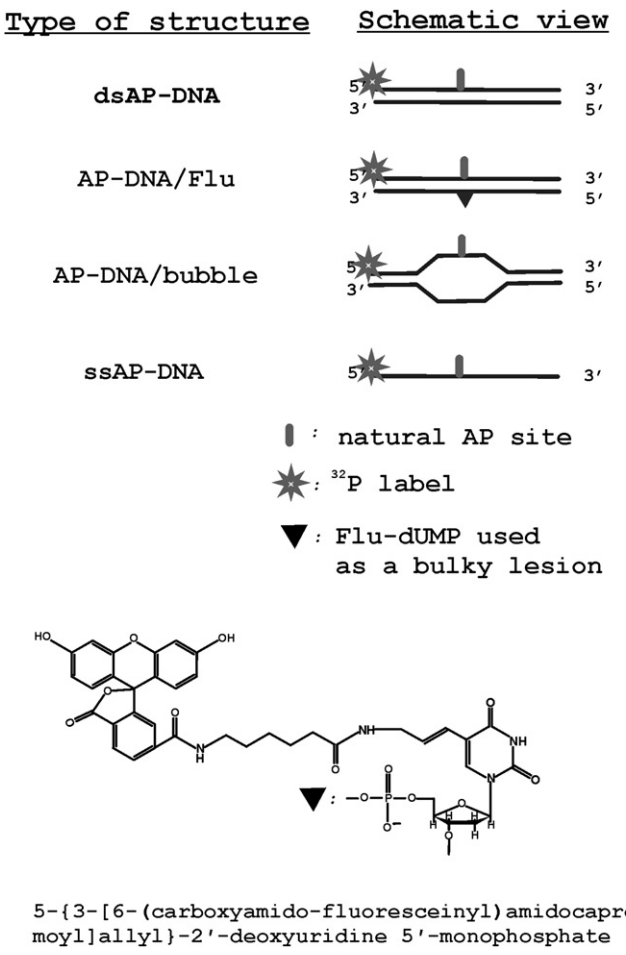

Fig. 1. Schematic view of DNA structures and nucleotide analog used in the experiments.

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), 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM dCTP, 1 mM ATP. Different combinations of Tdp1 (100 nM), Pol β (50 nM), XRCC1 (10 nM), PNKP (300 nM), and DNA ligase III (10 nM) or T4 DNA ligase (200 U/μl if indicated) were added as required. The mixtures were incubated at 37 °C for 30 min and analyzed as above.

2.5. Protein binding to DNA

Protein-DNA complexes were analyzed by gel retardation. The reaction mixture (10 μl) contained 50 mM Tris–HCl, pH 7.5, 50 mM KCl, 10 nM 5′-^{32}P-labeled DNA, and wild-type Tdp1 or mutants of Tdp1 at various concentrations. AP-DNA duplex was first incubated in reaction buffer with UDG (0.5 U/μl) for 15 min at 37 °C. The complexes Tdp1 with DNA were formed on ice. Then loading buffer (1:5 v/v) containing 20% glycerol and 0.015% Bromophenol Blue was added to the sample. Protein–nucleic acid complexes were electrophoresed under nondenaturing conditions. To separate the products of complex formation of Tdp1 or mutants, 5% polyacrylamide gel (acrylamide/bis-acrylamide = 60:1) was used. TBE was the electrode buffer. Electrophoresis was performed with voltage decrease 17 V/cm and at 4 °C. Positions of radioactively labeled oligonucleotide and protein–nucleic acid complexes were determined autoradiographically using a Molecular Imager FX Pro+ from BioRad.