DNA Repair 24 (2014) 1–9

Contents lists available at ScienceDirect

DNA Repair

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

Human DNA polymerases catalyze lesion bypass across benzo[*a*]pyrene-derived DNA adduct clustered with an abasic site

Lidia V. Starostenko[a,1], Nadejda I. Rechkunova[a,b,1], Natalia A. Lebedeva[a,b,1], Alexander Kolbanovskiy[c,2], Nicholas E. Geacintov[c,2], Olga I. Lavrik[a,b,*]

[a] *Institute of Chemical Biology and Fundamental Medicine, Lavrentiev av. 8, 630090 Novosibirsk, Russia*
[b] *Department of Natural Sciences, Novosibirsk State University, 2 Pirogova Street, 630090 Novosibirsk, Russia*
[c] *Department of Chemistry, New York University, 31 Washington Place/Suite 453 Brown New York, NY 10003-5180, USA*

ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form
10 September 2014
Accepted 3 October 2014

Keywords:
Base excision repair
DNA polymerases
Lesion bypass
Benzo[*a*]pyrene

ABSTRACT

The combined action of oxidative stress and genotoxic polycyclic aromatic hydrocarbons derivatives can lead to cluster-type DNA damage that includes both a modified nucleotide and a bulky lesion. As an example, we investigated the possibility of repair of an AP site located opposite a minor groove-positioned (+)-*trans*-BPDE-dG or a base-displaced intercalated (+)-*cis*-BPDE-dG adduct (BP lesion) by a BER system. Oligonucleotides with single uracil residue in the certain position were annealed with complementary oligonucleotides bearing either a *cis*- or *trans*-BP adduct. Digestion with uracil DNA glycosylase was utilized to generate an AP site which was then hydrolyzed by APE1, and the resulting gap was processed by X-family DNA polymerases β (Polβ) and λ (Polλ), or Y-family polymerase ι (Polι). By varying reaction conditions, namely, Mg^{2+}/Mn^{2+} replacement/combination and ionic strength decrease, we found that under certain conditions both Polβ and Polι can catalyze lesion bypass across both *cis*- and *trans*-BP adducts in the presence of physiological dNTP concentrations. Polβ and Polι catalyze gap filling translesion synthesis in an error prone manner. By contrast, Polλ selectively introduced the correct dCTP opposite the modified dG in the case of *cis*-BP-dG adduct only, and did not bypass the stereoisomeric *trans*-adduct under any of the conditions examined. The results suggest that Polλ is a specialized polymerase that can process these kinds of lesions.

1. Introduction

The genome is continuously subjected to damage induced by a variety of endogenous and exogenous chemicals. DNA lesions may block replication completely or cause a template nucleotide substitution, leading to mutations. In addition, accumulation of lesions in a non-replicated DNA region may inhibit gene expression and, thus, affect many cell processes.

Clusters where various lesions, including oxidized bases, apurinic/apyrimidinic (AP) sites, strand breaks, and bulky lesions, are within one or two DNA helix turns or occur in both of the DNA strands are the most dangerous for cells [1]. Benzo[*a*]pyrene (B[*a*]P) is the best known polycyclic aromatic hydrocarbons (PAH) that, after metabolic activation to toxic and reactive intermediates, reacts with DNA to form bulky adducts with distinct cytotoxic, mutagenic, and carcinogenic properties. Highly mutagenic AP sites form when the N-glycosidic bond is cleaved by DNA glycosylases or undergoes spontaneous hydrolysis. Error-prone DNA replication can lead to mutations, including deletions and frameshift mutations [2].

B[*a*]P and AP sites are repaired by appropriate repair mechanisms that include nucleotide excision repair (NER) and base excision repair (BER), respectively. AP sites are cleaved by apurinic/apyrimidinic endonuclease 1 (APE1) to generate single-strand breaks with the 3′-OH and 5′-deoxyribose phosphate (dRP) ends. The resulting intermediate can be processed via more than one BER pathway by different DNA polymerases [3]. Recent studies revealed that DNA polymerases have the remarkable ability to bypass DNA damage [4] by translesion synthesis (TLS) mechanisms.

The majority of TLS polymerases belong to the Y structural family [5]. Polι is member of Y-family DNA polymerases that bypasses

* Corresponding author at: Institute of Chemical Biology and Fundamental Medicine, Lavrentiev av. 8, 630090 Novosibirsk, Russia. Tel.: +7 383 363 5196; fax: +7 383 363 5153/333 3677.
E-mail addresses: ng1@nyu.edu (N.E. Geacintov), lavrik@niboch.nsc.ru (O.I. Lavrik).
[1] Fax: +7 383 333 3677.
[2] Tel.: +1 212 998 8407; fax: +1 212 998 8421.

http://dx.doi.org/10.1016/j.dnarep.2014.10.005
1568-7864/

AP sites and bulky lesions such as BPDE-dG adducts, although with low fidelity [6,7], and also possesses 5′-dRP lyase activity [8,9].

DNA polymerases belonging to other structural families may also play a role in TLS [10–13]. Polβ and Polλ, which function as repair polymerases in BER [14,15], are capable of translesion synthesis in certain cases, in particular, when filling single-strand gaps [16–19], and utilize their 5′-dRP lyase activity in the process [15,20]. Therefore all three polymerases (β, λ and ι) have characteristic features which are necessary to catalyze the bypass of lesion in the gap after the cleavage of AP site. All of these DNA polymerases have dRP lyase activity and the ability to effectively foster gap-filling reactions.

Recently, we studied DNA polymerases β and λ gap filling activities after cleavage of AP sites located opposite to a bulky lesion, the minor groove-positioned (+)-*trans*-, or the base displaced intercalated (+)-*cis*-BPDE-N^2-dG adducts in the opposite strand [21]. We found that only Polλ was able to introduce complementary dCMP into the one-nucleotide gap opposite to (+)-*cis*-B[*a*]P-dG, but not its *trans*-isomer, which is the main product of DNA modification by B[*a*]P diol epoxide metabolites *in vivo* [22]. Polβ failed to catalyze translesion synthesis through (+)-*trans*- or (+)-*cis*-BPDE-N^2-dG. However it was shown recently that Polβ is capable of incorporating a correct nucleotide opposite both (+)- *or* (−)-*trans*-BPDE-N^2-dA or (+)- *or* (−)-*trans*-BPDE-N^2-dG adducts at high (millimolar) dNTP concentrations. DNA synthesis was more efficient in the case of modified adenine than guanine. [19].

It is generally assumed that Mg^{2+} is physiological cofactor for replicative DNA polymerases in vivo. However, later studies suggest, that certain DNA polymerases, such as DNA polymerases λ and ι, may preferentially utilize Mn^{2+} in vitro [23,24].

In the present study, we analyzed *in vitro* the TLS gap-filling activities of DNA polymerases β, λ and ι in the case of gaps that resulted from the cleavage of AP sites located opposite to BPDE-N^2-dG adducts under different reaction conditions.

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 and DNA ligase were 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).

Human recombinant DNA polymerase λ was purified from *Escherichia coli* BL21(DE3) RP cells as described [15]. The recombinant plasmid DNA coding hPolλ was a kind gift of Dr. S.H. Wilson (NIEHS, USA). The recombinant DNA polymerase β was purified from *E. coli* as described [25]. Human recombinant DNA polymerase ι was purified from *E. coli* RW64 as described [26]. The recombinant plasmid DNA coding hPolι and producing strain were a kind gift of Dr. R.G. Woodgate (NICHD, USA). The recombinant purified UDG and APE1 were a generous gift from Dr. S.N. Khodyreva (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 [27]. Unreacted [γ-^{32}P]

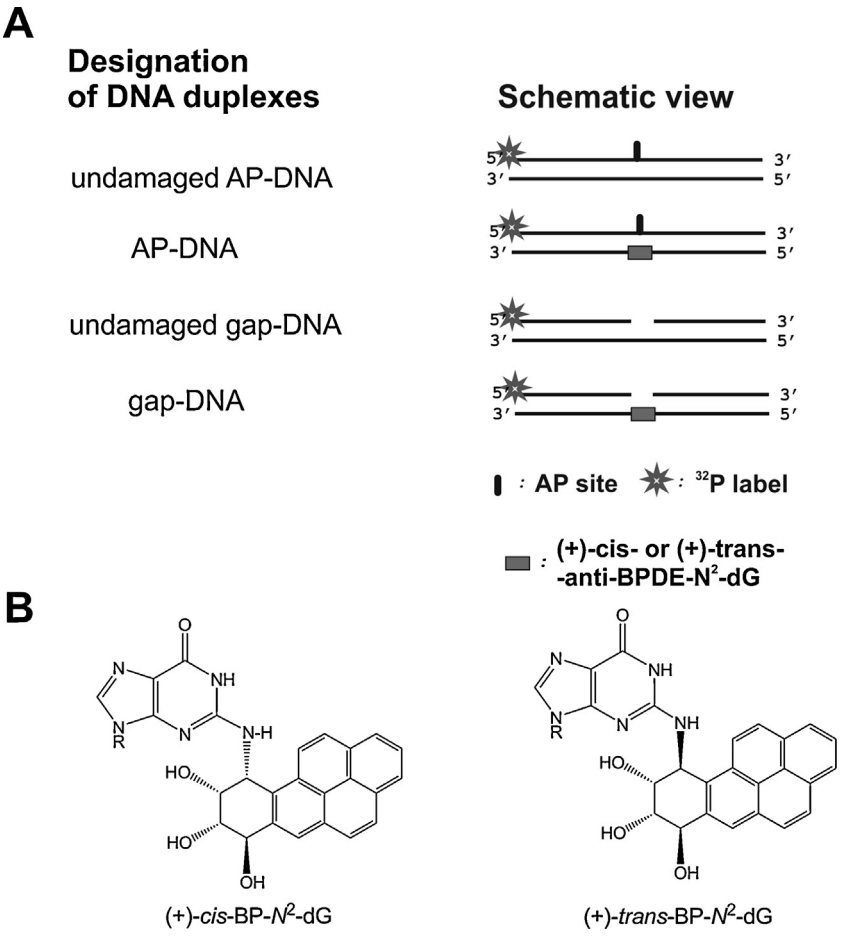

Fig. 1. Structures of DNA duplexes (A) and (+)-*cis*- and (+)-*trans*-anti-BPDE-dG adducts (B).