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Action of multiple base excision repair enzymes on the 2′-deoxyribonolactone☆

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Abstract

Free radical attack on the sugar-phosphate backbone generates oxidized apurinic/apyrimidinic (AP) residues in DNA. 2′-deoxyribonolactone (dL) is a C1′-oxidized AP site damage generated by UV and γ-irradiation, and certain anticancer drugs. If not repaired dL produces G → A transitions in *Escherichia coli*. In the base excision repair (BER) pathway, AP endonucleases are the major enzymes responsible for 5′-incision of the regular AP site (dR) and dL. DNA glycosylases with associated AP lyase activity can also efficiently cleave regular AP sites. Here, we report that dL is a substrate for AP endonucleases but not for DNA glycosylases/AP lyases. The kinetic parameters of the dL-incision were similar to those of the dR. DNA glycosylases such as *E. coli* formamidopyrimidine-DNA glycosylase, mismatch-specific uracil-DNA glycosylase, and human alkylpurine-DNA *N*-glycosylase bind strongly to dL without cleaving it. We show that dL cross-links with the human proteins 8-oxoguanine-DNA (hOGG1) and thymine glycol-DNA glycosylases (hNth1), and dR cross-links with Nth and hNth1. These results suggest that dL and dR induced genotoxicity might be strengthened by BER pathway in vivo.

Keywords: Reactive oxygen species; Ionizing radiation; Oxidative DNA damage; Protein/DNA cross-links; Apurinic/apyrimidinic site; Oxidized abasic site; Base excision repair; AP endonuclease; DNA glycosylase; AP lyase

DNA damage can be generated in cells by various mechanisms that involve radical oxygen species (ROS) attack and other chemical and/or physical agents. In addition to base damage, ROS are also able to generate oxidized abasic sites (OAS) including 2′-deoxyribonolactone (dL), which structurally corresponds to the 1′-oxidized form of the 2′-deoxyribose (dR) [1,2]. dL is produced after a radical abstraction of the C1′ hydrogen induced by UV and γ-radiations or antibiotics such as neocarzinostatin or organic compounds containing a metallic cation. Previously, we reported an efficient synthesis of dL using the quantitative photochemical conversion of a nitro-indole nucleoside precursor selectively incorporated in an oligonucleotide [3,4]. Using specifically modified DNA duplexes, we have shown that despite structural analogy between dL and dR, their repair and mutagenesis are different [5]. In *Escherichia coli*, dL codes mainly for thymidine, at variance to regular abasic site, which codes for adenosine. The dL is mainly processed by the

☆ *Abbreviations:* ROS, reactive oxygen species; AP site, apurinic/apyrimidinic site; dR, 2′-deoxyribose; dL, 2′-deoxyribonolactone; OAS, oxidized abasic sites; BER, base excision repair; TagI, *E. coli* 3-methyladenine-DNA-glycosylase I; AlkA, *E. coli* 3-methyladenine-DNA-glycosylase II; UNG, *E. coli* uracil-DNA-glycosylase; Fpg, *E. coli* formamidopyrimidine-DNA glycosylase; Nth, *E. coli* endonuclease III; Nfo, *E. coli* endonuclease IV; MUG, *E. coli* mismatch-specific uracil-DNA glycosylase; ANPG, human alkylpurine-DNA *N*-glycosylase; APDG, rat ANPG; hOGG1, human 7,8-dihydro-8-oxoguanine-DNA glycosylase; hNth1, human endonuclease III; Ape1, human AP-endonuclease; hTDG, human thymine-DNA glycosylase.

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AP endonucleases and is highly mutagenic in the absence of repair.

BER is thought to be the major pathway for oxidative DNA damage, and it requires sequential action of two enzymes for proper incision of DNA: a DNA glycosylase which excises abnormal base through glycosidic bond hydrolysis and an AP endonuclease which incises apurinic/apyrimidinic (AP) sites and removes 3′-blocking groups [6,7]. There are two types of DNA glycosylases: a mono-functional DNA glycosylase exclusively produces apurinic/apyrimidinic (AP) site and a bi-functional DNA glycosylase cleaves at the 3′-side of the AP site through a β-elimination reaction (AP lyase activity) leaving 3′-aldehyde and/or 3′-phosphate residues [8]. The AP endonucleases hydrolyze the phosphodiester on the 5′-side of the AP site creating a deoxyribose 5′-phosphate and a 3′-hydroxyl nucleotide to prime DNA repair synthesis. In *E. coli*, the enzymes that process AP sites and remove blocking 3′-termini, such as the α, β unsaturated aldehydes and 3′-phosphate, are the Xth protein (exonuclease III) which is the major constitutive AP endonuclease and the Nfo protein (endonuclease IV) which is induced by superoxide anion generators [9]. In yeast, the homolog of Nfo protein is Apn1 protein (AP endonuclease 1) [10]. Apn1 accounts for more than 90% of total AP endonuclease activity. Its main substrates are 3′-phosphoglycoaldehyde, 3′-phosphoryl groups, and 3′-α,β unsaturated aldehydes [11]. In human, the major AP endonuclease, Ape1 (Apex1, REF-1, HAP-1), is structurally homologous to *E. coli* Xth and oxidizing agents induce its expression [12].

BER pathway faces recognition problems during which some enzymes form tight complexes with their substrate and others, on the contrary, interact only for brief periods of time. In some cases, the intrinsic reactivity of the lesion may provoke a covalent cross-link with the interacting protein. Recently, Greenberg and colleagues [13] found that dL cross-links to the lysine-120 residue of *E. coli* Nth. In addition, following endonucleolytic incision of dL by Ape1, it cross-links to DNA polymerase β [14]. Such a covalent and thus irreversible binding is expected to inhibit the repair reaction and may potentiate genotoxicity of dL in vivo.

In the present study, we have used highly purified BER proteins to search for the enzymes specifically interacting with dL using 2′-deoxyribose (dR) for comparison. Our starting hypothesis is based on the structural analogy between dL and dR (represented in Fig. 1), a well-documented lesion, which is also an intermediate in the BER pathway. The strategy employed in this report consisted in incubating 31 mer oligonucleotides containing a single dL lesion with various BER enzymes. The aim was to identify enzymes specifically interacting with oligonucleotide duplexes containing dL and dR. The affinity and/or incision efficiency of various proteins towards dL and dR were quantified and compared via the measurement of binding and kinetic constants. In addition, we have searched for the proteins forming a covalent complex with dL and dR.

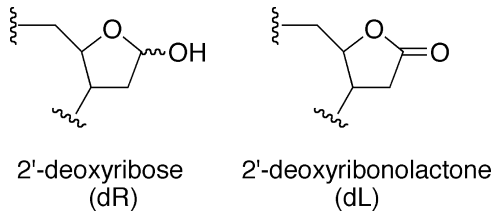

Fig. 1. Structure of the baseless sites 2′-deoxyribonolactone (dL) and 2′-deoxyribose (dR).

Materials and methods

Oligonucleotides. The 31 mer oligonucleotides d (GATGAATT CAGGCCTXGAGGAATCGCTGGTA) where X is either 2′-deoxyribonitroindole (dNi) or 2′-deoxyuridine (dU) were synthesized using standard solid-phase cyanoethyl phosphoramidite chemistry with an expedite DNA synthesizer. Nitroindole nucleoside phosphoramidite was synthesized according to the method described [3]. Oligonucleotides were purified using polyacrylamide gel electrophoresis (PAGE) and HPLC column as described [15].

Oligonucleotides were 5′-end labeled by T4 polynucleotide kinase (New England Biolabs, OZYME, Saint Quentin Yvelines, France) in the presence of [γ-^{32}P]ATP (4500 Ci/mmol, ICN Biomedicals, S.A.R.L., Orsay, France). The ^{32}P-labeled oligonucleotides were hybridized with 20% molar excess of the non-radiolabeled complementary strand by incubating at 70 °C for 5 min and then cooling slowly at room temperature for 60 min.

To obtain dR, the oligonucleotide containing dU was treated with 1 nM *E. coli* uracil-DNA glycosylase (UNG) at 37 °C for 60 min, under these conditions more than 90% conversion was obtained. To obtain dL, the oligonucleotide duplex containing the dNi residue was illuminated using 200 W Hg/Xe lamp (Oriel Instrument, Stratford) and a KNO_3 filter (2 M) as previously described [3,4]. In order to limit the degradation of the dL residue, the illumination was carried out at 4 °C. Nearly complete conversion of dNi was obtained after 90 min of illumination. The conversion efficiencies of dU into dR and dNi to dL were controlled by alkali treatment in 0.5 M KOH for 30 min at room temperature or for 5 min at 70 °C.

Enzymes. The *E. coli* Xth (exonuclease III) protein was purchased from Roche Diagnostics (Meylan, France). Purifications of the *E. coli* UNG, TagI (3-methyladenine-DNA glycosylase I), AlkA (3-methyladenine-DNA glycosylase II), MUG (mismatch-specific uracil-DNA glycosylase), Fpg (formamidopyrimidine-DNA glycosylase), Nth (endonuclease III), Nfo (endonuclease IV), glutathione *S*-transferase-tagged hTDG (human mismatch-specific thymine-DNA glycosylase), histidine-tagged hOGG1 (human 8-oxoG-DNA glycosylase), hUNG (human catalytic domain uracil-DNA glycosylase), ANPG (human truncated alkyl-*N*-purine-DNA glycosylase, 24.4 kDa, 220 AA), and APDG (rat alkyl-*N*-purine-DNA glycosylase) proteins were performed as described [16,17]. Purifications of *Saccharomyces cerevisiae* Apn1 (AP endonuclease 1) and human AP endonuclease 1 (Ape1) proteins were performed as described [18]. The hNth1 (human endonuclease III) protein was generously provided by Dr. R. Roy (American Health Foundation, Valhalla, USA). The activity of the various proteins was tested using their principal substrates prior to use.

Cleavage assays. Enzymatic activities were measured in the appropriate incubation buffer by cleavage of the duplex oligonucleotide containing either dL or dR at a defined position. The standard