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Regular paper

The influence of cdG on 8-oxodG excision by OGG1 and FPG glycosylases

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Abstract: Human genome is exposed to the variety of damaging factors, such as ionizing radiation. 5′,8-cyclo-2′-deoxypurines (cdPus) are well described unfavorable outcomes of DNA damage, especially devastating as a part of clustered DNA lesions (CDL). Since cdPus are not repaired by base excision repair (BER) and poorly repaired by nucleotide excision repair (NER), it is important to unveil the mechanisms of cdPus action within the genome. In this study the influence of both 5′*S* and 5′*R* diastereomers of 5′,8-cyclo-2′-deoxyguanosine (cdG) on the activity of OGG1 and FPG was examined. Synthetic oligonucleotides containing cdG and two molecules of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) were designed as model of single-stranded CDL. The activity of both enzymes increased in the presence of cdG, compared to the control DNA strands, and the increase was greater in the case of 5′R diastereomer. These results are supported by previous studies concerning cdPus and confirm the impact of lesions proximity on the DNA repair efficiency. Due to the biological importance of cdPus, it is necessary to understand the mechanisms of lesions recognition by repair proteins in further studies.

Keywords: 5′,8-cyclo-2′-deoxyguanosine (cdG), tandem lesions, base excision repair, 8-oxoguanine glycosylase, formamidopyrimidine glycosylase

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Abbreviations: CdPus, 5′,8-cyclo-2′-deoxypurines; CDL, clustered DNA lesions; BER, base excision repair; NER, nucleotide excision repair; cdG, cyclodeoxyguanosine; OGG1, oxoguanine glycosylase 1; FPG, formamidopyrimidine glycosylase; cdA, 5′,8-cyclo-2′-deoxyadenosine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FapyA, 4,6-diamino-5-formamidopyrimidine; SSB, single-strand break; ROS, reactive oxygen species; DSB, double-strand break; PAGE, polyacrylamide gel electrophoresis; TBE, tris-borate-EDTA buffer; EDTA, ethylenediaminetetraacetic acid; DTT; dithiothreitol; BSA, bovine serum albumin; ScdG, (5′S)-5′,8-cyclo-2′-deoxyguanosine; RcdG, (5′R)-5′,8-cyclo-2′-deoxyguanosine; UDG, uracil-DNA glycosylase; hAPE1, human AP-site endonuclease 1

INTRODUCTION

The DNA molecule, which is the biological foundation of life, is exposed to variety of external damaging factors. The exposure may lead to structural modification of DNA helix (Tomkova & Schuster-Böckler, 2018). Over 70.000 of unwanted and harmful DNA lesions occurs in each human cell a day giving about 3 x 10^{17} lesions in the whole human body every hour (Lindahl, 1993; Sudhir Ambekar, 2017; Gorini *et al.*, 2021; Tubbs & Nussenzweig, 2017). They are mostly removed by repair pathways. If not repaired, they disrupt the stability of the genetic information and may lead to, for example, mutagenesis and cancerogenesis (Chatgilialoglu *et al.*, 2019; You *et al.*, 2012). Among many types of lesions, specific clustered lesions (CDL) can be distinguished. They are referred to as two or more lesions per 1-2 DNA helix turns. CDL are especially unfavorable for repair and lead to a high risk of mutagenesis (Jaruga & Dizdaroglu, 2008). Another type of lesion that may be a part of clustered lesion is a tandem lesion. Tandem lesions appear as a result of damage of two adjacent nucleotides by a single radical event. Tandem lesions may also appear within a single nucleotide containing two separated impairments. In this case, cyclopurines (cdPus) are well-known examples (Sage & Shikazono, 2016; Cadet *et al.*, 2012) (Fig. 1). The cdPus yield as a result of hydrogen abstraction from the 5'-methylene group of 2'-deoxyribose by •OH radical (Dizdaroglu & Jaruga, 2012; Krokidis *et al.*, 2017; Brooks, 2017). In the case of 5',8-cyclo-2'-deoxyguanosine (cdG), studies indicate that its 5'R diastereomer is less likely to appear and is easier to remove from the genome, when compared to 5'S (Dirksen *et al.*, 1988). Similar conclusion is made for 5',8-cyclo-2'-deoxyadenosine (cdA). 5'R-diastereomer of cdA show higher affinity to NER pathway and its excision occurs faster (Brooks, 2017; Kropachev *et al.*, 2014). Moreover, recent studies indicate that the ionization potential of -GGG- stack is lower than single guanine in DNA strand. If guanine molecules are positioned next to each other, the one located closer to 5'-end of DNA strand is energetically favored for ionization (Kumar *et al.*, 2020).

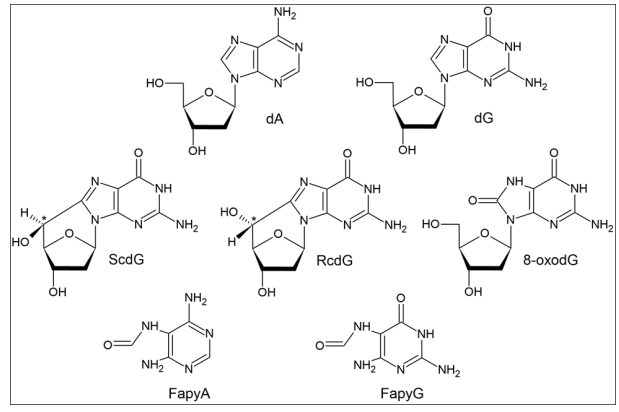

Figure 1. Chemical structures of DNA components: 2′-deoxyadenosine (dA), 2′-deoxyguanosine (dG) and their derivatives.

CdPus are poor substrates of nucleotide excision repair (NER) pathway, and they are not repaired by base excision repair (BER) system. The removal of 5'R-cdA and 5'S-cdA is 40 and 150 times slower, respectively, than cisplatin adducts (Kuraoka *et al.*, 2000). It is due to additional covalent C5'-C8 bond, which adds stiffness to the nucleotide structure (Bukowska & Karwowski, 2018). Thus, mono- and bifunctional glycosylases, specific for BER pathway, are not able to perform single base excision with subsequent AP-site formation. CdPus appearance lead to more extensive bulky distortions of DNA helix than in the case of single lesions, which are mostly removed by BER system (Bukowska & Karwowski, 2018; Kuraoka *et al.*, 2000). In this study it was examined if the presence of both diastereomers of cdG within clustered lesions reveals any impact on the activity of BER initial enzymes. Two DNA glycosylases (OGG1 and FPG) were chosen as they are responsible for the excision of typical BER substrate: 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (Boiteux *et al.*, 2017).

The above-mentioned enzymes OGG1 and FPG are members of bifunctional DNA glycosylases subclass and demonstrate both DNA glycosylase and AP-lyase activities. They excise 8-oxodG, FapyG and FapyA (FPG only) with subsequent AP-site incision, leading to single-strand breaks (SSB) formation (Wang *et al.*, 2018; Boiteux *et al.*, 1990; Hirano, 2008; Serre *et al.*, 2002; Boiteux *et al.*, 2017) (Fig. 1). However, the bifunctional nature of OGG1 has been questioned in recent years (Faucher *et al.*, 2012; Boiteux *et al.*, 2017). The difference between monofunctional and bifunctional glycosylases is the nucleophilic agent used to break N-glycosidic bond. Monofunctional glycosylases use water, while bifunctional use amine residue from the active site (Pro2 in FPG and Lys249 in OGG1) (Sowlati-Hashjin & Wetmore, 2014, 2015). OGG1 is specific for eukaryotic organisms, while FPG plays its role in bacteria (Držata *et al.*, 2013). Although FPG homologs are also present in some eukaryota, they do not show noticeable activity towards 8-oxo-dG due to structural differences (Duclos *et al.*, 2012). Both OGG1 and FPG search for damaged guanine moieties by 1-dimensional "sliding" down the DNA strand. However, the exact mechanism of lesion recognition remains the topic of scientific debate and has been widely considered (Li *et al.*, 2017; Faucher *et al.*, 2012; Boiteux *et al.*, 2017; Kreppel *et al.*, 2018). After the attack on N-glycosyl bond and the excision of damaged guanine, OGG1 cleaves the DNA strand towards 3' to the AP-site by β-elimination. This generates an unsaturated hydroxyl-aldehyde 3'-terminus and phosphate 5'-terminus (Hao *et al.*, 2020; Tyugashev *et al.*, 2019). On the other hand, FPG action reveals multistep mechanism involving formation of Schiff base as an intermediate and following β,δ-elimination. Unfortunately, no precise data concerning, for example, electron transfer chain is available (Popov *et al.*, 2020). However, there are evidences showing only β-elimination products of FPG activity and both β,δ-elimination products of OGG1 action (Yin *et al.*, 2015; Tesfahun *et al.*, 2021), which were also observed in this study. The explanation may be that due to the liability of the AP-site, the mechanism of its degradation differs depending on the reaction conditions. In the face of this research it is worth noting that both FPG and OGG1 active sites interact with the moiety of 8-oxo-dG and with three nucleotides towards 3'-end and 2 nucleotide towards 5'-end of the damaged strand of ds-DNA (Bruner *et al.*, 2000; Shigdel *et al.*, 2020; Gilboa *et al.*, 2002; Rogacheva *et al.*, 2006).

8-oxodG, as well as FapyG, is a single DNA lesion appearing as a result of UV radiation or reactive oxygen species (ROS) actions. Nucleotides are widely damaged by presence of Fenton reaction products, such as free hydroxyl radicals. Fenton reactions occurs under conditions which are rich in Fe^{2+} and superoxide dismutase. Fenton reaction products affect the structure of nucleotides as long as nucleotides chelate Fe^{2+} cations. Among all DNA bases, guanine is the most susceptible to oxidation due to its lowest ionization potential (Gruber & Walker, 2018; Imlay, 2013; Rush *et al.*, 1990). Therefore, despite the existence of more than 100 different purine and pyrimidine modifications, 8-oxodG appears and is investigated most frequently and is regarded as a cellular biomarker of oxidative stress (Pluskota-Karwatka, 2008; Gruber & Walker, 2018; Mirbahai *et al.*, 2010; Hao *et al.*, 2020). The appearance of 8-oxo-dG instead of guanine leads to negligible changes in DNA helix structure and stiffness. However, 8-oxo-dG molecules may lead to adenine mismatch during next round of replication. Mutations in the form of transversion of G:C into T:A may then occur as the most probable outcome (Mirbahai *et al.*, 2010; De Souza-Pinto *et al.*, 2001). It may potentially cause genomic instability and initiate or accelerate carcinogenesis. Studies proved that cancer tissues exhibit elevated levels of 8-oxo-dGTP (Smart *et al.*, 2006).

8-oxodG was chosen as a typical BER substrate to evaluate the activity of OGG1 and FPG in the presence of cdG. In order to achieve the above, clustered DNA lesions containing both cdG and 8-oxodG were designed within single-stranded oligonucleotides. Native complementary strands were used to create double-stranded DNA model fragments. Double-stranded clusters were not chosen due to the fact that in normal physiological conditions they may induce double-strand breaks (DSB), which are not a substrates of BER pathway (Bukowska & Karwowski, 2018).

Table 1. Sequences and obtained quantities of oligonucleotides.

Oligonucleotide	Sequence	Quantity	
		(OD)	(nmol)
Matrix H/A	5'-GCCTTTGGTGCGAGCATAGAGACAATATTCCTGACAAGAG-3'	40.7	93.61
Matrix H/G	5'-GCCTTTGGTGCGAGCACAGAGACAATATTCCTGACAAGAG-3'	32.3	74.29
–6/+6(H/dA)	3'-CGGAAACCACHCTCGTATCTCTHTTATAAGGACTGTTCTC-5'	24.4	56.12
–6/+6(H/ScdG)	3'-CGGAAACCACHCTCGTXTCTCTHTTATAAGGACTGTTCTC-5'	18.0	41.4
–6/+6(H/RcdG)	3'-CGGAAACCACHCTCGTYTCTCTHTTATAAGGACTGTTCTC-5'	2.7	6.21

Abbreviations mean the following: H, 8-oxo-7,8-dihydro-2'-deoxyguanosine; X, (5'*S*)-5',8-cyclo-2'-deoxyguanosine; Y, (5'*R*)–5',8-cyclo-2'-deoxyguanosine

MATERIALS AND METHODS

Substrate oligonucleotides preparation and analysis

The oligonucleotides synthesis, purification, concentration and mass spectroscopy were performed as previously reported (Karwowski, 2019; Szewczuk *et al.*, 2021a, 2021b; Boguszewska *et al.*, 2021). The phosphoramidite derivatives of (5'*R*) and (5'*S*) cdG were synthesized as described by Romieu *et al.* (Romieu *et al.*, 1999). The complete sequences of applied oligonucleotides and obtained quantities are listed in Table 1. The melting temperatures of oligonucleotides containing cdPus exceeded 70°C, providing their stability in experimental conditions (Karwowski, 2019). The online oligonucleotide properties calculator OligoCalc (Kibbe, 2007) was used for the extinction coefficient determination of the oligonucleotides. The calculated masses, found masses and mass spectra of analyzed oligonucleotides are available in Supplementary Materials (Figs S12–S16, Table S3 at https://ojs.ptbioch.edu.pl/index.php/abp/).

In order to obtain appropriate control for the experiments, –6/+6(H/dA) control strand with no cdG lesion was synthesized. The presence of adenine residue at 17th position (counting from 3'-end) instead of guanine residue has negligible influence on the overall experimental conditions. In the case of this study, the aim was to replace native purine (adenine within the control strand) with cyclodeoxypurine (cdG used as a lesion).

Substrate oligonucleotides labeling and hybridization

The oligonucleotides labeling and hybridization were performed as previously reported (Szewczuk *et al.*, 2021a). A 2-fold excess of the purified non-radiolabeled complementary strands were selected for hybridization as follows: matrix H/A for control oligo and matrix H/G for oligos containing cdG. The efficiency of the hybridization process and the purity of both single- and double-stranded radiolabeled oligonucleotides were examined by PAGE. The analysis was performed on 15% denaturing polyacrylamide gel containing 8 M urea in 1×TBE (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA) for 120 min at a constant power of 45 W. Obtained autoradiogram is available in Supplementary Materials (Fig. S1 at https://ojs.ptbioch.edu.pl/index.php/abp/).

OGG1 and FPG cleavage assay

OGG1 and FPG were purchased from Trevigen (Gaithersburg, MD, USA) and NEB (New England BioLabs, Ipswich, MA, USA), respectively. The general procedure of cleavage assay was as follows. The radiolabeled double-stranded oligonucleotides (2.3 pmol) were incubated in 5 μL of reaction buffer with 0.5 U OGG1 or FPG at 37°C. For OGG1 cleavage assay, the reaction buffer contained 20 mM Tris-Cl, 1 mM EDTA, 1 mM DTT, 100 μg/ml BSA (REC™ Reaction Buffer 6) and the reaction times were 0, 30, 60, 120, 180, 240 and 300 min. For FPG cleavage assay, the reaction buffer contained 10 mM Bis-Tris-Propane HCl, 10 mM $MgCl_2$, 1 mM DTT, 100 μg/ml BSA (NEBuffer™ 1) and the reaction times were 0, 1, 5, 15, 30, 60, 120 min. The reactions were stopped by cooling down the samples in an ice/water bath and addition of 5 μL of denaturing loading dye (95% formamide, 2 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol). The efficiency of the cleavage was determined by PAGE using 15% denaturing polyacrylamide gel containing 8 M urea in 1×TBE (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA) for 120 min at a constant power of 45 W. The results were visualized by autoradiography. Each set of data was quantified using Quantity One 1-D analysis software (Bio-Rad). All experiments were performed

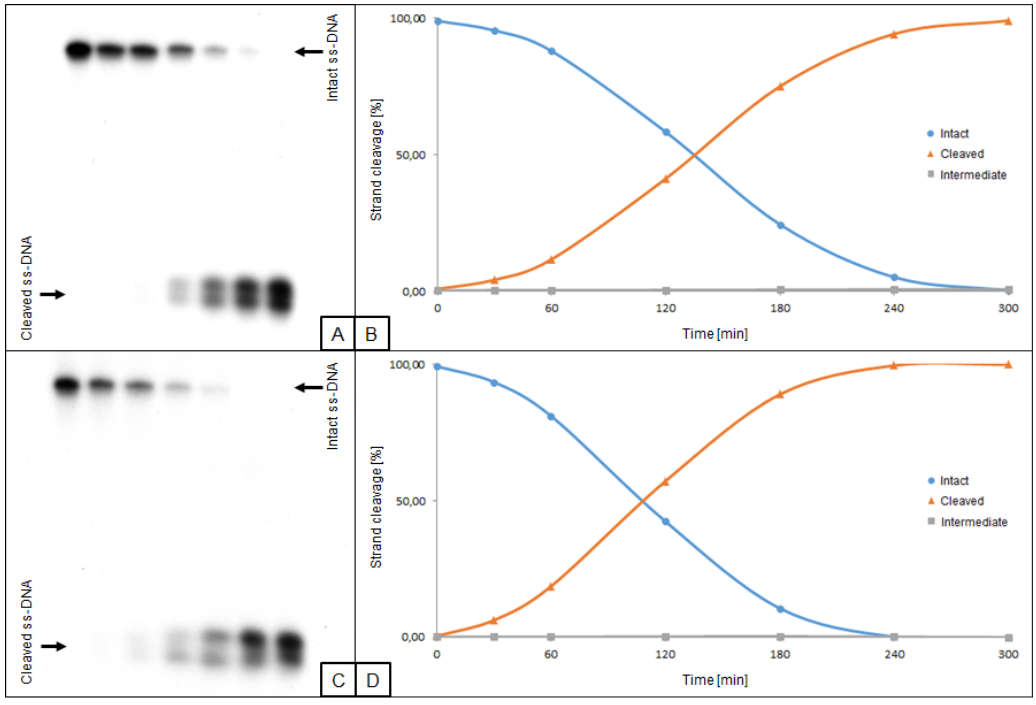

Figure 2. Cleavage of ds-DNA containing 8-oxodG and cdG by 0.5 U OGG1.
Double-stranded DNA fragments were obtained using native complementary Matrix H/G and -6/+6(H/ScdG) for (**A, B**) or –6/+6(H/RcdG) for (**C, D**); (**A, C**) show 7 lanes each, which correspond to reaction times 0, 30, 60, 120, 180, 240 and 300 min starting from the left. (**B, D**) show the quantity losses of intact ss-DNA (blue), the quantity increases of SSB-DNA (orange), and an intermediate oligo fragment (grey).

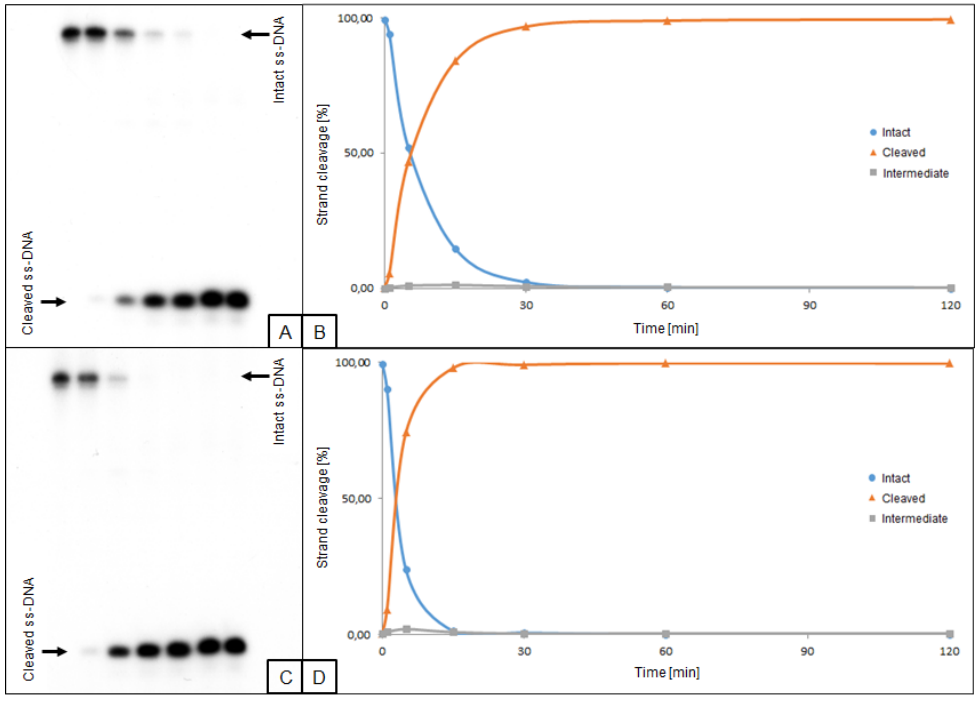

Figure 3. Cleavage of ds-DNA containing 8-oxo-dG and cdG by 0.5 U FPG.
Double-stranded DNA fragments were obtained using native complementary Matrix H/G and –6/+6(H/ScdG) for (**A, B**) or –6/+6(H/RcdG) for (**C, D**); (**A, C**) show 7 lanes each, which correspond to reaction times 0, 1, 5, 15, 30, 60 and 120 min starting from the left. (**B, D**) show the quantity losses of intact ss-DNA (blue), the quantity increases of SSB-DNA (orange), and an intermediate oligo fragment (grey).

three times to ensure that provided results are consistent and reliable.

Visualization of the results

The results were visualized by autoradiography. The time-dependent cleavage of ds-DNA was analyzed as the intensity of bands obtained from each sample. Every lane of bands represents different reaction time. The reaction times were established experimentally to allow to visualize 100% of strands cleavage. The length of oligo fragments determines the location of bands: upper band (intact 40-mer), middle band (29-mer) and lower band (17-mer). Due to bifunctional properties of both enzymes, the SSB formation indicates successful 8-oxo-dG excision and allows to quantify the time-dependent reactions. The quantification was performed using Quantity One 1-D analysis software (Bio-Rad). To obtain a percentage value of DNA cleavage, the intensity of each band was calculated as a percentage of the total intensity of all bands within one lane.

RESULTS

To evaluate the influence of both diastereomers of cdG on the activity of tested enzymes, cleavage assays were initially performed using –6/+6(H/dA) strand as a control. Radiograms and graphs showing control results are available in Supplementary Materials (Figs S3–S5 at https://ojs.ptbioch.edu.pl/index.php/abp/). In the case of OGG1, 50% of the control strand cleavage occurred after 180 min and lead to 79.24% after 240 min and 94.58% after 300 min. In the case of FPG, 50% of the control strand cleavage occurred after approximately 9 min and lead to 78.07% after 30 min and 96.81% after 120 min. Further results for examined strands containing cdG (Figs 2–3) were compared to the control.

The influence of cdG on OGG1 activity

The impact of ScdG and RcdG on the ability of OGG1 to excise 8-oxo-dG and cleave ds-DNA is shown in Fig. 2. Double-stranded oligonucleotides containing –6/+6(H/ScdG), or –6/+6(H/RcdG) were treated by 0.5 U OGG1 for up to 5 hours to obtain 100% of cleavage. For both diastereomers, the enzyme's activity increased in relation to control. The ds-DNA cleavage reached 50% after approximately 140 min (ScdG) and 110 min (RcdG), comparing to 180 min for control. After 240 min, the cleavage of –6/+6(H/ScdG) reached 94.28% and the cleavage of –6/+6(H/RcdG) reached 99.63% (control: 79.24%). Apart from elevated OGG1 activity, these results indicate that the presence of RcdG diastereomer force the increase more noticeable.

The influence of cdG on FPG activity

The impact of ScdG and RcdG on the ability of FPG to excise 8-oxo-dG and cleave ds-DNA is shown in Fig. 3. Double-stranded oligonucleotides containing –6/+6(H/ScdG), or –6/+6(H/RcdG) were treated by 0.5 U FPG for up to 2 hours to obtain 100% of cleavage. Received results are similar to those for OGG1 and indicate that the enzyme's activity is elevated (in relation to control) in the presence of both cdG diastereomers, especially for RcdG. The ds-DNA cleavage reached 50% after approximately 6 min (ScdG) and 3 min (RcdG), comparing to ~9 min (control). After 30 min, the cleavage of –6/+6(H/ScdG) reached 96.88% and the cleavage of –6/+6(H/RcdG) reached 99.13% (control: 78.07%). However, the difference between both diastereomers of cdG is more significant if results after 15 min are compared: 97.98% (RcdG), 84.08% (ScdG) and 57.24% (control). These results suggest that the presence of RcdG within the clustered lesion elevates the enzymes' activity more than the presence of ScdG in the same location.

DISCUSSION

This study continues the research in the field of cdPus influence on the activity of DNA glycosylases and endonucleases, considered in previous studies (Karwowski, 2019; Szewczuk *et al*., 2021b, 2021a; Boguszewska *et al*., 2021).

The most valuable observation denoted during these studies is the increase in both enzymes' activity in the presence of both cdG diastereomers within single-stranded clustered lesions, in relation to control ss-DNA strand. These results are in line with other studies on cyclopurines indicating the increase of UDG and hAPE1 activity in similar experimental conditions (Szewczuk *et al*., 2021a). However, the increase in UDG and hAPE1 activity was greater for 5'*S*-diastereomer of cdG, in comparison to 5'*R*, which is in opposite to the results obtained for OGG1 and FPG.

The increase in enzymes' activity in comparison to control may be explained as follows. First of all, the 6 base pairs distance between cdG and 8-oxo-dG may be enough to avoid the direct interaction between cdG and the active site of the enzyme. On the other hand, the disturbing structural modification of DNA helix propagated by cdG are compensated by the helix loosening in the appropriate distance from cdG. Thus, enzymes proceeding 8-oxo-dG excision may have easier access to the lesion and their activity may increase in comparison to the control strand. These conclusions are supported by the previous studies indicating that direct interactions between cdPus and the enzyme lead to decrease in its activity, and the decrease is not observed when the distance to the lesion is extended (Karwowski, 2019; Karwowski *et al*., 2014).

Another important finding from these studies is that there was no clear-cut middle band appearance after excision of the 8-oxo-dG located closer to the 3'-end of the oligonucleotide. During the reactions with OGG1, the middle band had the highest intensity of 0.61% (240 min, ScdG), 0.34% (180 min, RcdG) and 0.34% (300 min, control). In the case of FPG, these values were 1.26% (15 min, ScdG), 1.94% (5 min, RcdG) and 3.75% (15 min, control). Thus, it can be seen that for both enzymes, the middle band appeared faster and had a higher intensity for 5'*R* diastereomer. This indicates the priority of excision of cdG molecules located towards radiolabeled 5'-end of the DNA strand and the excision occurs faster for 5'*R* diastereomer.

These results, supported by previous studies concerning cdPus, indicate clear impact of the lesions proximity on the mechanisms of DNA repair pathways. Due to the fact that for many enzymes there are no specific data unveiling their performance in detail, it is relevant to undertake further studies in this field.

Supplementary Materials

Fig. S1: Efficiency of oligo labeling and hybridization, Figs S2: Visualization of ds-DNA cleavage by OGG1 or FPG, Figs S4–S45: Autoradiograms and graphs of cleavage assays of oligonucleotides, Figs S3–S11: Autoradiograms and graphs of cleavage assays of oligonucleotides, Figs S12–S16: Mass spectra of applied oligonucleotides, Table S1-S2: Raw numerical data of densitometry, Table S3: Calculated and found masses of oligonucleotides.

Author Contributions

Conceptualization, B.T.K.; methodology, B.T.K.; validation, M.S. and B.T.K.; analysis, B.T.K. and M.S.; investigation, B.T.K.; resources, B.T.K.; data curation, B.T.K. and M.S.; writing – starting draft preparation, M.S.; writing – review and editing, M.S. and B.T.K.; visualization, M.S.; supervision, B.T.K.; project administration, B.T.K.; funding acquisition, B.T.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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