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Base excision repair in non-canonical DNA structures

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Motivation and Aim: Maintaining genome integrity is a necessary condition for survival of all organisms. One of the most important DNA repair systems is base excision repair, which starts with recognition and cleavage of small non-bulky DNA modifications (AP sites, modified bases). Although genomic DNA in living cells mainly exists in the B form, many functionally important elements of the genome can adopt alternative structures *in vivo*. Such structures (thereafter referred to as "non-canonical DNA") include cruciform DNA, hairpins, triplexes, quadruplexes, intercalated motifs (i-motifs), single-stranded DNA, bulges, Z-DNA, DNA/RNA heteroduplexes, and displacement loops (D-loops) containing either RNA (R-loops) or DNA invading strands. Some of them are known as regulatory elements of gene activity and genome stability, others could be intermediates of harmful processes causing genome instability. Non-canonical DNA itself is highly sensitive to DNA damage, causing gene activity misregulation and severe diseases [1, 2]. Repair process in such structures remains poorly investigated.
Methods and Algorithms: In this work we applied qualitative and quantitative methods to estimate glycosylase activity at oligonucleotide substrates containing damaged bases or nucleotides – uracil, 5-hydroxyuracil (hoU), 8-oxoguanine (oG) or tetrahydrofuran – and forming non-canonical structures. Glycosylase activity assay was used for cruciform DNA and hairpins, steady-state kinetics was used for heteroduplexes DNA/RNA and 1–2-nucleotide bulges, single turnover and/or burst kinetics were used for bulges, heteroduplexes, i-motifs, and G-quadruplexes. Each type of substrate was tested with several prokaryotic and eukaryotic glycosylases.
Results: Uracil-DNA-glycosylases from *E. coli* and human showed activity on all non-canonical structures tested: i-motifs, G-quadruplexes, heteroduplexes and bulges, with no significant effect of the position in the structure or of an opposite base.
Functional but not structural homologs recognizing oxoguanine (Fpg from *E. coli* and human OGG1) were more disparate: they both showed activity on cruciform DNA, hairpins formed by $(CAG)_n$ triplet repeats, and regular hairpins (activity for both enzymes was detected only when oG was in the stem but not in the loop). Activity of OGG1 at i-motifs was very low whereas Fpg demonstrated cleavage depending the position of oG in the i-motif structure. Both glycosylases showed activity at oG in G-quadruplexes, but OGG1 was very sensitive to the position of oG while Fpg was not. DNA/RNA heteroduplexes and bulges were also cleaved by Fpg with higher efficacy than by OGG1. For these substrates kinetic parameters were obtained where possible. Fpg retained its specificity at hereroduplexes, discriminating substrate with adenine opposite to oG. OGG1 removed oG only from the oG/rC pair. The rate constants of glycosydic bond cleavage (k_2) for OGG1 on oG/rC and bulges were two orders of magnitude lower than k_2 for oG in canonical DNA. Fpg removed oG from a one-

nucleotide bulge with the same efficiency as from the oG/C pair, but for two nucleotide bulges the specificity constant (k_{cat}/K_M) was an order of magnitude lower.
Nei from *E. coli* and its mammalian homologs NEIL1 and NEIL2 remove the same damaged bases (mostly oxidized pyrimidines) but with different DNA structure specificity. They all cleaved substrates tested in this work but to varying degrees. NEIL2 worked expectedly better than others on hoU in the loop part of hairpins and in bulges, and much worse on DNA/RNA heteroduplexes.
Conclusion: The ability of most glycosylases to recognize and remove its specific damages from non-canonical structures depends of its location in the structure and its accessibility for the catalytic amino acids. In the cases when the glycosylases showed activity, the catalytic efficacy was usually much lower compared to their specific substrate structure.
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References

1. McMurray C.T. Mechanisms of trinucleotide repeat instability during human development. *Nat Rev Genet*. 2010;11:786-799.
2. Zhao X., Krishnamurthy N., Burrows C.J., David S.S. Mutation versus repair: NEIL1 removal of hydantoin lesions in single-stranded, bulge, bubble, and duplex DNA contexts. *Biochemistry.* 2010;49:1658-1666.