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The influence of 5',8-cyclo-2'-deoxypurines on the activity of glycosylases in the repair of clustered DNA lesions

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Abstract

The genetic material of living organisms is exposed to adverse external factors, such as ionizing radiation and reactive oxygen species. Under the influence of such factors, potentially mutagenic DNA damage can occur, which is removed from the genome by complex repair mechanisms. Dysfunctions of DNA repair systems can lead to the accumulation of damage and the appearance of mutations in the genetic material. This can result in the development of diseases, including cancer. A specific type of such damage is complex damage, an example of which are cyclodeoxypurines (cdPus). As part of my research, I attempted to estimate the impact of the presence of cdPus in DNA on the activity of enzymes in the base excision repair (BER) mechanism. In addition, I estimated the impact of the presence of cdPus on the activity of restriction endonucleases, which are part of the restriction-modification system in bacteria. The research work was divided into three stages:

- Stage 1: the evaluation of the influence of 5',8-cyclo-2'-deoxyadenosine and 5',8-cyclo-2'-deoxyguanosine on the activity of BER pathway enzymes (UDG and hAPE1) towards 2'-deoxyuridine (dU).
- Stage 2: the evaluation of the influence of 5',8-cyclo-2'-deoxyguanosine on the activity of BER pathway enzymes (OGG1 and FPG) towards 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxodG).
- Stage 3: the evaluation of the influence of 5',8-cyclo-2'-deoxyadenosine and 5',8-cyclo-2'-deoxyguanosine on the activity of BsmAI and SspI restriction endonucleases.

During my research, I used specially designed single-stranded oligonucleotide fragments with a length of 40 nucleotides, which contained damaged bases in their sequence. Single-stranded fragments were labeled with the radioisotope ^{32}P, and then hybridized with complementary oligonucleotides to obtain fragments of double-stranded DNA (dsDNA). I subjected dsDNA fragments to enzymatic reactions, and the progress of these reactions was evaluated by polyacrylamide gel electrophoresis (PAGE) using a 15% or 25% denaturing gel. I analyzed the results of electrophoretic separation of the products of enzymatic reactions by using the effect of exposure of X-ray films by β- radiation emitted by the 32P isotope. I quantified and further analyzed the results using Quantity One software and Microsoft Excel.

The results of the research work allow to conclude that the presence of cdPus affects the activity of the analyzed enzymes. The activity of the BER pathway enzymes (UDG, hAPE1, OGG1 and FPG) against the oligonucleotide substrates was higher than that of the cdPus-free controls. In the case of restriction endonucleases, I showed that the inhibition of their activity occurs primarily if cdPus are located near the site of substrate hydrolysis by the enzyme. If an enzyme hydrolyzes dsDNA within a sequence it recognizes, the presence of DNA damage within that sequence leads to complete inhibition of the enzyme. I also showed that the stereoisomeric form of cdPus, as well as its location in the dsDNA structure, affects the efficiency of reactions catalyzed by the enzymes studied.

The results of the research work carried out may in the future help to understand the mechanisms of action of individual enzymes involved in the protection of the human genome against the negative impact of complex DNA damage.