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Radio- and photolysis of labeled DNA.

**From synthesis of modified oligonucleotides to sequence dependence
of photodamage**

Radiotherapy is therapy employing ionizing radiation to kill malignant cells. Ionizing radiation works by damaging the DNA of cancerous tissue leading to cellular death. Unfortunately, this method of treatment causes several side effects, including secondary malignancies. Incorporation of halogenated nucleosides not only makes the DNA more sensitive to radiation but also in some cases toxic gamma ray can be replaced by less harmful UV radiation. Despite the fact that the sensitizing properties of halogenated nucleosides were determined in the 1950s, these compounds are still not used in clinical practice. It is because of incomplete knowledge about mechanisms that govern radio- and photosensitization of modified DNA.

The main goal of this doctoral dissertation was to explain the mechanism of radio- and photosensitization of labelled DNA. Moreover, a molecular tool was designed to allow quick and easy synthesis of DNA labelled with modified nucleosides.

It is commonly known that the formation of UV-induced damages is governed by long-range photoinduced electron transfer and its dependency on local DNA sequence. So far, experiments had focused on choosing the best electron donor and linker between unmodified and modified nucleotide, which resulted in definition of 5'-GAA^{Br}U-3' sequence as a "hot spot". The presence of that sequence in double-stranded DNA guarantees high biopolymer's photosensitivity. Unquestionably, the unclear issue was the effect of modification of the adjacent nucleotide (X or Y) on the ionization energy of the guanine and electron affinity of the modified nucleotide within 5'-XCAA^{Br}UY-3' motif. In this study, the mechanism linking the sensitizing properties of BrdU with local DNA sequence was elucidated and the sequence (5'-CCAABrUT-3') which provides the highest efficiency of photodamage formation in double stranded DNA was determined.

The second research was devoted to the γ -induced damage to model DNA fragments (TYT) labelled with iodinated nucleosides (Y = 5-iodo-2'-deoxyuridine/cytidine). The LC-MS analyses of radiolytic products reveal that the γ -irradiation of modified trimers results mainly in the formation of strand breaks – the presence of the following products was confirmed: HO⁺TX_{OH}/HO⁺XT_{OH}, P⁺XT_{OH}, O=XT_{OH}, dT=O, P⁺T_{OH}, HO⁺T_{OH}, HO⁺T_P, dTI. The comparison of the type and the yield of electron-induced degradation for particular trimers leads to the conclusion that iodinated pyrimidines (especially 5-iodo-2'-deoxyuridine) are better sensitizers than commonly used 5-bromo-2'-deoxyuridine.

Finally, the chemically-enzymatic method, employing a DNA polymerase and ligase, that enables a modified nucleoside, in the form of its 5'-triphosphate, to be incorporated into DNA fragment in a pre-determined site was optimized. Using this protocol two double-stranded DNA fragments – a long one, 75 base pairs (bp), and a short one, 30 bp in length – were pin-

point labelled with 5-bromodeoxyuridine. The photoreactivity of synthesized oligonucleotides was confirmed, which was an ultimate test showing that the product possesses an assumed nucleotide sequence and is sensitive to UV-radiation. We designed a general approach enabling precise labeling of DNA with any modified nucleoside.

The accomplishment of these three projects broadened scientific knowledge about radio- and photosensitivity of modified DNA. Moreover, the designed molecular tool not only enables testing of radio- and photosensitizing properties of all newly synthesized modified nucleosides but also their stability in a solution and resistance to elevated temperature.