Radiation-induced oxidation of proteins in DNA-protein complexes.

M. Spotheim-Maurizot[a], S. Mazier[a], S. Villette[a], S. Goffinont[a], D. Genest[a], M. Cadene[a] and M. Davidkova[b]

[a] *Centre de Biophysique Moléculaire, CNRS, rue C. Sadron, 45071 Orléans, France;* [b] *Dept. Radiation Dosimetry, Nuclear Physics Instit, Na Truhlarce 39, 18086 Prague 8, Czech Republic*

spotheim@cnrs-orleans.fr

A key step in the regulation of gene expression, DNA structuring and DNA repair is the binding of some proteins to specific DNA sequences. Previously we have shown that DNA-binding proteins are acting as efficient protectors against the attack of hydroxyl radicals produced by water radiolysis. They protect their binding sites on DNA by shielding and by radicals scavenging. They also modify the conformation of DNA (compaction, bending) thereby rendering DNA more resistant to radiolysis. But proteins are also vulnerable and get damaged under irradiation. The progressive accumulation of damages on the protein (mainly side chain modifications) firstly affects the configuration of the DNA-protein couple and finally renders the protein unable to play its protective role: the protein loses its ability to bind to its specific DNA sequence. We have studied the effect of irradiation on the E. coli lactose operator-repressor complex. At low doses the protein protects its specific binding site on DNA. At high doses, the complex is disrupted mainly due to the damage to the protein. CD data show that upon irradiation, the structure and the stability of the binding domain of the protein (the headpiece) changes. Fluorescence measurements reveal the degradation of tyrosine residues. Mass spectrometry data complemented by RADACK calculations allow identifying all the oxidized amino-acids of the DNA binding domains of the proteins. Molecular dynamics simulations reveal and characterize the structural changes induced by the oxidation of each amino-acid in the headpiece. Most of the identified oxidized amino-acids are essential for the DNA-protein interaction as revealed by the analysis of the NMR- or crystallography-based structures of the complexes. Thus, irradiation can critically affect proteins properties and consequently can hinder their binding to DNA, due to the oxidation of amino-acids of the DNA-binding domain and to the subsequent conformational changes of it.

Article published by EDP Sciences and available at http://www.radioprotection.org or http://dx.doi.org/10.1051/radiopro:2008526