Radiation damage to DNA-protein complexes

M Spotheim-Maurizot\textsuperscript{1} and M Davídková\textsuperscript{2}
\textsuperscript{1} Centre de Biophysiqe Moléculaire, CNRS, rue C. Sadron, 45071 Orléans, France -
\textsuperscript{2} Dept. of Radiation Dosimetry, Nuclear Physics Institute ASCR, Na Truhlářce 39/64,
18086 Prague 8, Czech Republic.

E-mail: spotheim@cnrs-orleans.fr

Abstract. We review here the advances in understanding the effects of ionizing radiations on
DNA, proteins and their complexes, resulting from the collaboration of the authors’ teams. It
concerns the preponderant indirect effect of low LET ionizing radiations, thus the attack of the
macromolecules in aqueous solution by the most aggressive product of water radiolysis, the
hydroxyl radical. A model of simulation of the reaction of these radicals with the
macromolecules (called RADACK) was developed and was used for calculating the
probabilities of damage of each constituent of DNA or proteins (nucleotide or amino-acid).
The calculations allowed to draw conclusions from electrophoresis, mutagenesis, spectroscopic
(fluorescence, circular dichroïsm) and mass spectrometry experiments. Thus we have shown
that the extent and location of the lesions are strongly dependent on the 3D structure of the
macromolecules, which in turns is modulated by their sequence and by the binding of some
ligands. Molecular dynamics simulation completed our studies in showing the consequences of
each lesion on the stability and structure of the proteins and their complexes with DNA.

1. Introduction

Ionizing radiations are damaging DNA, proteins and their complexes \textit{in vivo} (in cells) and \textit{in vitro} (in
aqueous solution) via their direct and indirect effects. The direct effects occur via the ionization and
excitation of the macromolecules, whereas the indirect ones occur via the products of ionization and
excitation of the water molecules surrounding the macromolecules.

The proportion of the two types of effects depends on the LET of the radiation, the proportion of
direct effects being higher for high LET radiation (alpha particles, carbon ions, etc). In the case of low
LET radiations such as gamma rays the main damaging species induced by the irradiation of
macromolecules in solution are the sparsely distributed hydroxyl radicals (OH\textsuperscript{.}) produced by water
radiolysis. The proximity of the radicals produced in the high density tracks of high LET particles
favors their recombination and thus reduces the proportion of indirect effects.

1.1 DNA radiolysis

The hydroxyl radicals are damaging DNA by abstracting H atoms from the sugar moiety
(deoxyribose) and bases or to the double bonds of certain bases. The final consequence of these
reactions is the formation of strand breaks (called frank strand breaks, FSB), abasic sites or modified
sugar and bases (figure1). Part of the nucleotides with modified bases is alkali labile and therefore
such lesions can be revealed as strand breaks in alkaline pH (called alkali revealed breaks, ARB).
Figure 1. Attack of DNA by the OH· radicals produced by the radiolysis of water and its consequences.

Figure 2. Sequencing gel electrophoresis applied to an irradiated double stranded (D) and quadruplex DNA (Q). 1. DNA is labeled with 32P at the 5’- end of one strand. 2. DNA is irradiated and breaks occur on both strands. 3. DNA is denatured and radioactive fragments of different length are obtained. 4. The radioactive fragments are separated in a sequencing gel. The radioactivity of each band is quantified (area under the peak) using a Phospholmager. It is proportional to the amount of radioactive fragments ending by a given (unlabelled) nucleotide. It is proportional to the probability of breakage at that nucleotide. On the example of gel, samples at neutral pH reveal FSB and samples incubated with piperidine (alkali) reveal FSB+ARB. The middle lane shows the bands corresponding to fragments ending by an unlabelled guanosine.
Their number and distribution along a DNA molecule can be determined by applying electrophoresis techniques. In the case of a circular supercoiled plasmid DNA, by taking into account that a single strand break converts the plasmid into a relaxed circular one, and that a double strand break leads to a linear one, the separation of the three forms by agarose gel electrophoresis allows to determine the number of single and double strand breaks [1]. In the case of a linear double stranded DNA, using sequencing gel electrophoresis of radioactively labeled irradiated fragments (figure 2) allowed to observe that the frequencies of FSB and ARB are not homogeneously distributed along the molecule, but are modulated by DNA sequence of nucleotides [2].

Using molecular modeling (figure 3) we were able to explain the observed radio-resistance of certain sequences of B-DNA (such as the 5’-AATT-3’ sequence) by the reduced accessibility (defined as the surface of the van der Waals sphere representing the H atom on which a sphere of the size of an OH· radical can roll over) of the H4’ and H5’2 atoms of sugars, atoms located in the narrow minor groove of such sequences3. The accessibility of the H5’1 atom situated at the edge of the minor groove is independent of the minor groove width.

It should be recalled that among all the H atoms of the sugar moiety, H4’ and H5’2 are the most accessible to OH· radicals and their abstraction is the main cause of breakage of the phosphodiester backbone of DNA (by a beta-elimination reaction) [3].

![Figure 3. Accessibility of H4’ and H5’ atoms to OH· radicals in a B-DNA fragment.](image)

In the top picture: OH· radical in red, H4’ in magenta, H5’1 in yellow and H5’2 in green.

Later on, an original model of simulation of radiolytic attack (RADACK) based on a Monte Carlo technique allowed to calculate the probability of breakage reactions at each nucleotide by taking into account not only the accessibility of attack sites, but also the chemical reactivity of the different bases and the efficiency with which the abstraction reaction leads to strand breakage [4]. The principle of the simulation model is presented in figure 4. The three dimensional structure of DNA was obtained either from structural databases such as PDB or was built by molecular modeling using the SYBYL software.
The same approach succeeded to account for the observed influence of more drastic variations of DNA structure (Z-DNA, quadruplexes) on the distribution of FSB and ARB [5-8]. Interestingly the efficiency with which the reaction of OH- radicals with sugar H atoms leads to strand breakage seems to be different in the left handed Z-form of DNA than in the canonical right handed B-DNA, probably because of differences in sugar conformation (for nucleotides containing guanines) and other structural parameters.

The consideration of local structural variations within studied biological macromolecules in the mentioned modeling approach is for the moment exclusive. Similar stochastic models usually apply track structures of incident radiation to calculate the spectrum of damages in large DNA fragments, chromosome domains or the whole cell nucleus, where biomolecules are represented by standard “average” molecular conformation (e.g. [9-10]).

Figure 4. Principle of RADACK model. DNA with atoms represented either by van der Waals spheres (non-reactive atoms) or Smoluchowski’s sphere (reactive atoms, spheres with radius proportional to their reactivity) in a box where OH- radicals are generated randomly. They can diffuse and either react with the encountered DNA atom or escape from the box. The number of encounters with the atoms of a nucleotide divided by the total number of generated OH- radicals represents the relative probability of reaction with that nucleotide.

2. Binding of small molecules affects DNA radiolysis
The use of the same combined electrophoresis and molecular modeling methodology allowed observing and understanding the protection or sensitization of certain regions of DNA by small positively charged molecules such as polyamines (putrescine, spermidine and spermine) [11-12] or some thiols (metabolites of the chemo- and radio-protective drug Ethyol (Amifostine) and related compounds, cysteamine, N-acetylcysteine, captopril) [13-16].

As revealed by molecular modeling calculations, the regions of DNA that have a narrow minor groove and thus a strongly negative electrostatic potential attract such molecules. Through binding in the minor groove of DNA, they mask and therefore protect the H4’ and H5’2 atoms whose abstraction would lead to strand breakage. When the most negative potential is located in the major groove, binding of the small ligands to such regions do not protect the critical sugar H atoms and therefore these regions are not protected. Moreover, the binding in the major groove can trigger in some cases the broadening of the minor groove and thus, can lead to the radiosensitization of the region (figure 5).

Thus the effects of polyamines and thiols are also dependent on the sequence of DNA via the modulation of DNA structure and electrostatic potential. It can be explained by the physical screening of the binding site, the scavenging of OH- radicals, the chemical repair of DNA radicals (only for thiols who donate the H atom of their SH function and thus repair the damaged sugar) and by the binding-induced structural changes of DNA (narrowing or broadening of the minor groove, or even compaction of DNA in the case of highly charged polyamines).

3. Binding of proteins affects DNA radiolysis
The DNA ligands of the highest biological interest are proteins. Their binding to DNA influences many critical cellular processes such as regulation of gene expression, DNA repair or DNA
compaction. We have observed the protection of the binding sites of proteins such as lactose repressor (a protein managing the negative regulation of genes involved in lactose metabolism of *E. coli*), CRP (the catabolite gene activator protein, a regulatory protein involved in the energy metabolism of *E. coli*), an estrogen receptor (a ligand-activated transcription factor regulating the expression of genes controlled by estrogens), MC1 (a chromosomal protein of the archaeabacterium Methanosarcina sp. involved in DNA bending and supercoiling) and histones (the octamer of proteins located in the center of nucleosome cores in chromatin) (figure 6). On the basis of this observation we have proposed the radiolytic footprinting as a useful method of determination of proteins binding sites on DNA [2].

![Figure 5](image)

**Figure 5.** Radioprotection and radiosensitization of different regions of a DNA fragment by spermine (electrical charge +3). I. Electrostatic potential at the surface of DNA: the sites with a highly negative potential are red. A, B, C and D sites are located in the minor groove, and A’ site is in the major groove. II. Experimentally determined values of $1/\text{PF}$ where PF (protection factor) = FSB in DNA irradiated in presence of spermine/FSB in free DNA. III. Molecular modeling showing the narrowing of the minor groove resulting from spermine binding at B site (minor groove) and broadening of the minor groove resulting from the binding of spermine at A’ (major groove).

RADACK calculations in which the three dimensional structures of the complexes were extracted from the PDB structural data base were performed (Figure 7). They were in good agreement with the experimental results and allowed showing that the protection by the proteins is due to the physical screening of the binding site, to the scavenging of OH radicals by the protein and to the binding-induced structural changes of DNA [17-22].
Figure 6. Variation of strand breaks frequency along a DNA irradiated in complex with lactose repressor (A), CRP (B) and with histone octamer in nucleosome core (C). The bleached zones correspond to the regions with low FSB yields due to the presence of the protein.

Figure 7. Comparison of RADACK calculations (red curves) with experimental results (FSB, histograms) for DNA irradiated in complex with the repressor headpieces. The three dimensional structures of the complex used in the calculations are 1LBG (K) and 1CJG (R).
4. Protein radiolysis destabilizes DNA-protein complexes

The protein cannot accomplish anymore its role of DNA “bodyguard” when irradiated with high doses of radiation because in reacting with the OH radicals, it gets much damage and consequently, loses its ability to bind DNA. The type of reactions and consequences are quite similar to those of DNA: abstraction of H atoms and binding to aromatic rings, leading to backbone breakage and modification of side chains (figure 8).

![Proteins: sites of OH radical attack and types of damage](image)

**Figure 8.** Attack of proteins by the OH- radicals produced by the radiolysis of water and its consequences.

Side chains oxidation of amino-acids are induced not only by radiation, but also by natural metabolic processes. These last ones are leading to deleterious effects when exceeding the detoxification ability of the organism (like in aging or strong oxidative stress conditions).

When irradiating DNA-protein complexes with high doses, the lactose repressor-operator complex, the complex between the formamidopyrimidine-DNA glycosylase (Fpg) and DNA bearing a model oxidative damage and the complex between estrogen receptor and the estrogen receptor element were destabilized [23-24]. In all cases we have proven that the loss of binding ability of the protein is the main cause of the complex destabilization. The results of a molecular modeling study in which 8-oxoG lesions were introduced into DNA were consistent with only a weak effect of DNA damage on the lactose repressor-operator interactions [25].

Moreover we have shown that when irradiated in complex with DNA, the protein loses its binding ability at doses higher than those necessary for inactivating the free protein. This means that when irradiated in the complex, the protein is less damaged than when irradiated free in solution. We have concluded that in the complex not only the protein is protecting DNA, but in turns, DNA protects the protein by masking (and thus rendering inaccessible for the OH- radicals) the aminoacids of the binding site. The results for the lactose repressor-operator complex are shown in figure 9.

RADACK calculations have predicted which amino-acids are most likely to be damaged by irradiation. Consistent with these predictions, mass spectrometry and fluorescence measurements have shown that the tyrosine residues are oxidized upon irradiation of the DNA binding domains (headpieces) of the lactose repressor [26]. Molecular dynamics simulation have shown that the replacement of tyrosine residues by their oxidation product 3,4-dihydroxyphenylalanine (DOPA) in the headpiece leads to a loss of its stability and to a reduction of its helical content of the protein.
These results are in good agreement with the unfolding and destabilization of the headpiece as revealed by circular dichroism measurements [27].

**Figure 9.** Decrease of DNA amount in DNA-repressor complexes upon the irradiation of the complex (magenta), of the free protein (red) or of the free DNA (blue).

**Figure 10.** Probability of reaction of OH- radicals with the amino-acids of the tetramerization domain of the lac repressor tetramer. In the bottom a schematic representation of the tetramer-to-dimer radio-induced transition.
After comparing a native and an oxidized headpiece, molecular dynamics simulations were performed for 1. the native complex formed by two headpieces and the operator DNA, and 2. the complex between the oxidized headpiece and DNA. The results have shown effects consistent with complex destabilization: increased flexibility, increased DNA bending, modification of the hydrogen bond network, decrease of the positive electrostatic potential at the protein surface and of the global energy of DNA-protein interactions [28].

Another modification of the repressor structure resulting from the irradiation concerns its oligomerization state. Lactose repressor is a tetrameric protein formed by two identical homodimers connected by a 4-helix bundle based on leucine zippers (called tetramerization domain). We have shown that upon high dose irradiation the tetramer splits into its constitutive dimers likely because of the damage of the leucines of the tetramerisation domain. RADACK calculations (figure 10) have shown that these leucines whose integrity is indispensable for the maintenance of the tetramerization have indeed a high probability of getting damaged by OH− radicals [29].

5. Conclusion
Our work allowed to observe and to explain the variations of radiation-induced damage yields and distribution in free DNA and in DNA involved in supramolecular complexes. It emphasized the critical role of structural parameters in the induction of damage. It also shows that the observed destabilization of DNA-protein interactions is mainly due to the chemical and structural damages of the protein.

Acknowledgments
All members of our two teams having contributed to the research on radiation damage to biomolecules as well as our co-workers are warmly thanked. Our research got the financial support of CNRS, INSERM, Electricity of France, League against Cancer, Association for Research against Cancer, Academy of Sciences of the Czech Republic (B1048901, A1048103, KJB4048401), MEYS CR (1P05OC085, OC09012), bilateral French-Czech programs (CNRS-ASCR, Barrande-Egide) and the European actions COST P9 and CM0603.

References
[1] Spotheim-Maurizot M, Charlier M and Sabattier R 1990 *Int. J. Radiat. Biol.* 57 301
[2] Franchet-Beuzit J, Spotheim-Maurizot M, Sabattier R, Blazy-Baudras B and Charlier M 1993 *Biochem.* 32 2104.
[3] Sy D, Savoye C, Běgusová M, Michalik V, Charlier M and Spotheim-Maurizot M 1997 *Int. J. Radiat. Biol.* 72 147.
[4] Běgusová M, Spotheim-Maurizot M, Sy D, Michalik V and Charlier M 2001 *J. Biomol. Struct. Dyn.* 19 141.
[5] Tartier L, Michalik V, Spotheim-Maurizot M, Rahmouni A R, Sabattier R and Charlier M 1994 *Nucleic Acids Res.* 22 5565.
[6] Michalik V, Spotheim-Maurizot M and Charlier M 1995 *J. Biomol. Struct. Dyn.* 13 565.
[7] Tartier L, Spotheim-Maurizot M and Charlier M 1998 *Int. J. Radiat. Biol.* 73 45.
[8] Běgusová M, Tartier L, Sy D, Michalik V, Spotheim-Maurizot M and Charlier M 1999 *Int. J. Radiat. Biol.* 75 913.
[9] Nikjoo H, O’Neill P, Terrissol M and Goodhead DT 1999 *Radiat. Environ. Biophys.* 38 31.
[10] Friedland W, Paretzke HG, Ballarini F, Ottolenghi A, Kreth G and Cremer C 2008 *Radiat. Environ. Biophys.* 47 49.
[11] Spotheim-Maurizot M, Ruiz S, Sabattier R and Charlier M 1995 *Int. J. Radiat. Biol.* 68 571.
[12] Sy D, Hugot S, Savoye C, Ruiz S, Charlier M and Spotheim-Maurizot M 1999 *Int. J. Radiat. Biol.* 75 953.
[13] Spotheim-Maurizot M, Franchet J, Sabattier R and Charlier M 1991 *Int. J. Radiat. Biol.* 59 1313.
[14] Spotheim-Maurizot M, Garnier F, Kieda C, Sabattier R and Charlier M 1993 *Radiat. Environ. Biophys.* **32** 337.

[15] Savoye C, Swenberg C, Hugot S, Sy D, Sabattier R, Charlier M and Spotheim-Maurizot M 1997 *Int. J. Radiat. Biol.* **71** 193.

[16] Sy D, Durand C, Hugot S, Savoye C, Swenberg C, Charlier M and Spotheim-Maurizot M 1999 *Theor. Chem. Acc.* **101** 114.

[17] Isabelle V, Franchet-Beuzit J, Sabattier R, Laine B, Spotheim-Maurizot M and Charlier M 1993 *Int. J. Radiat. Biol.* **63** 749.

[18] Běgusová M, Sy D, Charlier M and Spotheim-Maurizot M 2000 *Int. J. Radiat. Biol.* **76** 1063.

[19] Běgusová M, Eon S, Sy D, Culard F, Charlier M and Spotheim-Maurizot M 2001 *Int. J. Radiat. Biol.* **77** 645.

[20] Běgusová M, Giliberto S, Gras J, Sy D, Charlier M and Spotheim-Maurizot M 2003 *Int. J. Radiat. Biol.* **79** 385.

[21] Davidková M, Štísová V, Goffinont S, Gillard N, Castaing B and Spotheim-Maurizot M 2006 *Radiat. Prot. Dosim.* **122** 100.

[22] Štísová V, Goffinont S, Spotheim-Maurizot M and Davidková M 2006 *Radiat. Prot. Dosim.* **122** 106.

[23] Eon S, Culard F, Sy D, Charlier M and Spotheim-Maurizot M 2001 *Radiat. Res.* **156** 110.

[24] Gillard N, Běgusová M, Castaing B and Spotheim-Maurizot M 2004 *Radiat. Res.* **162** 566.

[25] Sy D, Flouzat C, Eon S, Charlier M and Spotheim-Maurizot M 2001 *Theor. Chem. Acc.* **106** 137.

[26] Gillard N, Goffinont S, Buré C, Davidková M, Maurizot J C, Cadene M and Spotheim-Maurizot M 2007 *Biochem. J.* **403** 463.

[27] Mazier S, Villette S, Goffinont S, Renouard S, Maurizot J C, Genest D and Spotheim-Maurizot M 2008 *Radiat. Res.* **170** 604.

[28] Aci-Sèche S, Garnier N, Goffinont S, Genest D, Spotheim-Maurizot M and Genest M 2010 *Eur. Biophys. J.* **39** 1375.

[29] Goffinont S, Davidková M and Spotheim-Maurizot M 2009 *Biochem. Biophys. Res. Commun.* **386** 300.