*Free Rad. Res. Comms.*, Vol. 6, No. 2-3, pp. 157-159
Reprints available directly from the publisher
Photocopying permitted by license only



# HYDROXYL RADICAL-INDUCED CROSSLINKING BETWEEN DOUBLE-STRANDED POLY(dA-dT) AND TRIPEPTIDES CONTAINING AN AROMATIC RESIDUE

VINCENT FAVAUDON

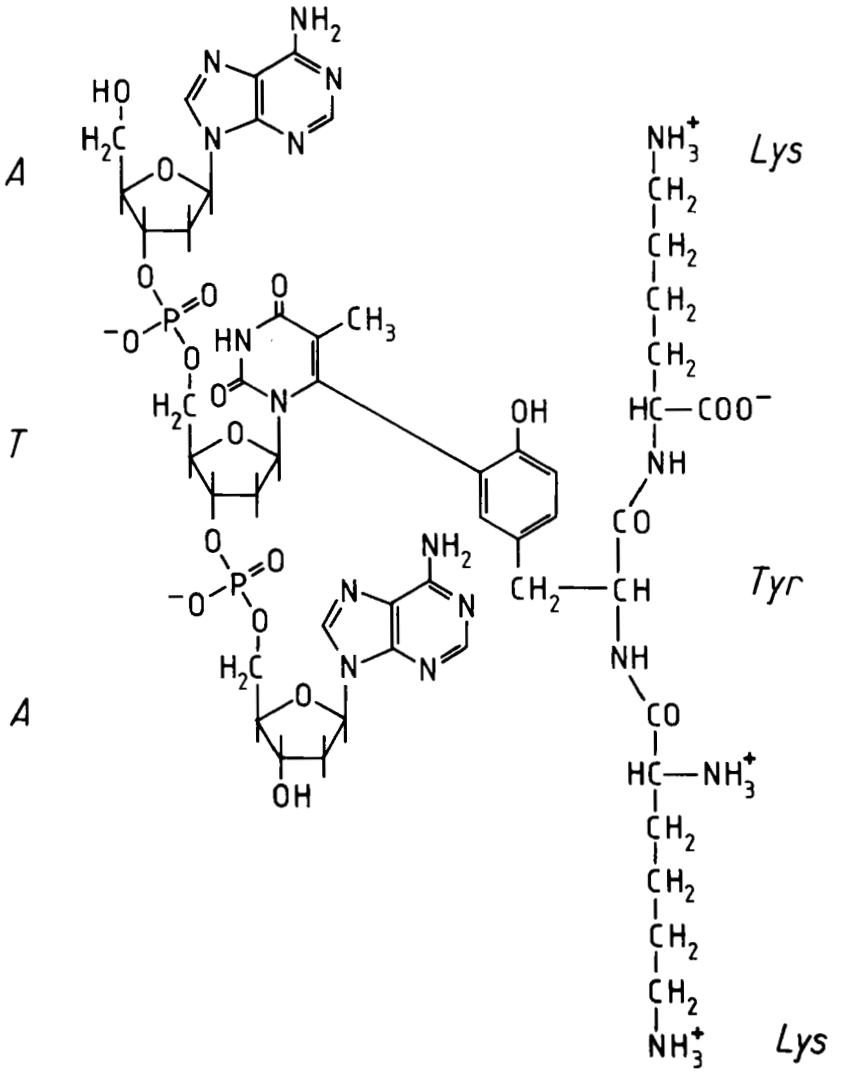
*Unité 219 INSERM, Institut Curie-Biologie, Labs. 110–112, Centre Universitaire, 91405-Orsay Cedex (France)*



It has been known since the pioneering studies of Alexander and Stacey[1] that ionizing radiations are able to induce crosslinking of nucleic acids and proteins.[2-8] The structure of chromatin is likely to determine the fate of radiation-induced DNA-protein crosslinks,[9-11] but in fact little is known about the formation and repair of these lesions, particularly with regard to the lethality or mutagenicity of protein-concealed double-strand breaks. How radical recombination occurs between peptides and bases or deoxyribose in DNA, and how this process could be modulated by oxygen or drugs, though also outstanding questions, are more amenable to *in vitro* model studies.

One such simple model consists of the double-stranded alternating copolymer poly(dA-dT) and of the tripeptides Lysyl-Tryptophyl-Lysine or Lysyl-Trosyl-Lysine. These positively charged oligopeptides are known to bind to DNA by both heteropolar and stacking interactions in an ionic strength-dependent equilibrium.[12] Complexation of the peptides to poly(dA-dT) was found to result in efficient protection of the peptide aromatic groups against oxidative damage by the $OH^{\cdot}$, $Br_2^{\cdot-}$ or $N_3^{\cdot}$ radicals, and of poly(dA-dT) against $OH^{\cdot}$-induced thymine release as well. Hydroxyl radical-induced covalent poly(dA-dT)-peptide adducts were produced in relatively good yield with Lys-Trp-Lys ($G \simeq 0.31$ mol/100 ev) or Lys-Tyr-Lys ($G \simeq 0.18$ mol/100 ev). These adducts were isolated by reverse-phase HPLC from the enzymic hydrolyzates of irradiated solutions. About ten different isomers were formed with both peptides, due presumably to different modes of radical recombination. All these compounds appeared to have the same general tripeptide-trinucleotide (Lys-Trp-Lys)-d(ApTpA) or (Lys-Tyr-Lys)-(ApTpA) structure, based on double-labeling experiments of poly(dA/T) with[*methyl*-$^3$H]dTTP or [8-$^3$H]dATP on the one hand, and [$\alpha$-$^{32}$P]dTTP or [$\alpha$-$^{32}$P]dATP on the other hand. It is thus proposed, from the known properties of the enzymes used in the digestion procedures, that crosslinking takes place mostly, if not uniquely, at that level of thymidine.

Upon acidic hydrolysis, the major (Lys-Trp-Lys)-d(ApTpA) adduct, purified to homogeneity by sequential reverse-phase and ion-exchange HPLC, released free adenine and a single thymine-containing product that did not co-migrate with thymine or 2'-deoxythymidine. Crosslinking by radical recombination at the C(5)-, C(6)- or 5-methyl-positions of thymine, such as shown in the accompanying figure, can reasonably be proposed for this particular adduct fraction in light of the results of Simic and Dizdaroglu.[13,14] Other adduct fractions, however, showed patterns of mild alkaline hydrolysis suggesting peptide addition on the deoxyribose moiety at thymidine sites.




SCHEME I



## *References*

1. Alexander, P. and Stacey, K.A. Crosslinking of deoxyribonucleic acid in sperm heads by ionizing radiations. *Nature*, **184**, 958, (1959).
2. Yamamoto, O. Radiation-induced binding of some protein and nucleic acid constituents with macromolecular components in cell systems. *Radiat. Res.*, **61**, 261, (1975).
3. Minsky, B.D. and Braun, A. X-ray mediated crosslinking of protein and DNA. *Radiat. Res.*, **71**, 505, (1977).
4. Fornace, A.J. and Little, J.B. DNA crosslinking induced by X-rays and chemical agents. *Biochim. Biophys. Acta*, **477**, 343, (1977).

5. Hawkins, R.B. Quantitative determination of crosslinkage of bacteriophage DNA and protein by ionizing radiation. *Int. J. Radiat. Biol.*, **33,** 425, (1978).
6. Giocanti, N. and Ekert, B. Radiochemical crosslinking between proteins and RNA within 70S ribosomal particles from *E. coli* MRE600. *Int. J. Radiat. Biol.*, **40,** 507, (1981).
7. Mee, L.K. and Adelstein, S.J. Predominance of core histones in formation of DNA-protein crosslinks in gamma-irradiated chromatin. *Proc. Natl. Acad. Sci. USA*, **78,** 2194, (1981).
8. Olinski, R., Briggs, R.C., Hnilica, L.S., Stein, J. and Stein, G. Gamma-radiation-induced crosslinking of cell-specific chromosomal non-histone protein-DNA complexes in HeLa chromatin. *Radiat. Res.*, **86,** 102, (1981).
9. Chiu, S.M., Friedman, L.R., Sokany, L.M., Xue, L.Y. and Oleinick, N.L. Nuclear matrix proteins are crosslinked to transcriptionally active genes by ionizing radiation. *Radiat. Res.*, **107,** 24, (1986).
10. Meyn, R.E., Van Ankeren, S.C. and Jenkins, W.T. The induction of DNA-protein crosslinks in hypoxic cells and their possible contribution to cell lethality. *Radiat. Res.* **109,** 419, (1987).
11. Ramakrishnan, N., Chiu, S.M. and Oleinick, N.L. Yield of DNA-protein crosslinks in gamma-irradiated chinese hamster cells. *Cancer Res.*, **47,** 2032, (1987).
12. Toulmé, J.J. Stacking interactions: the key mechanism for binding of proteins to single-stranded regions of native and damaged nucleic acids? *Chromosomal Proteins and Gene Expression*. Roeck, G.R., Goodwin, G.A. and Puigdomenech, P. (eds.), New York: Plenum, P. 263, (1986).
13. Simic, M.G. and Dizdaroglu, M. Formation of radiation-induced crosslinks between thymine and tyrosine: possible model for crosslinking of DNA and proteins by ionizing radiation. *Biochem.*, **24,** 233, (1985).
14. Margolis, S.A., Coxon, B., Gajewski, E. and Dizdaroglu, M. Structure of a hydroxyl radical induced cross-link of thymine and tyrosine. *Biochem.*, **27,** 6353, (1988).