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THE PRIMARY LESION INDUCED IN DNA BY RADIATION

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1 - DNA damage by radiation.

Exposure of living cells to moderate doses of electro-magnetic radiation diminishes their survival and increases the mutation rata. The alteration is of genetic nature and is due to molecular damage in DNA. The DNA is the "sensitive target".

The DNA damage results from the interplay of two processes. First, the induction of the "primary lesion" in DNA through a series of physico-chemical events brought about by the deposition of the radiation energy in living matter. Second, the incomplete or faulty operation of the repair enzymes which tend to restore the DNA integrity. The biological effect of radiation ultimately depends on how the "primary lesion" will be eliminated or modified and fixed as permanent DNA injury by the biochemical response of the cells.

The repair mechanisms are a variable property of the cells and are actually the determinants of their radiosensitivity. The "primary lesion" is also important because its precise molecular features determine which enzyme may start the specific sequence of the repair reactions.

2 - Interaction of radiant energy with living matter.

The physical interaction between radiation and matter has to be expressed in terms of dissipated energy. This defines thermodinamically the event inducible by a given radiation. We shall consider U.V. light with wavelength, λ, of more than 200 nm, the X-rays and the γ-rays of common use.

The U.V. radiation is absorbed by specific substances and its energy of 3 to 6 eV per incident photon with λ of 400 and 200 nm, respectively, is more than enough to produce excitation in the electronic state of the target molecules. Some light absorbing compounds act as photosensitizers by transferring their excitation state to other nearby molecules which become the true "sensitive targets".

DNA absorbs U.V. light in the spatial region around 260 nm but undergoes molecular damage through the photodynamic effect of sensitizing substances with absorption maxima outside the peak observed for nucleic acids (acetone, tryptophan, furocoumarins).

The transition and the physico chemical events induced by the absorption-excitation process as well as the energy transfer from a "photosensitizer" will be dealt with in the lecture of Crippa and more generally in the Section on Luminescence.

The X- and γ-rays exert their influence on biological materials by means of the high speed electrons generated from their interaction with matter (photoelectrical effect and Compton scattering). These electrons have kinetic potentials above 100-120 eV and mean pathways of 0.1 to 10 mm in soft tissues. Within such distance, the kinetic energy is dissipated by collisions with electrons in molecular orbitals and these hits are usually sufficient to ionize molecules. Thus, ion pairs consisting of molecular anions (often radicals) and free electrons are produced.

The electrons ejected from molecular orbitals excite other molecules but sometimes carry away enough energy to induce secondary ionizations (the ionization potential of water and of the most common biological substance is of 35-38 eV). Therefore, a "spur" of excitation and ionizations takes place in the small region where the energy of some tens of eV has been deposited by a fast electron. This is a rather explosive event which involves indiscriminately many many molecules and is well above the 14-16 eV energy of most covalent bond-making or covalent bond-breaking biochemical reactions.

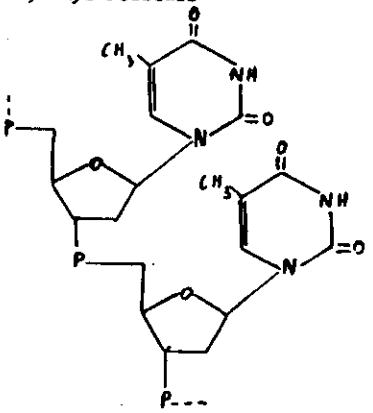
Chemical damage will then result for a number of biomolecules but it will be bound to have critical consequences only for those substances which cannot be replaced or repaired by the synthetic machinery of the cell. DNA remains a critical target of the complex interaction between ionizing radiation and living matter.

3 - U.V. damage in DNA

The 5-6 double bond of the pyrimidine bases is the most sensitive site of DNA. Because of energy transfer processes within the DNA chains, almost all primary lesions appear in the pyrimidines.

U.V. light dimerizes adjacent pyrimidines in DNA with a maximum efficiency for the thymines. The reaction occurs through the formation of a four carbon ring between the two 5-6 bonds, thus resulting in a cyclobutane-like structure.

Thymidyl residues



Dimerized thymidyl residues

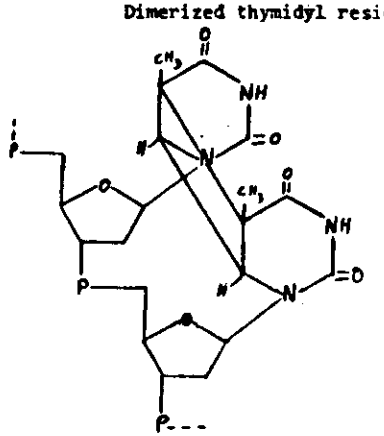


Fig. 1. Thymine dimer in DNA.

While in normal DNA the adjacent bases lie in directions diverging by an angle of 36°, the dimerized pyrimidines are parallel (one over the other) and represent a distortion in the double helix. The photoreaction is reversible, but equilibrium greatly favors dimerization because the dimers absorb much less U.V. radiation than the corresponding monomerized pyrimidines. For instance, with thymine the rate constant K_1 is 50 times less than K_2 but the absorption of monomer exceeds by a factor of 400 that of the dimer.

Monomerized pyrimidines in DNA $\underset{K_2}{\overset{K_1}{\rightleftharpoons}}$ Dimerized pyrimidines in DNA

A second DNA alteration caused by U.V. light is the hydration of cytosine. Although easily reversible, the process is of importance because hydrated cytosine codes in DNA like thymine and thus behaves chemically as a point mutation.

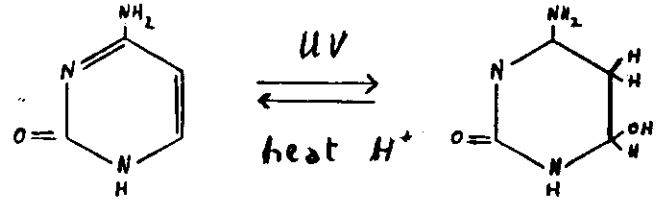
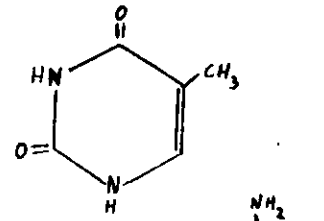
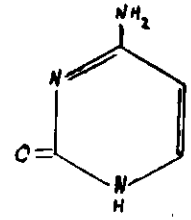


Fig. 2. Hydration of cytosine.

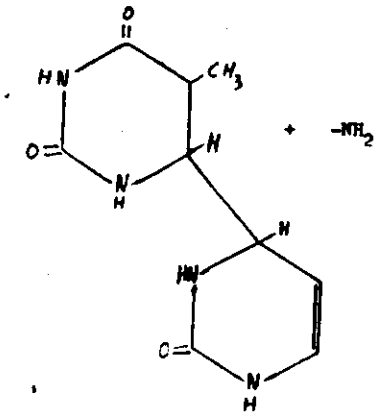
Less frequently, U.V. radiation produces a thymine-cytosine bridge. It may occur when the two bases are adjacent in DNA and a covalent linkage is formed between the C atoms in position 6 of thymine and the C atom in position 4 of cytosine. This involves the opening of the 5-6 bond in thymine and the loss of the $-NH_2$ group from the 4 position of cytosine.



Thymine



Cytosine



Thymine-cytosine bridge

4- The DNA damage by X-rays

The fast electrons released in manner by X- and -rays give rise to primary lesions in DNA by three distinct mechanisms. The high speed electrons may discharge their energy directly in the DNA itself and induce ionizations and excitations (direct action).

Alternatively, they may promote radiolysis of water in the vicinity of DNA with formation of the radicals $H^{\cdot}$, $OH^{\cdot}$, e^{-}_{aq}, of H_2O_2 and of the superoxide radical anion O_2^{-}. Then, the products of the water radiolysis will react with DNA in a more or less complex manner (indirect action).

Finally, organic radicals originated from the ionizations occuring nearby the chromosomes may attack the DNA and give rise to macromolecular damage (secondary attack).

In irradiated cells, radicals and the other reactive species diffuse only over a short distance. Therefore, the radiation processes damaging DNA are always localized to DNA and its microenvironment. For that reason, Block and Loman use the comprehensive term of "localized action" for defining the mechanism of DNA lesions by ionizing radiation under the "in vivo" conditions. The yield of various molecular damages may then be conveniently expressed as number of events per million dalton per megarad which corresponds to the number of molecular events per 104 eV of energy deposited in the sensitive DNA target. This parameter should be used in substitution of the G values which refer to the radiochemical yield and corresponds to the number of primary events per 100 eV of energy deposited in the molecules of given material.

Like for U.V. irradiation, the DNA damage by X- and -rays is mainly in pyrimidine nucleotides. Direct ionization in DNA seems to yield finally a relatively stable radical on thymine. On the other hand, $OH^{\cdot}$, and $H^{\cdot}$ radicals from water are easily added to the 5-6 double bond of the pyrimidine bases and many studies in this regard have been carried out with solutions of thymine and of its nucleoside and nucleotides derivatives.

It appears that the presence of O_2 leads to the formation of the various forms of hydroxy-peroxy-radicals of 5-6 dihydrothymine. These radicals will be reduced to the corresponding peroxydes or will decompose to a number of compounds (glycols, dihydrothymine, 5-hydroxymethyl uracil, 5-hydroxy-5 methyl barbituric acid and 5-hydroxy-5-methyl idantoin)

Molecular damage to the purine bases of DNA has been reported to occur in much lower yield than the pyrimidine lesions.

Primary injury of the deoxyribose residues of DNA will result in chain ruptures. Single strand breaks accumulate according to a linearly proportional relationship with the radiation dose. Double strand breaks are initially formed with a ratio of 1 to 20 with respect to the monohelical scissions and subsequently increase with the square of the dose. In fact, they may also be indirectly produced by two internucleotide scissions occurring nearby on the opposite helices of DNA.

The DNA breaks are not simple hydrolytic events but result from structural alteration of the nucleotide residue at the 3' site of the rupture. Many of them are concomitant with partial or total loss of a nucleotide residue. Therefore, not all the internucleotide scissions in irradiated DNA have the right 3'-OH//5'-PO_4 conformation for being proficiently sealed by DNA ligase.

Circustantial evidente suggests that, in DNA irradiated in vivo, a significant portion of the damage is due to the formation of DNA adducts involving a number of small biomolecules. These compounds are covalently linked to DNA after having been transformed by the ionizing rays in radicals or equally reactive chemical species. These DNA-adducts are proficiently recognized by specific enzymes devoted to repair the moelcular alterations of DNA.

Base and sugar damages often resulting in chain breaks are the most common primary lesions of DNA at moderate doses of ionizing rays. Exposure of cell to massive irradiation will cause other molecular types of DNA injury (for instance interchain and intrachain cross-links).

3

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