Probing biomolecules: Gas phase experiments and biological relevance

Sascha Gohlke and Eugen Illenberger
Institut für Chemie—Physikalische und Theoretische Chemie
Freie Universitär Berlin, Berlin, Germany

After the discovery of X-rays, radioactivity and ultimately nuclear fission—leading to the release of atomic bombs over Hiroshima and Nagasaki—it became clear that the exposure of living beings to high energy radiation (particles and photons) can result in fatal effects for the concerned individual. The result of such effects is subsumed under the all embracing term *radiation damage*. It includes damage of biological material on a short time scale, i.e. the immediate collapse of living cells eventually resulting in the death of the individual within hours or days but also describes effects appearing on much longer time scales since, instead of complete destruction of cells, radiation can also change the genetic expression of DNA. This may ultimately result in cancer and the appearance of tumours; but for prolonged periods after initial exposure the individual amy appear to have no obvious problems, however such dormant effects may appear as mutations in the individuals descendants (e.g the number of deformities in babies born in the Ukraine in the years after the Chernobyl accident).

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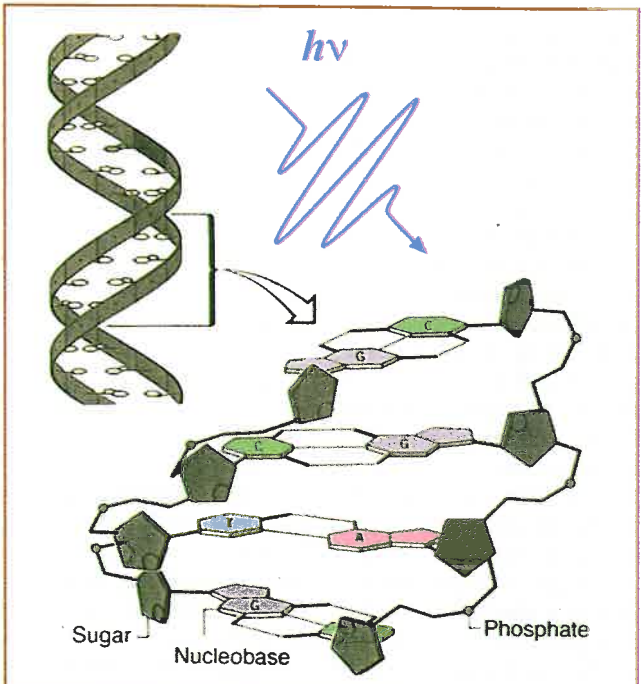
Radiation damage and radiosensitisers for tumour therapy

While high energy radiation can irreversibly damage biological material, radiation may also be successfully used in tumour therapy. The problem here is to only expose the cancerous material while keeping all other areas unirradiated. One method of treatment uses *radiosensitisers* with the effect that the sensitised cancerous cells will be destroyed with radiation dosages that leave the healthy material essentially unaffected. The necessary prerequisite for effective and controlled therapy strategies is the understanding of the molecular mechanisms of the underlying processes. In an effort to describe these effects on a molecular level, different laboratories have started programs to study the building blocks of biomolecules in the gas phase [1–4]. The advantage of gas phase studies is that experimental techniques like mass spectrometry or electron spectroscopy (eventually in combination with laser pump and probe techniques) can easily be applied. These techniques allow detailed information on the properties of molecules and the dynamics of reactions to be explored. The question then is to which degree these *intrinsic* properties



▲ **Fig. 1:** The DNA double helix and an extended view showing the base pairing through hydrogen bonding. The arrow represents a bunch of high energy photons in the MeV range interacting with DNA and its environment.

features

(as revealed by gas phase studies) can be transferred to their analogue in solution. This problem has been a longstanding issue in many areas of Physical Chemistry. One has to be aware that the solvent represents a dissipative environment and in the case of reactions where charged particles are involved, solvation can considerably modify the energy profile along the reaction coordinate.

The most important component of the cell nuclei is DNA in which genetic information is stored. DNA is a biopolymer consisting of two chains (strands) containing the 4 heterocyclic bases thymine (T), adenine (A), cytosine (C) and guanine (G), each of them bound to the DNA backbone which itself is composed of phosphate and sugar units. Both strands are connected through reciprocal hydrogen bonding between pairs of bases in opposite positions in the two strands. The geometry is such that adenine pairs with thymine (AT) and guanine with cytosine (GC) resulting in the well recognised double helix form. The DNA itself is surrounded by other biomolecules (proteins) and, of course, water.

Primary and secondary processes in living cells

To understand the effect of high energy radiation on DNA and its environment one may follow this interaction in terms of its chronological sequence. As an example consider a bunch of photons at energies in the MeV range interacting with DNA and its environment. The *primary* photon interaction (absorption, scattering) removes electrons from essentially any occupied state, from valence orbitals to core levels. Depending on the energy of these ionised electrons they induce further ionisation events thereby losing energy and being slowed down. The estimated quantity is 10^4 *secondary* electrons per 1 MeV primary quantum [5]. These electrons are usually assigned as *secondary* although they are the result from primary, secondary, tertiary etc. interactions, including electrons from Auger processes during relaxation of the core holes. Taking a snapshot a few femtoseconds after the primary interaction we will see multiple charged sites within the complex molecular network (eventually undergoing Coulomb explosion), single ionised and electronically excited sites and, last but not least, an exceedingly large number of low energy secondary electrons. Although the double and single ionised sites as well as electronic excitation can result in the rupture of chemical bonds, the major effects are induced by the large number of secondary electrons. In the course of successive inelastic collisions within the medium these are thermalised within picoseconds before they reach some stage of solvation.

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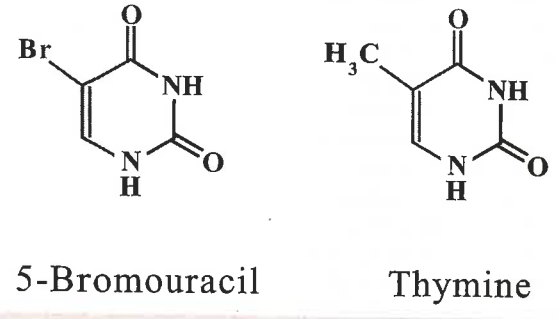
Damage of the genome in a living cell by ionising radiation is about one third a *direct* and two thirds an *indirect processes*. Direct damage concerns reactions directly in the DNA and its closely bound water molecules. Indirect damage results from energy deposition in water molecules and other biomolecules in the surrounding of the DNA. It is believed that almost all the indirect damage is due to the attack of the highly reactive hydroxyl radical OH^* on the DHA chain.



▲ **Fig. 2:** The nucleobases thymine (T) and bromouracil (BrU).

Electron initiated reactions in gas phase DNA bases

In order to reveal the mechanism of *direct* damage it appears straightforward to investigate the interaction of low energy electrons with single DNA bases representing the building blocks of the large polymer. Such experiments have been carried out in different laboratories [1–4] with crossed electron/molecular beam arrangements where a monochromatised electron beam interacts with an effusive molecular beam containing the DNA bases. The beam is generated by moderately heating the powder sample containing the DNA bases to 150 – 200°C and effusing the molecules through the collision region. The ions resulting from the electron – molecule collisions are extracted from the collision region and focused to a mass filter where they are mass analysed and detected. An alternative and partially complementary technique is to record the electron current transmitted through the gaseous target [2].

We shall consider here a prototype gas phase result to illustrate the effect on the DNA base thymine (T) and bromouracil (BrU). It has been known for many years that substitution of T by BrU in the genetic sequence of cellular DNA (Fig. 2) leads to a greater sensitivity to ionising radiation without changing the gene expression in unirradiated cells. Hence bromouracil potentially may be used as a tumour specific sensitiser in cancer therapy. On proceeding from higher energies to low energies the following features in electron impact to T and BrU become apparent:

A. The ionisation and excitation cross sections for both compounds behave in the manner usual for polyatomic molecules i. e. they rise gradually above the corresponding threshold with total values remaining below the geometrical cross section of the molecule. Ionisation and electronic excitation can also result in fragmentation but this area is still fairly unexplored.

B. However while T and BrU behave quite similarly at energies above electronic excitation there are remarkable differences in the subexcitation region. The common feature is that both molecules exhibit pronounced resonance features due to resonant electron attachment but such resonances have very different cross sections.

In thymine (T) the most abundant channel is identified as

$$e^- (\approx 2\ eV) + T \rightarrow T^{\#-} \rightarrow (T-H)^- + H^* \qquad (1)$$

with a resonance maximum near 2 eV and a threshold close to 0 eV [4]. $T^{\#-}$ represents the transient negative ion formed upon a Franck–Condon transition from the neutral molecule which decomposes into the closed shell fragment anion $(T-H)^-$ and a neutral hydrogen radical H^*. The absolute cross section for this dissociative electron attachment (DEA) cross section can be estimated as $\sigma_{DEA} \approx 2$ Å^2.

In contrast to that, the radiosensitiser bromouracil exhibits a very intense and narrow low energy resonances (Fig. 3) located close to 0 eV and associated with the processes

$$e^-(0\ eV) + BrU \rightarrow BrU^{\#-} \rightarrow (U\text{–}yl)^* + Br^- \quad (2a)$$

$$\rightarrow (U\text{–}yl)^- + Br^* \quad (2b)$$

which are complementary with respect to the negative charge. (U–yl) denotes the fragment formed by the loss of bromine. (2a) is the most abundant channel with an estimated cross section of 600 Å^2. Reaction (2b) is also open at zero eV (though at only 6% of the efficiency of (2a)). Due to the appreciable electron affinities of Br* and (U–yl)* exceeding 3 eV, reactions (2a) and (2b) have low energy thresholds. Figure 3 also shows that the undissociated anion weakly appears within the time scale of the experiment (ca. 50 μs).

It is interesting to note that both T and BrU are damaged at electron energies below 3 eV. The absolute cross sections, however, differ by more than two orders of magnitude. The conclusion then is that the initial mechanism for *direct* DNA damage is bond cleavage by low energy secondary electrons which is much more effective in the radiosensitisers.

Gas phase results and biological reality

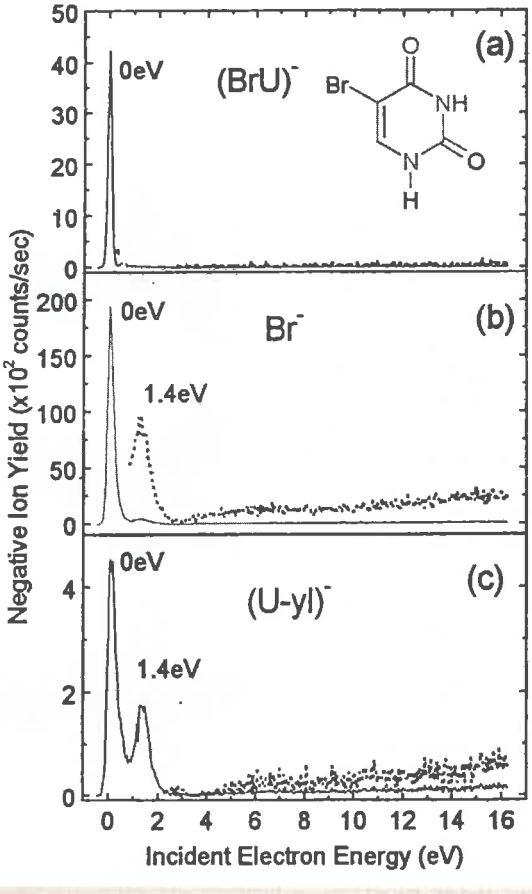
The general problem still remains on the question to which degree such gas phase results are relevant for a real (*in vivo*) biological system. DNA as a polymer is embedded in a medium while the present reactions are observed from isolated gas phase components. In the following we consider a few critical points that require further investigations:

1. Coupling of the nucleobases to the backbone and the opposite chain will certainly modify the energy of the involved molecular orbitals to some degree but can one assume the essential DEA features of the isolated bases will remain in the polymer?
2. In a condensed environment the reactivity (bond cleavage) is usually suppressed due to energy dissipation, but there are also situations where bond rupture via low energy attachment can be enhanced by the medium [6]. Irrespective of the phase condition, however, can one assume that BrU remains the more effective dissociative electron scavenger which respect to T which explains the mechanism by which BrU operates as radio sensitiser?
3. The reaction route from dissociative electron capture to single and double strand breaks is not directly obvious and has to be explored.

The importance of reactions of *presolvated* electrons with amino acids and nucleotides has already been pointed out more than 2 decades ago by time resolved pulse radiolysis experiments [7]. More recently, the ability of *free* ballistic electrons (3–20 eV) to efficiently induce single and double strand breaks in supercoiled DNA has clearly been shown [5]. In these studies it was demonstrated that the DNA strand breaks were initiated by the formation and decay of transient negative ion (TNI) states, localised on the various DNA components (phosphate, deoxyribose or hydration water). Unfortunately these experiments did not cover the energy region below 3 eV.

We finally mention that electrons in the solvated stage may not play any significant role. The binding energy of those electrons in water is above 3 eV and hence any dissociative electron transfer associated with reactions of the form (2a) and (2b) are associated with a large activation barrier and may not play any significant role.

To conclude it seems almost paradoxical that the damage of high energy radiation in the million eV range is actually the result of the interaction of secondary electrons at very low energies. Capture of electrons into antibonding molecular orbitals, however, is a very effective means to transfer energy of the light electron into motion of the heavy nuclei.



▲ **Fig. 3:** Negative ions observed in low energy electron impact to gas phase bromouracil.

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