Damage of DNA by Low Energy Electrons (< 3 eV)

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Abstract. Recent experiments on low energy electron attachment to DNA and its components in the condensed phase and in the gas phase are reviewed and analysed. From different condensed phase experiments the sensitivity of DNA towards low energy electrons is well documented and strand breaks in DNA are observed at subexcitation energies (< 3 eV) and also in ultrafast electron transfer experiments involving electrons in presolvated states. Gas phase experiments indicate that all building blocks of DNA (the nucleobases, the sugar and the phosphate moiety) undergo resonant dissociative electron attachment (DEA) in the subexcitation regime which may ultimately lead to strand breaks. From very recent gas phase experiments on an entire nucleotide it can be concluded that most strand breaks result from direct electron attachment to the DNA backbone, but also initial electron capture by the nucleobase following electron transfer to the backbone contributes.

1. Introduction

It has been widely accepted by now that low energy electrons (LEEs) play a crucial role in radiation damage to biological material [1-3]. This is based on the fact that in the course of the interaction of high energy radiation (particles and/or photons) with condensed matter (e.g. a living cell) in the first step electrons are removed from any atomic or molecular quantum level of the material accessible at these energies. These secondary electrons can initiate further ionisation or excitation processes thereby being slowed down. Within femtoseconds following the initial interaction of the material with the high energy quantum, the energy distribution of these ballistic electrons extends to a few tens of eV [4]. Further inelastic scattering events slow the electrons down until they are thermalized before they reach some state of solvation, chiefly becoming by then chemically inactive species.

Before considering in more detail the action of high energy radiation to a living cell we shall briefly recall some elementary facts concerning the structure of DNA which represents the most vital component of the cell nuclei. The DNA is a biopolymer (figure 1) consisting of two chains (strands) which contain the four heterocyclic bases thymine (T), adenine (A), cytosine (C) and guanine (G). Each of these four bases is bound to the DNA backbone which in turn consists of sugar and phosphate units. Both strands are coupled through hydrogen bonds between base pairs in opposite positions in the two strands (figure 1). The architecture is such that adenine pairs with thymine (A-T) and guanine with cytosine (G-C) are resulting in the well known right handed double helix form. The DNA itself is surrounded by proteins and embedded in water, which is by far the most abundant component in a cell.
High energy quanta interacting with a living cell can obviously interact directly with DNA or with the surrounding water or proteins, either separately or in combination. Water as the most abundant component generates the reactive OH radical which in turn can attack DNA and its surrounding biomolecules (e.g. proteins). Two types of damage may be induced. Direct damage refers to energy deposition and subsequent reactions directly in the DNA and indirect damage refers to energy deposition into the environment of DNA and hence chiefly energy deposition to water thereby creating the reactive OH radical. It has been assumed that by far most of the indirect damage results from the attack of the highly reactive hydroxyl radical to DNA. It has been assumed that the damage of the genome by high energy radiation is about one third direct and two third indirect [5]. However, this notion has recently been questioned in the course of ultrafast electron transfer experiments to DNA [6] proposing that two thirds of the damage are direct and one third is indirect (see also below).

Figure 1. Helical structure of DNA and molecular structures of the base pairs adenine (A) – thymine (T) and guanine (G)- cytosine (C) connected by hydrogen bonding (from reference [3]).

The particular role of ballistic LEEs became directly obvious by a series of seminal experiments performed by Sanche and coworkers [7] who irradiated plasmid DNA by a well-defined electron beam at variable energy in the range 3-20 eV. They observed the appearance of single strand breaks (SSBs) and double strand breaks (DSBs) also in the range below the ionisation threshold with the efficiency showing a resonant behaviour with respect to the electron energy. Moreover, in the energy range below the level of electronic excitation (< 3 eV) strand breaks were also detected within structured resonances in this case, however, only SSBs [8]. From the resonant structure of the damage profile it was proposed that resonant electron capture could occur at particular molecular components and sites of the DNA and thus may be the initial step towards the observed strand breaks. It is well known that at low energies, preferentially below the level of electronic excitation, electrons can be captured by molecules thereby creating transient negative ions (TNIs) which could subsequently decompose into several fragments (dissociative electron attachment, DEA) [9]. This means that a chemical bond can be cleaved by electrons at energies well below the corresponding bond dissociation energy and sometimes already at threshold (0 eV). Energetically, such a low energy bond-breaking process becomes accessible provided that the electron binding energy on the negatively charged fragment is sufficiently large to locally increase the available energy. By that mechanism, therefore, the secondary ballistic electrons, on their way to be slowed down (but before getting solvated) can be captured by
specific molecular sites within DNA and thereby trigger the chemical reactions that can eventually lead to cell apoptosis.

The sequence of reactions occurring along the ionisation track in the molecular network of a living cell may be divided into three major stages: (1) the physical stage which refers to processes and reactions in the time window from $10^{-15}$ s (fs) to $10^{-12}$ s (ps) after the primary interaction. This time window includes electronic excitation and ionisation with subsequent bond ruptures along repulsive potential energy surfaces, the formation of OH radicals and formation of abundant numbers of secondary electrons, (2) the chemical stage, which includes molecular relaxation and reorganisation including multiple bond cleavages and the subsequent formation of new molecules. Such reactions typically occur in the time window between picoseconds ($10^{-12}$ s) to microseconds ($10^{-6}$ s) (metastable decays), and (3) the biological stage, which finally refers to the alterations occurring on a longer time scale, including the overall response of the system which could extend over a time scale of several years and more.

Among this sequence of processes and reactions the fast and ultrafast initial events (driven to a large extent by LEEs) are considered as important and decisive steps for the evolution of the biomolecular system and hence for the chemical and biological effects occurring on a much longer time scale. In the following sections, we shall first describe a few experiments performed on DNA and its gas components in the condensed phase, and then, describe electron driven reactions on gas phase building blocks. From the latter experiments, the intrinsic properties of the DNA components with respect to LEE interaction can be studied. Together with larger units and the results from the condensed phase, a fairly detailed description of the molecular processes, in particular how low energy electrons damage DNA, can be obtained.

2. Results and discussion

2.1. Electron induced damage to DNA in the condensed phase

The landmark experiments by Sanche and coworkers demonstrated that LEEs can induce strand breaks (DSBs and SSBs) below the level of ionisation and also at subexcitation energies, albeit in the latter case only SSBs. While the resonant profile of the damage efficiency suggested resonant capture of electrons as the initial step, the detailed mechanism leading to strand breaks was not obvious. For the molecular mechanism inducing SSBs it was theoretically predicted [10,11] that LEEs are captured by the DNA bases and transferred to the backbone thereby rupturing the connection between the phosphate and the sugar unit representing a strand break (figure 1). Different experiments on model DNA in fact revealed the particular sensitivity of the biopolymer towards LEEs.

Particularly interesting results with respect to radiation damage have been obtained by low energy photoelectron transmission spectroscopy (LEPET) applied to self-organized layers of short single and double stranded oligonucleotides of known size (15 units) and sequence [12]. In LEPET electrons are detected, which are photo-ejected from the metallic substrate, transmitted through the film and are finally escaping into vacuum. It was found that the oligonucleotides readily capture electrons within the energy spectrum covered by the photo-injection technique which is in the subexcitation range, and that the transmitted current significantly depends on the presence of guanine (G), i.e., the number of G bases controls the capturing efficiency. These findings support the picture that the DNA bases act as antennae for the capturing of subexcitation electrons and that SSBs are induced via electron transfer to the backbone.

A high throughput method to study electron-induced damage in model DNA was established by immobilising single stranded short oligonucleotides on a gold surface in a microarray format and exposing them to electrons in the subexcitation energy range (1-3 eV) [13]. It was found that damage to single stranded oligomers (25 units) is detected at quite a low electron dosage of about 300 electrons per oligonucleotide [14] and that damage correlates linearly with the number of guanines [15] which in turn correlates with the results from LEPET mentioned above.
Recent experiments on plasmid DNA in water using femtosecond (fs) laser techniques revealed the particular role of presolvated electrons in radiation damage [6]. Water is ionised and/or excited by the absorption of two UV photons creating OH radicals and electrons. Within a time window of about 100 fs the electrons reach a kind of presolvated state. It was claimed that SSBs and DSBs induced by electron transfer from this presolvated stage (reductive damage) is twice as efficient as that of strand breaks induced by the OH radicals (oxidative damage [16]). The important role of presolvated electrons in radiation damage has also been pointed out before [17,18].

More direct information on the molecular mechanisms following electron attachment to DNA and its components can be obtained from gas phase experiments as described below.

2.2. Electron induced damage to gas phase building blocks of DNA

2.2.1. DNA bases. The first gas phase experiments on DNA bases were carried out in 1998 in the Berlin laboratory by means of a crossed electron-molecular beam experiment [19]. Negative ions created by the interaction of the electron beam with the effusive molecular beam (resulting from thermal evaporation) were extracted from the reaction volume and analysed by means of a quadrupole mass spectrometer.

Figure 2. Low energy DEA in the 4 DNA bases leading to the loss of a neutral hydrogen atom. (T-H) assigns the closed shell anion of thymine, formed by hydrogen loss from the transient anion (from reference [21]).

These earlier gas phase results clearly revealed the presence of low energy resonances in the DNA Bases T, C, G, A and U, namely in the energy range around 6-12 eV and in the energy range around 1 eV. While the negative ion fragments from T originating from the higher energy resonances indicated DEA reactions involving the opening of the cyclic structure, the low energy resonance yielded an ion at a mass close to that of the corresponding parent for each nucleobase (NB). In fact, it became soon obvious in experiments by the Innsbruck and Berlin laboratories [20,21] (see figure 2) that all NBs exhibit a low energy resonance associated with the loss of a neutral hydrogen atom according to the dissociative electron attachment (DEA) process.
$e^- + NB \rightarrow (NB-H)^- + H$  \hspace{1cm} (1)

where $(NB-H)^-$ describes the closed shell anion formed by ejection of a neutral hydrogen radical from the target molecule. The reaction is already operative at sub-excitation energies and is energetically driven by the appreciable electron affinity of the $(NB-H)$ radicals which in the case of T lies in the range between 3 and 4 eV.

More detailed studies in T revealed an unexpected bond and site selectivity for the hydrogen loss process in that only the N-H bonds are involved [21], and most remarkably, that the sharp peak in the $(N-H)^+$ ion yield located at 1 eV (figure 2) exclusively results in hydrogen loss from the N1 position, and the broader resonance peaking at 1.8 eV preferentially leads to hydrogen loss from the N3 position [22]. For T coupled in the DNA network the N1 position is involved in the coupling to the sugar unit and the N3 position in hydrogen bonding to the complementary base A (see figure 1).

The general situation in the isolated DNA bases is then the presence of low energy resonances at subexcitation energies exclusively associated with the loss of a neutral hydrogen atom, and more or less structured resonance features in the energy range between 6 and 12 eV, which are associated with complex DEA reactions leading to the deterioration of the cyclic structure [19,20,23] (not shown here). To which degree these resonances are involved in SSBs or DSBs depends on how these properties propagate when the bases are covalently coupled within the DNA network. While the molecular mechanisms leading to DSBs are presumably very complex and not obvious so far, a clearer picture on how subexcitation electrons induce single strand breaks (SSBs) is emerging from the gas phase studies. As mentioned above, quantum chemistry studies [10,11] predict capture of a subexcitation electron by the DNA bases and transfer to the backbone, ultimately leading to the rupture of the C-O bond between the phosphate and the sugar moiety. The question then arises on the sensitivity of the sugar and the phosphate moiety (representing the DNA backbone) towards low energy electrons and hence their possible contributions to direct SSBs.

2.2.2. Components of the DNA backbone. The DNA backbone is known to consist of alternating phosphate units and the sugar 2-deoxy-D-ribose, with the nucleobases connected via the glycosidic C-N bond (see figure 3, top). Obviously the cleavage of any of the P-O-C bonds (connecting the phosphate with the sugar moiety) but also cleavage of the C4-C5 bond within the sugar unit would represent a strand break. In addition, a decomposition of the sugar unit within DNA would result in both strand breaks and nucleobase release. The sensitivity of the components of the backbone towards low energy electrons is hence of particular interest as decomposition reactions on this site of the DNA could act as one of the most direct mechanisms for single strand breaks.

Electron attachment experiments to different six- and five-membered isolated sugar molecules showed a surprisingly high sensitivity of these compounds towards LEEs already at energies close to zero eV. Electron capture is associated with remarkably complex reactions leading to the loss of one to several H$_2$O and H$_2$CO molecules [24-27].

A more realistic view on the behaviour of the sugar and phosphate moiety in DNA may be achieved by using tetraacetylribose (TAR) and the phosphoric acid esters dibutylylphosphate (DBP) and triethylphosphate (TRP) as the corresponding surrogate (figure 3 bottom).

TAR possesses a five-membered ring structure and no free hydroxyl group, and the acetyl groups represent polar moieties with an electron affinity similar to the phosphate group and the nucleobases. The electron attachment and fragmentation reactions in TAR can specifically be assigned to resonances which are located at particular sites within the molecule [28]. A group of heavier fragment anions is exclusively generated through resonances peaking at 1.6 – 1.8 eV (not shown here), but also a resonance close to zero eV yielding a variety of fragments, some of the ion yields are shown in figure 4. These fragment ion yield curves mirror the decomposition of the ring and are also reminiscent of the yield curves observed for bare sugar molecules.

The first gas phase results on DEA processes involving phosphoric acid derivatives were obtained on dibutylphosphate (DBP) and triethylphosphate (TEP) [29]. Both compounds are subject to DEA
processes occurring at different energies, ranging from close to 0 eV up to 10 eV. The most important reaction in the context of DNA damage is the loss of a butyl group via C-O bond cleavage. This DEA reaction takes place within two broad resonances located at 2-4 eV and 7-10 eV (figure 5 left).

![Figure 3](image1.png)

**Figure 3.** Molecular structure of the DNA backbone consisting of the sugar and the phosphate units (top), and molecular structures of dibutylphosphate (DBP), triethylphosphate (TEP) and tetraacetyl-D-ribose (TAR), which serve as surrogates for the phosphate or the sugar unit in DNA, respectively.

Figure 4. Fragment ions at 70 amu, 84 amu and 100 amu arising from DEA to tetraacetyl-D-ribose (TAR). These ions are produced close to 0 eV and within a weaker resonance located at 7 – 9 eV and are hence reminiscent to the ones obtained from DEA to bare D-ribose (adapted from reference [28]).
A similar reaction was observed in TEP (figure 5 right), i.e., the loss of an ethoxy radical due to P-O bond cleavage. It should be emphasised that for the phosphate group in DNA both reactions would correspond to a direct single strand break.

Another remarkable set of reactions takes place in both DBP and TEP close to zero eV and is associated with the excision of the negatively charged central phosphate unit (not shown here), in TEP only PO$_3^-$ was observed at zero eV, driven by the unusually large electron affinity of PO$_3$ (4.95 eV). Obviously, the excision of these central units requires multiple bond cleavages which, in DNA, would certainly result in a strand break.

2.2.3. Larger DNA building blocks. Experiments on larger building blocks in the gas phase, namely thymidine (thymine coupled to sugar) [30] and D-ribose-5-phosphate [31] showed that the low energy resonances present in the separated molecules are still present in the covalently coupled compounds undergoing effective DEA reactions. From a very recent experiment on an entire nucleotide (2’-deoxycytidine 5’-monophosphate, dCMP) [32] it can be deduced that SSBs driven by subexcitation electrons predominantly occur by direct electron localisation at the backbone, but also electron transfer reactions involving initial electron localisation at the nucleobase are contributing. This can be concluded from the nature of the final ionic products and their resonance profiles which in turn carry the signature of the initial attachment process.
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