From DNA to nucleic bases – the effects of low-energy electron impact

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Abstract. Extensive results from measurements on short DNA strands impinged on by 1-30 eV electrons indicate that the damage they induce is due to the chemical nature of the nucleic bases and/or their sequence. The strong variation of effective cross sections for plasmid DNA single-strand breaks with incident electron energy and the resonant enhancement at 1 eV suggest that considerable damage is inflicted by very low-energy electrons to DNA, and it indicates the important role of π* shape resonances formed on different constituents of DNA. Recent results of vibrational and electronic excitation of thin condensed films of adenine and thymidine by electrons of energy from 1 to 12 eV are presented.

1. Introduction

Since the first experiments of low-energy electron scattering from condensed DNA [1] have been performed, the interest in studying low-energy electron-biomolecule interactions has been increasing considerably. It is not surprising that most studies following these first experiments are focused on gas-phase studies, as electron interactions with isolated molecules are easier to interpret and model theoretically than in condensed phase, especially if considering bulk samples. Methods devised to analyze the damage that low-energy electrons inflict on thin films of condensed DNA and its constituents provided interesting results. Knowledge of effective cross sections for single- and double-strand breaks of DNA and for vibrational and electronic excitation of nucleic bases and nucleosides are opening the door to better understanding of effects of radiation on live tissue and, furthermore, possibly indicating interaction pathways leading to mutations and cancer.

Extensive results from measurements on short DNA strands impinged on by 1-30 eV electrons indicate that the damage they induce is due to the chemical nature of the nucleic bases and/or their sequence. The strong variation of effective cross sections for DNA single-strand breaks with incident electron energy and the resonant enhancement at 1 eV suggest that considerable damage is inflicted by very low-energy electrons to DNA, and indicates the important role of π* shape resonances in the bond-breaking process. The complexity of DNA, even if studied as a short single-strand chain, imposes a need to perform measurements on its isolated constituents, such as nucleic bases and nucleosides, applying different experimental methods.

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Investigation of the vibrational and electronic excitation of nucleic bases and nucleosides by low-energy electron impact leading to dissociation through formation of temporary negative ions aims at understanding the correlation of nucleic base-sugar moiety conformational coupling and its consequences on the bond cleavage in DNA. The conformation of the 2'-deoxyribose moiety with respect to the base is expected to influence which species are formed upon exposure of nucleosides to ionizing radiation.

In this Progress Report, recent results from low-energy electron scattering from thin molecular films of adenine [2] and thymidine will be described and discussed in a view of our earlier study [3] of electron scattering from DNA.

2. Experiment

In our earlier experiments [3] the effective cross section, $\sigma(E)$, for SSB (single-strand break) in plasmid DNA by low-energy electrons was deduced from the loss of supercoiled DNA and the relative intensity of the fluorescence line from dye-marked DNA molecules inserted in the agarose gel. By recording the damage for each of the incident electron energies for the same period of exposure (10 s), and knowing how many electrons are arriving at the target, we obtained the effective cross section for the formation of circular form DNA from supercoiled plasmids. These measurements were performed under ultra-high vacuum conditions at room temperature and great care was taken to insure the purity of the sample and protection from other sources of damage. DNA molecules in our samples contained only structural water – on average 2.5 water molecules per base pair. The energy distribution of the electron beam had a full width at half maximum (FWHM) of 0.5 eV and the electron current was 2 nA. Procedures for preparation and analysis of the irradiated samples were delicate and time consuming and the information obtained from these measurements was limited to the global picture of the DNA damage. It did not provide us with the details of the mechanisms leading to SSBs.

To further investigate effects of low-energy electron interaction with DNA it was necessary to perform more sophisticated experiments on thin films of isolated DNA bases and nucleosides.

2.1. High Resolution Electron Energy-Loss measurements of condensed adenine and thymidine

Our measurements on thin films of nucleic bases and nucleosides were performed using a HREEL (High Resolution Electron Energy Loss) spectrometer [4, 5] placed in a cryogenically pumped UHV chamber (base pressure of $10^{-11}$ Torr).

The incident energy, $E_i$, of electrons in a beam produced by a electron monochromator containing a hemispherical energy selector was calibrated by measuring the onset of the electron current transmitted through the sample, within ±0.1 eV with respect to the vacuum level. The combined energy resolution of the spectrometer (monochromator and analyzer) ranged from 15 to 20 meV FWHM, for an incident electron current of 0.77 nA in the case of adenine and 1.3 nA for thymidine. In order to provide clean vacuum conditions for sublimation of sample molecules from the oven onto the tip of the sample carrier (ceramic finger), and from the carrier onto the substrate in the main UHV chamber, the oven is placed in the load-lock UHV chamber connected to the main chamber by the gate valve. The residual background gas and possible undesirable decomposition of the sample is monitored in the load-lock chamber with a quadrupole mass spectrometer. The substrate for sample deposition (in the main vacuum chamber) is made of polycrystalline platinum foil, which is cryogenically cooled to a temperature of 18 K. The deposition procedure for argon, adenine and thymidine was the same as in the case of thymine [4, 6] and pyrimidine [5] except for the sublimation time and temperature. Argon, with a stated purity of 99.9995% (Matheson Canada Ltd.), is condensed on a previously cleaned and cooled Pt substrate by introducing the amount sufficient to make 5-6 layers. The thickness of the film was estimated from the pressure drop in the calibrated volume. The crucible in the oven is filled with adenine or thymidine powder with the stated purity of 99.9% (Sigma Aldrich Canada) and heated to a temperature of approximately 50 - 60°C for several hours to eliminate water and other impurities from the sample powder. This temperature is well below the reported [7] temperatures for decomposition (250°C for adenine and 185°C for thymidine) of either of the two
molecules. Typical time for deposition of either of the two molecules onto the ceramic finger was 10 min. In order to transfer deposited molecules onto the substrate, the ceramic finger was introduced into the main vacuum chamber to approach the substrate at distance of 5 mm and heated to ~150 °C for 70 s for a sub-monolayer and 90 s for a monolayer of adenine or to ~100-110 °C for 2 min for a monolayer of thymidine. In most measurements, the thickness of adenine film was between 0.8 and 1.2 and for thymidine film around a monolayer. The actual number of adenine molecules in the film is estimated based on the results of several studies on orientation and molecule-surface interaction [8, 9]. Each of adenine molecules is fixed in a network of four hydrogen bonds of 2.94 ± 0.02 Å. In a LEED study [9], it was found that the same unit-cell vectors existed for different experiments under various conditions, i.e. vacuum sublimated films as well as adsorbates dried from the solution. There is no specific experimental evidence of a true position of adenine molecules deposited on solid argon. We assume the same cell structure, and even a weaker interaction with the substrate due to the chemical inertness of argon and a very low temperature of the substrate. Argon also proved to be a convenient substrate for the observed energy range from 1.5 to 12 eV because of a large energy gap between the elastic and the first electronically excited state (~12 eV).

However, in the case of thymidine, the situation is more uncertain and complicated as the orientation of these molecules is difficult to control. It highly depends on the respective orientation of the base and sugar moiety (α- or β- orientation). We assume that most thymidine molecules on the argon surface have their thymine base laying flat and the deoxyribose sugar ring oriented off-surface and that, in a monolayer, the structure of the thymidine network should be simple enough to allow us to employ the same method of analysis as in the case of adenine.

A well-collimated and focused incident electron beam of energy ranging from 1 to 12 eV was aimed at the target at an angle of 15° with respect to the direction perpendicular to the film surface. Energy-loss spectra were recorded by measuring the number of electrons backscattered from the film, at the fixed analyzer angle of 45°. Tuning of the entrance electron lens in the analyzer for each incident electron energy provided detection of all electrons scattered from the film at this angle. Exposure times for collecting individual EEL spectra were sufficiently small to exclude significant contribution to the signal from damaged molecules. All spectra which exhibited charging or damage effects were discarded.

2.2. Method of analysis.

As described in a previous study [4], both the characterization of the target sample and recording energy-loss (EL) spectra rely on the measurement of the transmitted and the backscattered electron current, \(I_s\) and \(J(\theta_d)\) respectively. From extrapolation of the linear relation between \(J(\theta_d)\) and \(I_s\) we can find values of the effective incident current \(I_{o,d}\)' and the incident current on the target \(I_o\), and then normalize the EL spectrum to obtain the values of the absolute reflectivity. Since the transmitted current, \(I_s\), changes with the incidence angle within only 10%, we consider the ratio between \(J(\theta_d) / I_{o,d}\) approximately constant over the angles of analysis. Consequently, this ratio is analogous to dividing the integral of \(J(E, \theta_d)\) over the half angular space by the \(I_o\), i.e. it is equal to the \(\frac{J(E)}{I_o}\).

Multiple-scattering theory [5] developed for the interaction of low-energy electrons with molecules in a condensed film can also be applied in the case of electron scattering on very thin films. The relation between the above ratio, calculated from the experimental data, and the scattering probability per unit length per unit solid angle and per unit energy range (SPUL) for an electron of energy \(E_o\) to lose \(E-E_o\) and be deflected from an incident direction \(\theta_i\) to a back-scattered direction \(\theta_d\), \(Q(E_o, E-E_o, \theta_d, \theta_i)\), is:

\[
\frac{J(E)}{I_o} = Q(E)L = \sigma(E)nL,
\]

where \(L\) is the thickness of the film, \(n\) is the molecular density of the target film and \(\sigma(E)\) is the absolute integral cross section. Therefore, to determine \(\sigma(E)\), we need to know the molecular
coverage of the target area, \( nL \). The above formula is valid for near-normal incidence on an azimuthally disordered molecular coverage, when we assume that the electrons scatter from the film into a near cylindrically symmetric space.

To estimate the thickness of thin condensed films, on the other hand, we used the fact that the specular reflectivity of the surface is very directional and very sensitive to surface conditions. Therefore, we relied on the attenuation of its value for the argon film after it has been covered by deposited molecules. As described in the previous work on thymine \([4]\), this attenuation is an exponential function of the surface density of the molecular film:

\[
I(0) = I_o \Re e^{-\langle n \rangle},
\]

where \( R \) is the specular reflectivity and \( \langle n \rangle = \frac{N}{M} \) is the average number of molecules per adsorption site. For the same incident current and the reflectivity inherent to a certain type of molecules for different coverage, we find that the function \( \ln \left( \frac{I}{I_o} \right) \) vs. \( \frac{I}{I_o} \) gives us the number of monolayers in the film.

3. Results

Effective cross sections for SSB damage of the plasmid DNA, induced by electrons of energy from 0.1 to 4.7 eV and at 10 eV, are shown in Figure 1 \([3]\). They are all higher than the values quoted in \([1]\) at 10 eV! The most striking features of the results in Figure 1 are: a) the electron energy threshold for formation of SSBs is practically at zero energy, and b) throughout the 0.1 to 4.7 eV energy range, the magnitude of the cross section is of the same order as at 10 eV. Considering previous measurements of the yield function for SSB between 5 and 100 eV \([10]\), this result implies that, over the energy from 0.1 to 13 eV range, electrons are capable of damaging plasmid DNA at a high rate with a magnitude similar to that for electrons of the energy between 30 and 100 eV.

Mechanisms that can lead to specific features, such as the strong peak at 1 eV in Figure 1, are not easy to clearly identify, but there are several factors that can be identified as playing a more or less important role in the SSB damage of DNA. At energies below 5 eV, we exclude the possibility of secondary electrons coming from ionization of the sample by the primary electron beam because the ionization of any of DNA constituents requires at least 5.7 eV (for the fully solvated GC pair and more for the unsolvated) up to 11eV \([11]\) (sugar-phosphate backbone fragments), and the formation of OH radicals from water molecules requires higher energy for direct dissociation; no dissociative electron attachment (DEA) signal arising from structural water has been observed from vacuum-dried DNA films \([11]\) either.

It has been shown \([12, 13]\), that the DNA backbone lesions caused by sub-excitation energy electrons are essentially a result of breaking of the C-O bond between the sugar and the phosphate moiety. In a study of low-energy electron impact on CGTA and GCAT tetramers \([14]\) HPLC analysis of fragments showed that the phosphodiester bond cleavage varied from site to site in a tetramer. It depended on the type of nucleobase and their sequence. According to numerous studies \([14\) and ref.s. therein], at energies below 3 eV, low-energy electron can attach to a \( \pi^* \) orbital of the phosphate group forming a TNI or, the resonance capture of the lowest \( \pi^* \) orbital of the bases followed by the electron transport to the \( \pi^* \) of the phosphate. Both mechanisms would lead to the transition state formed with
an extra electron in the usually unfilled P=O $\pi^*$ orbital that can further lead, via curve-crossing, to a $\sigma^*$ anion state, and to cleavage of the phosphodiester bond.

Since electronic excitation of the nucleic bases is not possible at such low energy (the lowest-lying triplet electronic state in thymine is around 3.7-4 eV), the transient anion is produced via a shape resonance, unless vibrational Feshbach resonances are involved. On the other hand, experiments on H ion desorption from gaseous DNA bases [7] and thymidine [15] show that the specific position from which the hydrogen atom can be extracted is the nitrogen (N1 or N3) site, which is participating in the base-pair bonding in the DNA. The $\sigma^*$ resonance exists in the N1-C character of the bond (antibonding character) and the cleavage of this bond can take place at 0.1 eV incident electron energy.

This interpretation is supported by numerous experimental studies of interactions between electrons and isolated DNA constituents, mostly in the gas phase [7, 16-18]. The most important result of these studies is the recognition of a low energy threshold for the electron attachment to the isolated DNA bases [16]. It turned out that the electrons of energy between 0.4 and 0.6 eV will attach efficiently to either purine or pyrimidine bases and form transient negative ions. There are also indications [19] of possible mixing of dipole-bound anion states of the bases and the anion states associated with the lowest $\sigma^*$ valence orbitals, resulting in the prominent vibrational Feshbach resonances, with the vibrionic coupling between the valence $\pi^*$ and the repulsive $\sigma^*$ anion states giving rise to DEA peaks at energies near those of the $\pi^*$.

3.1 Adenine
Adenine is a purine base (Figure 2) making a fundamental part of the DNA and the ATP (adenosine tri-phosphate) molecule, which is responsible for energy supply in the living cell. We have recorded and analyzed vibrational and electronic excitation from thirteen energy-loss spectra, at incident energy ranging from 1.5 to 12 eV. In order to calculate the integral cross sections for different vibrational modes, we integrated the Gaussians of deconvoluted peaks from the EEL spectra, corresponding to the most intensive vibrations up to the energy-loss of 0.5 eV. For electronic excited states, we applied the same method for the energy-loss ranging from 4.2 to 7.2 eV, at the incident energies from 8 to 12 eV.

The cross section (Figure 3) for asymmetric stretching of the amino group hydrogen atoms shows an enhancement via a possible resonance at 3 eV, which also appears as a weak structure in the other vibration cross sections. Around 5 eV, all vibrational modes seem to exhibit an enhancement in the cross section. This is not surprising since the coupling of different vibrations in adenine is very likely. Furthermore, there is much evidence for the internal conversion from the $\pi\pi^*$ to the ground state, which could result in vibrational excitation. The energy of this state is very close to the energy of the $\pi\pi^*$ state, at 5 eV. From the electron-transmission spectroscopy (ETS) measurements and calculations of Aflatooni et al [12], we would expect to see in our integral cross sections a shape resonance structure at the vertical attachment energy of 2.17 eV (at approximately the same position as in the (A-H) anion yield of Gohlke et al [22]) and a core excited resonance at 6.28 eV, both leading to DEA and the (A-H) anion yield. In thymine and uracil, for example, H-atoms are released from the N3 site via the coupling between the temporary anion states associated with the $\pi^*$ and $\sigma^*$ valence orbitals owing to out-of–plane vibrations. This mechanism produces anion yields near the energies of the $\pi^*$ temporary anion states. From the N1 site, on the other hand, hydrogen atoms are ejected via sharp Feshbach resonances created by mixing of the dipole bound negative ion states with the lowest unoccupied valence $\sigma^*$ molecular orbital. Considering that the polarization in the substrate is expected to shift the shape resonances positions downward for about 0.8 eV [5] with respect to those in the gas-phase, we would expect to see the resonances observed by Aflatooni et al [23] at 1.4 eV and 5.5 eV. Thus, the broad peak in Figure 4, around 5 eV could be associated with the

![Figure 2: Adenine](image-url)
gas-phase 6 eV core-excited resonance. On the other hand, in the present measurements, energy-loss spectra were taken in the 1 eV steps, implying that the measured position of the resonance at 3 eV could be, at an energy shifted for ± 0.5 eV. Therefore, comparison of the resonance positions, as seen in the gas-phase and condensed phase results, is qualitative and more of an indication than a definite interpretation of the origin of these structures.

The magnitudes of the electron excited states cross sections for the electronic excited states are in the range from 10^{-18} to 10^{-17} cm², an order of magnitude smaller or the same as in thymine. The largest cross section occurs at the EL of 6.1 eV. Recent results of negative ion formation in adenine in the gas phase [24, 25] confirmed the existence of increased probability for H⁻ [24] and CN⁻ and (A-HCN)⁻ ion production at 6 eV (with an energy resolution of 1 eV). The suggested interpretation was that it appeared as a consequence of the electron capture by the positive electron affinity of the π*ₚ, π*ₚ and π*ₜ states. According to a theoretical study [26] of the adenine dimer σ* orbitals are located at the peripheral N-H groups of the two adenine constituents, while no such anti-bonding orbitals exist between the individual parts along the H-bond. Detailed orbital assignments for the core-excited resonances are yet to be determined.

Triplet states have been investigated very little [27, 28]. In the previous HREEL study [29] of thymine, triplet states have been clearly observed at the EL of 3.7 eV (ππ*) and 4 eV (nπ*), but in adenine (according to the DFT calculation of Marian [28], the lowest lying excited states of adenine are triplet states at 3.63 and 4.4 eV) the positions of triplet states could not be accurately identified.

3.2 Thymidine
Thymidine is one of the most important nucleosides of DNA and an important component of antiviral compounds [31]. In the condensed phase, thymidine’s 2’-deoxyribose ring is in the pentose sugar ring form, which is a true conformation of this nucleoside in DNA (Figure 4). From Raman, IR and UV spectroscopic measurements, [32] it is known that thymidine vibrations are dominated by thymine modes. However, virtually all normal modes of thymidine involve some degree of vibrational coupling between the thymine base and the deoxyribose! Most deformation from the sugar moiety is attributed to a CH2 group, while the OH group vibration is represented at the highest energy-loss. Electron energy-loss spectrum in Figure 5 shows particularly strong peaks around 111 meV (with a large contribution to thymine ring deformations from C2’H2 rocking and sugar skeletal modes) and 220 meV, which is predominantly thymine-localized.

![Figure 3](image1.png)  
**Figure 3:** Effective cross sections for amino-group vibrations in adenine [2]

![Figure 4](image2.png)  
**Figure 4:** Thymidine
The gas-phase experiments on sugar analogs of deoxiribose [33] showed the existence of a resonance at the energy below 1 eV as a result of the dissociative electron attachment. Based on this fact and the resonances in DNA, we turned our attention to possible resonances in thymidine cross sections (Figure 6) at incident energy below 5 eV. There is an evident enhancement of the effective cross sections of certain vibrational modes in thymidine for incident electron energies of 1.5 and 2 eV as a possible contribution from deoxyribose.

In comparison with previous measurements on thymine [4], large cross sections for the vibrational excitation of the “breathing” mode and “ring deformations” are almost an order of magnitude smaller in thymidine. Possible dissociation of thymidine through transient electron attachment will probably proceed through hydrogen detachment from thymine and dissociation of OH from deoxyribose.

4. Conclusion

All deformations of the amino group are of particular importance for understanding the properties of complexes where adenine binds by means of the hydrogen bond. Vibrations of the azine group (NH), in particular, on the N9 site, are also important due to its role in binding to the sugar moiety in DNA and RNA. In thymidine, the N1 and N3 sites on thymine are sensitive to low-energy electron impact and this has consequences both on its base pairing with adenine in DNA and its bonding with the sugar moiety. There is no evidence that the C5’ of deoxyribose is a particularly sensitive site in thymidine. If an electron of less than 5 eV is temporarily attached to the deoxyribose in thymidine, it has a considerable potential to cause complex deformations of the entire molecule or/and H and OH release from the sugar moiety.

It is extremely difficult to disentangle all the channels and identify a specific vibrational motion or an electronic state leading to a specific bond breaking. It seems plausible that the bond breaking, as a result of formation of transient negative ions, is often a result of simultaneous action on different parts of the molecule. Improved experimental methods and theoretical models will be necessary to apply in order to fully explain the mechanism of strand-breaks in DNA.
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