

Desorption of small ionic fragments from oligonucleotides induced by low energy carbon ions

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Abstract. The degradation of oligonucleotide films containing differing base sequences induced by 4 keV C⁺ ions has been studied experimentally. The oligonucleotides were deposited onto a gold coated stainless steel substrate and the anions and cations released from the films were analyzed by a quadrupole mass spectrometer. The total ion desorption yield was recorded as a function of time using a constant C⁺ ion flux of 6×10^{14} ions cm⁻² s⁻¹. At low fluences the formation of small ionic fragments was observed, whilst for fluences greater than 1.2×10^{18} ions cm⁻² molecules were sputtered from the substrate. In addition to studies of the influence of a particular base to the total cation desorption yield, the effect of base substitution with bromine was measured for negative ion desorption. These results showed a strong degradation of oligonucleotide films during ion bombardment.

1 Introduction

Since the discovery that genetic modification can be induced within biological cells by low energy ion (LEI) implantation [1], the field of ion beam bioengineering has grown rapidly [2]. The transfer of genetic information carriers from outside a cell into it (gene transfer) is also governed by ions and has been investigated in living cells of both plants and bacteria [2,3]. In addition to mutation and gene transfer, LEIs can also induce direct DNA damage [4]. Experimental studies on the damage to DNA molecules both in vitro and in vivo induced by ion beams with energies below 200 keV, have been summarized in a recent review [4]. In spite of the short penetration depth of LEI's in DNA [5], the effectiveness of such ions in causing irreparable damage to the DNA has led to the development of ion beam therapy as a tool for cancer treatment. Indeed the clinical success of hadron therapy, where proton and ions with energies of below 150 MeV are used, has led to LEIs being used in treatment of many tumour types [6].

However, the mechanism of such ion beam therapy remains unclear. Along the radiation track of the primary heavy particle (MeV) significant numbers of ions lose their energy due to inelastic scattering, and many low energy secondary fragments (electrons and ions) are produced

within the tissue. If such secondary ions/electrons with energies of a few keV are formed in the close vicinity of the nucleus of a living cell they can cause significant DNA alteration.

In order to understand the direct effects of LEI's on DNA, a series of experiments have been performed [7–9]. In these studies, dehydrated samples of supercoiled plasmid DNA, placed under high vacuum conditions, were exposed to different ion beams (i.e. Ar⁺, C⁺ and N⁺) whose energies were up to 6 keV and subsequent damage was quantified using the gel electrophoresis technique. Due to changes in a topology of damaged DNA, supercoiled plasmid transforms into nicked circular and linear forms in the case of single and double strand breaks, respectively. Both types of damage have been detected as a function of ion exposure, energy and charge state [8]. In these studies, the observed changes in nicked circular and full length linear forms after ion irradiation were small, therefore the absence of deposited material was assumed to be due to the production and release of short linear fragments from the surface.

In order to detect the small ionic fragments of synthetic DNA (oligonucleotides) produced during ion irradiation, we have performed experiments on ion stimulated desorption using mass spectrometry. Since the pioneering work of Macfarlane, the desorption of ions from a surface by fast heavy ion bombardment, has had a major impact on biomolecular mass spectrometry [10]. It was found

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that the energy deposited into electronic excitation by fast heavy ions in solids is sufficient for the desorbed ions to overcome the surface binding and escape with few eV's of excess energy.

In this work, we have irradiated several deposited films of oligonucleotides, each with a different base composition, using a low energy (4 keV) C^+ beam. By recording the total ion yield, we have been able to identify the most susceptible base to C^+ ion bombardment. This would not be possible if we had used a plasmid target since this is composed of a mixture of nucleobases. Moreover, due to possible applications of LEI's in cancer treatment, we have studied ion bombardment of brominated oligonucleotides, since halogenated compounds are regularly used as radiosensitizers.

2 Experimental set-up

These experiments were performed using the Electron Cyclotron Resonance (ECR) ion source at Queen's University Belfast. The experimental chamber was coupled to a floating beamline of the ECR. The beamline was maintained at -4 kV whilst, for the purpose of this experiment, the source was maintained at several kV positive (typically 4 kV). The resulting ion beam was thus extracted, analysed and transported at ~ 8 kV maximising mass resolution and minimising beam transport losses. Upon exiting the 'floating' beamline, the ion beam was focussed using a 3 element decelerating lens and then collimated by 2 mm diameter apertures separated by ~ 300 mm. The current was kept at 30 nA, corresponding to a current density of 0.1 mA cm^{-2} . The ion beam was then incident on a stainless steel plate orientated at 45 degrees to both the ion beam and the central axis of a quadrupole mass spectrometer. The plate was maintained at approximately 50 V to extract desorbed ions, whilst the spectrometer entrance was equipped with electrostatic deflectors enabling the desorbed ions to be directed along the spectrometer axis.

Several single stranded oligonucleotides were purchased from Sigma-Aldrich, each contained 10 bases with following sequences (from 5' to 3'): AAAAAAAAAA, CCCCCCCCCC, GGGGGGGGGG, TTTTTTTTTT, GCATGCATGC and GCA(BrU)GCATGC. Aliquots of oligonucleotides in aqueous solution (0.5 $\mu g/\mu L$) were deposited onto the gold coated stainless steel substrate and dried under ambient conditions. The resulting thin films were then irradiated with C^+ ions and the desorbed ions (nominal mass range 0.4 to 110 Da) detected using a quadrupole mass spectrometer. In order that the recorded spectra contained sufficient numbers of ions to ensure good statistics, the detection time for this range of masses was set at eight minutes (one cycle). The total fragmentation yield of desorbed ionic fragments was measured up to fluences of about 2.3×10^{18} ions cm^{-2} . Due to the range of kinetic energies of the desorbed species, the obtained mass resolution was low and the intensity of all desorbed ions was summed, normalized to the total ion yield recorded in the first cycle and averaged.

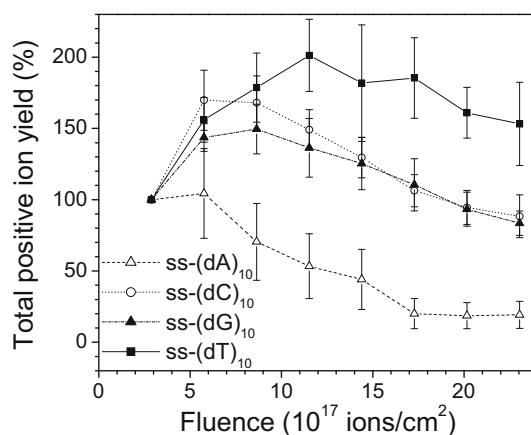


Fig. 1. Total positive ion yield for 4 'mono-nucleobase' oligonucleotide chains irradiated by 4 keV C^+ .

3 Results and discussion

3.1 Positive ion desorption

Figure 1 shows the total yield of positively charged ions desorbed from the films, plotted as a function of ion fluence. Each data point represents the mean of three irradiated samples where the error bar is calculated as one standard deviation from the mean. Due to non-uniformity in the prepared films, the absolute yield of the desorbed fragments cannot be estimated. All the samples were irradiated with 4 keV C^+ ions with an ion flux of 6×10^{14} ions $cm^{-2} s^{-1}$. A strong degradation of the film containing adenine bases was observed indicating that the adenine based oligonucleotide is particularly sensitive to carbon ion irradiation. In the case of oligonucleotides containing other nucleobases, the total ion yield increased more than 50% within 20–30 min then decreased once again to a yield comparable to that observed in the initial cycle. After ion exposure of 2.3×10^{18} ions cm^{-2} , the relative ion yields from each of the oligonucleotides was as follows: $T > C \approx G > A$ (Fig. 1). The most significant increase in the total ion yield was observed for oligonucleotides with thymine nucleobases and, even after 10^{18} ions of C^+ irradiation, the signal remained very high.

The general mechanism of ion desorption induced by keV ions is still poorly understood [11]. The lack of a deeper understanding of the interaction between organic films and ions is mainly due to an insufficient number of experimental studies. According to a recent calculation on ion implantation into DNA, a C^+ ion with kinetic energy in the range of 1–6 keV can penetrate a 5–60 nm thick layer of DNA before being completely stopped [5]. In our studies, knowing the volume of the deposited DNA solution; the density of solid DNA 1.7 g cm^{-3} [12] and assuming a uniform distribution of DNA on the surface, the average thickness of the films was estimated to be of the order of 20 nm. Therefore it is most likely that the primary ion will travel through the layer of deposited DNA molecules and transfer its kinetic energy into the substrate.

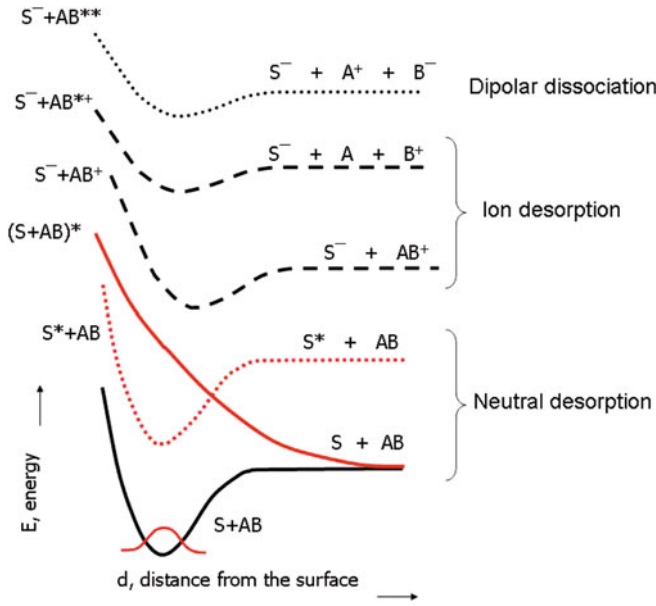
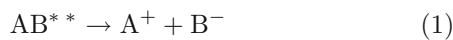


Fig. 2. (Color online) Potential energy diagram for ion stimulated desorption of ions based on the MGR model (adapted from [31]).

Here we propose the mechanism for ion desorption induced by LEI's based on two models of electron stimulated desorption. The first mechanism, proposed by Menzel, Gomer and Redhead (M-G-R model) [13,14], involves direct excitation of the valence electrons into a repulsive potential energy surface for the substrate (S) – adsorbed molecule (AB) complex leading to desorption of the ion species. The second mechanism, initially formulated by Knotek and Feibelman (the K-F model) [15,16] and extended into a more generalized mechanism is called “Auger stimulated desorption” [17]. The latter mechanism has been already suggested for ion desorption during LEI irradiation [18]. This process involves creation of holes in the inner orbitals of the ion-bombarded surface followed by intra-atomic Auger decay.

The mechanism for neutral and ion desorption according to the MGR model is illustrated by the schematic potential diagram in Figure 2. Initially the (S+AB) complex is excited from the ground state into the antibonding curve, (S+AB)* or the ion curve (S⁻+AB⁺). For neutral desorption, once the molecule is in the antibonding curve (S+AB)*, it can either return to the ground state (S+AB) or it travels down the curve until it crosses the curve (S*+AB). If the neutral intact molecule (AB) is in an electronically excited state (higher than the ionization energy) it can dissociate into two ionic fragments via dipolar dissociation:



For the cation case, the description of the desorption mechanism is similar to the above. The adsorbate-substrate complex is excited into a (bonding) ionic state

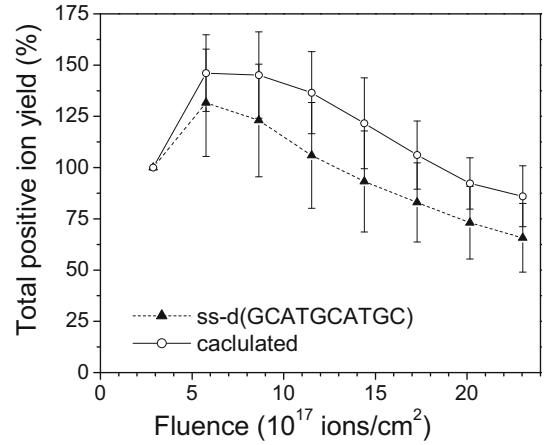


Fig. 3. Total positive ion yield for a ‘mixed nucleobase’ oligonucleotide chain irradiated by 4 keV C⁺ and the theoretical yield calculated from Figure 1.

(S⁻+AB⁺). An ion travelling down the ionic curve, can be recaptured or the positive ion will be desorbed. Depending on the internal energy of this ion it can further decay into fragments via the following reactions:



The processes presented above, based on the MGR model for desorption [19,20], are presented in Figure 2 by hypothetical potential energy curves for the ground state of (S+AB) and electronically excited states along the S+AB coordinate.

Figure 3 shows the total ion yield for oligonucleotides containing a mixture of nucleobases (mixed oligos) with the following sequence: GCATGCATGC. In this case, an increase in the ion signal was detected at low ion exposure up to 6×10^{17} ions cm⁻². If the contribution of each nucleobase in the oligonucleotides is assumed to be independent, the calculated total ion yield for mixed oligos can be calculated using the following formula:

$$\frac{\sum T_N}{n} \quad (5)$$

where T_N is the total ion yield of oligonucleotides containing the particular nucleobase and n number of nucleobases. In the case of GCATGCATGC, this formula is given by:

$$\frac{(3 \times T_G) + (3 \times T_C) + (2 \times T_A) + (2 \times T_T)}{10}. \quad (6)$$

The ion yields obtained using this calculation are also presented in Figure 3. The calculated and measured total ion yields show the same trend for a whole range of C⁺ fluences. Moreover, the calculated and measured values agree within the error bars.

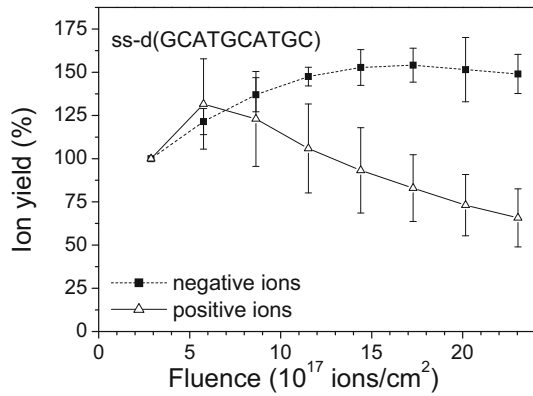


Fig. 4. Positive and negative ion yields for a ‘mixed nucleobase’ oligonucleotide chain irradiated by 4 keV C^+ .

3.2 Negative ion desorption

The desorption of negative ions from oligonucleotide films after irradiation by 4 keV C^+ was also investigated (Fig. 4). The anion yield strongly differs from that for cations for ion bombardment higher than $6 \times 10^{17} C^+$ ions. A comparison of the ion yields suggests that there are different mechanisms for the production of negative and positive ions. One of the possible mechanisms for the formation of negative ions has been shown in the dipolar dissociation process, reactions (1) and (2). Moreover, a positive ion with a kinetic energy of a few keV can eject an electron from any substrate [21]. The ejected electrons (with an average kinetic energy up to 20 eV) can then interact with the molecule (AB) adsorbed on the substrate and lead to the fragmentation of this molecule by the process of dissociative electron attachment (DEA). The DEA process has been extensively studied in the condensed phase [22]. The products of DEA are the neutral (radical) and anionic fragments:



or



The total ion yield from films of mixed oligonucleotides and oligonucleotides where one thymidine was substituted by bromouridine, i.e. $GCA(BrU)GCATGC$, as a function of ion fluence was measured and is shown in Figure 5. The total ion yield from the films of mixed oligonucleotides increases by about 50% after ion irradiation with a fluence of $2.3 \times 10^{18} \text{ ions cm}^{-2}$. However, the result with brominated oligonucleotides was completely different, the total ion yield decreases rapidly above the ion fluence of $5 \times 10^{17} \text{ ions cm}^{-2}$ and subsequently decreases more slowly reaching one quarter of the initial value after long term exposure. In this experiment, the presence of bromouridine in oligomers significantly modifies the behaviour of the oligonucleotides upon ion irradiation apparently leading to a much more rapid degradation of the sample. Due to the presence of many secondary electrons produced upon ionizing radiation, the prompt fragmentation (as revealed

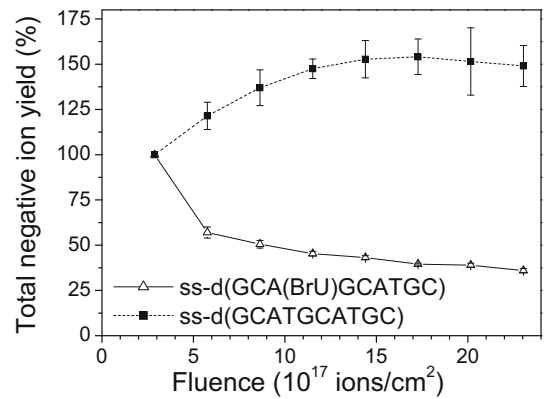


Fig. 5. Total negative ion yield resulting from 4 keV C^+ irradiation of 2 oligonucleotide chains, (i) mixed nucleobases and (ii) mixed nucleobases with a brominated base substitution.

by the rapid reduction in the ion yield) of the brominated oligomers can be attributed to the DEA process initiated by electron capture in the bromouridine unit. These results may be compared with previous studies on the damage induced by low energy electrons ($<30 \text{ eV}$) on oligonucleotides deposited on a gold substrate which also shown increased fragmentation in the case of brominated oligomers [23,24]. It was reported there that desorbed fragmentation yield increased by factor of about 3 over the entire range of low energy electrons.

4 Conclusion

Low energy ions (LEIs) with energies of a few keV, can be formed in a living organism as secondary products of ionizing radiation. We have investigated low energy carbon ion bombardment of oligonucleotides. In our approach we have detected the total yield for desorbed charged fragments by means of mass spectrometry. LEI irradiation of oligonucleotides is shown to result in distinct total ion desorption yields depending upon the base composition of the chain. The total positive ion yield for a mixed base nucleotide could be modelled, within experimental error limits, as a sum of the individual nucleobase components. This indicates that the fragmentation yield mainly depends on the nucleobase composition and not significantly on the other components of DNA, i.e. the sugar and phosphate groups. In future experiments it will be important to study high resolution mass spectra in order to identify masses of desorbed ions and thence deduce possible fragmentation pathways.

So far there is non-consistent theory concerning ion desorption induced by LEI's, mainly due to the lack of experimental data. Therefore we have proposed the mechanism for desorption of positive and negative ions based on the MGR model.

Finally, the effect of bromination of oligonucleotide chains was investigated revealing that the total negative ion yield decreases rapidly with ion irradiation, the opposite to that observed with a non-brominated chain.

The efficacy of radiosensitization of oligonucleotides by bromination has been observed previously during photon irradiation [25–29]. The studies presented here on ion interactions with modified oligonucleotides as well as studies with metallic atoms incorporated to DNA [30] may be applied to the ongoing effort of developing LEI's as a new technology for radiotherapy.

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