Effects of low energy carbon ions on plasmid DNA

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Abstract. The damage induced in supercoiled plasmid DNA molecules by low energy (< 1 keV u⁻¹) singly and doubly charged carbon ions has been investigated as a function of ion exposure. The production of short linear fragments through multiple double strand breakage is indicated and exponential exposure responses for each of the topoisomers are presented. The damage produced by C²⁺ is apparent at much lower ion exposures than with C⁺.

1. Introduction

The effects of high energy radiation on biomolecular systems have been linked to ionisation of the target material and production of reactive radical species. However, it has been recently demonstrated that low energy electrons (1-20 eV) [1] can cause DNA strand breakage through dissociative electron attachment. In radiotherapy, many secondary species, including low energy ions and excited neutral species are produced. The production of these heavier secondary species is of particular importance when the primary radiation is composed of heavy particles. Secondary species in various charge states, with energies up to ~ 1 keV, are produced along the incoming particle track [2].

There have been few studies of the interactions of heavy, low energy ions, with DNA. Deng et al [3] have studied singly charged argon ions at very low energies (less than 200 eV) and demonstrated fragment desorption from films of DNA components. It was shown that, even at these very low energies, the projectile ions were able to fragment the target molecules. An earlier study of plasmid DNA with singly charged argon ions [4] demonstrated that, at low energy (1 keV), single and double strand breakages occurred in plasmid DNA.

In the present work we have employed carbon ions because of their relevance to heavy ion therapy [5,6] and the abundance of carbon in biological matter. The irradiation target chosen for these studies was plasmid DNA (pBR322), a supercoiled circular DNA molecule comprising 4361 base pairs [7].

2. Experimental approach

The experimental setup has been described in detail elsewhere [8] and will only be briefly described here. The system comprises an electron cyclotron resonance (ECR) [9] ion source, fed with CO gas and coupled to a low energy ‘floating beamline’ ion accelerator. The ion beam was extracted from the source, analysed by a 90° double focusing magnet and focused by electrostatic lenses. A switching
magnet then directed the beam through two 4 mm diameter apertures and into the sample chamber. A custom designed sample holder, consisting of two concentric rings of sample wells with 8 sample positions in each ring, one of samples to be irradiated and the other a set of controls. The sample holder was mounted on a UHV XYZ rotatable manipulator.

pBR322 was replicated in *Escherichia coli* and subsequently extracted, purified and 6 µl of dilute solution (20 ng µl⁻¹) was deposited in each of the wells occupying their full volume. The samples were freeze dried, yielding a DNA film thickness approximately double the ion penetration depth. Samples were then transferred into the sample chamber which was operated at a pressure of ~1 × 10⁻⁶ mbar. After irradiation, samples were analysed by agarose gel electrophoresis.

The intensities of each DNA topoisomer were obtained from the area of the corresponding peak. A correction factor [10,11] was applied to the intensity of the supercoiled form to allow for the decreased binding of the DNA stain, ethidium bromide. The initial quantity of plasmid, \( I_c \), was determined according to:

\[
I_c = I_s + I_n + I_l
\]  

where \( I_s/ I_n/ I_l \) are the mean areas of the supercoiled, nicked and linear bands in the control samples. The percentage of each form in the irradiated samples was calculated from the corresponding band area as a percentage of \( I_c \). Preliminary experiments demonstrated that the total irradiated intensity varied as a function of the irradiation exposure. This was assumed to be due to the formation of multiple double strand breaks (MDSBs). The percentage conversion of control material, \( P_{Fr} \), was calculated from:

\[
P_{Fr} = 100 \left[ 1 - \frac{I_s + I_n + I_l}{I_c} \right]
\]  

where \( I_s/ I_n/ I_l \) are the intensities of supercoiled, nicked circle and full length linear in the irradiated samples.

### 3. Results and discussion

Figure 1 shows the percentage change in each of the forms of the plasmid with increasing ion exposure. Also shown (as lines) are model fits to the data. Each data point was the result of the mean of seven samples and the error bars were calculated as one standard deviation. A change was then defined by the subtraction of the control percentage from the irradiated percentage. The change in supercoiled form \( \Delta P^s \) was calculated by:

\[
\Delta P^s = 100 \left[ \frac{I_c^s - I_s^i}{I_c^i} \right]
\]

It is interesting to note that the data series reach ‘saturation’ at high ion exposures. The short linear fragments plot is of particular importance. The procedure used a quantity of DNA which would fill the well to approximately double the estimated ion penetration depth to avoid secondary electron production from primary ions striking the metallic surface. At very high exposures it is expected that all the plasmid molecules subject to ion irradiation will undergo MDSBs. The saturation level of approximately 56% of the starting material *i.e.* 44% of the plasmid DNA is ‘shielded’, is in good agreement with this interpretation. The fragment saturation level therefore corresponds to the fraction of the deposited DNA which was irradiated.

Over the entire exposure range, the fraction in the supercoiled form can be seen to decrease following irradiation. The change in this percentage follows an exponential decay (see figure 1) such that the dependence on ion exposure is given by:

\[
\Delta P^s = P^s_c (1 - e^{-b_{c,f}})
\]
where \( P_c^S \) is the percentage of the control material in a supercoiled conformation, \( b_s \) is the cross section for conversion of a supercoiled form to nicked, linear or fragmented form and \( f \) is the ion exposure. \( P_c^S \) is calculated to be \( 47 \pm 4 \% \) and the cross-section is calculated to be \((2.2 \pm 0.5) \times 10^{-14} \) cm\(^2\) where the errors are estimated from the curve fitting process.

![Figure 1](image)

**Figure 1** The effect of C\(^+\) ion exposure upon the change in each of the form. ● - supercoiled form, ○ - short linear fragments. The lines plotted are exponential growth/decay to saturation fitting the data sets.

The decay to a saturation level was also observed by Boudaiffa et al [12] with low energy electrons. The total destruction cross section for loss of supercoiling was calculated to be \( \sim 4 \times 10^{-15} \) cm\(^2\) (at an electron energy of \( \sim 50 \) eV). This value is five times smaller than that calculated for 2 keV C\(^+\) in the present work.

The induction of MDSBs as a function of ion exposure is also shown in figure 1 indicating a strong exposure dependence with 56% of the control material being converted to short linear fragments at higher exposures. A fit to the experimental data was obtained using an equation similar to equation 4 yielding a cross section of \((2.8 \pm 0.5) \times 10^{-14} \) cm\(^2\). However, the saturation yield for fragmented forms with C\(^+\) is much higher than with low energy electrons [12] at 10% compared to 56% with ions at saturation.

Figure 2 shows the percentage change in the plasmid forms when the ion charge state was increased. Both experiments were carried out with an acceleration potential of 5 kV resulting in C\(^+\) ions at 5 keV and C\(^{2+}\) at 10 keV. The low number of data points available means that only a qualitative comparison can be made. Two notable differences between the C\(^+\) and C\(^{2+}\) plots are visible, (i) the saturation levels with C\(^{2+}\) are higher than for C\(^+\) and (ii) the damage produced by C\(^{2+}\) is apparent at much lower exposures than with C\(^+\). The first of these points is explained by the increased kinetic energy possessed by the C\(^{2+}\) ion which resulted in a greater penetration depth with more DNA exposed to ion irradiation. This is also responsible for the different saturation levels observed in figures 1 and 2 (2 keV compared to 5 keV). The increased low exposure damage with C\(^{2+}\) has been observed in previous experiments [8] and results from the additional potential energy deposited by a C\(^{2+}\) ion in an electron capture event. Similar potential energy mediated effects were observed in our investigation of molecular synthesis and destruction induced by carbon ion irradiation of icy mixtures [13].
4. Conclusions
We have studied damage induced in plasmid DNA by low energy (< 1 keV\textsuperscript{u}\textsuperscript{-1}) carbon ions as a function of ion exposure. A strong exponential exposure response is observed suggesting that fragmentation dominates at higher exposures. The saturation levels with C\textsuperscript{2+} are higher than for C\textsuperscript{+} and the damage produced by C\textsuperscript{2+} is apparent at much lower exposures than with C\textsuperscript{+}.

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