Particle induced strand breakage in plasmid DNA

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We have investigated the damage of synthetic plasmid pBR322 DNA in dilute aqueous solutions induced by fast carbon ions. The relative contribution of indirect damage and direct damage to the DNA itself is expected to vary with linear energy transfer along the ion track, with the direct damage contribution increasing towards the Bragg peak. Therefore, $^{12}\text{C}$ ions at the spread-out Bragg peak (dose averaged LET = 189 ± 15 keV/µm) and in the plateau region of the Bragg curve (LET = 40 ± 5 keV/µm) were employed and the radical scavenger concentration in the plasmid solution was varied to quantify the indirect effect. In order to minimize the influence of $^{12}\text{C}$ fission fragments, a relatively low initial energy of 90 MeV/u was employed for the carbon ions. DNA damage has been quantified by subsequent electrophoresis on agarose gels. We find that single strand breaks and double strand breaks due to both indirect and direct effects are systematically higher in the plateau region with the difference being smallest in the Bragg peak region. In view of the fact that the relative biological effectiveness for many biological endpoints is maximum at the Bragg peak our findings imply that DNA damage at the Bragg peak is qualitatively most severe.

based on:

_Heavy ion induced damage to plasmid DNA: Plateau region versus spread out Bragg-peak_

(submitted)
4.1 Irradiation of plasmid DNA in aqueous solutions with energetic particles

When fast atomic ions like C$^{6+}$ at GeV energies interact with matter, a sizeable fraction of ion kinetic energy is deposited at the end of the track. For instance a 100 MeV/u $^{12}$C beam has a penetration depth in water of about 25 mm and looses the last 20% of kinetic energy on the last 1.5 mm. The resulting dose-maximum at this location is called the Bragg peak and it is the existence of this dose maximum that renders ions interesting for medical application in cancer treatment. The Bragg peak allows selective dose deposition in deeply seated solid tumors without excessive damage to surrounding tissue. Therefore, proton therapy and carbon therapy have the potential to significantly improve the therapeutic ratio of cancer therapy [92].

However, dose is not the only relevant quantity in this context. Leith et al. [93] for instance examined the inset of paralysis as a biological response of rat spinal cord irradiation and found that the relative biological effectiveness (RBE) of helium ions in the plateau and spread-out Bragg peak regions are comparable (a definition of plateau and spread-out Bragg peak will be given at the end of the introduction). On the other hand, Staab and co-workers [94] studied the influence of C irradiation on chinese hamster cell V79 spheroid volume growth under hypoxic conditions as a function of LET. They found an increase in RBE with increasing LET with the most efficient cell inactivation occurring at the Bragg peak whereas at the plateau, radiobiological mechanisms of C ion induced cell inactiviation seem to be similar to those observed for X-ray irradiation and lighter ions.

The reason of the peak in the dose deposition lies in the fact that the stopping power has a maximum for low velocities: In the range of the Bragg peak, ions start to reach the velocity range of target electrons involved in molecular binding. It is mainly the interaction with these outer electrons that dominates the stopping on a molecular level, in contrast to the high energy regime where interaction with inner-shell electrons is dominant [95]. Accordingly, on a molecular level damage mechanisms may be very different at the Bragg peak and at the plateau. De Vries et al. [96] for instance have shown, that for heavy ion at collisions energies typical for the very end of the Bragg peak molecular fragmentation can lead to formation of secondary ions with kinetic energies of several 10 eV. These fragments can subsequently induce damage in neighboring biomolecules and in particular in DNA. It was shown by Deng et al. [97] that the interaction of such low energy secondary ions with DNA bases is sufficient to induce their fragmentation. Similar effects were observed for fragmentation of small oligonucleotides by Sellami et al. [98]. These results indicate that complex DNA damage such as clustered strand breaks could be relevant at Bragg peak energies. This most severe type of damage could explain the high RBE of ions at Bragg
4.1 Irradiation of plasmid DNA in aqueous solutions with energetic particles

peak energies. Very recently, Psonka-Antonczyk et al. found evidence for formation of such clustered lesions in atomic force microscopy studies of heavy ion irradiated plasmid DNA thin films [99].

Finally, nuclear fragmentation of the incident C$^{6+}$ along the track is an important issue. For instance, at an initial energy of about 290 MeV/u, up to 50% of C$^{6+}$ are fragmented into secondary energetic ions like H$^+$ and He$^{2+}$, and to some extent into Li$^{3+}$, Be$^{4+}$ and B$^{5+}$ before they reach the Bragg peak [100–102]. The recoils of nuclear reactions are in the MeV to tens of MeV range depending on the depth of creation and the specific reaction. The fragment ion velocity thus is the same or higher as that of the primary ion at the same location, so the energy is in the tens to hundreds of MeV range rather than in the MeV range for most of their trajectory.

![Figure 4.1: Depth-dose distribution of the unmodulated (stars) and modulated (circles) 90 MeV/u $^{12}$C beam, featuring the regular Bragg peak and the spread-out Bragg peak (SOBP), respectively. Lines are drawn to guide the eye. The two arrows indicate the sample location for irradiation at the plateau of the Bragg curve (40 ± 5 keV/µm) and at the SOBP (189 ± 15 keV/µm). The shift in penetration depth is due to the presence of additional windows for the SOBP.](image)

Consequently, under therapeutic conditions the carbon beam at the plateau and the Bragg peak (fig. 4.1) is of rather different composition and this could result in variations of the DNA damage per radiation dose and, due to the higher energy of the fragments, in different molecular mechanisms underlying part of the damage.

In this article we compare plasmid DNA damage for 90 MeV/u C$^{6+}$ slowed down
Heavy ion induced damage to plasmid DNA: Plateau region versus spread out Bragg-peak

4.2 Results

4.2.1 Plasmid in dilute aqueous solution

Figure 4.2 shows an example of a gel electrophoresis image of a DNA sample irradiated in pure water with $40 \pm 5$ keV/µm carbon ions in the plateau region, demonstrating the dose-variation of the main plasmid forms. The relative yields of the three fractions (SC, OC and L), as quantified from the gel image in fig. 4.2, are plotted as a function of dose in fig. 4.3. The relative amount of native plasmids (SC) drops exponentially with dose as expected from Poisson statistics. The observed trend in the yield of the open circular plasmid form is due to the interplay of single and multiple damages of the same plasmid. At low dose, almost all damage is due to isolated events and accordingly the OC-fraction of the plasmids increases with dose. As the dose increases, plasmids containing a single...
4.2 Results

Figure 4.3: Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after $^{12}$C irradiation at the plateau region of the Bragg curve (LET = 40 ± 5 keV/µm) as a function of dose (without Mannitol). For the open symbols, short linear fragments (SL) have been included in the analysis. The solid lines are the fits to the data using the model of Cowan et al. [103].

Figure 4.4: Gel electrophoresis image of plasmid DNA after $^{12}$C irradiation at the SOBP at various doses (without mannitol). OC: Open circular, L: Linear, SC: Supercoiled, C: sample stored in refrigerator at 4°C and without any irradiation, D: Digestion.
SSB suffer additional SSB, which do not lead to a further increase of the OC fraction. On the other hand, when closely spaced sequential SSB lead to DSB formation from a relaxed plasmid, the latter is converted to the L form. This explains the observed increase of L forms accompanied by a decrease of OC forms. Direct DSB induction is a possible channel as well. We have used the model of Cowan et al. [103] to extract the yield of SSB and DSB per plasmid per dose from the relative intensities of each band in the gel image. This model (lines in fig. 4.3) which is described in next section reproduces the experimental data (symbols in fig. 4.3) accurately. For the data obtained in the absence of mannitol we have also estimated the yield of the SL fraction as described earlier and corrected the SC, OC and L fractions accordingly. The corrected data is also displayed in fig. 4.2 as open symbols. It is obvious that SL fraction increases up to 15% at 300 Gy.

Fig. 4.3 displays the results for the SSB and the DSB fraction in the plateau region of the Bragg curve. The fraction of supercoiled plasmid DNA decreases whereas the fraction of the linear (L) form for double strand breaks (DSB) increases as a function of dose. The fraction of open circular (OC, due to SSB) plasmids formed by single strand breaks shows a rapid increase with dose until a maximum of 80% is reached at about 30 Gy after which is monotonically decreases with dose. For higher doses, the SSB fraction decreases monotonically. The fraction of the L form (due to DSB) gradually increases up to 70% with dose.

Figure 4.4 shows the gel electrophoresis images of DNA irradiated by $^{12}$C ions at the SOBP for different doses in the absence of radical scavengers. Similar to the case of the plateau region, the fraction of supercoiled plasmids quickly drops as a function of dose. The OC fraction increases up to a dose of about 50 Gy and decreases again for higher doses. The fraction of linear plasmids is monotonically increasing over the whole dose range under study. The results of the quantitative analysis are shown in fig. 4.5. Again, the data corrected for the SL fraction is displayed as open symbols and again about 15% of SL are observed at 300 Gy.

4.2.2 The influence of radical scavengers

In a second experiment, the influence of a radical scavenger on the damage is investigated at the plateau region. The fractions of OC, SC and L plasmid conformations as a function of dose for a 600 mmol/l mannitol concentration are depicted in figure 4.6. Compared to the situation where no scavenger is present (Figure 4.3), the DNA damage yields are strongly reduced. Linear plasmids decrease to less than 15%. This implies that scavenging of $^\cdot\mathrm{OH}$ radicals plays an important role in preventing plasmid DNA damage under realistic cellular conditions. The radiation damage to the plasmids was also studied as a function of mannitol concentration for a fixed dose of 300 Gy. The quantitative results are displayed in figure 4.7. The scavenger effect
4.2 Results

Figure 4.5: Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after $^{12}$C irradiation at SOBP (dose averaged LET$_\infty$ = 189 ± 15 keV/µm) as a function of dose (in pure water). For the open symbols, short linear fragments (SL) have been included in the analysis. The solid lines are fit to the data using the model of Cowan et al. [103].

saturates above 200 mmol/l mannitol, i.e. at the same concentration where saturation was observed in our earlier study on $^{137}$Cs γ-photon irradiation of plasmids [104]. At 300 Gy the fraction of supercoiled plasmids is 20% at the plateau.

For plasmid DNA in a 600 mmol/l mannitol solution and irradiation at the SOBP, L formation (fig. 4.8) and also OC formation is stronger suppressed than for the plateau region. At 300 Gy, less than 10% L and about 60% OC are observed (fig. 4.9) which must largely be due to direct effects. For both cases the estimated SL fraction does not exceed the order of the uncertainty of the evaluation method. A (very poor) linear fit through the SL data gives an estimation of 7% SL for 300Gy at the SOBP and 3% at the plateau but these values have to be taken with caution. We can however clearly conclude that in the absence of scavengers, most of the SL plasmids are produced by indirect effects. Furthermore, at high scavenger concentrations the sum of L and SL fractions at 300 Gy is at most equal for the SOPB and the plateau region.

The results observed for irradiation in the absence of a scavenger are counterintuitive. Compared to the case of irradiation at the plateau, the damages at the Bragg peak region as a function of dose are less. In particular the L fraction due to DSB or multiple SSB is reduced below 10%. Moreover, as seen in figures 4.9 and 4.7, there is a peak in the OC plasmid fraction at a scavenger concentration of 10 mmol/l. This
Effect could be due to the fact that DSB at low concentration are to a large extent due to multiple radical induced SSB and thus scavengable. Thus, when the concentration of mannitol increases and L plasmid yields due to multiple SSB decrease, an increase in OC plasmids is expected.

Different from the plateau results, for plasmid DNA in a 600 mmol/l mannitol solution and irradiation at the SOBP, L formation (fig. 4.8) and also OC formation is stronger suppressed. At 300 Gy, about 0.1 L and about 0.6 OC are observed (fig. 4.9), indicating the relevance of direct effects. The results observed for irradiation in the absence of a scavenger are counterintuitive. Compared to irradiation at the plateau, the damages at the Bragg peak region as a function of dose are less. The maximum fraction of SSBs and DSBs are only 50%. An example of the differences in DSB yields obtained after irradiating plasmid DNA in the two regions is shown in figure 4.10.

4.2.3 Damage yields as a function of scavenging capacity

For the discussion of the experimental data, it is convenient to calculate the probability for induction of SSB \( \mu \) and DSB \( \phi \) on a plasmid per Gray. We employ the model by Cowan et al [103], originally developed to determine these probabilities for in-
4.2 Results

**Figure 4.7:** Relative yields of supercoiled (SC), open circular (OC) and linear (L) DNA after 300 Gy $^{12}$C irradiation at the plateau as a function of Mannitol concentration. The lines are to guide the eye.

**Figure 4.8:** Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after $^{12}$C irradiation at SOBP as a function of dose (with 600 mmol/l of Mannitol). The solid lines are the fits to the data using the model of Cowan et al. [103].
Heavy ion induced damage to plasmid DNA: Plateau region versus spread out Bragg-peak

Figure 4.9: Relative yields of supercoiled (SC), open circular (OC) and linear (L) DNA after 300 Gy $^{12}C$ irradiation at SOBP as a function of Mannitol concentration. The lines are to guide the eye.

Figure 4.10: DSB yields induced by $^{12}C$ irradiation. Comparison of DNA breakage yields measured at plateau region and SOBP. The lines are to guide the eye.
dependent action of nicking (SSB inducing) and cleaving (DSB inducing) enzymes, respectively. In [103] it was already pointed out that the model is also applicable to the action of ionizing radiation on plasmids. The damaging events are considered independent and thus Poisson statistics can be applied.

The yield of supercoiled plasmids as a function of dose \( D \) is:

\[
SC(D) = e^{-\phi D} e^{-(\mu D + \mu_0)}
\]  

(4.1)

\( \mu_0 \) is the yield of SSB per plasmid at zero dose. This parameter reflects the quality of the plasmid sample under study. Equation 4.1 describes the exponential decrease of supercoiled plasmids due to induction of SSB and DSB. The yield of open circular plasmids can be written as:

\[
OC(D) = e^{-\phi D} \left( 2e^{-(\mu D + \mu_0)/2} - 2e^{-(\mu D + \mu_0)} + (\mu D + \mu_0)X \right)
\]  

(4.2)

with

\[
X = \frac{1}{b} \sum_{k=1}^{\frac{1}{b}} e^{-(\mu D + \mu_0)(1+kb)/2} \left[ \frac{1}{2} (\mu D + \mu_0) (1 - kb) \right]^{2k-1} 2k!
\]

The DSB channel only leads to loss of OC plasmids described by an exponential decrease term. SSB of the SC form lead to filling of the OC channel and SSB of OC plasmids can lead to their loss if both SSB are on opposite strands and within a distance of less than \( b \times \text{plasmid length} \) base pairs. Correct application of Cowan’s model requires knowledge of the interaction distance between strand breaks with \( b = 10 \text{ bp/4361 bp} \). \( b \) is the correlation length between SSBs in fraction of the plasmid length. In our model calculations, we used an interaction distance of 10 bp for DSB. \( k \) number of SSB on one strand. In case of more than two SSB, even multiple DNA scission can occur, also leading to loss from the OC channel. The loss channels lead to the more complex structure of equation 4.2.

The last equation describes the dose dependence of the linear form due to plasmids that were subject to at least one DSB:

\[
L(D) < e^{-\phi D} \left[ \frac{\mu D + \mu_0}{2 - b(\mu D + \mu_0)} \left( (\mu D + \mu_0)X - Y + e^{-(\mu D + \mu_0)/2} - e^{-(\mu D + \mu_0)} \right) \right]
\]

\[
+ \phi D \left( e^{-(\mu D + \mu_0)} + (2e^{-(\mu D + \mu_0)/2} - 2e^{-(\mu D + \mu_0)} + (\mu D + \mu_0)X) \right)
\]  

(4.3)

with

\[
Y = \frac{1}{b} \sum_{k=1}^{\frac{1}{b}} e^{-(\mu D + \mu_0)(1+kb)/2} \left[ 2k + \frac{1}{2} (\mu D + \mu_0) (1 - kb) \right] \times \frac{1}{2k!} \left[ \frac{1}{2} (\mu D + \mu_0) (1 - kb) \right]^{2k-1} 2k!
\]
Here, DSB can lead to filling of the L channel from SC and OC and DSB can lead to loss from L into short linear fragments. SSB can fill the L channel from OC and also lead to loss into short linear fragments. There is no analytical solution for the loss process, since the definition of "short" depends on the question which fragment length will lead to a deviation from the L band in the gel-electrophoresis analysis. Cowan et al [103] give equation 4.3 as an upper limit for the L channel, which should approximate the correct results for the $b$ values relevant here.

<table>
<thead>
<tr>
<th>Yields (/plasmid/Gy)</th>
<th>Plateau region</th>
<th>SOBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>0.15 ± 0.03</td>
<td>0.052 ± 0.006</td>
</tr>
<tr>
<td>$\phi$</td>
<td>0.009 ± 0.001</td>
<td>0.0031 ± 0.0004</td>
</tr>
</tbody>
</table>

The parameters $\mu$ and $\phi$ in the above equations can now be used as adjustable parameters to obtain optimum agreement with the experimentally obtained yields. To this end, the model-predicted relative yields of the various plasmid forms, normalized to (SC+OC+L) = 100%, were employed since short fragments are only produced in negligible quantities in the experiment for scavenger concentrations exceeding a few 10 mmol/l. The fitting procedure was based on the Nelder-Mead [105] and Marquardt [106] algorithms, implemented in the Matlab software package (The MathWorks Inc., Natick, MA).

The yield of SSB and DSB per Gray is higher at the plateau than at the Bragg peak. When mannitol is added to the samples, the yields of SSB and DSB were strongly reduced. This finding illustrates that in dilute aqueous solution the DNA scissions are mainly induced by $\cdot OH$ free radicals from water radiolysis.

Considering the values in the table 4.1 and 4.2, it is easy to see that the number of SSB per gray decreased by about 96% in the presence of mannitol at both the plateau and at the SOBP. For the DSB the decrease when mannitol is added is somewhat smaller, around 88%. This finding is in agreement with previous results obtained with helium, carbon and iron ions [107–109] where the DSB induction was found less protected in the presence of mannitol than the SSB breaks induction so DSB are relatively less related to radicals or more to direct effect.
Table 4.2: SSB per plasmid per Gy (µ) and DSB per plasmid per Gy (φ) yields after C irradiation at plateau region and SOBP at different Mannitol concentrations.

<table>
<thead>
<tr>
<th>Mannitol concentration (mmol/l)</th>
<th>Plateau region</th>
<th>SOBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.054 ± 0.037</td>
<td>0.0043 ± 0.001</td>
</tr>
<tr>
<td>1</td>
<td>0.027 ± 0.025</td>
<td>0.0022 ± 0.0008</td>
</tr>
<tr>
<td>10</td>
<td>0.0145 ± 0.0009</td>
<td>0.0009 ± 0.0002</td>
</tr>
<tr>
<td>30</td>
<td>0.0075 ± 0.0005</td>
<td>0.0006 ± 0.0002</td>
</tr>
<tr>
<td>100</td>
<td>0.0066 ± 0.0002</td>
<td>0.0005 ± 0.00009</td>
</tr>
<tr>
<td>250</td>
<td>0.0056 ± 0.0004</td>
<td>0.00047 ± 0.00016</td>
</tr>
<tr>
<td>600</td>
<td>0.0055 ± 0.0004</td>
<td>0.0004 ± 0.0003</td>
</tr>
</tbody>
</table>
4.3 Discussion

As a first counter-intuitive conclusion, the yield of plasmid SSB ($\mu$) and DSB ($\phi$) induced by C-irradiation is higher in the plateau region than in the SOBP by more than a factor of 3 ($c_\mu = \frac{\mu_{\text{plateau}}}{\mu_{\text{SOBP}}} \approx 3$), see table 4.1 in the absence of scavengers. At high scavenger concentrations the factor between the DSB yields obtained for the two regimes reduces to $c_\phi = 1.4$ whereas the difference between the SSB exhibits a less pronounced drop to $c_\mu = 1.9$ (see table 4.2).

Figure 4.11 displays the $c$-ratios as a function of scavenging capacity.

The observation of higher DSB yields in the plateau is at variance with the results of Usami et al. [109] who studied C induced plasmid damage at much higher initial energies (276 MeV/u) and observed no differences in DSB induction between the two regimes. However, at such high energies substantial nuclear fragmentation occurs along the ion track transversing through the plasmid DNA. This is leading to a more than 50% contamination at the SOBP with light nuclear fragment ions. These light ions have deeper penetration depths and are responsible for the "tail" of the Bragg-peak. Accordingly, for the more than 50% light nuclear fragments, the $^{12}$C Bragg-peak region still is a plateau region and therefore a comparison between the two regimes is very difficult. Also, Usami et al. have a lower LET (13.1 keV/µm) in their entrance location (plateau region) since they work with higher initial energies. In
our experiments, the energy of the $^{12}$C ions beam is 90 MeV/u and about 95% of the beam are $^{12}$C ions at the SOBP. Hence, the contribution of light fragments in the track is negligible. Note that the ion energies used by Usami et al. are closer to the energy range usually encountered in heavy ion therapy. However, for an investigation of the underlying processes here we purposely chose a beam with negligible fragmentation.

Figure 4.12: SSB and DSB yields induced by $^{12}$C irradiation. Comparison of DNA breakage yields measured at plateau region and SOBP. The lines are to guide the eye.

The results from table 4.2 are plotted in fig. 4.12. Note that the data in the figure were converted from a (plasmid)$^{-1}$ to a Da$^{-1}$ scale to allow easy comparisons to existing literature on irradiation of different plasmids (e.g. [58] for $\gamma$-photons and fast protons on pHAZE). pBR322 has a mass of approximately $2.834 \times 10^6$ Da. The mannitol concentration has been converted into a scavenging capacity $\sigma = k_s \times c_s$ with $c_s$ being the scavenger concentration as given in the table and $k_s = 1.8 \times 10^9$ mol$^{-1}$ls$^{-1}$ the reaction rate coefficient for mannitol with $\cdot$OH radicals [110]. It is
obvious that for the plateau region and for the SOBP, the dependence of both $\mu$ and $\phi$ on the scavenging capacity follows a similar trend. Briefly, the scavenging capacity can only influence the radical mediated indirect damage. At very high capacities, virtually all radicals are scavenged and the indirect effect is essentially switched off. This is clearly seen in the curves for both $\mu$ and $\phi$ in fig. 4.12: Damage yields are high at low scavenging capacity and are found to decrease roughly following a power law. For high scavenging capacities exceeding $10^8 \text{ s}^{-1}$, damage yields deviate from the power law and asymptotically approach a saturation value. The constant damage at high scavenging capacity can be considered non-scavengable and is thus probably due to direct damage. Note that the saturation region starts approximately at the scavenging capacity expected within the cell nucleus ($4 \times 10^8 \text{ s}^{-1}$ [111]). This value is indicated in the figure.

We can now extract the ratios between the damage yield at scavenging capacities where saturation is reached and at negligible scavenging capacity for SSB and DSB at the plateau and at the SOBP:

$$d_{\text{plateau,SSB}} = \frac{\mu_{\text{plateau}}(10^9 \text{ s}^{-1})}{\mu_{\text{plateau}}(2 \times 10^5 \text{ s}^{-1})} = 3.7\%$$

$$d_{\text{plateau,DSB}} = \frac{\phi_{\text{plateau}}(10^9 \text{ s}^{-1})}{\phi_{\text{plateau}}(2 \times 10^5 \text{ s}^{-1})} = 6.5\%$$

$$d_{\text{SOBP,SSB}} = \frac{\mu_{\text{SOBP}}(10^9 \text{ s}^{-1})}{\mu_{\text{SOBP}}(2 \times 10^5 \text{ s}^{-1})} = 5.7\%$$

$$d_{\text{SOBP,DSB}} = \frac{\phi_{\text{SOBP}}(10^9 \text{ s}^{-1})}{\phi_{\text{SOBP}}(2 \times 10^5 \text{ s}^{-1})} = 10.3\%$$

(4.4)

Obviously, the parameter $d$ also equals the ratio between direct damage (the only contribution at high scavenging capacity) and the total damage (being the sum of indirect and direct damage at negligible scavenging capacity), i.e. we can conclude that at the plateau 4% of the SSB and 7% of the DSB are due to direct damage whereas at the SOBP 5% of the SSB and 10% of the DSB are due to direct damage. Note, that for determination of the numbers, the SL fraction was ignored because of the large uncertainties involved.

Three interesting conclusions can be drawn from fig. 4.12: 1) for scavenging capacities similar to the cellular environment, the indirect effect is already fully suppressed. Extrapolation of this conclusion to the cellular is certainly not straightforward as our dilute aqueous solution differs significantly from the cellular environment. 2) for DSB and SSB in the plateau and SOBP region, only 10 % or less of the total damage at low scavenging concentration are due to direct damage. 3) sur-
prisingly, at both plateau and SOBP region, a sizable fraction of SSB is due to direct effects. This fraction is only about half as big as for DSB but clearly not negligible.

Whereas scavengable (indirect) damage is clearly due to diffusing radicals produced relatively far from the plasmid DNA, non-scavengable (direct) damage can either be due to direct modification of the plasmid DNA itself or due to production of $\bullet$OH radicals or radical-clusters in the direct vicinity of the plasmid DNA (or a combination of both). The scavengable damage is most probably due $\bullet$OH radicals stemming from primary water radiolysis along the track. Non-scavengable radicals or radical-clusters close to plasmid DNA can for instance also stem from radiolysis induced by Auger-electrons from DNA constituent atoms (P, C, N, O) [109].

In this picture, reduced $\mu$ values at the Bragg-peak as compared to the plateau region could be explained by suppression of indirect damage: at the SOBP due to the very high LET the $\bullet$OH concentration in the track increases, leading to increasing probability for radical recombination [112]. Jones et al. [113] invoked the same scenario to interpret their data on fast He induced SSB and DSB damage of SV40 DNA at different concentrations of DMSO.

This scenario explains our observed decrease of $c_\mu$ and $c_\phi$ when the scavenging capacity increases: If more radicals are scavenged, their density decreases and radical-radical interactions are suppressed.

There are however at least two facts, which suggest that this scenario is incomplete. First of all, the damage curves for the plateau are higher than those at the SOBP for both SSB and DSB over the whole range of scavenging capacities ($c > 1$, see fig. 4.12). If radical-radical interactions were the only relevant processes here, a reduction of the $c_\mu$ and $c_\phi$ to values smaller than 1 would be expected. Secondly, from in vitro and in vivo heavy ion irradiation studies, it is known that for most biological endpoints, the RBE at the SOBP is higher than at the plateau: In rat spinal cord irradiation studies with fast C-ions and symptoms of paresis in 50 % of the animals as biological endpoint, Karger et al. [114] observed difference factors in RBE between SOBP and plateau of 2.2 for 6 fractionations and of 3.5 for 18 fractionations. Lücke-Huhle and co-workers [115] investigated survival rates of Chinese hamster V79-spheroids cultures and found an RBE increase factor of 4.2 when comparing SOBP and plateau. For the same system, Staab et al. [94] only found a ratio of 1.5 for oxygen rich conditions and a ratio of 3 under normal circumstances. Suzuki et al. [62] studied fast C ion induced killing of different human cell lines and found on average a 2 times higher RBE in the SOBP than at the plateau.

In contrast to these finding, the $c$ values measured in our study are clearly larger than 1, implying systematically larger plasmid damage in the plateau region! An explanation for this finding could be the generation of types of DNA damage at the SOBP which are more difficult to repair by the cell [31]. Radical-radical neutraliza-
tion however would render the additional dose lost and accordingly could not explain the observed increase in RBE.

Clearly, in addition to the radical-radical neutralization effect, an additional mechanism plays a crucial role here. We propose that at the high LET values at the SOBP more complex clustered plasmid damage is induced as compared to the lower LET situation at the plateau. The electrophoresis technique is not sensitive to such a change in damage quality since a cluster of close DSBs will lead to an only insignificantly shortened L plasmid. At the same time due to higher LET the dose at the SOBP is due to fewer tracks than at the plateau and lower $\phi$ values are observed. This effect is even amplified by the fact that at Bragg peak energies, less energetic secondary electrons are produced as compared to the plateau region. This leads to smaller track radius and thus volume at the Bragg peak, implying an even higher local dose. Last but not least, there is a small contribution of SL plasmid fragments that proved very difficult to quantify.

To get a better insight into the problem it is useful to look into the damage on a per track level. At identical dose, the number of tracks is a factor of 4.3 larger at the plateau ($40 \pm 5$ keV/\(\mu\)m) than at the SOBP (dose average LET of 176 keV/\(\mu\)m). Under the assumption that the dose effect on the plasmid in the SOBP is already lowered due to changes in damage quality and due to radical-radical neutralization, the number of tracks per unit volume becomes a relevant quantity: Probably, the higher SSB and DSB yields in the plateau region are to a large extent an effect of the 4.3 times larger number of tracks. Last but not least, the radial dose distribution along a heavy ion track, which to a large extent reflects the energy spectrum of the produced secondary electrons, varies as a function of ion velocity [116]. The local dose along a track is thus higher for the SOBP.

### 4.4 Conclusion

In this chapter, we have investigated the SSB and DSB induction in plasmid DNA upon irradiation with fast C ions. SSB and DSB yields per plasmid per dose were found to be lower in the SOBP than in the plateau region. In view of the fact, that in biological systems, for cell killing the RBE of C ions is usually found to be higher in the SOBP than in the plateau region, we conclude that C ion at SOBP energies induce DSBs that are qualitatively different from those induced in the plateau region. Clustered strand breaks as recently identified for the first time by Psonka et al. [54] are a likely explanation for this quality-difference.

From our data, we could also determine the amount of non-scavengable plasmid damage as high scavenging capacities. Not only DSB but also a sizable fraction of SSB are due to direct effects.