Particle induced strand breakage in plasmid DNA
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Interaction of ionizing radiation with plasmid DNA can lead to formation of single strand breaks, double strand breaks and clustered lesions. We have investigated the response of the synthetic plasmid pBR322 in aqueous solution upon irradiation with $^{12}\text{C}$ ions under spread-out Bragg peak conditions (densely ionizing) and with $^{137}\text{Cs}\gamma$-photons (sparsely ionizing) as a function of dose. To evaluate the relevance of indirect effects, i.e. influences of diffusion limited radical induced DNA damage triggered by water radiolysis, the experiments were performed at various concentrations of the radical scavenger Mannitol. Agarose gel electrophoresis was employed to quantify the DNA damage. At low scavenger concentration for a given dose DNA damage is higher for $\gamma$-photons than for $^{12}\text{C}$. For the latter, the microscopic dose distribution is inhomogeneous, with very high dose deposited along the few tracks through the solution. This is in agreement with the concept that scavengers efficiently reduce damage for $\gamma$-photons, implying that the underlying damage mechanism is single strand break induction by OH radicals. For $^{12}\text{C}$ induced damage, the fraction of SSB and DSB that is unaffected by radical scavengers and thus due to direct effect is quantified.

based on:

Plasmid DNA damage by heavy ions at spread-out Bragg peak energies
5.1 Introduction

Radiation-induced damage to DNA in dilute aqueous solution can be due to energy deposition, i.e. ionization of the DNA itself by the primary quantum of radiation and secondary particles produced along the track. This is usually referred to as the direct effect [117]. Ionization of water molecules in the direct vicinity of DNA, i.e. in its solvation shell leads to immediate hole-transfer to the DNA and is considered as damage of the direct-type as well. Energy is also deposited into the bulk water outside the DNA solvation shells. DNA damage due to attack by radicals produced in the bulk water is usually referred to as indirect effect [117]. Most reactive species originate from water radiolysis into hydrated electrons (e\textsubscript{aq}\textsuperscript{−}), free hydrogen atoms (H\textsuperscript{∙}) and hydroxyl (\textsuperscript{∗}OH) radicals [17,118,119].

DNA damage can be influenced by radiation quality, dose, dose rate, etc. [120]. In contrast to low Linear Energy Transfer (LET) radiation such as γ-photons, high LET heavy-ions at Bragg peak energies lead to more complex track structures leading to biological havoc in organisms, tissues, cells, and DNA [120,121]. Milligan et al. [122,123] have measured SSB yields induced by sparsely ionizing radiation (662 keV photons) for doses ranging from 0 to 100 Gy and low or high scavenger concentrations. Their results showed that SSB are due to single energy deposition events manifesting in a linear dose response over a wide dose range.

Jones and coworkers [113] have compared SSB and DSB induction by low LET (\textsuperscript{137}Cs γ-photons) and high LET (MeV \textsuperscript{4}He ions) irradiation as a function of scavenger concentration. At high scavenger concentration and equal dose they observed higher DSB yields for the high LET radiation, suggesting a qualitatively different damage mechanism active in case of \textsuperscript{4}He irradiation. This result suggests that high LET radiation leads to formation of locally multiply damaged DNA (clustered lesions). Similar results have been found by Stankus \textit{et al.} [124] for neutron irradiation of plasmid DNA in aqueous solution. In a very comprehensive study Taucher-Scholz and Kraft [125] measured plasmid SSB and DSB for irradiation of a variety of fast ions and for X-rays as a function of energy. (Note that in this study the influence of scavenger concentration was not investigated.) It was later shown that the key features observed in their survey could be explained using a track structure model based on the local dose deposited [57]. Most recently, Elsässer \textit{et al.} [126] introduced an improved local effect track structure model to accurately describe the biological effects of carbon ion beams \textit{in vivo} and \textit{in vitro}. On the other hand, it was shown by Beuve [127] that a model strictly based on the local dose cannot reproduce all features of cell survival curves for low LET irradiation. Fuquan and coworkers [128] could show that a random breakage model can conveniently describe the length distribution of short linear plasmid fragments at high LET only in the high dose regime.

It is the goal of this study, to quantify the "non-scaveng-able" part of plasmid DNA damage by heavy ions at spread-out Bragg peak energies.
DNA damage induced by $^{12}$C ions under spread-out Bragg peak conditions (high LET radiation) or by $^{137}$Cs $\gamma$-photons (low LET radiation). The non-scavengable DSB yield is due to direct effects and can thus be attributed e.g. to clustered lesions.

To this end, DNA damage is quantified as a function of scavenger concentration up to levels where saturation of the damage is observed i.e., for doses of 300 Gy. The dose dependency of the damage is then compared for an essentially scavenger-free solution and for the high scavenger concentration where saturation is observed. The experimentally observed plasmid damage is then analyzed using the same model as Leloup and coworkers did [58].

5.2 Results

5.2.1 $\gamma$ photon irradiation

![Figure 5.1: Gel electrophoresis image of DNA after irradiation by $\gamma$ photons at various doses without mannitol. OC: Open circular, L: Linear, SC: Supercoiled, D: Digestion.](image)

Figure 5.1 shows typical results from the gel electrophoresis analysis for 0-300 Gy plasmid irradiation by $\gamma$ photons irradiation (low LET) without the presence of radical scavengers. The digested sample (D) and the control sample (0) are included for comparison. For the irradiated samples, the dose in Gy is given. It is obvious that the supercoiled plasmids (SC) are completely lost already above 10 Gy. Above 60 Gy, the overall fluorescence is strongly decreasing.

Figure 5.2 displays a quantitative analysis of the data in fig. 5.1. 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 sharp increase with dose until a maximum is reached at around 10 Gy. For higher doses,
the SSB fraction decreases monotonically. Moreover, short linear DNA fragments appear for doses exceeding 180 Gy. The overall decrease of the SC, OC and L signals with dose in fig. 5.1 shows that at high dose short linear fragments are efficiently formed which are not detectable on the gel. This is due to the statistically distributed fragment sizes which result in a very broad, low intensity fluorescence signal. Further more, these fragments will move more rapidly than full length linear strands and may be lost due to migration out of the studied area of the gel. The fraction of the OC form dramatically increases from 5% to over 90% and the fraction of the L form increases up to 80% with the increasing dose in the absence of mannitol.

In a second experiment, with 600 mmol/l mannitol, the fractions OC and SC forms are shown as a function of dose. The results are depicted in figure 5.3. The radiation damage to the plasmids was also studied as a function of mannitol concentration for a fixed dose. The quantitative results are displayed in figure 5.4. Compared to the situation where no scavenger is present (Figure 5.2), the DNA damage yields are very strongly reduced. No linear plasmids are observed at all for 600 mmol/l mannitol. This implies that efficient scavenging of *OH radicals completely suppresses the formation of L to undetectable levels. The trend saturates above 200 mmol/l mannitol. Furthermore, also induction of OC is strongly reduced. At 300 Gy the fraction

Figure 5.2: Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after gamma irradiation as a function of dose (without Mannitol). The solid lines are the fits to the data using the model of Cowan et al. [103].
5.2 Results

**Figure 5.3:** Relative yields of supercoiled (SC) and open circular (OC) plasmid DNA after gamma irradiation as a function of dose (with 600 mmol/l of Mannitol). The lines are to guide the eye.

**Figure 5.4:** Relative yields of supercoiled (SC), open circular (OC) and linear (L) DNA after 300 Gy gamma irradiation as a function of Mannitol concentration. The lines are to guide the eye.
of supercoiled plasmids still exceeds 80%. Previous studies showed that for dilute aqueous solutions as studied here, almost 100% of the $\gamma$ induced DNA damage is due to indirect effects, i.e. mainly the action of $\cdot OH$ radicals [129, 130]. (Note, that for the high scavenger concentration present in cellular systems, the indirect effect only accounts for 60-70 % of the induced damage [131, 132].) The fact that our results show incomplete suppression of the OC channel thus implies incomplete scavenging at the 600 mmol/l mannitol (about 1 mannitol molecule per 100 water molecules).

5.2.2 $^{12}$C irradiation (spread out Bragg peak)

![Figure 5.5: Gel electrophoresis image of DNA irradiation by $^{12}$C ions at various doses in the absence of mannitol. OC: Open circular, L: Linear, SC: Supercoiled, D: Digestion, * : sample stored in refrigerator at 4°C and without any irradiation.](image)

The experiments of the plasmid DNA under C$^{6+}$ irradiation are similar to the ones mentioned in previous chapter. Only different is the maximum dose rate with 60 Gy/min. Figure 5.5 shows the gel electrophoresis images of DNA irradiated by $^{12}$C ions at different doses in the absence of radical scavengers. Similar to the case of $\gamma$ rays, the fraction of supercoiled plasmids quickly drops and is virtually zero around 60 Gy. 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 as made for $^{12}$C irradiations are shown in fig. 5.6.

In contrast to the photon results, a 600 mmol/l mannitol solution does not fully suppress L formation (fig. 5.7) and also OC formation is suppressed to a lesser extent. At 300 Gy, about 0.1 L and about 0.6 OC are observed (fig. 5.8), indicating the relevance of direct effects. The scavenger effect saturates again above 200 mmol/l
Figure 5.6: Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after $^{12}$C irradiation as a function of dose (without Mannitol). The solid lines are fit to the data using the model of Cowan et al. [103].

Figure 5.7: Relative yields of supercoiled (SC), open circular (OC) and linear (L) plasmid DNA after $^{12}$C irradiation 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].
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Figure 5.8: Relative yields of supercoiled (SC), open circular (OC) and linear (L) DNA after 300 Gy $^{12}$C irradiation as a function of Mannitol concentration. The lines are to guide the eye.

mannitol. It is the same observation for the case irradiation of plasmid DNA with carbon beam in plateau region (chapter 4) and gamma rays.

The results observed for irradiation in the absence of a scavenger are counterintuitive. Whereas for photon irradiation already at 180 Gy only linear or short linear fragments are observed, $^{12}$C irradiation even at 300 Gy only leads to 50% linear fragments and 50% of plasmids in the OC form. The apparent relatively weaker effect of the $^{12}$C ions is due to the inhomogeneous (spiked) dose distribution of heavy ions [133, 134]. Due to the high LET, the energy deposition is localized in the small volume of the relatively few tracks. A plasmid crossed by a $^{12}$C track thus suffers substantial damage that often involves multiple sites. On the other hand, a large fraction of plasmids are not crossed by a track and remain intact.

5.3 Discussion and conclusion

In the following, the yields of SSB and DSB per plasmid per Gy from the experiment data are intergrated from the fitting curves using the model by Cowan et al. [103] as described in chapter 4.

Fitting the data for photon and carbon irradiation (see figs. 5.2 and 5.6) in the
5.3 Discussion and conclusion

Table 5.1: SSB per plasmid per Gy (µ) and DSB per plasmid per Gy (φ) yields after irradiation with photons and C ions.

<table>
<thead>
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<th>Yields/(plasmid/Gy)</th>
<th>Photon irradiation</th>
<th>Carbon irradiation</th>
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<tbody>
<tr>
<td>µ</td>
<td>0.46 ± 0.03</td>
<td>0.062 ± 0.008</td>
</tr>
<tr>
<td>φ</td>
<td>0.006 ± 0.002</td>
<td>0.0029 ± 0.0003</td>
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absence of mannitol by the model of Cowan et. al with those equations 4.1, 4.2 and 4.3 leads to µ and φ as shown in table 5.1. Clearly, photon irradiation is very efficient in single strand break induction µ = 0.46/plasmid/Gy whereas for carbon ions at Bragg peak energies, µ = 0.062/plasmid/Gy, i.e. almost one order of magnitude less single strand breaks are induced. Despite this difference between both radiation modalities, the values of φ differ by only a factor of 2.

In the presence of mannitol, DNA damage induced by high-LET radiation is more serious than that by low-LET radiation. The results for γ rays at zero concentration (fig. 5.2) show that DNA is very efficiently damaged since the SC form of DNA disappears at the dose of about 10 Gy and the fraction of linear form DNA increases with increasing dose. This is probably the result of the interaction between DNA and free radical ∙OH. Our samples contain about 5.5×10⁹ plasmids per 10²⁰ water molecules. We are thus dealing with a dilute solution where a plasmid on average occupies 1 (µm)³. For typical ∙OH diffusion length of about 10 nm [133] in the absence of scavengers, we can thus assume that each plasmid only interacts with radicals in its direct neighborhood.

As is obvious from fig. 5.3, for high scavenger concentration the linear plasmid fragments vanish in the dilute aqueous solution even for high dose, and the percentage of OC plasmids decreases strongly (OC (10 Gy) = 0.9 at zero scavenger concentration and OC (10 Gy) = 0.1 for 600 mmol/l Mannitol). This implies that the interaction with free radicals is the main cause of DNA damage for low LET radiation. DSB induction, i.e. formation of linear fragments is even completely quenched at high mannitol concentrations.

In table 5.2, the values for µ and φ are tabulated for different concentrations of scavenger for both γ and carbon irradiation. In case of γ irradiation with mannitol concentration exceeding 250 mmol/l, no L forms are observable. Thus, we do not fit the equations to these data.

Comparing figures 5.2 and 5.6, it is found that in the absence of scavengers the radiation damage leading to OC and L plasmids after irradiation by gamma photons is higher than for irradiation with ¹²C ions. The maximum fraction of L plasmids...
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<th>Mannitol concentration (mmol/l)</th>
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<tr>
<td></td>
<td>µ (/plasmid/Gy)</td>
<td>φ (/plasmid/Gy)</td>
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Table 5.2: SSB per plasmid per Gy (φ) and DSB per plasmid per Gy (κ) yields after irradiation with photons and C ions at different mannitol concentrations.

Plasmid DNA damage by heavy ions at spread-out Bragg peak energies.
achieved by high-LET at 60 Gy is about 10% which is 4 times smaller than in case of low-LET radiation. For low LET gamma irradiation the dose is uniformly distributed over the whole sample [135]. Under \(^{12}\)C ions radiation, the situation is different due to the confined track structure of high-LET \(^{12}\)C ions [133]. The secondary electrons and free radicals induced by \(^{12}\)C ions are localized near the tracks [74]. The concentrations of secondary electrons and free radicals reduce exponentially with the radial distance from the path of the ions. Thus the probability of interaction between DNA and ions reduces exponentially with the radial distance from the tracks and only DNA close to the track structure can be damaged. For high-LET radiation, as shown in Figures 5.5 and 5.8, the interaction with DNA is a combined effect of direct damage and the indirect effect due to free radicals [54,136,137] in contrast to the situation for low LET irradiation. In the presence of a high concentration of scavengers, also for \(^{12}\)C ions the radical action is almost totally suppressed. However, OC and L plasmids induced by direct action still occur and increase with dose. The direct action accounts for 15% because \(L_{\text{max}} = 0.53\) without scavenger and \(L_{\text{max}} = 0.08\) with 600 mmol/l scavenger at 300 Gy.

To compare our data with existing results, we have converted the \(\phi\)-values and \(\mu\)-values from tables 5.1 and 5.2 to a \(\text{Gy}^{-1}\text{Da}^{-1}\) scale and the scavenger concentration to a scavenging capacity. This way data obtained with different plasmids and different scavengers can be directly compared (see fig. 5.9). Note that we estimated the scavenging capacity at zero mannitol concentration as \(2 \times 10^5 \text{s}^{-1}\) due to remaining buffer in the samples. It is obvious that for lower LET radiation (\(^{60}\)Co \(\gamma\)-radiation and \(^{137}\)Cs \(\gamma\)-radiation, but also 26 MeV He ions and fission neutrons) single strand break yields (\(\mu\)) and double strand break yields (\(\phi\)) follow roughly the same linear dependence on scavenging capacity, respectively. The dashed lines are drawn just to guide the eye. As pointed out before this implies that in dilute aqueous solution almost all damage induced by lower LET radiation can be scavenged.

For SOBP carbon ions, the damage dependence on scavenging capacity is completely different. \(\phi\) and \(\mu\) decrease with scavenging capacity only up till capacities of about \(10^8 \text{s}^{-1}\). Higher scavenging capacities leave the yields unaffected. The dotted lines indicate the asymptote of both curves. Obviously the very high LET of the SOBP carbon ions induces a large fraction of damage that cannot be scavenged (\(\mu \approx 10^{-9} \text{Gy}^{-1}\text{Da}^{-1}\) and \(\phi \approx 10^{-10} \text{Gy}^{-1}\text{Da}^{-1}\)). This clearly implies that the respective damage is of direct nature and could e.g. be due to clustered DNA lesions induced by single SOBP carbon ions.

In conclusion, plasmid DNA was irradiated by \(^{12}\)C ions and \(\gamma\) rays, either in pure water or pure water with different scavenger concentrations, and analyzed by gel electrophoresis. The results show that at low scavenging capacity DNA is substantially damaged by both low- and high-LET radiations. In the presence of the scavenger
mannitol, only for SOBP carbon ions a substantial yield of L plasmids remains and increases with increasing dose. This yield is due to direct DNA damage, e.g. clustered lesions. For low LET radiation, the L plasmids formation is found to be completely due to radical action and can thus be efficiently suppressed by scavengers.

Figure 5.9: SSB and DSB yields induced by $^{60}$Co, $^{137}$Cs, helium nuclei, carbon, and fission neutron irradiation. Comparison of DNA breakage yields measured by a: our data; b: Leloup et al. [58]; c: Stankus et al. [124]. The lines are to guide the eye.