Particle induced strand breakage in plasmid DNA

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CHAPTER 7

Summary

This thesis aims at a better understanding of heavy ion induced biological damage on the molecular level, with DNA being the cellular component most relevant for radiation induced cell-killing, plasmids (see fig. 7.1) were chosen as ideal objects for this endeavor.

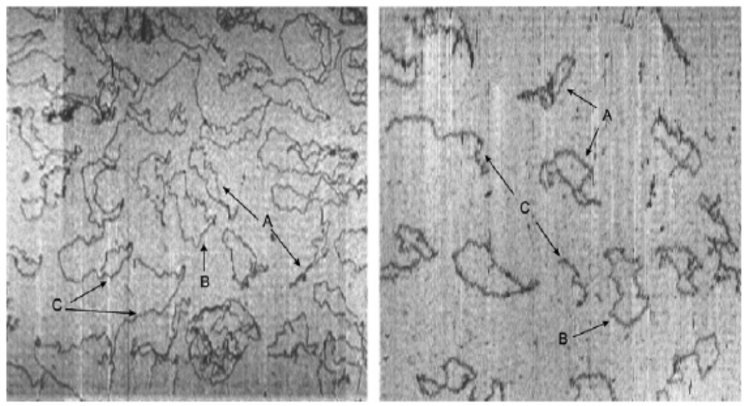

Figure 7.1: *Typical AFM images ($3\times3\,\mu m^2$) of supercoiled ΦX174 plasmid DNA irradiated with 250 Gy: X-rays (left panel) and 3.5 MeV/u Ni ions (right panel). Molecules in different conformations are indicated with arrows: supercoiled, A; relaxed, B; linear, C [54].*

In the course of this thesis, heavy ion irradiations at MeV energies have been performed using the AGOR cyclotron (Accélérateur Groningen-ORsay) whereas for keV

energies on electron cyclotron resonance ion source was employed. Complementary studies on ^{137}Cs active on plasmids were performed at the University Medical Center Groningen (UMCG). The pBR322 plasmid DNA was used which is suitable for irradiation experiments because of its well defined size and relatively easy preparation in milligram quantities at high purity. As observables for DNA damage, single strand breaks (SSBs) and double strand breaks (DSBs) were chose, whose yields can be conveniently quantified by means of gel electrophoresis. Fig. 7.1 displays an atomic force microscopy image of the different plasmid configurations (intact–super coiled, SSB–circular, DSB–linear).

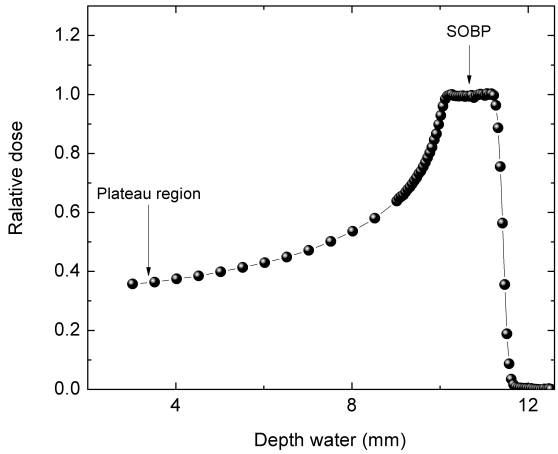

Figure 7.2: *Depth-dose distribution of modulated (circles) 90 MeV/u ^{12}C beam, featuring the spread-out Bragg peak (SOBP). Lines are drawn to guide the eye. The two arrows indicate the sample location for irradiation at the plateau of the Bragg curve (40 $\pm$ 5 keV/μm) and at the SOBP (189 $\pm$ 15 keV/μm).*

First series of experiment were carried out for C kinetic energies corresponding to two different regions of the Bragg curve, either in the entrance (plateau) location or in the Spread Out Bragg Peak–SOBP (fig. 7.2). 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. Moreover, we could also determine the amount of non-scavengable plasmid damage at high scavenging capacities from our data. Surprisingly not only DSB but also a sizable fraction of SSB are due to direct effects.

Chapter 5 presents a comparative study of heavy ion and γ-photon induced damage to plasmid DNA. Plasmid DNA was irradiated by ^{12}C ions and γ rays, either in pure water or pure water with different scavenger concentrations, and analyzed by gel electrophoresis. At low scavenging capacity DNA is substantially damaged by both low- and high-LET radiations. In the presence of the scavenger mannitol mimicking cellular conditions, only for SOBP carbon ions a substantial yield of linear–L plasmids remains that 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.

In chapter 6 it is shown that keV ions can efficiently induced SSB and DSB in plasmid DNA thin films. Even though for a given ion kinetic energy, H^+ and He^{2+} ions have similar LETs, at identical dose about 5 times more DSBs are induced by He^{2+} ions. This finding hints at a strong contribution of nuclear stopping, since simulations showed that He^{2+} ions are about 5 times more efficient in direct atom knock out from the sample. For C^{q+} ions on the other hand, simulations predict very similar rates of vacancy production as compared to He^{2+}. However, DSB induction is found to increase by 1–2 orders of magnitude. We tentatively attribute this effect to the higher LET which could lead to increase complexity of local damage and higher DSB yields. Also, for C^{q+} ions, energetic recoils could induced further damage.

Thin film production, controlled irradiation, collection and analysis of the irradiated sample can be performed in a reproducible fashion. For the future, systematic studies on the influence of charge state and kinetic energy would certainly allow a deeper understanding on the damage process and in particular the interplay of electronic and nuclear stopping.

This thesis has not only resulted in a deeper insight into the molecular mechanisms underlying heavy ion therapy, it has also opened up new questions.

- Here, we investigated the response of plasmid DNA upon heavy ion impact. This simplified model helps understanding the first steps of DNA damage. However, to have a link to the real biological environment, experiments in vitro and in vivo are required to study for instance the influence of repair mechanism.

- Experiments at few keV or sub-keV ion kinetic energies could allow investigation of the role of nuclear stopping in more detail.

- Atomic Force Microscope (AFM) analysis of irradiated plasmids in order to analyze the fraction of short linear fragments due to multiple double strand break or clustered damage for more complete quantification of the damage.

- It is thought that the introduction of heavy atoms into a tumor prior to irradiation changes the resulting damage due to significant increases in the induction

of clustered lesions. To look closer into this issue, high atomic number (Z) materials such as gold or titanium could be added to the plasmid solvent.