1.1. General introduction

When ionizing radiation crosses a living cell, ionization of biologically relevant molecules such as water as well as DNA constituents occurs. These processes are the initial steps in biological radiation damage [1]. Secondary particles such as low energy electrons, radicals and singly/multiply charged ions are formed along the track. To a large extent the biological damage induced is due to the action of these secondary particles. Processes where the secondary particles are radicals (e.g. water radiolysis) that chemically attack DNA are classified as indirect damage. Whereas most of all other (primary and secondary) particle induced damage is classified as direct. This thesis deals exclusively with direct damage. In this context, ions can be of relevance not only as primary but also as secondary particles. As secondary species, for instance it can happen that molecules within a cell are subject to core ionization by a primary particle. Subsequent Auger-cascades lead to formation of multiply charged ions. These ions in turn can interact with DNA constituents.

As primary particles, high energy proton and heavy ion beams are becoming a particularly effective tool in cancer therapy [2, 3]. For example fast protons are successfully used in the treatment of small tumors localized near critical structures, e.g. uveal melanoma [4] and olfactory neuroblastoma [5]. On the other hand, \(C^{q+}\) ion radiotherapy offers an effective treatment option for skull based chordomas [6] and localized prostate cancer [7]. A technical advantage of heavy–ion therapy is the smaller beam widening in tissue, leading to better control. Other sorts of light-ions like lithium or beryllium have been proposed as an even better treatment because they cause less detrimental biologic effects to normal tissues [8]. Furthermore, radiation in the form of heavy particles is also found in the natural environment. From environmental studies, it is known that inhaled radon and its decay products emit energetic alpha particles (He\(^{2+}\)) which in turn have large damage potential to tissue [9, 10]. In space,
Figure 1.1: Comparison of the depth–dose distribution of photons and carbon ions. With photons the dose decreases exponentially with increasing depth. Carbon ions dispose of an inverse dose profile, i.e. the dose increases with increasing penetration depth. This profile can be shifted by energy variation over the target volume. The figure is courtesy of D. Schardt, GSI.

the alpha particle and proton components of solar flares represent a hazard for manned space missions [11, 12]. For example the solar particle event of August 1972 could have delivered severe doses in an extended period of time to the astronauts even inside a spacecraft [13].

Depth–dose distributions for photons at different energies and $^{12}$C-ions are shown in fig. 1.1. Compared to other kinds of ionizing radiation such as electrons or photons, atomic particles such as $^{12}$C-ions and protons have a different depth distribution of the deposited dose, peaking at an energy-dependent depth (the Bragg peak) at the end of the particle’s track and dropping to zero beyond this peak. It is the volume selectivity given by the existence of a well localized Bragg-peak region, where the deposited dose is maximum, that makes proton and heavy ion therapy such a promising technique in cancer treatment.

The shape of the Bragg peak can be understood by looking at the velocity term $V$ in the non-relativistic stopping power equation of Bethe [14]:

$$-\frac{dE}{dx} = \frac{4\pi e^4 Z^2 N}{mV^2} \ln \left( \frac{2mV^2}{I} \right)$$

(1.1)

where $e$ and $m$ are the charge and mass of the electron, $Z$ and $V$ are the charge and velocity of the incident particle, and $N$ and $I$ are the electron density and the mean excitation potential of the medium. It can be seen that with decreasing $V$ the stopping power increases to a maximum and then decreases sharply at lower velocities. This stopping power formula accounts for
1.1 General introduction

inelastic atom-electron collisions. These collisions are statistical in nature, and they occur with a certain quantum mechanical probability. Eq. 1.1 gives the average rate of energy loss per unit path length for a non-relativistic charged particle. Taking the average is valid since the number of collisions per macroscopic path length is large, so that the total energy loss is small. The stopping power was first calculated by Bohr using classical arguments and later by Bethe, Bloch and others using quantum mechanics. Eq. 1.1 was obtained under the first Born approximation and under the assumption that the projectile velocity is much larger than the orbital speeds of the target electrons. In reality, the stopping of heavy ions is a much complex process and the Bethe formula in eq. 1.1 does not reflect the microscopic nature of the process. Additional terms have to be added in order to get an accurate description. Nevertheless the formula shown gives a good phenomenological approximation [15].

In the Bragg-peak region, the ions are slowed down to MeV, keV energies and below. Due to the high linear energy transfer of the particles at those energies, the density of ionization events is very high along the particle’s track resulting in a reduced cellular repair rate. Accordingly, not only the deposited dose is maximum but also the relative biological effectiveness (RBE) is enhanced at the Bragg peak [16]. It is generally accepted that the main target for radiation damage to cells is the DNA. DNA is a large double stranded macromolecule made up of a large number of deoxyribonucleotides. Each of them is composed of a base, a sugar and a phosphate group. The nucleobases in DNA carry the genetic information whereas the sugar and the phosphate group constitute the backbone of each strand. The complementary strands are linked by selective hydrogen bonds between the nucleobases, forming Watson–Crick pairs. A schematic view of the double stranded DNA is shown in the upper part of fig. 1.2. When ionizing radiation interacts with DNA in a direct or indirect way (via radicals) several lesions can occur in the form of base damage or strand breaks. Within the living cell a complex repair machinery operates in order to maintain the integrity of DNA. Some of the lesions can be repaired but if the damage is very severe, it can be lethal to the cell. The connection between the initial lesions and the cellular end points is not fully understood [17].

The damage inflicted upon DNA can be classified as either Single Strand Breaks (SSBs), Double Strand Breaks (DSBs) or clustered lesions. In living cells, SSBs have large probability of being repaired without further consequences leading to a healthy cell, but they can also be mis-repaired or completely not repaired leading to cell death. DSBs are more difficult to repair, their mis-repair can cause mutations or even cell death. Furthermore, multiple close-lying ionization events along the particle’s track can form complex clusters of lesions which are even more difficult to repair [18]. A scheme with the different kinds of strand breaks and the possible pathways that can follow is shown in fig. 1.2. It was shown that low doses of radiation can induce clustered lesions in human cells [19]. Studies on plasmid DNA have revealed that keV Ar$^+$ ions can induce SSBs and DSBs [20] and, very recently it was shown that C$^+$ ions of keV energies can induce multiple DSBs in plasmid DNA [21].

A first approach to understand strand breaks at the molecular level is to study the fragmentation and energetics of individual building blocks under different kinds of particle irradiation in the region of the Bragg-peak. The velocity range relevant for the Bragg peak corresponds to projectile energies from 0 to a few hundred keV/amu. In this range, collisions involving atomic particles are very complex, since their velocities are similar to the typical velocities of molecular valence electrons. Until very recently, it was commonly believed that the main
differences between irradiation with atomic particles and with electrons/photons lied in the different track structures and in the higher density of ionization events along the track in the case of atomic particles. For a biologically relevant medium such as liquid water, the effect of a (sub)-keV atomic particle moving through that medium implies screening and charge exchange processes which dynamically alter the particle’s effective charge. Monte-Carlo track-structure calculations for full stopping of low energy alpha particles in water including all interactions present in the media predict that at keV energies, helium is predominantly found in its neutral state [22]. Cross sections for protons and hydrogen in water vapor reveal the importance of the interplay between electron capture and electron loss in this range of energies [23, 24]. Furthermore, it was very recently shown in calculations that at low kinetic energy, neutralized protons have a large contribution to the direct ionization of H₂O molecules in the liquid phase [25].

Recent studies have focussed on interactions of ions with kinetic energies in the keV range since these energies are relevant for heavy ion induced biological radiation damage in the region of the Bragg peak. There are indications that in the Bragg peak region, heavy ions have the potential to induce very complex damage to DNA [26]. Even at keV ion energies (typical for the Bragg-peak region) collisions with isolated nucleobases lead to fragment ions whose kinetic energies can easily exceed 10 eV [27,28]. Such energetic secondary ions in turn can induce further molecular fragmentation of DNA building blocks [29,30]. The interactions of singly and multiply charged ions with the isolated nucleobases uracil [31, 32], thymine [27,28] and adenine [33] have been addressed by means of gas-phase collision studies. It has been observed that the dissociation dynamics depend strongly on the projectile ions’ electronic structure and velocity as well as on the properties of the target molecule. Until now, such gas-phase studies have been limited to charged projectiles. In this thesis for the first time, results on fragmentation induced by neutral projectiles will be presented, too.
Gas phase studies allow a detailed exploration of the molecular mechanisms involved, but by nature neglect any effects of the chemical environment such as modified ionization energies or dissipation of the excitation energy. The relevance of gas phase studies for biomolecular radiation damage, however, relies on the assumption that the fundamental ionization and dissociation dynamics are similar for isolated molecules and for molecules embedded in a biological environment. In comparison in vivo and in vitro experiments of biomolecular radiation damage inherently include the environment effects. In this thesis a first step towards a realistic environment was done by investigation of ion irradiation of clusters of biomolecules.

1.2. Outline

The general motivation of our work has been addressed. In the following chapter an overview of the different experimental techniques used will be given. In chapter 3, the theoretical concepts underlying processes studied in this thesis will be briefly described and some methods used to study the electronic structure of the molecules will be summarized. In chapter 4–7, the experimental results that gave rise to several publications will be presented and extensively discussed. More specific, in chapter 4 the different dissociation channels of water molecules after the impact of alpha particles and protons will be discussed. In chapter 5 experimental results on collisions of neutral projectiles with nucleobases will be shown. In chapter 6 the statistical fragmentation of deoxyribose molecules after ion impact will be discussed. Chapter 7 deals with the effects of ion interactions with clusters of nucleobases. Chapter 8 summarizes the conclusions and outlook of this thesis. Finally the summary of the thesis is given in Dutch and Spanish.