Particle induced fragmentation of nucleobases

The first gas phase studies on molecular mechanisms underlying biological radiation damage were performed using nucleobases. Together with the deoxyribose and phosphate groups, the nitrogenous bases constitute the basic components of DNA. The DNA bases thymine, adenine, guanine and cytosine together with the RNA base uracil codify the genetic information in cells. In this chapter, for the first time experimental data on molecular fragmentation of the nucleobase adenine following neutral projectile impact are presented. The results obtained for atomic particle collisions are compared to measurements with ionic projectiles. The appearance of surprisingly long lived metastable adenine cations, particularly strong for neutral projectile collisions, is reported for the first time and a two-step fragmentation process is suggested to explain their appearance.

based on:

*Interactions of neutral and singly charged KeV atomic particles with gas phase adenine molecules*
F. Alvarado, S. Bari, R. Hoekstra, and T. Schlathölter.

*Ion-induced ionization and fragmentation of DNA building blocks*
T. Schlathölter, F. Alvarado, S. Bari and R. Hoekstra.

*Ion-biomolecule interactions and radiation damage*
T. Schlathölter, F. Alvarado, and R. Hoekstra.
5.1. How it started

In their pioneering studies on the effects of secondary particles on DNA, Sanche and coworkers showed that low energy electrons (3-20 eV) can cause single and double strand breaks of plasmid DNA [82, 83]. They also demonstrated a strong correlation between the formation of these strand breaks, particularly their energy dependence, and the decays (via electron detachment or dissociation along one or several bonds) of transient molecular anions produced by electron attachment on the basic DNA components [84, 85]. It was shown that even electrons with kinetic energies down to zero were able to induce single strand breaks with pronounced maxima between 0 and 3 eV [86]. Those results were in good agreement with resonances found for dissociative electron attachment to gas phase nucleobases [87–89]. From there on it became clear that nucleobases are a relevant model target in the context of biological radiation damage.

It is well known that low energy electrons are the most abundant secondary species produced along a particles track. However, other secondary processes occur along the path of a charged particle, such as radical production and formation of slow secondary ions. Whereas low energy electrons mainly induce easily repairable SSBs in DNA, slow ions carry sufficient potential and kinetic energy to induce more relevant biological damage e.g clustered lesions.

Our studies on the interaction of keV ions with isolated DNA/RNA building blocks started with the nucleobases thymine (C₅H₆N₂O₂) and uracil (C₄H₄N₂O₂). These studies revealed that fragmentation patterns and fragment formation are strongly influenced by the projectile characteristics. The dependence of ionization and fragmentation of thymine and uracil on type, charge state q and velocity v of the projectiles have been intensively studied [27, 28, 31, 90]. It was shown that the electronic structure of the projectile plays an important role in molecular fragmentation. On the other hand the dependence of the fragmentation cross sections on projectile velocity was found to be weak. Our previous studies also showed that the fragmentation processes depend strongly on the target molecule [91].

For the isolated nucleobases uracil and thymine the response to the interaction can be divided into three regimes: i) non-dissociative ionization (mainly due to electron capture), ii) two-body break up and iii) multi-fragmentation. The fragments originating from the collisions can have energies in the order of 10 eV for C⁹⁺ projectiles [27] and of the order of 100 eV for Xe²⁵⁺ projectiles [90]. Deng et al. found that even ions with kinetic energies in this order (10-200 eV) can produce damage to fundamental building blocks of DNA in films [29]. Their results indicate that usual models of biological radiation damage might underestimate the damage at the end of the Bragg peak [26].

In fig. 5.1 the molecular structures of the five nitrogenous bases are given. Adenine and guanine belong to the class of purine derivatives while thymine, cytosine and uracil are pyrimidine derivatives. Being aromatic compounds, the molecules are mainly planar. The main difference between the structure of purines and pyrimidines is the number of rings present. In living systems, the main difference between purines and pyrimidines is the way they are synthesized from simple precursors: in order to form the purines rings 5 precursors are needed but to synthesize the pyrimidines ring, only two precursors are needed [92].
5.1 How it started

**purines**

- Adenine
- Guanine

**pyrimidines**

- Thymine
- Cytosine
- Uracil

Figure 5.1: Schematic representation of the molecular structure of the four DNA nucleobases adenine, thymine, guanine and cytosine and the RNA base uracil.
5.2. The nucleobase adenine

Adenine (C₅H₅N₅) is a purine derivative. Purines differ from pyrimidines because they contain not only the aromatic 6-membered ring characteristic for pyrimidines but fused to it also an imidazole ring. Previously studied nucleobases (thymine and uracil) were pyrimidines. The components of nucleobases are carbon, nitrogen, hydrogen and oxygen atoms. Adenine is the only nucleobase without oxygen atoms in its structure.

Gas phase adenine molecules were studied using ultraviolet photoelectron spectroscopy. There, the ionization potentials of the adenine orbitals were determined and conclusions on the most stable tautomeric structures were obtained [93]. Early studies using electron impact ionization of pyrimidines [94] and purines [95] gave the first hints of differences in fragmentation paths between purine and pyrimidine nucleobases. Studies on site selectively isotope labelled adenine compounds gave a better inside on the specific adenine fragmentation pathways [96]. Negative fragment ions have been investigated in high resolution experiments of electron attachment to gas phase adenine at electron energies up to 15 eV [97]. The dehydrogenation processes due to electron attachment for very low energies (<3 eV) were also studied [98]. To our knowledge the only studies of interactions of ions with adenine molecules were carried out very recently using 36 keV F²⁺ [33], 56 keV Ar⁸⁺ [99] and 100 keV protons [100].
5.3 Adenine fragmentation by alpha particles

Fig. 5.2 shows two typical fragmentation spectra of adenine after collisions with He\(^+\) and He\(^{2+}\). The main characteristics of nucleobase spectra in general are 1) the relatively strong peak of the surviving parent molecule (in this case ade\(^+\) at \(m/z = 135\)) and 2) the clearly defined groups of fragment peaks numbered from 1 to 10 on top of the figure. Each of these groups is due to fragments with a fixed number of “heavy” atoms (carbon and nitrogen for adenine) and a variable number of hydrogen atoms. Similar characteristics were observed for uracil [31] and for thymine [27] after irradiation with carbon and other projectile ions [91].

A remarkable fact that has been also observed for other nucleobases is the selectivity in bond breakage. As can be seen, the yields of the group containing 7 heavy atoms (\(m/z \approx 92\)) are very low whereas fragments containing 9 heavy atoms (\(m/z \approx 118\)) are not observed at all. This implies that losing only one nitrogen or carbon atom with or without different numbers of hydrogens is not a viable fragmentation path. Note that while these groups are weak or absent for the isolated nucleobase, they are observed in the fragmentation spectra of adenine clusters where intermolecular hydrogen bonds weaken intramolecular bonds and thus influence fragmentation (chapter 7). On the other hand, although weak, the loss of one hydrogen was observed for adenine molecules after interaction with the different projectiles, in contrast to uracil where it was not observed at all. For thymine molecules the loss of one and even two protons was observed.

It can be seen that for He\(^+\) the fragmentation is more extensive as compared to He\(^{2+}\). This can be attributed to the projectile electronic structure. For He\(^+\) the resonant electron capture channel is closed. Electron capture can only occur accompanied by the deposition of large amounts of energy that will make the collision very violent. On the other hand He\(^{2+}\) has available levels into which resonant or quasi-resonant capture of electrons from the target molecule can occur. The possibility of gentle capture gives rise to larger amounts of surviving molecules. This will be discussed in detail later in this chapter.

Doubly charged surviving molecules appear as a strong channel for both projectiles. They have also been observed for other nucleobases. This reflects the capacity of the molecule to redistribute the charge without breaking any bonds. Small peaks of doubly charged nitrogen and oxygen observed in both cases are attributed to ionization of residual gases (N\(_2\), O\(_2\) and water vapor).

5.4 From charged to neutral projectiles

In fig. 5.3 mass spectra for 14 keV charged and neutral projectiles are presented. The spectra are normalized to the peak maximum of the parent molecule. Although intensities differ, the same groups of peaks appear in all spectra. No peaks or groups of peaks are specific to one of the projectiles which indicates that the possible fragmentation pathways depend neither on the type of projectile nor on the projectile’s electronic structure. Only the intensity of the different peaks, \textit{i.e.} the branching ratios, depend on the projectile. Note that the total ionization cross section is much larger for charged projectiles as compared to neutral ones but as the spectra are normalized to the parent peak, this effect does not show in fig. 5.3.

The shapes of the adenine spectra shown in fig. 5.3 are typical for nucleobases as pointed
Figure 5.3: Mass spectra of adenine after collisions with 14 keV $H^+$ and $H^0$, 14 keV $He^+$ and $He^0$ and 14 keV $C^+$ and $C^0$. The numbers on top of the upper panels represent the number of heavy elements (C or N) in each group of peaks.
out for the case of alpha particles. The groups of peaks for charged and neutral projectiles are labelled on top of the panels in fig. 5.3. A quantitative view on the details of the spectra will be given in the following sections.

5.4.1. Formation of adenine$^{2+}$

In all the spectra for adenine, remarkably enough also in those obtained from collisions with neutral projectiles, the doubly charged parent molecule is observed, indicating a high stability of the doubly charged adenine molecule. Zooms into the spectra around the doubly charged adenine peak appearing at 67.5 (group 5) are displayed in fig. 5.4. The spectra are normalized to the ade$^{+}$ yield. It can be seen that for hydrogen projectiles, the peaks for the ade$^{2+}$ are of equal relative intensity ($\approx 0.05$, see grey dotted stripe in fig. 5.4). Similar relative intensities around 0.05 are found for C$^{0}$ and C$^{+}$, and He$^{0}$ projectiles. The only exception is the He$^{+}$ projectile, for which the relative ade$^{2+}$ yield is three times higher.

5.4.2. The dominant fragments

In contrast to pyrimidine nucleobases, such as uracil and thymine, the fragmentation of the purine adenine mainly proceeds along specific pathways. This is partly due to the more homogeneous molecular composition of adenine: it lacks oxygen atoms. For H$^{+}$ and H$^{0}$ and C$^{+}$ and C$^{0}$ the dominant peaks in the spectra shown in fig. 5.3 correspond to the adenine parent molecule (ade$^{+}$) and ions with $m/z = 28$. A strong peak at $m/z = 28$ was also observed in early electron impact studies [95] and in photoionization studies [101] in which it was identified as the molecular fragment HCNH$^{+}$. A strong contribution of surviving adenine molecules was also observed in collisions with keV F$^{2+}$ ions, where a restriction to single electron capture events was made [33]. The contribution of residual gas in our experiments
Table 5.1: Relative yield of HCNH\(^+\) as compared to the parent molecular adenine ion for 14 keV ionic and neutral projectiles.

<table>
<thead>
<tr>
<th>Projectile</th>
<th>HCNH(^+)/ade(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(^+)</td>
<td>1.20±0.01</td>
</tr>
<tr>
<td>H(^0)</td>
<td>1.03±0.02</td>
</tr>
<tr>
<td>C(^+)</td>
<td>0.57±0.01</td>
</tr>
<tr>
<td>C(^0)</td>
<td>1.28±0.02</td>
</tr>
<tr>
<td>He(^+)</td>
<td>4.90±0.03</td>
</tr>
<tr>
<td>He(^0)</td>
<td>5.00±0.04</td>
</tr>
</tbody>
</table>

is negligible. We can thus rule out an appreciable contribution of N\(^2\)+ or CO\(^+\) from residual gas to the peak at \(m/z = 28\).

For He\(^+\) and He\(^0\) the HCNH\(^+\) peak clearly dominates the spectra, in contrast to the results for H and C projectiles where the ade\(^+\) also is a dominant peak in the spectra. In table 5.1 the ratios of the total integral of HCNH\(^+\) in comparison with the yield of the adenine parent molecule (ade\(^+\)) are given. It can be seen that for He projectiles the intensity of the HCNH\(^+\) peak is \(\sim5\) times larger than the parent molecule, hinting at a more violent fragmentation for this projectile both in its neutral and charged state.

It can already be seen from table 5.1 that when changing the charge state of the projectile from singly charged to neutral, the effect on HCNH\(^+\) formation is the strongest for carbon projectiles. After collisions with neutral carbon projectiles, the yield of this fragment is about two times larger than after collisions with the correspondent charged projectile. For H projectiles, when changing the charge state the intensity of this fragment slightly decreases with respect to the parent molecule.

5.4.3. The different groups

The neutral/charged projectile ratios of the total integrals of all the peaks within each of the groups labelled on the top panel in fig. 5.3 and normalized to the parent molecule (group 10) are plotted in fig. 5.5. From this figure, it is clear that the strongest difference between neutral and charged projectiles is observed for carbon projectiles. The interaction of a neutral carbon projectile with the adenine produces on average 3 times more small fragments (groups 1–5) than the interaction of a charged carbon projectile. For helium and hydrogen projectiles, the ratios are close to one for all groups, the only exception being the group containing 8 heavy atoms after collisions with the He projectiles (marked with a dashed circle in fig. 5.5). This group together with particular other fragments will be discussed in detail later.
5.4 From charged to neutral projectiles

5.4.4. Delayed fragmentation

In earlier adenine studies [95], it was shown that one of the characteristic fragmentation pathways for adenine after collisions with 70 eV electrons is the sequential loss of neutral HCN. Although the initial HCN loss requires at least two bond ruptures within the six-membered ring, this channel is known to be a major fragmentation pathway in many purine derivatives [96].

In fig. 5.3, the sequential loss of HCN is indicated with arrows. The channels corresponding to each HCN loss were assigned in photoionization studies [101] as follows:

\[
C_5H_5N_5^+ \rightarrow HCN + C_4H_4N_4^+ \quad (m/z = 108) \tag{5.1}
\]
\[
C_4H_4N_4^+ \rightarrow HCN + C_3H_3N_3^+ \quad (m/z = 81) \tag{5.2}
\]
\[
C_3H_3N_3^+ \rightarrow HCN + C_2H_2N_2^+ \quad (m/z = 54) \tag{5.3}
\]
\[
C_2H_2N_2^+ \rightarrow HCN + HCN^+ \quad (m/z = 27) \tag{5.4}
\]

Due to the neutral nature of the HCN, a direct observation of these channels is difficult. However, the complementary ion can be directly measured.

For the fragments likely to be associated with HCN loss, fig. 5.6 shows the ratio of their production by neutral and charged projectiles. It can be seen that for the fragments produced after collisions with H\(^0\) and H\(^+\) the ratios stay close to 1, i.e. the respective channels seem unaffected. The same trend was observed for all groups corresponding to these projectiles in fig. 5.5. For carbon projectiles, as already expected from fig. 5.5, an enhancement in the lower
mass fragments is observed. The fragments HCN\(^+\) \((m/z = 27)\) and C\(_2\)H\(_2\)N\(_2\)\(^+\) \((m/z = 54)\) are respectively 3 and 2 times stronger for the neutral projectiles than for the charged ones. For He\(^0\) the formation of the fragments C\(_4\)H\(_4\)N\(_4\)\(^+\) \((m/z = 108)\) and C\(_3\)H\(_3\)N\(_3\)\(^+\) \((m/z = 81)\) is respectively 2.5 and 1.8 times more probable than for He\(^+\). This could already be observed in fig. 5.5 especially for group 8 which strongly deviated from the general trend.

A zoom into group 6 and group 8 for the mass spectra after collisions with He projectiles is shown in fig. 5.7. The solid line corresponds to collisions with He\(^+\) while the dashed line shows the spectra after collisions with neutral He. It is clear that for collisions with neutral projectiles peaks appear at non–integer masses.

The appearance of such non–integer peaks at \(m/z = 81.7\) and \(m/z = 108.7\) is a characteristic signature of delayed fragmentation in reflectron-type TOF systems (see section 2.2.4). It originates from fragmentation processes that occur in the first field-free drift region of the spectrometer. After fragmentation in this region, the fragments will continue to propagate with an unchanged center-of-mass velocity until the electrostatic mirror is reached. Here, the change in ion mass manifests in a way that a given fragmentation process anywhere in the first field free region will lead to the same, shifted peak position in the TOF spectrum, \textit{i.e.} in the \(m/z\) spectrum.

For our reflectron system, the additional peak at \(m/z = 108.7\) can only be explained if the fragments arriving delayed to the detector stem from a metastable adenine fragment with \(m/z = 109\), C\(_4\)H\(_5\)N\(_4\)\(^+\)\(^*\) probably originating from the (prompt) loss of a neutral CN fragment. During its flight through the first drift region, the C\(_4\)H\(_5\)N\(_4\)\(^+\)\(^*\) then loses a neutral hydrogen. The two-step process should look like:

\[
C_5H_5N_3^+ \rightarrow CN + C_4H_5N_4^{++} \ (m/z = 109)
\] (5.5)
5.4 From charged to neutral projectiles

Figure 5.7: Zoom into group 6 and group 8 of the adenine mass spectra after collisions with 14 keV He\(^+\) and He\(^0\) presented in fig. 5.3. The solid line corresponds to the charged projectile and the dashed line to the corresponding neutral one.

\[
C_4H_5N^+ + \tau \sim \mu s \rightarrow H + C_4H_4N^+ (m/z = 108) \tag{5.6}
\]

For the non-integer mass peak at \(m/z = 81.6\), similar TOF estimations of the different fragments’ arrival times indicate that the shifted peak originates most probably from an excited fragment with \(m/z = 82\) that decays after a few microseconds by losing a neutral H. The precursor fragment with \(m/z = 82\) is produced promptly in the collision region. After reaction (5.1), the following steps are therefore:

\[
C_4H_4N^+ \rightarrow CN + C_3H_4N^+ (m/z = 82) \tag{5.7}
\]

\[
C_3H_4N^+ + \tau \sim \mu s \rightarrow H + C_3H_3N^+ (m/z = 81) \tag{5.8}
\]

In both cases, the excitation energy in the intermediate complex is thus statistically distributed over its vibrational modes. Depending on how far the excitation energy exceeds the energy needed to detach the respective H atom, this detachment will be either prompt or delayed.

For electron [95, 96] and photon impact [101] direct HCN loss was observed as the preferred fragmentation channel. This could mean that also in those studies fragmentation proceeds via eqs. (5.5)-(5.6) or (5.7)-(5.8) but: 1) their measuring system did not allow to study metastable decay of fragments or 2) the excitation energy was high enough to reduce average lifetimes \(\tau\) to the ns regime and made the effect not measurable.
Figure 5.8: Zoom into three groups of islands from a correlation plot of fragment ions produced in collisions of 20 keV He$^{2+}$. 

(HCNH$^+$, C$_4$H$_3$N$_4^+$) 

(HCNH$^+$, C$_3$H$_2$N$_3^+$) 

(HCNH$^+$, C$_2$HN$_2^+$)
5.4.5. Coincidence studies

For double ionization events, the precursor is ade\textsuperscript{2+}. Intuitively one expects that the channel from eq. (5.1) or rather the ones from eqs. (5.5)-(5.6) are then switching over to

\[C_3H_5N_5^{2+} \rightarrow HCNH^+ + C_4H_3N_4^+\]  \hspace{1cm} (5.9)

in which two closed shell fragments rather than radicals are formed. In our coincidence studies with singly and doubly charged projectiles, the formation of HCNH\textsuperscript{+} in coincidence with \(C_4H_3N_4^+\) \((m/z = 107)\) is observed, implying the two body break up of the adenine molecule. Also the fragments \(C_3H_2N_3^+\) \((m/z = 80)\) and \(C_2HN_2^+\) \((m/z = 53)\) are observed in coincidence with HCNH\textsuperscript{+}. In fig. 5.8 a zoom into specific islands from a coincidence plot for adenine after collisions with 20 keV He\textsuperscript{2+} projectiles is shown. The coincidence islands corresponding to the fragments formed in coincidence with HCNH\textsuperscript{+}, namely \(C_4H_3N_4^+\) \((m/z = 107)\), \(C_3H_2N_3^+\) \((m/z = 80)\) and \(C_2HN_2^+\) \((m/z = 53)\) are marked with an arrow.

The encircled areas in the figure, close to the coincidence islands, show the position of tails attached to the islands. As for adenine single ionization, here again we observe signs of delayed fragmentation. If occurring within the extraction region of the spectrometer, delayed fragmentation manifests in tails rather than additional peaks.

Delayed fragmentation of doubly charged adenine produced in collisions with 100 keV protons was recently reported by Franceries \textit{et al.} \cite{100} for the same two channels that we observed. The authors estimated lifetimes for the respective metastable states of ade\textsuperscript{2+} of the order of 200 ns.

5.5. Discussion

As it has been pointed out previously \cite{102} in general, in molecular fragmentation by charged projectiles, the appearance of intact parent ions can often be related to resonant electron capture at relatively large projectile–target distances - a relatively gentle process. This is in contrast to the formation of small fragments, which are often generated in more violent close collisions, involving not only electron capture but also direct ionization and electronic and vibrational excitation. We can thus divide the interaction processes in two regimes: 1) close collisions where ionization, capture and excitation occur simultaneously and 2) distant collisions where electron capture is the dominant mechanism.

The relative fragmentation yield for a molecule after impact with different projectiles can be assumed to be inversely proportional to the electron capture cross section. Bacchus-Montabonel \textit{et al.} showed that semiclassical calculations based on \textit{ab initio} potential energy curves can explain the experimentally observed trends in resonant charge transfer between uracil and C\textsuperscript{9+} ions \cite{103, 104}.

For neutral projectiles, obviously the main capture channel is closed as compared to the charged projectile case. Only regime 1) contributes and formation of smaller fragments is expected to become preferred if for the ionic projectile resonant capture was a relevant process in the first place. Such a closure of the capture channel seems to happen for carbon projectiles. For helium and hydrogen the changes in the ratios of the fragments are very weak.
Fig. 5.9 displays the energy levels of the adenine’s highest occupied molecular orbitals (HOMOs) [93] in comparison with the levels of the three projectile ions in which an electron can be captured [105]. The He$^{2+}$ is added for comparison. The crucial value in fig. 5.9 is the vertical ionization potential of adenine (IP=8.48 eV). Obviously, for C$^+$ resonant electron capture is possible into several energy levels (regime 2). The suppression of regime 2 (the case for neutral projectiles) increases clearly the average amount of energy deposited to the molecule which increases the fragmentation into small cations as seen in fig. 5.5.

For He$^+$ projectiles resonant capture from the adenine HOMO is energetically ruled out, inhibiting the contribution of regime 2). This explains the more severe fragmentation for He$^+$ as compared to C$^+$ where gentle collisions from regime 2) do contribute (fig. 5.3). For He$^+$, we thus look at events of high energy deposition, only.

To a smaller extend the same argumentation holds for H$^+$ projectiles. Here, the only energetically accessible state lies higher than in the He$^+$ case but still about 6 eV below the adenine HOMO. Resonant electron capture is unlikely for distant collisions but might become possible for close collisions. Since these belong to regime 1), switching off regime 2) will not have a strong effect on adenine fragmentation.

The strong effect for neutral Carbon can be observed once more in table 5.2 where the relative fragmentation cross sections for the spectra shown in fig. 5.3 are displayed. The relative fragmentation cross section ($\sigma_f$) is defined as $1 - Y_{ade}/Y_{total}$ where $Y_{ade}$ is the yield of the adenine peak and $Y_{total}$ is the sum of the yields of all adenine fragments excluding H$^+$ and H$_2^+$ that could also stem from the residual water; but including the adenine parent molecule yield $Y_{ade}$. The conclusion that for hydrogen and helium projectiles $\sigma_f$ are very similar for the charged and the neutral projectiles whilst for carbon projectiles, the difference in $\sigma_f$ is around 18%, summarizes the idea that electron capture plays an important role in these collision systems.
### Table 5.2: Relative fragmentation cross sections ($\sigma_f$) of adenine for the different projectiles shown in fig. 5.3

<table>
<thead>
<tr>
<th>Projectile</th>
<th>$\sigma_f$</th>
<th>ratio $X^0/X^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$^+$</td>
<td>0.82±0.01</td>
<td>1.03</td>
</tr>
<tr>
<td>H$^0$</td>
<td>0.80±0.01</td>
<td></td>
</tr>
<tr>
<td>C$^+$</td>
<td>0.72±0.01</td>
<td>1.18</td>
</tr>
<tr>
<td>C$^0$</td>
<td>0.85±0.01</td>
<td></td>
</tr>
<tr>
<td>He$^+$</td>
<td>0.95±0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>He$^0$</td>
<td>0.95±0.01</td>
<td></td>
</tr>
</tbody>
</table>

But why is fragmentation so pronounced for He$^+$ and He$^0$ projectiles? It was already hinted when we compared the fragmentation of adenine after He$^+$ and He$^{2+}$ projectiles. The large energy gap ($\approx 13$ eV) between adenine HOMOs and the accessible He$^+$ ground state is the most important reason. If this energy is put within the molecule, fragmentation is unavoidable. So for the neutral He projectiles as well as for the charged ones regime 1) is the only possibility. One possible charge transfer mechanism is Auger-type neutralization, a process well known from ion–solid interactions [106]. Auger neutralization may also explain the extraordinarily strong relative yield in adenine$^{2+}$ for He$^+$ projectiles: an electron from one of the adenine HOMOs Auger neutralizes the He$^+$ while the excess energy is used to release a second adenine electron.

The electronic excitation of a molecular target such as C$_{60}$ by an atomic projectile was shown to be a phenomenon that depends on the projectile atomic number, being very low for $Z = 1$ and reaching a first maximum (10 times larger) for $Z = 6 \sim 7$ (the so called $Z$-oscillations) [107]. In the case of adenine molecules, this does not seem to be the predominant process but could explain the increase in cross sections when going from H$^0$ ($Z=1$) to C$^0$ ($Z=12$). These $Z$ oscillations will definitely not explain the strong fragmentation observed for He$^0$.

Relative fragmentation cross sections for all the projectiles used in the studies of neutral vs. charged projectiles are plotted in fig. 5.10. It can be seen that for “light” projectiles such as H$^+$ and H$^0$ and He$^+$ and He$^0$, small differences in $\sigma_f$ for charged and neutral projectiles only start to appear at lower energies (less than 5 keV/amu for He for example). But for “heavier” projectiles such as C$^+$ and C$^0$ a difference in energy of a few keV/amu will influence only the fragmentation due to the charged projectile.

### 5.6. Conclusions

The interaction of ions with different nucleobases has been an emerging research field in the last years. Very significant dependence of the fragmentation patterns on the projectile electronic structure has been found. Also relatively strong dependence of the fragment
production on the different target molecules was observed when comparing the pyrimidines thymine and uracil with the purine adenine. In this chapter new results for the fragmentation of the nucleobase adenine induced by ionic or neutral projectiles were shown. Although it was found that nucleobases share similar characteristics in their fragmentation spectra the new delayed fragmentation process was observed for the first time in this thesis and for the case of adenine.

The ionization and fragmentation of gas-phase adenine molecules by keV neutral and singly charged H, He and C impact was quantitatively studied in this chapter. The largest effect on relative fragmentation was observed for the C\(^0\) projectiles in comparison with \(\text{C}^+\). This can be understood in terms of gentle resonant electron capture, only possible for \(\text{C}^+\). For H and He projectiles the changes when going from singly charged to neutral were negligible, since in these cases, electron capture cross sections are expected to be small even for charged projectiles. Delayed fragmentation with \(\mu\)s lifetimes was observed for fragments usually assigned in the purines cases to HCN loss. This effect is particularly pronounced for He projectiles but was also observed in a smaller scale for the other neutral projectiles used.

One open question remains: Why is the delayed loss of an H atom from adenine\(^+\) only observed for neutral projectiles? In recent studies of electron impact ionization of uracil molecules, transient fragment ions with microsecond lifetimes have been observed [108]. The fact that such long lived metastable fragments are observed also for electron impact can imply that also in our studies they are produced via a collisional process, more specifically via direct ionization.