Ion induced radiation damage on the molecular level
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Clusters of nucleobases

The relevance of studies on isolated gas phase molecules for processes occurring in biological systems is often questioned because of the lack of a real chemical environment. There are several ways to address this issue and still keep the system under study sufficiently simple to explore the underlying fundamental processes. One of the most straightforward approaches is to give the isolated molecule a chemical environment by surrounding it with analogue molecules or other molecules present in a realistic biological environment. In this chapter, results on the interactions of ions with neutral nucleobase clusters will be compared to previous results on isolated nucleobases. The experiments were performed at CIRIL/GANIL, Caen. The neutral clusters were produced in an aggregation source and ionized using multiply charged projectiles at keV energies. Experiments with homo-clusters and hetero-clusters were carried out. Structured mass spectra with prominent magic numbers are observed for clusters of pyrimidine bases but not for the purine adenine. When comparing fragmentation of one isolated single molecule and the same molecule within a cluster, significant changes are observed. New fragmentation channels open–up already for very small cluster sizes. Preliminary results on hetero-clusters show preferential formation of clusters containing bases which form Watson–Crick pairs within the DNA.

based on:

Ion-Induced Biomolecular Radiation Damage: From Isolated Nucleobases to Nucleobase Clusters.
7.1. From isolated nucleobases to nucleobase clusters

The unique potential of studies on isolated molecules in the gas-phase as discussed in the previous chapters lies in the possibility of studying ionization and fragmentation dynamics in finite systems without the perturbing effects of a surrounding medium. The relevance of these 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. This is not a priori the case. For instance, in aqueous solution the vertical ionization energies for DNA building blocks are altered [129–132]. The variation of adenine and thymine ionization potentials by hydration in the cluster phase has been shown to depend on the number of water molecules surrounding the nucleobase [133]. Furthermore, vibrational excitation of the molecules might be transferred to the environment before fragmentation can occur. Protonation of intact molecules or their fragments, as observed for instance in ion stimulated desorption experiments on thin films of thymine [29], is obviously ruled out for isolated molecules in the gas phase.

The radiobiological significance of gas phase studies is therefore often questioned because of the lack of a chemical environment. Preliminary studies on gas phase nucleobases clusters were performed by Dey et al. [134]. They used laser desorption of neutral molecules into a supersonic beam expansion in order to study pair formation of free unsubstituted nucleobases and mononucleosides. Pure clusters of pyrimidines produced in a supersonic expansion were studied by photoionization TOF mass spectrometry by Kim et al. [135, 136]. They observed magic numbers for thymine and uracil clusters. Pairing of DNA bases in the absence of the backbone has been extensively studied by means of spectroscopic techniques. Guanine-guanine and guanine-cytosine pairs were studied using resonance enhanced multiphoton ionization (REMPI) [137]. By using double resonance spectroscopy (R2PI) the studies were extended to cytosine-cytosine pairs [138] and later compared to theoretical calculations [139–141]. The same kind of spectroscopic studies combined with theoretical assignment of the peaks were performed on the adenine-thymine pair [142].

Very recently cold helium droplets have been used to study free electron attachment to pure and mixed nucleobases clusters [143, 144]. These studies have shown preferential formation of protonated clusters and long lived anions which can not be formed from isolated molecules in the gas phase.

The experimental approach used in this chapter is based on a very well–known technique to produce alkali clusters. The technique has been successfully used to investigate sodium clusters after ion impact [41]. Using this approach DNA building blocks can be surrounded by other molecules to mimic a biological environment. This technique allows to form neutral nucleobases clusters of a wide size distribution. The dynamics of a single molecule can be investigated within a cluster.

In the following, spectra obtained after the interaction of keV multiply charged ions with isolated nucleobases and their clusters will be compared in detail. In particular, the pyrimidine nucleobases thymine, uracil and cytosine and the purine nucleobase adenine will be discussed. Then, for the thymine case correlation between fragment cations will be used to investigate the molecular fragmentation within the cluster in more detail. To close the chapter preliminary results on gas phase mixed clusters will be presented and discussed. The structures of the molecules under study can be found in chapter 5.
7.1 From isolated nucleobases to nucleobase clusters

Figure 7.1: Typical mass spectrum for collisions of 60 keV C$_5^+$ with isolated thymine molecules. The assignment of the fragment peaks essentially follows the work of Deng et al. [29] thy indicates the parent thymine molecule.

7.1.1. Thymine

Fig. 7.1 displays a typical mass spectrum obtained for collisions of 60 keV C$_5^+$ ions with isolated thymine molecules. In a recent work on low energy (10-200 eV) Ar$^+$ ion stimulated desorption from thymine thin films, Deng et al. have identified the most prominent peaks in the spectrum by means of an isotope labelling technique [29]. The peak assignment in the figure largely follows their work. However, in the desorption studies, protonation of parent molecules and fragments is an important channel that is ruled out for isolated molecules in the gas-phase. Some of the dominating peaks (for instance the parent ion at mass 126) are therefore one mass unit shifted when comparing the spectrum in fig. 7.1 with condensed phase results. Almost all fragments observed in the spectrum correspond to products involving fragmentation of the ring. Only the loss of one or two hydrogens from the parent molecule does not imply destruction of the ring.

Fig. 7.2 shows a wide range spectrum of the fragment cluster-ion distribution (thy$_q^+$) obtained in collisions of 50 keV O$_5^+$ with neutral thymine clusters. Under the experimental conditions used, thymine clusters with more than 50 constituent molecules could clearly be identified. Cluster ions with charge states up to 4 were observed. In fig. 7.2, the series for $q = 1, 2, 3$ are indicated. Note, that for singly charged clusters, prominent peaks indicating magic numbers are found at $n = 7, 10$ and 13 (marked with a star in the figure). For the other pyrimidines uracil (section 7.1.2) and cytosine (section 7.1.3), qualitatively similar results are observed, although the magic numbers are less pronounced for the cytosine case. Kim et al. [136] have already observed the magic numbers 7, 10 and 13 in photoionization...
Figure 7.2: Wide scale mass spectrum for collisions of 50 keV O$^{5+}$ with neutral thymine clusters. The masses are given in multiples of the thymine mass (n). Peak series due to singly, doubly and triply charged clusters are indicated. The starred marked peaks are discussed in the text.

One of the main incentives of the experiments, was the investigation of nucleobase fragmentation within a cluster. In this context, the spectrum in fig. 7.2 already reveals one issue of particular importance: at masses higher than the thymine monomer, almost exclusively peaks due to thy$^+_n$ can be observed. This indicates that the ion impact does not lead to polymerization but only to dissociation of one or more single molecules within the cluster. Whether the molecular fragments from the cluster are protonated or not, cannot be answered due to the insufficient resolution of the mass spectrometer. However, in fig. 7.3 a zoom into the low mass region of the spectrum in fig. 7.2 is shown. The lower masses are not displayed since this range is overwhelmed by contributions coming from the condensation gas (He).

The fragmentation in the cluster case is obviously different from what is observed in the gas phase (see fig. 7.3). Not only the relative peak intensities are very different. Particularly the structures around $m/q \approx 109$ and $m/q \approx 97$, basically due to loss of OH and NH$_2$CH (or HCO) respectively, are not observed at all for ion induced fragmentation of isolated thymine molecules. For comparison, $m/q \approx 109$ has also not been observed in photoionization and electron impact ionization studies of isolated thymine molecules and for $m/q \approx 97$ only traces were found [101]. However, the peaks around $m/q \approx 109$ appeared in the mass spectra from ion collisions with condensed phase thymine [29]. Apparently the finite environment of a cluster is sufficient to allow “characteristic” fragmentation channels normally only present...
Figure 7.3: Zoom into the low mass region of fig. 7.2 with masses in amu. The assignment of the peaks essentially follows the work of Deng et al. [29] thy indicates the parent thymine molecule. The peaks around \( m/q = 97 \) have not been observed by Deng et al. For them, the assignment of Jochims et al. [101] is used. For comparison, the data for isolated molecules is added in grey.

in the condensed phase with the OH-loss peak at \( m/q \approx 109 \) being one of the fingerprints. OH-loss is the only channel observed that can solely be due to exocyclic bond cleavage.

This finding entails the question if the appearance of the peaks around \( m/q = 109 \) and \( m/q = 97 \) (loss of an OH and an NH\(_2\)CH (or an HCO) group, respectively) depends on the parent cluster size. This issue will be discussed in greater detail in the section 7.3.

7.1.2. Uracil

Fig. 7.4 shows a typical mass spectrum acquired for collisions of 48 keV C\(^5\)+ ions with isolated uracil molecules. Our peak assignment follows the photoionization studies of Jochims et al. [101]. As in photoionization and electron impact ionization studies [94], the prominent peaks are the parent ura\(^+\) (\( m/q = 112 \)), C\(_3\)H\(_3\)NO\(^+\) (\( m/q = 69 \)), C\(_2\)H\(_2\)O\(^+\) (\( m/q = 42 \)) and HCNH\(^+\) (\( m/q = 28 \)). As for thymine all fragment ions are formed by breaking the six-membered ring.

Fig. 7.5 displays a wide range spectrum of the fragment cluster-ion distribution (ura\(_n^{q+}\)) obtained in collisions of 100 keV O\(^3\)+ with neutral uracil clusters. The characteristics of the spectrum are similar to the ones for thymine. Note that due to a somewhat lower resolution, only cluster ions with charge states 1 and 2 could be identified unambiguously. In fig. 7.5, the series for \( q = 1 \) and 2 are indicated. As in the thymine case, for \( q = 1 \) prominent peaks corresponding to the magic numbers \( n = 7 \) and 10 are observed. As for thymine, there is no evidence for polymerization: for \( n \geq 1 \) almost exclusively peaks due to ura\(_n^{q+}\) can be.
Figure 7.4: Typical mass spectrum for collisions of 48 keV C$_5^+$ with isolated uracil molecules. The assignment of the peaks essentially follows the work of Jochims et al. [101] ura indicates the parent uracil molecule.

Figure 7.5: Wide scale mass spectrum for collisions of 100 keV O$_5^+$ with neutral uracil clusters. The masses are given in multiples of the uracil mass. Peak series due to singly and doubly charged clusters are indicated.
observed. In the region $n \leq 1$ however, molecular fragmentation channels specific to uracil within the cluster are evident. The respective zoom into this part of the spectrum, can be found in fig. 7.6.

Again fragmentation differs strongly for clusters and isolated molecules. The large fragments at around $m/q \approx 95$ and $m/q \approx 83$, due to loss of OH and HCO (or NH$_2$CH) respectively, are not observed at all for ion induced fragmentation of isolated uracil molecules. The fragment at $m/q \approx 96$ has been observed as a weak channel in photoionization, and $m/q \approx 83$ has neither been observed in photoionization studies [101] nor in electron impact ionization, where the biggest cation fragment observed for uracil was at $m/q \approx 69$ [145].

### 7.1.3. Cytosine

A mass spectrum obtained for collisions of 50 keV O$^5+$ ions with isolated cytosine molecules is displayed in fig. 7.7. Our peak assignment follows very recent electron collision studies by Shafranyosh et al. [146]. It has to be mentioned that cytosine gas phase molecules have not been studied as extensively as thymine and uracil; therefore the peak assignment is less certain. As for other nucleobases, the most prominent peak is due to surviving cytosine parent molecules cyt$^+$ ($m/q = 111$). The lack of isotopic labelled studies leaves the assignment of $m/q = 69$, $m/q = 42$ and $m/q = 28$ fragments tentative. The various possibilities are given in the figure. The cytosine nucleobase is the only pyrimidine that has an external NH$_2$ group. It can be seen in fig. 7.7 that exocyclic and endocyclic bond breaks are allowed. The fragmentation pathways involving groups outside the 6-membered ring (loss of oxygen or NH$_2$) are very weak.
Figure 7.7: Typical mass spectrum for collisions of 50 keV O$_{5}^{5+}$ with isolated cytosine molecules. The tentative assignment of the peaks follows a recent work of Shafranyosh et al. [146] cyt indicates the parent cytosine molecule.

Figure 7.8: Wide scale mass spectrum for collisions of 50 keV O$_{5}^{5+}$ with neutral cytosine clusters. The masses are given in multiples of the cytosine mass. Peak series due to singly, doubly and triply charged clusters are indicated. The marked peak at $n < 1$ is an experimental artifact.
7.1 From isolated nucleobases to nucleobase clusters

A wide range spectrum of the fragment cluster-ion distribution \((\text{cyt}_{n}^{q+})\) measured after collisions of 50 keV \(\text{O}^{5+}\) with neutral cytosine clusters is shown in fig. 7.8. The characteristics of the spectrum are similar to the ones for thymine and uracil. In fig. 7.8, the series for \(q = 1, 2\) and 3 are indicated. Hints of quadruply charged clusters are observed. As for the thymine and uracil cases, some indication of magic numbers at \(n = 7\) and 10 is observed for \(q = 1\) clusters.

As for the previous nucleobases discussed, there is no evidence for polymerization: for \(n \geq 1\) almost exclusively peaks due to \(\text{cyt}_{n}^{q+}\) can be observed. However, molecular fragmentation of a single cytosine molecule \(\text{within}\) the cluster can be studied. A zoom into the region \(n \leq 1\) of the spectrum can be found in fig. 7.9. In this case the fragmentation patterns differ less between clusters and isolated molecules as compared to the previous cases. Although the large fragments at \(m/q \approx 95\) and \(m/q \approx 83\) were also observed for isolated molecules in the gas phase, the relative intensities change very strongly. An extraordinary decrease in intensity for the fragments marked as \([\text{cyt-CO}]^{+}\) or \([\text{cyt-CNH}_2]^+\) is observed when going from the isolated molecule to the molecule \(\text{within}\) the cluster. On the other hand, the pathway related to exocyclic fragmentation is stronger for the cluster case than for the isolated molecules. Variations in relative intensities are also observed for other groups of fragments.

7.1.4. Adenine

A mass spectrum resulting from collisions of 65 keV \(\text{C}^{5+}\) ions with isolated adenine molecules can be found in fig. 7.10. The peak assignment used in the figure follows the photoionization studies of Jochims \textit{et al.} [101]. As in photoionization and in electron impact
Figure 7.10: Typical mass spectrum for collisions of 65 keV C\(^{5+}\) with isolated adenine molecules. The photoionization work of Jochims et al. [101] is followed for the assignment of the peaks. ade indicates the parent adenine molecule.

ionization [95], the prominent peaks are the parent ade\(^+\) (\(m/q = 135\)), the peaks corresponding to HCN loss: [ade-HCN]\(^+\) (\(m/q = 108\)) and [ade-2HCN]\(^+\) (\(m/q = 81\)) and the fragments C\(_3\)H\(_2\)N\(_2\)\(^+\) (\(m/q = 66\)), C\(_2\)HN\(_2\)\(^+\) (\(m/q = 53\)), and HCNH\(^+\) (\(m/q = 28\)). The strong peak at C\(_2\)N\(^+\) (\(m/q = 38\)) is only observed as a minor contribution in photoionization studies and the doubly charged parent ade\(^{2+}\) (\(m/q = 67.5\)) is not produced at all by photoionization or electron impact. An extensive discussion on adenine fragmentation can be found in chapter 5.

The results for 50 keV O\(^{5+}\) induced fragmentation of adenine clusters are displayed in fig. 7.11. Pronounced differences to the results for the pyrimidines thymine, uracil and cytosine are obvious. The cluster intensities drop continuously with cluster size \(n\). No obvious magic numbers are observed. In fig. 7.11, the series for \(q = 1\) and \(2\) are clearly seen. The series of triply charged cluster peaks is barely visible. Even though for isolated adenine molecules, formation of the doubly charged parent cation is clearly observed, adenine cluster dications have a larger appearance size (\(n_{app} = 15\)) than those of thymine (\(n_{app} = 11\)), uracil and cytosine (\(n_{app} = 13\)). Before discussing the stability of the different clusters in more detail, also for adenine the following question emerges: How does molecular fragmentation change when moving from the isolated molecule to the cluster case? The differences between the two spectra displayed in fig. 7.12 are smaller than in the cases of thymine and uracil, but comparable to the differences in cytosine. In line with the previous results for thymine and uracil a strong group of fragment ion peaks due to the loss of a NH\(_n\) group (main peak at \(m/q = 118\) [ade-NH\(_3\)]\(^+\)) is only observed in the cluster case and here also the peak around \(m/q = 92\) (C\(_4\)H\(_2\)N\(_2\)\(^+\)) is much stronger. Note, that peaks at \(m/q = 118\) and 119 also appear as weak channels in photoionization studies of isolated adenine [101].
7.1 From isolated nucleobases to nucleobase clusters

Figure 7.11: Wide scale mass spectrum for collisions of 50 keV $O_{5}^{5+}$ with neutral adenine clusters. The masses are given in multiples of the adenine mass. Peak series due to singly and doubly charged clusters are indicated.

Figure 7.12: Zoom into the low mass region of fig. 7.11 with mass given in amu. For comparison, the data for isolated molecules is added in grey.
Figure 7.13: Cluster cation yields taken from figs. 7.2, 7.5, 7.8 and 7.11. The data has been normalized to the monomer yield.

7.2. Cluster stability

The mass spectra for clusters from the nucleobases under study were shown in figs. 7.2, 7.5, 7.8 and 7.11. Whereas the pyrimidine cluster spectra exhibit magic numbers (7, 10 and 13), in the adenine cluster spectrum a monotonic decrease of cluster yields with the cluster size is observed. The yields vary only weakly after \( n = 4 \). Fig. 7.13 shows a direct comparison of the cluster yields for four different nucleobases. Obviously, for adenine clusters, the yields for small \( n (< 5) \) drop fastest with \( n \) whereas for thymine, the slowest decrease is observed. For cytosine clusters, on the other hand, the yields after \( n = 4 \) drop very fast, giving very small yields for larger \( n \).

The stability of the clusters is also reflected in the appearance sizes for dications. Small appearance sizes usually correspond to greater stability. The smallest doubly charged systems are found to be \( \text{thy}_{11}^{2+} \), \( \text{ura}_{13}^{2+} \), \( \text{cyt}_{13}^{2+} \) and \( \text{ade}_{15}^{2+} \). The thymine, uracil and adenine dications appearance size is in line with the cation yield distributions depicted in fig. 7.13. This is not the case for the cytosine clusters which according to appearance size should be similar to uracil but the experimental results show a very fast decrease in intensity with increasing \( n \).

Formation of cluster cations is a complex process that strongly depends on binding energies and ionization potentials. A number of recent \textit{ab initio} studies focused on the stability of neutral nucleobase dimers. Generally, two different types of dimers (and larger clusters) can be distinguished: i) planar systems based on hydrogen bonds with deep minima in the potential energy surface (PES), leading to well defined geometries and ii) stacked systems stabilized by the dispersion energy with very shallow minima in the PES [147]. The hydrogen bonded systems generally have higher binding energies as calculated for all stable cluster
geometries by Kelly et al. [148–150]: The most stable dimers are respectively bound by 0.99 eV (cytosine), 0.86 eV (adenine), 0.82 eV (thymine) and 0.71 eV (uracil). This ordering does not reflect the relatively low yields and large appearance sizes observed for adenine and cytosine, as compared to thymine and uracil (fig. 7.13). The ionization potentials of isolated adenine, thymine and uracil are 8.2 eV, 8.82 eV and 9.15 eV, respectively [101] and around 8.8 eV for cytosine [132]. For adenine and thymine, upon dimerization the monomer IPs have been found to drop by 0.5 eV, respectively [133], indicating that for small clusters the relative ordering of the monomer IPs is retained. This implies that the IP ordering is also not reflected directly in our experimental findings.

7.3 Coincidence data

In this section, a more detailed look into the fragmentation of thymine clusters will be presented. Thymine clusters were chosen because for them a comparison to the condensed phase data from Deng et al. [26, 29] is possible. The crucial question in this context is from which cluster size on mass spectral features that are similar to the condensed phase ones appear. In other words: At which cluster size does the transition from the isolated molecule to the molecule embedded in a chemical environment take place?

The cluster aggregation source used in our experiments produces neutral clusters with a rather broad size distribution. Only the average cluster-size can be influenced by parameters such as oven temperature and pressure of the He-atmosphere. The determination of the cluster-size is thus done a posteriori from the experimental data.

For multiple ionization of small clusters and coincident detection of the fragment ions, the cluster size determination is possible under the following assumptions: i) the majority of the fragmentation products are (positively) charged, and ii) transmission and detection efficiency of the spectrometer are close to 100%. The linear Wiley-McLaren TOF spectrometer used for the cluster studies meets the second requirement. To fulfil the first requirement, this study is limited to double ionization events, i.e. correlated detection of exactly two singly charged fragments, after collisions with 50 keV O$_5^+$ ions. From studies on collisions of multiply charged ions with C$_{60}$, it is known that close collisions lead to multiple ionization of the target. Single and double ionization, on the other hand, take place purely by means of electron capture at large impact parameters. For thymine clusters, this implies that 50 keV O$_5^+$ induced double ionization is also mainly due to gentle electron removal processes. Coincident detection of two fragment cations formed after a rather gentle ionization implies that the fragments stem from the subsequent charge separation. Neutral fragments are possible as well but they are expected to be of comparable size as the ionic ones. The sum of the masses of the fragment cations is then equal to or smaller than the mass of the respective parent cluster - in any case the order of magnitude of the original cluster size can be determined from the coincidence data.

Fig. 7.14 shows spectra for double ionization and fragmentation into one cation with a mass in the range $m/q = 30 – 120$ and a thy$_n^+$ ($n = 1 – 7$) entity (the lower resolution as compared to fig. 7.3 is due to larger bins required by the poorer statistics in the coincidence plots). All spectra have been scaled to an equal intensity of the $m/q \approx 109$ peak. Even though the poor statistics does not allow a detailed analysis of the spectra, a number of important
conclusions can be drawn: i) the $m/q \approx 109$ peak is prominent even for the smallest cluster ($n = 1$, i.e. the parent is a dimer); ii) the overall shape of the spectra is largely independent on $n$, and iii) for certain channels, variations with the cluster size can be observed: for instance the $m/q \approx 97$ peak (marked in grey) is weak at $n = 1$, increases to a relative maximum at $n = 4, 5$ and then decreases again.

A more quantitative representation of the coincidence data can be found in fig. 7.15. Plotted are the integrals of thy$_n^+$-cluster ions observed in coincidence with different fragments: [thy-OH]$^+$, [thy-NH$_2$CH]$^+$, [thy-OCNH$_2$]$^+$ and HNC$_3$H$_4^+$. The latter two (full symbols) are also present for isolated thymine, whereas the two others (open symbols) are cluster-specific. From fig. 7.15 it is obvious that the yield for cluster ions observed in coincidence with cluster-specific fragments systematically exceeds the yields for cluster ions observed in coincidence with the non-specific fragments. Since the data is normalized this implies that cluster-specific fragments, particularly [thy-NH$_2$CH]$^+$, are becoming relatively more important at larger $n$.

From the coincidence data therefore two conclusions can be drawn: i) “characteristic” fragmentation channels, forbidden for the isolated molecule, already open up for the smallest possible clusters i.e. dimers and trimers, and ii) the relative importance of these channels slightly increases with cluster size. Fragmentation channels considered to be typical for the condensed phase thus occur already in very small clusters, however, their relative strengths increase with the size of the cluster system.

This implies that for example the mechanism underlying [thy-OH]$^+$ formation is already active in very small clusters. We can thus try to link this mechanism to the calculated structures of thy$_2$ dimers. Kelly and Kantorovich [150] have shown that the most stable dimers are bound by two hydrogen-bonds between an H donor and an O acceptor. The most stable dimer structure is shown in fig. 7.16.
7.3 Coincidence data

Figure 7.15: Coincidence data for 50 keV O\(^{5+}\) induced double ionization of neutral thymine clusters. Yields of thy\(_n^+\) cluster ions observed in coincidence with fragments corresponding to the 4 peaks containing [thy-OH]\(^+\), [thy-NH\(_2\)CH]\(^+\), [thy-OCNH\(_2\)]\(^+\), and HNC\(_3\)H\(_4^+\), respectively. The data has been normalized to the thymine monomer cation peak. (For \(n=8\), the [thy-OCNH\(_2\)]\(^+\) could not be distinguished from the background.)

Figure 7.16: Geometry of the most stable thy\(_2^+\) dimer [150]. Hydrogen-bonds are indicated by non-continuous lines.
The chemical interaction between the two thymine units is to a large extent limited to the two hydrogen-bond regions, i.e. in these regions the electronic structure of the involved molecules is strongly altered. For any O or H atom subject to hydrogen bonding, the presence of an additional intermolecular OH bond will always weaken the intramolecular bonding. It is thus only straightforward that during molecular fragmentation of one thymine within the dimer, particularly these O and H atoms will be affected by the presence of the second thymine. This suggests that the characteristic peak observed in the cluster data \( m/q = 109 \) is probably due to loss of oxygen and hydrogen atoms involved in the hydrogen bonds. This is also supported by the results of fig. 7.15: the intensity of the peak due to OH (or O+H) loss is considerable and independent of the cluster size. With increasing cluster size, the fraction of thymine molecules that contain O-atoms that are involved in hydrogen bonds is increasing. Those O-atoms are then subject to an stronger weakening of the intramolecular C=O bond and the probability of forming the fragment \([\text{thy-OH}]^+\) should increase, but according to the experimental results, it keeps on average constant with cluster size.

The hydrogen-bond line of argumentation can also be used from another angle: Observation of OH or O+H loss indicates hydrogen-bonding between cluster constituents (planar clusters) rather than dispersive force driven bonding (stacked clusters). To confirm this, we performed quantum-chemical calculations with the Gaussian 03 package [49]. Thymine clusters with up to 5 constituents were geometry-optimized on the B3PW91/6-31G(d) level for a variety of different starting geometries. The stabilization energy, e.g. the total energy of the optimized combined clusters minus the total energy of its optimized components [150], has then been computed on the B3PW91/6-311+G(2df,2p) level. Figure 7.17 shows the geometries of the most strongly bound neutral thymine trimers, tetramers and pentamers found in the calculations. Similar to the observations for dimers, the geometry optimization for these small clusters almost always leads to geometries of planar nature. Stacked geometries have not been found. However, with increasing cluster size, the geometries deviate more from the perfect planarity of the dimer. In any case the calculations show that not only dimers but also the larger systems are based on hydrogen bonds between O and H atoms. Because of the dominant role hydrogen bonding also plays in condensed phase thymine, it is therefore possible to conclude in a straightforward manner that the characteristic fragmentation channels in the cluster and in the condensed phase are fingerprints of these hydrogen bonds.

### 7.4. Mixed clusters

The question whether DNA base pairs found in cellular DNA are also formed preferentially in clustering processes in the gas phase was addressed by Dey et al. in 1994 [134]. They showed that in a supersonic beam expansion base pairs of complementary molecules, namely adenine-thymine and cytosine-guanine are energetically favored as compared to homo–pairs and non-complementary pairs.

Extensive theoretical investigations on the different possibilities for nucleobase pairing have been performed by Hobza and co-workers [151] and very recently the results have been summarized by Kelly and Kantorovich [152]. Pair formation in the gas phase for different tautomers of complementary DNA bases was studied by means of double resonance laser spectroscopy and complemented with theoretical calculations [142, 153]. It seems that the
Figure 7.17: Relaxed thymine cluster geometries of high stability for cluster sizes $n = 3, 4, 5$. The dotted lines indicate hydrogen bonds.
Watson-Crick pair conformation of the cytosine-guanine dimer is formed as the most stable configuration in the gas phase but this is not the case for the adenine-thymine pair where two other isomers were found to fit best the IR spectra. The stability order for hetero- and homo-pairs of DNA nucleobases as given by the experimental results from Dey et al. [134] and complemented with theoretical calculations for stabilization energies in eV given by Kelly et al. [149] is as follows: Cyt–Gua(−1.21) [152] > Cyt–Cyt(−0.99) [148] > Gua–Gua(−1.12) [148] > Ade–Thy(−0.82) [152] > Ade–Ade(−0.79) [149] ≈ Thy–Thy(−0.82) [150] ≈ Ade–Gua(−0.87) [152] ≈ Ade–Cyt(−0.89) ≈ Cyt-Thy(−0.93) ≈ Gua–Thy(−0.94) [152]. The energetic ordering is in good agreement with experiment with exception of the Gua–Gua value. Other theoretical approaches have found lower values (−0.96) [154] for this configuration. Formation of the last three hetero-pairs was not observed experimentally.

A fragmentation pattern of clusters formed after collisions of O$^{5+}$ projectiles with mixed neutral clusters of adenine and thymine molecules is shown in fig. 7.18. The spectrum is normalized to the total yield of the thymine monomer. Each group of peaks belongs to a nucleobase number $n_{total} = m_{thy} + (n-m)_{ade}$. In the first group the adenine and thymine monomers can be clearly distinguished. In the second group, thy$_2$, thy$_1$ade$_1$ and ade$_2$ are visible. In the third group, the ade$_3$ is barely visible whereas thy$_3$ and thy$_2$ade$_1$ are the dominant peaks. From the fourth group on the clusters containing several thymine molecules and one adenine: thy$_n$ade$_1$ are dominant. In group 4 and 5, clusters containing only adenine molecules are not visible and the peaks corresponding to several adenine molecules and one thymine: thy$_1$ade$_m$ are barely visible. The intensity distribution of the fragments is clearly non-statistical. A statistical distribution should resemble Pascal’s triangle. An explanation for this phenomenon would be that a strongly bound thy$_1$ade$_1$ pair is present in the core of the cluster. The pair stabilizes the clusters and favors the growth by adding H-bonded thymine...
molecules. In contrast to the experiments with homo–nucleobases clusters, it seems that for hetero–nucleobases clusters polymerization is possible i.e. clusters containing fragments of molecules are visible to the left of the thy\(_n\) with \(n = 2 - 5\).

In order to get a better understanding of the cluster formation, and to explore the question whether nucleobases which form Watson-Crick pairs within the DNA helix will form preferentially pairs in the gas phase, experiments with molecular mixtures of nucleobases which do not form Watson-Crick pairs on DNA were performed. Fig. 7.19 shows a mass spectrum of clusters containing adenine and cytosine after O\(^{5+}\) ion impact. The groups represent nucleobase numbers: \(n\)\(_{total} = m_{cyt} + (n - m)_{ade}\). It can be seen that all cluster intensities decrease with \(n\), hinting at an statistical distribution of intensities. Intensity distribution follows the Pascal’s triangle. A possible explanation for this different behavior in comparison to the Ade-Thy clusters, is that for cytosine and adenine the molecules are joined together by van der Waals forces instead of H-bonding. Then stabilization of a selected pair does not occur.

Although preliminary, several conclusions can be drawn from the mixed clusters data:

- preferential formation of clusters containing \(m\) thymine molecules and one adenine molecule is evident.
- the intensities of clusters containing cytosine and adenine molecules follow a statistical distribution pattern.
- polymerization is possible for mixed clusters: small peaks in between clusters of entire molecules.

Following the discussion for homo–clusters of nucleobases, the clusters formed involving thymine molecules in our experimental conditions seem to be held together by hydrogen bonds. For cytosine-adenine clusters, the formation mechanism seems to involve only weak van der Waals forces.

**Figure 7.19:** Mass spectra of mixed clusters of cytosine and adenine, after collisions with O\(^{5+}\) 50 keV. cyt represents the cytosine molecule and ade represents the adenine molecule.
7.5. Conclusions

Biomolecular radiation damage studies on clusters can be seen as a step towards molecules embedded e.g. in aqueous solution. Using the cluster approach it is feasible to surround radiobiologically relevant molecules such as DNA building blocks by a realistic environment and still be able to investigate the dynamics of a single biomolecule.

As a first step in this direction, a comparison between fragmentation patterns of isolated uracil, thymine, cytosine and adenine molecules with the corresponding data obtained for clusters of these molecules has been presented in this chapter. For all systems, except cytosine it was found that characteristic additional fragmentation channels are observed in the cluster case. The strongest of these channels are loss of O and H (thymine and uracil) or loss of N and H (adenine). For thymine, the fragmentation was studied by means of coincidence techniques and it was compared to existing data on condensed phase thymine, where the respective characteristic channels observed in the cluster case are observed as well. The characteristic channels already open up for the smallest clusters but become relatively more important with increasing cluster size. These channels are probably due to the hydrogen bonding between O and H atoms in thymine, i.e. the clusters apparently favor planar geometries over stacked ones.

Regarding the stability of nucleobase clusters, the strongest decrease of cluster yield with cluster size $n$ was observed for adenine. There, also the appearance size for cluster dications is largest and no magic numbers are observed in the spectrum. For thymine and uracil, cluster yields drop much slower with $n$. Appearance sizes for cluster dications are generally smaller and the cluster exhibits clear magic numbers $n = 7, 10, 13$. These results are neither in line with the expectations from dimer binding energies nor with ionization potentials of nucleobase dimers and trimers.

Preliminary studies on ion induced dissociation of Watson-Crick pairs in the gas phase have been performed. Preferential formation of clusters containing $m$ thymine molecules was observed. When substituting the thymine molecules for cytosine molecules, the distribution was found to be statistical. According to spectroscopic and theoretical studies, several nucleobases tautomers can be present in the gas phase; therefore it is of importance to spectroscopically investigate the clusters formed with our technique to clearly identify the present tautomers.