

Multiple ionization and fragmentation of the DNA base thymine by interaction with C^{q+} ions

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Abstract. Ionization and fragmentation of the DNA base thymine upon interaction with keV C^{q+} ions ($q = 1 - 6$) has been studied. By means of time-of-flight spectrometry of two or more thymine fragments in coincidence with an ejected electron we could investigate particular dissociation channels by means of their associated kinetic-energy-release. The fragmentation dynamics are strongly influenced by the C^{q+} charge state: for low q values mainly fragmentation due to direct collisions is observed. With increasing q , electron capture becomes more important. For larger q we could identify several Coulomb explosion channels, leading to very energetic fragments.

PACS. 33.15.Ta Mass spectra – 34.50.Gb Electronic excitation and ionization of molecules; intermediate molecular states (including lifetimes, state mixing, etc.) – 61.80.Jh Ion radiation effects – 82.39.Pj Nucleic acids, DNA and RNA bases – 82.80.Rt Time of flight mass spectrometry

1 Introduction

An important fraction of the effects of ionizing radiation on cells is due to modification of the cellular DNA [1]. Double strand breaks of DNA lead to the most significant biological consequences and are the main underlying mechanisms for cancer risk as well as controlled cell killing action in radiotherapy. Clusters of strand breaks and damages to DNA bases occur when radiation tracks cross DNA, for instance in heavy ion therapy with carbon ions. Most of the biological damage occurs when these ions are slowed down to velocities of a few a.u. and below. However, also in case of other types of ionizing radiation (*e.g.* α - and γ -particles), singly and multiply charged ions of low kinetic energies are formed as secondary particles. In particular core ionization of target atoms and the subsequent emission of Auger electrons can lead to multiply charged ions which in turn interact with molecules of biological relevance, such as DNA.

The DNA base thymine has benzene-like structure, where the carbon atoms 1' and 3' are replaced by N atoms. Additionally the H attached to 2' and 4' is replaced by an O atom respectively and the H attached to 5' is replaced by a methyl-group. Up to now, only very limited research on particle induced ionization/fragmentation of single DNA bases has been done.

The response of nucleic bases - which can be considered as the chromophores of DNA - to femtosecond UV photo-

ionization has recently been studied by Reuther *et al.* [2] and the lifetimes of excited states involved have been measured. In dissociative electron attachment studies on DNA bases [3], the RNA base uracil [4] as well as bromouracil [5] it was shown that even resonant capture of electrons at kinetic energies well below the ionization energy of the nucleic base can induce substantial fragmentation. The direct importance of these electrons in DNA strand breaking has also been demonstrated very recently [6]. Only few studies involving nucleic base interaction with atomic particles have been performed. Nelson and McCloskey investigated collision induced dissociation of 30 eV uracil and discovered fragmentation pathways not present in electron impact ionization [7]. Fragmentation studies of gas phase uracil by means of keV protons have been performed as well [4, 8].

In the following we present a comparison of experimental results on the interaction of gas phase thymine molecules with 4 keV/amu C^{q+} ions of different charge states ($q = 1 - 6$). The Bragg peak of carbon ions in water has its maximum at ion kinetic energies of 3 MeV and a tail down to very low energies. The energy range studied here therefore deals with collisions taking place in the low energy part of the Bragg-peak.

Relative fragmentation cross sections as well as relative cross sections for various dissociation products are obtained from the measured coincidence time of flight (TOF) spectra. The results are discussed in terms of the electronic structure of the different carbon ions.

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2 Experiment

All experiments are performed using C^{q+} ($q = 1 - 6$) ions extracted from the electron cyclotron resonance (ECR) ion source located at the KVI in Groningen. The source is floated on potentials ranging from 8 kV to 24 kV in order to obtain the same velocity for all ions except C^{1+} . The plasma of the ECR source is fed with gaseous carbon monoxide. Helium is used as a mixing gas to increase the beam current at higher charge states. To avoid charge-over-mass ambiguities between C^{3+} and He^+ as well as C^{6+} and He^{2+} , for these charge states the source is operated with ^{13}CO . Mass over charge selection is accomplished by deflection of the ion beam in a 110° magnet. A 45° magnet is used to deflect the ions from the central beam line into the setup. The base pressure in the beam transport sections is about 1×10^{-8} mbar.

The ion beam is then collimated by two 1 mm diaphragms which are 205 mm apart and focussed into the extraction region by means of an electrostatic lens system. In the collision chamber, the ion beam is crossed with a gaseous thymine target evaporated from an oven at $170^\circ C$. This ensures sufficient target density without thermal fragmentation of the thymine. Molecules effuse through a $500 \mu m$ nozzle placed at a distance of ≈ 20 mm from the collision center. To keep the base pressure in the collision chamber at 3×10^{-8} mbar, a stainless steel plate, mounted close to the collision region and opposite of the oven, is kept at liquid nitrogen temperature. This way, the contribution of residual gas species is negligible.

Charged particles are extracted from the collision region by means of an electric field (600 V/cm) provided by two 50 mm diameter stainless steel discs, mounted 10 mm apart. Field distortions due to coverage of the plates with a thick layer of insulating thymine require frequent heating of the extraction system to about $100^\circ C$ to clean the system. Positively charged collision products are extracted through a 5 mm diaphragm into a reflectron time-of-flight (TOF) spectrometer (resolution $\frac{\Delta m}{m} = 1500$ at $m = 720$ amu [9]) and detected on a multi sphere plate (MSP) detector. For masses up to the uracil parent ion ($m = 112$ amu) the detection efficiency varies by less than 0.5% [9]. In opposite direction of the fragment ions, electrons are extracted through a 5 mm diaphragm onto an MSP and detected without further discrimination. In the chopped beam mode, the trigger for the ion beam pulse also starts the TOF measurement. In the coincidence mode, the TOF measurement is started by the detection of an electron. The time-to-digital converter used (FAST 788, 1 ns resolution) allows multiple stops as necessary for electron-ion-ion coincidence measurements.

3 Results and discussion

Sets of fragment mass-spectra measured in chopped beam as well as in electron-ion coincidence mode are displayed in Figure 1. Spectra obtained with a chopped beam do not discriminate any positively charged collision products

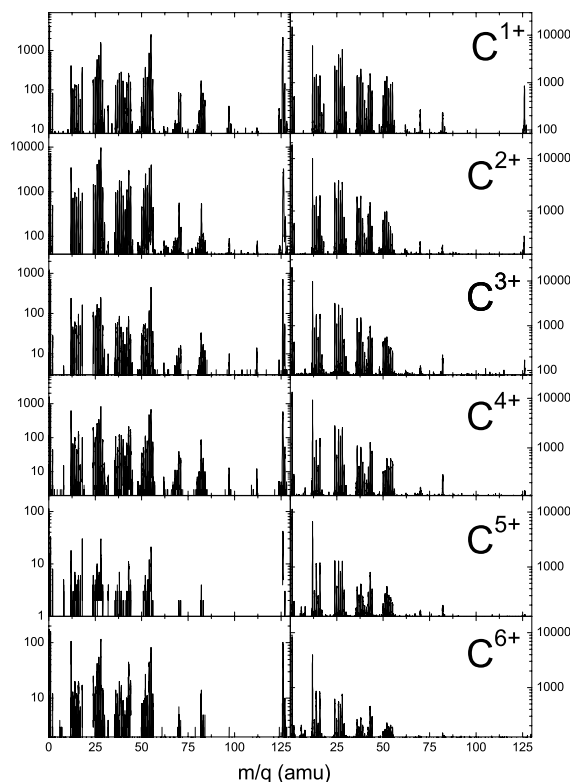


Fig. 1. Mass spectra resulting from the interaction of 4 keV/amu C^{q+} ($q = 1 - 6$) with thymine (chopped beam: left column, electron-ion coincidences: right column).

and are therefore the best characterization of the relative strengths of all interaction processes. The characteristic features of the spectra obtained with the different C^{q+} ions are quite similar. Dominating structures are the 4 groups of peaks at $m/q = 12-16$ amu, $24-28$ amu, $36-44$ amu and $50-55$ amu, representing fragments based on 1, 2, 3 or 4 heavy atoms (C, O, N) respectively. Furthermore, 2 groups of weaker peaks around 70 amu and 82 amu, as well as single peaks at 97 amu and 112 amu are observed. The parent ion at 126 amu is intense and exhibits the characteristic isotope distribution. Albeit the spectra are quite similar to our recent results with the RNA base uracil [10] the fragmentation yield is higher in the thymine case. Spectra acquired in the electron-ion coincidence mode on the other hand show an inherently incomplete picture of the fragmentation dynamics. Only those events are represented, in which at least one slow electron is emitted. In recent studies on ion-fullerene collisions we could show, that such events are mainly due to close collisions [11]. Gentle electron capture only takes place at large distances without major excitation of the target [12-14]. The latter class of collisions however, is of minor importance in biological radiation damage. In that sense, electron triggered spectra reflect the biologically relevant processes much better. From the right column of spectra of Figure 1 it is obvious that in particular the high m/q region is strongly suppressed in electron-ion coincidence spectra as compared to the non-coincidence spectra

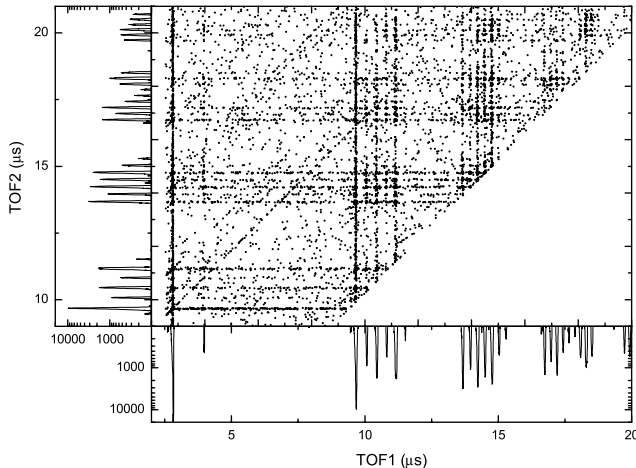


Fig. 2. A section of the triple coincidence map (electron-fragment ion-fragment ion) for interactions of 4 keV/amu $^{13}\text{C}^{3+}$ with thymine.

obtained with the chopped beam (on the left). The parent ion ($m/q = 126$) is only observed for small q values and even completely absent for $q = 5, 6$. This is due to the fact, that it is almost always formed by resonant single electron capture which is not accompanied by electron emission. Also a large fraction of the bigger fragments is due to fragmentation induced by electron capture. The differences between the left and the right column of Figure 1 therefore increase with q which reflects the increasing importance of electron capture processes. For the extreme case of $q = 6$ by far the most prominent fragments are H^+ and C^+ .

More detailed information on the electron triggered fragmentation events can be drawn from triple coincidence measurements. For each event at least two positively charged fragment ions are detected and the respective flight times are recorded. A resulting coincidence map is displayed in Figure 2 for 4 keV/amu $^{13}\text{C}^{3+}$. The window shows coincidences of ions of $m = 1 - 51$ (TOF1) with ions of $m = 12 - 55$ (TOF2). The resulting electron-ion coincidence spectrum is shown as a projection next to both axes. Horizontal, vertical and diagonal lines within the pattern are artifacts due to random coincidences. Coincidences such as *e.g.* C^+ with C^+ are suppressed due to the dead time of the detector. It is obvious from Figure 2, that maxima occur for almost all combinations of fragment ions. It is thus difficult to do a quantitative analysis. For a more thorough analysis of the patterns as a function of q we have chosen three islands within the coincidence map. The H^+ - C^+ coincidences involve the two most abundant species within the molecule. For higher q the H^+ - C^+ peak is the strongest in the coincidence map since this pair is probably formed in almost all fragmentation processes. The O atom present in the C^+ - O^+ island stems from a location on the outside of the thymine ring. Relatively high kinetic energy release (KER) values for O^+ ions have already been observed for C^{q+} interaction with uracil [10]. As a third example, C^+ - N^+ coincidences were chosen since a heavy atom within the ring is involved.

Figure 3 displays the respective islands as contour plots for $q = 1 - 6$. For all three examples an increase of the island size with q is observed. This broadening reflects the kinetic energy of the respective fragments obtained in the fragmentation process. In the extreme case, an ion with initial velocity v_0 can be emitted towards the detector (forwards, *f*) or away from it (backwards, *b*). The backwards emitted ion has to turn around in the extraction field and therefore suffers a time delay Δt . This is for example clearly seen in Figure 3a already for the C^{3+} case. The upper left part of the island is due to a backwards emitted C^+ ion and a forward emitted O^+ ion, denoted with $\text{C}_f^+/\text{O}_b^+$, whereas the lower right part is due to a $\text{C}_b^+/\text{O}_f^+$ situation. For a given ion, Δt and initial velocity v_0 are related by the formula

$$\Delta t = \frac{2mv_0}{E} \quad (1)$$

with the electric field in the extraction region E and the ion mass m . If the fragmentation process is isotropic in space, Δt is the maximum extension of an island on the respective TOF axis. Other orientations of the fragmentation axis lead to smaller time differences between the correlated fragments. The events are expected to be uniformly distributed over an island of rectangular shape if no angular correlation between triggering electron and fragment exists [15] and if the fragmentation cross section does not depend on the molecular orientation. A transmission smaller than unity could cause blocking of fragment ions emitted perpendicular to the spectrometer axis leading to a minimum of the island at small time differences. An extraction field of 600 V/cm rules out such transmission effects. However, we almost always observe a splitting of the island for a given pair. For a thymine plane oriented perpendicularly to the spectrometer axis almost no coincidences are observed. This implies that the C^{q+} -thymine interaction cross section for this orientation is small or/and electrons are emitted preferably within the thymine plane and have a small transmission for this orientation. This is particularly obvious from the H^+ - C^+ coincidences in Figure 3c: double islands are observed with a clear separation in TOF1 (H^+) and a broad distribution in TOF2 (C^+). This indicates a clear forward/backward situation for the H^+ , indicating that it is emitted in a first fragmentation step, *i.e.* in a two-body breakup process, where almost all of the kinetic energy goes into the light H^+ . The C^+ is formed in subsequent fragmentation processes and can originate from various locations within the molecule. It has a broad TOF distribution and kinetic energies from 0.3 eV ($q = 1$) to 7 eV ($q = 6$). From Figure 3c we extract the KER of the first step to lie between 6 eV ($q = 1$) and 24 eV ($q = 6$). From these numbers we can estimate the initial charge r removed from the thymine by assuming a Coulomb repulsion of the H^+ and a second charge located in the center of the thymine ring: $r \approx 2$ ($q = 1$) and $r \approx 5$ ($q = 6$).

Similar considerations hold for the C^+ - O^+ pair. Coulomb explosion of O^+ from the remaining parent ion leads to similarly high energies as for the H^+ . For this

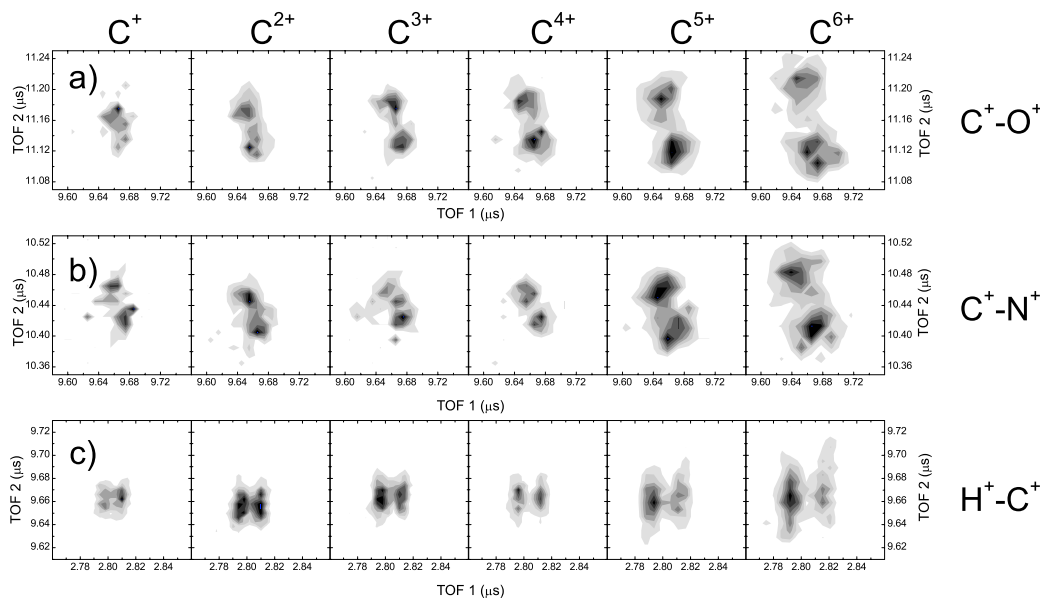


Fig. 3. Contour plots of the correlated flight times for triple coincidences. (a) $e^-C^+-O^+$; (b) $e^-C^+-N^+$; (c) $e^-H^+-C^+$. The respective projectile ion C^{q+} is given on the top.

step we extract from Figure 3a KERs of 2 eV ($q = 1$) up to 29 eV ($q = 6$) implying initial thymine charge states of $r \approx 2$ ($q = 1$) to $r \approx 6$ ($q = 6$). Here, again, the kinetic energy of the subsequently formed C^+ is much lower [0.3 eV ($q = 1$) – 1.2 eV ($q = 6$)] and depends only weakly on q .

The case of the C^+-N^+ pair, however, is different. Being a thymine ring atom the N cannot easily take part in a first-step Coulomb explosion. Indeed from Figure 3b we obtain N^+ kinetic energies from 3 eV ($q = 1$) to 16 eV ($q = 6$). Surprisingly these energies are again much higher than the corresponding C^+ values (1 eV ($q = 1$), 3 eV ($q = 6$)). An explanation could be the subsequent Coulomb explosion of a multiply charged thymine fragment. Such multiply charged fragments with intermediate masses can indeed be identified in the spectra as peaks with non-integer m/q . If a multiply charged thymine fragment is formed and an N atom is located in an exposed position, the fragment can Coulomb explode, producing a kinetic N^+ cation. The remaining fragment ion can be subject to further fragmentation leading to the observation of low energy C^+ in coincidence with N^+ .

4 Summary

By means of a multiple coincidence technique the fragmentation of thymine upon interaction with C^{q+} ($q = 1 - 6$) was studied. Comparable to our recent studies on uracil [10], we observe extensive fragmentation. In particular the dissociation into smaller fragments is accompanied by electron emission of the excited and ionized thymine complex. This allows the measurement of electron-ion-ion coincidences. From the respective correlation plots surprisingly high kinetic energies of O^+ and H^+ can be extracted which are well described by a two body Coulomb explosion in the first step of the fragmentation process. The peculiar shape of the islands in the correlation plots can be explained by angular correlation of emitted electrons and charged fragments.

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